Effect of anthocyanins and their microbiota metabolites on monocyte adhesion to endothelial cells at physiologically relevant concentrations

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▶ **To cite this version:**

Irèna Krga, Laurent-Emmanuel Monfoulet, Sylvie Mercier, Christine Morand, Marija Glibetic, et al.. Effect of anthocyanins and their microbiota metabolites on monocyte adhesion to endothelial cells at physiologically relevant concentrations. 7. International Conference on Polyphenols and Health (ICPH2015), Oct 2015, Tours, France. 322 p., 2015, 7th International Conference on Polyphenols and Health. hal-01282565

HAL Id: hal-01282565
https://hal.science/hal-01282565

Submitted on 3 Jun 2020

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WELCOMING MESSAGE

Dear friends and colleagues,

It is our great pleasure to welcome you for the 7th International Conference on Polyphenols and Health (ICPH 2015) on 27-30 October 2015 in Tours.

ICPH is a biennial international conference that has been hosted all around the world for more than 10 years. Building on the success of previous ICPHs in Vichy (2003), Davis (2005), Kyoto (2007), Harrogate (2009), Barcelona (2011) and Buenos Aires (2013), ICPH 2015 has attracted several hundreds delegates including renowned speakers from all over the world.

Polyphenols are now widely recognized as bioactive compounds of plant foods demonstrating a potential role in the protection of human health from chronic diseases through multiple mechanisms of action. Knowledge in the field of polyphenols has greatly evolved in the past years, however much remains to be done to identify the levels of intake to optimize their health benefits for everyone and to support the development of new functional foods.

ICPH is the most important international forum to present and discuss the latest trends, topics and developments in the field of polyphenols, food science and health.

We wish you a very fruitful conference and hope that you will enjoy your stay in Tours.

The Organizing Committee

Castle of Chenonceau

y Hall of Tours © Anaïs Dut

COMMITTEES

ORGANIZING COMMITTEE

- **Chair: Christine Morand (INRA - Clermont-Ferrand - France)**
- Claudine Manach (INRA - Clermont-Ferrand - France)
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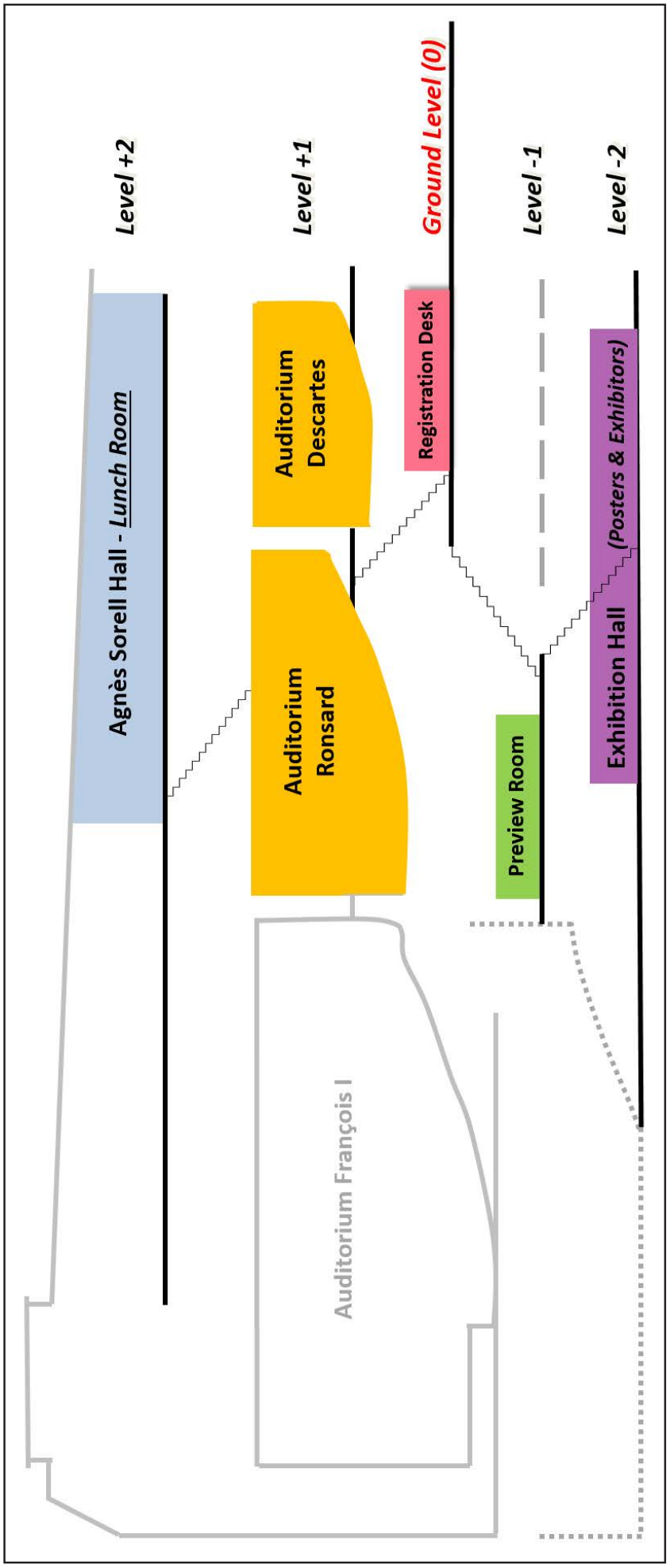
- Helmut Sies (University of Düsseldorf - Germany)
- Jeremy Spencer (University of Reading - UK)
- Junji Terao (University of Tokushima - Japan)
- Francisco Tomas-Barberan (CEBAS-CSIC of Murcia - Spain)
- David Vauzour (University of East Anglia - UK)
- Andrew Waterhouse (UC Davis - USA)
- Gary Williamson (University of Leeds - UK)

PROGRAM AT A GLANCE

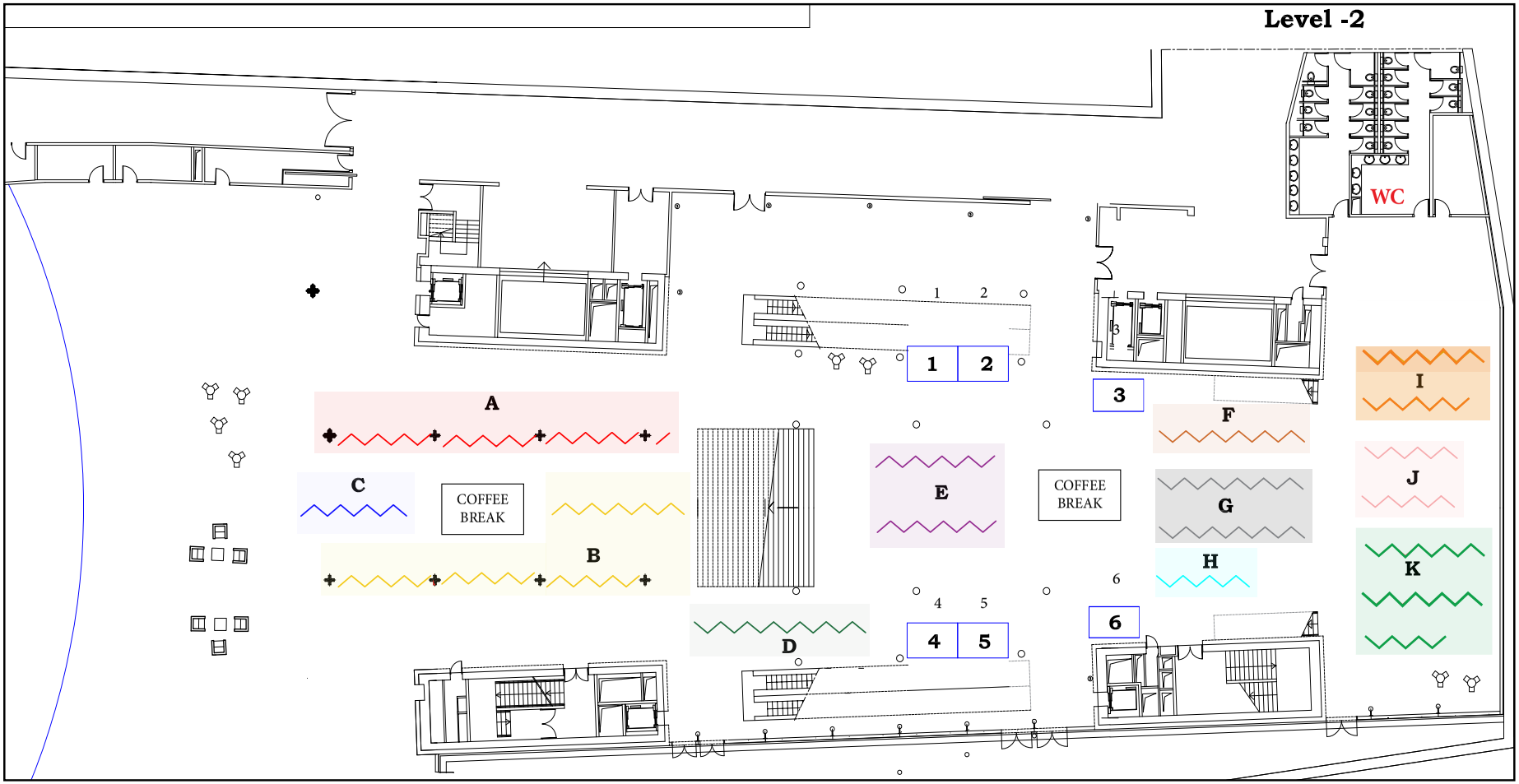
ICPH2015 - October 27-30, 2015 - Tours, France

TUESDAY		
15:00-15:30	WELCOME *Auditorium Ronsard*	
15:30-17:30	**PLENARY OPENING SESSION** *Auditorium Ronsard*	
WEDNESDAY		
08:30-10:00	**PLENARY SESSION 2** Introductory lectures for sessions A&B *Auditorium Ronsard*	
10:30-12:30	**Session A: Innovative aspects in polyphenol bioavailability and exposure assessment** *Auditorium Descartes*	**Session B: Advances in polyphenol targets for obesity, metabolic syndrome and diabetes** *Auditorium Ronsard*
	Lunch	
13:30-14:30	Poster session	
14:30-15:30	**PLENARY SESSION 3** Introductory lectures for sessions C&D *Auditorium Ronsard*	
15:30-17:45	**Session C: Neuro-cognitive effects of polyphenols: at the cutting edge** *Auditorium Descartes* PEPSICO	**Session D: Recent breakthroughs in cancer prevention** *Auditorium Ronsard*
17:45-19:00	Poster session	
THURSDAY		
08:30-10:00	**PLENARY SESSION 4** Introductory lectures for session E&F *Auditorium Ronsard*	
10:30-12:45	**Session E: Update for properties of polyphenols in cardiovascular diseases** *Auditorium Ronsard*	**Session F: Innovative approaches in polyphenol research** *Auditorium Descartes*
	Lunch	
13:45-14:30	Poster session	
14:30-15:30	**PLENARY SESSION 5** Introductory lectures for sessions G&H *Auditorium Ronsard*	
15:30-17:30	**Session G: New insights into mechanisms of action of polyphenols** *Auditorium Ronsard*	**Session H: Hot topics in polyphenols and gut microbiota interactions** *Auditorium Descartes*
17:30-18:30	Poster session	
FRIDAY		
08:30-10:00	**Flavan-3-ols and cardiovascular health - journey to dietary recommendations** *Auditorium Ronsard* Unilever	**Cranberry Polyphenols: A Nutritional Approach to Combating Antimicrobial Resistance** *Auditorium Descartes* Ocean Spray
10:30-12:30	**CLOSING SESSION** Innovation and perspectives in polyphenol research: The future is now *Auditorium Ronsard*	
12:30-13:00	AWARDS / CLOSING	

CONFERENCE CENTER PLAN



PLAN EXHIBITION HALL FOR POSTERS



Exhibitors

1 - JUICE PLUS
2 - INDENA
3 - NATUREX
4 - EXTRA SYNTHESE
5 - ACTIV INSIDE
6 - UNILEVER

Posters

Session A: Polyphenol bioavailability and exposure assessment
Session B: Polyphenols in obesity, metabolic syndrome and diabetes
Session C: Neuro-cognitive effects of polyphenols
Session D: Polyphenols and cancer prevention
Session E: Polyphenols in cardiovascular diseases
Session F: Innovative approaches in polyphenol research
Session G: Mechanisms of action of polyphenols
Session H: Polyphenols and gut Microbiota interactions
Session I: Polyphenols in other diseases
Session J: Polyphenos and Food Process

SCIENTIFIC PROGRAM

TUESDAY 27, OCTOBER

13:00-15:00 ICPH REGISTRATION

15:00-15:30 **WELCOME**
Christine Morand - ICPH 2015 chair

15:30-17:30 **PLENARY OPENING SESSION - Auditorium Ronsard**

Chairs: **Ramaroson Andriantsitohaina** - *INSERM - France*
Paul Kroon - *Institute of Food Research of Norwich - UK*

15:30 Bioavailability of polyphenols as an essential step to understanding efficacy
Gary Williamson - *University of Leeds - UK*

16:00 Dietary flavonoids and healthy ageing - recent advances from epidemiological and clinical research.
Aedin Cassidy - *University of East Anglia – UK*

16:30 Inflammaging and epigenetic age.
Paolo Garagnani - *University of Bologne – Italy*

17:00 Dietary Modulation of brain plasticity: Implications for Mental Health.
Sandrine Thuret - *King's College London – UK*

18:30 **WELCOME COCKTAIL - City Hall, Tours**

WEDNESDAY 28, OCTOBER

08:30-10:00 **PLENARY SESSION 2 - Auditorium Ronsard**

Introductory lectures for sessions A&B

Chairs: **Cristina Andres Lacueva** - *University of Barcelona - Spain*
Junji Terao - *University of Tokushima - Japan*

08:30 Polyphenol metabolism by the gut microbiota and nutrikinetics modeling.
John Duynhoven - *University of Wageningen – Netherlands*

09:00 Polyphenols and risk of new-onset diabetes in the PREDIMED trial.
Rosa Maria Lamuela-Raventos - *University of Barcelona – Spain*

09:30 Preclinical and clinical evidence for the beneficial effects of the cacao flavonols on metabolic dysregulations.
Israel Ramirez-Sanchez - *Instituto Polytecnico Nacional – Mexico*

10:00-10:30 *COFFEE BREAK*

10:30-12:30 **PARALLEL SESSION A - Auditorium Descartes**

Innovative aspects in polyphenols bioavailability and exposure assessment

Chairs: **Glaucia Maria Pastore** -*UNICAMP-Campinas - Brazil*
Peter Hollman - *Wageningen University - The Netherlands*

10:30 Metabolomic approach for improving assessment of exposure to polyphenols-rich foods through combined multi-metabolite models.
Rafael Llorach - *University of Barcelona - Spain*

11:00 Effects of biotransformation and food matrix on the bioactivities of polymethoxyflavones.
Hang Xiao - *University of Massachusetts - USA*

Selected communications

11:30 Multiple-approach studies to assess anthocyanin gastric absorption – potential role of GLUT1.
Iva Fernandes - *University of Porto – Portugal*

11:45 Role of sulfotransferases in resveratrol bioavailability and adipocyte metabolism.
Nele Gheldof - *Nestlé Institute of Health Sciences - Switzerland*

12:00 Neuroprotective potential of phenolic sulfates, abundant bioavailable metabolites.
Claudia Nunes dos Santos - *iBET - Portugal*

12:15 Linking metabolism and bioactivity of curcumin.
Claus Schneider - *Vanderbilt University Medical School – USA*

10:30-12:30 **PARALLEL SESSION B - Auditorium Ronsard**

Advances in polyphenols targets for obesity, metabolic syndrome and diabetes

Chairs: **Rosa Maria Lamuela-Raventos** - *University of Barcelona - Spain*
Israel Ramirez Sanchez - *Instituto Polytécnico Nacional - Mexico*

10:30 A novel mechanism for the anti-diabetic action of green tea polyphenols.
Jeonga Kim - *University of Alabama - USA*

11:00 Fruit polyphenols improve type 2 diabetes and the metabolic syndrome through alleviating inflammation and modulating the gut microbiota.
André Marette - *University of Laval - Canada*

Selected communications

11:30 Regularly consuming a green/roasted coffee blend shows benefits against metabolic syndrome.
Laura Bravo - *ICTAN-CSIC – Spain*

11:45 Flavan-3-ols and n-3 fatty acids prevent non-alcoholic fatty liver disease in a high fat-high fructose fed mouse model.
Anne M Minihane - *University of East Anglia - UK*

12:00 Estrogen receptor α triggers most of the beneficial effect of red wine polyphenols intake of obesity-induced metabolic and vascular disorders.
Raffaella Soleti - *INSERM - France*

12:15 The metabolism enhancement effect of cyanidin-3-glucoside through skeletal muscle, adipocyte and their communications.
Toshiya Matsukawa - *University of Tsukuba – Japan*

12:30-13:30 *LUNCH*

13:30-14:30 **POSTER SESSION**

14:30-15:30 **PLENARY SESSION 3 - Auditorium Ronsard**

Introductory lectures for sessions C&D

Chairs: **Cesar Fraga** - *Universidad de Buenos Aires - Argentina*
Barbara Shukitt-Hale - *University of Tufts - USA*

14:30 Potential mechanisms by which flavonoids mediate improvements in cognitive function. *Lecture sponsored by Pepsico*
Jeremy Spencer - *University of Reading - UK*

PEPSICO

15:00 Epidemiological evidence for protection against gastrointestinal cancers by polyphenol intake.
Raul Zamora-Ros - *International Agency for Research on Cancer – France*

15:30-17:45 **PARALLEL SESSION C SPONSORED BY PEPSICO - Auditorium Descartes**

Neuro-cognitive effects of polyphenols: at the cutting edge

Chairs: **Jeremy Spencer** - *University of Reading - UK*
Sandrine Thuret - *Institute of Psychiatry, Psychology and Neuroscience of London - UK*

15:30 Polyphenols found in berry fruit improve age-associated changes in cognitive function and brain inflammation.
Barbara Shukitt-Hale - *University of Tufts - USA*

16:00 Cognitive and cerebral blood-flow effects of polyphenols in humans.
David Kennedy - *University Northumbria, UK*

Selected communications (non-sponsored)

16:30 Characterization of brain-bioavailable bioactive polyphenol metabolites in pre-symptomatic Alzheimer's disease.
Giulio Maria Pasinetti - *Icahn School of Medicine at Mount Sinai – USA*

16:45 The acute effects of a polyphenol-rich blueberry beverage on cognitive function in healthy younger and older adults.
Georgina Dodd - *University of Reading – UK*

17:00 Beneficial effects of the Neurophenol extract on age-related cognitive decline.
Julien Bensalem - *Activ'Inside – France*

17:15 Green tea promotes positive effects on redox status of different regions in the central nervous system of obese and lean rats.
Rosemari Otton - *Universidade Cruzeiro do Sul – Brazil*

17:30 Phenolic acid intake, delivered via moderate Champagne wine consumption, improves spatial working memory via the modulation of hippocampal and cortical protein modulation in aged animals.
David Vauzour - *University of East Anglia - UK*

15:30-17:45 **PARALLEL SESSION D - Auditorium Ronsard**

Recent breakthroughs in cancer prevention

Chairs: **Augustin Scalbert** - *International Agency for Research on Cancer - France*
Kayoko Shimoï - *University of Shizuoka - Japan*

15:30 Advances in cancer chemoprevention – lessons from resveratrol.
Karen Brown - *University of Leicester - UK*

16:00 Stress, breast cancer and flavonoids - A role of quercetin conjugate as a beta2-adrenergic receptor antagonist.
Kayoko Shimoï - *University of Shizuoka - Japan*

Selected communications

16:30 Dietary polyphenols and cancer incidence: a comprehensive systematic review and dose-response meta-analysis.
Giuseppe Grosso - *University of Catania – Italy*

16:45 Preclinical efficacy of pterostilbene against human melanoma: a stress-related indirect mechanism reconciles bioavailability and anticancer activity in vivo.
Jose M. Estrela - *Faculty of Medicine Valencia – Spain*

17:00 Analysis of Normal and Malignant Colon Tissue Specific MicroRNAs in Response to Dietary Intervention with a Pomegranate Extract: Modulatory Effects vs. Potential Artefacts.
María Teresa García Conesa - *CEBAS-CSIC of Murcia – Spain*

17:15 Exploring the anti-tumoral effect of citrus polyphenols using a 3D cell model of colorectal cancer.
Ana Teresa Serra - *iBET – Portugal*

17:30 Bioavailability and bioactivity of raspberry phytochemicals using in vivo digestion coupled to ex vivo simulated colonic fermentation.
Chris Gill - *Ulster University - Ireland*

17:45-19:00 *REFRESHMENTS* - **POSTER SESSION**

THURSDAY 29, OCTOBER

08:30-10:00 **PLENARY SESSION 4 - Auditorium Ronsard**

Introductory lectures for sessions E&F

Chairs: **Kevin Croft** - *University of Western Australia - Australia*
Ramaroson Andriantsitohaina - *INSERM - France*

08:30
Dietary flavanols, endothelial homeostasis and cardiovascular health: results from FP7 FLAVIOLA project.
Christian Heiss - *University of Düsseldorf - Germany*

09:00
Beneficial effects of phenolic compounds on HDL functionality.
Montserrat Fito - *Institute de investigacion medica de Barcelona - Spain*

09:30
Innovative targets and innovative routes in the study of dietary polyphenol putative biological activity.
Daniele Del Rio - *University of Parma - Italy*

10:00-10:30 *COFFEE BREAK*

10:30-12:45 **PARALLEL SESSION E - Auditorium Ronsard**

Update for properties of polyphenols in cardiovascular diseases

Chairs: **Christian Heiss** - *University of Düsseldorf - Germany*
Montserrat Fito - *Institute de investigacion medica de Barcelona - Spain*

10:30
Flavonols and their metabolites in the vascular wall.
Francisco Perez-Vizcaino - *Universidad Complutense of Madrid - Spain*

11:00
Cardiovascular benefits of dietary polyphenols.
Kevin Croft - *University of Western Australia - Australia*

Selected communications

11:30 Increased microparticle production and impaired microvascular endothelial function in aldosterone-salt-treated rats: protective effects of polyphenols.
Ramaroson Andriantsitohaina - *INSERM - France*

11:45 Comparative acute effects of soy isoflavones and orange juice-derived flavanones on markers of cardiovascular risk in men at moderate risk of cardiovascular disease.
Peter Curtis - *University of East Anglia – UK*

12:00 Sustained effects of wild blueberry consumption on vascular function in healthy individuals.
Rodrigo Féliciano - *University of Düsseldorf - Germany*

12:15 Increased bioavailability of phenolic acids and enhance vascular response following intake of an enzymatically generated, high free ferulic acid flatbread.
Alice Turner - *University of Reading – UK*

12:30 Do anthocyanins in purple tomatoes reduce the risk of cardiovascular disease?
Sebastian Achterfeldt - *Institute of Food Research – UK*

10:30-12:45 **PARALLEL SESSION F - Auditorium Descartes**

Innovative approaches in polyphenol research

Chairs: **Daniele Del Rio** - *University of Parma - Italy*
Claudine Manach - *INRA Clermont-Ferrand - France*

10:30 Shotgun Metabolomics and lipidomics for discovery of polyphenol bioactivity.
Jan Frederik Stevens - *University of Oregon State - USA*

11:00 Novel controlled-release technologies to enhance efficacy of polyphenolics.
Ramesh Gupta - *University of Louisville - USA*

Selected communications

11:30 Identification of the metabolomic fingerprint associated with flavonoid consumption level.
Kerry Ivey - *Harvard T. H. Chan School of Public Health – USA*

11:45 Fluid shear stress: a modulator of the vascular protective effects of curcumin?
Laurent-Emmanuel Monfoulet - *INRA Clermont-Ferrand– France*

12:00 Exploring the biological activity of flavonoid metabolites.
Colin Kay - *University of East Anglia – UK*

12:15 A novel matrix rich in flavan-3-ols: hazelnut skin. From phytochemical characterization to evaluation of biological activities.
Pedro Mena - *University of Parma – Italy*

12:30 C. elegans as a model to elucidate the mechanisms of action of flavonoids: a transcriptomic approach.
Celestino Santos-Buelga - *Universidad de Salamanca – Spain*

12:45-13:45 *LUNCH*

13:45-14:30 **POSTER SESSION**

14:30-15:30 **PLENARY SESSION 5 - Auditorium Ronsard**

Introductory lectures for sessions G&H

Chairs: **Alan Crozier** - *University of california - UK*
Patricia Oteiza - *University of California - USA*

14:30 New insights on the complex molecular mechanisms of polyphenols through nutri(epi)genomics.
Dragan Milenkovic - *INRA Clermont-Ferrand - France*

15:00 Human gut microbiota metabolism of ellagic «acid». Interindividual variability for urolithins production is correlated with health status: cause or consequence.
Juan Carlos Espin - *CEBAS-CSIC of Murcia – Spain*

15:30-17:30 **PARALLEL SESSION G - Auditorium Ronsard**

New insights into mechanisms of action of polyphenols

Chairs: **Mayte Garcia Conesa** - *CEBAS-CSIC of Murcia - Spain*
Dragan Milenkovic - *INRA Clermont-Ferrand - France*

15:30 Dietary flavonoids induce autophagic degradation in macrophages.
Yoshichika Kawaï - *University of Tokushima - Japan*

16:00 Procyanidin-membrane interactions in the regulation of cell signaling.
Patricia Oteiza - *University of California - USA*

Selected communications

16:30 Soy isoflavones and membrane steroid receptors: a new horizon.
Vladimir Ajdžanović - *Institute for Biological Research "Siniša Stanković" – Serbia*

16:45 Delphinidin inhibits tumor growth by acting on VEGF signalling in endothelial cells.
Claire Lugnier - *INSERM – France*

17:00 Potent inhibition of VEGF activity by pomegranate polyphenols: A novel mechanism for the atheroprotective effects observed in epidemiology.
Rebecca Edwards - *Institute of Food Research – UK*

17:15 (Poly)phenols from quasi-isogenic raspberries: attenuation of neuroinflammation.
Gonçalo Garcia - *iBET – Portugal*

15:30-17:30 **PARALLEL SESSION H - Auditorium Descartes**

Hot topics in polyphenols and gut microbiota interactions

Chairs: **Francisco Tomas-Barberan** - *CEBAS-CSIC of Murcia - Spain*
David Vauzour - *University of East Anglia - UK*

15:30 A role for polyphenols in shaping the structure and function of the gut microbiome.
Kieran Tuohy - *Foundation Edmund Mach of Trento – Italy*

16:00 Dietary modulation of gut microbial metabolome.
Wendy Russell - *Rowett Institute of Nutrition and Health - UK*

Selected communications

16:30 Identification of novel flavonoid O- and C-glycosidases in gut bacteria.
Annett Braune - *German Institute of Human Nutrition Potsdam-Rehbruecke – Germany*

16:45 Quantification of microbial polyphenol uptake, kinetics and metabolism using mass spectrometry.
Nikolai Kuhnert - *Jacobs University Bremen – Germany*

17:00 Importance of the colonic microbiota in the bioavailability of orange juice (poly)phenols: in vivo and in vitro studies.
Gema Pereira-Caro - *Andalusian Institute of Agricultural and Fishering Research and Training – Spain*

17:15 Short chain fatty acids affect hesperetin transport and phase II metabolism in Caco-2 cells.
Evelien Van Rymenant - *Ghent University – Belgium*

17:30-18:30 *REFRESHMENTS* - **POSTER SESSION**

18:30 **GALA DINNER** - *Departure in bus in front of the Congress Center*

FRIDAY 30, OCTOBER

08:30-10:00 **PARALLEL SPONSORED SESSION BY UNILEVER - Auditorium Ronsard**

Flavan-3-ols and cardiovascular health - Journey to dietary recommendations

08:30 Introduction
Aedin Cassidy - *University of East Anglia - UK*

08:35 Review of current science on tea flavan-3-ols.
Peter Hollman - *Wageningen University - Netherlands*

08:55 Review of totality of the evidence for flavan-3-ols.
Janet Novotny - *U.S. Department of Agriculture - USA*

09:15 Process for achieving dietary recommendations & current gaps in flavan-3-ol evidence.
Mario Ferruzzi - *Purdue University - USA*
Lecture sponsored with the support of ILSI NORTH AMERICA

09:35 Panel of discussion moderated by **Aedin Cassidy**

08:30-10:00 **PARALLEL SPONSORED SESSION BY OCEAN SPRAY - Auditorium Descartes**

Cranberry Polyphenols: A Nutritional Approach to Combating Antimicrobial Resistance

08:30 Antimicrobial Resistance and Urinary Tract Infections.
Kalpana Gupta - *Boston University School of Medicine, USA*

08:50 Cranberry Polyphenols and Bacterial Mechanisms.
Christina Khoo - *Ocean Spray's Director of Research Science, USA*

09:10 Bioactive Dietary Polyphenols, Cardiometabolic and Cognitive Outcomes.
Peter Howe - *University of Newcastle, Australia*

09:30 Chemo-protective effectiveness of cranberry phenolic fractions.
Luis Goya - *ICTAN-CSIC, Spain*

10:00-10:30 *COFFEE BREAK*

10:30-12:30 **CLOSING SESSION - Auditorium Ronsard**

Innovation and perspectives in polyphenols research: The future is now

Chairs: **Christine Morand** - *INRA Clermont-Ferrand - France*
Gary Williamson - *University of Leeds - UK*

10:30 The exposome in cancer epidemiology – Polyphenols as a case study.
Augustin Scalbert - *International Agency for Research on Cancer - France*

11:00 Polyphenols, cardiovascular disease and health claims.
Paul Kroon - *Institute of Food Research – Norwich, UK*

11:30 Microbiome - a window into food microbe - host interactions.
Joël Doré - *INRA - Jouy en Josas, France*

12:00 The polyphenols and health dialogue: the genome is the interpreter.
Jose Ordovas - *Tufts University - Boston, USA*

12:30-13:00 **AWARDS / CLOSING**

GUIDELINES FOR SCIENTIFIC COMMUNICATIONS

All participants are invited to come and view the posters, which will be displayed in the Exhibition Hall (level -2) from **Tuesday 27th October to Friday 30th October.**

Session	Title	Abstract
A	Polyphenol bioavailability and exposure assessment	P001-P043
B	Polyphenols in obesity, metabolic syndrome and diabetes	P100-P165
C	Neuro-cognitive effects of polyphenols	P200-P215
D	Polyphenols and cancer prevention	P300-P325
E	Polyphenols in cardiovascular diseases	P400-P433
F	Innovative approaches in polyphenol research	P500-P521
G	Mechanisms of action of polyphenols	P600-P641
H	Polyphenols and gut Microbiota interactions	P700-P712
I	Polyphenols in other diseases	P800-P832
J	Polyphenols and Food Process	P900-P927
K	Other topics	P1000-P1047

The presenting authors should be in front of their poster as scheduled:

	13:30 to 14:30	17:30 to 19:00
Wednesday 28th	Sessions A & I	Sessions B, F & K
Thursday 29th	Sessions C, E & H	Sessions D, G & J

Guidelines for poster presenters

Please refer to the abstract book (Authors index) to find your poster number and fix your poster on the board marked with the same number (refer to the plan of the exhibition hall).
Tacks and technical equipment will be available for hanging the posters. Please be aware that you should be in front of your poster during the time of the session assigned (see schedule above).

Guidelines for invited speakers and selected oral presentations

Only Powerpoint presentations will be accepted. The use of personal laptop is not allowed.
You are kindly requested to go to the Preview room at the level -1 (see plan). Your presentation will be checked and transferred from the preview room to the computer in your presentation auditoriums.
We kindly recommend that you go to the preview room the day before your presentation. If this is not possible, please provide your presentation the morning before your presentation as early as possible. Please make sure to be in the auditorium at least 10 minutes before the beginning of the session to meet the chairs.

Guidelines for chairs

Please make sure to be in the auditorium at least 10 minutes before the beginning of the session. We are very grateful to you for ensuring that the timing assigned to the session and to each presentation is respected.

SOCIAL PROGRAM

WELCOME COCKTAIL

Tuesday 27th October - 18:30

Welcoming cocktail for all ICPH attendees at the Tours City Hall kindly offered by the City of Tours.
5 minute walk from the Congress center.

TOURS

GALA DINNER (upon registration)

Thursday 29th October

Gala dinner will take place at the Clos Lucé Castle - Renaissance meal

> Departure by bus in front of the Congress Center at 18:30

> Return time at 23:30 at the Congress Center

Clos Lucé Castle (last residence dwelling place of Leonardo da Vinci)

The spirit of Leonardo da Vinci remains in the Château du Clos Lucé, mingling with the memory of all the famous guests who contributed to the richest times enjoyed by the building. Times for royalty, times for genius, times for chivalry and times for bloodshed, looking back over a place loaded with history ...

The dinner

Sieur Sausin will introduce you to Renaissance cuisine. He will explain cooking with spices in the Mediaeval period, and the variety of flavours during the Renaissance and at the time of Catherine de Medici. «Lovers of fine meats, eager explorers of taste – bon voyage»

FOR ACCOMPANYING PERSON

The Tourism Office of Tours is located close to the Congress Center.
Many visits are organized, see the website: www.tours-tourism.co.uk

GENERAL INFORMATION

PRACTICAL INFORMATION

Venue and time
7th International Conference on Polyphenols and Health will start on Tuesday 27th October at 15:00 and will end on Friday 30th October at 13:00.
Vinci Center Congress
26 Boulevard Heurteloup
37042 TOURS CEDEX 1
www.tours-evenements.com

Conference Secretariat
M&O Agency +33 (0)6 73 61 32 87
Isabelle Prévot
Clémence Lemaigre
Sandra Eisenberg
Hostesses will be at your disposal during the conference for help you if necessary.
We thank the Lycée Saint-Ursule (Tours) for their precious collaboration.

Registration Desk and Hours
The registration desk is located at the ground level (see the plan) of the Vinci Center.
Registration will be open:

Tuesday 26th October	13:00-18:00
Wednesday 27th October	08:00-18:00
Thursday 28th October	08:00-18:00
Friday 29th October	08:00-13:00

Badges
All delegates and speakers are kindly requested to wear their name badge during all Conference activities.
The entrance to the venue is restricted to registered participants wearing their name badge.

Conference badges are distinguished as follows:

Delegates	No color
Invited Speakers	Red
Scientific Committee	Purple
Organising Committee	Orange
Exhibitors	Blue
Accompanying person	Brown

Certificate of Attendance
A certificate of attendance can be obtained upon request from the registration desk.

Message
Messages for delegates may be left at the Registration desk for display on the message board.

Wifi
Delegates can access the internet using the Vinci Center's Wifi network.
The Wifi is available on the Ground Level (0), on the Agnès Sorell Hall (level +2) and on the Exhibition Hall (level -2).

Liability and insurance
When registering for the 7th International Conference on Polyphenols and Health, delegates agree that the organizers do not assume any liability whatsoever. It is highly recommended that all participants carry their proper travel and health insurance.

Lunches

Wednesday 28th October	Sitting buffet in the Agnès Sorell Hall (Level +2)
Thursday 29th October	Sitting buffet in the Agnès Sorell Hall (Level +2)
Friday 30th October	Lunch box will be distributed at the Ground Level

PUBLICATION OF ICPH 2015 COMMUNICATIONS
Speakers selected for short communications at the 7th ICPH have been invited to publish a paper in a special issue of the journal Archives Biochemistry and Biophysics (ABB) – Helmut Sies and Christine Morand are the guest editors of this special issue on « Polyphenols and Health ».

CONFERENCE AWARDS

ICPH 2015 is pleased to announce the following awards for young investigators and students:

Travel awards
20 awards: 500€ each
Abstracts submitted by Travel Award applicants for the 7th International Conference on Polyphenols and Health have been reviewed and scored by the organizing and scientific committees. The applicants receiving the highest scores have been given financial support to attend the meeting and present their work.

Poster prizes
One ABB (Archives Biochemistry and Biophysics) prize and 7 ICPH prizes, 250€ each
All legible posters (submitted by young scientists and students) will be reviewed and scored by the ICPH 2015 scientific committee and renowned scientists. The 8 posters with the highest scores will be awarded the poster prizes.

All awards will be presented at the closing session on Friday 30th October.

OUR SPONSORS

The organizing committee would like to warmly thank the following companies for their support.

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BIOGRAPHIES

PLENARY OPENING SESSION - Tuesday 27 October, 2015 - From 15:30 to 17:30

Gary Williamson - Biography

Professor and Chair at the University of Leeds, UK; previously Head of Group at Nestle Research Center, Lausanne, and Institute of Food Research, Norwich. Research interests in how dietary polyphenols are absorbed and the mechanisms by which they reduce the risk of cardiovascular disease/diabetes.

Has published more than 330 peer-reviewed scientific papers, and is ISI/Thomson Reuters highly cited researcher with h index of 73. Has supervised 46 previous and current PhD students, and involved in over 25 human intervention studies. Holder of a prestigious European Research Council Advanced Grant, Fellow of the Royal Society of Chemistry, previously Editor-in Chief of the Royal Society of Chemistry journal, Food and Function, and organiser and chairman of 4th ICPH in Harrogate, UK.

His talk - Bioavailability of polyphenols as an essential step to understanding efficacy.

The bioavailability and bioefficacy of polyphenols is intricately linked. The pathways and molecular mechanisms of absorption of polyphenols will be summarised, together with how this affects biological activity in vivo and in vitro. The focus will be on the underlying principles governing absorption from the small intestine, and how polyphenols affect diabetes risk by acting at various sites in the body.

Aedin Cassidy - Biography

Aedin Cassidy's research focuses on understanding the impact of plant bioactives on cardiometabolic health. She completed her PhD and post-doctoral work at Cambridge University. Prior to joining UEA she was Head of Molecular Nutrition in the Biosciences Division of Unilever Research, UK.

She has been awarded the Nutrition Society Medal (1999), 'Outstanding Contribution to Soy Research' in Tokyo, Japan for her contribution to isoflavone research (2008), Finalist, Times Higher Education Awards for 'International Collaboration of the Year' 2013, awarded 'Flavonoid Research Award for her contribution to Clinical research on Flavonoids' at the International Conference on Polyphenols, Buenos Aires, Argentina (2013). She was awarded the Royal Society Wolfson Research Merit Award in 2013.

Her talk - Dietary flavonoids and healthy ageing - recent advances from epidemiological and clinical research.

Recent findings identifying the relative importance of the different flavonoids present in the habitual diet to a range of health outcomes (with a focus on cardiometabolic health) will be presented, together with the emerging evidence from clinical trials and risk biomarkers.

Paolo Garagnani - Biography

Dr. Paolo Garagnani was born in Bologna (Italy) in May 1973.

In 2003 Master Degree in Biology at the University of Bologna (UNIBO) and in May 2007 he obtained the PhD in Biodiversity and evolution. Since 2007 is deeply involved on several research projects focused on the identification of genetic, epigenetic and glycomic biomarkers of longevity, ageing and age-related diseases.

From September 2011 is the scientific manager of a research grant, sponsored by Pfizer Pharmaceutical Industry, on the "Identification of genetic determinants of Psoriatic Arthritis". From October 2012 he is shareholder of the Personal Genomics enterprise, whose prevalent market activity is in the fields of: i) genetic biomarker of age-related disease, ii) in the application of next generation sequencing techniques to personalized genomics and iii) in the point-of-care solutions for nucleic acid based diagnostic testing. Dr. Garagnani is author of 41 papers in peer review Journals.

His talk - Inflammaging and epigenetic age.

Dr. P. Garagnani will present a quick overview of inflammaging theory, a more detailed description of the age-related epigenetic changes in humans. Finally, he will present an overview on the possible on how polyphenols can interact with inflammaging and age related epigenetic derangement towards anti-ageing strategies.

Sandrine Thuret - Biography

Dr. Thuret is a neuroscientist with a background in food science, molecular, cellular, behavioural and ageing biology. She has been appointed Lecturer in 2011 following on her RCUK Academic Fellow in Neural Stem Cell Research award and is the head of the Nutrition, Neurogenesis and Mental Health laboratory at King's College London, Institute of Psychiatry, Psychology & Neuroscience. She graduated from the University of Heidelberg with a PhD in neuroscience studying the development of dopaminergic neurons.
She then did her postdoctoral work at the Salk Institute with Prof. F.H. Gage, CA, USA, where she investigated the role of stem cells in the mammalian central nervous system.

Her talk - Dietary Modulation of brain plasticy: Implications for Mental Health.

Research over the last 10 years has firmly established that learning and memory abilities as well as mood can be influenced by diet. Although the underlying mechanisms by which diet modulates mental health are not well understood. One of the brain structures associated with learning, memory and mood is the hippocampus. Interestingly, the hippocampus is one of the two structures in the adult brain where the formation of newborn neurons -or neurogenesis- persists. The level of neurogenesis in the adult hippocampus has been linked directly to cognition and mood: In the rodent, an increase of neurogenesis in the hippocampus is associated with improved learning/memory abilities, whereas a decrease is associated with symptoms of depression. Therefore modulation of adult hippocampal neurogenesis by diet emerges as a possible mechanism by which nutrition impacts on mental health. During this talk I will present evidence of cognition and mood being modulated by dietary parameters, which are also responsible for adult hippocampal neurogenesis regulation. This will inform a discussion on the important translational concept that diet, a modifiable lifestyle factor, holds the ability to modulate brain plasticity and function.

PLENARY SESSION 2 - Wednesday 28 October, 2015 - From 8:30 to 10:00

Introductory lectures for sessions A&B

John Van Duynhoven - Biography

John van Duynhoven is heading the Spectroscopy & Imaging group at Unilever Vlaardingen where he develops and deploys a wide range of analytical techniques for the Unilever food and nutrition R&D programme. In 2010 he was appointed as part-time professor at Wageningen University.

His current research activities are in application of analytical methods for resolving complex food compositions and structure as well as assessment of the metabolic impact of diet on humans. In these areas he has initiated and led a range of academic-industrial collaborative projects such as in the Netherlands Metabolomics Centre.

His talk - Polyphenol metabolism by the gut microbiota and nutrikinetics modeling.

In order to study the complex interactions between dietary polyphenols, the gut microbiota and the human host, he developed and implemented a suite of enabling profiling/identification/quantification platforms and nutrikinetic modelling tools. This allowed us to capture the large chemical diversity of circulating conjugated polyphenol catabolites. Thus he could pave the way for personalization of polyphenol-rich diets based on gut microbial functionality of individuals or populations.

Rosa Maria Lamuela-Raventos - Biography

Dr. Lamuela-Raventos is Associate Professor at the Department of Nutrition and Food Science, in University of Barcelona, and leader of a CIBEROBN group. She has more than 200 papers with and h-index of 47.

Rosa is currently working in important European and National projects, related to the nutritional interest of food bioactive compounds.

Her talk - Polyphenols and risk of new-onset diabetes in the PREDIMED trial.

Increasing evidence demonstrates a positive relationship between consumption of polyphenol-rich foods and chronic disease prevention. Some epidemiological, in vitro and animal studies support the beneficial effects of dietary polyphenols on glucose homeostasis, thus they could be used in the prevention of diabetes. She has found inverse associations between different polyphenols subgroups and the incidence of diabetes within the PREDIMED trial.

Israel Ramirez-Sanchez - Biography

Ph. D. by the National Autonomous University of Mexico. Postdoctoral training in the laboratory of Dr. Villarreal at the School of Medicine of the University of California, San Diego. Research is focused on the effects of the flavanol (-)-epicatechin on several biological processes involved in obesity and metabolic disorders, in humans as wells as in animal models.

His talk - Preclinical and clinical evidence for the beneficial effects of the cacao flavonols on metabolic dysregulations.

Obesity has been associated with increase in cardiometabolic risk (CMR). CMR-therapeutic interventions such lifestyle changes and/or pharmacologic agents are often limited by poor compliance and/or side effects. Consumption of dietary products, such as cacao products, exerts positive effects on CMR factors. (−)-Epicatechin (EPI), the most abundant flavonoid in cacao has been reported to replicate such effects. He is focused on the mechanisms by which EPI exerts such beneficial effects.

PARALLEL SESSION A - Wednesday 28 October, 2015 - From 10:30 to 11:30

Innovative aspects in polyphenols bioavailability and exposure assessment.

Rafael Llorach - Biography

Dr. Rafael Llorach is assistant professor in the Biomarkers & Nutrimetabolomic Research Group (XARTA, INSA, Campus Torribera, Pharmacy School, University of Barcelona). He got a PhD with European mention in Biology (CEBAS-CSIC/University of Murcia). He performed postdoctoral studies in the Human Nutrition Unit at the INRA in Clermont-Ferrand (France) and the Aberystwyth University (UK). Currently, his main research focuses on the application of the nutrimetabolomics strategies to study the metabolome changes in response to dietary exposure, with particular attention in identifying potential markers of food intake (Food Metabolome).

His talk - Metabolomic approach for improving assessment of exposure to polyphenols-rich foods through combined multi-metabolite models.

The main aim of this presentation is to show the state of the art of the identification of biomarkers related to the polyphenols-rich foods intake such as walnuts, cocoa or wine. Throughout this presentation, it will be shown that multi-metabolite models are a precise, specific and accurate measurement of polyphenols-rich foods intake as nutritional biomarkers, which seek to be an objective and accurate tool for determining the dietary exposure.

Hang Xiao - Biography

Dr. Hang Xiao is an Associate Professor at the Department of Food Science at the University of Massachusetts. He received a PhD degree in Food Chemistry from the University of Wisconsin-Madison, and had a post-doc training at Rutgers, the State University of New Jersey.

His current research is focused on biological activities and molecular mechanisms of bioactive food components in disease prevention. He has published more than 80 manuscripts in peer-reviewed journals. Dr. Xiao has received multiple research awards such as Institute of Food Technologist (IFT) Samuel Cate Prescott award, American Society for Nutrition (ASN) Mary Swartz Rose Investigator Award, and International Life Sciences Institute (ILSI) North America Future Leader Award.

His talk - Effects of biotransformation and food matrix on the bioactivities of polymethoxyflavones.

Orally ingested flavonoids undergo complex physiochemical changes in the gastrointestinal tract, which in turn dictates the biological activities of flavonoids. This talk offers an insightful summary on the biological fate of polymethoxyflavones, a unique class of flavonoids found in citrus fruits, in the gastrointestinal tract, and its impact on their bioactivities.

PARALLEL SESSION B - Wednesday 28 October, 2015 - From 10:30 to 11:30

Advances in polyphenols targets for obesity, metabolic syndrome and diabetes

Jeonga Kim - Biography

Jeonga Kim is Assistant Professor at division of Endocrinology, Diabetes, and Metabolism - *University of Alabama at Birmingham, England.*

My research focuses on the beneficial effects and mechanisms for the metabolic actions of green tea polyophenol, EGCG.

Her talk - A novel mechanism for the anti-diabetic action of green tea polyphenol.

A green tea polyphenol, epigallocatechin gallate (EGCG) ameliorates high fat diet-induced insulin resistance and endothelial dysfunction. We recently observed that EGCG stimulates autophagy which contributes to reduction of lipid accumulation. I will present more detailed mechanisms by which EGCG stimulates autophagy and its consequences in intracellular lipid metabolism.

André Marette - Biography

Dr. André Marette is full professor in the department of Medicine and Scientific Director of the Institute of Nutrition and Functional Foods at Laval University, Québec, Canada. He is an international expert on the pathogenesis of inflammation, type 2 diabetes and cardiovascular diseases in obesity.

He has published over 165 papers in high-impact journals and 20 reviews or book chapters, and received several awards including the prestigious Charles Best Lectureship Award of the University of Toronto in recognition for his outstanding contribution to diabetes research.

His talk - Fruit polyphenols improve type 2 diabetes and the metabolic syndrome through alleviating inflammation and modulating the gut microbiota.

Dr. Marette will speak about the mechanisms by which fruit polyphenols improve type 2 diabetes and the metabolic syndrome. He will present evidence that polyphenols exert their beneficial actions on inflammation and intestinal homeostasis through the regulation of the gut microbiota.

PLENARY SESSION 3 - Wednesday 28 October, 2015 - From 14:30 to 15:30

Introductory lectures for sessions C&D

Jeremy Spencer - Biography

Prof. Spencer received his PhD from King's College London in 1997 and is currently Professor of Nutritional Biochemistry and Medicine at the University of Reading. His initial work focused on the cellular and molecular mechanisms underlying neuronal death in Parkinson's and Alzheimer's disease. His recent interests concern how flavonoids influence brain health through their interactions with specific cellular signaling pathways pivotal in protection against neurotoxins, in preventing neuroinflammation and in controlling memory, learning and neuro-cognitive performance.

His talk - Potential mechanisms by which flavonoids mediate improvements in cognitive function.

His presentation will focus on the acute effects of flavonoids and flavonoid-rich foods on human executive function (attention, sustained attentiveness and task responsiveness) and how such effects may be mediated by changes in peripheral and cerebrovascular blood flow and by potential direct modulation of neuronal and glial signalling.

Raul Zamora-Ros - Biography

Raul Zamora-Ros, International Agency for Research on Cancer, Lyon, France

Raul Zamora-Ros qualified in both Nutrition and Food Science and also obtained a master on Statistics and Epidemiology. He earned his Doctorate from the University of Barcelona in 2008.

He did his PosDoc in the Catalan Institute of Oncology, in the International Agency for Research on Cancer (IARC) and in the MRC Epidemiology Unit, University of Cambridge, before joining his current position as a visiting scientist in the Biomarker group, Nutrition and metabolism unit, IARC. He is interested in whether dietary factors, particularly polyphenols and polyphenol-rich foods, are causally associated with the development of chronic diseases. He has published over 50 peer-review articles and above 10 book chapters in his area.

His talk - Epidemiological evidence on polyphenols and the risk of gastrointestinal cancers.

The aim of this talk will be to summarize available epidemiological data on the health effects of polyphenol classes, particularly flavonoids, on the risk of gastrointestinal cancers, such as esophageal, gastric, colorectal and liver cancers.

PARALLEL SESSION C - Wednesday 28 October, 2015 - From 15:30 to 16:30

Neuro-cognitive effects of polyphenols: at the cutting edge - sponsored by PEPSICO

Barbara Shukitt-Hale - Biography

Dr. Barbara Shukitt-Hale is the USDA Lead Scientist in the Laboratory of Neuroscience and Aging, USDA-ARS, Human Nutrition Research Center on Aging (HNRCA) at Tufts University in Boston, MA. She received her Ph.D. in Experimental Psychology from Boston University in 1993.

Dr. Shukitt-Hale has been involved in research for 30 years. Her current work involves determining the factors responsible for age-related behavioral and neurochemical changes and possible amelioration of these effects with various nutritional treatments. She has published more than 200 articles and selected papers.

Her talk - Polyphenols found in berry fruit improve age-associated changes in cognitive function and brain

Research has demonstrated, in both human and animals, that cognitive functioning decreases with age, to include deficits in processing speed, executive function, memory, and spatial learning. The cause of these functional declines is not entirely understood; however, neuronal losses and the associated changes in the activity of neurotransmitters, secondary messengers, and their receptors may be caused by long term increases in and susceptibility to oxidative stress and inflammation. Therefore, one approach to improving neuronal functioning might be to alter the neuronal environment to reduce the impact of oxidative and inflammatory stressors. Research conducted in our laboratory has shown that consumption of fruits and vegetables high in polyphenolics can prevent and even reverse age-related cognitive deficits. The polyphenolic compounds found in these foods may exert their beneficial effects indirectly, through their ability to lower oxidative stress and inflammation, or directly, by altering neuronal structure and signaling involved in neuronal communication. Therefore, dietary interventions with polyphenolic-rich foods may be one strategy to forestall or even reverse age-related neuronal deficits.

David Kennedy - Biography

David O. Kennedy, PhD, is Professor of Biological Psychology and the Director of the Brain Performance and Nutrition Research Centre at Northumbria University in the United Kingdom (www.nutrition-neuroscience.org).

His own research involves the effects of nutritional interventions, including vitamins and minerals, omega-3 fatty acids, amino acids, and a host of plant-derived extracts and compounds, including polyphenols and caffeine, on human brain function. His recently published book, Plants and the Human Brain (Oxford University Press), describes the psychopharmacology and plant ecological 'secondary metabolite' roles of a multitude of plant-derived compounds.

His talk - Cognitive and cerebral blood-flow effects of polyphenols in humans.

Polyphenols may exert a number of effects within cellular signal transduction pathways that are relevant to the beneficial modulation of brain function. Previous research involving healthy non-elderly adults conducted in our own laboratory has established acute cognitive improvements following cocoa-flavanols, and demonstrated consistent increases in cerebral blood-flow and the modulation of haemodynamic responses to task performance in the frontal cortex following single doses of the stilbene resveratrol. These latter effects were seen in the absence of any evidence of improved cognitive function. These results and data from a current trial will be discussed.

PARALLEL SESSION D - Wednesday 28 October, 2015 - From 15:30 to 16:30

Recent breakthroughs in cancer prevention

Karen Brown - Biography

Karen Brown (BPharm, PhD) is Professor of Translational Cancer Research in the Department of Cancer Studies at the University of Leicester. Her work centres on the development of therapeutic agents for the prevention of cancer, particularly improving the strategies employed for identifying active agents, along with accompanying pharmacodynamic biomarkers, and optimising the design of early phase clinical trials.

Her interests cover both naturally derived compounds such as resveratrol and curcumin, and repurposed drugs. Karen is also Co-Chair of the UK Therapeutic Cancer Prevention Network that brings together scientists, clinicians and epidemiologists with the overall aim of rapidly advancing effective agents from the bench to bedside and then widespread use in high-risk populations.

Her talk - Advances in cancer chemoprevention - lessons from resveratrol.

Trials of drugs such as tamoxifen and celecoxib provide proof of principle that the prevention of cancer through pharmaceutical intervention is feasible and cost effective; however, their use in this context is severely hampered by an increased risk of serious side effects. Diet-derived compounds with good safety profiles are considered attractive alternatives to synthetic drugs but despite extensive preclinical data indicating that phytochemicals and micronutrients can protect against cancer, these findings have largely failed to translate to successful clinical outcomes. In an attempt to improve the development process for dietary chemopreventive agents, using resveratrol as a model compound for the prevention of colorectal cancer, we have addressed issues of poor bioavailability and the role of metabolites, how to choose the optimal dose for clinical translation and the influence of dietary fat on efficacy.

Kayoko Shimoi - Biography

She is professor at the Laboratory of Biochemistry and Toxicology, Graduate Division of Nutritional and Environmental Sciences - *University of Shizuoka, Japan.*

- Research Assistant Professor (1983) - *Shizuoka College of Pharmacy.*
- Research Assistant Professor, School of Food and Nutritional Sciences (1991) - *University of Shizuoka.*
- Associate Professor, Institute for Environmental Sciences (2001) - *University of Shizuoka.*
- Professor, Institute for Environmental Sciences (2005) - *University of Shizuoka.*
- Professor, Graduate Division of Nutritional and Environmental Sciences (2013) - *University of Shizuoka.*

Her talk - Stress, breast cancer and flavonoids - A role of quercetin conjugate as a beta2-adrenergic receptor antagonist.
Effects of quercetin-3-O-glucuronide (Q3G), a circulating metabolite of quercetin on β2-adrenergic receptor-mediated responses such as invasion in human breast cancer MDA-MB-231 cells will be shown and a new role of Q3G in stress-induced breast cancer progression will be discussed.

PLENARY SESSION 4 - Thursday 29 October, 2015 - From 8:30 am to 10:00 am

Introductory lectures for sessions E&F

Christian Heiss - Biography

PD Dr. Heiss is an attending physician, interventional cardiovascular specialist, and heads the vascular medicine section of the Division of Cardiology, Pulmonology and Vascular Medicine, University Duesseldorf.
His research interests include the (patho)physiology of endothelial dysfunction and therapeutic approaches with dietary bioactives in the context of primary and secondary prevention.

His talk - Dietary flavanols, endothelial homeostasis, and cardiovascular health: Results from FP7- FLAVIOLA project.
Dietary flavanols and procyanidins in general, and cocoa flavanols in particular, have gained major public attention over the past years. However, it is noteworthy that the published studies were almost exclusively undertaken in populations at increased cardiovascular disease risk or manifest cardiovascular disease. Consequently, there exists a dearth of data from controlled dietary interventions in healthy individuals of both sexes and across a broader age-range, which limits our ability to comprehensively assess the potential role of dietary flavanols and procyanidins in the context of health and nutrition, evidence-based dietary guidance, and primary prevention. The FLAVIOLA research project entitled 'Targeted delivery of dietary flavanols for optimal human cell function: Effects on cardiovascular health' was funded by the European Union's 7th framework program to address these gaps. The lecture will provide an overview of the clinical outcomes obtained in FLAVIOLA.

Dr. Montserrat Fito - Biography

She is Head of - Cardiovascular Risk and Nutrition Research Group (CARIN)
- IMIM-Hospital del Mar Medical Research Institute
- CIBEROBN of Obesity Physiopathology and Nutrition

Dr. Montserrat Fitó, Medical Doctor, PHD in Medicine, Specialist in Clinical Analyses. Her main field of work is in: nutrition and protective/risk factors for cardiovascular disease, with special focus on the Mediterranean diet, olive oil, wine, and their phenolic compounds. She has worked in several nutritional intervention studies with olive oil, as well as with dietary patterns.

Her talk - Beneficial effects of phenolic compounds on HDL functionality

My talk will treat about the diet polyphenols and high density lipoprotein (HDL) funcionality. Beyond the cardiovascular protection afforded by a high concentration of HDL-cholesterol, the improving of the HDL function may explain the benefits. A healthy diet, rich in single- and poly-phenol antioxidant compounds, may contribute to improve the HDL biological properties. A polyphenol-rich olive oil intake has resulted in an increment in the HDL cholesterol efflux, in the monolayer fluidity, an enhancement of the HDL composition, an increment of the large-HDL and a decrease of the small ones, and incorporation to the HDL of the biological metabolites of olive oil phenolic compounds. The olive oil phenolic compounds linked to HDL or surrounding the lipoprotein, confer an improvement in the oxidative status and functionality of the particle.

Daniele Del Rio - Biography

Daniele Del Rio, is an Associate Professor of Human Nutrition at the University of Parma. He is running the Laboratory of Phytochemicals in Physiology at the Department of Food Science and is the co-founder of the LS9 «Bioactives & Health» Interlaboratory Group, where the biological activity of human and microbiota derived phytochemical metabolites represents one of the core research topics. Daniele is an Honorary Visiting Scholar at the UK Medical Research Council Human Nutrition Research Unit in Cambridge, a Visiting Fellow of the Wolfson College, University of Cambridge and the Scientific Director of the Need for Nutrition Education/ Innovation Programme (NNEdPro), an independent knowledge generation and research platform overseen by the British Dietetic Association. Dan is also the Commissioned Reviews Editor of the Journal of Human Nutrition and Dietetics (the official Journal of the British Dietetics Association) and Associate Editor of the International Journal of Food Sciences and Nutrition. Daniele has been recently listed among the Thomson-Reuters Highly Cited Researchers.

His talk - Innovative targets and innovative routes in the study of dietary polyphenol putative biological activity

The presentation will deal with novel mechanisms of action of polyphenolic metabolites in the framework of chronic disease experimental models, with a particular focus on the cardiovascular context. Hepatic, microbial, and systemic metabolites will be taken into consideration, highlighting many pitfalls in the process used to date to unravel potential biological activities attributed to polyphenolic substances. In vitro, as well as in vivo results will be presented.

PARALLEL SESSION E - Thursday 29 October, 2015 - From 10:30 to 11:30

Update for properties of polyphenols in cardiovascular diseases

Francisco Perez Vizcaino - Biography

Francisco Perez Vizcaino (born 1966, BPharm 1989, PhD 1992) is Professor of Pharmacology and Vice-dean of Research at the School of Medicine, Universidad Complutense de Madrid. He has authored 137 indexed papers (cumulative impact factor ≈ 600, h-index 31).

His scientific career has been devoted to vascular pharmacology and pathophysiology. He has studied the vascular effects of flavonoids and its role in endothelial dysfunction and hypertension.

His talk - Flavonols and their metabolites in the vascular wall.

The talk will address the new advances in flavonols and flavanols on arterial health, focusing on their effects on arterial tone, endothelial function, NADPH oxidase and NFR2 and the role of flavonoid metabolites and their deconjugation.

Kevin Croft - Biography

Prof Kevin Croft is a Professorial Fellow in the School of Medicine and Pharmacology at the University of Western Australia. He is co-director of the Biomedical Mass Spectrometry unit and has active research interest in nutritional biochemistry, biomarkers of oxidative damage, the pathogenesis of atherosclerosis and the mechanism of vascular protection by dietary polyphenols.

He serves as the editor-in-chief of the Royal Society of Chemistry journal Food and Function. He has over 250 peer reviewed publications. He was on the advisory group that put together the National Heart Foundation of Australia position statement on "Antioxidants in foods, drinks and supplements for cardiovascular health" in 2010.

His talk - Cardiovascular benefits of dietary polyphenols.

He will focus on the evidence from clinical trials that dietary polyphenols can improve cardiovascular health by reducing blood pressure and improving vascular function. He will also discuss possible mechanisms for these cardiovascular effects from studies in cultured human endothelial cells and mouse models of heart disease.

PARALLEL SESSION F - Thursday 29 October, 2015 - From 10:30 to 11:30

Innovative approaches in polyphenol research

Jan Frederik Stevens - Biography

Jan Frederik Stevens received his M.Sc. in pharmacy (1988), pharmacy license (1990), and Ph.D. in medicinal chemistry (1995) from Groningen University, The Netherlands. He received postdoctoral training at Oregon State University (1995-1999), the Free University of Amsterdam (1999-2000), and the Leibniz Institute for Plant Biochemistry, Halle/Saale, Germany (2000-2002). He is now a Professor of Medicinal Chemistry in the College of Pharmacy and a Principal Investigator in the Linus Pauling Institute at Oregon State University. Mass spectrometry-based metabolomics is a new direction in his laboratory for discovery of biological effects of vitamins and phytochemicals.

His talk - Shotgun Metabolomics and lipidomics for discovery of polyphenol bioactivity.

Dietary polyphenols exert a broad spectrum of beneficial effects in various models of disease, but the underlying mechanisms of action are still not well understood. His laboratory explores the use of untargeted, mass spectrometry-based metabolomics and lipidomics approaches to elucidate mechanisms of polyphenol bioactivity. This lecture will demonstrate how anti-obesity mechanisms of the hops polyphenol, xanthohumol, can be deduced from metabolomic and lipidomic profiles.

Ramesh Gupta - Biography

Dr. Gupta is Professor and Endowed Chair at the James Graham Brown Cancer Center, University of Louisville, Louisville, USA. His major research focus is on prevention and treatment of lung, breast and cervical cancers with natural compounds, including polyphenolics, alone and in combination with standard chemotherapeutic drugs. Polymeric implants and natural exosomes are being used to circumvent the oral bioavailability issues of polyphenolics.

His talk - Novel controlled-release technologies to enhance efficacy of polyphenolics.

Dr. Gupta will discuss strategies to enhance health benefits of polyphenolics by incorporating innovative controlled-release drug delivery technologies.

PLENARY SESSION 5 - Thursday 29 October, 2015 - From 2:30 pm to 3:30 pm

Introductory lectures for sessions G&H

Dragan Milenkovic - Biography

Dr Dragan Milenkovic is senior research scientist at the French Institute of Agricultural Research (INRA). He received his master degree in genetics and his PhD from University of Versailles (France) in molecular genetics. The aim of his research is identification of molecular and cellular mechanisms underlying cardiovascular protective effect of bioactive plant compounds, particularly polyphenols. This research is based on nutrigenomic analyses, including of polyphenols in animal models of atherosclerosis as well in clinical trials in humans. He is currently developing studies on the impact of polyphenol metabolites on endothelial cell function and interaction with immune cells.

His talk - New insights on the complex molecular mechanisms of polyphenols through nutri(epi)genomics.

It is now accepted that polyphenols exert their health properties by exerting nutrigenomic effects. This talk will present complex nutri(epi)genomic effects of polyphenols underlying particularly their cardiovascular protective effects, including their impact on cell signalling pathways, expression of genes and miRNA as well as epigenetic modulations.

Juan Carlos Espín - Biography

Juan Carlos Espín completed his PhD at the University of Murcia (1997) and his postdoctoral training at ATO-DLO (The Netherlands) in 2000. He is full Research Professor in CEBAS-CSIC (Murcia), belonging to the Spanish National Research Council (CSIC). His research mainly focuses on understanding the role of dietary polyphenols in human health. His h-index is 46 and has been cited over 6.000 times.

His talk - Human gut microbiota metabolism of ellagic acid. Interindividual variability for urolithins production is correlated with health status: Cause or consequence?

The polyphenol ellagic acid is metabolized by the gut microbiota to yield urolithins, small bioavailable molecules that exert anti-inflammatory activity. Not all individuals produce the same metabolic profile of urolithins. Subjects can be stratified into three urolithin-producing groups, the so-called phenotypes A, B and 0. Interestingly, phenotype B is more abundant in overweight-obese people, metabolic syndrome and colorectal cancer and seems to be related to gut dysbiosis. These phenotypes could show differences in the gut microbioma associated to the health status and should be considered in intervention trials dealing with health benefits of ellagitannins or ellagic acid. Whether this phenotypic variation could be a biomarker related to differential health benefits or illness predisposition deserves further research.

PARALLEL SESSION G - Thursday 29 October, 2015 - From 15:30 to 16:30

New insights into mechanisms of action of polyphenols

Yoshichika Kawaï - Biography

He is Associate Professor at the Univerisity of Tokushima in the Laboratory of Food Science.
From 2011 to 2015, he was Associate Professor at Nagoya University, in 2008 and 2009, he was Special Volunteer at NHLBI/NIH and from 2003 to 2011, Yoshichika Kawaï was Assistant Professor at the University of Tokushima.

His talk - Dietary flavonoids induce autophagic degradation in macrophages.

I will present our recent finding that structurally related flavonoids could induce the gene expression of autophagy-related and lysosomal genes in macrophages.

Patricia Oteiza - Biography

Patricia I. Oteiza is a Professor at the Departments of Nutrition and of Environmental Toxicology at the University of California, Davis, USA. She obtained her Doctorate degree in Biochemistry from the University of Buenos Aires Argentina. Her research interests are focused on the impact of diet on early development and on chronic disease states. With regard to flavonoids, using molecular modeling, biophysics and molecular biology approaches, she studies the basic mechanism of action of flavan-3-ols and procyanidins, in particular those involved in their capacity to act as anti-inflammatory, antioxidant, and anticancer compounds.

Her talk - Procyanidin-membrane interactions in the regulation of cell signaling.

We have previously observed that procyanidins, basically composed of more than 4 (-)-epicatechin subunits, can modulate oxidant production and cell signaling in intestinal epithelial cells. These procyanidins are not absorbed as intact compounds, and can still exert biological actions through their interactions with the cell membrane. I will present our latest findings showing the specificity of those interactions, and discussing their consequences on membrane associated processes, downstream signaling and cell fate.

PARALLEL SESSION H - Thursday 29 October, 2015 - From 15:30 to 16:30

Hot topics in polyphenols and gut microbiota interactions

Kieran Tuohy - Biography

KIERAN TUOHY received his PhD from the University of Surrey (UK) in 2000. Between 2000 and 2006 he worked as a post doctoral researcher in the Food Microbial Sciences Unit of Professor Glenn Gibson, University of Reading and in 2006, was appointed lecturer in the Department of Food Science and Nutrition, University of Reading. He now leads the Nutrition and Nutrigenomics Group at Fondazione Edmund Mach, Trento, Italy. His research focuses on the health effects of diet:microbe interactions within the gastrointestinal tract and has expertise in microbial ecology, fermentation technologies, nutrition, functional food design and testing, metabolomics and metagenomics. He has over 84 international, peer reviewed publications in the area of gut microbiology, a H index of 23, and is co-editor of the book "Diet-Microbe Interactions in the Gut", Elsevier, in press.

His talk - A role for polyphenols in shaping the structure and function of the gut microbiome

Plant polyphenols have long been considered to play an important role in the health promoting effects of foods and diets rich in whole grain cereals, fruit and vegetables. Due to their complex structure, glycated native forms, and the fact that they are often complexed within lowly digested food matrices and fiber compounds, the majority of ingested polyphenols reach the large intestine, home to the colonic microbiome. The colonic microbiome, comprises the bulk of the gut microbiota and is now thought of as an integral part of human physiology, if not an organ in its own right capable of important physiological effects locally in the gut and systemically, throughout the body via the neuroendocrine system, immune system and the flux of metabolites absorbed from the gut. Although, it has been recognised for some years that the intestinal microbiome plays an important role in the biological availability and biological activity of ingested polyphenols, recent studies are also showing that these polyphenols themselves may actively shape microbiome structure and function. This presentation will summarise recent findings demonstrating microbiome modulation by plant derived polyphenols and discuss the implications for human health and our understanding of the mechanisms of effect of whole plant foods and their derivatives.

Wendy Russell - Biography

Wendy Russell is a principal investigator in natural product chemistry at the University of Aberdeen; Rowett Institute of Nutrition and Health researching the complex interplay between diet and health. With a BSc in Chemistry and PhD in Natural Product Chemistry (University of Bristol) she has specialised in phytochemical analysis, metabolism, bio-availability and bio-activity. Current research focuses on establishing the metabolic phenotype for different population groups and the impact of dietary modulation. This work has involved several human dietary interventions including macronutrient modulation, weight loss, animal vs. plant protein, benefits of plant-based diets and the nutritional control of type 2 diabetes.

Her talk - Dietary modulation of gut microbial metabolome.

The gut microbiota plays an important role in the development of many human disorders (obesity, type-2-diabetes, cardiovascular disease and cancer). Products of microbial metabolism are considered to have an important impact on immune function and inflammation, yet these, and the bacteria responsible for their formation are mostly unknown. This talk will focus on results obtained from tightly controlled human dietary interventions, where we have shown that consumption of plant products, rich in phytophenols are likely to be an important factor in maintaining gut and systemic health.

CLOSING SESSION - Friday 30 October, 2015 - From 10:30 to 12:30

Innovation and perspectives in polyphenol research: The future is now

Augustin Scalbert - Biography

Dr. Augustin Scalbert is head of the Biomarkers Group at the International Agency for Research on Cancer (IARC) in Lyon, France (www.iarc.fr). His current research focuses on the discovery of novel biomarkers of exposure for cancer risk factors through metabolomic approaches and their implementation in cohort studies to improve understanding of the mechanisms by which diet and metabolic factors affect cancer risk. His research on plant polyphenols has received large attention from the scientific community. He has been identified as a Highly Cited Researcher (Thomson Reuters, 2001, 2014). He has notably developed Phenol-Explorer, the first comprehensive database on polyphenols in foods and their metabolism. He was the founder of the International Conference on Polyphenols and Health.

His talk - The exposome in cancer epidemiology – Polyphenols as a case study.

Cancer aetiology is largely governed by lifestyle and environmental factors. Exposome-wide association studies opens exciting avenues to identify novel risk factors for cancer and better understand the aetiology of these diseases. Dietary polyphenols measured in large-scale epidemiological studies will be taken as an example to illustrate possibilities offered by these agnostic approaches.

Paul Kroon - Biography

Dr Paul Kroon is a Research Leader at the Institute of Food Research and is head of the Polyphenols and Health group. His group focusses on basic research to understand the mechanisms by which dietary polyphenols are absorbed and exert health benefits.

This knowledge is also used to inform the development of foods with health benefits that are supported by scientific evidence. Dr Kroon is coordinator of the EU FP7 BACCHUS project which is focussed on developing the science to support health claims for food products containing bioactive peptides and polyphenols (http://www.bacchus-fp7.eu/). He is an editor for the Molecular Nutrition and Food Research and Nutrition and Ageing journals and has published more than 130 refereed publications and book chapters and currently has a H-index of 43.

His talk - Polyphenols, cardiovascular disease and health claims.

He will cover the following:

- What is the strength of scientific evidence supporting the notion that there are cardiovascular health benefits associated with consuming dietary polyphenols?
- What is the landscape for health claims in Europe, and how well have polyphenol-based health claims fared?
- What are the challenges to successful health claims for polyphenols, and how can these be overcome?
- How is the EU FP7 BACCHUS project addressing the issues facing polyphenol-based health claims?

Joël Doré - Biography

Joël Doré, PhD, Scientific Director of the Industrial Demonstrator MetaGenoPolis (mgps.eu), Institut National de la Recherche Agronomique, Jouy-en-Josas, France. Research Director at INRA, Joël is Scientific Director of MetaGenoPolis, a Unit of the Micalis Institute "Food and Gut Microbiology for Human Health".

Joel joined INRA in 1983. He trained as gut microbial ecologist and developed expertise in intestinal metagenomics towards diagnostic and prognostic applications as well as a window into food-microbe-host interactions. He aims to contribute to a better understanding of the intestinal ecosystem in order to support therapeutic choices in the medical area, as well as science-based recommendations in health nutrition. Joël Doré chairs the gutmicrobiotaforhealth.com scientific web-platform.

His talk - Microbiome - a window into food microbe-host interactions.

In spite of our inability to culture many of the dominant microbes of the human intestine, functional metagenomics open this new window for the exploration of fine interactions between food constituents, microbiota and host, which leads to the identification of enzymes, signals and intimate mechanisms of crosstalk between bacteria and food constituents as well as between bacteria and human cells. One outcome is personalization of medicine and nutrition.

Jose Ordovas - Biography

Jose M. Ordovas Ph.D., is Professor of Nutrition at the Friedman School of Nutrition Science and Policy, Professor of Genetics at the Sackler School of Graduate Biomedical Sciences at Tufts University and Senior Scientist at the USDA Human Nutrition Research Center on Aging at Tufts University in Boston Massachusetts where he is also the Director of the Nutrition and Genomics Laboratory. He is a Senior Collaborating Scientist at the CNIC, and IMDEA Alimentation, both in Madrid (Spain).

Dr. Ordovas' major research interests focus on the genetic factors predisposing to cardiovascular disease and obesity and their interaction with the environment and behavioral factors with special emphasis on diet. He has published about 700 scientific articles in peer review journals and written numerous reviews and books on these topics.

His talk - The polyphenols and health dialogue: the genome is the interpreter.

The polyphenols and health dialogue: the genome is the interpreter.

During the last few decades we have gained substantial knowledge about genes associated with the most common age-related diseases and their risk factors (i.e., CVD, diabetes, plasma lipids, obesity, blood pressure, cancer). Variants at these genes and others still to be uncovered define our predisposition to disease; however, the expression of this legacy depends on the environment to which we are exposed and our own habits. Multiple reports describing gene-environment interactions support this concept and whereas these interactions have included the analysis of environmental factors such as tobacco smoking, physical activity, drugs, etc., the most commonly researched environmental factor has been nutrition. Hence, there are hundreds of studies showing statistically significant interactions between genetic polymorphisms and various components of the diet including plant bioactives, such as polyphenols, modulating disease risk factors and even disease itself. Consolidating this knowledge is key to move into personalized medicine and effective disease prevention approaches.

7th ICPH International Conference on Polyphenols and Health

INVITED LECTURES

PARIS

TOURS

October 27-30, 2015

PLENARY OPENING SESSION - *Auditorium Ronsard*

I01
Bioavailability of (poly)phenols as an essential step to understanding efficacy

Gary Williamson
University of Leeds, Leeds, UK. Email: g.williamson@leeds.ac.uk

The pathways of absorption and metabolism of the most common dietary (poly)phenols are now mostly understood. The enzymes involved, their site of action, essential transporters and exact conjugating enzymes have been characterised, and *in vitro* mechanistic experiments have been supported by numerous studies, using healthy volunteers, ileostomists, intestinal perfusion, and animal models. Given this increased understanding in recent years, it should now be possible to gain insight into the reasons for inter-individual differences in bioavailability, which include dietary habits, genetic disposition and microbiota composition.

Since the types of metabolites in blood have been identified and some synthesised, we can more carefully design *in vitro* experiments and translate the findings into human intervention studies. We have demonstrated, for example, that quercetin-3′-*O*-sulfate is an effective inhibitor of xanthine oxidase *in vitro*, and that quercetin supplementation, bioequivalent to ~100 g red onion daily, for one month, decreases plasma uric acid (the product of xanthine oxidase) by 8% in mildly hyperuricemic males.

In addition to the biological activity of (poly)phenol conjugates, we propose that the action of (poly)phenols *in vivo* includes modulating nutrient absorption and metabolism. High glucose concentrations show deleterious cellular effects such as mitochondrial dysfunction and lipid accumulation in hepatic cells, and are thought to mediate much of the damage in (pre-)-diabetes, where both fasting blood glucose and post-prandial glucose are elevated. Certain dietary (poly) phenols attenuate blood glucose after a high carbohydrate meal, and this is shown using *in vitro* optimised enzyme and ^{14}C-sugar transport assays, together with studies *in vivo* on healthy volunteers consuming a high-(poly)phenol fruit spread together with a carbohydrate source. In addition, the absorbed (poly)phenols also help to repair or prevent the damage cause by high glucose. The combination of effects are essential for the overall actions of dietary (poly) phenols, and determining these types of effects require understanding of both the metabolism of (poly)phenols and the mechanisms of interactions with nutrients. The end result is maintenance of normal body metabolism and reduction in the risk of developing type 2 diabetes.

Financial support: ERC advanced grant 322467; EU BACCHUS 312090; EU PlantLIBRA 245199; British Commonwealth; China Scholarship Council

I02
Recent advances in flavonoids and health – evidence from prospective studies and clinical trials

Aedín Cassidy
Department of Nutrition & Preventive Medicine, Norwich Medical School, University of East Anglia, UK

The impact of flavonoids on health requires an integrated scientific approach drawing together our growing knowledge base on absorption, metabolism, impact on risk biomarkers and incident disease (from prospective studies and optimally designed randomised clinical trials) and investigating the physiological and molecular mechanisms of action of key metabolites. Recent data from prospective cohort studies highlight the beneficial impact of habitual intakes of specific sub-classes on both biomarkers of cardiovascular risk and disease outcomes including myocardial infarction and type 2 diabetes. Emerging data suggest that intake of specific flavonoids may also help with weight maintenance, depression, lung function and erectile dysfunction. In contrast, limited associations are currently being observed between habitual intakes of the different sub-classes and cancer risk in prospective studies. The focus of the presentation will be on the developing evidence base for flavanones and anthocyanins, the two sub-classes present in fruits/vegetables commonly consumed in the diet, comparing clinical trial data with the available data from epidemiological studies.

Although recent database advances have allowed researchers to more accurately capture the diverse range of flavonoids present in the diet than previous studies there is measurement error in dietary assessment and wide variability in the flavonoid content of foods so the key next step is to develop validated biomarkers which integrate intake and the subsequent extensive metabolism these compounds undergo following ingestion. The parent flavonoid compounds present in the diet are unlikely to be responsible for bioactivity; instead this is likely to be mediated by metabolites present in the systemic circulation. Absorption occurs in both the small and large intestine and the microflora are likely to play a key metabolic role, catabolising unabsorbed flavonoids into smaller molecules including phenolic and aromatic acids, which can then also be absorbed. Whether the microbiome impacts on the health effects of flavonoids directly or via their effects on metabolism is currently unknown and is a major focus of current research.

Data from the available trials conducted to date provide evidence to suggest that when flavonoids are consumed there is extensive inter-individual variability in the levels of metabolites measured in biological samples. This heterogeneity in responsiveness to intake may relate to a number of factors, including variability in Phase 1 and Phase 2 metabolism, but the microbiome is likely to be critical as it plays a key role in flavonoid metabolism. This wide inter-individual variability in metabolism suggests that metabolism may be critical in explaining the differential responses observed in clinical trials (responders v non-responders).

Dietary intervention trials (acute and chronic) are now needed to understand the importance of flavonoid metabolism (and the key role played in this by the microbiome) in clinical efficacy. These studies will also be critical in identifying and developing validated and robust biomarkers of flavonoid intake/metabolism that can be used to examine associations of bioavailable flavonoids with health outcomes in future prospective cohort studies. These studies are critical for more clearly defining the relevance of different flavonoids to public health.

I03
Inflammaging and epigenetic age.

Paolo Garagnani
University of Bologna, Italy

INFLAMMAGING can be conceptualized as a chronic auto-inflammatory process triggered and sustained by progressive increase with age of the production of self-generated inflammatory stimuli (self-garbage) accompanied by the increased exposure to bacterial (e.g. gut microbiome) and viral (e.g. CMV and other persistent infections) inflammatory stimuli. Inflammaging can be assumed as a paradigmatic example of unifying Bio-Medical hypothesis being involved in the aging process as well as in most if not all chronic age-related diseases, all sharing a common inflammatory pathogenesis. Besides inflammaging another major molecular feature of ageing is the epigenetic remodelling. In these years we devoted effort to study the age related changes in the DNA methylation architecture in human being. We focused our attention to informative human models including centenarians. In particular we studied whole genome DNA methylation data (HumanMethylation450 platform) on whole blood from donors representing two opposite model of aging, *i.e.* semi-supercentenarians (105+) and their offspring (decelerated aging) and Down syndrome persons (accelerated aging) and their unaffected siblings. I will also present whole genome DNA methylation data of human livers (used for transplants) from donors of different age (age range: 12-90 years). Finally, I will present data on stochastic epigenetic mutations (SEMs), i.e. those epimutations that randomly arise in the genome, and that are not shared among subjects. Using a newly developed analytical strategy able to detect rare SEMs in methylation data (Infinium HumanMethylation27 and HumanMethylation450 platforms) applied to a cohort of 178 subjects ranging from 3 to 106 years, I'll present data showing that the number of SEMs is low in childhood and increases exponentially during aging. In females the number of SEMs is significantly correlated to the degree of X chromosome inactivation skewing, this correlation being independent from age. These unexpected results allow to propose the new hypothesis that the mechanism underpinning the age-related X chromosome inactivation skewing is the accumulation of SEMs. It worth noting that there is a growing body of evidences that showed that polyphenols, especially those from fruits, vegetables and plant-derived beverages, can counteract both inflammation and epigenetic remodeling. Accordingly I will present an overview on polyphenols as potential modulator of these two major features of aging process.

I04
Dietary Modulation of brain plasticity: Implications for Mental Health

S. Thuret
*Institute of Psychiatry, Psychology & Neuroscience, Department of Basic and Clinical Neuroscience, King's College London, United Kingdom. *Sandrine.1.thuret@kcl.ac.uk*

Research over the last 10 years has firmly established that learning and memory abilities as well as mood can be influenced by diet. Although the underlying mechanisms by which diet modulates mental health are not well understood. One of the brain structures associated with learning, memory and mood is the hippocampus. Interestingly, the hippocampus is one of the two structures in the adult brain where the formation of newborn neurons -or neurogenesis- persists. The level of neurogenesis in the adult hippocampus has been linked directly to cognition and mood: In the rodent, an increase of neurogenesis in the hippocampus is associated with improved learning/memory abilities, whereas a decrease is associated with symptoms of depression. Therefore modulation of adult hippocampal neurogenesis by diet emerges as a possible mechanism by which nutrition impacts on mental health.

During this talk I will present evidence of cognition and mood being modulated by dietary parameters, which are also responsible for adult hippocampal neurogenesis regulation. This will inform a discussion on the important translational concept that diet, a modifiable lifestyle factor, holds the ability to modulate brain plasticity and function.

Financial Support: Research Council UK

PLENARY SESSION 2 - *Auditorium Ronsard*
Introductory lectures for sessions A&B

I05
Assessment of the metabolic fate of polyphenols: critical analytical and modelling methodologies

J. van Duynhoven[1,2*] E. van Velzen[2], N. de Roo[2], D, Jacobs[2]
[1] *Laboratory of Biophysics, Wageningen University, The Netherlands*
[2] *Unilever R&D, Vlaardingen, The Netherlands, * john-van.duynhoven@unilever.com*

The introduction of metabolomics and microbiomics technologies has widened our view on the complex interactions between polyphenols, the gut microbiota and the human host. The important role of the gut microbiota as a versatile converter of polyphenols is now widely recognized, but many mechanisms remain to be unravelled. We currently still lack insight in the interplay between gut microbial catabolism and host conjugative metabolism, and in particular also how these bioconversion routes differ between individuals. In order to unravel these interactions for black tea polyphenols we developed a suite of identification and quantification platforms for polyphenol metabolites in urine and plasma. Here we strongly relied on joint and complementary deployment of LC-MSn and LC-SPE-NMR-MS techniques. This allowed us to capture the large chemical diversity of circulating conjugated gut microbial catabolites of black tea polyphenols. The combined use of LC-MS and qNMR also allowed us to quantify the concentrations of newly identified metabolites in plasma for which no authentic standards were available. In our intervention studies we used longitudinal designs to obtain a dynamic view on the appearance of conjugated gut microbial catabolites in plasma and urine. By using nutrikinetic modelling approaches we could decode signatures that point towards phenotypes that differ in gut microbial bioconversion capacity of and host conjugative metabolism.

Financial Support: Unilever, Netherlands Genomics Initiative/Netherlands Metabolomics Centre

I06
Polyphenols and risk of new-onset diabetes in the PREDIMED trial.

R.M. Lamuela-Raventós[1,2], A. Tresserra-Rimbau[1,2], M. Guasch[2,3], J. Salas-Salvadó[2,3], M.A. Martínez-González[2,4], D. Corella[2,5], R. Estruch[2,6], on behalf of the PREDIMED Study Investigators.
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[3] *Human Nutrition Unit, School of Medicine, IISPV, University Rovira i Virgili, Reus,*
[4] *Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra, Pamplona,*
[5] *Department of Epidemiology, Preventive Medicine and Public Health, School of Medicine, University of Valencia, Valencia,*
[6] *Department of Internal Medicine, Hospital Clínic, IDIBAPS, University of Barcelona, Barcelona.*

Increasing evidence demonstrates a positive relationship between consumption of polyphenol-rich foods and chronic disease prevention. Some epidemiological, *in vitro* and animal studies support the beneficial effects of dietary polyphenols on glucose homeostasis, thus they could be used in the prevention of diabetes.

We worked with data from the PREDIMED cohort (ISRCTN 35739639). From 7447 participants, 3430 were selected on the basis they were free of diabetes at baseline. Polyphenol intake was calculated by matching food consumption data from yearly food frequency questionnaires with the Phenol explorer database on the polyphenol content of each reported food. Time-dependent Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for diabetes according to tertiles of polyphenol intake.

During a median of 5.51 years of follow-up (18900 person-years), a total of 313 incident cases of diabetes were diagnosed (9.1%). After adjusting for potential confounders, significant inverse associations were found for total polyphenols [HR 0·67 (95% CI, 0·45 to 0·99), P-trend=0·04], stilbenes [HR 0·59 (95% CI, 0·36 to 0·96), P-trend=0·02], lignans [HR 0.64 (95% CI, 0·43 to 0·95), P-trend=0.02], and dihydroflavonols [HR 0·51 (95% CI, 0·32 to 0·83), P-trend=0·02

We have found inverse associations between different polyphenols subgroups and the incidence of diabetes within the PREDIMED trial.

I07

Preclinical and clinical evidence for the beneficial effects of the cacao flavonols on metabolic dysregulations.

I. Ramirez-Sanchez
Escuela Superior de Medicina, Instituto Politecnico Nacional, Mexico.
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Obesity is a chronic disease, currently recognized as the triggering agent for the development of the metabolic syndrome. Obesity rises from an energy imbalance due to excessive food ingestion and insufficient physical activity.

Overweight and obesity have been associated with increase in cardiometabolic risk. Therapeutics includes life-style changes and/or pharmacologic agents. However, such interventions are often limited by poor compliance and/or significant side effects. The consumption of certain dietary products, such as cocoa, has been demonstrated to exert positive effects on cardiometabolic risk factors, such as hyperglycemia, hypertriglyceridemia, and hypertension. (-) Epicatechin (Epi), the most abundant flavonoid in cacao has been reported to replicate such effects. However, the Epi mechanisms of action have not been fully elucidated. Mitochondria has an important role in energy balance and, interestingly, recent findings have found an association between obesity and mitochondrial dysfunction due to a defective network among regulator proteins such as peroxisome proliferator-activated receptors (PPARs), sirtuins (SIRTs), and PPAR coactivator 1 alpha (PGC-1α). These molecules are currently under extensive research in aims of finding new agents that could be used in the treatment of obesity and metabolic syndrome. In animal models, obesity has been developed after the administration of the high-fat diet for several weeks. Epi significantly decreased on the obesity model the rate cardiometabolic risk factors such as of weight gain, glycemia and hypertriglyceridemia. Epi restored the obesity-induced decreases in the levels of skeletal muscle and abdominal tissue sirtuins (SIRTs), peroxisome proliferator-activated receptor coactivator (PGC-1α), mitofilin, transcription factor A mitochondrial (TFAM), uncoupling protein 1 (UCP1), and deiodinase (DIO2). Epi has been showed as a treatment yielded beneficial effects on high fat diet-induced endpoints thus may be considered as a potential agent for the treatment of obesity and its cardiometabolic associated abnormalities. Mechanism of action may be attributed to the modulation of cellular/mitochondrial function, thus improving overall metabolism.

I08

Metabolomic approach for improving assessment of exposure to polyphenols-rich foods through combined multi-metabolite models

R. Llorach-Asunción[1], M. García-Aloy[1], M. Urpi-Sarda[1], R. Vázquez-Fresno[1], and C. Andres-Lacueva[1]
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Nutrimetabolomics is a powerful tool to analyze the metabolome changes associated to the polyphenols-rich foods exposure. The main aim of this presentation is to show the state of the art of the identification of biomarkers related to the polyphenols-rich foods intake, as well as their potential association with health in a high risk of cardiovascular disease population from the Mediterranean region through the application of an untargeted metabolomics approach.

Several studies have shown that the diet-related differences in urinary metabolome are associated with food digestion, microbiota metabolism and endogenous metabolism. Throughout this presentation, it will be shown that generating exposure biomarkers models composed by more than one metabolite we obtain better results to measure the food exposition than using the measurements obtained from each marker individually. These multi-metabolite models are precise, specific and accurate measurements of food intake as nutritional biomarkers, which seek to be an objective and accurate tool for determining the dietary exposure. As far we known, only very few studies have attempted to work with combinations of nutritional biomarkers. This represents an important innovation in the field of nutrimetabolomics opening an alternative way for the further discovery of dietary exposure biomarkers as determinants of compliance in long-term intervention trials conducted in free-living individuals.

I09
The effects of biotransformation and food matrix on the bioactivities of polymethoxyflavones

Hang Xiao
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Orally ingested flavonoids undergo complex physiochemical changes in the gastrointestinal tract, which in turn dictates the biological activities of flavonoids. We investigated the gastrointestinal biotransformation pathways of major polymethoxyflavones, a unique class of citrus flavonoids, in rodents. In general, the major sites of biotransformation for polymethoxyflavones are small intestine and colon. In the small intestine, polymethoxyflavones undergo extensive phase I metabolism to produce multiple demethylated metabolites. These phase I metabolites further undergo phase II metabolism to produce sulfate and/or glucuronide conjugates. Therefore, the majority of polymethoxyflavones exist as phase II conjugates in the small intestine. However, in the colon, the phase II conjugates are converted back to phase I metabolites by the action of gut microbiome, thus only phase I metabolites can be found in the colon. Most interestingly, our results demonstrated that phase I metabolites of polymethoxyflavones have potent biological effects such as anti-inflammation, anti-proliferation, pro-apoptosis, anti-angiogenesis, anti-oxidation, etc. and these effects are even stronger than those produced by their parent polymethoxyflavones. Overall, our findings indicated that gastrointestinal biotransformation of polymethoxyflavones results in high levels of phase I metabolites in the colon that may elicit enhanced biological effects.

PARALLEL SESSION B - *Auditorium Ronsard*
Advances in polyphenols targets for obesity, metabolic syndrome and diabetes

I10
A novel mechanism for the anti-diabetic action of green tea polyphenol

Hae-Suk Kim[1], Jeong-a Kim[1, 2]
Department of Medicine, Division of Endocrinology, Diabetes, and Metabolism[1], UAB Comprehensive Diabetes Center[2], University of Alabama at Birmingham, Birmingham, Alabama 35294

Vascular endothelium plays an important role in energy metabolism by regulating transport of nutrients and hormones to metabolic tissues. Endothelial dysfunction is observed in subjects with obesity, diabetes, and cardiovascular diseases. We have shown that green tea polyphenol, epigallocatechin gallate (EGCG) has vasodilator actions by stimulating endothelial nitric oxide synthase (eNOS) and NO production, which contributes to prevention of insulin resistance and improvement of glucose homeostasis.

Autophagy is one of the protective mechanisms by which cells degrade organelles, lipids, and long-lived proteins. We recently observed that EGCG stimulated autophagy and reduced accumulation of lipid droplets in endothelial cells. Elevated levels of fatty acids are manifested in the patients with cardiometabolic syndrome, which cause ectopic lipid accumulation. Since autophagy is one of the mechanisms for reduced lipid accumulation, we investigated the mechanisms by which EGCG stimulates autophagy and its role in prevention of lipid accumulation. We observed that EGCG stimulates autophagy through a CAMKKβ/AMPKdependent mechanism. Moreover, EGCG-stimulated autophagy promoted the degradation of lipid droplets in endothelial cells. EGCG increased the co-localization of lipid droplets and lysosome. In summary, autophagy is a novel mechanism by which EGCG prevents accumulation of lipid, which may contribute to the beneficial health effects of green tea polyphenol in metabolic syndrome, including diabetes, dyslipidemia, endothelial dysfunction, and insulin resistance.

I11

Fruit polyphenols improve type 2 diabetes and the metabolic syndrome through alleviating inflammation and modulating the gut microbiota.

A. Marette, Laval
University, Quebec, Canada

The worldwide epidemic rise in type 2 diabetes (T2D) and cardiovascular disease (CVD) calls for novel treatments of these obesity-linked diseases. Visceral obesity is also associated with the development in nonalcohol fatty liver disease (NAFLD), which can lead to liver cirrhosis and cancer. Current pharmacological treatments for T2D and NAFLD have limited efficacy or unwanted side effects. I will present growing evidence that the gut microbiota is a key determinant of diet-induced obesity and T2D. I will show that new polyphenol-rich extracts from cranberry and other wild berries can protect against obesity-linked inflammation and alleviate T2D and NAFLD in high fat-fed mice. This is associated with a shift in the gut microbiota and in particular the increased abundance of the mucin-degrading bacterium *Akkermansia muciniphila*. Increase in the *Akkermansia* population may therefore contribute to the anti-inflammatory and beneficial effects of fruit polyphenols in obese mice. Finally I will present recent data showing that dietary supplementation with a polyphenol-rich supplement from strawberry and cranberry extracts improves insulin sensitivity in overweight and obese insulin-resistant men and women.

PLENARY SESSION 3 - *Auditorium Ronsard*
Introductory lectures for sessions C&D

I12
Flavonoids and neuro-cognitive improvements: The invovlement of the vascular system as a mediator of benefits

Lecture Sponsored by Pepsico

Jeremy P E Spencer, Caroline Saunders, Catarina Rendeiro, Claire Williams
University of Reading, Reading, UK

Evidence suggests that dietary phytochemicals, in particular flavonoids, may exert beneficial effects on the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation and by improving cognitive function. Historically, they were believed to do this via an ability to express classical antioxidant activity in the brain. However, their poor brain bioavailability and extensive metabolism means that this is unlikely. Instead, their actions on the brain appear to be mediated by effects on both the peripheral and cerebro-vascular system that lead to changes in improve blood flow to the brain capable of inducing angiogenesis, neurogenesis and changes in neuronal morphology. Such vascular effects may lead to the activation of critical protein and lipid kinase signalling cascades in the brain, leading to a suppression of neuroinflammation and the promotion of synaptic plasticity. This paper will focus on the acute effects of flavonoids and flavonoid-rich foods on human executive function (attention, sustained attentiveness and task responsiveness) and how such effects may be mediated by changes in peripheral and cerebrovascular blood flow, measured using flow-mediated dilatation and fMRI. Through such a mechanism, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent loses in cognitive performance. In addition, flavonoids may represent important precursor molecules in the quest to develop a new generation of brain enhancing drugs.

I13
Epidemiological evidence for protection against gastrointestinal cancers by polyphenol intake

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Cancers of the gastrointestinal tract are common and leading causes of cancer deaths. Many polyphenols exert anti-carcinogenic activities in *in vitro* and in animal studies, especially against gastrointestinal tumors. These beneficial effects may be either local on the alimentary tract or systemic after their absorption and metabolism. However, results from observational epidemiologic studies are still limited and are often inconsistent. This is partially due to the difficulties in the estimation of polyphenol exposure. Improved food composition databases, such as USDA and Phenol-Explorer databases, and the discovery of several polyphenol biomarkers have allowed the study of a wider range of polyphenol subclasses in epidemiological studies. In this talk, epidemiological evidence on the associations between polyphenol exposure, using questionnaires and biomarkers, and risk of gastrointestinal cancers is comprehensively reviewed. Protection by some individual flavonoids and flavonoid subclasses against esophageal, gastric and colorectal cancer risks has been observed. The talk will also stress the main limitations of the work conducted so far, both in terms of polyphenol coverage and for pooling results in reviews and meta-analyses, because the estimation of polyphenol intake is often not comparable between epidemiological studies. The need for replication of the results in other epidemiological settings to check their reproducibility using populations with different lifestyle, dietary and environmental factors will also be emphasized. These findings will also need to be confirmed in in long-term randomized trials, and administration of pure compounds will be important to establish which specific polyphenols and doses are effective.

Financial Support : Institut National du Cancer, Paris (INCa grants 2011-105)

I14

Polyphenols found in berry fruit improve age-associated changes in cognitive function and brain inflammation

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Research has demonstrated, in both human and animals, that cognitive functioning decreases with age, to include deficits in processing speed, executive function, memory, and spatial learning. The cause of these functional declines is not entirely understood; however, neuronal losses and the associated changes in the activity of neurotransmitters, secondary messengers, and their receptors may be caused by long term increases in and susceptibility to oxidative stress and inflammation. Therefore, one approach to improving neuronal functioning might be to alter the neuronal environment to reduce the impact of oxidative and inflammatory stressors. Research conducted in our laboratory has shown that consumption of fruits and vegetables high in polyphenolics can prevent and even reverse age-related cognitive deficits. The polyphenolic compounds found in these foods may exert their beneficial effects indirectly, through their ability to lower oxidative stress and inflammation, or directly, by altering neuronal structure and signaling involved in neuronal communication. Therefore, dietary interventions with polyphenolic-rich foods may be one strategy to forestall or even reverse age-related neuronal deficits.

I15
Cognitive and Cerebral blood-flow effects of polyphenols in humans

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Polyphenols may exert a number of effects within cellular signal transduction pathways that are relevant to the beneficial modulation of brain function. To date the evidence of direct improvements in cognitive function from human controlled trials is still limited.

Previous research involving healthy non-elderly adults has demonstrated improvements to cognitive function following cocoa-flavanols. Examples include a study from our own laboratory showing acute cognitive improvements during the performance of demanding cognitive tasks. Our own research has also demonstrated consistent increases in cerebral blood-flow and the modulation of haemodynamic responses to task performance in the frontal cortex following single doses of the stilbene resveratrol. However, these haemodynamic effects have not been associated with any evidence of improved cognitive function. These results and data from a current human trial assessing cerebral blood-flow and cognitive function in normoxic and mildly hypoxic environments following resveratrol will be discussed in the context of the broader literature relating to polyphenols and the human brain.

PARALLEL SESSION D - *Auditorium Ronsard*
Recent breakthroughs in cancer prevention

I16
Stress, breast cancer and flavonoids - A role of quercetin conjugate as a β_2-adrenergic receptor antagonist

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Epidemiological and animal studies indicated that daily stress including nocturnal light exposure promote the development of breast cancer. Endogenous noradrenaline (NA) are released from the adrenal medulla and sympathetic nervous system during exposure to stress. It has been reported that patients receiving β-AR(adrenergic receptor) blocker treatment had a reduced risk of breast cancer-associated mortality. The results obtained from these studies have suggested that the β2-adrenergic receptor signaling pathway can modulate breast cancer progression and metastasis. On the other hand, epidemiological studies also suggested an inverse association between a higher intake of flavonoids and breast cancer risk.

In order to clear the chemopreventive action mechanism of flavonoids on stress-promoted development of breast cancer, we have focused on adrenergic receptor-mediated responses in human breast cancer MDA-MB-231 cells expressing β_2-AR. It was found that quercetin-3-*O*-glucuronide (Q3G, 0.1 μM), a circulating metabolite of quercetin, suppressed the induction of *MMP-2* and -9 gene expression through the inhibition of NA mediated β_2-AR signaling which involves cAMP-PKA, MAPK, and ROS-NF-kB pathways. Further, Q3G also suppressed NA-promoted invasion of MDA-MB-231 breast cancer cells and MMP-9 induction. It was also found that Q3G inhibited the binding of [^{3}H]-NA to β_2-AR.

These results suggest that Q3G may function as a β_2-AR antagonist in breast cancer cells, and may play a role in stress-induced breast cancer progression as a dietary anti-metastatic agent. Antagonistic effect of other metabolites will also be shown.

Financial Support: JSPS KAKENHI Grant-in-Aid for Scientific Research (B)

I17
Advances in chemoprevention – lessons from resveratrol

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Despite extensive preclinical data indicating that phytochemicals and micronutrients can protect against cancer, these findings have largely failed to translate into successful clinical outcomes; this may be attributed to a failure to identify the optimal dose for clinical assessment. In an attempt to improve the development process for dietary chemopreventive agents, using resveratrol as a model compound for the prevention of colorectal cancer, we have addressed issues of poor bioavailability and the role of metabolites, how to choose the optimal dose for clinical translation and the influence of dietary fat on efficacy. Our findings demonstrate that the major human metabolite resveratrol sulfate may contribute to efficacy *in vivo*, by delivering resveratrol to target tissues in a stable conjugated form, enabling gradual regeneration of the parent within selected cells. We also compared the pharmacokinetics and activity of a low dietary relevant dose of resveratrol, equivalent to the amount contained in a large glass of red wine with an intake 200-fold higher. The lower dose inhibited intestinal adenoma development in a mouse model of colorectal carcinogenesis more effectively than the pharmacological dose. Efficacy correlated with increased activation of AMP-activated protein kinase and further *in vitro* examination indicated a bell-shaped dose response for AMPK signalling, leading to autophagy and senescence. Analysis of tissue from resveratrol-treated patients and experiments with human explant cultures provided further evidence that low dietary exposures can elicit biological changes relevant to chemoprevention and should be included in preclinical testing strategies.

Financial support: Cancer Research UK programme (C325/A13101) with assistance from the Leicester Experimental Cancer Medicine Centre (C325/A15575 Cancer Research UK/UK Department of Health).

PLENARY SESSION 4 - *Auditorium Ronsard*
Introductory lectures for sessions E&F

I18
Dietary flavanols and cardiovascular health: Results from the FP7- FLAVIOLA project

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Evidence from dietary intervention studies suggests that diets rich in flavanols are causally related to cardiovascular health benefits in humans at increased cardiovascular risk. Within the pan-European research consortium FLAVIOLA, we assessed the efficacy, relevance, and applicability of cocoa flavanol (CF) intake to improve cardiovascular health in various cohorts representing a broad segment of the healthy population. While considering important pharmacokinetic characteristics of CF, we investigated the cardiovascular effects of CF from a gender- and age perspective in healthy low risk humans. Our data demonstrate that CF intake exerts beneficial effects on various accredited cardiovascular endpoints including endothelial function, arterial stiffness, blood pressure, and blood lipids in healthy men and women across all the ages and groups investigated. Collectively, our results provide direct evidence for population-based health benefits, thus advocating a potential role for cocoa flavanols in primary cardiovascular disease prevention and support that these compounds should be considered in future dietary guidelines.

Financial Support: European Union

I19
Beneficial effects of phenolic compounds on HDL functionality

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High-density lipoprotein (HDL) cholesterol (HDL-C) levels are inversely and independently associated with cardiovascular disease (CVD). Pharmacologically raised HDL-C concentration has not always led to a decrease in cardiovascular risk. In addition, genetic variants predisposing to high HDL-C levels are not associated with lower risk of suffering a coronary event. Thus the physiological role of HDL seems, therefore, to be better reflected by its function than HDL-C.

An increase in cholesterol efflux capacity (CEC) after the consumption of a real-life dose of virgin olive oil was reported. A randomized, crossover, controlled trial with 47 healthy European male volunteers was performed. Participants ingested 25 mL/d of polyphenol-poor (2.7 mg/kg) or polyphenol-rich (366 mg/kg) raw olive oil in 3-week intervention periods, preceded by 2-week washout periods. HDL cholesterol efflux capacity significantly improved after polyphenol-rich intervention versus the polyphenol-poor one (+3.05% and -2.34%, respectively; P=0.042). Incorporation of olive oil polyphenol biological metabolites to HDL, as well as large HDL (HDL2) levels, was higher after the polyphenol-rich olive oil intervention, compared with the polyphenol-poor one. Small HDL (HDL3) levels decreased, the HDL core became triglyceride-poor, and HDL fluidity increased after the polyphenol-rich intervention. On the one hand, an increase in the OOPC biological metabolites bound to the HDLs was observed. OOPC could exert a local antioxidant protection in HDLs that may prevent oxidative modifications of the apolipoprotein A-I, the main HDL protein involved in CEC. On the other hand, we found a decrease in the relative content of triglycerides in the HDL core. A triglyceride-poor HDL core is related to a more stable conformation of ApoA-I in the lipoprotein which could lead to better HDL capacity to perform CEC.

The consumption of olive oil phenolic compounds increased the ability of HDLs to pick up cholesterol excess in peripheral cells. These changes can respond to an improvement of HDL oxidative status and composition. Our results provide for the first time a first-level evidence of an enhancement in HDL function by polyphenol-rich olive oil.

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I20
Innovative targets and innovative routes in the study of dietary polyphenol putative biological activity.

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In recent years, the use of tandem mass spectrometry for the analysis of flavonoids and related compounds in foods and biological samples has significantly enhanced our understanding of (poly)phenol bioavailability. These advancements have led to improvements in the understanding of how dietary polyphenols are modified by human and microbial enzymes upon consumption. This has, in turn, led to the development of new synthetic strategies and to the application of such metabolites to experimental models at concentrations coherent with dietary intake, an approach surprisingly forgotten until the most recent years. This approach has then paved the way to identify new targets and putative mechanisms of action exerted by this wide class of phytochemical compounds. This presentation will deal with recently published and on-going experiments where phenolics and phenolic metabolites have been observed as able to modulate physiological and pathological functions in a preventive direction, including yeast and mammal models of neurodegeneration, cardiovascular disease (including processes involving the action of a recently discovered marker of cardiovascular risk, namely trimethylamine-N-oxide, TMAO), inflammation and diabetes.

Financial Support : The works presented have been made possible thanks to many different grants, public and private, that will be disclosed during the presentation**.**

PARALLEL SESSION E - *Auditorium Ronsard*
Update for properties of polyphenols in cardiovascular diseases

I21
Flavonols and their metabolites in the vascular wall

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Flavonoids exert beneficial effects on vascular health, reducing arterial tone and reverting endothelial dysfunction, which are proposed as main mechanisms in the prevention of cardiovascular disease. Paradoxically, most flavonoids are not found in plasma after oral administration because they are extensively metabolized into methylated and glucurono- and sulfo-conjugated metabolites, which are the main plasma circulating forms. Conjugation with glucuronide is a reversible process involving β-glucuronidase and glucuronoconjugates can be hydrolyzed at the vascular level, yielding the parent aglycone which accumulates in tissues. Thus, a key question is whether the in vivo effects of dietary flavonoids results from the direct action of metabolites or requires deconjugation. This raises the issue of the importance of the activity of β-glucuronidase as a determinant of the biological activity of flavonoids. Importantly, β-glucuronidase is an inducible enzyme whose activity increases under inflammatory conditions. Additionally, the activity of β-glucuronidase is genetically determined explaining the inter-patient variability in response to flavonoids. In this talk, the vascular effects of flavonoids on the vascular wall will be reviewed, focusing on the oxidative pathways (e.g. NADPH oxidase and NRF2), the impact on vascular tone and endothelial function and the role of the conjugation-deconjugation cycle.

I22
Cardiovascular benefits of dietary polyphenols

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Population studies suggest cardiovascular health benefits of consuming fruits and vegetables, which may be due in part to their high content of polyphenolic compounds. Population studies however can only indicate that there is an association between certain foods or components and human health. To truly evaluate the impact of foods or food components on human health or disease risk factors, controlled human intervention studies are necessary.

Since it is extremely difficult to conduct long term (years) nutritional trials with endpoints such as disease outcome or mortality, we have focused on acute and short-term studies to determine effects on risk factors for cardiovascular disease such as vascular function and blood pressure. Since whole foods and beverages contain many different components we have also conducted studies with isolated compounds to determine if these are responsible for the effects seen with whole foods.

Some examples will be presented from a 6 month, randomised parallel designed trial to determine the effect of black tea polyphenols on blood pressure in subjects with high normal blood pressure; an acute, cross-over design study of apple polyphenols on vascular function in normal healthy subjects; dose-response studies using pure polyphenolic compounds such as chlorogenic acid, epicatechin or quercetin glycosides on vascular function in healthy subjects.

New data from human aortic endothelial cell studies and animal models will be presented to provide some insight into the mechanism of action of dietary phenolic compounds.

Financial Support : National Health and Medical Research Council of Australia, Unilever, Nestle, Fruit West.

PARALLEL SESSION F - *Auditorium Descartes*
Innovative approaches in polyphenol research

I23
Shotgun metabolomics and lipidomics for discovery of polyphenol bioactivity

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Xanthohumol (XN) is a prenylated flavonoid found in hops, beer, and in dietary supplements. There is substantial evidence in the literature that XN improves glucose and lipid metabolism of mammalian cells *in vitro,* but relatively little is currently known about the potential benefits/risks of chronic exposure to XN *in vivo.* Even less is known about the underlying mechanisms of action.

Using Zucker fa/fa rats and diet-induced obese C57BL/6J mice as models of obesity and metabolic syndrome, we treated animals with XN at three dose levels 6-12 weeks (n=12-16/dose group). The XN-treated animals had significantly lower plasma glucose levels and smaller body weight gain compared to the control groups. Using untargeted metabolomics and lipidomics approaches, we found a dose-dependent decrease of hepatic triglyceride content and metabolic products of dysfunctional lipid metabolism (medium-chain acylcarnitines, dicarboxy fatty acids, hydroperoxy and hydroxy fatty acids). Untargeted metabolomics analyses in combination with oxygen consumption measurements of mouse skeletal C2C12 muscle cells treated with XN revealed that it increased basal oxygen consumption and induced an adaptive stress response.

Collectively, these findings are consistent with XN acting as a mild mitochondrial uncoupler of oxidative phosphorylation. Because XN has undesired electrophilic and pro-estrogenic properties, we prepared two hydrogenated derivatives of XN that lack electrophilicity and pro-estrogenicity. The derivatives were tested in diet-induced obese C57BL/6J mice and in C2C12 cells. They showed comparable activity as the parent XN but at lower doses and concentrations.

These data suggest that hydrogenated XN derivatives are promising candidates for further development to treat metabolic syndrome.

Financial Support: National Institutes of Health & Hopsteiner Inc.

I24
Novel Controlled-Release Technologies to Enhance Efficacy of Polyphenols

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Polyphenols represent one of the largest classes of plant bioactives and have been a subject of innumerous investigations as to their health benefits. Curcumin (CUR) and green tea polyphenols (GTPs) are the most investigated polyphenols in clinical studies. However, the outcomes have been less than rewarding as both these polyphenolics and others (e.g., ellagic acid) suffer from poor oral bioavailability issues. Clearly new effective approaches are warranted to overcome the bioavailability issues and recognize their fullest potential and health benefits in clinical setting. This laboratory has developed two novel approaches for polyphenolics and other compounds: 1) polymeric implant devices to provide continuous systemic and local delivery; and 2) exosomal delivery orally.

In polymeric implant delivery, CUR, GTPs and punicalagins (PC) were embedded in polycaprolactone and the polymer formulation was extruded into small cylindrical implants. These implants were found to furnish continuous release for long durations (months to >1 year) both *in vitro* and *in vivo*. While subcutaneous polymeric implants of CUR, GTPs and PC showed significant inhibition of DNA adducts in rats treated with the carcinogen, benzo[*a*]pyrene, no adduct inhibition occurred when these polyphenols were administered orally despite 10 - 100-fold higher doses. Furthermore, tissue CUR levels were found to be several-fold higher with the implant delivery than oral delivery. These data indicate that the implant delivery overcomes the oral bioavailability issues and enhances efficacy.

Exosomes are endogenously produced nanovesicles, detected in all the bodily fluids, shuttle in and out of the cells, and are being explored as vehicle to deliver small molecules. In exosomal delivery, exosomes isolated from bovine milk were loaded with both lipophilic (CUR) and hydrophilic (anthocyanidins - anthos) compounds. Exosomal formulations of CUR and anthos were found to exhibit highly enhanced anti-proliferative and anti-inflammatory activities against lung and ovarian cancer cells. Exosomal-anthos formulation when administered orally also showed significantly enhanced anti-tumor activity against lung and ovarian tumor xenograft in nude mice compared with anthos alone. This is the first demonstration indicating that milk-derived exosomes can serve as efficient vehicle for oral delivery of polyphenols and other compounds.

Financial Support: NCI grants R01-CA-118114 and R01-CA-125152, R41-CA-189517, Agnes Brown Duggan Endowment and Helmsley Funds.

PLENARY SESSION 5 - *Auditorium Ronsard*
Introductory lectures for sessions G&H

I25
New insights on the complex molecular mechanisms of polyphenols through nutri(epi)genomics

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Epidemiological, human and animal studies suggest a protective role of dietary polyphenols against cardiovascular diseases. Their capacity to modulate genes expression and signaling pathways may be involved in their cardiovascular protective effects but the mechanisms remain still unknown. The aim of our research is to investigate the cardiovascular protective property of polyphenols and decipher underlying molecular mechanisms using holistic nutrigenomics approach.

In murin models of atherosclerosis, 16-week supplementation with nutritionally-relevant doses of polyphenols (catechin, bilberry anthocyanins, naringin or curcumin) decreased progression of atherosclerosis. Nutrigenomic studies of aorta indicated that polyphenols modified the expression of hundreds genes and functional analysis of these data identified a cluster of common pathways related to cell-cell adhesion, cell junctions, focal adhesion, and cell cytoskeleton. These processes regulate adhesion and transendothelial migration of monocytes into the intima of blood vessels, the initial step of atherosclerosis development. Immunofluorescence analysis of the aortic roots showed a reduction of the number of macrophages in intima.

To deepen molecular mechanisms of polyphenols, we investigated the impact of plasma metabolites of polyphenols on cell signaling pathways and miRNA expression in endothelial cells. The nutrigenomic effect in endothelial cells was associated with modulation of the phosphorylation level of several transcription factors and signaling pathway proteins, such as p38, p65 or Akt. We also observed changes in expression of miRNA in endothelial cells and bioinformatic analysis suggests that miRNA-target genes are also involved in processes of adhesion, transendothelial migration, focal adhesion or cytoskeleton organization. More recent studies have revealed that polyphenols can also modulate DNA methylation in endothelial cells, genes that are overrepresented in the pathways also regulating adhesion and transendothelial migration. Using different in-vitro assays, we have shown that these nutri(epi)genomic effects are associated with lowed monocyte adhesion to endothelial cells as well as transendothelial migration when exposed to polyphenol metabolites at physiologically-relevant concentrations.

The role and impact of dietary polyphenols on endothelial function and gene expression in humans was investigated. We showed that orange juice decreases diastolic blood pressure and significantly improves postprandial microvascular endothelial reactivity and that hesperidin could be causally linked to the observed beneficial effect of orange juice. Nutrigenomics study revealed that orange juice and hesperidin consumption commonly modulated expression of 1,582 genes. Many of these genes were involved in chemotaxis, adhesion, infiltration and lipid transport, which is suggestive of a lower recruitment and infiltration of circulating cells to vascular wall and lower lipid accumulation.

In conclusion, these results provide both evidence for cardiovascular protective effect of polyphenols and a global integrated view of the potent mechanisms by which plasma metabolites of polyphenols works at the endothelial level.

I26

Human gut microbiota metabolism of ellagic acid. Interindividual variability for urolithins production is correlated with health status: Cause or consequence?

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Urolithins are metabolites produced by the gut microbiota from dietary ellagitannins and ellagic acid. This metabolism has been conserved through phylogeny. *Gordonibacter genus* from *Actinobacteria* phylum is critically involved in urolithins production. Urolithins mainly exert anti-inflammatory and cancer chemopreventive activities in preclinical research. Interestingly, urolithins target malignant and normal colonic mucosa from colorectal cancer (CRC) patients. Not all individuals produce the same amount and type of urolithins. Human subjects can be stratified into three urolithin-producing groups. Individuals belonging to the so-called 'Phenotype A' produced only urolithin A conjugates (around 67% of the subjects, range 25-80%).

'Phenotype B' produced isourolithin A and/or urolithin B plus urolithin A (24%, range 10-50%). In 'Phenotype 0' (13%, range 5-25), urolithins were not detected. These phenotypes were observed independently of the subjects' health status and characteristics (age, gender, BMI), and of the ellagitannin food source ingested (waltnus, pomegranates, strawberries, raspberries, etc.). Individuals belonging to Phenotype B usually present higher blood lipid levels than those from Phenotype A. Interestingly, a higher percentage of Phenotype B was observed in those subjects with chronic illness (metabolic syndrome or CRC) associated to gut microbial imbalance (dysbiosis) but also in overweight-obese individuals which was correlated to changes in gut microbiota. Our results nourish the hypothesis that links Phenotype B with gut dysbiosis. Whether phenotype B could be a biomarker related to metabolic illness predisposition in overweight-obese healthy individuals and whether *Gordonibacter* could have a beneficial role in preventing chronic illness associated to obesity are under intensive research in our group.

Financial Support: AGL2011-22447 (MINECO, Spain), FP7-KBBE-2012-6-single stage (BACCHUS Project, European Commission Grant Agreement 312090) and 201370E068 (CSIC, Spain).

PARALLEL SESSION G - *Auditorium Ronsard*
New insights into mechanisms of action of polyphenols

I27
Dietary flavonoids induce autophagic degradation in macrophages

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We have previously found that macrophages could be one of the potential targets of dietary flavonoids *in vivo*, whereas the molecular actions of flavonoids, leading to the prevention of a wide array of diseases, still remain unclear. We noticed the fact that autophagy, a major intracellular lysosomal degradation system, is involved in maintaining cellular homeostasis and therefore impairment of autophagy could be implicated in a variety of age-related diseases. In other words, it is expected that the induction of autophagy could be, at least in part, a common mechanism underlying the prevention of multiple diseases by flavonoids. To investigate this hypothesis, we examined the effects of 65 polyphenolic compounds, including flavonoids, on the expression of autophagy-related genes and proteins in J774.1 macrophage-like cell line. We found that the expression of an autophagic adaptor protein p62/SQSTM1 was significantly enhanced upon treatment with quercetin, butein, or eriodictyol. It is of interest that butein and eriodictyol also significantly induced the mRNA expression of a transcriptional factor CHOP, which contributes to the expression of multiple autophagy-related genes. Furthermore, we also found that methoxylated quercetins (isorhamnetin and tamarixetin) significantly induced lysosomal degradation of BODIPY-labeled bovine serum albumin. These methoxyquercetins also induced lysosomal gene expression, probably through the activation of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis. These results in this study, including the structure-activity relationships, could provide useful information to prevent multiple chronic diseases through the induction/maintenance of autophagic degradation activity by dietary intake of flavonoids and other polyphenols.

I28
Procyanidin-membrane interactions in the regulation of cell signaling.

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Large procyanidins (PAC) composed by the flavan-3-ols (-)-epicatechin and (+)-catechin, are not absorbed by intestinal epithelial cells (IEC), however they can exert biological action through their interactions with the cell membrane. We previously showed that PAC interact with membrane lipid rafts, interactions that are favored by cholesterol. Lipid rafts compartmentalize select cellular processes including endocytosis, cell signaling, and in IEC their "cross-talk" with tight junctions (TJs) participating in the regulation of intestinal permeability. Accordingly, in differentiated Caco-2 cells PAC prevented: i- lipid raft structure disruption and associated signaling induced by cholesterol depletion/redistribution; ii- bile salt-induced raft disruption, signaling activation and TJs permeabilization. In proliferating Caco-2 cells, a model of colorectal cancer cells (CRC), PAC modulated signaling cascades initiated upon activation of the lipid raft-located epidermal growth factor (EGF) receptor (EGFR), a major player in oncogenesis. PAC promoted CRC apoptosis and cell cycle arrest in part through the inhibition of EGF-induced EGFR activation and of the downstream PI3K/Akt and Raf/MEK/ERK1/2 cascades. Supporting a role of PAC-lipid raft interaction in these effects, in CRC cells PAC: i) did not act interacting with the EGFR ligand (EGF), ii) did not affect EGFR localization at lipid rafts, iii) affected lipid raft membrane fluidity; and iv) inhibited EGF-mediated activation of the EGFR, including receptor dimerization and promotion of EGFR internalization. The above results will be discussed in terms of the relevance of membrane interactions on the capacity of PAC to prevent CRC and mitigate gut inflammation and associated pathologies.

PARALLEL SESSION H - *Auditorium Descartes*
Hot topics in polyphenols and gut microbiota interactions

I29
"A role for polyphenols in shaping the structure and function of the gut microbiome"

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Plant polyphenols have long been considered to play an important role in the health promoting effects of foods and diets rich in whole grain cereals, fruit and vegetables. Due to their complex structure, glycated native forms, and the fact that they are often complexed within lowly digested food matrices and fiber compounds, the majority of ingested polyphenols reach the large intestine, home to the colonic microbiome. The colonic microbiome, comprises the bulk of the gut microbiota and is now thought of as an integral part of human physiology, if not an organ in its own right capable of important physiological effects locally in the gut and systemically. These interactions with host physiology are mediated via the neuroendocrine system, immune system and the flux of metabolites absorbed from the gut. Although, it has been recognised for some years that the intestinal microbiome plays an important role in the biological availability and biological activity of ingested polyphenols, recent studies are also showing that these polyphenols themselves may actively shape microbiome structure and function. For example, both red wine and de-alcoholised red wine modulate the gut microbiota and concomitantly, improve biomarkers of metabolic disease (1). Others have shown that cocoa flavanols change the relative abundance of bacteria seen as beneficial within the human gut microbiota and improve some of the same disease biomarkers (2). Our initial studies with high polyphenol apples and apple juice, show that intake of apple polyphenols also modulates the activity of the gut microbiome and impact on physiological parameters linked to metabolic disease risk. Similarly, in collaboration with the FLAVURS team at the University of Reading, U.K., we have shown that not only does increased consumption of fruit and vegetables impact on biomarkers of cardiovascular disease, but it modulates the gut microbiome (3). This presentation will summarise recent findings demonstrating microbiome modulation by plant derived polyphenols and discuss the implications for human health and our understanding of the mechanisms of effect of whole plant foods and their derivatives.

1. Boto-Ordóñez M, Urpi-Sarda M, Queipo-Ortuño MI, Tulipani S, Tinahones FJ, Andres-Lacueva C. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. Food Funct. 2014 Aug;5(8):1932-8.
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I30
Dietary modulation of gut microbial metabolome

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The gut microbiota plays an important role in the development of many human dietary related disorders such as obesity, diabetes, cardiovascular disease and cancer. The link between microbial diversity and metabolic functionality is far from clear and although the products of metabolism are considered to have an important impact on immune function and inflammation, the metabolites and the bacteria responsible for their formation are mostly unknown.

Tremendous advances have been made towards determining the genetic identity of the many commensal organisms that colonise the human gut and work at the Rowett Institute of Nutrition and Health has generated a unique collection of human colonic bacteria, many genome-sequenced through international projects. Research from our laboratory has identified key molecular transformations performed by these bacterial isolates and this is allowing us achieve our overall aim; to elucidate the complex interplay between diet, the intestinal microbiota and health.

Through tightly controlled human dietary interventions we have shown that macronutrient composition can impact on both gut and systemic health. In particular, high-protein (animal-based) diets were found to be detrimental if used in the longer-term, and that replacement of animal protein with an energy equivalent amount of plant protein ameliorated this risk. As we look towards the use of plant products to support global protein production in the human diet, an understanding of how these products are metabolised by the gut microbiota becomes essential.

Financial Support: Scottish Government Food, Land and People Programme / World Cancer Research Fund

PARALLEL SPONSORED SESSION BY UNILEVER - *Auditorium Ronsard*
Flavan-3-ols and cardiovascular health - Journey to dietary recommendations

I31
Tea and Cardiovascular Health

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Meta-analyses of observational studies showed that tea is inversely associated with a 10-20% lower cardiovascular risk. Especially with stroke, a consistent inverse association has been reported. Beneficial effects were found for black as well as green tea, although more studies with black tea have been reported. In addition, meta-analyses of observational studies also showed a 15% lower risk of diabetes with higher tea consumption. Different types of tea, including green tea were evaluated.

The reduced risk of cardiovascular disease observed among tea drinkers might be explained by the fact that randomised clinical trials with tea or tea extracts reported beneficial changes in cardiovascular disease risk factors, including vascular function, blood pressure (BP), blood lipids, and insulin sensitivity. A meta-analysis of 9 randomised controlled trials found that tea (green and black) increased endothelial-dependent vasodilation (FMD) with 2.6% (absolute). It was concluded that moderate consumption of tea already enhanced FMD substantially. A Cochrane meta-analysis analysed black and green tea together and reported a pooled decrease of 0.48 mmol/L in LDL cholesterol, and lowering of systolic (-2.25 mmHg) and diastolic (-2.81 mmHg) blood pressure. When analysing the effects of black tea and green tea on these risk factors separately, significant effect were also found, but the smaller number of trials increased the risk of bias. Therefore these results should be treated with some caution. More recent meta-analyses with green tea or green tea extracts could include more clinical trials, and showed an average reduction in systolic BP of 2.08 mm Hg, and lowering of total and LDL cholesterol. The beneficial effect of tea on diabetes type 2 found in observational studies, is consistent with the outcome of a clinical trial with a decaffeinated green tea extract. It was found that this extract improved insulin sensitivity in obese patients, whereas body weight did not change.

I32
Flavanols and Cardiovascular Health

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Dietary sources of flavan-3-ols have been associated with reduced risk of cardiovascular disease. These associations extend to both monomeric flavan-3-ols and procyanidins forms. In addition, dietary clinical intervention studies support these associations. Clinical studies with tea, apples, cranberries, and cocoa have demonstrated cardiovascular benefits of including these foods in the diet.

The variety of bioactives in any particular food item and the varying content of bioactives that can occur across a food item can result in mixed findings among association studies as well as clinical intervention studies. Therefore, to generate a complete understanding of the role of a given bioactive compound or class of bioactive compounds in health, it is imperative to investigate the health benefits of specific, well characterized compounds in purified form.

The compelling evidence for an inverse association between consumption of foods rich in flavan-3-ols and cardiovascular disease, combined with the promising results from intervention studies, has driven investigations of cardiovascular benefits of purified flavan-3-ols. A limited number of studies have been conducted to date with encouraging results for biomarkers such as flow mediated dilation and blood pressure. As the body of evidence expands for the role of flavan-3-ols in reducing risk of cardiovascular disease, it is important to begin to consider a methodical approach to filling in the knowledge gaps such that the available evidence can ultimately be used to provide dietary guidance.

I33
What is the best approach for building dietary recommendations for flavan-3-ols

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Lecture sponsored with the support of ILSI NORTH AMERICA

With the evidence base increasing for the beneficial health effects of flavan-3-ol rich foods, a strategy for providing consumers with sound dietary recommendations regarding the role of these polyphenols and their dietary sources remains elusive. In the United States, current frameworks for establishing recommended intakes for essential nutrients are not fully suited to capturing the complexity provided by broad classes of non-essential bioactive flavonoids. Gaps in knowledge relative to variation in dietary forms/intake and evidence built primarily on surrogate biomarkers of disease risk broadly challenge development of true dietary reference intakes (DRI) such as adequate intake (AI) values for "flavonoids" overall. While this complexity generally suggests the appropriateness of a broad food based guidance approach for flavonoids, consideration of the flavan-3-ol subclass and its role in promotion of cardiovascular health provides an opportunity to move toward an AI level that would provide a basis for improved dietary guidance and more consistent communication across sources of these specific flavonoids. The evidence base for flavan-3-ols in relation to cardiovascular health has been built through observational studies and strengthened by a number of randomized control trials. Additionally, the specific abundance of flavan-3-ols in the diet and similarity between sources is conducive to documentation of specific foods within composition databases and facilitating estimation of dietary intake across population groups. Information on bioavailability and metabolism of flavan-3-ols from dietary sources have also provided evidence of absorption and defined plausible and testable biological metabolites and mechanisms. While additional insights into toxicology and safety of flavan-3-ols is needed to establish risk assessments or tolerable upper limits (UL), the evidence base for flavan-3-ols suggest that consideration of a DRI like framework for this subclass of flavonoids may be plausible and could enhance the quality of general food based recommendations providing a path for improved guidance and products.

PARALLEL SPONSORED SESSION BY OCEAN SPRAY - *Auditorium Descartes*
Cranberry Polyphenols: A Nutritional Approach to Combating Antimicrobial Resistance

I34
Antimicrobial Resistance in UTI and Approaches to Prevention

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UTI is an exceedingly common outpatient problem among young healthy women, resulting in considerable morbidity and health care costs. In addition to the cost burden, increasing antibiotic resistance is making treatment of these infections more problematic. Resistance among uropathogens is now not only an issue for complicated hospital-associated UTIs, but for simple community-acquired infections as well. Multidrug resistant pathogens such as extended spectrum beta lactamase producing (ESBL) *E. coli* are now occurring in patients with no discernable healthcare exposure or risk factors. With rising resistance across multiple classes of drugs, oral options for UTI are often limited and sometimes nonexistent.

Given the frequency of UTI and the propensity for recurrence, prevention of symptomatic events is increasingly important. Although antibiotic prophylaxis is very effective for prevention of recurrent UTI, it requires prolonged exposure to antibiotics and is associated with alterations of normal endogenous flora as well as other adverse effects. Thus, the use of antibiotic-sparing approaches to UTI prevention has become very appealing.

One preventive approach that has been employed for generations is ingestion of cranberry products. The results from some, but not all, clinical trials suggest that cranberry may reduce the incidence of symptomatic UTI, particularly in healthy women with acute uncomplicated cystitis. However, many of these studies had inadequate statistical power due to lower than anticipated UTI event rates or small sample size. Thus a well powered, randomized clinical trial of cranberry for prevention of symptomatic UTI is still needed. With a growing appreciation of the importance of preservation of the gut microbiome and rising rates of resistance, understanding the potential benefits from a non-antimicrobial approach to prevention of UTI with cranberry is critical.

I35
Cranberries: From Polyphenols to Polysaccharides

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The North American cranberry, *Vaccinium macrocarpon*, has one of the highest polyphenol content of commonly consumed fruits. Due to the rich polyphenol and naturally low sugar content, cranberries are also tart and astringent, requiring sweetener for palatability. Cranberries are rarely consumed raw so most studies were conducted with cranberry juice and powders.

Characterization of cranberry compounds in these products is important to understand the well- known health effects including urinary tract, heart and gastrointestinal health. These benefits are thought to be conferred by bioactive flavonoids such as flavan-3-ol, anthocyanins, flavonols and polymeric proanthocyanidins; the Type A and Type B proanthocyanidins. The health promoting properties of cranberries linked to urinary tract health are thought to be due to the activity of proanthocyanidins, especially type A proanthocyanidins (PACs).

Urinary tract infection is one of the leading infections globally leading to increased use of antibiotics, contributing to antibiotic resistance. This is a significant public health issue. The mechanism by which cranberries are efficacious in preventing UTIs has yielded many hypotheses, including preventing bacterial adhesion in the bladder and gut, immune modulation and effects on bacterial fimbriae expression and motility as well as biofilm formation. Knowledge regarding the etiology of UTIs and the effects of cranberry components will be discussed in this talk.

There is active ongoing research and new publications exploring the bioavailability and activity of cranberry metabolites as well as metabolites of gut flora metabolism to understand their contribution to the benefits attributed to cranberries. In addition, a new and exciting avenue of research is focused on cranberry oligosaccharides, recently characterized and identified. More research is needed to understand the contribution of these compounds and their synergies in support of cranberry's health benefits, particularly in urinary tract infections.

Financial Support: Christina Khoo is an employee of Ocean Spray Cranberries, Inc.

I36
Bioactive Dietary Polyphenols - Cardiometabolic and Cognitive Outcomes

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Increasing evidence links cardiovascular disease with cognitive decline and depression and it appears that endothelial dysfunction may be an underlying pathogenic factor. Flow mediated dilatation, a surrogate measure of endothelial function which is impaired in obesity and metabolic syndrome, is inversely related to cognitive performance. Certain nutrients can improve flow mediated dilatation following ingestion. We have observed sustained improvements with regular consumption of diverse sources of plant polyphenols, including cocoa flavanols, soy isoflavones and resveratrol, and postulate that these and other *vasoactive* nutrients may deliver not only cardiovascular but also metabolic and cognitive benefits. We have found improvements in mood and/or cognition following chronic supplementation with omega-3 fatty acids and isoflavones and are now using transcranial Doppler ultrasound to explore associations between cognitive effects of nutrients and endothelial function in cerebral arteries. This new approach is enabling us to identify nutrients with the potential to boost brain blood flow to meet cognitive demands and thereby sustain mental health in an ageing population and counteract cognitive decline in hypertension, diabetes and related chronic disorders. Cranberries are an abundant source of potentially vasoactive polyphenols; rigorous clinical trials should be conducted to see whether cranberry extracts can deliver dose-dependent sustained improvements of cardiometabolic and cerebrovascular functions.

Financial Support: Ocean Spray Cranberries supported my participation in this conference

I37
Chemo-protective effectiveness of cranberry phenolic fractions.

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Background/Objectives: Oxidative stress and reactive oxygen species (ROS)-mediated cell damage are implicated in various chronic pathologies. Emerging studies show that polyphenols may act by increasing endogenous antioxidant defence potential. Cranberry has one of the highest polyphenols content among commonly consumed fruits. In this study, hepato-protective activity of a cranberry juice (CJ) and cranberry extract (CE) powders against oxidative stress was screened using HepG2 cells.

Methods: cells were tested for ROS production, intracellular non-enzymatic and enzymatic antioxidant defences by reduced glutathione concentration (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR) activity and lipid peroxidation biomarker malondialdehyde (MDA). Involvement of major protein kinase signaling pathways was also evaluated.

Results: Both powders in basal conditions did not affect cell viability but decreased ROS production and increased GPx activity, conditions that may place the cells in favourable conditions against oxidative stress. Powder pre-treatment of HepG2 cells for 20h significantly reduced cell damage induced by 400 µM *tert*-butylhydroperoxide (t-BOOH) for 2 hours. Both powders (5-50 mg/ml) reduced *t*-BOOH-induced increase of MDA by 20 % (CJ) and 25 % (CE), and significantly reduced over-activated GPx and GR. CE, with a significantly higher amount of polyphenols than CJ, prevented a reduction in GSH and significantly reduced ROS production. CJ reversed the *t*-BOOH-induced increase in phospho-c-Jun N-terminal kinase.

Conclusion: This study demonstrates that cranberry polyphenols may help protect liver cells against oxidative insult by modulating GSH concentration, ROS and MDA generation, antioxidant enzymes activity and cell signaling pathways.

Financial support: work funded by Ocean Spray Cranberries Inc.

CLOSING SESSION - *Auditorium Ronsard*
Innovation and perspectives in polyphenols research: The future is now

I38
The exposome in cancer epidemiology – Polyphenols as a case study

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Cancer aetiology is largely governed by lifestyle and environmental factors to which an individual is exposed all along life. Various risk factors have already been identified but the causes of many cancers still remain largely unknown. Modern analytical techniques can be used to measure in blood, urine and other biospecimens a considerable number of metabolites directly derived or influenced by environmental exposures. These metabolites constitute altogether the blood or urine exposome. The systematic comparison of the exposome in exposome-wide association studies (EWAS) involving individuals at varying risk of cancers opens exciting avenues to identify novel risk factors for cancers. Dietary polyphenols with so diverse chemical structures and biological properties, scattered in a large diversity of foods, offer a good example to illustrate how EWAS studies may be used to characterize and measure exposures to polyphenols and to study their associations with cancer outcomes. These biomarker-based approaches are complementary to the more classical approaches utilizing dietary questionnaires and food composition data. However the use of biomarkers as more objective measurements should limit biases and errors commonly met with questionnaires. Their implementation in large-scale epidemiological studies should help identifying polyphenols that may best limit risk of cancers and other diseases.

I39
Polyphenols, cardiovascular disease and health claims

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Cardiovascular disease (CVD) is the biggest cause of morbidity and mortality worldwide and is a major target of (inter)national programmes towards healthier diets and a driver for innovation in the food industry. There is a clear need and a substantial market opportunity for developing new foods and beverages that have proven benefits for CV health. The potential for dietary bioactives to deliver beneficial effects in humans has already been established; e.g. polyunsaturated fatty acids and plant sterols have proven health benefits and have resulted in several health claims approved by European Food Safety Authority (EFSA).

Pre-clinical research with polyphenols reported over the past 20 years has demonstrated that they possess tremendous potential in terms of exerting beneficial physiological effects on the cardiovascular system. Within the last 15 y, a substantial number of reports of significant improvements in biomarkers related to CV health following consumption of polyphenols and polyphenol-rich foods have been published[1]. Similarly, there are numerous reports of clinically significant effects of consuming bioactive peptides on blood pressure in humans which are supported by a large number of reports of strong bp-lowering effects in animal models of hypertension[2]. The accumulating evidence to support the notion that dietary polyphenols and bioactive peptides can beneficially affect CV health and reduce risk has been accompanied by significant investment in R&D by the food industry and SMEs to develop foods that benefit consumer health.

Despite numerous dossiers concerned with health claims for products containing polyphenols and peptides being submitted, only two have been given a favourable opinion by the European Food Safety Authority (EFSA)[3,4]. In this presentation, the barriers that have hindered efforts to secure health claims for polyphenol-rich foods will be discussed, and the areas where future research needs to advance understanding and provide high quality evidence of beneficial effects in humans will be highlighted. Some of the tools, resources and approaches adopted within a large collaborative project (FP7-BACCHUS) concerned with overcoming barriers to health claims for polyphenols will be presented. The activities include fundamental research to understand the stability, metabolism and absorption of these bioactives from the gut, characterising plausible mechanisms underlying observed beneficial cardiovascular health effects, and randomised controlled trials in human subjects to demonstrate the health benefits.

This research was funded by the European Union 7th Framework Programme (BACCHUS-312090) with additional support from the Biotechnology and Biological Sciences Research Council UK (Institute Strategic Programme Grant 'Food and Health' BB/J004545/1 to IFR).

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I40
Microbiome – a window into food-microbe-host interactions

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The recent developments of metagenomics permit an extremely high resolution molecular scan of the intestinal microbiota giving new insights and opening perspectives for clinical applications.

Beyond the unprecedented vision of the intestinal microbiota given by quantitative metagenomics studies, such as EU MetaHIT and ANR MicroObese, functional metagenomics tools allow the exploration of fine interactions between food constituents, microbiota and host, which leads to the identification of enzymes, signals and intimate mechanisms of crosstalk between bacteria and food constituents as well as between bacteria and human cells.

Cloning of large genome fragments, either from complex intestinal communities or from selected commensal bacteria or bacteria used in fermented foods, allows the screening of these biological resources for bioactivity towards complex plant polymers or simpler prebiotic molecules. This permitted the identification of novel glycohydrolase enzyme families and highlighted unsuspected bacterial players at the very top of the intestinal microbial food chain.

Similarly, exposure of fractions from genomic and metagenomic clones onto human cells engineered with reporter systems to track modulation of immune response, cell proliferation or cell metabolism has allowed the identification of bioactive clones modulating NFkB or AP1 pathways, or the production of TLSP, Fiaf or PPAR-gamma. This in turn opens the possibility to decipher mechanisms by which commensal or candidate probiotic bacteria can modulate the activity of human cells in the intestinal epithelium or even in distal organs such as the liver, adipose tissue or the brain.

Hence, in spite of our inability to culture many of the dominant microbes of the human intestine, functional metagenomics open this new window for the exploration of food-microbe-host crosstalk. One outcome is personalization of medicine and nutrition.

I41
Personalized nutrition is about what we eat and what we drink

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After decades of global dietary recommendations for the population at large, the notion of personalized nutrition is becoming more embraced by scientists and health professionals and more sought after by the general public. The current research is showing how genetic information can facilitate early prediction of a number of common, chronic diseases (i.e., cardiovascular diseases, diabetes, obesity), as well as more personalized dietary and behavioral approaches to prevent the expression of these diseases. To date the focus of this research has been of defining the right mix of macro- (proteins, fats and carbohydrates) and micronutrients (vitamins, minerals) for different genetic makeups. However, the foods we eat contain thousands of other chemical compounds that, although are not classified as traditional nutrients, may have a profound effect over our health status. Among them, polyphenols are the subject of increasing interest and these complex chemicals can be found not only in what we eat but also in what we drink (i.e., wine, beer, coffee, tea). In this regard, coffee is an excellent proof of concept of the relevance of these common drinks to health and the intricacies associated with the interpretation of the scientific findings.

Coffee is the second traded commodity after oil and we drink over 500 billion cups of coffee every year. The history of this beverage is long and convoluted and from its beginnings it has been preached or demonized. Like other common drinks, it owes some its organoleptic and health properties to a process of fermentation, which is one of the many steps that take the coffee fruit from the fields to the cup. As a natural product containing over 1000 different chemicals, coffee does not have a fixed formulation and the composition of the beverage when we drink it will depend on the origin of the beans, their processing and the way that is brewed before we drink it.

Conversely, most of the information regarding its healthy and unhealthy properties comes from subjective information obtained from questionnaires that cannot capture all the important details mentioned above. Currently the reported benefits of coffee drinking, including cardiovascular protection, lower risk of diabetes, improved cognitive performance, decreased total mortality and even protection against certain cancers, outweigh the potential downsides (i.e., transient increases in blood pressure, anxiety, insomnia). However, we need to be careful with the claims placed at both sides of the balance given the fact that most of the evidence has been obtained from observational studies and no long term randomized clinical trials have been performed in order to provide these claims with the highest level of scientific evidence. To this caveat we need to add the interindividual variability in response resulting from human genetic variation. Thus, we know that coffee drinking has a strong genetic component and so are the metabolic responses and thus, the reported disease associations.

In summary, the complex composition of coffee and the interindividual variation in the physiological exposure or response to caffeine or other coffee constituents challenge the traditional epidemiological approach to studies of coffee and health. In looking at the future, systems biology approaches will provide more solid basis about causality, explain the constituents of coffee responsible for health effects, and identify the subgroups most likely to benefit from increasing or decreasing coffee consumption.

7th ICPH International Conference on Polyphenols and Health

SELECTED COMMUNICATIONS

PARIS

TOURS

October 27-30, 2015

PARALLEL SESSION A - *Auditorium Descartes*
Innovative aspects in polyphenols bioavailability and exposure assessment

S01
Multiple-approach studies to assess anthocyanin gastric absorption – potential role of GLUT1

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The health effects associated with the consumption of anthocyanins-rich foods is unquestionable. Besides the low amounts detected in human samples, anthocyanins are rapidly detected in plasma in their parent forms, which may suggest their absorption at the gastric level.

The anthocyanins gastric absorption mechanism was evaluated by gathering the results from in vitro studies together with the results from human intervention studies. The absorption of anthocyanins through the gastric barrier was evaluated using a gastric adenocarcinoma cancer cell line MKN-28. Our results suggest that anthocyanins are absorbed through the gastric epithelium. This absorption was incubation time dependent, reaching a maximum around 6% after 3 hours. The transport of anthocyanins was reduced in the presence of glucose, suggesting a transport competition between anthocyanins and glucose. To understand anthocyanin gastric absorption mechanism a new stable transfected MKN-28 cell line with human glucose transporter hGLUT1 (pCMV6-SLS2a1) was developed. Also, a computational study was performed in which anthocyanins showed affinity for hGLUT1 forming stable complexes and similar conserved binding mechanism.

In the human study, the volunteers ingested a blackberry juice, containing mainly cyanidin-3-glucoside (Cy3glc). Cy3glc was detected in plasma samples, with a C_{max} located within the 2h period. The early appearance of Cy3glc methylated metabolites (peonidin-3-glucoside and isopeonidin-3-glucoside) in plasma reinforces the absorption and metabolism of Cy3glc at the gastric level, resulting in their detection after only 15 min of ingestion with the 3' isomer being favored. This metabolism pattern of Cy3glc was already observed with MKN-28 cellular lysates.

Financial support: This work was supported by FCT (Fundação para a Ciência e Tecnologia) (POCI, FEDER, Programa Comunitário de Apoio) by one Doc grant (SFRH/BD/93073/2013), two Post-Doc grants (SFRH/BPD/86173/2012 and SFRH/BPD/75294/2010), one starting grant (IF/01355/2014) and one project grant (PTDC/AGR-TEC/2227/2012).

S02
Role of sulfotransferases in resveratrol bioavailability and adipocyte metabolism

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Polyphenols such as resveratrol have received a lot of attention over the last years for their potential to trigger and/or improve weight loss in obesity, mimicking effects of caloric restriction with reduced body fat and increased energy expenditure. However polyphenols are rapidly metabolized in the human body, and it is not clear in which metabolic form and how they precisely act intracellularly within the target tissues. One of the main enzymes responsible for metabolizing these compounds is the phase II sulfotransferase enzyme SULT1A1, that sulfates and thereby inactivates polyphenols. We hypothesized that lower activity of this enzyme could augment the bioactive form of resveratrol intracellularly. We used two human cell models to explore and compare resveratrol metabolism, namely intestinal caco2 as well as SGBS adipocyte cells. By UHPLC-MS/MS, we found that in both cell models resveratrol is mainly metabolized into its monosulfated form, with dose-dependent changes. The expression of the SULT1A1 sulfotransferase enzyme is upregulated upon differentiation of both cell lines. Knocking down SULT1A1 in adipocytes to ~30% by siRNA resulted in lower amounts of resveratrol-sulfate and increased parent resveratrol, both intra- and extracellularly. This lower SULT1A1 activity resulted in an increased antilipolytic effect of resveratrol on SGBS adipocytes. Dysregulation of sulfotransferase expression in adipose tissue might thus play an important role in altered bioavailability and metabolic action of its substrates.

S03
Neuroprotective potential of phenolic sulfates, abundant bioavailable metabolites

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Over the past few decades, epidemiological studies have revealed that diets rich in fruits and vegetables promote health benefits, preventing against age-related disorders. Such pathologies are still without a cure but berries, rich in (poly)phenols, emerge amongst the most promising fruits to be effective protectors. (Poly)phenols and their metabolites must be bioavailable to exert biological effects. Evidences indicate that (poly)phenol metabolites can enter in the central nervous system in very small amounts but knowledge about their mechanisms of action are scarce.

Phenolic sulfates were identified in plasma samples from a human intervention study and the most relevant were chemically synthesized. The putative transport across the blood-brain barrier (BBB) of bioavailable (poly)phenol metabolites, detected in human plasma, was studied. Physiological concentrations were applied to a cellular model of the BBB and we observed, by LC Orbitrap-MS, that these metabolites are differently transported. Moreover, the neuroprotective effects of these metabolites was explored in different neuronal cells, in 2D and 3D systems, and a protective potential was observed. This work highlights the pertinence of these of metabolites a valuable biomarkers of (poly) phenol consumption and reinforces its value due to the BBB permeability and ultimate potential as neuroprotective and anti-inflammatory agents.

Financial support: This study and individual fellowships were supported by FCT Project (PEst-OE/EQB/LA0004/2011; IF/01097/2013); EuBerry Project (EU FP7 KBBE-2010-4 265942) and BacHBerry (FP7-KBBE-2013-613793).

S04
Linking metabolism and bioactivity of curcumin

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A remarkable feature of curcumin is its polypharmacology, a term used to refer to its ability to affect >100 distinct cellular targets. While this pleiotropy contributes to the popularity of curcumin as a dietary supplement and alternative therapeutic agent it creates a problem from the medicinal biochemistry point of view: that of specificity and mechanism – how can an apparently simple molecule like curcumin affect so many and diverse cellular targets? We hypothesize that metabolic transformation underlies the polypharmacology of curcumin. We have found that the well-recognized instability of curcumin at physiological pH is due to spontaneous autoxidation resulting in a dioxygenated bicyclopentadione derivative of curcumin. En route to the bicyclopentadione are quinone methide and spiroepoxide electrophilic intermediates that form adducts with cysteine residues of small molecule thiols or proteins. Michael adduction of reactive metabolites with critical cysteine sensors in target proteins provides a mechanism by which curcumin can affect a multitude of cellular targets. Using [$^{14}C_2$]curcumin as a tracer we have isolated and identified eight chemically distinct products formed in the autoxidation of curcumin at physiological pH. We argue that curcumin is not a single molecule but instead a pro-drug that gives rise to a multitude of chemical species with a wide spectrum of functional moieties and reactivities, including free radical, peroxyl radical, quinone methide, endoperoxide, spiroepoxide, vinylether, hemiacetal, cyclopentadione and other moieties. The chemical diversity of its spontaneous transformation products provides a novel paradigm to understand the polypharmacology of curcumin.

Financial support: NIH awards CA159382 and AT006896

PARALLEL SESSION B - *Auditorium Ronsard*
Advances in polyphenols targets for obesity, metabolic syndrome and diabetes

S05
Regularly consuming a green/roasted coffee blend shows benefits against metabolic syndrome

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Protective health effects of coffee could have a widespread impact on public health considering its high intake in industrialized countries. Coffee has been associated to weight loss, although its effects on hypercholesterolemia and type-2 diabetes are contradictory. Green coffee is richer in phenols and thus presents greater health potential than roasted, although its bitterness limits acceptance. This work evaluated the effects of regularly consuming a green/roasted coffee blend (G/R:35/65) on cardiovascular, diabetes and obesity biomarkers in humans.

A crossover, randomized, controlled study was performed in 25 normocholesterolemic and 27 hypercholesterolemic men and women, aged 18-45y, non-smoker, non-vegetarian with BMI 18-25kg/m2. Three servings/day of G/R:35/6 in water were consumed versus not consuming any coffee (8 weeks each). Polyphenol and methylxanthine rich foods were restricted. At the beginning and end of each intervention, blood samples were collected, anthropometric parameters, heart rate, diastolic and systolic blood pressure were taken, and dietary records and physical activity questionnaires completed. Biomarkers analyzed in serum were: glucose, total-C (cholesterol), HDL-C, LDL-C, triglycerides, antioxidant capacity (FRAP and ORAC methods, and lipid oxidation by malondialdehyde levels); C-peptide, ghrelin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1, glucagon, insulin, leptin, plasminogen activator inhibitor-1, resistin and visfatin using a multiplex kit. HOMA-IR, HOMA- and QUICKI were calculated. Statistical study: a general linear model of the variance for repeated measures was used and a Bonferron test within each group.

In conclusion, regularly consuming G/R:35/65 decreased insulin resistance, dyslipidemia, weight, and hypertension, thus reducing the risk of obesity, type 2 diabetes and metabolic syndrome.

Financial support: Funded by the Spanish Ministry of Science and Innovation AGL2010-18269, CSD2007-00063, AGL2014-58205-REDC, and JAE Program (CSIC and European Social Funds).

S06
Flavan-3-ols and n-3 fatty acids prevent non-alcoholic fatty liver disease in a high fat-high fructose fed mouse model

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Between 20-30% of EU adults present with non-alcoholic fatty liver disease (NAFLD), which is referred to as the hepatic component of the metabolic syndrome. An imbalance of lipid partitioning and a pro-inflammatory state are defining features of the pathology. Here based on knowledge of their molecular mechanisms of action and likely complementarity, we aim to demonstrate that the combination of marine n-3 fatty acids and flavan-3-ols will reduce steatosis and its associated metabolic dysregulation through altered expression of lipid metabolising and inflammatory genes and their regulatory transcription factors. In a mouse model of NAFLD, we examined the independent and interactive impact o n-3 fatty acids and flavan-3-ols on histologically defined NAFLD, insulin sensitivity hepatic gene expression, and fatty acid and flavan-3-ol profiles.

All treatments were effective in reducing steatosis and hepatic damage, with use of the human NAFLD activity score (NAS), indicating the greatest efficacy for the combination of n-3 fatty acids and flavan-3-ols which reduced both steatosis and hepatocyte ballooning by >90% ($p < 0.05$). Flavan-3-ol intake resulted in a 20-23% lower weight, and lower fasting and insulin responses during the intra-peritoneal glucose tolerance test ($p < 0.05$). Impacts of intervention on the expression of lipogenic genes and fatty acid transporters, SREBP1c and PPAR along with modulators of extracellular matrix were evident.

The combination of n-3 fatty acid and flavan-3-ols was established as an effective diet-based preventative strategy for NAFLD in a rodent model. Its ability to prevent progression or reverse pathology in existing NAFLD remains to be established.

Financial support: Minihane's research is supported by a BBSRC ISP Programme Grant (BB/J004545/1

S07

Estrogen receptor triggers most of the beneficial effect of red wine polyphenols intake of obesity-induced metabolic and vascular disorders

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OBJECTIVES: Extracts of red wine polyphenols correct cardiovascular and metabolic disorders associated with obesity. In addition, anthocyanins possess the same pharmacological profile than the total extract of red wine polyphenols exerts the protective effects by activating the alpha isoform estrogen receptor (ER). Here, we tested the investigated the contribution of ER on the effects of red wine-derived polyphenols (RWP) on cardiovascular and metabolic alterations associated with obesity.

METHODS: We used ovariectomized mice lacking or not ER receiving standard die (SD), western diet (WD), or SD and WD containing RWP at the dose of 20 mg/kg (SD+RWP and WD+RWP, respectively) during 12 weeks. Body weight was measured during treatment. Echocardiography examination was performed before sacrifice. Blood and tissues were sampled for biochemical and functional analysis with respect to NO and oxidative stress. Vascular reactivity and liver mitochondrial complexes were analysed.

RESULTS: In mice receiving WD, RWP reduced body weight gain, visceral and subcutaneous adipose tissue accumulation, plasma triglycerides and the decreased oxidative stress in aorta, heart, adipose tissues and liver. Also RWP improved activities of hepatic citrate synthase and complex II of mitochondrial chain. Deletion of ER prevented or reduced the beneficial effects of RWP especially aortic and liver oxidative stresses but it did not correct the increase of glycemia and cholesterolemia induced by WD.

CONCLUSIONS: Dietary supplementation of RWP remarkably attenuates features o metabolic syndrome most of these effects require ER -dependent mechanisms in mice fed with WD. Therefore, this study points for the therapeutic potential of this extract in metabolic and cardiovascular alteration linked to excessive energy intake.

Financial support: This study was support by grants from OSEO (Bpifrance).

S08

The metabolism enhancement effect of cyanidin-3-glucoside through skeletal muscle adipocyte and their communications

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Decreased metabolic activity is closely related to the epidemic of obesity, making th enhancement of metabolic activity is an effective way for prevention of obesity.

Cyanidin-3-glucoside (Cy3G), an anthocyanin, is contained in black soybean and blueberry and has been known to have anti-obesity effect. In this study, we investigated the effect of Cy3G on metabolism using skeletal muscles and adipocyte and by exploring the interaction between their two cell types.

First, black soybean seed coat extract (BSSCE) rich in Cy3G were fed to db/db mice (five week-old) for 30 days and the mice body weight was measured daily. Furthermore, to evaluate the effect of Cy3G on skeletal muscles and adipocytes *in vitro*, C2C12 myotubes and 3T3-L1 cells were treated with Cy3G.

BSSCE-administration inhibited the gain in body weight and white adipose tissue weight of db/db mice. Furthermore, Cy3G-treated 3T3-L1 cells had increased adiponectin secretion and decreased TNF- secretion by differentiating into smaller adipocytes. C2C12 myotubes cultured with spent medium of 3T3-L1 adipocyte cultures treated with Cy3G, also showed significantly increased expression of metabolism-related genes such as *PGC-1*. In addition, Cy3G enhanced the metabolic activity of C2C12 myotubes through the activation of mitochondrial activity.

The results of this study indicate that Cy3G has an anti-obesity effect through the enhancement of metabolic activity of skeletal muscles and adipocytes.

Financial support: This research is supported by the Center of Innovation Program from Japan Science and Technology Agency, JST.

PARALLEL SESSION C SPONSORED BY PEPSICO - *Auditorium Descartes*
Neuro-cognitive effects of polyphenols: at the cutting edge

S09
Characterization of Brain-Bioavailable Bioactive Polyphenol Metabolites in Pre-Symptomatic Alzheimer's Disease

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Alzheimer's disease (AD) is one of the top public health challenges facing the Western world and developing countries due to ever-increasing population aging. Currently there is no effective means to prevent or treat AD. Since 1998, there have been over 100 drug trials targeting specific AD pathogenic mechanisms, all of which have failed. AD is a complex disease involving multiple interrelating pathogenic mechanisms. We hypothesized that strategies to simultaneously target multiple pathogenic mechanisms may increase the likelihood of therapeutic success.

Polyphenols are receiving increasing attention for their potential role in preventing the onset and/or progression of age dependent preclinical AD. We previously reported that select grape-derived polyphenols can reduce AD-type neuropathology and attenuate cognitive decline in various mouse models of AD. More recently, we identified polyphenols metabolites, either individually or in combination, that can selectively influence specific AD pathologic features for translational therapeutic strategies. In particular, we identified 16 brain-bioavailable bioactive polyphenol metabolites with drug-like properties and in vivo safety profiles that can beneficially influence major AD-related neurodegenerative mechanisms following treatments wit polyphenols. Following brain isolation and structural identification of these metabolites, we are currently investigating their brain target engagement and mechanisms of action in mouse models of AD genetically engineered to develop AD-like synaptic degeneration, blood-brain barrier damage, and tau pathology. Moreover, new preliminary clinical evidence supporting the clinical development of brain-bioavailable bioactive polyphenol precursors, capable of delivering bioactive metabolites for pre-symptomatic AD subjects to delay/prevent the onset AD dementia, will be discussed.

Financial Support: NCCAM Grant 5P01AT004511 (to GMP)

S10
The acute effects of a polyphenol-rich blueberry beverage on cognitive function in healthy younger and older adults

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Blueberries, a rich source of polyphenols, have been shown to result in cognitive benefits such as improved spatial memory in rodents. Also, chronic supplementation has been shown to improve memory in older adults with early memory changes.

However, little is known about potential cognitive benefits in healthy adults. As such, acute cross-over RCTs were conducted to investigate whether a polyphenol-rich (579mg) blueberry beverage would lead to improvements in vascular and cognitive function compared to a control drink in healthy younger (N=19) and older (N=18) adults. Stiffness index (SI) and blood pressure (BP) were recorded at baseline and a hour post consumption of the study beverages, whereas cognitive performance was measured at baseline then 2 and 5 hours post drink. Whilst there was no effect of the intervention on SI or BP, the blueberry beverage resulted in improved executive function in the younger sample and memory in the older adults. In addition, a fMRI study (N=12 younger adults) found increased cerebral blood flow (CBF) to an area o the frontal lobes, a region thought to be important for executive function. This research suggests that cognitive benefits following blueberry supplementation are evident in healthy populations and can be seen following a single dose of polyphenol-rich blueberries. Furthermore, polyphenols may interact with nitric oxide pathways, improving endothelial function and hence increasing vasodilation, ultimately improving blood flow to the brain. Increasing CBF may result in acute benefits to cognitive function whilst in the longer term promote neurogenesis and lea to chronic cognitive benefits.

Financial support: This research was funded by a research studentship from the Graduate School of Social Sciences at the University of Reading.

S11
Beneficial effects of the Neurophenol extract on age-related cognitive decline

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It is now well accepted that aging is linked to the onset of cognitive impairments. Several studies have highlighted the beneficial role of polyphenols on memory functions and particularly on age-related memory decline.

Thus, the Neurophenol project aims at developing nutritional assets rich in polyphenols from grape and blueberry, and to objectify their beneficial role on the age-related cognitive decline in humans and pets. The preclinical part of this project aims to highlight beneficial effects of a polyphenol mix from grape and blueberry, the Neurophenol™ extract, on learning and memory performances in aged mice and to better understand the neurobiological mechanisms underlying these performances. More specifically, we have focused on the hippocampus, a key brain structure involved in learning and memory processes that is particularly altered during aging.

Our main results show that hippocampal-dependent learning and memory alterations during aging are recovered by a polyphenol supplementation. We also show that these age-related memory deficits are linked to a decrease of gene expression of proteins involved in signaling pathways underlying memory processes and to a decrease of hippocampal neurogenesis. The polyphenol supplementation can restore the expression level of some genes and up-regulate neurotrophin expression in the hippocampus. Moreover, this supplementation has a beneficial impact on hippocampal neurogenesis in aged mice. Finally, we show that a Neurophenol™ supplementation can reduce mortality in aged mice.

These results demonstrate the efficiency of the Neurophenol™ extract on the maintenance of memory performances in mice during aging, through their action on synaptic plasticity and neurogenesis.

These promising results in aged mice provide positive arguments on the benefits of polyphenols on memory during aging. Thus, it was also developed a clinical study aiming at supplementing elderly subjects with the Neurophenol™ extract in order to evaluate their memory performances.

Financial support: This work is part of the Neurophenol project selected in the 12th call for FUI (Fonds Unique Interministériel) project. It has been supported by BPI France, the Conseil Régional d'Aquitaine, and by the European Regional Development Fund (ERDF).

S12
Green tea promotes positive effects on redox status of different regions in the central nervous system of obese and lean rats

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The concept that obesity likely affects the brain has emerged recently. Much attention has been dedicated to understanding redox control in nerve cells to identify new therapeutic targets to sustain a neuroprotective therapy based on oxidant protection. The effects of green tea (GT) polyphenols against neurodegenerative disorders have been reported. This study aimed to evaluate some indicators of oxidative stress, inflammation and endoplasmic reticulum stress on different regions of the central nervous system (CNS) of obese rats treated with GT extract. The cafeteria diet provided to the rats for 2 months had little effect on oxidative stress (antioxidant enzyme activity, oxidative damage in biomolecules, GSH/GSSG ratio and FRAP) an inflammation (cytokine release, TLR4 mRNA level) in the cerebral cortex, cerebellum and hypothalamus; however, the brainstem was more sensitive to cafeteria-diet induced oxidative stress. It was evident that GT treatment (500 mg/kg of body weight) for 60 days had numerous protective effects against oxidative damage to biomolecules in all brain regions analyzed. In the hypothalamus, GT had beneficial oxidative effects, including increasing antioxidant enzymes and transcription factor Nrf2 and reducing inflammation. Different CNS regions did not respond the same way when exposed to a cafeteria diet. We conclude that, different a high fat diet, the cafeteria diet imposed a mild imbalance in the redox status of the cerebral cortex, cerebellum and hypothalamus, while showing greater effect on the rat brainstem. GT treatment could be a good strategy for improving and maintaining brain function under both healthy and pathological conditions.

Financial support: FAPESP 2011/19216-8 and 2012/19681-5, National Council for Scientific and Technological Development (CNPq) and Cruzeiro do Sul University.

S13

Phenolic acid intake, delivered via moderate Champagne wine consumption, improves spatial working memory via the modulation of hippocampal and cortical protein modulation in aged animals.

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While much data exist for the effects of flavonoid-rich foods on spatial memory in rodents, information related to foods/beverages predominantly containing hydroxycinnamates and benzoic acids is scarce. To address this knowledge gap, we investigated the effects of moderate Champagne wine intake, which is rich in these components, on spatial memory and related mechanisms relative to the alcohol- and energy-matched controls.

In contrast to the isocaloric and alcohol-matched controls, supplementation with Champagne wine (1.78 ml/kg BW, alcohol 12.5% vol.) for 6 weeks led to an improvement in spatial working memory in aged rodents. Targeted protein arrays indicated that these behavioural effects were paralleled by the differential expression of a number of hippocampal and cortical proteins, including those involved in signal transduction, neuroplasticity, apoptosis, and cell cycle regulation. Western immunoblotting confirmed the differential modulation of brain-derived neurotrophic factor, cAMP response-element-binding protein (CREB), p38, dystrophin, 2,3-cyclic-nucleotide-3-phosphodiesterase, mammalian target of rapamycin (mTOR) and Bcl-xL in response to Champagne supplementation compared to the control drink, and the modulation of mTOR, Bcl-xL, and CREB in response to alcohol supplementation.

Our data suggest that smaller phenolics, such as tyrosol, gallic acid and caffeic acid, in addition to flavonoids, are capable of exerting improvements in spatial memory vi the modulation in hippocampal signaling and protein expression. Changes in spatial working memory induced by the Champagne supplementation are linked to the effects of absorbed phenolics on cytoskeletal proteins, neurotrophin expression, and the effects of alcohol on the regulation of apoptotic events in the hippocampus and cortex.

Financial support: The BBSRC partly funded these experiments

PARALLEL SESSION D - *Auditorium Ronsard*
Recent breakthroughs in cancer prevention

S14
Dietary polyphenols and cancer incidence: a comprehensive systematic review and dose-response meta-analysis

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Background. Dietary polyphenols have been suggested to reduce the risk of certain cancers. The aim of this study is to systematically review the current evidence on dietary polyphenols and incidence of various cancers.

Methods. A systematic search was conducted in electronic databases to identify studies that satisfied inclusion/exclusion criteria. Relative risks (RRs) and 95% confidence intervals (CIs) of highest vs. lowest exposure were extracted and pooled using either a fixed-effects or a random-effects model. Generalized least-squares trend estimation, corrected linear trends, fractional polynomials, and restricted cubic splines analysis methods were used to evaluate dose–response relationships.

Results. A total of relevant 165 studies included the following datasets: 31 from prospective and 44 from case-control studies on breast cancer; 11 and 19 on prostatic cancer; 7 and 11 on ovarian cancer; 3 and 11 on endometrial cancer; 13 and 9 on lung cancer; 4 and 2 on renal cancer; 2 and 12 on upper aero-digestive cancers; 9 and 11 on gastric cancer; 16 and 15 on colorectal cancer; 1 and 6 on pancreatic cancer; 4 an 2 on liver cancer; 5 and 1 on bladder cancer; 5 and 2 on blood cancers; 2 and 1 on thyroid cancer; 2 from prospective studies on urinary organs cancer; 1 from a case-control study on central nervous system cancer; 1 from prospective study on ski cancer; 1 from a case-control study on testicular cancer. Intake of isoflavones was associated with significant decreased risk of hormone-related cancers, such as breast (RR 0.65, 95% CI 0.57, 0.75 for case-control, RR 0.87, 95% CI 0.79, 0.96 for prospective studies), ovarian (RR 0.62, 95% CI 0.46, 0.83 for case-control), endometrial (RR 0.83, 95% CI 0.74, 0.91 for case-control), and prostate cancer (RR 0.85, 95% CI 0.77, 0.93 for case-control). Pooled analyses of studies on lignans revealed non-significant decreased risk for the aforementioned cancer types. Total flavonoids intake was not associated with any cancer whereas individual flavonoid classes (such as flavonols, flavones, catechins, flavan-3-ols, proanthocyanidins) showed decreased risk of certain cancers, including colon, lung, and stomach cancers.

Conclusions. The present study suggests that evaluation of total flavonoid intake ma lead to inconclusive result but an association between certain flavonoid classes and decreased risk of various cancers is relevant.

Financial support: None.

S15
Preclinical efficacy of pterostilbene against human melanoma: a stress-related indirect mechanism reconciles bioavailability and anticancer activity in vivo.

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Abundant information is available on cellular mechanisms by which phytochemicals of polyphenolic structure, commonly named natural polyphenols (PFs), may interfere with carcinogenesis, tumour growth and dissemination. However a main problem with PFs is their short half-life and low bioavailability under in vivo conditions. Indeed the main discrepancy between health claims versus clinical observations is the frequent use of non-physiologically relevant concentrations of these compounds in mechanistic studies. This discrepancy represents a fundamental question which has remained unsolved.

Different PFs have been shown to inhibit growth of different cancer types in vivo. Nevertheless it is unclear how potential underlying mechanisms can be correlated with bioavailable concentrations and biological half-life. We have addressed this question using different human melanomas growing in mice as xenografts and pterostilbene (trans-3,5-dimethoxy-4'-hydroxystilbene; Pter), a natural dimethylated analog of resveratrol but with higher biological half-life. Our results indicate that a) in vivo administration of Pter (i.p. or i.v.) inhibits melanoma growth (oral administration was found useless); b) the use of bioavailable concentrations of Pter, under in vitro conditions, does not affect tumour cell growth or viability; c) Pter treatment modulate the Nrf2-dependent antioxidant system in in vivo growing melanomas; d) Pter-induced melanoma growth inhibition involves an indirect mechanism where physiological glucocorticoids play a key role.

Our results show that, under in vivo conditions, the main anti-melanoma effect elicited by Pter is indirect and depends on neuroendocrine signaling mechanisms. These findings, if generalized to other cancers and PFs, could explain the apparent lack of correlation between bioavailability and anticancer activities.

Financial support: This research was supported by grants from the MINECO (SAF2012-31565), the ISCIII-FEDER (PI13/01848), and by Green Molecular, Spain.

S16

Analysis of Normal and Malignant Colon Tissue Specific MicroRNAs in Response t Dietary Intervention with a Pomegranate Extract: Modulatory Effects vs. Potential Artefacts

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MicroRNAs (miRs) are proposed as colorectal cancer (CRC) biomarkers. Dietary components modulate miRs expression. Pomegranate ellagic acid and their microbiota metabolites urolithins exert anticancer effects in preclinical models of CRC and are detected in normal (N) and malignant (M) colon tissues from CRC patients. Herein, we investigated whether the consumption of a pomegranate extract (PE) modified the expression of representative miRs in colon samples from CRC patients.

We conducted a randomized, controlled, double-blind study. Thirty-five CRC patient consumed 900 mg PE daily before surgery. A control group (n=10) was included.

Using RT-PCR, we compared the levels of 15 miRs in M and N colon tissues. Our results revealed: 1) significant differences for specific miRs between M and N tissue that can be modified by the surgical protocol; 2) an opposed trend between -5p and -3p isomolecules; 3) the induction of most analyzed miRs attributable to the surgery; 4) various miRs that were likely altered by the intake of the PE some of which have been associated with CRC and might be involved in the anticancer effects of the PE components. No apparent relationship between the pomegranate metabolites detected in colon tissues and miRs levels was detected. The PE components responsible for these effects and the potential clinical endpoint of these observations deserve further research. Our study confirmed a critical effect of surgery on miRs levels which hinders the discrimination of changes caused by dietary factors and the establishment of actual differences between M and N tissues as biomarkers.

Financial support: AGL2011-22447 (MINECO, Spain).

S17

Exploring the anti-tumoral effect of citrus polyphenols using a 3D cell model of colorectal cancer

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Polymethoxylated flavones (PMFs) found in citrus fruits are very attractive natural chemopreventive and chemotherapeutic agents. Several studies showed that PMFs inhibit cancer development through various mechanisms (e.g. cell cycle modulation, antiangiogenic effect, apoptosis induction) and present promising effect in combination with chemotherapeutic drugs. However, the majority of these studies were performed on cells grown on monolayers (2D) that present several limitations i mimicking cancer tissue architecture. Recent advances in cellular models show that 3D in vitro cultures of human cancer cell lines are a promising tool to address specific mechanisms and pathways in cancer development but their application in the Nutrition&Health field is very limited.

The aim of this work was to use a 3D cell model of colorectal cancer to evaluate the anticancer potential of PMF-rich extracts produced from orange peels using supercritical fluid technology. For this purpose, HT29 cells were cultured as cell aggregates in stirred-tank bioreactors that allowed the generation of 3D cellular spheroids with typical characteristics of in vivo tumors (e.g. presence of hypoxic core, exhibition of dedifferentiated outer rim and presence of cancer stem cells).

Results showed that PMF-rich extract inhibited cell proliferation and decreased HT2 spheroid size in a dose dependent manner. It is important to highlight that the antiproliferative response was significantly lower ($p<0.05$) than that obtained in 2D cell model ($EC50_{2D}$=0.43mg/ml;$EC50_{3D}$=1.24mg/ml) which could be explained by diffusion limitations and/or chemoresistant phenotype of cellular spheroids. The effect of PMF-rich extract in inducing cell cycle arrest, promoting apoptosis and targeting cancer stem cell population was also evaluated and compared with standard PMFs. The HT29 spheroids provide a cellular context closer to actually occur in cancer microenvironments being a valuable pre-clinical model to study and evaluate the anticancer effect of food bioactives, including polyphenols.

Financial support: The authors acknowledge the FA COST Action TD1203 - Food waste valorisation for sustainable chemicals, materials & fuels (EUBis) (2012-2016)

S18

Bioavailability and bioactivity of raspberry phytochemicals using in vivo digestion coupled to ex vivo simulated colonic fermentation.

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The anticancer properties of bioactive phytochemicals within berries are of interest given the inverse correlation of fruit and vegetable consumption with the incidence o colorectal cancer (CRC). Post-consumption, raspberry phytochemicals undergo digestion and microbiota-mediated alterations in their structure and function. As suc the importance of in vivo digestion cannot be over-estimated when considering the potential anticancer activity of berry phytochemicals. We investigated, the fate of colon-available raspberry (poly)phenolic compounds following the acute ingestion a raspberry puree, made from 300 g of berries, by by volunteers with an ileostomy.

Ileal fluid was collected for up to 8 h post-supplementation and analyzed by targeted and untargeted $LCMS^n$ to identify novel components entering the colon. The colonic microbiota has a key role to play in the catabolism of phytochemicals that are not absorbed in the small intestine, giving rise to a diversity of phenolic and aromatic acids which may reduce risk of CRC through direct interaction with the colonic epithelium. Therefore, we simulated the impact of the colonic microbiota on in vivo digested berry phytochemicals by subjecting ileal fluid to fermentation (24 h) with human faecal inocula (from a common donor) and determined the effects on phenoli composition and whether breakdown products retained their ability to modulate DNA damage in normal CCD 841 CoN and transformed (HT29) colonic cell lines.

Financial support: National Processed Raspberry Council Grant.

PARALLEL SESSION E - *Auditorium Ronsard*
Update for properties of polyphenols in cardiovascular diseases

S19
Increased microparticle production and impaired microvascular endothelial function in aldosterone-salt-treated rats: protective effects of polyphenols.

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We aimed to characterize circulating microparticles in association with arterial stiffness, inflammation and endothelial dysfunction in aldosterone-salt-induced hypertension in rats and to investigate the preventive effects of red wine polyphenols Uninephrectomized male Sprague-Dawley rats were treated with aldosterone-salt (1µg.h(-1)), with or without administration of either red wine polyphenols, Provinols™ (20 mg.kg(-1).day(-1)), or spironolactone (30 mg.kg(-1).day(-1)) for 4 weeks.

Microparticles, arterial stiffness, nitric oxide (NO) spin trapping, and mesenteric arterial function were measured. Aldosterone-salt rats showed increased microparticle levels, including those originating from platelets, endothelium and erythrocytes.

Hypertension resulted in enhanced aortic stiffness accompanied by increased circulating and aortic NO levels and an upregulation of aortic inducible NO-synthase NFB, superoxide anions and nitrotyrosine. Flow-induced dilatation was reduced in mesenteric arteries. These effects were prevented by spironolactone. Provinols™ did not reduce arterial stiffness or systolic hypertension but had effects similar to those o spironolactone on endothelial function assessed by flow-mediated vasodilatation, microparticle generation, aortic NO levels and oxidative stress and apoptosis in the vessel wall. Neither the contractile response nor endothelium-dependent relaxation in mesenteric arteries differed between groups. The in vivo effects of Provinols™ were not mediated by mineralocorticoid receptors or changes in shear stress. In conclusion vascular remodelling and endothelial dysfunction in aldosterone-salt-mediated hypertension are associated with increased circulating microparticles. Polyphenols prevent the enhanced release of microparticles, macrovascular inflammation and oxidative stress, and microvascular endothelial dysfunction independently of blood pressure, shear stress and mineralocorticoid receptor activation in a model of hyperaldosteronism.

Financial support: This work was supported by grants from the “Fondation de France” (2005005375) and the “Agence Nationale de la Recherche”.

S20
Comparative acute effects of soy isoflavones and orange juice-derived flavanones on markers of cardiovascular risk in men at moderate risk of cardiovascular disease

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Background/Objectives: Prospective studies and randomised controlled trials (RCTs) suggest cardioprotective effects of flavanones and isoflavones, however the impact o inter-individual variability in metabolism remains unclear. In vitro and retrospective RCT data suggest vascular effects of the microbiota-derived isoflavone metabolite equol, yet equol producers (EPs) have never been prospectively recruited to confirm acute vascular responses. Similarly, the vascular effect of equol supplements are unknown in non-EPs. We assessed flavanone and isoflavone metabolism and associated acute vascular function, and determined vascular responses according to EP phenotype.

Methods: 44 male participants at moderate risk of cardiovascular disease undertook a soy-challenge; n=14 EPs and n=14 matched non-EPs were subsequently prospectively recruited to the isoflavone intervention. A further n=16 participants were recruited for the acute flavanone intervention. Two cross-over design RCTs were performed (isoflavone versus control; with equol supplementation assessed in non-EPs; flavanone: orange juice (OJ) versus hesperidin supplement versus control). Endothelial function, blood pressure, arterial stiffness and cardiac autonomic functio were assessed, together with plasma metabolites.

Results: Plasma Isoflavone and flavanone metabolites were significantly elevated (6 and 24h for isoflavones; 5h for

flavanones), with a range of previously identified and novel flavanone metabolites quantified. Whilst no acute vascular effects followed flavanone or equol supplement intake in non-EPs, EPs had significantly lower pulse wave velocity after soy isoflavone intake, which correlated with plasma equol concentrations.

Conclusions: Whilst acute flavanone intake did not elicit vascular effects, our data from prospectively recruited EPs shows, for the first time, the vascular benefits of th microbiota-derived EP phenotype.

Financial support: The Biotechnology and Biological Sciences Research Council (BBSRC, UK) funded this project

S21
Sustained effects of wild blueberry consumption on vascular function in healthy individuals

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Recent studies have shown that blueberries can improve endothelial function in healthy individuals acutely, but little is known on the chronic effects of daily consumption of blueberries. In a double-blind randomized controlled parallel study we investigated the effects of daily intake of a wild blueberry (the equivalent to 100 grams of fresh blueberry twice per day) or a placebo beverage for 1 month on vascular function (n=40). The results showed a significant increase in flow-mediated dilation (FMD) after 1 month supplementation (-0.28% vs 2.11%, $p<0.0001$) and acutely after 2 h post-consumption (0.02% vs 1.48%, $p<0.005$) when the intervention group was compared to the placebo. Polyphenols in urine and plasma were quantified using micro-elution SPE and targeted metabolomics by UPLC-Q-TOF MS. A multivariate regression analysis showed that the plasma concentrations of hippuric acid ($r=0.57$, $p<0.01$), catechol-O-sulfate ($r=0.56$, $p<0.01$), 4-methylcatechol-O-sulfate ($r=0.42$, $p<0.05$), 3-hydroxyphenylacetic acid ($r=0.46$, $p<0.05$), 4-hydroxyphenylacetic acid ($r=0.40$, $p<0.05$), 3-(4-hydroxy-3-methoxyphenyl)propionic acid ($r=0.47$, $p<0.05$), 1-methylpyrogallol-O-sulfate ($r=0.50$, $p<0.01$), dihydro caffeic acid 3-O-sulfate ($r=0.63$, $p<0.001$), homovanillic acid ($r=0.52$, $p<0.05$), ferulic acid 4-O-sulfate ($r=0.43$, $p<0.05$), isoferulic acid 3-O-ß-D-glucuronide ($r=0.48$, $p<0.05$) and the sum of total phenolics ($r=0.40$, $p<0.05$) were significantly correlated with changes in FMD detected after 1 month, while other compounds as dihydro ferulic acid 4-O-ß-D-glucuronide ($r=0.42$, $p<0.05$) and chlorogenic acid ($r=0.44$, $p<0.05$) were correlated with acute FMD increases 2h post consumption. These results suggest that blueberry polyphenol metabolites positively affect vascular function and may be, at least in part, involved in mediating the observed increases in FMD after 1 month blueberry supplementation.

S22
Increased bioavailability of phenolic acids and enhance vascular response following intake of an enzymatically generated, high free ferulic acid flatbread.

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Whole-grain contains a high amount of phenolic acids (PA), predominantly ferulic acid (FA), which have been found to prevent cardiovascular disease risk through their actions on the vascular system. However, whole-grain is covalently bound to arabinoxylans limiting bioavailability.

The objective of the present study was to investigate the impact of flatbread, treated to contain increased free FA, on endothelial function and phenolic absorption/metabolism.

A randomised, placebo-controlled, cross-over intervention trial was conducted on 20 healthy, male volunteers. The impact of a high free-ferulate flatbread (produced via enzymatic processing) on vascular response compared to standard wholegrain and white bread was assessed using flow-mediated dilatation (FMD) and Laser Doppler Iontophoresis (LDI) at baseline, 2, 5 and 7 hours. Urine samples were collected for 2 hours to assess FA absorption and metabolism.

A significant increase % FMD was observed at 5 h ($p<0.05$) post consumption of free ferulate-rich flatbread, relative to control (trend for increase at 2h; $p<0.1$). Changes i FMD response were paralleled by vascular improvements measured using LDI with iontophoresis, with increased endothelial-dependent blood flow improving at 5h (trend at 5h; $p<0.1$). Vascular changes were matched temporally by the appearance of FA metabolites in the urine: 3-fold higher after consumption of ferulate rich flatbread compared to the standard whole-grain and white after 2 hours. FA levels decreased at 7-24 h, corresponding with an increase in vanillic acid, 3,4- dihydroxybenzoic acid, 4-hydroxybenzoic acid and 3-(4-hydroxyphenyl) propionic acid.

In conclusion, enzymatic processing of whole-grain bread products increases the bioavailability of FA, and its benefits towards the vascular system.

S23
Do anthocyanins in purple tomatoes reduce the risk of cardiovascular disease?

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Background and rationale: Epidemiological studies have shown an inverse relationship between consumption of anthocyanins (ACN) and CVD risk while supplementing animal diets with ACN-rich food/extracts reduced atherosclerosis and altered gene expression. However, due to the presence of other bioactives, these studies cannot prove that ACNs cause the observed effects. To determine this, suitable control foods (i.e. similar foods lacking ACNs) are needed, but these do not occur naturally.

Approach: An animal feeding study using genetically modified 'purple tomatoes' expressing high levels of ACN and equivalent ACN-free red tomatoes was undertaken which allowed direct assessment of the ability of dietary ACNs to affect CVD risk. We used increasing concentrations of purple tomatoes (2.5, 5 and 10%) in purified diets containing a total of 10% w/w/ tomato and compared the effects with a 10% red tomato and no tomato diets. This highly controlled intervention in the ApoE$^{-/-}$ mouse model assessed the dose-response of ACNs from tomato on atherosclerosis and gene expression.

Results and conclusion: The 10% purple tomato diet caused a 45% mean reduction in aortic lesion size compared to the red tomato diet ($p<0.001$). Further, there was a clear linear dose-response ($R^2=0.999$, $p<0.0001$ using average data) demonstrating the ACNs had beneficial effects by reducing atherosclerosis in this mouse model. Gene expression data from livers and aorta of the mice showed an effect of the anthocyanin-rich tomatoes on reverse cholesterol transport. In conclusion, it is show for the first time that consumption of tomato anthocyanins significantly reduces atherosclerosis in the ApoE$^{-/-}$ mouse model.

Financial support: This research was funded by the EC 7th Framework Programme (Grant No. 245121; ATHENA) and by the Biotechnology & Biological Sciences Research Council through an Institute Strategic Programme Grant (BB/J004545/1) to IFR.

S24
Identification of the metabolomic fingerprint associated with flavonoid consumption level

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Objective: to determine whether there is a metabolic signature that is associated wit level of flavonoid consumption.

Methods: 184 circulating metabolites were profiled using liquid chromatography-tandem mass spectrometry in plasma samples of 584 men and women.

Results: Using Canonical Discriminant Analysis (CDA), we were able to develop a model that characterized low and high flavonoid consumers. In unadjusted and multivariate-adjusted analyses, the Canonical Variable resulting from the CDA was capable of differentiating participants with differing levels of flavonoid consumption

We then deconstructed the metabolome data to create the Metabolite Difference Score, which successfully described the pattern of 26 metabolites that characterized participants with different levels of total-flavonoid intake ($P<0.001$). The predominant characterizing features of high flavonoid consumers were their relativel low concentrations of 52:1 and 50:30 triacylglycerols and 20:3 lysophosphatidylcholines, and their relatively high concentrations of 58:9, 50:2 and 50:4 triacylglycerols and 22:6 lysophosphatidylethanolamines.

Conclusion: This study identifies metabolomic discrimination between low and high flavonoid consumers. These results suggest that metabolomic profiling may have a wide range of possible applications in the exploration of diet-metabolism relationships, particularly in relation to relationships with flavonoid-class consumption.

Financial support: Project grant from the US Highbush Council

S25
Fluid shear stress: a modulator of the vascular protective effects of curcumin?

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A growing number of evidences suggest that dietary polyphenols exhibit vascular protective properties by preventing the early stages of vascular dysfunction and atherosclerosis development. Using holistic nutrigenomic approaches, we have previously demonstrated that polyphenols may protect the integrity of vascular endothelium by reducing interactions between monocytes and endothelial cells. One important determinant of these inter-cellular interactions is the hemodynamic forces associated with blood flow, namely shear-stress. Usually, in-vitro studies aiming to decipher the impact of polyphenols on the molecular and cellular processes, are performed under static conditions. As chronic exposure of endothelial cells to lamina flow is known to affect endothelial cell functions, the aim of this work is to evaluate the impact of shear stress on the response of endothelial cells to polyphenols exposure.

Primary human endothelial cells were grown under a 1Hz-pulsatile aortic shear-stress of 7 dyne/cm² to mimic as closely as possible physiological conditions, or under static conditions. Confluent cells were exposed to low concentrations of curcumin (0.5-1M for 3 hours prior to their stimulation by TNF-alpha for 4 hours and then cell adhesion assays were performed. Our first results demonstrate that the pre-exposure of endothelial cells to 0.5µM curcumin under a physiological flow reduced by 40% the adhesion of THP1 monocytes while only a 25% decrease is observed in static condition. This observation suggests that shear-stress can modulate the cellular effects of polyphenols. Further experimentations are ongoing to identify the impact o this shear-stress on the nutrigenomic and molecular responses to curcumin in endothelial cells.

Financial support: INRA

S26
Exploring the biological activity of flavonoid metabolites

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Previous in vitro studies exploring flavonoid bioactivity primarily utilized precursor structures, while metabolites are found in much higher concentration in the circulation. In addition, most studies utilise supraphysiological concentrations (25-100 μM). We aimed to establish the potential mechanism of action of flavonoids by exploring physiologically relevant concentrations of metabolites. We screened 0.1 1 and 10 μM of 6 flavonoids (quercetin, hesperetin, naringenin, epicatechin, cyanidin and peonidin), and 14 common metabolites (phase II conjugates of benzoic, protocatechuic and vanillic acid), both in isolation and as mixtures, in THP-1 human monocytes, for cytotoxicity, Nrf2 & NF-B activity, and LPS-induced TNF-, IL-1 and IL-10 protein and mRNA expression. No cytotoxicity was observed. Treatments were subsequently screened for effects on Nrf2 protein expression. Despite previously reported activity of flavonoids, only queretin activated Nrf2 and its downstrem target gene, heme oxygenase-1. We then explored anti-inflammatory activity of the metabolites by assessing effects on LPS-induced TNF- secretion. Here two anthocyanins, 5 metabolites and four combination treatments significantly reduced TNF- protein, while there was no effects on TNF- mRNA, suggesting a post-transcriptional mechanism of action. Interestingly, the four bioactive mixtures contained compounds which were not active in isolation, suggesting additive or synergistic effects. The active treatments were subsequently explored for modulation of LPS-induced IL-1 or IL-10 secretion. No effects were observed. These data suggest that effects on TNF- protein are independent of NF-B, which was confirmed by NF-B luciferase assay. This study provides novel evidence suggesting flavonoid bioactivity results from cumulative or additive effects of circulating metabolites.

Financial support: Biological Sciences Research Council Diet and Health Research Industry Club (BBSRC-DRINC) (BB/I006028/1; BB/J500100/1).

S27
A novel matrix rich in flavan-3-ols: hazelnut skin. From phytochemical characterization to evaluation of biological activities.

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Nut skins are extremely rich in phenolic compounds with potential biological activity and may contribute to the benefits of a diet rich in nuts. Hazelnut (*Corylus avellana* L.) skin is a very rich source of monomeric and oligomeric flavan-3-ols, whose potential health effects – or those derived from their *in vivo* circulating metabolites – have not been studied in detail so far. The aim of this set of experiments is to fill this gap by applying an inter-disciplinary, comprehensive approach.

First, the (poly)phenolic profile of the raw material was fully assessed, and the stability of phenolic during its storage was evaluated. Subsequently, an experimental hazelnut skin extract (HSE) was developed and its cytotoxicity, genotoxicity, and safety were assessed. A pilot study was designed to identify the metabolites of human/microbial origin produced after consumption of a HSE drink and a yogurt containing hazelnut skin. The main circulating metabolites identified by LC-MS were chemically synthesized and their biological activity was evaluated in different *in vitro* models dealing with neurodegeneration (proteopathy-induced cell death in *S. cerevisiae* cells expressing either human -synuclein or an artificial -amyloid peptide) atherosclerosis (cell adhesion and migration), and cancer related molecular targets (EphA2-ephrinA1 protein-protein interaction). Finally, an intervention study targeting the endothelial function of healthy subjects after HSE consumption is currently ongoing. This scientific path allowed the identification of the responsible components, the involved mechanisms of action and the modulated physiological functions linked to the putative health effects of hazelnut skin extracts in humans.

Financial support: PM has been funded by a grant of the Postdoctoral Fellowship Program from Fundación Séneca (Murcia Region, Spain). LC's fellowship has been partly supported by "Progetto AGER" (grant no. 2011-0283).

S28

Polyphenols in physiological matrices: Where do they go and how do they get there?

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Polyphenols and their metabolites in order to be bioactive must be transported to cell and either bind to the cells or enter the cells. We have investigated several classes of polyphenols and their metabolites by *ex vivo* spiking of pig blood (human surrogate) with µM concentrations and measuring their concentrations in various fractions by HPLC.

Plasma contains more of the compounds than associates with red blood cells. Polyphenols and their metabolites were found bound to the surface and inside the red blood cells. We fractionated plasma by our published lipoprotein affinity column method. All classes of polyphenols, except phenolic acids, were present more in the LDL+VLDL fraction than in the albumin + HDL fraction. This in spite of the fact that the LDL+VLDL was 1.8µM in pig plasma and albumin + HDL was 690 µM(66µM albumin).

In later experiments using an albumin affinity column we were able to separate HDL from albumin and found that HDL bound more polyphenol than albumin which was greatly in excess. Thus the major transport proteins for most classes polyphenols and their glucuronide metabolites in our *ex vivo* model are shown to be the lipoproteins. We and others have published that polyphenols and their metabolites bound to lipoproteins protect them from oxidation both in the plasma and in the sub-endothelial space where oxidation takes place leading to atherosclerosis.

Since all cells need cholesterol and thus have cholesterol receptors, we hypothesize that lipoproteins are the major transport system for polyphenols and their metabolites to cells.

Financial support: University of Scranton Undergraduate and Graduate Research Support

PARALLEL SESSION G - *Auditorium Ronsard*
New insights into mechanisms of action of polyphenols

S29
Soy isoflavones and membrane steroid receptors: a new horizon

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Soy isoflavone's (especially genistein and daidzein) health effects have been studied for decades, while their mechanisms of action remain complex, involving modulation of gene expression and enzyme activity. Currently there is a need for refreshed approach in this field considering identification of isoflavone's new molecular target The cell membrane constituents-mediated effects of soy isoflavones are worthy of special attention from the perspective of cancer metastasis or cardiovascular diseases management. Specifically, the expanding concept of membrane steroid receptors and rapid signalling from the cell surface may include the prominent role of these steroid-like compounds.

We have shown in vitro that genistein inhibits LNCaP prostate cancer cells invasiveness by decreasing the membrane fluidity. This is complemented with genistein-caused immobilization of the androgen receptor containing membrane lipid rafts and down regulation of the androgen receptors/Akt signalling in mentioned metastatic prostate cancer cell line. On the other hand, it was observed that daidzein strongly couples with membrane estrogen receptors in adrenal medullary cells. At lo doses (10 – 1000 nM) daidzein was found to stimulate catecholamine synthesis via extracellular signal-regulated kinase 1/2 or protein kinase A pathways, but at higher doses it inhibited catecholamine synthesis and secretion induced by acetylcholine.

Given that catecholamine excess can contribute to the cardiovascular pathologies and that catecholamine lack may lead to depression, daidzein application promises to have a wide range of therapeutic effects.

These data are supportive in development of the molecular pharmacotherapy pertinent to balanced soy isoflavone treatment of steroid-related malignancies as well as cardiovascular and psychiatric diseases.

Financial support: This work was supported by the Ministry of Science, Education and Technological Development of the Republic of Serbia, Grant number 173009.

S30
Delphinidin inhibits tumor growth by acting on VEGF signaling in endothelial cells

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The vasculoprotective properties of delphinidin are driven mainly by its action on endothelial cells. Moreover, delphinidin displays anti-angiogenic properties in both i vitro and in vivo angiogenesis models and thereby might prevent the development of tumors associated with excessive vascularization. This study was aimed to test the effect of delphinidin on melanoma-induced tumor growth with emphasis on its molecular mechanism on endothelial cells. Delphinidin treatment significantly decreased in vivo tumor growth induced by B16-F10 melanoma cell xenograft in mice. In vitro, delphinidin was not able to inhibit B16-F10 melanoma cell proliferation but it specifically reduced basal and VEGF-induced endothelial cell proliferation. The anti-proliferative effect of delphinidin was reversed in the presence either of the MEK1/2 MAP kinase inhibitor, U-0126, or the PI3K inhibitor, LY-294002. VEGF-induced proliferation was reduced either by U-0126 or LY-294002. Under these conditions, delphinidin failed to decrease further endothelia cell proliferation. Delphinidin prevented VEGF-induced phosphorylation of ERK1/2 and p38 MAPK and decreased the expression of the transcription factors, CREB and ATF1. Finally, delphinidin was more potent in inhibiting in vitro cyclic nucleotide phosphodiesterases (PDEs), PDE1 and PDE2, compared to PDE3-PDE5. Altogether delphinidin reduced tumor growth of melanoma cell in vivo by acting specifically on endothelial cell proliferation. The mechanism implies an association between inhibition of VEGF-induced proliferation via MAPK, PI3K and at transcription level on CREB/ATF1 factors, and the inhibition of PDE2. In conjunction with our previous studies, we demonstrate that delphinidin is a promising compound to prevent pathologies associated with generation of vascular network in tumorigenesis.

Financial support: This work was supported in part by the French Centre National de la Recherche Scientifique, the INSERM, the University of Strasbourg, the University of Angers and APRIFEL/Ligue Contre le Cancer, France.

S31

Potent inhibition of VEGF activity by pomegranate polyphenols: A novel mechanism for the atheroprotective effects observed in epidemiology

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Vascular Endothelial Growth Factor (VEGF) is a potent angiogenesis stimulator. Increased VEGF signalling is associated with pathological angiogenesis within multiple diseases including cancer, wet age-related macular degeneration and atherosclerosis. The crucial role of VEGF in atherosclerosis progression has been demonstrated in multiple animal models, but targeting VEGF to reduce atherosclerosis is largely unexplored. A range of polyphenols have been shown to inhibit VEGF signalling, but at supra-physiological concentrations. Recently, we reported strong, polyphenol-specific inhibition of VEGFR-2 phosphorylation at physiological concentrations mediated by direct interactions of EGCG and a procyanidin tetramer with the VEGF protein. Pomegranate polyphenols have been reported to inhibit VEGF signalling in cancer models. The overall aim of this project is to determine if VEGF is a target for pomegranate polyphenols and to demonstrate the impact on VEGF-induced angiogenesis and atherosclerosis.

After screening multiple polyphenols for their ability to inhibit VEGF-dependent VEGF signalling, punicalagin and ellagic acid were identified as two of the most potent inhibitors (50% inhibition (IC_{50}) achieved at 30 and 300 nM, respectively). Colonic microbial catabolism produces, in consecutive order, urolithin D, C, A and B Urolithin D exhibited inhibition comparable to ellagic acid, while the inhibitory potency of the other urolithins declined along the urolithin transformation pathway. We show that inhibition of VEGF signalling by ellagic acid and urolithins is due to direct binding to the VEGF protein. A study to determine if ellagic acid dietary supplementation can reduce VEGF induced pathological angiogenesis and in turn reduce atherosclerosis progression in vivo is ongoing.

Financial support: The research leading to these results received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under Grant Agreement 312090 (BACCHUS). The IFR receives funding from the Biotechnology and Biological Sciences Research Council through Institute Stretegic Programme Grant (BB/J004545/1)

S32

(Poly)phenols from quasi-isogenic raspberries: attenuation of neuroinflammation

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Many neurologic disorders are associated with chronic neuroinflammatory processes Microglia are the innate immune cells of the central nervous system (CNS). However once chronically activated, these cells become deleterious by the uncontrolled releas of diverse molecular mediators such as inflammatory cytokines.

The identification of bioactive compounds from food that can attenuate the pro-inflammatory cascade or improve the resolution of the inflammation process in CNS turns out as a major factor for prevention of neurologic disorders. Among all dietary phytochemicals, (poly)phenols represent one of the classes with the strongest anti-inflammatory bioactivity. However, because gastro-intestinal digestion strongly modifies the structure and bioactivity of (poly)phenols, it is imperative to test the effect of digested (poly)phenols.

In this study we evaluated the bioactivity of raspberry digested fractions as inhibitors of inflammation in microglia stimulated with lipopolyssaccharide. The fractions were obtained by in vitro digestion of five quasi-isogenic raspberry accessions and cultivars, each of them with different profiles of (poly)phenols. Some of the fractions not only attenuated microglial pro-inflammatory response by decreasing the expression of CD40, and the production of nitric oxide, tumor necrosis factor- and intracellular superoxide; but also increased anti-inflammatory factors such as interleukin-10.

A yeast model of inflammation was used to elucidate the molecular mechanisms underlying the anti-inflammatory activity of digested raspberry fractions. The fractions inhibiting the yeast Crz1/calcineurin pathway (homologous to the mammalian nuclear factor of activated T-cells (NFAT)/calcineurin) were those that diminished the production of pro-inflammatory factors by microglia. This suggested that (poly)phenols present in these fractions could attenuate microglial pro-inflammatory processes through transcription factor regulation. To explore this hypothesis, activation of different molecular pathways involved in canonical inflammatory response including NF-kB, MAPK and NFAT, were accessed in microglia.

Financial support: This study and individual fellowships were supported by FCT Project (PEst-OE/EQB/LA0004/2011; IF/01097/2013); EuBerry Project (EU FP7 KBBE-2010-4 265942) and BacHBerry (FP7-KBBE-2013-613793)

PARALLEL SESSION H - *Auditorium Descartes*
Hot topics in polyphenols and gut microbiota interactions

S33
Identification of novel flavonoid O- and C-glycosidases in gut bacteria

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Bioactive plant-derived flavonoids are ingested by humans with diet and dietary supplements mostly as *O*- or *C*-coupled glycosides. The deglycosylation of dietary flavonoids is usually a prerequisite for their absorption and/or further metabolism and, thus, impacts the bioavailability and biological activity of these polyphenolic compounds. Gut bacteria play a crucial role in this deglycosylation and may thereby affect the proposed health-promoting effects of flavonoids in the host. In particular the highly stable *C*-glucosides appear to be exclusively cleaved by bacteria such as the intestinal *Lachnospiraceae* strain CG19-1 and *Eubacterium cellulosolvens*. However, the enzymes catalyzing the *C*-deglycosylation were unknown.

By cloning and functional expression of the encoding genes in *Escherichia coli*, we identified an enzyme system in *E. cellulosolvens* that affords the deglycosylation of -coupled isoflavone and flavone glucosides. Two of the five proteins catalyzing the deglycosylation of *C*-glucosides also cleave the corresponding flavonoid *O*-glucosides. They were also found in strain CG19-1. These *O*-deglycosylating enzymes lack similarity to known -glycosidases and presumably conduct hydrolysis by an unusual redox-assisted mechanism. Heterologous expression of the identified genes enabled even intact *E. coli* cells to cleave both *C*- and *O*-coupled flavonoid glucosides to their aglycones. The presence of similar genes in the genome of other *Lachnospiraceae* suggests that the described enzyme systems are widely distributed among intestinal bacteria with deglycosylating activities.

Financial support: Grant from the German Research Foundation (DFG, BR 2269/4-1).

S34
Quantification of microbial polyphenol uptake, kinetics and metabolism using mass spectrometry

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The gut microbiota play a significant role in metabolising, modulating the bioavailability and, thus, the biological activities of key dietary polyphenols metabolites. Additionally, evidence is emerging on the role of dietary polyphenols o the modulation of the colonic microbial population composition or activity through their antimicrobial activity. Which polyphenols are taken up by which gut microorganisms, what is their intracellular concentrations, how fast are they metabolized and how fast are they excreted define a set of important scientific questions crucial to understanding the interactions between dietary polyphenols and the gut microbiota. All of these questions remain open until now.

In this communication we will show proof of principle experiments using various selected mutants of Escherischia Coli and selected xenobiotics, demonstrating the capability of mass spectrometry to quantify bacterial compound uptake of down to 500 molecules per cell using high resolution single ion monitoring. Additionally we will present validation experiments using synchrotron fluorescence experiments demonstrating that cell uptake measured by two independent methods are in the same order of magnitude.

Following proof of principle studies we present quantitative data on the uptake of selected flavonoids and their glycosides in E.coli, Bifidobacterium and Lactobacillus under anaerobic conditions using typical dietary concentrations. We show that after an initial uptake phase elimination and metabolism leads to a reduced intracellular concentration. Selected flavanoid metabolite concentrations were as well quantified. Different bacteria show vastly different selectivities and uptake and metabolism rates for flavonoids of different structural types. The work forms the basis for a detailed understanding of polyphenol microbe interactions.

Financial support: DAAD, EU innovative medicine initiative (ND4BB)

S35

Importance of the colonic microbiota in the bioavailability of orange juice (poly)phenols: in vivo and in vitro studies

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Orange juice (OJ) flavanones are bioactive (poly)phenols that are absorbed principally in the large intestine. We investigated the bioavailability of flavanones, and their colonic transformation following acute ingestion of OJ by humans. Product formed during anaerobic in vitro fecal fermentation of flavanones were also investigated. In addition, in an acute study volunteers drank OJ with and without a microencapsulated probiotic (Bifidobacterium longum) while the chronic effects were examined when OJ was consumed after daily supplementation with probiotic over 4 weeks. Urinary metabolites and phenolic acid catabolites were analyzed by HPLC-MS^2 and GC-MS. After ingestion of OJ by humans, the main urinary metabolites were hesperetin-O-glucuronide, naringenin-O-glucuronides and hesperetin-3´-O-sulfate, corresponding to 22% of flavanone intake. Urine also contains substantial amount of colon-derived flavanones equivalent to 21% of ingested OJ (poly)phenols. In vitro anaerobic incubation of OJ flavanones demonstrated the involvement of the colonic microbiota in the overall bioavailability of OJ (poly)phenols though the production of hydroxyl- and methoxyphenyl propionic acids. In addition, acute OJ consumption following chronic probiotic intake over 4 weeks resulted in the excretion of 27% of flavanone intake while excretion of selected phenolic acids also increased significantly to 43% of (poly)phenol intake. These findings demonstrated that OJ flavanones were principally and extensively transformed by colonic microflora yielding phenolic acid catabolites which are absorbed into the circulatory system prior to excretion. In addition, these results showed positive effects of a chronic, but no acute, intake of microencapsulated probiotic (B. longum) on the absorption and colonic transformation of OJ flavanones by humans.

Financial support: The investigations were partially supported by the Coca Cola Company and CSIRO Preventative Health National Research Flagship, Australia. G.P.-C. was supported by a postdoctoral fellowship from Foundation "Alfonso Martín Escudero". AC was the recipient of a McMaster Fellowship

S36

Short chain fatty acids affect hesperetin transport and phase II metabolism in Caco-2 cells.

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Sort chain fatty acids (SCFA) constitute gut microbiota metabolites naturally present at very high levels in the colon, the main site of absorption of dietary hesperetin and phenolic acids. We investigated how chronic treatment of Caco-2 cells with SCFA affected hesperetin phase II metabolism and transport.

Caco-2 cells were pretreated with1 mM butyrate, propionate, acetate or lactate for 14 days and transport and metabolism of hesperetin (20 M) added apically was examined. Hesperetin (HT), sulphated (HT-3-sul, HT-7-sul) and glucoronidated metabolites (HT-7-glu, HT-3-glu1 and 2, presumably R and S forms) were identified and quantified in apical and basolateral samples by LC-MS/MS. mRNA expression o transporters ABCG2, MCT1 and MCT4 was monitored by ddPCR. Changes of p0.01 were considered significant (n=12).

Lactate decreased metabolism/transport of HT-3-sul and HT-3-glu (R and S) both apically and basolaterally, indicating downregulation of UGT(s) and SULT(s). Apica HT-7-glu and HT-7-sul were also lower implying transcriptional/translational effects on transporter(s) responsible for 7-subsituted HT. Both butyrate and propionate increased HT-7-glu apically and basolaterally pointing to an up-regulation of UGT(s HT-7-sul was also higher on the basolateral side with butyrate and propionate, suggesting up-regulation of a basolateral transporter involved, possibly ABCC1 or C3. Acetate suppressed HT-3-glu2 in both compartments and decreased apical HT-3-sul.

Butyrate stimulated expression of MCT1, but decreased MCT4. Propionate decrease the expression of the apically located efflux transporter ABCG2, but increased expression of MCT1 and 4, which are mainly located at the basolateral membrane. These changes might affect transport of phase II metabolites to the basolateral and apical compartments. The results demonstrate that SCFAs affect the metabolism of hesperetin. Each SCFA acts independently, suggesting that the profile of SCFA produced by the gut microbiota, as influenced by diet, can strongly affect flavonoid metabolism. Information from protein analysis experiments planned will give mechanistic insight on the observed changes.

Financial support: EVR is supported by the Agency for Innovation by Science and Technology in Flanders (IWT) and results were obtained during a COST INFOGEST Short-Term Scientific Mission.

7th ICPH International Conference on Polyphenols and Health

POSTERS

PARIS

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October 27-30, 2015

Poster Session A: Polyphenols bioavailability and exposure assessment

P001

Comprehensive identification of phenolic compounds in red wine extract and zebrafish embryos through HPLC-ESI-LTQ-Orbitrap-MS

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The Mediterranean diet is rich in natural antioxidants such as polyphenols, whose main dietary sources are fruits, vegetables, red wine, coffee, olives and virgin olive oil. Considerable interest has been focused on the bioactive phenolic compounds in wine and wine extracts, notably anthocyanins, flavanols, flavonols and resveratrol, since they possess many biological activities, such as antioxidant, cardioprotective, anticancer, anti-inflammation, antiaging and antimicrobial properties. Although positive effects of polyphenols have been demonstrated in humans and animals, the chemical and in vitro biological techniques regularly used to evaluate their antioxidant capacity are not useful for determining their underlying mechanisms of action. The zebrafish embryo is a highly interesting biological model with numerous advantages, among them, a rapid embryonic development and antioxidant systems analogous to humans. We selected the ZF embryo model as a potential method to study the effects of natural bioactive compounds from dietary sources.

In this study, we used liquid chromatography/electrospray ionization-linear ion trap quadrupole-Orbitrap-mass spectrometry (HPLC/ESI-LTQ-Orbitrap-MS) to identify the polyphenol compounds in a red wine extract and zebrafish embryos. Phenolic compounds and anthocyanin metabolites were determined in zebrafish embryos previously exposed to the red wine extract.

Compounds were identified by injection in a high-resolution system (LTQ-Orbitrap) using accurate mass measurements in MS, MS^2 and MS^3 modes. The HPLC-LTQ-Orbitrap-MS has permitted the identification of polyphenols in red wine extract and in zebrafish embryos. To our knowledge, this research constitutes the first comprehensive identification of phenolic compounds in zebrafish by HPLC coupled to high-resolution mass spectrometry.

Financial support: Funding for this work was provided by the Institute of Nutrition and Food Safety (INSA) of the University of Barcelona, FRI2012. A.V.-Q thanks the postdoctoral fellowship from the Foundation Alfonso Martín Escudero for carrying out research in foreign countries.

P002

Preparation of sulfated metabolites of flavonoids and flavonolignans by arylsulfotransferase from Desulfitobacterium hafniense

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Background: To study the bioavailability of plant food bioactive compounds, standards for the determination of their metabolites are needed. The metabolism of foreign compounds involve mainly oxidation reactions in Phase and conjugation wit hydrophilic moieties (glucuronate, sulfate, glutathione, glycin, taurine etc.) in Phase of their biotransformation. In the case of polyphenols, sulfate and glucuronate conjugations are the major metabolic biotransformations in their metabolism. But standards of polyphenolic Phase II metabolites are not easy to prepare and usually no commercially available.

The objective of this study was the chemoenzymatic preparation, purification and characterization of the sulfates

of important food bioactive flavonoids and flavonolignans silybin, dehydrosilybin, isosilybin, silychristin, silydianin, quercetin, taxifolin, rutin and isoquercitrin.

Methods: Arylsulfotransferase (EC 2.8.2.22) from *Desulfitobacterium hafniense* heterologously expressed in *E. coli* and cheap *p*-nitrophenyl sulfate as sulfate donor were used for the preparation of the sulfated metabolites. The products were purified by gel filtration on Sephadex LH-20 and their structures were elucidated by HRMS, H NMR, ^{13}C NMR, gCOSY, gHSQC, and gHMBC. Reaction progress and product purities were assessed by analytical HPLC-PDA.

Results: Pure (95%) silybin A 20-*O*-sulfate, silybin B 20-*O*-sulfate, isosilybin A 20-*O*-sulfate, isosilybin B 20-*O*-sulfate, dehydrosilybin 20-*O*-sulfate, dehydrosilybin-7,20-*O*-disulfate, silychristin-20-*O*-sulfate, silydianin-19-*O*-sulfate, taxifolin-3´-*O*-sulfate, quercetin-3´-*O*-sulfate, quercetin-4´-*O*-sulfate, isoquercitrin-4´-*O*-sulfate and rutin-4´-*O*-sulfate were obtained in quantitative yields fully characterized by HRMS and NMR methods and will be used as authentic standards in *in vivo* metabolic experiments.

Conclusion: Sulfation of flavonoids by arylsulfotransferase from *Desulfitobacterium hafniense* is an excellent method for the production of authentic sulfated metabolites usable as standards for bioavailability studies and for evaluation of their biological activity.

Financial support: This work was supported by the Czech Science Foundation 15-03037S; COST actions FA1403 POSITIVe (Interindividual variation in response to consumption of plant food bioactives and determinants involved) and CM1303 Systems Biocatalysis (MŠMT LD13041 and LH13097).

P003
Characterization of phenolic compounds of Algerian Rosmarinus tournifortii leaves by UHPLC-DAD-QTOF–MS

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In south-west of Algeria, leaves of (Elyazir) Rosmarinus tournefortii are widely used in folk medicine as a sedative, antispasmodic, treating menstrual disturbances, stomach ache, cough, influenza, colic, fever, pain, hepatic and intestinal problems. The healthy effect of these leaves could be attributed to their composition, especially, phenolic compounds. In this sense, the objective of this study was a detailed qualitative characterization of phenolic compounds in Rosmarinus tournefortii leaves using sophistical analytical tools.

In order to achieve this purpose, samples of Rosmarinus tournefortii were collected from the south-est of Algeria. To isolate and characterize the phenolic fraction, extraction with hydromethanolic solvent (80:20, v/v) followed by ultra-high-performance liquid chromatography (UHPLC) coupled to diode-array detection (DAD) and quadrupole-time of flight (QTOF)-mass spectrometry (MS) via electrospray ionization were used. The tentative identification was based on the UV-Vis data and the accurate mass measurements (mass error, 5 ppm), isotope distribution, and fragmentation pattern. All data were contrasted with previous literature on Rosmarinus and available databases, since in major cases no commercial standards were available. In this study, 128 phenolic compounds were characterized. The tentatively identified compounds belonged to different phenolic families such as, flavonols (quercetin, kaempferol, isorhamnetin and derivatives), flavanones (hesperidin, hispidulin), flavones (genkwanin, luteolin), hydroxycinnamic acids (chlorogenic, rosmarinic, ferulic, caffeic acids and derivatives), hydroxybenzoic acids (syringic acid, vanillic acid and protocatechuic acid) and phenolic diterpenes (carnosic acid, carnosol, rosmanol and derivatives). It is important to highlight that, to our knowledge, this is the first time that a Rosmarinus tournefortii phenolic extract has been characterized.

Financial support: Centre de Recherche en Biotechnologie (CRBt) Constantine – Algeria & Excellence Project P11-CTS-7625 from the Andalusian Regional Government, Council of Innovation and Science

P004
Identification of bioavailable polyphenols from black carrots (Daucus carota) using an in vitro digestion/Caco-2 absorption model

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Nowadays, black carrots have gained interest due to their substantial content of polyphenols, especially anthocyanins. Black carrot is often consumed after processing into various products. As a result of processing, large amounts of by-products having the potential to be used as valuable resources of bioactive compounds are generated. As such, the aim of this study was to monitor the stability of polyphenols in black carrot and its by-products during in vitro digestion and absorption.

Black carrot and its by-products were collected in triplicate from an established processing plant in Konya, Turkey (Erkonsatre Co.). A combined in vitro digestion/Caco-2 absorption model was used to mimic the physiological conditions in the gastrointestinal tract and to identify black carrot polyphenols transported across the intestinal epithelium. The experimental set-up of the in vitro digestion model involved the enzymatic digestion followed by a microbial colon model. The identification and quantification of polyphenol metabolites was carried out by UPLC-ESI-MS^E and HPLC-DAD, respectively.

The results showed that black carrot and its by-products accumulate five major anthocyanin pigments, among them two were the non-acylated cyanidin-3-xylosylgalactoside and cyanidin-3-xylosylglucosylgalactoside, and three derivatives of cyanidin acylated with sinapic acid, ferulic acid and coumaric acid. During the course of digestion, the loss of anthocyanins was observed while cyanidin-3-feruloyl-xylosyl-galactoside remained the most abundant compound. Digestion increased the amount of caffeic acid available in the colon, due to decomposition of chlorogenic acid by esterase activity of the colonic microflora.

Overall, the current study highlighted that the combined in vitro digestion/Caco-2 absorption model is useful for investigating the bioavailability of black carrot polyphenols.

Financial support: S. Kamiloglu is supported by a PhD fellowship from Ghent University research fund (BOF; 01SF0214). The authors would like to thank the Istanbul Technical University scientific research projects unit (BAP) for the financial support and the Hercules project (AUGE028 and AUGE014) for funding the UPLC-ESI-MS^E.

P005
Chemical characterization of pulp oil of avocado Persea American “Hass” variety.

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This study aimed to determine the compounds present in the pulp oil avocado (Perse americana) “Hass” variety. Gas chromatography and Mass Spectrometer through an Electro Spray Ionization Source EASI-MS (+) was used to determine the profile of fatty acids. Thin-layer gas chromatographic method used to the determination phytosterols. Major family of the phenolic compounds from the samples of the pulpoil were determined in the Mass Spectrometer through an Electro Spray Ionization Source EASI-MS(-). The peroxide value of the oil was determined by international oficial method of the Association of Analytical Communities (AOAC).

Avocado oils showed 43.2% of oleic acid, 14.9% linoleic acid, 25.3% palmitic acid and 14.5% palmitoleic acid. Radio of polyunsaturated fatty acids/saturated fatty acid is the 2.8%. Pulp oil showed 614,55mg sterols/100g of oil where in 81,6% this oil pulp is beta-sitosterol plus sitostanol. The families of phenolic compounds of oils showed lignans, anthocyanins, flavonones and methoxyflavonols. The peroxide index value (1 mEq.G/kg) did not exceed the limit established by Brazilian legislation (10 mEq.G /kg).The avocado pulp oil showed chemical properties such as fatty acid composition, especially oleic acid, rich source of phytosterols with attention to beta-sitosterol plus sitostanol and important presence of classes of phenolic compounds.

Financial support: This work was supported by Conselho Nacional de Desenvolvimento Científico e Teconológico (CNPq) (protocol nº 3159-1) and Jaguacy Brasil Company to donate the oil.

P006
Characterization of the tea leaves of avocado Persea American variety "Hass".

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The purpose of this study was to determine the compounds present in the tea of avocado leaves (*Persea americana*) from "Hass" variety. Avocado leaves were collected in November 2013 and dried in an oven with air circulation at 40°C for 4 days. The tea was prepared by aqueous extraction (1g/25ml of water at 100°C). The major phenolic compounds were characterized by HPLC, the total phenols and flavonoids by colorimetric method; and antioxidant capacity by FRAP, DPPH and DPPH-IC^{50}. Minerals were analyzed in the dry leaves by Inductively Coupled Plasm Optical Emission Spectrometry (ICP-OES).

Major phenolic compounds from the tea leaves were quercetin, kampeferol, epicatechin and catechin (284.63mg.L^{-1}, 51.59 mg.L^{-1}, 2.18 mg.$L^{-1}$and 1.87 mg.L^{-1}, respectively). The flavonoids concentration was 0.92 mgCE.L^{-1} and total phenols 0.3± 0.03 mgGAE.mL^{-1}. DPPH-IC^{50} was 0.0045g.ml^{-1}, the total capacity of free radical scavenging, DPPH 9.40 ± 0.79 µmol trolox equivalent.ml^{-1} and the ability to reduce the iron 7270.92 Trolox equivalent umol.L^{-1} of tea. As the concentration of toxic metals and minerals sheet in dried leaves showed trace amounts of cadmium selenium, calcium and potassium. In addition, to minerals such as phosphorus, sodium, copper, Iron and zinc showed important quantity: 657 ± 7 mg.kg^{-1}, 199 ± 9 mg.kg^{-1}, 155 ± 8 mg.kg^{-1}, 148 ± 3 mg.kg^{-1}, 35 ± 1 mg.kg^{-1}, respectively.

Tea leaves of the avocado showed great antioxidant property and important source or polyphenols.

Financial support: This work was supported by Conselho Nacional de Desenvolvimento Científico e Teconológico (CNPq) (protocol nº 3159-1) and Jaguacy Brasil Company to donate the leaves.

P007
Association of flavonoids and flavonoid-rich foods with risk of all-cause mortality

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Background: Flavonoids are bioactive compounds found in foods such as chocolate red wine, fruits and vegetables. Higher intakes of specific flavonoids and flavonoid rich foods have been linked to reduced mortality from specific vascular diseases and cancers. However, the importance of flavonoids in preventing all-cause mortality remains uncertain.

Methods: We examined the association of flavonoid and flavonoid-rich food consumption with subsequent total and cause-specific mortality among 93,145 women in the Nurses' Health Study II. Dietary intake was assessed at baseline and updated every 4 years.

Results: During 1,838,946 person-years of follow-up, 1,808 participants died. The age-adjusted and multivariate-adjusted hazard ratios (95% CI) for death among participants who were in the highest quintile of total-flavonoid intake, as compared with those in the lowest quintile were 0.82 (0.72, 0.95) and 0.94 (0.82, 1.09), respectively. In age and multivariate-adjusted models, frequent consumption of many individual flavonoid-rich food items, such as celery, blueberries and strawberries, were associated with reduced risk of all-cause mortality. When compared to non-consumers, frequent chocolate and red wine consumption showed the greatest magnitude of reduction in risk; multivariate-adjusted hazard ratios (95% CI): 0.76 (0.63, 0.92) and 0.63 (0.54, 0.73), respectively.

Conclusion: In a large cohort of young and middle-aged women, the frequency of flavonoid-rich food consumption was inversely associated with all-cause mortality, independently of other predictors of death.

Financial support: Project grant from the US Highbush Council

P008

Symbiotic versus conventional red wine: phytochemical profile, antioxidant and anti-hemolytic properties following oxygen exposure

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Wines produced using an environmentally sustainable approach, such as organic, biodynamic and biological practices have recently enjoyed increasing popularity due to the growing demand for healthy products.

The present study was performed to evaluate the effect of a bio-friendly approach on the quality of wines undergoing to the same winemaking process. In particular, we investigated whether the use of a microbiological consortium, in particular mycorrhizal fungi, can lead to the production of high-quality wines with improved nutritional and nutraceutical value.

Thus, the aim of this work was to evaluate the effect of the microbiological consortium on the phytochemical composition and the antioxidant properties of Sangiovese wines with respect to wines from conventional agricultural, paying particular attention to their oxidative stability following oxygen exposure.

We determined by spectrophotometric methods and HPLC-DAD their phytochemical composition and we tested by ORAC (Oxygen Radical Antioxidant Capacity) assay their antioxidant activity. Besides, in order to evaluate the beneficial effects of both symbiotic and conventional wines, we investigated on human erythrocytes the cellular antioxidant activity (CAA) and the hemolysis inhibition.

As a general result, based on the concentration of the bioactive compounds determined by spectrophotometric methods and HPLC-DAD, our data demonstrated that symbiotic wines showed both a better oxidative stability and a significantly higher level of bioactive compounds compared to the conventional ones. However, despite the bioactive compounds variation, no difference in antioxidant capacity was found. Besides, symbiotic wines exerted a better biological activity on human erythrocytes with significantly increased value of cellular antioxidant activity and a better anti-hemolytic effect than the co-respective conventional one.

In conclusion, the use of a microbiological consortium represents an ecologically and economically relevant solution in vineyards cultivation able to obtain high-quality wines, with improved nutritional and nutraceutical content, reducing the use of chemical treatments.

P009

Plasma fingerprinting of mixed nuts exposure in subjects with metabolic syndrome. New insights into diet and host-microbial interactions in the protection against cardiometabolic disease risk

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Nuts have a recognized role in the improvement of the metabolic syndrome (MetS), but the mechanisms implied in the health effects are not fully characterized. Urolithins are gut microbiota products of ellagitannins highly abundant in walnuts, and currently implicated in the protection of cardiometabolic disease risk. However, obesity is known to be associated to microbial dysbiosis, thus the urolithin-producing capacity may be affected in the presence of this MetS diagnostic component.

We firstly applied a LC-ESI-qToF-MS-driven untargeted metabolomic approach to identify the most discriminant biomarkers of nuts exposure in fasted plasma of subjects with MetS following 12-week intake of mixed nuts (30 g/d), compared to a matched control group. Urolithin A glucuronide (nivel I identification) was the most discriminative dietary biomarker (high predictive capacity [ROC_AUC=89.6% (80.8–98.4)]) despite the inter-individual variation expected for a host-microbial metabolite.

We then investigated the eventual correlation with the MetS diagnostic traits, at baseline and post-intervention. Plasma levels of urolithin A glucuronide showed a significant inverse correlation with abdominal adiposity (waist circumference: r=-0.550; waist-hip ratio: r=-0.409) and impaired glycaemic control (fasting insulin: r=-0.414; HO-

MA-IR: r=-0.417) at baseline, suggesting the presence of a more urolithin prone-to-produce microbiota, while positively associated with the reduction of adiposity following the nut intake (change in % body fat: r=0.456). Significant changes in circulating medium-chain dicarboxylic acids (nivel I identification), recognized as alternative energy substrates particularly relevant in the case of glycaemic control impairment, were also associated with nut consumption, confirming that unexplored metabolic mechanisms may be contributing to their health effects.

As far as we know, this is the first study suggesting a higher urolithin-producing capacity (and therefor exposure) in subjects with less severe MetS traits, particularly those known to be associated with gut microbial dysbiosis. Although further studies are highly recommended to verify the generated hypotheses.

Financial support: AGL2009-13906-C02-01 from the Spanish Ministry of Economy and Competitiveness (MINECO).

P010

Health benefits of diets rich in sustainable sources of plant protein: Bioavailable phytophenol metabolites and their implications for human health

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Currently, the major protein source in most higher-economy countries is meat Our previous research showed that high-protein (predominantly animal-based) diets are likely to be detrimental to gut health in the long term, as hazardous metabolites are increased and cancer-protective compounds are reduced in the colon.

Plant-protein (predominantly soya-based) diets ameliorated against this risk, increased satiety and improved the biomarkers of metabolic syndrome.

It is now important that current non-sustainable protein sources such as soya and meat are partially replaced with sustainable protein-rich products. The aim of this work is to assess the benefits of a diet rich in these foods on human health.

Flours produced from high-protein plants (lupin, buckwheat, hemp, pea and fava bean) were characterised for their macronutrient, micronutrient and biologically-active phytophenol content before and after processing into bread rolls. These products were then consumed in an acute human dietary intervention study with ten healthy subjects (42 ± 11.8 years; BMI of 26 ± 5.8 kg/m²) wherepostprandial bioactive phytophenol metabolites were measured along with other postprandial health biomarkers.

In addition to their rich macro- and micronutrient content, non- and anti-nutritional bio-active compounds were identified and quantified. The phytoyphenol content ranged from 43.80 mg/kg (lupin) to 601.58 mg/kg (buckwheat Following consumption, many of these polyphenol metabolites were bioavailable in the systemic circulation and may be responsible for the beneficial effects on health.

Protein-rich plants represents sustainable source of bioavailable phytophenol metabolites, which are increasingly being recognised for their health benefits.

Financial support: Scottish Government's Strategic Partnership on Food & Drink Science

P011
Assessment of (poly)phenol intake: identified gaps and suggestions for harmonization

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Background: Several evidences suggest that a continuous and prolonged intake of (poly)phenols help in prevention of degenerative pathologies such as diabetes, cardiovascular diseases, neurodegenerative diseases and cancer, and to prevent symptoms associated to aging and menopause. However, prediction of health effects of (poly)phenols in epidemiological studies is dependent on calculating (poly)phenol daily intake. This, on one hand relies on the correct estimation of food intake and, on another hand, on the correct estimation of (poly)phenol content of foods.

Methods: A review of the literature was conducted to gather Information on polyphenol intake in different countries all over the world. Total polyphenol intake, main food contributors to polyphenol intake, methods used for assessment of food intake and polyphenol quantification were evaluated.

Results: We are far from knowing global daily polyphenol intake since few countries have available consistent data. Several gaps underline the wide variability observed on reported (poly)phenol intake: large time period between year of diet assessment and year of publication (10 to 20 years); type of instrument used to assess food intake (FFQ, 24h R, DHQ, FBS, DFR) and food included in the assessment; database used for estimation of (poly)phenols content in foods (USDA, Phenol Explorer, food analysed by authors), class and type of (poly)phenol studied (extractable versus non-extractable (poly)phenols).

Conclusion: Harmonization of methods for correct estimation of polyphenol intake are essential to compare different world regions. Twenty-four hour reports for assessment of food intake should be preferred and both extractable and non-extractable polyphenols should be quantified.

Financial support: EuBerry Project (EU FP7 KBBE-2010-4 265942)

P012
Bioavailability of anthocyanins and ellagitannins metabolites following acute intake of a polyphenol-rich juice of grumixama (Eugenia brasiliensis Lam)

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The grumixana, belongs to Myrtaceae family, is a native fruit of the South and Southeast regions of the Atlantic Forest of Brazil. The fruit, also known as Brazilian cherry, is a rich source of phenolic compounds, mainly anthocyanins and ellagitannins. We investigate the bioavailability of the main flavonoids, and their colonic transformation following acute intake of a grumixama juice by humans.

Fifteen healthy subjects consumed the juice at single dose (0.052 mg eq. cyanidin 3-glucoside/ Kg of body weight), and urine and plasma were collected at different time points over 24 h period post-ingestion. Urinary metabolites and phenolic acid were analyzed by LC-ESI-MS^n and also by LC-Q-TOF. Four anthocyanins were detected in urine. Although cyanidin 3-glucoside was identified as the main anthocyanin presented in the juice, peonidin, the methylated form of cyanidin, was the main anthocyanidin metabolite detected in urine collected after consumption (1 – 6 h). In addition, four urinary phenolic acids were excreted in significantly levels.

Hippuric acid followed by the vanillic acid were the main metabolites, which took place 4-14h after ingestion. The overall urinary excretion corresponded to 99.5% of phenolic acids and 0.5% of anthocyanin metabolites. In plasma, only phenolic acids were detected. It was also detected ellagitannins metabolites in urine, as urolithins, after 4 h of intake. These finding shows that poliphenols presents in grumixama juice were absorbed and metabolized but they are also extensively transformed by colonic microflora yielding phenolic acid.

Financial support: This research was supported by CNPq (scholarship 153453/2012-5) and FAPESP (2013/07914-8).

P013

Relative validation of 24-h urinary hippuric acid excretion as a biomarker for dietary flavonoid intake from fruit and vegetables in healthy adolescents

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Background and Objective: Studies on the relevance of flavonoids for health promoting activities of fruit and vegetables (FV) would be facilitated by a biomarker for dietary intake of flavonoids from FV (FlavFV). Urinary hippuric acid (HA), which was already shown to be a useful biomarker of FV intake at different age groups, is a major metabolite of flavonoids and may therefore reflect FlavFV exposure. Thus, we investigated the relative validity of urinary HA excretion as a biomarker for FlavFV intake in healthy adolescents.

Methods: Analyses were based on data from 287 healthy adolescents (aged 9-16 years, 52% females) comprising at least two pairs of HA measurements from 24-h urine samples and FlavFV intake estimated from 3-d weighed dietary records.

Spearman correlation and cross-classification analyses were used to assess the agreement between both methods. Linear regression models served for identification of possible confounders of the association. Using a split-sample approach in all analyses allowed for consecutive exploration (n = 192) and confirmation (n = 95) of results.

Results: Highly significant positive correlations between urinary HA excretion and FlavFV intake were found in both exploratory (r=0.47) and confirmatory samples (r=0.64, p<0.0001 for both). A high proportion of subjects were classified into same+adjacent quartiles in the exploratory (79%) and confirmatory (88%) samples, and maximally 5% were misclassified into opposite quartiles. Although BSA (body surface area) emerged as the only relevant covariate in the exploratory sample, its adjustment improved cross-classification estimates in both subsamples.

Conclusion: BSA-adjusted 24-h urinary HA is a useful biomarker of FlavFV intake i healthy adolescents.

Financial support: Analysis funded by the Wereld Kanker Onderzoek Fonds (WCRF NL), as part of the WCRF international grant programme (grant no. 2013/975); Basic funding by the Ministry of Science and Research of North Rhine Westphalia, Germany.

P014

Blackberry digested metabolites are transported across blood-brain barrier and protect human brain microvascular endothelial cells, NTera-2/cloneD1 cells and cerebellar granule cells

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An increasing and emerging contribution has been attributed to nutrition in the preservation of cognitive function. Neurodegenerative diseases represent a large and heterogeneous group of neurological disorders with increasing incidence associated with aging, to which oxidative stress and glutamate excitotoxicity are considered main contributing factors. Fruits, specially berries, constitute a rich source of (poly)phenols. (Poly)phenols have shown to exert cytoprotective effects on neuronal cells injured with an oxidative stress and seem to vary on their ability to cross and/or interact with the blood-brain barrier (BBB).

This work aimed to assess the transport of blackberry digested polyphenols (BDP) across BBB and investigate their neuroprotective effects in human brain microvascular endothelial cells (HBMEC), NTera-2/cloneD1 (NT2) [IC1] and mice cerebellar granule cells (MCGC). Blackberry (poly)phenols[IC2] were submitted to an in vitro model of digestion.[IC3]HBMEC were used as a simplified in vitro model of BBB to assess BDP transport. BBB integrity was assessed by TEER measurements and permeability studies and transport was quantified by LC-Orbitrap MS analysis. Cytoprotection of BDP was also evaluated in HBMEC. Moreover, neuroprotection was also assessed in NT2 cells and MCGC challenged with tert-But hydroperoxide and glutamate, respectively.

The results indicated that some BDP metabolites were able to be transported across HBMEC suggesting some BBB permeability. Moreover, BDP metabolites at physiological concentrations, were also able to protect HBMEC and NT2

cells from oxidative stress, and MCGC from glutamate excitotoxicity. These results reveal very promising potential of blackberry (poly)phenols, which justifies a future evaluation on the transported BDP metabolites on different neuronal models.

Financial support: FCT (PEst-OE/EQB/LA0004/2011; PEstOE/SAU/UI4013/2012 ; SFRH/BD/86584/2012; SFRH/BPD/84336/201; IF/01097/20132) and EU FP7 KBBE-2010-4 265942

P015

German chamomile extract and its major polyphenols inhibit carbohydrate digestion and absorption in vitro

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Excessive post-prandial glucose and fructose spikes are associated with an increased risk of diabetes and obesity. Attenuating this response by dietary means is seen as a potentially beneficial preventive strategy. We assessed the acarbose-like activity of German chamomile extract (GCE) and related polyphenols on intestinal -glycosidase and on glucose and fructose absorption.

Qualitative and quantitative analysis of GCE was performed using HPLC-ESI-MS and HPLC-DAD. Four major polyphenols were identified as the key components; cis and trans-ferulic acid hexoside, apigenin 7-O-glucoside and apigenin. Structural characterisation of ferulic acid isomers was confirmed by accurate mass TOFMS and ^{1}H and ^{13}C NMR. The in vitro studies revealed that GCE and individual polyphenol inhibit salivary human -amylase (HSA) and -glycosidase in a dose-dependent manner and the effect was greater on HSA. Apigenin exhibited the most potent inhibition on HSA with K_i of 17.2 μM. However, when tested at concentrations quantified in the extract, apigenin 7-O-glucoside showed the highest contribution. For -glycosidase a dose response inhibition was evidenced and apigenin 7-O-glucoside was the most effective, but neither GCE alone nor any of the individual polyphenols reached 50% inhibition. In human intestinal Caco-2/TC7 cells, the addition of GCE and apigenin 7-O-glucoside apically caused a significant dose-dependent inhibition of glucose and fructose absorption across the monolayer.

The anti-diabetic effect of German chamomile has been reported in the literature but the underlying mechanism is yet elusive. Our experimental findings suggest the bioactivity could partly be mediated by the inhibition of intestinal glycosidases and interactions with glucose transporters.

Financial support: Authors are grateful to E.U. for funding support. J. A. Villa-Rodriguez is grateful to CONACYT Mexico for PhD Funding

P016
Strategies for enhancing phenolic compounds bioavailability: synergistic interactions

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Preclinical Like pharmaceuticals, dietary phenolic compounds are recognized as xenobiotics by our organism and subjected to high rates of metabolism and excretion. Their resulting low bioavailability is a concern as it can limit their potential health effects. Investigations conducted in our laboratory, involving phenol-rich fruits extracts, demonstrated the enhancement of specific phenolic compounds absorption through synergistic activities. Using targeted metabolomics approach, with the use o UHPLC-MS/MS, phenolic metabolites were identified and quantified in plasma and faeces of rodents supplemented with fruits extracts. In some cases, and for specific compounds, the bioavailability of phenolic compounds from a first extract was greatly increased when co-administered with a second one (from 2 to 6 fold), the second extract acting as an absorption enhancer. Furthermore, the appearance of these phenolic compounds in faeces was decreased in equivalent proportions following the co-supplementation. Similarly, the absorption of some phenolic compounds was improved following a co-supplementation with a probiotic.

Such enhancement of bioavailability is likely resulting from several modulatory activities of phenolic compounds. These compounds are not only substrates for enzymes taking part in xenobiotic metabolism but also act as regulators. They have been demonstrated to act as prebiotics, modulating gut microbiota, as well as modulators of efflux transporters. Interestingly, phenolic compounds are thus able, in some conditions, to modulate the mechanisms that govern their own bioavailability, and may be provided to synergistically increase their absorption for full exploitation of their preventive and therapeutic benefits. Specific examples of our investigations will be discussed.

Financial support was provided by the Institute of Nutrition and Functional Foods of Laval University, the Quebec Industrial Bioprocess Research Consortium (CRIBIQ) of the Ministry of Economic Development, Innovation and Export Trade (MDEIE), Nutra Canada and Atrium Innovations.

P017
Functional hydroxytyrosol-enriched biscuits: evaluation of hydroxytyrosol bioavailability and effects on oxLDL in humans

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The recent health claim approved by EFSA on the capacity of olive oil polyphenols, namely hydroxytyrosol (HT) and its derivatives, to protect low-density lipoprotein (LDL) against oxidative stress has enticed the usage of HT as a bioactive food ingredient in the food industry. Although olive oil polyphenols are highly bioavailable and extensively recovered in urine, the food matrix could play a crucial role modifying HT bioavailability.

The aim of this study was to evaluate the bioavailability of HT after consuming HT-enriched biscuits (HT-B) in comparison with control biscuits (C-B) in a crossover, randomized, double-blind study. On two separate days, 13 healthy subjects consumed 30 g of B-C or B-HT (5.25 mg HT) after an overnight fast. Blood samples were taken at 0, 0.5, 1, 2, 3, 4, 5 and 6 h after intake and 24 h urine was collected in several intervals. After HT-B consumption, HT-sulfate and 3,4-dihydroxyphenylacetic acid (DOPAC)-sulfate were the main metabolites found in both plasma and urine, followed by DOPAC and homovanillic acid (HVA), HVA-sulfate and HVA-glucuronide. HT was rapidly absorbed; plasmatic metabolite peaked between 0.5-1 h and were extensively excreted in urine. No differences were observed in postprandial antioxidant activity after C-B and HT-B intake, except for oxLDL concentration which significantly decreased with HT-B.

In conclusion, HT is a promising functional ingredient in biscuits as it is highly bioavailable and decreases postprandial oxLDL levels.

Approved by Ethics Committee of Hospital Puerta de Hierro (Madrid).

Financial support: Nutrexpa S. L. The Spanish Ministry of Science and Innovation (CSD2007-00063, AGL2014-58205-REDC, BES-2011-047476) and JAE Program (CSIC and European Social Funds) are acknowledged.

P018
Absorption and metabolism of yerba mate phenolic compounds in humans

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Mate, a tea-like beverage prepared from the dried leaves of Ilex paraguariensis, is widely consumed in South America for its stimulating properties, having also health properties. Mate is rich in hydroxycinnamates, mainly mono- and dicaffeoylquinic acids, in addition to flavonols. This study aimed to evaluate the bioavailability of mate polyphenols in healthy humans.

Plasma samples of twelve fasting men and women were taken before (0h) and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, and 12h after consuming a mate infusion containing 396mg (1118mol) chlorogenic acids. Urine was collected at baseline (-2-0h) and at intervals 0–2, 2–5, 5–8, 8–12, 12–24h. Samples were analysed by LC–MS-QToF.

Up 32 metabolites were identified in biological fluids, mainly sulfated conjugates of caffeic and ferulic/isoferulic acids, in addition to unmetabolized caffeoyl-, feruloyl-, and p-coumaroilquinic acids, with rapid appearance and clearance in plasma consistent with small intestinal absorption; these compounds amounted 13.1% of the urinary metabolites. Delayed absorption of dihydrocaffeic, dihydroferulic and dihydrocoumaric acids and their phase II derivatives, in addition to feruloylglycine, showed their microbial origin and colonic absorption, accounting for 81.0% of excreted metabolites. Phase II flavonol metabolites (0.2%) derived mainly from rutin after colonic transformation and absorption have been identified. Additionally, dihydroferuloyl, dihydrocaffeoyl- and dihidrocoumaroylquinic acids (5.7%) have been identified for the first time, showing the most delayed kinetics. Total phenolic excretion (147.6mol) corresponded to 13.2% of ingested phenols.

In conclusion, mate polyphenols are partially bioavailable and extensively metabolized, the colonic microbiota playing a key role in their metabolization.

Approved by Ethics Committee of Hospital Puerta de Hierro (Madrid).

Financial support: Spanish Ministry of Science and Innovation (AGL2010-18269, CSD2007-00063, AGL2014-58205-REDC, BES-2011-047476) and JAE Program (CSIC and European Social Funds).

P019
Absorption, metabolism and excretion of raspberries anthocyanins and ellagitannins by human subjects

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Raspberries are a rich source of anthocyanins and ellagitannins. There is growing evidence linking consumption of these compounds to health benefits including cardioprotective and anti-cancer effects. However, the mechanisms involved remain poorly understood principally because of a limited understanding of their absorption, disposition, metabolism and excretion (ADME). This study aims to explore the identity and amounts of the phenolic metabolites of anthocyanins and ellagitannins i human plasma and urine following acute ingestion of raspberries.

Nine healthy volunteers were feed 300 g of blended raspberries containing in total 293 mol anthocyanins and 250 mol ellagitannins. All urine excreted over 48 h after the ingestion of raspberries was collected and blood samples were collected before (0h) and after raspberry consumption up to 24 h. Metabolites were identified and quantified by UHPLC-MS^n and UHPLC-QqQ-MS/MS.

Fourteen anthocyanin- and 8 ellagitannin-derived metabolites were identified in urine Analysis of plasma samples led to the identification of 9 anthocyanin metabolites and 6 ellagitannin metabolites. The main anthocyanin metabolites were methyl-, glycine- glucuronide- and sulfate conjugates of phenolic acids as well as free phenolic acids. Metabolites derived from ellagic acids and ellagitannins were mainly conjugates from the colonic ellagic acid degradation products urolithin A and B.

The results obtained in this study show an extensive degradation of anthocyanins and ellagitannins as they pass through the body with a great variety of metabolites. Anthocyanin and ellagitannin bioactivity is likely to be mediated by their phenolic metabolites as opposed to the parent structures.

P020

Pharmacokinetic study of the main bioactive compounds of Garcinia madruno: A comparative study between biflavonoids and their respective flavonoids monomers.

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Garcinia madruno (Kunt) Hammel, is an endemic plant species of Central and South America belongs to the Clusiaceae family. The main phytochemicals are biflavonoid compounds (morelloflavone, fukugiside, volkensiflavone and amentoflavone) recognized in previously works by its high hypocholesterolemic, neuroprotective and atheroprotective activities in vivo. However, there was a lack of pharmacokinetic a bioavailability information about G. madruno biflavonoids after oral administration specifically of morelloflavone and volkensiflavone (major aglycones from G. madruno). Thus, the present study aimed to develop and validate an UHPLC-MS/MS method for simultaneous determination in rat plasma of the biflavonoids: morelloflavone, fukugiside, volkensiflavone and amentoflavone and their respective flavonoids monomers: luteolin, apigenin, and naringenin and utilize it in a pharmacokinetic study of the seven compounds after oral administration of a biflavonoids mix, flavonoids mix and a G. madruno extract (GME) to rats. Among the main results, the biflavonoids have similar concentration–time curves intra-group and differ significantly inter-groups with double absorption peaks related to enterohepatic cycle or colonic absorption. Morelloflavone was the compound with the major $T_{1/2}$ and MRT, but volkensiflavone was the biflavonoid with the major AUC normalized by dose. Additionally, the bioavailability of the G. madruno biflavonoids increased in all cases when these were administered through GME compared to the administration of the pure compounds and was significantly lower in comparison to the flavonoids monomers. In conclusion, the results obtained gave us the first information about pharmacokinetics of G. madruno biflavonoids in order to understand the in vivo pharmacological effects that have been found until now.

Financial support: The authors thank the CODI and CAEPT of the Universidad de Antioquia for the logistic and financial support (Project code CPT 1219)

P021

Antioxidant activity and in-vitro availability of phenolics from infusion of some health tea, coffee and cocoa drinks

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Tea, cocoa and coffee drinks have been claimed to have therapeutic activity due to the presence of phenolic compounds, however the health effects of tea depend both on the amount of phenolics consumed and their availability. The objectives of this study were to investigate the content, antioxidant activity and in-vitro availability of phenolics from the infusion of the tea, cocoa and coffee with or without milk or sugar.

This was done by modeling Pepsin-HCL digestion in stomach and Pancreatin-bile extract digestion in small intestine on a set of dialysis tubing (12,500 molecular weight cut off). The infusion and dialysate were assayed for pH, titratable acidity, total phenolics and antioxidant activity using Folin-Coicateau reagent and 1, 1-diphenyl-2-picryl-hydrazil (DPPH), respectively, the activity of the drinks were compared with activity of ascorbic acid and catechin.

The results indicated that pH and titrable acidity ranged from 4.5 to 5.6 and 0.167 to 0.837 (as maleic acid), respectively. Total phenolics ranged from 1.15 to 1.17 mg/g galic acid equivalent, availability of phenolics was significantly higher ($P< 0.05$) in black tea which also recorded highest antioxidant activity that corresponds to 60 and 80 % of activity of 10 µg ascorbic acid and catechin, respectively. Sugar inhibited availability of phenolics from black tea and cocoa whereas milk enhanced its availability from all the drinks except coffee.

The study showed that health teas, cocoa and coffee drinks contain phenolic compounds which are moderately available, hence justified the claim that tea could ameliorate free radical induced health defects.

P022
Deconjugation of quercetin metabolites at sites of inflammation

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Quercetin is the major flavonoid included in human daily diet. After oral intake, absorbed quercetin is metabolized to glucuronides and/or sulfates, and some metabolites are additively methylated. We have previously reported the deconjugation of the glucuronides into the aglycone (non-metabolized quercetin), in the inflammatory macrophage cells, and that anti-inflammatory actions have exerted by the aglycone. In this study, we investigated the deconjugation sites of quercetin metabolites in mice, and explored the effects of quercetin on the -glucuronidase and sulfatase activity.

First, to examine the tissue distribution of deconjugating enzymes (-glucuronidase and sulfatase), mice tissue homogenates were mixed with fluorescent substrates, and the fluorescence intensity was measured after incubation at 37 °C. The -glucuronidase activity was observed in various organs, especially in spleen. In contrast, the sulfatas activity was hardly detected in most of organs. Next, we examined the deconjugation of quercetin metabolites in tissues of the normal diet-fed mice upon lipopolysaccharide (LPS)-induced inflammatory response. In some tissues, the content of quercetin aglycone was increased and the expression of -glucuronidase protein was enhanced by LPS treatment. These results indicated the in vivo deconjugation of quercetin metabolites by -glucuronidase in inflamed sites.

Finally, the effects of quercetin on the -glucuronidase and sulfatase activity were examined by measuring fluorescent intensities of the reaction mixtures of quercetin, commercially available enzymes, and respective fluorescent substrates. We have found that quercetin inhibited the both -glucuronidase and sulfatase activities. These results suggest that quercetin metabolites are deconjugated by these enzymes and the resultant aglycone levels might be controlled by feedback inhibition.

Financial support: This study was supported by a Grant-in-Aid for Young Scientists (A.I.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

P023
Absorption of chlorogenic acid in the stomach of mice

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Chlorogenic acid (5-caffeoylquinic acid) is present in almost every plant being the coffee the major source. The daily intake of this compound including coffee and other dietary sources can be higher than 100 mg/d. Chlorogenic acid presents antioxidant properties in vitro, and to evaluate the possible health effects is important to evaluate its absorption and disposition in vivo.

In spite of the absorption is not the main function of the stomach, some substances like glucose, simple sugars, amino acids, fat soluble substances and ethanol are readily absorbed. Moreover the pH of the gastric contents controls the absorption of certain ionisable substances such as aspirin and ranitidine which are readily absorbed in its unionized form.

The aim of this study was to study the absorption and metabolism of chlorogenic acid in the stomach of mice using an in situ intestinal perfusion technique.

Anesthetized CD1 mice fed with a polyphenol-free diet for 7 days before the experiment were used. The perfusion solution containing 32 ng/mL of chlorogenic acid and 0.1 mg/mL of phenol red was perfunded (0.16 mL/min). The outflow perfusate, a 60 min blood sample and the gallbladder were collected and analysed by HPLC-MS/MS. Perfusion samples were also analysed by LTQ-Orbitrap.

Chlorogenic acid was absorbed in 55.54 ± 9.24% and neither caffeic acid nor quinic acid were found in the samples using HPLC-MS/MS. However chlorogenic glutathione, dihydrocaffeic acid-3-O-sulfate, carboxylated quinic acid, hydroxylated quinic acid, ferulic acid, 3-hydroxyphenylacetic acid and 3-(3,4-dihydroxyphenyl)-propionic acid were identified in the gastric lumen when th Orbitrap technique was used.

Financial support: Spanish Ministerio de Economía y Competitividad (AGL2013-49083-C3-1-R) and the Instituto de Salud Carlos III, ISCIII (CIBEROBN) and by the Generalitat de Catalunya (2014 SGR 773).

P024

Co-administration of naringenin and quercetin in mice: study on the effect in their absorption and metabolism

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Naringenin and quercetin are two of the major flavonoids in tomato with reported health-promoting properties against many diseases. The benefits in human health are assumed to be in part related with their oral bioavailability, however low bioavailability has been reported for both: 10% for naringenin and 0.04-0.1% for quercetin after oral administration. Bioavailability depends basically on the permeability of the compound but also on how extensive is the intestinal and hepatic metabolism. When there is a co-administration a synergistic effect could appear related to a competition between them for membrane transporters or metabolic enzymes, causing changes on their absorption and metabolism.

The aim of this work was to study the influence of their co-administration in their absorption and intestinal first pass metabolism using an in situ intestinal perfusion technique in mice.

Anesthetized CD1 mice fed with a polyphenol-free diet for 7 days before the experiment were used. Perfusion solution containing naringenin and phenol red (3.5µg/mL and 0.1 mg/mL, respectively), quercetin (2.36 µg/mL) with phenol red, or bot polyphenols together, were perfunded (0.20 mL/min). The outflow perfusate, a 60 min blood sample and the gallbladder were collected and analysed by UPLC-MS/MS. Both naringenin and quercetin shows a decrease on its absorption when were administered together 94.82 ± 3.15% to 74.04 ± 6.57% for NAR and 85.02 ± 1.37% to 65.48 ± 6.15% for quercetin. The concentration of metabolites found in perfusion samples was higher when the co-administration, and in plasma and bile samples the number of metabolites identified were also higher.

Financial support: Spanish Ministerio de Economía y Competitividad (AGL2013-49083-C3-1-R) and the Instituto de Salud Carlos III, ISCIII (CIBEROBN) and by the Generalitat de Catalunya (2014 SGR 773).

P025

Characterisation of the global fate of polyphenols by analysis of the Phenol-Explorer database and measurement of 34 metabolites in the urine of European subjects.

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More than 500 polyphenols are commonly consumed in European diets. Most are extensively metabolised, both in human tissues and by gut microflora, to form conjugated metabolites and small phenolic acids. Here, two approaches were used to characterise the global fate of polyphenols consumed from European diets. Firstly, metabolism data from Phenol-Explorer, a database on the polyphenol composition of foods and their metabolites in humans and experimental animals, were analysed.

Studies in which pure polyphenols were administered to humans or rats were used to visualise known routes from ingested compound to urinary metabolite. In some cases the administered polyphenol was recovered in urine or plasma as glucuronide or sulfate conjugates, but many larger polyphenols were also metabolised to small phenolic acids that are products of microbial metabolism. Secondly, 34 polyphenol metabolites were quantified in 24-hr urine samples collected from 475 subjects from four countries in the European Investigation into Nutrition and Cancer (EPIC) cohort using a novel isotope dilution method. Amounts of metabolites excreted in 24-hr collections spanned several orders of magnitude (median metabolite excretion 0.05-157 mol/24 hr). By far the most abundant polyphenols in urine were small phenolic acids formed by microbiota, and profiles of polyphenol metabolites reflected differences in intake of polyphenol-rich foods in each of the four countries. These profiles could be mined to identify new dietary biomarkers of food intake or combined with biological information to predict possible health effects.

Financial support: Wereld Kanker Onzerdoek Fonds, French National Cancer Institute (INCa)

P026
Naringenin-O-glucuronide as a biomarker of tomato consumption

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Background/Objectives: Naringenin a characteristic polyphenol of citrus fruits it is also one of the most abundant flavonoid found in tomato and tomato products. Previous studies have demonstrated that the administration of a single dose of tomato sauce enriched with refined olive oil enhanced the bioavailability of naringenin and especially its metabolite, naringenin-O-glucuronide in plasma and urine.

The aim of the present work was to study the correlations between long-term consumption of tomato juices enriched with refined olive oil and the concentrations of naringenin and its metabolite, naringenin-O-glucuronide, in spot urine samples of participants at high cardiovascular risk.

Methods: Thirty-four patients, mean age 70.2 ± 3.7 years, and mean BMI 30.7 ± 3.8 kg/m^2 participated in an open, randomized, crossover and controlled clinical trial to assess the effects of daily consumption of three different doses of tomato juice (0 mL/day, 200 mL/day, and 400 mL/day) after four weeks of intervention. After a clean-up procedure, an Acquity UHPLC coupled to an API 3000 triple-quadrupole mass spectrometer with a TurboIon spray source used in negative mode and an UHPLC-LTQ-Orbitrap-MS was used for the analysis of urine samples.

Results: The concentration of naringenin-O-glucuronide in urine was 25 times higher than the naringenin, resulting the major metabolite in urine and indicating that naringenin is highly bioavailable and extensively metabolized before excretion. Both, naringenin and naringenin-O-glucuronide, showed a clear dose-response relationship.

Conclusion: There was a clear dose-response of naringenin and naringenin-O-glucuronide with tomato juice consumption. This last one can be used as a valid biomarker of tomato and tomato products consumption; however the interferences from citrus fruit consumption have to be considered.

Authors would like to express their gratitude for financial support from CICYT's (AGL2013-49083-C3-1-R), the Instituto de Salud Carlos III, ISCIII (CIBERobn) from the Spanish Ministry of Science and Innovation (MICINN) and Generalitat de Catalunya (GC) 2014 SGR 773.

P027
Bioavailability of (poly)phenolic compounds in humans after consumption of different food items containing coffee.

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Background/objectives: Chlorogenic acids (CGAs) are the most representative coffee phenolics, while flavan-3-ols are typical of cocoa-based products. Although the metabolism of these phenolics is well established in the literature, the influence o the food matrix in their bioavailability is far to be clarified. The aim of this study was studying the bioavailability of phenolic compounds in volunteers who consumed decaffeinated coffee, espresso coffee, a chocolate praline filled with coffee, a coffee drink enriched in liquid chocolate, and a coffee-soaked chewing-gum.

Methods: A single-blind, randomized, cross-over dietary intervention was conducted where 10 healthy volunteers randomly consumed each coffee-based product. Urines were collected at different time periods up to 24 hours. Samples were analyzed using UHPLC-MS^{n}analyses (linear ion trap MS for identification and triple quadrupole Mfor quantification purposes).

Results: The phenolic content of the products was approximately150µmol CGAs per serving, except for the coffee drink enriched in liquid chocolate (25µmol/serving).Thepralinecontainedadoubleamountofcocoaderivedflavan-3-ols (catechin, epicatechin, and procyanidin dimers) with respect to the coffee drink enriched in liquid chocolate. Based on the excretion patterns, both direct absorption of phenolics and gut microbiota metabolism / human phase II conjugations were observed. Dihydrocaffeic acidsulphate and isoferulic acid-glucuronide showed biphasic excretion profiles, possibly indicating both upper and lower gastrointestinal production/absorption. High levels of different cocoa-derived hydroxyphenyl--valerolactones were excreted after consumption of the praline filled with coffee.

Conclusion: Coffee phenolics show a lower 24-h urinary excretion when present in matrixes other than espresso coffees. Products containing both cocoa and coffee had the most interesting profile of phenolic metabolites with potentially health-promotin effects.

Financial support: Soremartec s.r.l.

P028

Stereoselective synthesis of (poly)phenol human and microbial metabolites and related phase II conjugates

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Polyphenols and related compounds are a group of plant-derived secondary metabolites which epidemiology indicates play a role in the protective effects of a fruit and vegetable-based diet. Flavan3ols are a heterogeneous group of phenolics comprising from single monomers to oligomeric structures, present in cocoa, green tea, red wine and different nuts such as hazelnut, in which skin they are particularly concentrated. Their intake has been associated with a lower risk of fatal cardiovascular diseases and colorectal cancers. In the human body, through various metabolic pathways, these molecules generate many metabolites, including phenyl-valerolactone derivatives. An asymmetric synthesis represents the only way to obtain these compounds in an enantio-pure form, considering the fact that -valerolactones are not commercially available.

We report here an efficient and enantio selective synthetic route toward these metabolites with the aim of subsequently testing their bioactivity. The stereodivergen plan leads through five steps to a series of differently substituted phenyl--valerolactones in both enantiomeric forms (R and S configured). The key chemical manoeuver in this process is an asymmetric vinylogous Mukaiyama–Aldol reaction (VMAR) between the donor (TMSOF) and an (poly)hydroxylated benzaldehyde, which affords two different diastereoisomers. Appropriate transformations of each diastereoisomer allowed the entry to both enantiomeric form of valerolactones, which were handled to obtain related phase II conjugates.

The selective synthesis of valerolactones represents an outstanding starting point for the study of their bioactivity, a key point to understand the health effects of diets rich in cocoa, green tea, and nut-derived foodstuffs, among other flavan-3-ol-containing products.

Financial support: Università degli Studi di Parma and “Progetto AGER” (grant n° 2011-0283)

P029

Flavonoid intake from black and green tea around the globe.

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Background: Flavonoids may reduce the risk of cardiovascular disease and positively influence surrogate cardiovascular endpoints. While tea accounts for a significant pa of dietary flavonoid intake, little is known about the inter-country comparability of intake and about intake in younger adults. This knowledge has important implication for public health messaging.

Objectives: Understand global intake of flavonoids from black and green tea in younger adults.

Methods: An online cohort was recruited consisting of a representative sample from United States of America, France, United Kingdom, Poland, Australia, China, India, Russia, Turkey, Indonesia and Saudi Arabia (n = 10,960; aged 18–45 years). Participants completed a comprehensive questionnaire on tea intake. Flavonoid intak was calculated using USDA databases.

Results: Globally 33% of the cohort consumed black tea daily and 14% green tea, ranging from 9% in France to 72% in Turkey. Amongst tea drinkers globally, on average black tea contributed 74.9mg/100g flavon-3-ols and 356.0mg/100g total flavonoids and green tea contributed 231.1mg/100g flavon-3-ols and 250.3mg/100g total flavonoids to the diet. Flavonoid intake from black tea intake varied greatly between countries, ranging for total flavonoids from 213.5mg/100g in China to 535.6mg/100g in Turkey. Flavonoid intake from green tea varied less, ranging for total flavonoids from 198.4mg/100g in India to 273.2mg/100g in China.

Conclusion: Among consumers flavonoid intake from green tea was comparable across countries, however black tea showed wide variation. In countries with low tea intake and low flavonoid intake from total diet, tea could be seen as a good additional source of flavonoids.

Financial support: This work was fully funded by Unilever Research & Development Vlaardingen.

P030
Matrix impact on the bioaccessibility of plant polyphenols in the gastric tract

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The consumption of fruit and vegetables (F&V) is inversely associated to the development of the main chronic diseases, and in particular cardiovascular diseases. Moreover, the cardiovascular protection could be ascribed to flavonoids, a class of polyphenols largely distributed in F&V. Polyphenols are poorly absorbed from the gastrointestinal tract (GIT) and undergo extensive metabolization by colon microflora before reaching plasma. Thus, before their absorption, polyphenols could exert a health benefit directly in the GIT. As a matter of fact, they were shown to protect polyunsaturated lipids from oxidation during gastric digestion. The bioaccessibility of polyphenols, defined as their release from the food matrix and solubilization into the bolus, is a prerequisite step.

This study aims at assessing the bioaccessibility of polyphenols in the gastric tract after the ingestion of two different matrices, F&V and the corresponding polyphenol extract.

Six mini-pigs equipped with gastric cannula were fed a meal containing principally sunflower oil and ground beef meat and enriched with either cubed F&V or the corresponding hydroacetonic extract containing polyphenols. Samples of the gastric digesta were taken up, centrifuged and the content and structure of the polyphenols in the aqueous phase assessed.

Less than 10% of the hydroxycinnamic acids and dihydrochalcones were recovered after both F&V and extract consumptions although the levels were higher after the ingestion of the extract. Flavanol monomers and dimers were not recovered. Most polyphenols interact with proteins and fibers and are lost upon centrifugation o the gastric digesta. Last, this matrix effect was F&V dependent, crude apple releasing more polyphenols than crude plum.

Financial support: INRA grant

P031
Interactions of isoflavonoids with human liver microsomal cytochromes P450 as one of determinants of their availability and safety

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Isoflavonoids are polyphenolic compounds widely distributed in plant kingdom known to be important for their health benefits. As they after ingestion interact with enzymes of biotransformation of foreign substances inc. drugs, they may be not only metabolized but also may act as a source of drug interactions. Six isoflavonoids (daidzein, genistein, biochanin, formononetin, glycitein, equol- daidzein metabolite), as well as their glycosides, daidzin, puerarin, genistin, sissotrin, ononin and glycitin) were studied to see whether may influence the function of the enzymes of drug metabolism, cytochromes P450 (abbrev. CYP).

The liver microsomal fraction is an established system for studying the drug interactions. Activities of seven drug-metabolizing microsomal CYP enzymes were studied with the corresponding prototypic substrates: 7-ethoxyresorufin (CYP1A2), testosterone (CYP3A4/5), midazolam (CYP3A4/5), coumarin (CYP2A6), 7-ethoxy-4-(trifluoromethyl)coumarin (CYP2B6), diclofenac (CYP2C9), bufuralol (CYP2D6) and chlorzoxazone (CYP2E1).

Experiments have indicated that genistein and daidzein are relatively potent non-competitive inhibitors of CYP2C9 in vitro enzymatic activity, with Ki values 36 and 61 μM, respectively. Also, a moderate inhibition effects on CYP3A4 were observed. Namely, genistein, equol, and biochanin A revealed a non-competitive type of inhibition with Ki 23 μM, 38 μM and 58 μM, respectively. The second prototypical CYP3A4 activity, midazolam 1´-hydroxylation, was also inhibited, however, the inhibition was less pronounced.

To predict whether pharmacokinetic interactions with drugs are elicited by dietary supplements or foods containing CYP inhibitors, extensive studies should be conducted to evaluate all factors contributing to the drug-food supplement interaction. The results presented here however indicate that there may be an interaction of isoflavonoids from soy food and drugs metabolized through CYP3A4 or CYP2C9 only under conditions e.g. of high intake of this kind of food. In other words, levels of isoflavonoids should reach approx. 50 μM in plasma which makes clinically important drug interactions less probable (the levels are ten times lower after usual meal).

Financial Support: Grant Agency of the Czech Republic P303/12/G163; Universitygrant IGA_LF_2015_004

P032
Intestinal absorption pathway of quercetin-O-3-glucoside in rats

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Quercetin is a popular dietary flavonoid and its bioavailability has been investigated in detail. We previously found that quercetin aglycone was partly transported into the lymph as its metabolites in rats, and the lymphatic transport was enhanced with lipid co-administration. In this study, qurcetin-O-3-glucoside (Q3G), a typical quercetin glycoside, was administrated to the thoracic lymph-cannulated rats, and its lymphatic absorption was investigated.

Male Wistar/ST rats with thoracic lymph duct cannulation and intraduodenal tubing were used. Q3G was administrated into rat duodenum as 10 mg quercetin aglycone equivalent/kg BW, as either solubilized in propylene glycol (PG) or suspended with lecithin, taurocholate, and with or without oleic acid. Lymph fluid was continuously collected from 1 h before to 6 h after administration, together with the peripheral blood from tail vein at the desired time points during the experimental period.

Obtained plasma and lymph fluid were analyzed with HPLC-UV (365 nm) with or without deconjugation treatment and with LC-MS/MS for individual metabolites determination. Quercetin conjugates were simultaneously detected both in the plasma and in the lymph after administration of Q3G in PG. When Q3G was administrated as suspension, the coexistence of oleate decreased the lymphatic quercetin metabolites compared with the sample without oleate.

In conclusion, Q3G was partly absorbed in the small intestine via the lymph, similarly to quercetin aglycone. However, coexistence of dietary lipids did not enhance the lymphatic transport of Q3G metabolites, probably because lipophilicity of Q3G was low and its accessibility to the intestinal epithelium was suppressed by fatty acids.

Financial support: JSPS KAKENHI Grant#26292072 (K.M. and S.I.)

P033
Effects of consumption timing of soybean isoflavones on blood lipid levels in mice

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Soybeans are almost exclusive dietary sources of isoflavones, which recognize to have many beneficial effects including reduction of blood lipid levels. Meanwhile, many of the biological processes have been reported to follow diurnal rhythms recurring every 24 hours, and consequently some beneficial effects might be difference depending on the consumption timing of functional ingredients. In this study, we evaluated the effects of consumption timing of soybean isoflavones on blood lipid levels in mice.

Genistein, daidzein, and glycitein amounts in nine kinds of soybeans cultivated in Miyazaki area, Japan, were analyzed using our previous method (Sakakibara et al., 2003, 51, 571-81). Female C57BL/6 mice were housed in the animal room (12 h/12 light-dark cycle, light on at ZT0). After acclimatization, mice were consumed AIN-93M diet containing 10% Kiyomidori, which was the isoflavone-rich soybean i this study, as following schedule; ad libitum consumption of normal AIN-93M diet (C/C) or 10% Kiyomidori diet (I/I) during whole of the day, consuming 10% Kiyomidori diet during ZT0-12 (I/C) or during ZT12-24 (C/I). After 2 weeks, blood samples were collected at ZT0 without starvation, and analyzed their lipid levels.

Isoflavone amounts were range from 8.6-11.6 mg as aglycones/g, and Kiyomidori contained 11.6 mg/g. There were not remarkable differences in the body weight gain and total food consumption among four groups. Food consumptions in the groups C/C, C/I, I/I were greater in the morning (ZT12-24) than that of the dinner (ZT0-12), but its amount of I/C group was same between the morning and dinner (ZT12-24 and ZT0-12). Serum triglyceride and free cholesterol (Chol) levels were significantly higher, and HDL-Chol was lower in I/C group than that of C/I, although serum total Chol level was similar between these two groups. We are now continuing the further study to elucidate these differences.

Financial support: Grant-in-Aid for Young Scientists (A) from the Japan Society for the Promotion of Science (No. 23310011, to H.S.)

P034
In vitro glucuronidation of -viniferin, a resveratrol dimer

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Stilbenes are naturally occurring phenolic compounds mainly found in grapes and re wine. These compounds have been reported to have potential preventive activities against human diseases. Among these stilbenes, *trans*-resveratrol has been widely investigated. Numerous studies have shown that after its ingestion *trans*-resveratrol is submitted to various metabolic conversion. Indeed, *trans*-resveratrol is conjugated to glucuronic acid or sulfate in various organs such as liver, intestine and brain. However little is known about the metabolism of the oligostilbenes especially -viniferin which is also a very active stilbene.

In this study, we investigated the glucuronidation of -viniferin, a *trans*-resveratroldimer which shows anti-inflammatory and anti-amyloid aggregation activities *in vitro*. First, the previously purified -viniferin has been submitted to chemical O-glucuronidation leading to the formation of the four different monoglucuronides. These compounds have been separately purified and fully characterized by HPLC-M and NMR spectroscopy. Second, -viniferin was also incubated with rat or human microsomes, in presence of UDP-glucuronic acid. This reaction led to the formation of only one glucuronide using rat microsomes, whereas human microsomes produce one major and two minor glucuronides.

This work, by combining chemical synthesis and biological reaction, allowed to determine the structure of the different glucuronides formed by rat and human liver. Since it is likely that beneficial effects attributed to polyphenols are linked to biological activities of their metabolites, further activity assays are under progress using these metabolites.

Financial support: University of Bordeaux

P035
Urinary excretion of chlorogenic acids and metabolites after acute coffee consumption in humans: intra- and inter-variability and correlation with food components' consumption.

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Coffee consumption is associated with benefits to human health, mainly due to the bioactivity of chlorogenic acids (CGA). Although these compounds have been extensively studied in recent years, data on variability of CGA metabolism are still limited.

The objective of this study was to investigate the intra- and inter-individual variability in the urinary excretion of CGA and metabolites after acute coffee consumption as well as to evaluate possible associations with regular diet components.

In three occasions, after 48h wash-out diet and 8h fasting, each subject (n=33) consumed a single dose of coffee containing 0.64 mmol CGA. Sequential urine samples were collected at baseline and at intervals up to 24h after test beverage consumption. Six CGA and 16 metabolites were analyzed by HPLC-DAD-MS. Food intake data were obtained by means of a Food Frequency Questionnaire and a 72h Food Intake Report. Results were treated by ANOVA followed by Fisher's test and Pearson correlation test (95% significance level).

Considering the 24h average urinary excretion of CGA and metabolites equivalent to 63% of the amount of CGA consumed, individual results from three tests varied up t 20% in relation to mean values, with great similarity in metabolites profile.

Differences among subjects varied considerably, from 30 to 150% in relation to mean values and were driven mainly by the excretions of colonic metabolites, such as 3,4-dihydroxyphenylacetic, 3,4-dihydroxyphenylpropionic, dihydroxybenzoic, p-coumaricacids, which were positively correlated with regular fiber intake (r^2 =0.74; 0.70; 0.60; 058 and p = 0.02; 0.03; 0.01;<0.001, respectively). This indicates that regular fiber intake increases the production and uptake of colonic CGA metabolites after coffee consumption probably by stimulation of colonic bacterial growth. Hippuric, benzoic and vanillic acids were positively correlated with protein intake (r^2 =0.89; 0.71; 0.68; p = <0.001; 0.02, 0.02, respectively). No correlation witpolyphenols intake was found.

Financial support: National Research Council (CNPq) and Carlos Chagas Filho Research SupportFoundation (FAPERJ), Brazil

P036

Enhances antioxidant / antiapoptotic activity of human peripheral blood mononuclear cells cultured in nitro with wine polyphenols

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Objectives: The effect of wine polyphenols, resveratrol and catechine (concentration range: 5-50 M) , was investigated on apoptotic cell death and on the oxidative metabolic status of human peripheral blood mononuclear cells (PBMNC), isolated ex vivo from healthy donors, and cultured in presence of 1,1diphenyl-2-picryl-hydrazyl(DPPH),a stable free apoptogenic radical.

Methodology: Apoptosis was detected with flow cytometry by recording changes of membrane permeability to propidium iodid (PI) and of phosphatidylserine (PS) exposure on the plasma membrane. Oxidative status was assessed by measuring the intracellular level of glutathione [GSH], the intracellular activity of gamma-glutamyltransferase [-GT] and glutathione-S-transferase [GST] antioxidant enzymes and the cellular content of malondialdehyde [MDA] produced from the lipid peroxidation.

Results: apoptosis nor cellular oxidative parameters were affected by culturing PBMNC in medium containing RS or catechine alone up to 20 M for 5 days, while the frequency of cells with Neith intermediate permeability to PI (apoptotic cells) increased twice at 50 M RS or catechine with respect to the level measured in untreated cell populations. In another series of experiments, PBMNC were first grown in medium containing 20 M RS or catechine alone for 24 h, and then for 96 h in medium containing 20 M of each polyphenol supplemented with 20 M DPPH radical. Pre-treatment of PBMNC with Polyphenols, RS or catechine, for 24 h counteracted the oxidative effect triggered by DPPH, as identified by the increase of GSH level and the activity of [-GT] an [GST], the reduction of MDA content and the dramatic decrease of apoptotic cells frequency, when compared to values of the same parameters measured in cells cultured without pre-treatment.

Conclusions: PBMN cells acquired an efficient antioxidant and antiapoptotic capacity in vitro when they were pre-incubated for 24 h with polyphenols RS or catechine alone and after then further co-cultured by addition of the free radical DPPH, a strong oxidant agent.

P037

Phenolic acid content in oats and their human bioavailability/metabolism

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Background/Objectives: Epidemiological data suggest that a diet rich in wholegrain i associated with a 21% reduced cardiovascular disease risk. In addition to beta-glucan wholegrain oats are rich in phenolic acids with potential health benefits. We assessed types/contents of phenolic acids in oat varieties and are examining human bioavailability/metabolism of phenolic acids following oat intake.

Methods: After grinding 10 oat samples, phenolic acids in the soluble and bound fractions were extracted using liquid extraction and alkaline hydrolysis and were identified/quantified using HPLC-DAD. In a bioavailability 2-arm crossover trial, after following a 2-day low-(poly)phenol diet, six healthy men received 66.8g oat bran porridge or control, and provided urine at baseline and during specific time intervals over 48h post-intake. Following solid-phase extraction, urinary phenolic acid metabolites are being identified/quantified using UPLC-MS/MS.

Results: Whilst 8 oat phenolic acids were identified in the soluble and bound fractions, 2 avenanthramides were only detected in the soluble fraction. Total oat phenolicacidcontentsrangedfrom314to579µg/g, of which 20-30% were present in the soluble fraction and ferulic acid was the major phenolic acid. Preliminarily human trial data will be presented.

Conclusions: Our data show that consuming 2 oat servings provides up to 40 mg of phenolic acids and may be a significant dietary source of penolic acids. Whilst our ongoing human trial will establish the bioavailability/metabolism of oat phenolic acids, future studies need to examine if phenolic acids contribute to the cardiovascular benefits of a diet rich in oats.

Financial support: The Biotechnology and Biological Sciences Research Council (BBSRC, UK) andPepsiCo funded this project.

P038
Polyphenols bioavailability and exposure assessment

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Background: Olive oil (OO) consumption is associated with cardiovascular disease prevention because of both its oleic acid and phenolic contents. The capacity of OO phenolics to protect against low density lipoprotein oxidation is the basis for a health claim by the European Food Safety Authority. OO phenolic compounds in vivo effects are related with their bioavailability and it is known that OO phenolics undergo Phase I and II metabolism reactions. The majority of bioavailability studies conducted in the area have focused on hydroxytyrosol and tyrosol - phenolic alcohol of virgin olive oil (VOO).

Objective: to study the impact of acute VOO intake on phenolic compounds metabolism and select plasma metabolites for VOO exposure.

Design: Self-reported healthy participants (n=9) ingested 50 mL of virgin olive oil (VOO) in a single dose with bread. Fasting venous blood was collected at baseline (before VOO ingestion) and at 0.5h, 1h, 2h, 4h and 8h after VOO ingestion. The obtained plasmas were stored at -80ºC until analysis.

Methods: Plasma samples were submitted to liquid-liquid extraction. Plasma screening of phase I and II metabolites of typical VOO phenolic compounds (phenolic acids and alcohols, flavonoids, lignans and secoiridoids) was done using LC-ESI-Orbitrap MS in negative mode.

Results and Conclusion: Statistical approach using principal component analysis enabled us to discriminate between plasma samples before and after VOO intake. Eight compounds were selected as plasma biomarkers for VOO exposure and included Phase I (hydration, hydrogenation and hydroxylation) and Phase II (methyl and glucuronide) metabolites mainly derived from secoiridoids.

Financial support: QREN 12228, Azeite+Global

P039
Dietary exposure of polyphenol-containing foods within the Irish population

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Background: Evidence from epidemiological studies suggests that polyphenols reduce the risk of cardiovascular disease (CVD) and cancer when consumed in sufficient quantities. One of the main barriers to setting dietary recommendations for polyphenol intakes i that they are found in different quantities in foods.

Aim: To estimate current dietary exposure of the predominant polyphenol-containing food in a representative sample of the Irish population including adults, adolescents and children.

Methods: Data from National food consumption surveys, including: adults (19-90y; N = 1500) teenagers (13-17y; N = 441) and children (5-12y; N = 594) were incorporated into a probabilistic modelling system using Crème Global software© to perform exposure assessments. The eBASIS and Phenol-Explorer databases identified polyphenol quantities in foods. Total polyphenol mean daily intakes (MDI), main polyphenol sub-classes and average portion sizes of the predominant food contributors were calculated.

Results: The major polyphenol-containing foods were identified in Irish adults (N= 34), teens (N=19) and children (N=18). The main foods contributing to polyphenol intakes wer tea, coffee and green tea. The major polyphenol sub-classes contributing to intakes were flavanols, ellagitannins and anthocyanins. Tea mean (SD) daily intake 521(386 provided 531mg/total polyphenols/d) amongst adults and adolescents and orange juice (mean (SD) daily intake 102(94); provided 47mg/total polyphenols/d) in children.

Conclusion: Tea and orange juice are the main contributors to polyphenol intakes in Irish adults and children. The identification of the principal polyphenol-containing foods provide useful consumption data which is of value to the food industry and the development of dietary recommendations for polyphenol intakes.

Financial support: Irish Department of Agriculture, Food and the Marine under its Food Institutional Research Measures (FIRM) initiative (2007-2013).

P040
Bioaccessibility of phenolic compounds in jabuticaba residue and guava bread

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Jabuticaba (Myrciaria jaboticaba) and guava (Psidium guajava) are Brazilian fruits rich in phenolic compounds, highlighting their potential application of use in food products. This study aimed to investigate the bioaccessibility of phenolic compounds in jabuticaba residue (peel and seed) and bread incorporated with 30% of guava powder.

Bioaccessibility was evaluated using a simulated in vitro gastrointestinal digestion model, which consisted of three-steps to mimic the digestive process in the mouth, stomach (gastric digestion) and small intestine (duodenal digestion). Digested samples were evaluated for phenolic compounds using HPLC-DAD.

Ten phenolic compounds (gallic, ellagic, m-coumaric and 3,4-dihydroxybenzoic acid and quercetin, myricetin, quercetin-3-O-rutinoside, myricetin-3-O-rhamnoside, cianidin-3-O-glucoside and delphinidin-3-O-glucoside) were identified in jabuticaba residue. Only m-coumaric and 3,4-dihydroxybenzoic acids, presented in low content in jabuticaba residue, were not identified in digested samples.

Cianidin-3-O-glucoside, delphinidin-3-O-glucoside and myricetin were more bioaccessible after gastric digestion. Other phenolic compounds were more bioaccessible after duodenal digestion. In guava bread fourteen phenolic compounds (4-hydroxyphenylacetic, 3,4-dihydroxybenzoic, 3,4-dihydroxyphenylacetic, benzoic, ellagic, ferulic, kaempferol, gallic, rosmarinic and syringic acids and quercetin-3-O-rutinoside, myricetin-3-O-rhamnoside, quercetin and myricetin) were identified. Only 3,4-dihydroxyphenylacetic acid, presented in low contents in guava bread, was not identified in digested samples. While benzoic and gallic acids were found in all digested samples, other phenolics compounds were observed only after duodenal digestion. Salicylic and m-coumaric acids, not identified in guava bread, were also observed after duodenal digestion

Our results indicate that most of the phenolic compounds are more bioaccessible afte duodenal digestion, suggesting that their release from the food matrix occurred due t the action of bile and/or pancreatic enzymes.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), ConselhoNacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação deAmparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

P041
Quantification of Curcumin Metabolites in Human Plasma by Isotope DilutionLC-MS

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Curcumin, the active polyphenol of the spice turmeric, is widely used in traditional Asian medicine and is currently evaluated in 116 clinical trials. Turmeric extract contains curcumin as the most abundant compound, while demethoxycurcumin and bisdemethoxycurcumin are present in lower amounts. Curcumin is metabolized in vivo by reduction and/or by conjugation with glucuronic acid and sulfate. However, quantification of plasma levels of curcumin metabolites has given inconsistent result which could be due to lack of an accurate and robust method of quantification. We have synthesized 8 deuterated standards and used them to develop a sensitive stable isotope-dilution LC-MS method to quantify curcumin and 10 relevant metabolites. Plasma samples are extracted using paper strip absorption and elution or liquid-liqui extraction. LC-MS/MS analysis is performed using selected reaction-monitoring.

Curcumin, demethoxycurcumin, bisdemethoxycurcumin, oxidized curcumin (bicyclopentadione), and curcumin-glucuronide are analyzed in positive ion mode. The reduced metabolites, di-, tetra-, hexa- and octa-hydrocurcumin, together with curcumin sulfate and ferulic acid (cleavage product) are analyzed in negative ion mode. The new method allowed the direct quantification of free curcumin and curcumin-glucuronide/sulfate in human plasma. After hydrolysis (-glucuronidase) curcumin levels increased up to 20 fold. This increase was accounted for by curcumin-glucuronide as the major conjugate. The levels of curcumin-sulfate were very low, whereas the most abundant metabolites were tetra- and hexa-hydrocurcumin which were quantified after hydrolysis.

The method will be applicable for reliable quantification of plasmatic levels of curcumin and its metabolites in pharmacokinetic studies, guiding dose regimen in the design of future clinical trials.

Financial Support: NIH awards AT006896 and CA159382.

P042

Assessment of different doses of polyphenols from dealcoholized red wine on endothelial function in subjects with metabolic syndrome.

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Background: Several studies pointed out that mortality and risk from cardiovascular disease (CVD and coronary heart disease (CHD) are higher in subjects with Metabolic Syndrome (MetS), which is considered a cluster of cardiovascular risk factors.

Epidemiological studies and intervention clinical trials have shown that dealcoholize red wine (DRW) and moderate consumption of red wine (RW) are inversely associated with cardiovascular risk factors to reduce the expression of cell adhesion molecules (CAM) indicating a possible mechanism by which the non-alcoholic fraction of red wine prevents vascular disease.

Methods: A randomized, open, prospective, and controlled clinical trial, running in parallel was performed in 36 subjects with MetS to evaluate the effects of polyphenols; 375 mL/day of DRW, dealcoholized red wine with grape extract (DRWEx) or water were administered during three months.

Anthropometric measurements and blood biochemical analysis were carried out at baseline and after each intervention. Levels of endothelial progenitor cells (EPC), circulating endothelial cells (CEC) and leukocyte cell membrane receptors were analyzed by flow cytometry.

Results: After DRW and DRWEx interventions, we observed a decrease on body weight, bod mass index, waist circumference, total cholesterol concentration and an improvement on blood pressure. Moreover, there was an increase in the number of EPCs and a significant decrease in levels of CECs and T-lymphocyte expression.

Conclusion: We conclude that the non-alcoholic fraction of wine, rich in polyphenols, may reduce CAM and CEC, known markers of severity of CVD, in a population at high risk of CVD due to MetS. This reduction could be an improvement in the condition of the vascular endothelium and in the development of atherosclerotic plaques. An increase number of EPC is inversely associated with risk factors of CVD, as a marker of endothelial regeneration.

Financial support: Authors would like to express their gratitude for financial support from INCOMESproject supported by Ministerio de Economía y Competitividad through theINNPRONTA program. And CICYT (AGL2013-49083-C3-1-R), Generalitat de Catalunya FI-DGR 2013/FI-DGR 2014, the Instituto de Salud Carlos III and ISCIII (CIBERObn) from the Spanish Ministry of Science and Innovation (MICINN).

P043

Biofunctional properties of blueberries

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Blueberries are an edible fruit known for their health beneficial properties. They are one of the most popular fruits rich in phytochemicals. These benefits are attributed to the polyphenols, in particular the anthocyanins, which work as antioxidants and are considered preventive against cardiovascular disease.

The aim of this study was to investigate the biofunctional properties of blueberry. The objectives were to evaluate the content of phenolic compounds: total phenolic content (TPC), total flavonoids content (TFC), as well as antioxidant activity.

Phenolic compounds were estimated spectrophotometrically and showed a TPC value of 0.680 mg gallic acid (GA) equivalents/mg dry weight (DW) and TFC value of 0.970 mg GA equivalents/mg DW.

Antioxidant activity of blueberry extract was evaluated in a linoleic acid emulsion model system using ferric thiocyanate (FTC) and thiobarbituric acid reactive substance (TBARS) methods to monitor the formation of lipid peroxides and malondialdehyde, respectively. Tests were performed every 24 hours over a period o six days, using butylated hydroxyanisole (BHA) as the positive control. The FTC an TBARS assay results (taken on the day showing highest oxidation) revealed that blueberry extract showed antioxidant activity with 49.87 % and 78.22 % inhibition when using FTC and TBARS, respectively. Antioxidant scavenging activity was also determined using DPPH, reducing power, hydrogen peroxide, and chelating affect assays. These results will be presented at the conference.

Financial support: University of Surrey

P044

The impact of *in vitro* digestion on bioactive compounds and antioxidant activity of anthocyanin rich extract from black bean seed coats

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Anthocyanins (ANTs) are flavonoid pigments whose consumption is associated with health benefits. This positive effect is closely related to its antioxidant activity (AA), which has been reported in several studies. However these compounds present poor bioavailability and instability in intestinal pH conditions. Thus, the reported AA may be lower than the real activity after digestion. The present study evaluated the effect of in vitro digestion in total phenols (TPs), monomeric ANTs (MAs) and AA (TEAC - Trolox Equivalent Antioxidant Capacity and ORAC - Oxygen Radical Absorbance Capacity assays) of an ANTs rich extract from black bean seed coats, through the simulation of chemical and enzymatic conditions of digestion gastrointestinal. Extracts were submitted to gastric digestion with the addition of pepsin and the intestinal digestion was simulated with the addition of pancreatin-bile solution. The MAs content was determined through the differential pH method and the TPs by a Folin-Ciocalteu assay. AAPH and ABTS radicals were respectively utilized in hydrophylic ORAC and TEAC assays. The simulated digestion was responsible for decrease of 45% in TPs and 90% in MAs. The AA was also reduced after digestion with 28% and 35% reduction for the TEAC and ORAC assays, respectively. The reduction of AA was attributed mainly to the degradation of ANTs during the digestive process. Through a relatively simple approach compared with in vivo studies, we found that digestion can alter the biological effects of ANTs present in black beans, influencing their AA.

Financial support: CNPq - National Counsel of Technological and Scientific Development; CAPES- Coordination for the Improvement of Higher Level -or Education- Personnel

Poster Session B: Polyphenols in Obesity, metabolic syndrome and diabetes

P100

Citrus industrial residue as a source of biotransformed flavanones with anti lipogenic effects in 3T3-L1 cells

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Flavonoid extract from citrus peel has biological properties, and biotransformation could improve its potential. We aimed to evaluate flavonoids from citrus residues biotransformed by solid state fermentation for its antioxidant activity in vitro, and also its lipogenesis and lipolysis activity in 3T3-L1 cell line. According to HPLC analysis, In Natura residue (control extract) had higher content of naringin and hesperidin, while Biotransformed residue had higher level of naringenin and hesperetin. Biotransformed residue presented smaller amount of total flavanones, however the antioxidant capacity of both extracts were similar according to DPPH and ORAC assays. MTT assay indicates that the extracts were not toxic to cells (0.01 – 1.00 mg/mL). According to Oil Red O staining, when samples were added in differentiation medium (in pre-adipocytes), there was little effect in lipid accumulation. In Natura extract had the best result, with lipid accumulation reduction of 18%. On the other hand, the addition in maturation medium (in adipocytes) cause a dose-dependent decrease in lipid accumulation, reaching a diminution of 22% and 48% for Biotransformed and In Natura extracts. Total glycerol concentration was below limit of detection with In Natura extract treatment. Biotransformed residue extract, on the other hand, presented some effect on glycerol release (2.39 ± 0.17 - 5.24 ± 0.29 mg/mL of glycerol). Thus, biotransformation was able to modify flavanones profile, increasing the aglicones. All extracts could be used in obesity treatment, In Natura extract seemed be useful to reduce new adipocytes synthesis a lipid accumulation, and Biotransformed extract could be used to induce lipolysis.

Financial supports: CNPq/FAEPEX/FAPESP

P101

Protective effects of ginger and pomegranate peel extracts against streptozotocin-mediated oxidative stress in diabetic rats.

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The efficacy of ginger extract (GE) and pomegranate peel extract (PPE) as a traditional medicine for diabetes is well reported, however there is negligible reports on the biochemical aspects of these extracts against oxidative stress-mediated diabetes. This study evaluated the antioxidant potential properties of GE and PPE in an experimentally streptozotocin (STZ)-induced diabetic model.

Male Sprague Dawley rats were allocated into groups: non-diabetic control that received chow diet; non-diabetic control group that received chow diet plus oral feeding of either GE or PPE; diabetic group that received chow diet; and diabetic group that received chow diet plus oral feeding of either GE or PPE. Serum was collected to measure the following: glucose, insulin, and oxidative stress indices. The pancreas tissues were homogenized for measuring cellular antioxidants (glutathione, GSH and total antioxidant capacity, TAC).

Results showed that STZ induce hyperglycemia and oxidative stress in rats of diabetic groups, as evidenced by high serum levels of advanced oxidation protein products, protein carbonyls and nitrates plus nitrites. Treatment with GE and PPE have significantly abrogated the observed STZ insults and lowered the levels of oxidative stress markers. The same observation was reported for the antioxidants measurement (GSH and TAC) in the pancreas tissues, where GE and PPE treatment combat the STZ-mediated depletion of GSH and impairment of TAC.

GE and PPE might be considered as effective dietary antioxidants in attenuating the STZ-induced diabetes and oxidative stress. It is recommended to be used for controlling diabetes, and preventing oxidative stress-induced pancreas damage.

Financial support: Internal grant fund (IG/AGR/FOOD/13/05) and His Majesty Strategic Fund (SR/AGR/FOOD/11/01) offered by Sultan Qaboos University.

P102
Dietary chia oil (Salvia hispanica L) improves antioxidant status in diet-induced obese rats

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Background: The chia seed oil contains the highest proportion of omega-3-linolenic acid of any known natural source and possess a high antioxidant potential in vitro, being considered a new source of natural antioxidants, due to the content of tocopherols, phytosterols, carotenoids and phenolic compounds. **Objectives:** This study investigated the effects of chia oil intake on plasma and liver lipid peroxidation antioxidant capacity and oxidative stress markers in diet-induced obese rats.

Methods: Thirty-two Wistar rats were divided in four groups: lean control (Control) high-fat and high-fructose diet (HFF), HFF with chia oil in short (6-wk) and long (12-wk) treatments. Plasma and hepatic indicators of lipid peroxidation, enzymatic and non-enzymatic endogenous antioxidant system and antioxidant potential were determined. The limit of significance was set at $P<0.05$. **Results:** The HFF diet induced weight gain, oxidative stress and lipid peroxidation in plasma and liver of the animals. The ingestion of chia oil diet (12 and 6 wk) increased plasma thiol reduced levels, plasma catalase, plasma glutathione peroxidase (GPx) and liver glutathione reductase activities (GRd), with no changes in liver catalase and GPx activities, plasma and liver superoxide dismutase activity. The ingestion of chia oil diet did not modify the liver lipid peroxidation, but was able to reduce plasma malondialdehyde and 8-isoprostane levels. The plasma and hepatic antioxidant capacity values were increased in chia oil groups about 30% and 42%, respectively. **Conclusion:** Chia oil intake reduced oxidative stress in vivo, since it improved antioxidant status and reduced lipid peroxidation in diet-induced obese rats.

Financial support: Fundação de Amparo à Pesquisa do Estado de Sao Paulo (FAPESP, 2012/23813-4) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 140386/2012-2 and 300533/2013-6).

P103
In vitro and in vivo antioxidant potential of freeze-dried jaboticaba peel and jaboticaba peel tea

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Jaboticaba (*Myrciaria spp.*) is a typical Brazilian fruit with high levels of polyphenols. The chemical characteristics of jaboticaba peel show possible health applications and the jaboticaba peel tea can be a viable intake alternative.

This study evaluated the phenolic compounds and antioxidant capacity *in vitro* of freeze-dried jaboticaba peel (FJP) and jaboticaba peel tea (JPT) and oxidative stress in diet induced obese rats.

The phenolic compounds, total anthocyanins, flavonoids and antioxidant capacity (DPPH, FRAP, ABTS and ORAC assays) in the FJP and JPT were measured. The ellagic acid, gallic acid and cyanidin-3-*O*-glucoside were measured by HPLC. In the biological assay, twenty-four *Wistar* rats were divided in four groups (= 6): AIN-93M normal control diet; HFF (obese control) feed high-fat and fructose diet; HFF-FJP diet with 2% of FJP powder; JPT feed HFF diet and with water substituted by JPT. After 12 experimental weeks, plasma and liver FRAP, TBARS, GSH levels and antioxidant enzymes activity (GPx, GR, CAT and SOD) were determined. The limit of significance was set at $P < 0.05$.

The FJP showed higher levels of total phenolics and anthocyanins, ellagic acid and DPPH, FRAP and ORAC assay, but JPT showed higher levels of cyanidin-3-*O*-glucoside, gallic acid and ABTS assay. In the animals, the antioxidant potential of the FJP and JPT was confirmed by important markers, as TBARS, GSH levels and CAT activity, but not by FRAP assay, and SOD, GR and GPx enzymes. Thus, FJP and JPT showed an important antioxidant potential *in vitro* and *in vivo*

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

P104
Passion fruit (Passiflora edulis) by product improves oxidative stress induced by high fat diet in Sprague-Dawley rats

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Background/objectives: Obesity is associated with chronic low grade inflammation with increased oxidative stress (OS). Passion fruit (Passiflora edulis f. floricarpa) pee has been considered a good source of fiber and antioxidants compounds, which can improve certain health parameters. Therefore our research aimed to investigate the effects of passion fruit peel in oxidative stress induced by high fat diet in rats.

Methods: A commercial passion fruit peel flour (PFPF) was used. Animals and diets Thirty-two Sprague-Dawley (male) were divided into 4 groups, they was remained seven days in acclimatization and for 10 weeks in experiment. Two Control diets: AIN-93G, with protein concentration of 12% and a high-fat control diet, AIN 93G-modified with 35% (g) of fat. Two Experimental diets: AIN-93G with 50% dietary fiber (cellulose) replacement by PFPF fiber, the same replacement was done for AIN 93G-modified. Lipid peroxidation was measured by thiobarbituric acid-reactive substances (TBARS) and lipid hydroperoxides. The activities of glutathione peroxidase, gluthatione reductase, superoxide dismutase, catalase and glutathione total were measured by colorimetric assays.

Results: The PFPF intake protected the animals from high fat diet group against weight gain, which also showed decreased markers of lipid peroxidation in liver and adipose tissue and increased glutathione peroxidase, glutathione reductase and superoxide dismutase activities.

Discussion: This result showed that the ingestion of PFPF was effective in decreasing oxidative stress and positively modulates the activity of antioxidant enzymes.

Synergistic action of antioxidants, the soluble fiber and antioxidant enzymes, may have contributed to the improvement of antioxidant status of animals fed high-fat diet.

Financial support: The authors thank the financial support of FAPESP, CAPES and CNPq.

P105
Dietary flavonoid intake and weight change

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Background: Higher flavonoid consumption may be associated with less weight gain over time through mechanisms related to energy balance, glucose uptake in tissues, fat oxidation, and adipokine secretion.

Objective: To examine whether intake of specific flavonoid sub-classes is associated with weight change over time, including flavonols, flavones, flavanones, flavan-3-ol anthocyanins, and polymeric flavonoids.

Methods: We examined the association between change in intake of the range of flavonoid sub-classes and change in weight in three large prospective cohorts of 124,102 men and women participating in the Health Professionals Follow-up Study (HPFS), Nurses' Health Study (NHS), and Nurses' Health Study II (NHS II). From 1986 to 2006, these associations were examined within multiple 4-year time interval adjusting for simultaneous changes in other lifestyle factors including other aspects of diet, smoking status, and physical activity. Results were combined using a fixed effects meta-analysis.

Results: Increased consumption of all flavonoid sub-classes was inversely associated with weight change over 4-year time intervals. The greatest magnitude of association was observed for anthocyanins (-0.52 lbs, 95% CI -0.74 to -0.30 lbs per additional SD/day, 22 mg), flavones (-0.32 lbs, 95% CI -0.47 to -0.17 lbs per additional SD/day

1.9 mg), and flavonols (-0.28 lbs, 95% CI -0.44 to -0.12 lbs per additional SD/day, 1 mg).

Conclusion: These data may help to refine previous dietary recommendations for the prevention of obesity and its potential sequelae.

Financial support: NIH, the Danish Council for Strategic Research, and the Rose Traveling Fellowship.

P107
Sorghum flours fractions: In vitro and in vivo antioxidant potential

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Background: Sorghum grains present high in vitro antioxidant activity due to the presence of antocyannins, tannins and flavonoids. The presence of these compounds and their antioxidant activity could improve oxidative stress in vivo related to obesity.

Objective: This work aimed to evaluated antioxidant activity of whole sorghum flour (WF), decorticated sorghum flour (DF) and sorghum bran (SB) of the genotype SC 21 and their effects on antioxidant capacity in diet-induced obese rats.

Methods: Antioxidant activity was determined by DPPH and ORAC assays. Biological assay was carry out by 12 weeks with thirty *Wistar* rats divided in five groups: learn control (AIN-93M), high-fat and high-fructose diet (HFF), HFF added with WF, DF and SB Sorghum diets were provided for 6 weeks. Plasma and liver antioxidant enzymes activity (GPx, GR, CAT and SOD) and GSH levels were determined. The limit of significance was set at $p<0.05$.

Results: SB showed the highest antioxidant activity while DF was the lowest by both assay. There was no difference on plasma CAT activity. GPx, GR and SOD levels were higher in AIN-93M. GSH level in DF and SB were lower than AIN-93M and did not differ from HFF. In liver CAT, SOD, GSH levels were higher in AIN-93M. GPx level was lower in HFF group and AIN-93M did not differ from WF and SB. GR levels were higher in sorghum flour fractions diets than in HFF and lower than AIN-93M.

Conclusion: Sorghum bran showed the highest antioxidant activity. Sorghum flours fractions did not affect plasma antioxidant enzymes levels. However, liver GPx and GR activities were improved.

Financial support: National Counsel of Technological and Scientific Development CNPq/Brazil.

P108
Higher polyphenol intakes are associated with a decreased risk of diabetes: study of the PREDIMED cohort.

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The increasing incidence of type 2 diabetes (T2D) is a challenging health problem. Most cases could be prevented by the adoption of healthier lifestyle, including healthier diets. A higher consumption of some polyphenols ha been associated with T2D due to the beneficial effects of these compounds on glucose homeostasis.

However, no studies have evaluated the relation between all polyphenol subclasses and the incidence of T2D.

We used data from the PREDIMED study (ISRCTN 35739639). This cohort include 7447 elderly participants at high cardiovascular risk. Polyphenol intake was calculated by matching food consumption data from yearly food frequency questionnaires with the Phenol explorer database on the polyphenol content of each reported food. Hazard rations and 95% confidence intervals between polyphenol intake and new-onset diabetes were estimated using time-dependent Cox proportional hazard models.

During a median of 3.65±1.93 years of follow-up (12545 person-years), a total of 31 incident cases of diabetes were diagnosed (9.1%) among the 3430 participants that were free of T2D at baseline. After adjusting for anthropometric, sociodemographic, lifestyle and dietary variables (fully-adjusted model), significant inverse associations were found for total polyphenols, lignans, stilbenes and dihydroflavonoids.

Those who reported a high polyphenol intake, and especially some classes, showed a reduced risk of T2D compared with those with lower intakes. These results could be useful to establish new dietary guidelines to prevent T2D.

Financial support: CICYT (AGL2013-49083-C3-1-R) from the Spanish Ministry of Science and Innovation (MICINN), the Generalitat de Catalunya (GC) 2014 SGR 773 and the Instituto de Salud Carlos III, ISCIII (CIBEROBN-CB06/03).

P109
Whole sorghum flour improves glucose tolerance test in diet-induced obese rats

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Background:Sorghum grains are rich in antocyannins, tannins and flavonoids and dietary fiber. These compounds could improve glucose and insulin metabolism in obese rat.

Objective: This work aimed to evaluated the effect of whole sorghum flour (WF), decorticated sorghum flour (DF) and sorghum bran (SB) of the genotype SC 21 on fasting glucose and insulin levels such as their effect on glucose tolerance test (GTT) and insulin tolerance test (ITT) in high fat-fructose diet-induced obese rats.

Methods: 40 Wistar rats, divided in five groups, were 12-wk feed: regular chow (AIN-93M), high-fat and high-fructose diet (HFF), HFF added with WF, DF and SB. Sorghum diets were provided for 6 weeks. GTT and ITT were done. Animals were decapitated and blood was collected to serum glucose and plasma insulin determination by commercial kit and radioimmuneassay, respectively. The islets were collected immediately after decapitation using the collagenase method. Insulin level was measured after 1 h of incubation in the presence of glucose at 2.8 and 16.7 mM. The limit of significance was set at $p<0.05$.

Results: Fasting glucose and insulin levels of sorghum flour fractions groups did not differ from both, AIN-93M and HFF. However, AIN-93M and WF groups showed lower area under the curve of GTT compared to HFF. The decay rate of glucose curve in kITT was higher in AIN-93M. Sorghum flour fractions groups did no differ from both, AIN-93M and HFF in static insulin secretion (16.7mM of glucose).

Conclusion: Sorghum flour fractions did not affect the fasting glucose and insulin levels and ITT. However, WF improves the GTT.

Financial support: National Counsel of Technological and Scientific Development CNPq/Brazil.

P110
Antioxidant and antidiabetic effects of polyphenols-rich extracts of Myrianthus arboreus

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Myrianthus arboreus is a small tree growing in tropical regions of Africa. Its root bar aqueous extract is traditionally used in Congo to treat diabetes. In order to evaluate the therapeutic potential of polyphenolic extracts from root bark of M. arboreus, the various polyphenols contents of its aqueous and ethanol extracts (EtOH) and the EtOH two fractions, ethyl acetate (EAc) and hexane, were determined. The total phenols (PT), flavonoids (Flv), hydroxycinnamic acids (HCA) and proanthocyanidin were determined by spectrophotometric methods. Both TP (331.5±2.5mg GAE/g) an HCA content (201±1.5mg CAE/g) were determined to be the highest in EAc fraction The results of the DPPH free radical and $ORAC_{Fl}$ assays showed no significant difference between the EAc fraction and commercial extract Oligopin®, while the EAc fraction had the highest antioxidant capacity by the phosphomolybdenium method (PM). The EtOH extract was determined to have the same antioxidant efficiency as Oligopin® by PM. The inhibition of glucose-6-phosphatase enzyme (G6Pase) and stimulation of glucose uptake were determined for all studied extracts, in cultured H4IIE hepatocytes and C2C12 myocytes respectively. For both assays, cells were treated at the maximum non-toxic concentration for 18 h. Insulin (100 nM served as the positive control for both assays. Metformin (400µM) was used as another positive control for the glucose uptake assay. The obtained results indicate that the EtOH extract and its two sub-fractions, especially the polyphenols richest EAc fraction, significantly decrease the G6Pase activity. Research is in progress to isolate and identify the antioxidant and antidiabetic components from the EtOH extract and its EAc fraction.

Financial Support: CRMR and INAF (Université Laval, Canada) /Society of Jesus (Jesuits of Congo)

P112

Green tea extract induces thermogenic changes and relieves the redox state by modulating parameters related to endoplasmic reticulum stress

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Background/Objective: There is a lack of information on how green tea (GT) extract modulates endoplasmic reticulum stress (ER stress), especially in adipose tissue (AT The aim of this study was to evaluate the effect of supplementation with GT on insulin resistance (IR), inflammation and ER stress in the AT of mice induced to obesity by a cafeteria diet (Ethic Committee nº 135/2013).

Methods/Results: Male C57Bl/6 mice (3 months) were divided into 4 groups: Contro (chow diet), GT (chow diet), Obese and Obese+GT. The mice were fed with cafeteria diet for 4 weeks. After this period, the animals received gavage with GT (500 mg/Kg of BW) over 12 weeks (5 days/week). After 16 weeks of experimentation, the mice were killed and AT was properly stored. The treatment with GT decreased fat depots improved insulin sensitivity (GTT and ITT) and increased GLUT4 expression which were modified by the cafeteria diet. In addition, GT increased the expression of thermogenic genes (PRDM16 and UCP-1), and modulated energy expenditure acutely, regardless the type of diet. Obese mice treated with GT decreased inflammatory response as assessed by expression of MCP-1, F4/80 and AP-1 and GSH/GSSG content. GT induced the transcription factor Foxp3 and GATA3 and increased GSH content. ER stress was induced by obese condition and GT was able to enhance BIP and reduce the expression of CHOP and PDI.

Conclusion: GT extract promoted a positive effect in the IR, inflammation and ER stress axis induced by obesity and may represent a good strategy to improve health.

Financial support: Fapesp (2012/20415-8).

P113

Effects of quercetin on uric acid metabolism

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High blood uric acid (hyperuricemia) is a common phenomenon in populations with hypertension, hyperglycemia, obesity and/or dyslipidemia. This study was to investigate the effects of quercetin supplementation on blood uric acid level and the biochemical mechanism behind it.

A pilot trial confirmed the delivery of quercetin from a supplement tablet in healthy males (n=6). Randomized, double-blind, crossover, placebo-controlled 4-week dietary intervention trial with the same supplement tablet containing 500 mg quercetin d^{-1} was conducted in selected healthy males (n=22, with higher blood uric acid but within normal range). Changes of uric acid and glucose were analysed in fasting blood plasma at 0, 2 and 4 weeks. Plasma metabolomics were profiled by ^{1}H-NMR. Where quercetin and its metabolites may affect in the pathway of uric acid metabolism was investigated in vitro and ex vivo.

At the end of the 4-week trial, plasma uric acid levels were significantly reduced (mean change -26.5 μM, 95% CI -45.5 to -7.5, P=.008, n=22), as were diastolic blood pressures in normotensive subjects (-3.1 mm Hg, -5.8 to -0.5, P=.048, n=10). Paired plasma ^{1}H-NMR spectrum showed lowered glutamine (P=.008), acetoacetate (P=.005) and lactate (P=.03) after quercetin treatment. A dose-dependent inhibition o quercetin, quercetin-3'-O-sulfate and 3,4-dihydroxyphenylacetic acid on xanthine oxidase was found in vitro and a mild inhibitory effect of quercetin on plasma adenosine deaminase.

Quercetin supplementation can maintain blood uric acid level and blood pressure within a low-risk range. It is probably a result of regulated purine metabolism by quercetin, its microbial derivatives and their metabolites.

Financial support: Scholarship of China Scholarship Council and University of Leeds

P114
The effect of the pulp-oil and avocados leaves tea in development of rats.

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MUFA and polyphenols, present in avocado oil and avocado leaves tea are reported to reduce some negative effects from obesity. This study aimed to determine the effect of Hass avocado pulp oil and avocado leaves tea treatment on body weight of Wistar male rats.

Seven first weeks, the animals were divided between 2 groups: Standard (S) (n=4) and high fat (HF) (n=15). The treatment lasted five weeks. HF group was divided in three another groups (n=5, each). The first one kept the high fat diet (HF), and the second one was replaced by standard diet plus avocado oil with 2% omega-9 (TO) instead of high fat diet. For the last one, water was replaced by avocado leaves tea with standard diet (TC). During the experiment, the rats was weighted once a week. The hydric and dietary consumption were monitored two and three times a week, respectively. There was a statistic difference in animals' growth between the sixth and seventh weeks in the S and HF groups ($p<0,05$), However in treatment period the animals did not show distinction. About the weight gain, TO and S group decreased in 15% and 9% respectively, while TC group increase 14 %, when compares with HF. After the eighth week, the animals received avocado oil (TO) and tea (TC) differed statistically from HF group ($p<0,05$). The total TO and TC groups consumption showed statistically difference than the S group, but not in the HF group ($p<0,05$). In the fourth first treatment weeks, there were significant difference consumption of tea ($p<0,05$) when S and HF groups were compared.

Just the avocado oil was effective compared to the avocado leaves tea to reduce weight gain, not compromising the animals growth.

Financial support: Conselho Nacional de Desenvolvimento Científico e Teconológico (CNPq) (protocol nº 3159-1) and Jaguacy Brasil company to donate the oil and leaves.

P115
Effect oil pulp and tea leaves of avocado Hass associated with swimming exercise in rats.

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Bioactive compounds, as polyphenols, and exercise can modulate weight gain. We investigated the effect of the treatment with 2% omega-9 from Hass avocado pulp oil or avocado leaves tea plus swimming on body weight of male Wistar rats.

During the first seven weeks, the animals were divided into 2 groups: Standard (SE) (n=4) and high fat (HE) (n=15). After this period the treatment was started for five weeks: exchange of high fat diet for standard diet and/or addition of bioactive compounds and swimming for 2 minutes/days in the last two weeks. HE group was divided into 4 groups (n=5). The first remained with the high-fat diet plus exercise (HE); for the other, high fat diet was changed to standard diet plus exercise (HTE), the TEO group received avocado oil-pulp with 2% omega-9, and for TEC group water was replaced by avocado leaves tea. During the experiment the rats was weighed once a week. The hydric and dietary consumption were monitored two and three times a week, respectively. As for growth, HE group had the lowest growth compared to the others ($p <0.05$) in all weeks. From the 8th week, HTE and TEO groups showed the same growth as the SE, but TEC group showed 35% of differed from HE at 8 until 10 week ($p <0.05$).The groups SE, TEO and TEC had a reduction in weight gain, 16% , 9% and 11%, respectively and only the HTE group showed 6% weight gain in comparison of the group HE ($p <0.05$).TEO consumed an average 45 more diet in all weeks in relation to HE group ($p <0.05$). There was no significant difference of tea consumption ($p <0.05$). Oil pulp and tea leaves reduced weight gain when associated to physical exercise.

Financial support: Conselho Nacional de Desenvolvimento Científico e Teconológico (CNPq) (protocol nº 3159-1) and Jaguacy Brasil company to donate the oil and leaves.

P116

Protective effect of polyphenol-rich extract from Antirhea borbonica medicinal plant on adipose cells exposed to Porphyromonas gingivalis LPS endotoxin

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Background/Objective: Obesity induces oxidative stress and chronic inflammation in adipose cells, leading to insulin resistance and type 2 diabetes. Several factors including lipopolysaccharide (LPS) endotoxin from gut microbiota could be involve Periodontal disease may also represent a risk factor for type 2 diabetes, and has been associated with obesity. The biological properties of plant polyphenols, including antioxidant, anti-bacterial and anti-inflammatory effects, are of high interest. Here, we evaluated the impact of LPS from *Porphyromonas gingivalis* (a major periodonta bacteria) on adipocytes and the potential protective effect of polyphenols from *Antirhea borbonica*, a medicinal plant from Reunion Island.

Methods: Polyphenol-rich extract from *Antirhea borbonica* was analyzed by UPLC-MS method. Then, the effect of polyphenols on 3T3-L1 adipose cells exposed to *P. gingivalis* LPS was determined by measuring cell viability (MTT assay), ROS production (DCFH-DA test), adipokine secretion (ELISA), and expression of genes coding for antioxidant enzymes and key factors involved in inflammatory signaling pathways (RT-qPCR).

Results: *Antirhea borbonica* exhibited a high level (4%, w/w) of polyphenols comprising chlorogenic acids, dicaffeoylquinic acids and kaempferol. Plant polyphenols limited the production of ROS and pro-inflammatory molecules such as IL-6 and MCP-1 induced by LPS in adipocytes. Several molecular targets have been investigated, including TLR2 innate immunity receptor, NFB transcription factor as well as superoxide dismutase and catalase antioxidant enzymes.

Conclusion: This study showed that *Antirhea borbonica* polyphenols prevented oxidative stress and inflammation mediated by periodontal bacteria LPS in adipose cells. It should be of interest to assess their impact on obesity-related insulin resistance.

Financial support: French Ministry of Education and Research

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Curcuma longa polyphenols improve insulin-mediated lipid accumulation and inflammatory response of 3T3-L1 adipose cells exposed to oxidative stress

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Background/Objective: Obesity induces an over-production of reactive oxygen species (ROS) such as H_2O_2, leading to oxidative stress and inflammation in adipose cells responsible for fat storage. Such disorders contribute to insulin resistance and type 2 diabetes. Several studies have reported health benefits of polyphenols like curcumin from *Curcuma longa* spice. However, there is still a lack of data regarding the impact of polyphenol-rich extract from Curcuma longa on adipose cells. Here, w aimed to evaluate the effect of polyphenol-rich extract from *Curcuma longa* produce in France on 3T3-L1 adipose cells exposed to H_2O_2.

Methods: Polyphenol-rich extract from *Curcuma longa* was analyzed by UPLC-ESI-MS method. Then, we determined the impact of polyphenols on adipocyte differentiation and lipid accumulation (Oil Red O staining), ROS production (DCFH-DA assay), adipokines' secretion (ELISA), and the expression of genes coding for key transcription factors and antioxidant enzymes (RT-qPCR).

Results: *Curcuma longa* contained a high level (6%, w/w) of polyphenols identified as curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcuminoid-rich extract protected cells against H_2O_2-induced ROS production and improved insulin-mediated lipid accumulation. Moreover, it reduced the production of pro-inflammatory mediators including IL-6, TNF-, MCP-1 and NFB. Such effects were associated with an up-regulated expression of genes coding for superoxide dismutase and catalase antioxidant enzymes, as well as PPAR and adiponectin know as insulin-sensitizing factors.

Conclusion: This study led to identify French *Curcuma longa* as a relevant source or polyphenols that improve oxidative stress and inflammation in adipose cells. It will be important to assess *in vivo* effects against obesity and related insulin resistance.

Financial support: French Ministry of Education and Research, and the Région Réunion

P118

Green Tea Extract Restores Obesity-Related Dysfunction of White Adipose Tissue by Remodeling its Metabolism – a supposed AMPK-dependent mechanism

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Beneficial effects of green tea polyphenols against obesity have been reported. However, until this moment the molecular mechanisms of how green tea can modulate obesity and regulates fat metabolism, particularly in adipose tissue, remain poorly understood. The purpose of this study was to evaluate the role of green tea extract in the adipose tissue of obese animals and its effect on weight gain, metabolism and function (de novo lipogenesis and lipolysis) and the involvement of AMP-activated protein kinase (AMPK) activation. Here, we show that obese-induce rats with cafeteria diet for 2 months that were treated with a green tea extract (500 mg/ kg of body weight) for 60 days, showed a significant reduction in indicators of obesity such as hyperlipidemia, fat synthesis, body weight and fat depots through activation of AMPK as compared with control standard diet. AMPK was induced in adipose tissue of animals that were treated with green tea and restored insulin sensitivity, increased mRNA expression of GLUT4, reducing the concentrations of plasma and liver lipid content, also stimulating fatty acid oxidation in the adipose tissue. Importantly, AMPK represses de novo lipogenesis in adipose tissue, but also reverted lipid droplets in the liver and the development of insulin resistance in

diet-induced obese rats. Our study identified that metabolic changes caused by green tea intake, induced AMPK-activity which, in turn, modulates the expression of genes involved in metabolism, particularly in adipose tissue, thus offering a therapeutic strategy to combat insulin resistance, dyslipidemia and obesity.

Financial support: FAPESP (2011/19216-8) and Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq (139307/2012-5)

P119

Anti-inflammatory effects of polyphenol-rich Araça (Psidium Cattleianum Sabine) extract on macrophages J774.

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Phenolic compounds are molecules with potential anti-inflammatory and antioxidant effects that neutralize free radicals. Researches have shown the benefits of phenolic compounds rich diets, preventing or ameliorating damages of metabolic diseases, such as obesity, diabetes and insulin resistance. Araçá (Psidium cattleianum Sabine) is a native Brazilian fruit which has significant amounts of polyphenols. This study aimed to demonstrate an anti-inflammatory effect of the polyphenol-rich Araçá extract (PAE) in vitro.

Macrophages J774 were treated with different concentrations (100, 500 or 1000 g/mL) of PAE in the absence or presence of 1 g/mL lipopolysaccharide (LPS) during 24 hours. The following techniques were used to reach our objectives: High Performance Liquid Chromatography (HPLC), flow cytometer, Griess method and Western Blotting.

Cellular viability was not reduced at any concentration. The nitric oxide (NO) production was significantly reduced by PAE at 100 g/mL, as measured by Griess method. This result correlates with the phosphorylation of p38 and the decreased expression of inducible nitric oxide synthase (iNOS). LPS-stimulated macrophages were treated with PAE, rutin (16.7 nM), ellagic acid (43.35 nM), catechin (264 nM) or polyphenol equimolar mixture at concentration equivalent to 100 g/mL treatment. Catechin was able to decrease NO production, p38 and iNOS; rutin increased proinflammatory actions and the polyphenol mixture was able to reproduce the effects of PAE in all measured parameters.

Our results support the benefits of catechin-containing foods and nutraceuticals on inflammatory status. Therefore, the polyphenol-rich extract of Araçá could be considered as a putative nutraceutical to prevent and treat metabolic diseases.

Financial support: Fapesp / CNPq

P120

Chalcones suppress differentiation of preadiopocytes to adipocytes via AMPK and MAPK pathways

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Adipocytes differentiation is involved in the onset of obesity through accumulation of fat in adipose tissue. In this study, we searched for polyphenols capable of inhibiting lipid accumulation in 3T3-L1 cells and attempted to clarify the underlying molecular mechanism. Among 50 polyphenols tested, 7 compounds including four chalcones namely 4-hydroxyderricin, xanthoangelol, cardamonin and flavokawain B were effective for inhibition of lipid accumulation in 3T3-L1 cells without cytotoxic effect at a concentration of 5M. The inhibition of accumulation accompanied by

down-expression of adipocyte-specific transcription factors,

CCAAT/enhancer-binding protein- (C/EBP-), C/EBP-, and peroxisome proliferator-activated receptor (PPAR-). To obtain insights into the underlying mechanism, the activation of AMP-activated protein kinase (AMPK) and mitogen-activated protein kinase (MAPK) pathways was investigated.

4-Hydroxyderricin and xanthoangelol promoted phosphorylation of AMPK and acetyl CoA carboxylase during differentiation of 3T3-L1 adipocytes accompanied by down-expression of glycerol-3-phosphate acyl transferase-1 and up-expression of carnitine palmitoyltransferase-1. These chalcones also promoted phosphorylation of extracellular signal-regulated kinase (ERK) and Jun aminoterminal kinase (JNK), but not p38. Moreover, the inhibitors for AMPK and ERK abolished the chalcones-caused down-expression of C/EBP-, C/EBP-, and PPAR-. An inhibitor for JNK also abolished the down-expression of C/EBP- and PPAR-, but not C/EBP-. In the case of cardamonin and flavokawain B, they also increased phosphorylation of ERK, and the inhibitors for ERK restored expression of C/EBP- and PPAR-. In conclusion, chalcones 4-Hydroxyderricin, xanthoangelol, cardamonin and flavokawain B inhibit adipocytes differentiation through the activation of AMPK and MAPK pathways, resulting in the down-expression of adipocyte-specific transcription factors.

Financial Support: This study was partly supported by JSPS KAKENHI Grant Number 25292072 and Coordination Funds for Promoting Science and Technology, Creation of Innovation Centers for Advanced Interdisciplinary Research Areas (Innovative Bioproduction Kobe), MEXT, Japan.

P121

Effects of phenolic compounds from Araça (Psidium cattleianum Sabine) on proteins activated upon oxidative stress-induced cell death in pancreatic beta

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The development and complications of diabetes involve increased oxidative stress and inflammatory activity in several tissues, especially in pancreatic beta cells.

Studies have shown protective effects of phenolic compounds present in the diet, through mechanisms that modulate inflammatory signaling and antioxidant activity. The purpose of this work was to study the effects of phenolic extract from Araçá (*Psidium cattleianum* Sabine) on oxidative stress-induced death in pancreatic beta cells (INS-1E).

INS-1E were treated with purified extract in 3 concentrations (100, 500 or 1000 g/mL) for 1 or 24 hours. We performed Flow cytometry, Western Blotting and High Performance Liquid Chromatography (HPLC) to effort our hypothesis. Catechin, rutin, ellagic acid were found (7.5: 1: 1.3). Regarding DNA fragmentation and membrane integrity, only the concentration of 1000 g/mL was significantly toxic. Cells previously treated with extract at 100 g/mL for 24h were protected from DNA fragmentation induced by short incubation with H_2O_2 (40M), a well-known reactive oxygen species (ROS). Reduced JNK phosphorylation was also observed in the same treatment. The treatment with extract at 100 g/mL increased phosphorylation of ERK1/2 and AKT1. The treatment with catechin at equimolar concentration used in the 100 g/mL extract reduced H_2O_2–induced DNA fragmentation, while rutin, similarly at equimolar concentration, potentiated the mentioned H_2O_2 effect.

The results point out to a protective effect of Araçá extract against oxidative stress in pancreatic beta cells, through activation of ERK1/2 and AKT signaling. This study *in vitro* confirms Araçá extract as a potent nutraceutical extract to treat and prevent diabetes.

Financial support: FAPESP, CNPq/PIBIC

P122

Green tea extract changes the profile of inflammatory cytokine release from lymphocytes of obese and lean rats and protects against oxidative damage

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This study aimed to investigate whether green tea extract (GT) modulates some functional parameters of lymphocytes from obese rats. Male Wistar rats were treated with GT by gavage (12 weeks/5 days/week; 500 mg/kg of body weight) and obesity was induced by cafeteria diet (8 weeks). Lymphocytes were obtained from mesenteric lymph nodes for analyses. In response to the cafeteria diet, activity of the metabolic enzyme hexokinase was increased, together with ROS production, which was accompanied by a increases in MnSOD, CuZnSOD and GR enzyme activities. Proliferation capacity of cells at baseline was increased due obesity, whereas IL-10 production was decreased. Obese rats treated with GT showed decreased cell proliferation under ConA stimulation, hexokinase and G6PDH activity, ROS production and MnSOD, CuZnSOD, GPx and GR enzymes remained increased, accompanied by an increase in Nrf2 mRNA level. Regarding cytokine release, decreases in pro-inflammatory IL-2, IL-6, IL-1, TNF– were accompanied by a decrease in the mRNA level of TRL4 and restoration of IL-10 production in obese rats treated with GT. GT treatment of lean rats showed similar results to that of obese rats, thereby indicating that the effects of GT are independent of diet. Foxp3 and IRF4 mRNA levels were increased by GT. In conclusion, cafeteria diet modulated lymphocyte function, mainly by increasing ROS production and decreasing anti-inflammatory IL-10, which could contribute to the inflammatory stat in obesity. GT was efficient at reducing ROS production, improving the redox status and reducing pro-inflammatory cytokine production by lymphocytes, suggesting that GT treatment may be driving lymphocytes to a more anti-inflammatory than pro-inflammatory microenviroment.

Financial support: FAPESP (2011/19216-8)

P123

Anti-inflammatory effects of polyphenol-rich Araça (Psidium cattleianum Sabine) extract on macrophages J774.

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Phenolic compounds are molecules with potential anti-inflammatory and antioxidant effects that neutralize free radicals. Researches have shown the benefits o phenolic compounds rich diets, preventing or ameliorating damages of metabolic diseases, such as obesity, diabetes and insulin resistance. Araçá (*Psidium cattleianum* Sabine) is a native Brazilian fruit which has significant amounts of polyphenols. This study aimed to demonstrate an anti-inflammatory effect of the polyphenol-rich Araçá extract (PAE) *in vitro*.

Macrophages J774 were treated with different concentrations (100, 500 or 1000 g/mL) of PAE in the absence or presence of 1 g/mL lipopolysaccharide (LPS) during 24 hours. The following techniques were used to reach our objectives: High Performance Liquid Chromatography (HPLC), flow cytometer, Griess method and Western Blotting.

Cellular viability was not reduced at any concentration. The nitric oxide (NO) production was significantly reduced by PAE at 100 g/mL, as measured by Griess method. This result correlates with the phosphorylation of p38 and the decreased expression of inducible nitric oxide synthase (iNOS). LPS-stimulated macrophages were treated with PAE, rutin (16.7 nM), ellagic acid (43.35 nM), catechin (264 nM) or polyphenol equimolar mixture at concentration equivalent to 100 g/mL treatment. Catechin was able to decrease NO production, p38 and iNOS; rutin increased proinflammatory actions and the polyphenol mixture was able to reproduce the effects of PAE in all measured parameters.

Our results support the benefits of catechin-containing foods and nutraceuticals on inflammatory status. Therefore, the polyphenol-rich extract of Araçá could be considered as a putative nutraceutical to prevent and treat metabolic diseases.

Financial support: Fapesp / CNPq

P125

Polyphenols have a highly significant effect on postprandial glucose; a randomized, controlled, single blind crossover intervention.

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We tested the hypothesis that dietary polyphenols with the ability to inhibit carbohydrate digesting enzymes and glucose transporters in vitro would attenuate post-prandial blood glucose in healthy volunteers. We designed a polyphenol rich food mixture (PRM) consisting of green tea, blackcurrant, blackberry, strawberry a green tea; constituent polyphenols were analysed by HPLC and total polyphenols by Folin (gallic acid equivalents, GAE).

The polyphenols from the PRM were first assessed in vitro. Green tea (EGCG 19.6, EGC 11.9, ECG 3.3 and EC 2.1 %) gave IC_{50} values of 14, 1000 and 1500 µg GAE/mL for maltase, iso-maltase and sucrose inhibition respectively. The IC_{50} values for -amylase inhibition using amylose as substrate were 4, 35, 55 and 75 µg GAE/mL for green tea, blackberry, blackcurrant and strawberry respectively. Apple and strawberry polyphenols have previously been shown to inhibit glucose transport across Caco-2 cells.

A randomized, controlled, single-blind intervention study was conducted on 16 healthy volunteers to compare glycemic response after a reference meal (white bread compared to test samples (2 doses of PRM with white bread). Plasma incremental area under the glucose (hexokinase) curve was calculated using the trapezoid rule. The lower dose gave a decrease in blood glucose AUC of 27.4±7.52 % (mean ± SD; $p<0.0001$) and the higher dose of 49.0±15.3 % ($p<0.0000001$). We conclude that dietary polyphenols have a pronounced and highly significant effect on postprandial blood glucose response in humans, and that a panel of in vitro assays can be used to predict effects in humans in vivo.

Financial support: Commonwealth Scholarship Commission, UK.

P126

Effect of soy isoflavone extract bioconverted by free beta glucosidase from Aspergillus niger LBA 02 and immobilized in Lentikats® and sol-gel in adipocyte differentiation

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In recent decades, the prevalence of obesity increased and turned a significant social and economic challenge. The scientific studies already established that phytochemicals may be beneficial in reducing the risk of obesity. Genistin and daidzin, are found in soy in major amount however the glycosidic form isn't well absorbed by our body.

The aim of this work was bioconverted soy isoflavones to achieve larger amount of the aglycone form by three different methods, using free beta glucosidase, immobilized in Lentikats® and sol-gel. Then we examined the effects of bioconverte extracts on adipocyte differentiation. The 3T3-L1 cells were treated with the extracts before differentiation and in the mature cells, the lipid accumulation was measured by oil red staining.

Our results indicate that the extracts did not significantly affected cell viability in the concentrations tested. Therefore, 0.1 – 1.0 mg/mL was used in al experiments. We found that when the cells were treated before differentiation the extracts did not affect significantly the lipid accumulation compared with the control group. However the extract bioconverted with the enzyme immobilized in sol-gel inhibited adipogenesis in 20 % at 0.4 mg/mL concentration. In the cells treated after differentiation with the extracted bioconverted by enzymes immobilized in Lentikats® and sol-gel, the lipid accumulation decreased in lower concentrations (0.1; 0.2 mg/mL) and adipogenesis was stimulated mainly at 1.0 mg/mL compared with the control group. In the extract bioconverted with free enzyme the lipid accumulation increased in all concentrations. These results suggest that the bioconversion method could influence in adipogenesis.

Financial support: Fapesp

P128

Polyphenol intakes in healthy men and women following current UK dietary guidelines compared to the average UK diet: the CRESSIDA study

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Dietary guidelines recommend increased consumption of plant-based foods. Adherence should increase dietary intakes of phenolic compounds that may have favourable effects on health.

We investigated whether a free-living population following advice to adhere to current UK dietary guidelines (DG) increased polyphenol intakes compared to an average UK diet (control). CRESSIDA (ISRCTN92382106) was a parallel-designed, randomized, controlled trial in healthy men and women aged 40-70 y. Dietary polyphenol intake was quantified from 322 x 4-day food diaries at baseline and endpoint of the 12-wk intervention (n=161; 64 M, 97 F). A polyphenol food composition database was created using Phenol-Explorer and USDA databases, and extended using recipes and polyphenol retention factors.

At baseline, median total polyphenol intake was 1.19 g/d (IQR 0.86). At endpoint, dietary intakes were increased following DG ($P = 0.033$): DG, median 1.29 g/d, IQR 1.13; control, median 0.98 g/d, IQR 0.98. The difference was no longer significant when adjusted for baseline total polyphenol intake (ANCOVA estimated marginal mean difference in total polyphenol intake was 4.7% greater following DG, 95% CI -9.2, 18.7). ANCOVA analysis of polyphenol subclasses and individual polyphenols revealed higher intakes of cyanidin, malvidin, flavanones, isoflavonoids, lignans and some phenolic acids ($P < 0.05$) following 12-wk DG compared to control.

Results demonstrate that following advice to conform to current UK dietary guidelines does not increase total polyphenol intake when adjusted for baseline intakes, but does increase intake of specific polyphenols that may confer cardiometabolic protective effects.

Financial support: UK Food Standards Agency/Department of Health

P129

Polyphenols from cambuci (Campomanesia phaea O. Berg) fruit attenuate glucose intolerance and adipose tissue inflammation induced by a high-fat, high-sucrose diet

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Elevated intake of certain dietary components, such as polyphenols of fruits and vegetables, reduces the risk of developing obesity and other metabolic diseases characterized by a chronic, low-grade inflammation. Cambuci (Campomanesia phae

O. Berg) is a Brazilian Atlantic Coastal Forest fruit rich in polyphenols that were previously shown to display anti-inflammatory actions. Herein we investigated whether the administration of polyphenols-rich extract from cambuci (CBC) at two different doses protects mice from diet induced obesity, insulin resistance, and chronic peripheral inflammation. In order to verify this, C57BL/6J mice fed either with a chow or a high-fat, high-sucrose (HFHS) diet were daily treated by gavage with water or CBC (18 and 32 mg gallic acid equivalent, GAE/kg body weight) for 8 weeks and evaluated for body mass, adiposity, glucose tolerance, and adipose tissue inflammation. Despite the absence of changes in body weight gain and adiposity, administration of CBC at two different doses protected mice from the disarrangements in glucose homeostasis induced by HFHS feeding as evidenced by the reduced fasting glycemia and insulinemia and improved glucose tolerance featured by CBC treated mice. Along with glucose homeostasis, CBC administration also protected mice from adipose tissue inflammation induced by HFHS feeding as evidenced by the lower expression of proinflammatory cytokines tumor necrosis factor-alpha (TNF-) and interleukin-6 (IL-6) and macrophage markers CD11b, F4/80 and CD86 in retroperitoneal adipose tissue. Furthermore, the higher dose of CBC increased plasma HDL-cholesterol while reducing LDL-cholesterol levels. Altogether, our findings indicate that polyphenols from cambuci have potentially beneficial actions protecting from HFHS diet-induced glucose intolerance and adipose tissue inflammation.

Financial support: São Paulo Research Foundation (FAPESP 2013/19797-6 and 2013/50394-5) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PEC-PG 5554-11-0 and CAPES/DFAIT 007/2013)

P130

Effect of fruit juices from Myrtaceae family on postprandial glycemia in individuals with metabolic syndrome and dysglycemia

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Incidence of Metabolic Syndrome and type 2 diabetes has taken epidemic proportion in recent years. Accordingly, approaches to control and/or treat these diseases, become relevant to mitigate their impacts on public health, considering the low adherence to therapy. Some native Brazilian fruits can be considered excellent sources of phenolics. Among them, phenolics found in fruits of the Myrtaceae family are able to inhibit the enzymes of carbohydrate metabolism, a-amylase and a-glucosidase, and the juices are capable of reducing postprandial glucose levels by about 20 to 60% in healthy subjects.

The aim of this work was to extend the previous study evaluating the effect of cagait (Eugenia dysenterica DC) fruit juices, on postprandial glucose and insulin in pre-diabetic human patients after consumption of white bread (about 50 g). Three different meals were consumed by volunteers. The first one consisted of white bread (50 g) plus water (300 mL) as a control; the second one, white bread (50 g) plus clarified cagaita fruit juice (300 mL), and the last one white bread (50 g) plus non-clarified cagaita fruit juice (300 mL). The results showed that both cagaita fruit juices reduced the total amount of glucose absorbed (AUC) by 56% (clarified juice) and 71% (non-clarified) and insulin released by 59% (clarified juice) and 69% (non-clarified). Although glucose incremental velocity (GIV) did not show significant differences, absolute increase of glucose (AIg), glucose incremental percentage (GIP and peak values of glucose (GPV) and insulin (IPV) were significantly lower than those of control ($p < 0.05$).

Financial support: FAPESP

P131

Consumption of acai beverage (Euterpe oleracea Mart.) in metabolically challenged individuals

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Acai polyphenolics (*Euterpe oleracea* Mart.) have been reported to have anti-inflammatory properties both in vitro and in short-term clinical studies. However the longer-term effects of acai consumption have not yet been investigated. The objective of this study was to determine the anti-inflammatory activities of acai consumption on levels of inflammatory risk markers in individuals with metabolic syndrome. Volunteers consumed 2 x 10 oz/day acai beverage (n=18) or placebo (n=19) for 12 weeks. After 0, 4, 8, and 12 weeks, measurements of body composition dietary intake, and blood and urine samples were collected. Participants in the acai beverage group experienced changes after 12 weeks in TNF- (Control 16.87 ± 31.78 pg/ml; Acai -3.83 ± 22.00, p=0.0651) and IFN- (Control -1.61 ± 5.03; Acai -6.68 ± 6.03, p=0.0308). Selected biochemical parameters in blood and urine were analyzed by ANCOVA with body composition and nutritional intake as covariates revealed changes: Insulin (-14.4559 ± 7.6974 ulU/ml, p=0.0758), C-peptide (-1650.78 ±762.19 pg/ml, p=0.0458), hs-CRP (-3.08 ± 0.85 mg/L, p=0.002) and IL-6 (-28.5178 9.072 pg/mL, p=0.0807) were decreased, and resistin (2280.06 ± 1059.18 pg/ml, p=0.0479) was increased by acai beverage intake compared to the control group. Results indicate that acai beverage improved the levels of pro-inflammatory markers compared to the control group. These results suggest that the consumption of acai beverage may be beneficial in the mitigation of risk factors relevant to the metabolic syndrome.

P132

Effect of polyphenols from Eugenia dysenterica DC fruit juice on postprandial glycemia in subjects with metabolic syndrome and dysglicemia

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Brazil has several native fruits, some of them are considered potential sources of phenolic compounds. Cagaita (*Eugenia dysenterica* DC) is a native fruit from Cerrado region belonging to the *Myrtaceae* family. Some polyphenols presents in fruits of this family may be able to act on inhibition of enzymes related to carbohydrates such as and -glucosidase in vitro. The inhibition of those enzymes activities delays blood glucose absorption, thereby reducing postprandial glycemia and insulinemia. This study aimed to assess the effect of cagaita fruit juices, clarified and non-clarified, rich in phenolic compounds including ellagitannins and proanthocyanidins, on the postprandial blood glucose and insulin responses from a bread meal (50 g), in prediabetic humans who were not taking medications known to influence glucose or insulin metabolism. Three different meals were consumed by volunteers (n=14). The first one consisted of white bread (50 g) plus water (300 mL) as a control; the second one, white bread (50 g) plus clarified cagaita juice (300 mL), and the last one white bread (50 g) plus non-clarified cagaita juice (300 mL). The results showed that both cagaita juices reduced the total amount of glucose absorbed (AUC) by 56% (clarified) and 71% (non-clarified) and insulin released by 59% (clarified) and 69% (non-clarified), after the ingestion of white bread. Although glucose incremental velocity (GIV) did not show significant differences, absolute increase of glucose (AIg), glucose incremental percentage (GIP) and peak values of glucose (GPV) and insulin (IPV) were significantly lower than those of control (p 0.05).

Financial support: CAPES; FAPESP

P133

(-)-Epicatechin improves hepatic insulin sensitivity and redox-signaling in in vitro and in vivo obesity models

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Background: Obesity is becoming a major health problem because of the associated pathologies, including the development of insulin resistance and type 2 diabetes (T2D). Our previous results showed that the flavan-3-ol (-)-epicatechin (EC), the most abundant flavonoid present in fruit and vegetables, improves inflammation and insulin sensitivity in adipocytes in culture and in high fructose-fed rats. **Objective:** To investigate the capacity of EC and its metabolites (ECM) to improve hepatic redox-signaling and insulin sensitivity in a mouse model of obesity-induced T2D and in human hepatocytes (HepG2 cells). **Material and methods**: Mice were fed a high fat diet (HFD) (60% total calories from fat) for 15 weeks developed obesity and insulin resistance. Underlying mechanisms were investigated in HepG2 cells incubated with palmitate (Pal) (0.25 mM), and in the absence/presence of EC and ECM (0.25-1 μM). **Results:** EC supplementation (20 mg EC/kg BW/d) mitigated HFD-induced alterations in the insulin pathway and in glucose homeostasis (ITT, GTT, fast/fed glucose and insulin). EC inhibited events involved in hepatic insulin resistance: oxidative stress, increased expression/activation of the superoxide anion ($O_2^{\cdot-}$) generating enzyme NADPH oxidase (NOX) (no changes in mitochondria $O_2^{\cdot-}$ production were associated to HFD), activation of redox-sensitive signals, and inflammation. In HepG2 cells, Pal caused an increased oxidant production, increased NOX expression/activity, and activation of redox-sensitive signals (JNK and IKK) known to down regulate the insulin cascade. EC and ECM partially/completely inhibited all these actions. **Conclusion:** EC and ECM improved obesity-associated insulin resistance in part through the modulation of redox-regulated events.

Consumption of EC-rich foods may provide benefits against obesity-induced T2D.

Financial support: USDS USA

P134
Antioxydant and antihyperlipidic activities of rhamnus alaternus polyphenol

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The effectiveness of Rhamnus Alaternus, an herb distributed along the Mediterranean, has been proven in traditional medicine. This plant is used as a purgative, laxative, diuretic and detoxifying antihypertensive. These applications led us to quantitatively analysis some of its bioactive components such as polyphenols and to study antioxidant and antihyperlipidemic activities of polyphenolic extract. Antioxidant activity was effected by DPPH and ABTS tests and antihyperlipidemia activity was tested on hyperlipidemia induced by a single dose of TritonWR-1339 (300 mg/kg) in Wistar rats. The results of this study indicate that Rhamnus alaternus polyphenolic extract has an anti-radical high efficiency and causes a good lipid-lowering effect where the most effective dose is 200mg/kg. The analysis of histological sections of the liver and aorta of rats with induced hyperlipidemia and treated with plant polyphenol extract shows a gradual disappearance of fat which is plant extract doses-dependent. The obtained results indicate that the bioactive constituents of Rhamnus alaternus have potential in reducing the outbreak of atherosclerosis and hepatic steatosis.

Financial support: national

P135
Fat reducing effect of wine lees

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The World Health Organization establishes the overweight and the obesity as the fift leading risk for global deaths, as they are the origin of CVD, diabetes, and some cancers. This reality demands the discovery of new bioactive compounds, and food by-products can be used as a viable source. Lees, polyphenol rich wine by-product formed at the bottom of wine containers, are assessed for their potential health benefits for the first time. Therefore, lees polyphenolic extract effectiveness is tested on zebrafish lipid metabolism.

Firstly, polyphenolic extract was extracted from lees, and its toxicity was evaluated to establish non-toxic doses. Afterwards, fat deposit of embryos was stained with Nile Red and the reduction of fluorescence emission after different dose and time expositions to the lees was measured. Later, metabolic rate, an indicator of energy expenditure, was individually analyzed. Finally the impact on the expression of gene implicated in lipid metabolism was studied.

Fat reduction effect was very pronounced (reached 40% compared to control) and was dose and time dependent. Furthermore, lees were able to alter expression of genes related with lipogenesis (FASN and SREBP), lipolysis (CPT1b) and lipid transport (MTP). Changes in metabolic rate could explain, at least in part, the oxidation of fat reserve.

Polyphenolic extract from wine lees is a potential ingredient for the development of new healthy products targeted to weight management therapies, and it deserves further research in zebrafish and higher animal models.

Financial Support: MC is recipient of a DAPA grant. JA is a recipient of an AZTI grant. This work has been supported by the Basque Government, Ministry for Economic Development and Competitiveness.

P136

Targeting mitochondrial biogenesis and function by dietary polyphenols as an approach to prevent non-alcoholic fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD) which affects 24% of the general population can progress to nonalcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma. Mitochondrial dysfunction and generation of reactive oxygen species (ROS) have recently been identified as important contributors to NAFLD and progression to NASH. We hypothesized that dietary polyphenols can help protect against mitochondrial dysfunction in NAFLD. In an in vitro model of NAFLD using cultured human HepG2 hepatocytes exposed to 1.5 mM oleic acid, we studied the effects of five dietary polyphenols (catechin, resveratrol, quercetin, berberine, cyanidin) on mitochondrial functions and ROS generation.

Cells were pre-treated with polyphenols (1, 5, 10 μM) for 2 h, exposed to oleic acid (1.5 mM) for 24h, and then evaluated for fat accumulation (Nile Red staining), intracellular ROS generation (using DCFH-DA), and relative mRNA expression (using RT-qPCR) of genes involved in mitochondrial biogenesis (SIRT1, NRF1, TFAM) and mitochondrial respiratory complex I-V subunits (NDUFS8, SDHb, UQCRC1, COX6B1, and ATP5G1).

Oleic acid significantly induced intracellular steatosis, ROS generation (127%), and decreased mitochondrial biogenesis and the expression of some of the mitochondrial respiratory complex subunits. Catechin, quercetin, and cyanidin significantly decreased intracellular lipid (33-34%), and all of the polyphenols significantly decreased oleic acid-induced ROS (by up to 77% at 10 μM). Although oleic acid decreased expression of mitochondrial biogenesis and respiratory complex genes, several polyphenols (catechin, quercetin, berberine, and resveratrol) prevented the decrease and in many cases increased it to significantly more than control cells.

Cyanidin was unique in although significantly decreasing oleic acid-induced ROS a steatosis, it had no effects on genes controlling mitochondrial biogenesis or respiratory complex expression. Together the data suggest that dietary polyphenols can prevent steatosis and ROS production in hepatocytes, at least in part by inducing mitochondrial biogenesis, and should be studied further as possible dietary treatments for NAFLD.

Financial support: Saskatchewan Health Research Foundation (SHRF)

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In-vivo glucose- and cholesterol- lowering effects and body weight gain control in mice fed a high fat diet supplemented with a Red Orange and LEmon EXtract (ROLEEX®)

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Citrus fruit are rich sources of high value bioactive compounds and their consumption has been associated to health-promoting effects on humans. Red oranges are particularly rich in anthocyanins (95% being represented by cyanidin-3-glucoside an cyanidin-3-6"-malonyl-glucoside), flavanones (hesperidin, narirutin, didymin) and hydroxycinnamic acids (caffeic, coumaric, sinapic, ferulic acids). Lemon fruit are also rich in flavanones (eriocitrin, hesperidin, diosmin) and other polyphenols. All these compounds present health benefits as dietary antioxidants for their capacity to scavenge free radicals. Anthocyanins have also been associated with beneficial effects against diabetic retinopathy and for the control of fat accumulation in mice fed a high fat diet. The extraction of bioactive compounds by reusing wastes of citrus processing industry is industrially quite useful, other than being a profitable and environmental-friendly process. Furthermore, there is at present a great interest in the development of new nutraceuticals of natural origin to be employed within the pharmaceutical field and/or as dietary supplements. The expertise of CRA-ACM have been used to develop a method for the recovery of anthocyanins and other polyphenols from red orange processing wastes and eriocitrin and other flavanones from lemon peel. Both the extracts have been quali-quantitatively characterized by UPLC-PDA-ESI/MS^n. Finally a standardized new extract called ROLEEX® (Red Orange and LEmon EXtract), obtained by properly mixing the red orange and the lemon extracts, have been powdered by spray-drying and conveniently used for in-vivo assays aimed at testing its glucose-and cholesterol-lowering activity and its role in the body weight gain control. In-vivo experiments performed in male CD1 mice fed with high fat diet have shown that a 8-week treatment with ROLEEX® is able to induce a significant reduction in glucose and cholesterol blood levels besides de-

termining positive results in the body weight gain control. These results suggests a possible use of the new phytoextract for nutraceutical purposes.

Financial support: National grant –POR FESR Sicilia 'Functional Food and nutraceutical supplements with bioactive compounds from citrus fruit and lupin-based ingredients' - ALIFUIDEA Project.

P138
Redox modulation on EA.hy926 cells by phenolic extract of Pleurotus albidus

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Diabetes mellitus (DM) is a chronic disease for which the prevalence has risen to epidemic proportions worldwide. It is characterized by elevated blood sugar levels. There is growing evidence that excess generation of reactive oxygen species due to hyperglycemia causes oxidative stress in a variety of tissues. Endothelial dysfunction underlies both micro- and macrovascular complications of diabetes. Studies have shown modulation of the hyperglycemic state by mushroom extracts. Pleurotus albidus is an edible mushroom recently described, so there is no biological redox studies yet. Therefore, the aim of this study was to evaluate the antioxidant activity of P. albidus through 2,2 diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays and in EA.hy92 endothelial cells exposed to hyperglycemic condition. It was observed that the extract presented high level of phenolic (742.80 ± 0.26 mg% of gallic acid equivalent) and flavonoid (471.25 ± 1.25 mg% of quercetin equivalent) contents. This agrees with DPPH (IC50 = 21.62 ± 1.02 mg of extract) and ABTS (12.47 ± 0.16 mg of extract) radical scavenger activity. P. albidus extract was able to reduce the lipid and protein oxidative damage and the imbalance in the activities of the antioxidant enzymes superoxide dismutase and catalase induced by high glucose levels (35mM) in EA.hy926 treated cells. An important find was the ability of the extract to avoid the inhibition of the electron transport chain found in EA. Hy926 cells treated with high levels of glucose. Although another studies are need, our data showed that P. albidus present promising anti diabetic activity through modulation of mitochondrial redox status.

Financial support: Fapergs, CAPES and CNPq.

P139
Functional properties of ellagitannins from pomegranate peels along the gastro-intestinal tract

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Pomegranate fruits (Punica granatum L.) are a rich source of polyphenols, mainly anthocyanins and ellagitannins (ETs), with demonstrated healthy properties. Pomegranate fruits are majorly processed by food industry to obtain the juice from the arils, while the pomegranate peels (PPe) are discarded. PPe are a nutritionally valuable by-product due their high content of ETs and dietary fiber. The aim of this study was to assess the bioaccessibility of ETs from PPe and their potential effects on digestive enzymes along the gastrointestinal tract (GIT).

To this purpose, PPe were submitted to a sequential *in vitro* enzymatic digestion. Soluble fractions from each digestion step were collected and the concentration of ETs was measured by HPLC/UV-VIS analysis. Soluble extracts from the salivary, gastric and intestinal steps were tested *in vitro* to assess their effects towards -glucosidase, -amylase and lipase activity.

Results showed that punicalins, punicalagins, castalagin and ellagic acid were the most abundant phenolic compounds released during gastrointestinal digestion. ETs-rich extracts released during digestion showed a strong inhibitory activity again -glucosidase and a weaker inhibitory activity against lipase and -amylase.

In conclusion, the data demonstrated that PPe may modulate post-prandial response at carbohydrates and fats in humans thus supporting its use as functional food ingredient. Future studies are warranted to evaluate the *in vivo* efficacy of PPe for energy intake and blood glucose control in humans.

Financial support: National fellowship

P140

Protocatechuic acid diminishes inflammation and improves insulin signalling in obese visceral adipose tissue

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An inverse association between polyphenol-rich food intake and incidence of chronic-degenerative diseases has been evidenced. The phenolic protocatechuic acid (PCA) has a great nutritional interest because it is recognized as a main metabolite o complex dietary polyphenols. We have previously demonstrated that PCA exerts insulin-like activities in human adipocytes. This work is aimed at defining the role of PCA in modulating insulin signalling and inflammatory status in visceral adipose tissue (VAT).

VAT biopsies were obtained from obese subject (BMI30 Kg/m^2) undergoing abdominal surgery for benign conditions. Total tissues or isolated adipocytes were incubated or not with 100 M PCA for 24 h. After incubation, control and PCA-treated tissues were acutely stimulated with insulin (20 nM, 20 min). Immunoblotting analysis was carried out to determine Tyr-IRS-1 and Ser-Akt phosphorylations in control and PCA-treated tissues, in basal conditions and after insulin stimulation. Furthermore, activated NFB was evaluated in control and PCA-treated VAT and isolated adipocytes. PCA-treated VAT, acutely stimulated with insulin, showed increased Tyr-IRS-1 and Ser-Akt phosphorylation compared to insulin-stimulated control VAT (two- and three-fold increase, respectively). Additionally, a reduction in NFB phosphorylation was observed after PCA treatment in both VAT and isolated adipocytes.

PCA improves the response of obese VAT to insulin by activating IRS-1 and Akt. Furthermore, PCA is able to diminish inflammation in isolated adipocytes and VAT. These results support the role of polyphenol-rich foods, key components of Mediterranean Diet, as important metabolic modulators by virtue of their ability to influence cellular pathways.

Financial support: Grant ISS/USA 11US/23

P141

Effects of grape consumption on gene expression of inflammatory mediators in peripheral blood mononuclear cells of obese individuals

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Obesity is a pro-inflammatory state involving activation of inflammatory mediators and increased oxidative stress. Polyphenols have been associated with protection against obesity-associated chronic diseases. We investigated whether consumption o polyphenol-rich whole grapes alters expression of genes related to inflammation and oxidative stress in peripheral blood mononuclear cells (PBMC) in obese individuals following 4 weeks of grape consumption and acutely following a high fat, high carbohydrate (HFHC) meal challenge. Using a crossover design, 20 obese subjects (BMI 37.0 ± 9.9) were randomized to consume either 60 g of freeze-dried whole grape powder (Grape; 297 mg polyphenols) or matched placebo powder (Control; 0 mg polyphenols) daily in 500 ml of water for 4 weeks separated by a 2-week washout. PBMC were isolated from fasted blood before and after each intervention. Meal challenge days consisted of a HFHC meal accompanied by Grape or Control consumption with PBMC isolated at time points 0, 1, 3, and 5 hr. Results show no changes in fasting expression of TNF, NF-B, IB, CREB, IL6 or Nrf2 following 4 weeks of either treatment. Following the HFHC meal, there were no significant differences between treatments, however effects of time were observed. Postprandial NF-B expression differed at each time point relative to baseline within Grape and Control treatments ($p<0.0001$). CREB and Nrf2 expression differed at 1 hr and 3 hr relative to baseline following Grape treatment ($p<0.05$). Results indicate activation o inflammatory and oxidative stress response following HFHC meal, however no differences between Grape and Control treatments were observed.

Financial support: California Table Grape Commission and USDA CRIS CA-S*-NTR-6316-H

P142

Epigallocatechin gallate and its metabolites promote glucose uptake through translocation of GLUT4 in muscle cells

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Epigallocatechin gallate (EGCG) is reported to possess anti-diabetic activity. We have reported that EGCG promotes translocation of glucose transporter type 4 (GLUT4) in both L6 myotubes and skeletal muscle of mice. However, underlying molecular mechanism is not fully understood, and there is no information about the effect of catechin metabolites on translocation of GLUT4. In this study, we searched for the effective catechin metabolites and investigated the mechanism of EGCg on GLUT4 translocation in L6 myotubes.

L6 myoblasts were differentiated to myotubes and treated with EGCG and catechin metabolites. Glucose uptake was measured using 2-deoxyglucose by an enzymatic method. Phosphorylation of proteins in insulin-signaling pathway and AMPK was evaluated by Western blotting.

EGCG increased glucose uptake in L6 myotubes expectedly. In addition to EGCG, several metabolites also increased glucose uptake. Of these, we focused on 5-(3',5'-dihydroxyphenyl)--valeroactone (BM6), because this compound is one of the major metabolites and found in the body after intake of EGCG. BM6 dose-dependently increased glucose uptake by 3 M accompanied by promoting translocation of GLUT4 in the plasma membrane of the cells. As the underlying molecular mechanism of GLUT4 translocation, EGCG induced PI3K, aPKC and AMPK phosphorylation without affecting IR and Akt phosphorylation. BM6 also showed almost the same mechanism as EGCG. In conclusion, BM6, the EGCG metabolite, has the potential to reduce postprandial blood glucose level by promoting GLUT4 translocation in skeletal muscle and may improve hyperglycemia in type 2 diabetes mellitus.

Financial support: This study was partly supported by JSPS KAKENHI Grant Number 2529207and Coordination Funds for Promoting Science and Technology, Creation of Innovation Centers for Advanced Interdisciplinary Research Areas (Innovative Bioproduction Kobe), MEXT, Japan.

P143

Grape-seed proanthocyanidins improve hepatic lipid metabolism in healthy rats by a concomitant increase in cellular NAD+ content and SIRT1 activity

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Proanthocyanidins (PAC) have been reported to modulate multiple targets controlling simultaneously many pivotal metabolic pathways in both liver and adipose tissue. However, the precise mechanism for the action of PAC on the regulation of genes controlling lipid metabolism in liver remain to be clarified.

Here, we use NMR and MS-based metabolomics to evaluate the hepatic changes induced by different doses of grape-seed PAC in liver of healthy rats. Furthermore, all these changes in metabolites were validated and complemented with additional gene-expression analysis of selected genes.

We report that PAC consumption during 21 days increased significantly the hepatic NAD+ content in a dose-dependent manner by specific inhibition of gene expression of major NAD+-consuming enzymes such as poly(ADP-ribose) polymerase 1 (PARP1) and cyclic ADP-ribose synthases (CD38). The hepatic metabolites of NAD+ cycle and the mRNA levels of those genes involved in NAD+ de novo biosynthesis pathway were also elevated. Notably, PAC consumption also up-regulated significantly the mRNA levels of hepatic SIRT1. The increase of both, NAD+ availability and Sirt1 gene expression, in turn, resulted in a hepatic increase o SIRT1 activity, which was associated significantly with an improved protection against hepatic lipid accumulation after an acute fat challenge.

Our data suggest that PAC consumption could be a valid tool to enhance hepatic SIRT1 activity through the modulation of NAD+ levels, and consequently, could be potentially therapeutic approach to prevent hepatic fat accumulation.

Financial support: grant number AGL 2008-00387/ALI and AGL2011-25831 from the Spanish Ministry of Economy and Competitiveness and b the Universitat Rovira i Virgili under grant number 2014LINE-07.

P144

Protocatechuic acid attenuates inflammation in differentiated THP-1 macrophages exposed to human visceral adipose tissue lipids

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Obesity is accompanied by low-grade inflammation and macrophage infiltration in visceral adipose tissue (VAT). Excessive body fat accumulation is associated with clinical manifestations, such as type 2 diabetes and cardiovascular disease. NLRP3 inflammasome activity is one of the major contributors to VAT inflammation and dysfunction during obesity. It has been reported that a diet rich in vegetables and fruits can improve health. Particularly, evidence showed that protocatechuic acid (PCA), a main metabolite of ACNs, might exert anti-inflammatory activity.

The present study was addressed to assess the anti-inflammatory activity of PCA on differentiated THP-1 monocytes treated with a lipid mixture derived from human VAT biopsies. We investigated the activation status of the inflammatory markers, NF-B, NLRP3 inflammasome, and IL-1 after lipid treatment, and the ability of PCA to counteract these effects.

THP-1 cells were differentiated with 40nM PMA for 24h and kept overnight in RPM 1640, then stimulated with 0,1% of lipids collected from human VAT biopsies in the presence or absence of 25μM PCA for 18h. VAT biopsies were obtained from overweight/obese subjects (BMI25) undergoing abdominal surgery for benign conditions. After the treatment, activated NF-B and NRLP3 were evaluated in whole cell lysates, and IL-1 in the supernatants by immunoblotting. We found that THP1 treatment with VAT lipids increases p-NF-B (+84%), NLRP3 (+56%) and IL-1 (+51 %) protein expressions with respect to untreated cells. PCA was able to completely prevent such events.

In conclusion, we suggest a possible role of PCA as anti-inflammatory compound representing a new therapeutic approach to reduce obesity-associated inflammation.

Financial support: ISS

P145

Acute anti-hyperglycaemic effects of standardized maqui berry extract Delphinol®

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Berry polyphenols, predominantly anthocyanins, are well described to improve blood sugar in humans. Maqui berry (Aristotelia chilensis) extract Delphinol® (provided by MNL Chile), standardized to bear 25% delphinidin anthocyanins, was previously described to inhibit SGLT activity in rat small intestine and demonstrated in humans to diminish post-prandial glucose following rice ingestion.

We describe acute Delphinol dose-response effects on post-prandial glucose in OGT with 36 pre-diabetic volunteers, men and women, aged 19-50 years. All subjects participated in four OGTT with minimum 8 days intermission between experiments. In each experiment fasting Blood was drawn, Delphinol taken, 60 minutes later blood was drawn, 75 g glucose ingested, then blood in 30 min intervals until two hours pas OGGT.

Unexpectedly we found that with dosages beginning at 60 mg single intake, the maqui extract significantly lowers fasting glucose and -insulin within 60 min. Following glucose challenge, postprandial blood glucose and -insulin were significantly lower with 60, 120 and 180 mg Delphinol after 30 minutes, as compare to same experiments carried out in absence of Delphinol. While post-prandial blood glucose is lowered with increasing amounts of Delphinol 30 minutes past OGTT, as compared to untreated control, blood sugar is higher at later time points until end of the observation 180 min past OGTT. Our findings point to novel blood sugar control mechanisms related to Delphinol.

Financial support: Corfo, Chile

P146
Understanding the anti-glyceamic activity of a polyphenol rich olive leaf extract

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Introduction: A six week chronic dietary intervention with olive leaf extract recently reported improvements in insulin sensitivity in overweight men; the mechanisms for this effect remain uncertain. We hypothesise that regulation of glucose and fat uptake in the gut may explain this observation.

Aims: We set out to investigate the effects of olive leaf extract on the activity of porcine pancreatic -amylase and lipase in vitro.

Methods: Olive leaf extract, at doses equivalent to those achievable in the gut with supplementation, and oleuropein, hydroxytyrosol, luteolin and rutin were used to inhibit the activity of -amylase relative to acarbose , and to inhibit lipase relative to orlistat.

Results: Olive leaf extract inhibits -amylase activity with an IC_{50} value of 1.749 ±0.176 mg/mL when compared with acarbose (IC_{50} value 0.051 ± 0.003 mg/mL). It also inhibits pancreatic lipase 0.82±0.02 mg/mL when compared with orlistat (IC_{50} value 0.6±0.01 mg/mL). In addition, the olive polyphenols oleuropein, hydroxytyrosol, luteolin and rutin individually showed an ability to inhibit –amylase and/or lipase.

Discussion/Conclusion: Elevated post-prandial plasma glucose, together with relate hyperlipidaemia is associated with morbidity. Our data add weight to growing evidence that olive leaf and its phenolics may be useful as supplements for the prevention of metabolic disease.

P147
The consumption of tea made of the Hass avocado leaves - instead of its pulp oil – le to the increase of enzyme markers of liver injury in rats fed with high-fat diet.

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The purpose of this research is to verify what is the effect of both tea of the Hass avocado leaves, Hass avocado pulp oil and exercise on the hepatic enzymes and on the lipid oxidation.

Animals were divided into 2 groups for seven weeks: Standard (SE) (n=4) and High-Fat 35% (HE) (n=15). After this period, a treatment of five weeks, in which the high-fat diet was replaced by a standard diet (AIN93M) and/or with addition of bioactive compounds and swimming exercises for 5 minutes/day on the last two weeks.

HE group was divided into 4 groups (n=5). First group was fed with high-fat diet plus exercises (HE). Second one had AIN93M diet plus exercises (HTE). TEO group had AIN93M diet plus avocado oil-pulp with 2% w-9. Lastly, TEC group, had AIN93M diet and water was replaced by tea made of avocado leaves (45g/l). Analysis of hepatic enzymes (AST/ALT) were performed using commercial kits and analyses of malondialdehyde by the TBARS method.

TEO group ingested 32±7,69 ml/day of tea. TEC has shown an increase of lipid oxidation when compared to the HTE and TEO groups ($p<0,05$), whilst there was no significant difference when compared to the HE group ($p<0,05$). Only the group that consumed avocado oil pulp (TEO) has shown decrease of the hepatic enzymes AST and ALT in comparison to the HE and HTE groups ($p<0,05$). TEC showed significant increase of ALT enzyme when compared to HTE and TEO groups, and there was no variance in relation to HE group ($p<0,05$).

Frequent consumption of tea made of avocado leaves has lead to development, and not to the recovery, of hepatic injuries caused by high-fat diet. Animals that practice exercises and had addition of avocado pulp oil in the diet showed more substantial liver improvement than those who only practiced exercises.

Financial support: National Council of Scientific Development (in Brazilian Portuguese: Conselho Nacional de Desenvolvimento Científico e Teconológico – CNPq, protocol n° 3159-1). It was developed with collaboration of Lund University, Swedish Research Council (nº C0618901) for the collaborative grant. The avocado oil and leaves used in the research were donated by Jaguacy Brasil Company.

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Protective effect of dietary polyphenols preconditioning on cerebral microvascular endothelial cells exposed to H2O2-mediated oxidative stress

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Background/Objective: Cerebral microvascular endothelial cells are the major components of the blood brain barrier that forms a dynamic wall between the blood and the central nervous system. During pathological conditions such as type 2 diabetes, oxidative stress associated with a pro-inflammatory state is a key event in the endothelial dysfunction, which may alter the blood brain barrier functions. We investigated the impact of a preconditioning with polyphenols, namely caffeic acid, chlorogenic acid, kaempferol, quercetin, and catechin on cerebral microvascular endothelial cells exposed to H_2O_2-mediated oxidative stress.

Methods: Murine bEnd3 endothelial cells were preconditioned with each polyphenol during 24 h, then rinsed to eliminate the polyphenol from the medium, and exposed t H_2O_2 during 24 h. We evaluated ROS production (DCFH-DA assay), the expression of genes encoding antioxidant enzymes (RT-qPCR) and the secretion of cytokines (ELISA).

Results: Most of the compounds significantly decreased ROS production induced by H_2O_2. Their antioxidant capacity could be explained by their marked free radical-scavenging activity, and ability to modulate antioxidant enzymes production. Indeed, all the polyphenols protected the pool of catalase during oxidative stress.

Remarkably, quercetin up-regulated superoxide dismutase gene expression level, and both quercetin and chlorogenic acid elevated glutathion peroxidase gene expression level. Moreover, caffeic acid and catechin exhibited anti-inflammatory properties by reducing H_2O_2-induced IL-6 secretion.

Conclusion: Our findings demonstrate that polyphenols protected cerebral microvascular endothelial cells exposed to H_2O_2, and highlight their potential benefits in strategies aiming to improve oxidative stress during pathological conditions such as diabetes.

Financial support: French Ministry of Education and Research, and the Région Réunio

P149

Low bioavailable polymeric procyanidins from apples alleviates lipids metabolism in C57BL/6J mice fed Western diet

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Various studies both in animals and human subjects have suggested that procyanidin exert a biological function in the prevention of various diseases, such as cardiovascular disease and lifestyle related diseases. However, polymeric procyanidins are a low bioavailability and their role in the biological functions remains ambiguous. In this study, we examined the effect of highly polymeric procyanidin (PP) on lipid metabolisms in mice fed high-fat high-sucrose Western die (WD).

Male ten week-old C57BL/6J mice were divided into four groups, normal diet (ND) group, WD (high-fat high-sucrose diet) group, WD + PP group and WD + oligomeri procyanidin (OP) group. After 20 weeks, we deconstructed the mice and analyzed blood biochemical findings, gene expression by quantitative real-time PCR and gut microbiota by next-generation sequencing.

Body weights, plasma glucose, triglyceride and total cholesterol in PP and OP group were significantly decreased in comparison with WD group. Gene expression of lipid metabolism-related factors (Ppar-, Ampk, Cpt) was significantly increased in OP group compared with WD group. In contrary, expression of lipid composition and cholesterol synthetic factor (Srebp1c, Hmg-coar) was significantly reduced in OP and PP groups compared with WD group. In addition, PP reduced the effects of gut microbiota by WD ingestion and the ratio of Firumicutes and Bacteroides of PP group showed an almost similar level to that of ND group. In conclusion, polymeric procyanidins showed to affect on lipid metabolism due to improvement of gut microbiota.

Financial support: Research Project on Development of Agricultural Products and Foods with Health-promoting benefits (NARO)

P150

Metabolomics of polymeric procyanidins in C57BL/6J mice fed Western diet by Liquid Chromatography–Quadrupole Time-of-fright/Mass Spectrometry (LC-QTOF/MS)

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Apple consumption is considered to promote beneficial effects on several human chronic diseases such as cardiovascular disease and lifestyle related diseases. Various studies have demonstrated that apple procyanidins possess various physiological functions such as anti-obese. In this current study, we investigated the effect of highly polymeric procyanidin (PP) on urine metabolites in mice fed high-fat high-sucrose Western diet (WD).

Male ten week-old C57BL/6J mice were divided into four groups, normal diet (ND) group, WD (high-fat high-sucrose diet) group, WD + polymeric procyanidin (PP) group and WD + oligomeric procyanidin (OP) group. After 20 weeks, metabolites in the urine were analyzed on the time-dependent by a high performance liquid chromatography-quadrupole time-of-fright mass spectrometry (HPLC-QTOF/MS) in negative ionization mode. Principal component analysis (PCA), t-test and PCA-discriminant analysis (PCA-DA) on the data were performed using MarkerView® software. Makers contributing to the discrimination of the different groups of mice were identified using the Extracted Ion Chromatogram (XIC) manage add-on for PeakView® software.

PCA-DA scores plots were separated between the groups and several markers were found in loading plots. In OP group, flavan-3ols and their metabolites degraded by gut microbiota were conjugated by glucronic acid and sulfate and methylated. In contrast, various tryptophan derivatives were found in PP group. In conclusion, PP ingestion affects on gut microbiota and suppressed lipid metabolism by improvement of various endogenous metabolites, such as tryptophan derivatives.

Financial support: Research Project on Development of Agricultural Products and Foods with Health-promoting benefits (NARO)

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Modulation of intestinal glucose uptake by phenolics rich Concord and Niagara grap juices in a coupled in vitro digestion/Caco-2 model system

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Grape juice (GJ) is rich in health promoting phenolics. However, GJ also contains natural sugar, which should be consumed in moderation. While the ability of phenolics to mitigate glycemic response has been proposed, the extension of these activities to GJ is unclear. Phenolic profiles of 100% GJ samples from traditional white and purple American grapes (*V. labrusca*; Niagara and Concord) were defined by LC-MS. Initially, the ability of GJ phenolics to inhibit starch digestion (α-amylase, α-glucosidase activity) and deuterated glucose and fructose (d7-glu/d7-fru) transport were assessed in vitro and in a Caco-2 human intestinal cell model, respectively. 100% GJ and sugar-matched control were then subjected to in vitro digestion prior to assessing impact of bioaccessible phenolics on Caco-2 transport of d7-glu. Both Niagara and Concord GJs were rich in total phenolics (359-825 μM) with anthocyanins highest in Concord (~61.1 μM). GJ extracts showed limited ability to inhibit amylase, however, all extracts showed modest inhibitory activity on α-glucosidases (6.2-11.5%; $P<0.05$). Additionally, all GJ extracts similarly reduced transport of d7-glu/d7-fru by Caco-2 monolayers in a dose-dependent fashion at GI relevant doses of 25-100μM total phenolics. 60 min d7-Glu/d7-fru transport was reduced by 10-38% by co-administration of GJ extracts compared to control. Similarly, transport of d7-glu was reduced by 26-38% from digesta of both Concord and Niagara GJ compared to control. These data suggest that monosaccharides naturally present in GJ may be absorbed more slowly compared to a non-grape sweetened beverage, in part, due to the action of grape phenolics.

Financial support: Welch's Foods Inc. and USDA National Needs Fellowship (INDW-2011-03876)

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Influence of cranberry extract on activity of cytochromes P450 (CYP) in mouse liver microsomes of obese and normal mice

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Obesity can result in several serious diseases. For treatment, food supplements are often used. These compounds are ingested without care of their possible interactions with concomitantly taken drugs. As many drug interactions are based on interference with drug metabolizing pathways, studies are needed to evaluate the safety of these products. Here, the effect of American cranberry (Vaccinium macrocarpon Ait.) on activities of obese and normal mouse liver microsomal cytochromes P450 (CYP) is presented.

Male NMRI mice were either obese (MSG mice, after administration of monosodium glutamate to newborn animals) or normal. Cranberry extract was administered in concentration 2% in the standard diet for 28 days. Both groups were fed ad libitum. At the end of experiment, all mice were fasted for 12 h and were sacrificed by cervical dislocation (all the mice on the same day).

Specific substrates typical for individual human CYP enzymes (1A2, 2A6, 2B6, 2C9 2C19, 2D6, 2E1 and 3A4) were used to evaluate the influence of cranberry extract o CYP enzymes. Protein expression in the liver microsomal fraction was determined by Western blotting, level of mRNAs was determined by RT-PCR using specific primer of CYPs enzymes of mice orthologous to human CYPs.

While the administration of 2% cranberry extract to control mice resulted in a significant increase of the mRNA levels of CYP1A2, CYP2E1, CYP3A11 and CYP3A13, in obese mice only a slight increase of mRNA levels in all studied enzymes were found. CYP protein expression in normal mice was increased in forms CYP2A6, 2C19, and 3A4; obese mice did not show any significant changes. An increase of enzyme activity in normal mice was observed with mice orthologs of human CYP2E1 and CYP3A4; on the other hand, a slight decrease was detected in activity of mice CYP2A5 (orthologous to human CYP2A6) after treatment with cranberry extract.

Financial support: Grant Agency of the Czech Republic

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Gene expression patterns in C57BL/6J mice fed a high-fat fed mice and raspberry puree, raspberry juice, ellagic acid, and raspberry ketone: a comparison study using focused microarrays.

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To evaluate hepatic gene expression patterns during metabolic syndrome and the health benefits of raspberry phytochemicals, a microarray approach was used to examine the effects of raspberry juice concentrate (RJC), raspberry puree concentrat (RPC), raspberry ketone (RK), or ellagic acid (EA) consumed in diet at typically-consumed levels. A custom-designed focused gene array was used; the array contained genes relevant to carbohydrate and lipid metabolism, biliary and xenobiotic transport, and inflammation.

Male C57BL/6J mice (n=8 each) were provided: low-fat diet (LF, 10% kcal fat), high-fat diet (HF, 45% kcal fat), or HF supplemented with RJC (HF+RJC), RPC (HF+RPC), EA (HF+EA), or RK (HF+RK). After 10 weeks, hepatic RNA was isolated. Gene expression was measured using a RT^{2}Profiler Focused Gene Array (Qiagen) and array analysis was performed to evaluate expression of 88 mRNAs.

The intake of RJC, RPC, EA, and RK improved metabolic parameters of mice. Gene expression patterns revealed regulation of pathways associated with the cholesterol, carbohydrate, lipid, and xenobiotic metabolism, along with antioxidant protection and modulation of inflammation. Compared to HF-fed mice, other diets altered gene expression as follows: HF+RK: 30/88 mRNAs regulated, HF+EA: 21/88 mRNAs, HF+RJC: 31/88 mRNAs, and HF+RPC 33/88 mRNAs, ($P < 0.01$-0.10). Analysis indicates each diet produces a unique expression pattern. Further, our results conclusively demonstrate that in HF-fed mice, little-studied RK is a bioactive compound, in that consumption of RK modulated hepatic gene expression. These results allow for the identification of candidate mechanisms by which raspberry phytochemicals may act in metabolic syndrome.

Key words: raspberry; ellagic acid, raspberry ketone, phytochemical; microarray; metabolic syndrome

Financial support: National Processed Raspberry Council (USA)

P154

Suppressive effect of caffeic acid phenethyl ester on adipocyte differentiation through control of reactive oxygen species related with regulation of NADPH oxidase 4complex in 3T3-L1 cell model

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In some studies, nontoxic amounts reactive oxygen species (ROS) generation may play a crucial role as secondary messenger in signaling pathways of differentiation process of various cells. Also, it was reported that ROS production increased during the differentiation of adipocyte. In this study, the suppressive effect of CAPE on 3T3-L1 differentiation to adipocytes through the inhibition of generation of ROS was investigated. The expression of adipogenic transcription factors such as CCAAT/enhancer binding protein (C/EBP) and peroxisome proliferator activated receptor (PPAR) was analyzed by western blot. Lipid accumulation during differentiation of adipocyte was significantly suppressed by CAPE treatment between 10 and 50 M in a dose-dependent manner. Consistent with this, the CAPE treatment does-dependently decreased the protein expression level of adipogenic transcriptional factors such as PPAR and C/EBP. The intracellular ROS generation increased to its highest level within 2 days and dose-dependently attenuated via regulation of NADPH oxidase 4 (NOX 4) complex formation by CAPE treatment between 10 and 50 M. These results imply that the inhibitory effect of CAPE on differentiation of 3T3-L1 cells into adipocyte can be accomplished by decreasing ROS generation via regulation of NOX 4 complex. Consequently, the regulation of ROS generation may be the determining factor of inhibitory effect of CAPE on adipogenesis, further implying ROS as a prime target of obesity control.

Financial support: BK21 Plus project of the National Research Foundation of Korea Grant

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Silibinin induces the differentiation of adipose tissue-derived mesenchymal stem cell into functional adipocytes.

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Background and aims. Silibinin, a flavonolignan contained in silymarin, an extract from the seeds of milk thistle, has been recently demonstrated to exert beneficial metabolic effects in animal models of obesity. The aim of this study was to examine the in vitro effects of silibinin on adipose tissue-derived mesenchymal stem cell (ASC) differentiation into mature adipocytes.

Methods: Human ASC were treated with 10 μM silibinin during and after 14 days of differentiation with an adipogenic coctail. After treatment lipid accumulation was assessed by Oil Red O staining; gene expression of was assessed by RealTime PCR and Western Blot analysis.

Results. Oil Red O staining revealed that treatment with silibinin reduces accumulation of lipid droplets in ASC. Quantitative Real Time PCR showed that silibinin decreases expression of key adipogenesis-related factors: PPAR, Diacylglycerol O-acyltransferase 1, FAS and FABP4. By contrast, silibinin promotes gene expression of PPAR, PPAR, C/EBP, transcription factors involved in lipid oxidation. Furthermore, treatment with silibinin was able to significantly increase expression of PGC-1, SIRT1 and UCP genes.

Conclusions. Our results indicate that silibinin induces the differentiation of ASC int smaller, insulin-sensitive adipocytes, thus suggesting that silibinin may be a novel agent used in the prevention and treatment of obesity and metabolic syndrome.

P156
Inhibitory effect of the leaf extract of pomegranate on postprandial glucose level inmice

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Glucosidase inhibitors including acarbose and voglibose are well known as an oral antidiabetic agent. In a survey of non-sugar based glucosidase inhibitors from nature sources, we investigated inhibitory effects of several food extracts using in vitro glucosidase inhibition assay. Among the tested samples, the leaf extract of pomegranate showed potent inhibitory activity. In vivo maltose tolerance test was performed with BALB/c mice to evaluate the reducing effect of pomegranate leaf extract (62.5, 125 and 250 mg/kg) compared with voglibose as a positive control on blood glucose levels after a single oral administration of maltose (2 g/kg). The treatment with pomegranate leaf extracts revealed significant suppression on glucose level with dose dependent in comparison with that of control group. The results led u to identify the glucosidase inhibitors in pomegranate leaf extracts. The 70% aqueous acetone homogenate of the leaves of pomegranate was extracted with diethyl ether, ethyl acetate, and water-saturated n-butanol to obtain the respective extracts.

Glucosidase inhibition assay-guided fractionation led to isolation of six hydrolysable tannins and related polyphenols (granatins A and B, corlagin, punicafolin, pentagalloylglucose, and ellagic acid) and three flavonoid glycoside (luteolins 4'-*O* -glucoside and 3'-*O*-xyloside, and quercetin 3-*O*-glucoside). The structures ofisolated compounds were characterized based on spectral analyses such as NMR andMS. Pentagalloylglucose and punicafolin showed strong inhibitory effects on glucosidase.

Financial support: National Grant

P157
Acute effects of apple and blackcurrant extract on postprandial glucose, insulin and glucose-dependent insulinotropic polypeptide concentrations.

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Polyphenols, such as phloridzin found in apples, inhibit intestinal glucose absorption but the effects of consumption of apple extracts on postprandial glucose concentrations have not been fully clarified. We investigated postprandial responses to apple extract, consumed with and without blackcurrant extract.

Twenty-five healthy subjects (20 M, 5 W, mean age 32 y, SD 14) completed a randomised, double-blind, crossover study. Three matched fruit cordial drinks contained either 1) 1.2 g apple polyphenols (A); 2) 0.6 g apple polyphenols + 0.6 g blackcurrant anthocyanins (1.4 g blackcurrant polyphenols) (B); or, 3) no added polyphenols (C; control). The drink was administered immediately before a high-carbohydrate meal (41 g starch, 22 g sucrose, 1.3 MJ). Plasma glucose, insulin and glucose-dependent insulinotropic polypeptide (GIP) were measured 0-120 min.

Early (iAUC 0-30 min) response, peak concentration (Cmax) and time until maximum concentration (Tmax) in plasma glucose were reduced by treatments A and B compared with C ($P<0.0001$). Mean differences in plasma glucose iAUC 0-30 min were: A-C, -25.3 mmol/L·min (95% CI -33.6, -16.9), B-C, -33.1 mmol/L·min (95% CI -42.4, -23.8); B-A, -7.8 mmol/L·min (95% CI -12.5, -3.2). Plasma insulin and GIP concentrations were similarly significantly reduced in all parameters.

Consumption of apple polyphenols equal to 3-4 large apples (containing 100 mg phloridzin) dampened the glycaemic response to a starch and sucrose-containing meal; this inhibitory effect was slightly greater following consumption of a lower dose (equivalent to 2 large apples) containing 50 mg phloridzin in combination with blackcurrant extract equivalent to 1-2 portions fresh weight.

Financial support: King's College London

P158

Resveratrol increases the expression of leptin receptor in the hypothalamus of health mice after short-term treatment.

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BACKGROUND: Leptin is the principal regulator of body weight and its functionality is disrupted in obesity. This condition is known as leptin resistance (LR), a state of elevated plasma leptin levels that fail to suppress appetite and reduce body weight. There are evidences that LR is due, at least in part, to alterations in the gene expression of some components of leptin signalling pathway in central nervous system, mainly in the hypothalamus. The role of resveratrol on health has gained significant interest. Interestingly, resveratrol has showed to affect components of cell signalling cascades improving parameters of metabolic syndrome and adipokine secretion. Consequently, the aim of this study was to analyse the effect of resveratrol on leptin signalling cascade in the hypothalamus to assess its potential to improve leptin sensitivity.

METHODS: Male NMRI mice were fed with standard diet supplemented with 100mg/Kg of resveratrol (RSV group, n=10) or vehicle (STD group, n=10) for 15 days. Gene expression of the long form of leptin receptor (Obrb), orexigenic regulators neuropeptide Y (Npy) and agouti related protein (Agrp) and anorexigenic neuropeptide proopiomelanocortin (Pomc) were quantified by RT-PCR.

RESULTS: Obrb was highly overexpressed in the hypothalamus of mice after 15 days of resveratrol consumption ($p=0.024$) compared to the control group. However, resveratrol consumption did not alter the mRNA levels of orexigenic neuropeptides Npy and AgRP and anorexigenic Pomc.

CONCLUSIONS: Resveratrol is able to modulate Obrb mRNA levels in the hypothalamus of healthy mice, suggesting that resveratrol could regulate leptin signalling cascade. Nevertheless, further studies of factors involved on leptin pathway are needed to elucidate the potential role of resveratrol on the improvement of leptin signalling.

Financial support: grant AGL2013-40707-R

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Dipeptidyl peptidase Inhibitory polyphenols from Irish and international blackberries

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Diabetes is considered an epidemic that affects 3-7% of western society, where type- being the most prevalent today. It is however treatable, using drugs like metformin which have existed since the 1950's. The widely distributed physiological enzyme dipeptidyl peptidase 4 (DPP-4) has been implicated in the regulation of the biological activity of multiple hormones, including the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 and GIP help stimulate the release of insulin in the pancreas triggered by food intake, and it has been proven that inhibition of DPP-4 leads to increased insulin levels in blood plasma and lower plasma glucose levels. This study seeks to find potent DPP-4 inhibitory polyphenol fractions and associate compounds from Irish blackberries and also from imported blackberries sourced to supplement the growing market during the off-season of native fruits. Blackberries are rich in polyphenols such as anthocyanins flavanols and phenolic acids. Numerous scientific and epidemiological studies have linked consumption of polyphenols with anti-diabetic effects. Using an aqueous-acetone mixture to extract polyphenols from fresh blackberries and fractionating these extracts through flash chromatography, several fractions high in polyphenols were identified using the total polyphenol content assay. Fractions with the highest inhibitory activity were identified using a commercially available DPP-4 activity assay. Through the use of UPLC MS-MS the compounds within these fractions were identified and quantified. This study has identified potent DPP-4 polyphenol compounds which when consumed may have a positive effect on patient suffering from type-2 diabetes.

Financial support: Food Institute Research Measure (FIRM 13F539, Cardio-RUBUS project), Department of Food, Agriculture & Marine, Ireland.

P160
Sucrase inhibition by polyphenols using Caco-2/TC7 cell model

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The prevalence of Type 2 Diabetes has doubled over the last thirty years and affects an estimated 1 in 10 worldwide. Growing evidence from both animal and human studies indicates that polyphenols reduce postprandial glucose response and improve insulin sensitivity. One mechanism of action is through the inhibition of carbohydrate digestion in the gut by a-glucosidases. As sugar intake has increased over the last few decades and a recent study demonstrated that the availability of sugar is a significant statistical determinant of Diabetes, this research focuses specifically on the sucrase enzyme.

The limitation of current research on sucrase inhibition is that many of these studies use non-human enzyme sources and also at high polyphenol concentrations at which they are either insoluble or not physiologically achievable. Furthermore, polyphenols can interfere with the determination of the product, glucose, and many reported data fail to take this into account. Most investigations thus far have looked at acute effect and so the chronic effect on enzyme activity and expression is unknown. The aim here is to investigate the true inhibition of a-glucosidases by polyphenols taking into account these issues.

An enzyme assay was set up using human Caco-2/TC7 cells, a commonly used mode for the small intestine, as a source of sucrase. Sucrase activity, $K_m$and V_{max}were determined and the assay was used to design inhibition studies. Results will be presented for polyphenols and polyphenol rich extracts. Cells were also treated chronically with polyphenols and the mRNA, protein and sucrase activity were determined.

Financial support: European Research Council Advanced Grant POLYTRUE? (322467)

P161
Assessment of a dietary supplement based on plant polyphenols for weight management in overweight volunteers

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Lippia citriodora (LC) and *Hibiscus sabdariffa* (HS) polyphenolic extracts have shown to reduce lipid accumulation, oxidative stress and inflammation in adipose cel model and prevent fatty liver disease (FLD) and improve lipid metabolism in animal hyperlipidemic models. The objective of this study was to evaluate the effects of a combination of these extracts on body weight as well as on several metabolic and hematological parameters associated with metabolic syndrome on overweight volunteers.

Overweight women with total cholesterol above 200 mg/dL were recruited into the study and included individuals followed an isocaloric diet for two months. The control group (L1) received two capsules of placebo and treatment group (L2) received 500 mg of MetabolAid® (LC extract + HS extract). Anthropometric and biochemical measurements, systolic and diastolic blood pressure and heart beat were taken at baseline, 30 and 60 days of the intervention period. The group having the dietary supplement significantly reduced body weight, waist circumference and body fat compared to the control group. This group also decreased significantly the heart rate and the systolic blood pressure compared to the placebo. Biochemical data also exhibited a global decrease of blood sugar level, total cholesterol and LDL cholesterol in both groups, but a bigger decrease was observed in the L2 group, although for these particular parameters differences were not statistically significant.

In conclusion, the consumption of MetabolAid® for two months in overweight women improved the anthropometric parameters, decreased systolic blood pressure and heart beat and improved blood lipid profile. The consumption of 500 mg/day of MetabolAid® in combination to isocaloric diet may be considered as a dietary supplement for weight management and the prevention of metabolic syndrome and may deserve further clinical studies.

Financial support: PROMETEO/2012/007, CIBER (CB12/03/30038, CIBERobn, Instituto de SaludCarlos III) and Monteloeder S.L. (Elche-Alicante, Spain).

P162
Effect of polyphenols on fructose transport by GLUT5

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Prevalence of diabetes has been linked to increasingly high intakes of sugars through the diet. Excess fructose, in particular, increases serum triglyceride and LDL-cholesterol in humans, and promotes hepatic insulin resistance, hyperinsulinemia and hyperglycemia. Polyphenols may have a positive impact on carbohydrate metabolism, by helping to control glycemic responses. In order to examine the effects of polyphenols and food extracts on fructose transport, we looked at GLUT5, the only transporter which can transport fructose but not glucose or galactose. GLUT5 was expressed in X. laevis oocytes through microinjection with mRNA. Expression of this protein was demonstrated using automated Western blotting, and uptake of^{14}C-fructosewas consequently enhanced as shown by liquid scintillation counting. The fructose analogue, L-sorbose-Bn-OZO inhibited^{14}C-fructose uptake as expected. However, nopolyphenols or food extracts exhibited a significant effect on fructose uptake into oocytes by GLUT5. Other GLUTs might play a role in fructose uptake inhibition, and so GLUT2 and GLUT7 are now being investigated using the same methodology. In summary, we now have robust system the study the direct effects of polyphenols on fructose transport.

Financial support: European Research Council Advanced Grant POLYTRUE? (322467)

P163
Xanthohumol and 8-Prenylnaringenin modulate angiogenic and metabolic pathways in diabetic animals

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Diabetes mellitus (DM) is responsible for metabolic deregulation leading to inflammation and oxidative stress, causing angiogenic derangements. An angiogenic paradox is present, with distinct patterns in different organs, namely an increase of kidney neovascularization and the opposite on the left ventricule (LV), but the mechanism has not been described. Beer polyphenols, as xanthohumol (XN) and 8-prenylnaringenin (8-PN), modulators of angiogenesis, could ameliorate DM relate complications.

This study aimed to verify if polyphenols consumption affects angiogenic paradox and if this is related with metabolic changes in diabetic mice.

For this purpose, C57Bl/6 mice were divided in 5 groups: standard diet, high fat diet (HFD), HFD and ethanol, HFD and XN, and HFD and 8-PN during 20 weeks. Kidney and LV were collected to evaluate microvessel density (MVD) and the expression of angiogenic receptors and related pathways. Liver and Muscle were collected to analyse metabolic pathways. Statistically significance was assessed by ANOVA followed by Bonferroni test.

Results demonstrated that XN reduced MVD and VEGFR-2 activation in kidneys, and 8PN raised both parameters in LV. Besides the polyphenol improvement in angiogenic paradox, polyphenols also ameliorate metabolic mediators. Polyphenol prevent the decrease in AMPK and ACC phosphorylation, key enzymes responsible for the DM imbalance on lipid metabolism accompanied by a reduction of PFKFB3, glycolytic activator in endothelial cells.

Beer polyphenols had benefits on DM angiogenic paradox with distinct effects on different tissues. It would be of great interest to clarify the interplay of angiogenesis and metabolism for the development of novel therapeutic strategies.

Financial support: SFRH/BD/86895/2012, UID/BIM/04293/2013 and PEst-OE/SAU/UI0038/2014 andERAB EA 14-23

P164
Dietary (-)-epicatechin mitigates podocyte abnormalities induced by high-fructose diet in rats

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High-fructose consumption is associated with impaired renal function. We evaluated the effects of dietary (-)-epicatechin to prevent the loss of podocyte integrity and impaired renal function associated with high fructose consumption in rats. Male Sprague Dawley rats were divided into 3 groups: Control (C), Fructose (F), and Fructose - EC (FEC). Fructose was administered in the drinking water (10% w/v) an (-)-epicatechin was supplemented in the diet (20 mg/kg BW/d) for 8 w. Body and kidney weights, and creatinine and urea plasma levels were non different among the groups. The amount of urinary excreted protein was higher in F compared to C and FEC (C=2.4±0.4; F=4.1±0.6* and FEC= 3.0±0.2 mg protein/mg creatinine, *$p < 0.05$). Collagen deposition, an index of fibrosis, was evaluated by Sirius Red staining in the renal cortex tissue. The degree of fibrosis was higher in F compared to C and FEC (4- and 2-fold increase respectively; $p < 0.05$). Nephrin, synaptopodin, and WT1, proteins associated with the normal structure and function of the glomerular filtration barrier, were measured as podocyte markers. Evaluation by immunohistochemistry of these proteins showed lower staining (% staining per glomeruli) in F respect to C (nephrin: 4.5±0.5% vs 16±1%, synaptopodin: 3.9±0.8% vs 30±2%, and WT1: 2.4±0.5% vs 5.7±0.1% in F vs C respectively). In FEC these parameters were restored to control values. These observations are in agreement with our previous results showing that dietary (-)-epicatechin also attenuated high-fructose induced increase in NADPH-dependent superoxide anion production, altered NO metabolism, and inflammation in the renal cortex.

Overall (-)-epicatechin emerges as a bioactive which consumption, as part of a flavanol-rich diet, could help to mitigate the renal alterations found in subjects with metabolic/inflammatory diseases.

Financial support: UBACyT 20020120100177 and 20020130100760BA, PIP0612, PICT-2012-0765

P167
A comparison of the effect of black currant with its fiber-rich extracts on lipid metabolism and inflammation-related gene expression of liver in diabetic ob/ob mice

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Many polyphenols and other bioactive substances are contained in black currant. We developed a novel type of dietary fiber-rich black currant extract (FRBE). Diabetic ob/ob mice exhibit dyslipidemia and an elevate expression of inflammation-related proteins. In this study, we tried to compare the difference of functionality between FRBE and black currant (BC) in diabetic ob/ob mice. Mouse macrophage RAW264 cells (2×10^5cells/well) were treated with various concentrations of anthocyanins in LPS-containing DMEM for 24hr. After treatment, nitric oxide in the each medium were measured by Griess method. 10% FRBE or 10% BC-containing diet were given to male diabetic ob/ob mice for 10-12 weeks. The mRNA levels of lipid metabolism and inflammation related genes were measured by real time PCR. We showed a significant decrease in the nitric oxide in the culture medium following anthocyanin treatment after LPS-stimulation. FRBE, but not BC, significantly reduced body weight gain and serum total cholesterol compared to the control diet-fed ob/ob mice. Hepatic AMPK mRNA level was decreased in control diet-fed diabetic mice and restored in FRBE diet-fed group. The overexpressed revel of MCP-1 in control diet-fed mice was reduced in FRBE diet-fed mice. These findings suggest that FRBE rather than BC may have the potential for amelioration of lipid metabolism and inflammation.

Financial support: Strategic International Collaborative Research Program (SICORP) from JapanScience and Technology Agency (JST)

Poster Session C: Neuro-cognitive effects of polyphenols

P200

Impacts of polyphenol-rich berry intake on cognition and brain insulin resistance

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High-fat diets impair insulin signaling in brain, which could harm the survival of neurons and cognitive function. Polyphenols intake could improve insulin sensitivity preventing neurodegeneration. The aim of this study was to investigate the effects of a high-fat diet, containing Syzygium malaccense as a polyphenols source, in working memory and insulin resistance.

The polyphenols of the freeze-dried S. malaccense berry (SMB) were analyzed using high-performance liquid chromatography. Adult Swiss mice were divided in groups according to their diets composition: normal-fat (N), N+5% SMB (NS), high-fat with 31% lard (HF), and HF+5% SMB (HS), during 10 experimental weeks. The Morris Water Maze (MWM), Glucose and Insulin Tolerance Tests (GTT and ITT) were performed. The Insulin-GSK3-Tau axis in hippocampus was analyzed using Wester Blot (WB).

Among the polyphenols found, cyanidin and quercetin derivatives were highlighted. The HF group demonstrated decreased escape latency in the MWM acquisition and lesser times crossing the point of the platform in the probe trial, when compared to N The HS-fed animals showed 176% improvement in the memory task. The ITT showed that the HF animals were insulin resistant and the HS diet increased insulin sensitivity. The WB results of HF showed a significant decrease in phosphorylation of insulin receptor substrate and GSK3- and higher Tau phosphorylation. The opposite pattern was found in the HS animals, demonstrating more insulin sensitivity and reduced risk for neurodegeneration.

The polyphenols of SMB were strongly related to the prevention of high-fat diet-induced insulin resistance, which corroborated the improvement in learning/memory function.

Financial support: Brazilian foundations - CNPq (206092/2014-8 and 300533/2013-6) and CAPES.

P201

The acute effect of flavonoid-rich apples and nitrate-rich spinach on cognitive performance and mood in healthy men and women

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Background/Objectives: Flavonoids and nitrate in a fruit and vegetable diet may be protective against cardiovascular disease and cognitive decline through effects on nitric oxide (NO) status. The circulating NO pool is increased via distinct pathways by dietary flavonoids and nitrate. Our aim was to investigate the acute effects of apples, rich in flavonoids, and spinach, rich in nitrate, independently and in combination on NO status, cognitive function and mood.

Methods: In a randomised, controlled, cross-over trial with healthy men and women (n=30), the acute effects of four energy-matched treatments (control, apple, spinach and apple+spinach) were compared. Endpoints included plasma nitric oxide status (determined by measuring S-nitrosothiols+other nitroso species (RXNO)), plasma nitrate and nitrite, salivary nitrate and nitrite, urinary nitrate and nitrite as well as cognitive function (determined using the Cognitive Drug Research (CDR) computerized cognitive assessment battery) and mood.

Results: Relative to control, all treatments resulted in higher plasma RXNO. A significant increase in plasma nitrate and nitrite, salivary nitrate and nitrite as well as urinary nitrate and nitrite was observed with spinach and apple+spinach compared to control. No significant effect was observed on cognitive function or mood.

Conclusions: Flavonoid-rich apples and nitrate-rich spinach augmented NO status acutely with no concomitant improvements or deterioration in cognitive function and mood.

Financial support: NH&MRC, ARC and DAFWA

P202

Anti-stress effects of Trans-cinnamic acid on H2O2-stressed SH-SY5Y cells and tail suspension test

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Stress is known to induce alterations in various physiological responses. In particular hydrogen peroxide (H_2O_2) was considered as a major source of reactive oxygen species (ROS) and induces oxidative stress in cell lines. Polyphenols are the most wide spread secondary metabolite in plant kingdom. It has been reported that their antioxidant activity is mainly due to their redox properties.

This study aimed to investigate the anti-stress effects of trans-cinnamic acid (TA) on H_2O_2-induced overproduction of ROS, ATP depletion, in concordance with heat shock protein expression (HSP27 and HSP90) in human neuroblastoma SH-SY5Y cells. Additionally, we investigated the effect of TA on mice in tail suspension test (TST).

Our results demonstrated that pretreatment of SH-SY5Y cells with TA significantly reversed mRNA expression of HSP27 in H_2O_2-treated SH-SY5Y. In addition, pretreatment of the cells with TA prevented the accumulation of ROS relative to $H_{2\ 2}$-treated cells. Moreover, our results showed that H_2O_2 at 150 μM significantly decreased intracellular ATP levels in SH-SY5Y cells to reach 67%. Contrariwise, TA significantly increased the levels to reach 90%. Daily oral administration of TA was conducted to ICR mice for 2 weeks, was resulted a significant decrease of immobility time in TST. In addition, our results demonstrated that TA did not cause the death of any mouse following treatment.

By increase of ATP levels, and decrease of ROS production and immobility time in TST, we can consider that TA possesses anti-stress properties and may be of interest as a therapeutic agent for the treatment of stress disorders.

Financial support: University of Tsukuba

P203

Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: an 8-week randomised double-blind placebo-controlled trial in healthy older adults

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Background: Research indicates that chronic consumption of flavonoids is associated with cognitive benefits in adults with mild cognitive impairment and neurodegenerative disease, although, there has been no such studies in healthy older adults. Furthermore, the effects of commonly consumed orange juice flavanones on cognitive function remain unexplored. The objective was to investigate whether eight weeks of daily flavanone-rich orange juice consumption was beneficial for cognitive function in healthy older adults.

Methods: High flavanone (HF: 305mg) 100% orange juice and equicaloric low flavanone (LF: 37mg) orange flavored cordial (500ml) were consumed daily for eight weeks by thirty seven healthy older adults (mean age 67 years) according to a crossover, double blind, randomized design separated by a four week washout. Cognitive function, mood and blood pressure were assessed at baseline and follow with standardized validated tests.

Results: Global cognitive function was significantly better following eight week consumption of flavanone-rich juice relative to eight week consumption of the low flavanone control. No significant effects on mood or blood pressure were observed.

Conclusions: Chronic daily consumption of flavanone-rich 100% orange juice over eight weeks is beneficial for cognitive function in healthy older adults. The potential for flavanone-rich foods and drinks to attenuate cognitive decline in ageing and the mechanisms which underlie these effects should be investigated.

P204

Anthocyanins modulation of microglia M1/M2 phenotype and effect on neuronal differentiation and synaptic plasticity

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Flavonoids have been related with the prevention of cognitive function decline characteristic of ageing and also to improve several memory dependent tasks. In response to environment, microglia is capable of acquiring different phenotypes and flavonoids have been pointed out as inhibitors of microglia activation and preventers of some pro-inflammatory cytokines release. This work hypothesis was that flavonoids may decrease the classical M1 microglia activation phenotype inducing a switch to M2 phenotype that could affect neurons differentiation and their synaptic communication.

N9 microglia cell line was pre-treated for 24 h with 1μM of cyanidin, cyanidin-3-glucose, 3,4-methyl-cyanidin-3-glucose or vehicle, and with an M1 inducer (Lipopolysaccharide (LPS)) or an M2 inducer (IL-4) for another 24 hours. Transcription of M1 (TNF-, IL-6, iNOS) and M2 (Ym1, CCR2 and Arg1) markers were measured by qRT-PCR. SHSY-5Y, a neuronal cell line, was then treated with the conditioned medium of N9 cell line for 24 hours. Differentiation and mRNA expression of synaptic related proteins (fractalkine, synapsin, synaptophysin) were tested.

Anthocyanins treatment reduced TNF- levels (M1 marker) on basal conditions and seems to increase Ym1 expression (M2 marker). LPS was able to induce a sharp increase of all M1 markers tested that were partially prevented by flavonoids treatment. LPS conditioned media decreased SH-SY5Y differentiation and this effect was reverted by 3,4-methyl-cyanidin-3-glucose. Also, cyanidin-3-glucose and 3,4-methyl-cyanidin3-glucose increased synapsin expression in these conditions.

Concluding, anthocyanins improved cytokines profile in basal conditions, and, although expression of M2 markers was only slightly affected by anthocyanins, it seems they may affect neuronal differentiation and synaptic transmission. These results reinforce the role of polyphenols on prevention of neuronal ageing.

Financial support: Fundação para Ciência e Tecnologia through POPH/FSE (Programa Operacional Potencial Humano/ Fundo Social Europeu), (1 grant project PTDC/AGR-TEC/2227/2012 and 2 grants SFRH/BPD/75294/2010 and SFRH/BD/78367/2011).

P205

Pterostilbene and cognitive performance in the aged rat model: molecular and behavioral effects.

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Pterostilbene is a major polyphenolic component of blueberries. Recent papers report that Pterostilbene is potentially very beneficial for health care, improving, for example, cognitive performance and short-term memory impaired by old age or neurodegeneration. While the literature reports effects of Pterostilbene in prevention/treatment of cognitive impairment in old individuals, no studies have investigated the detailed mechanisms underlying these observations.

The intent of this work is to identify novel downstream effectors of Pterostilbene treatment in aged rats. In an ongoing study we are evaluating whether autophagy and epigenetic modulation could be the mediators of Pterostilbene effects.

Aged (18 months) rats were initially evaluated through behavioral tests. Half cohort was then subjected to Pterostilbene treatment for 20 consecutive days at the end of which the cognitive assessment was repeated. Ventromedial prefrontal cortex, perirhinal cortex, dentate gyrus and the rest of hippocampus were then collected and analyzed by RT-qPCR or western blot.

Behavioral data indicate that Pterostilbene-treated animals show an improvement in memory and learning tests. Gene expression studies reveal that in general the dentate gyrus is the area most solicited by the treatment. In vitro experiments on HeLa cells suggest that these effects might be downstream of an increase of cAMP levels and TFEB nuclear translocation.

Concluding, gene and protein analysis suggests that behavioral improvement may be related to: 1. a rearrangement in the synaptic architecture 2 change in the acetylation level of histones and 3. an increase in autophagy.

Financial support: Research Projects of National Interest (PRIN, Research Italy).

P206
Neuroprotective Potential of Peach Fruit Phenolic Extracts

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Polyphenols are the most abundant antioxidants in the diet. Fruit and beverages represent the main sources of polyphenols. Epidemiological studies have suggested associations between the consumption of polyphenol rich food or beverages and the prevention of diseases.

Peaches are a popular summer fruit and there has been an increasing interest in their nutritional value. Phenolic compounds represent the main phytochemical antioxidants present in peaches. Phenolic compounds have recently received particular attention because of their possible preventive role in neurological disorder Because A and S are the primary components of amyloid plaques and Lewy bodies, respectively, the aggregation of these proteins is considered to be a critical step during neurodegenerative processes associated with Alzheimer's disease (AD) and Parkinson's disease (PD), respectively. The purpose of this study is to assess the inhibitory properties of fruit extracts of seven peach varities cultivated in Algeria against A and S aggregation.

Several phenolic peach fruit extracts (Prunus persica L.) were investigated in order to assess their inhibitory property against A and S fibril formation and neuroprotective capacity against A-induced toxicity on PC12 cells were evaluated. The effect of each peach extract on the A_{2535} aggregation was monitored by using an in vitro assay based on UV-visible measurements, curcumin being used as positive control. The inhibitory property of peach extract on the S aggregation was studied by using thioflavin T binding assay. And the neuroprotective capacity against A-induce toxicity on PC12 cells was monitored by determination of cell viability by MTT assay and intracellular ROS production measurements by DCFH-DA assay.

Results showed that all peach phenolic extracts inhibit A and S fibril formation and protect PC12 cells against A-induced toxicity. Concerning A fibril formation, of the seven varieties of peaches studied, Red Top and Cardinal extracts had the strongest inhibition percentage comparing to the other cultivars. Peach extracts are more effective on S aggregation. These data suggested that the constituents of each cultivar might have some influences on the inhibitory process. Incubation of PC12 cells with 100 and 200 g/mL of peach extracts of all cultivars significantly prevented the cytotoxic effect of A_{25-35}. The extracts increased the cell viability in dose dependent manner. For ROS production, treating cells with the different fruit peach extracts permitted to reduce ROS generation induced by A.

In conclusion, these results suggest that peach fruit could serve as a good source of natural antioxidant compounds and might contribute for nutritional prevention of certain chronical diseases linked to oxidative stress such as neurological disorders (Alzheimer and Parkinson diseases).

Financial support: La Ligue contre le cancer

P207
Concord grape juice, cognitive function and driving performance: a 12 week, placebo controlled, randomised crossover trial in mothers of pre-teen children

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Background: Daily consumption of Concord grape juice (CGJ) over three months ha been shown to improve memory function in adults with mild cognitive impairment, and reduce blood pressure in hypertensive adults. These benefits are likely due to the polyphenols in CGJ. Increased stress can impair cognitive function and elevate blood pressure. Thus we examined the effect of CGJ in individuals experiencing stressful demanding lifestyles.

Methods: Twenty five healthy mothers of pre-teen children, aged 40-50 years, who were employed for > 30 hours/week consumed 12oz CGJ (777mg polyphenols) or an energy, taste and appearance matched placebo daily for twelve weeks according to a randomised, crossover design with a four week washout. Verbal and spatial memory, executive function, and attention were assessed at baseline, six and twelve weeks. A subsample (n=17) completed a 25 minute

driving assessment in the University of Leeds Driving Simulator requiring participants to match the speed and direction of a lead vehicle.

Results: Significant improvements in immediate spatial memory and driving performance were observed following CGJ relative to placebo. There was evidence o an enduring effect of CGJ such that participants who received CGJ in arm 1 maintained better performance in the placebo arm.

Conclusions: Cognitive benefits associated with chronic consumption of flavonoid-rich grape juice are not exclusive to adults with mild cognitive impairment Moreover, these cognitive benefits are apparent in complex everyday tasks such as driving. Effects may persist beyond cessation of flavonoid consumption and future studies should carefully consider the length of washout within crossover designs.

Financial support: Welch Foods Inc. Concord, MA, USA

P208

Neuroprotective effects in human SH-SY5Y neuroblastoma cells of selected microbial-derived phenolic metabolites and aroma compounds from wine

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Beneficial health effects issued from moderate wine consumption have been mainly associated to the occurrence of bioactive molecules in wine, such as polyphenols and some aroma-active compounds. However, the beneficial health properties of polyphenols are mainly attributed to the compounds derived from their metabolism. In the colon, gut bacteria can convert wine polyphenols into different low molecular weight metabolites (in particular phenolic acids) that are able to be absorbed in situ.

Neuroinflammation process underpins the basis of neurodegenerative disorders, as Alzheimer's or Parkinson's diseases. Moderate wine consumption has been reported to exert a positive effect in prevention of neuronal disorders as well as to improve cognitive function. Furthermore, recent evidences suggest that wine components could exert their actions by interacting with different signalling routes, such as mitogen-activated protein kinase (MAPK) pathway. MAPK are a family of serine/threonine kinases that translate extracellular signals into intracellular responses, leading to changes in enzyme activity and gene expression involved in neuroprotection. To go deep into the knowledge of which mechanisms are involved in the protective role of wine in neuroinflammation, we have assessed changes in the expression of MAPK (ERK1/2, p38 and JNK) and STAT-1 proteins in SH-SY5Y neuroblastoma cells, after incubation with phenolic acids derived from the colonic microbial metabolism of wine polyphenols [3,4-dihydroxyphenylacetic acid, 3-(4-hydroxyphenyl)propionic acid, 3-(3-hydroxyphenyl)propionic acid, 3-hydroxyphenylacetic acid, 2-hydroxybenzoic acid (salicylic acid) and its -D-O-glucuronide] and wine aroma compounds (linalool and 1,8-cineole). The peroxynitrite generator SIN-1(3-morpholinosydnonimine), was used to cause a cell injury situation. After discarding a neurotoxic effect of the assayed compounds, the study of the protective effect of these compounds (0.1-10μM) against neuronal induced injury was carried out using Alamar Blue assay. The results indicated that al the phenolic acids and their derivatives were able to protect neurons from SIN-1-induced injury; meanwhile aroma compounds did not exert a clear protective effect.

Inmunoblotting technique revealed a significant modulation ($p<0.05$) of the phosphorylation of p38 and ERK1/2 by both type of compounds, phenolic acids and aroma compounds, depending of the compound and concentration. On the other side, no clear effects were observed in the case of JNK or STAT-1 expression for both types of compounds. To sum up, our work means an advance in the characterization of the molecular mechanisms responsible of the neuroprotection effects of wine.

Financial Support: A.E.F is a recipient of FPI contract from Spanish Ministry of Economy and Competitiveness (Project AGL2012-40172-C02-01).

P209
The acute effects of wild blueberry (V. angustifolium) on cognition: a dose-response study

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Blueberry polyphenols have been shown to improve cognition (Krikorian et al 2011; Whyte & Williams 2014), with vasodilator effects leading to increased cerebral blood flow (Francis et al. 2006; Dodd et al. 2015) implicated as a possible mechanism of action. Importantly, Rodriguez-Mateos et al. (2014) recently observed a dose-dependent relationship between blueberry polyphenols and vascular reactivity.

To investigate the relationship between dose-dependent vascular changes and cognition, this double-blind, placebo-controlled, crossover study investigated the cognitive effects of five wild blueberry doses. 42 healthy adults (aged 18-40) consumed blueberry drinks (500ml), containing 319, 639, 766, 1278, and 1791mg total polyphenols. Drinks (matched to the natural sugar and vitamin C content of the highest dose, 40g and 9.5mg respectively) were administered over six test days, separated by a one week washout. Episodic memory, mood, and executive function were assessed at baseline and 1 hour postprandially.

Blueberry supplementation produced improvements in performance for measures of executive function and mood, with performance decreases observed for episodic memory measures. Importantly though, none of these changes were significantly different from those also observed following consumption of a placebo.

Cognitive effects did not match previously reported dose-related, polyphenol induce changes in vascular reactivity. This suggests that alternative mechanisms of action may be responsible for published blueberry polyphenol benefits. Data from an ongoing glycaemic response study indicate differences in the glycaemic profiles between the blueberry and placebo drinks. Such differences may convey cognitive benefits at later time points or in glucoregulatory impaired populations, which are areas for further research.

Financial support: ESRC studentship / Blueberry powder donated by Wild Blueberry Association of North America.

P210
Effects of dark chocolate 72% cacao intake in healthy Japanese persons (Part2)

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Recently, much attention has been focused on the correlation between the intake of cocoa with high polyphenol and improvement of the mental and chronic fatigues. Several reports showed that intake of cacao products with high polyphenol improve the cognitive function. Cacao products with high polyphenol have been reported to increase cerebral blood flow in the healthy elderly subjects. These data indicated that cacao polyphenols increase the development of cognitive function related the cerebra blood flow. The decrease of the Brain Derivative Neurotrophic Factor (BDNF) concentration in brain and plasma was reported in the patients with depression or Alzheimer's disease, but physical exercise increased BDNF concentration. This stud aimed to evaluate correlation between dark chocolate intake and quality of life (QOL was assessed with the SF-36v2 and BDNF as cognitive marker.

Methods: Intervention clinical trial includes 385 healthy Japanese without taking no medicine regularly, suffering from no allergy of cacao, aged 45 to 69 years. All subjects took 25 g dark chocolate 72% cacao per day for 4 weeks. The study protocol was approve by the Human Ethics Committee of the Gamagori City hospital (Aichi Japan).

Results: Three hundred forty seven subjects completed the study. The scores of bodily pain, general health perceptions, vitality, role emotional, mental health and mental component score at 4 weeks were significantly higher than at baseline with the SF-36v2. The concentration of BDNF increased significantly at baseline and at 4 weeks.

Conclusion: The intake of dark chocolate may improve the quality of life with improving of cognition.

Financial support: Ryoshoku Research Fund

P211
Neural effects of cocoa flavanols during letter memory encoding

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Human research has shown benefits of cocoa flavanol consumption for memory and learning. This pilot study employed blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (FMRI) during letter memory encoding to explore the neural changes following an acute dose of cocoa flavanols with a view of understanding the mechanisms behind these cognitive benefits.

BOLD was performed in 12 participants (mean age: 64 (+/-4), 5 female) who performed a memory task before and 2 hours after consumption of low (23mg) or high (494mg) 330ml equicaloric flavanol drinks (matched for caffeine, theobromine, taste and appearance) in a randomized, counterbalanced, double-blind, crossover design. Using a block-design, participants were asked to remember individual consonants (1 consonant during easy 'current' trials and 4 consonants during harder 'update' trials). Changes in BOLD activation during memory encoding from pre- to post-drink consumption for the contrast update-current trials were compared between drinks using FSL 5.08.

Preliminary analysis showed increased BOLD activation during memory encoding for the contrast update-current trials in the dorsolateral prefrontal cortex (DLPFC), a key mediator of working memory, and in the hippocampus following consumption of the high flavanol relative to the low flavanol drink (unthresholded, $p<0.001$). This is in line with the current literature showing a green tea-induced increase in DLPF activation during a letter-based memory task and flavanol-induced hippocampal plasticity in animals linked to improvements in spatial working memory tasks.

This pilot study revealed acute cocoa flavanol-induced increases in neural activation in the hippocampus and DLPFC, which could have mechanistic relevance for the memory benefits attributed to cocoa-flavanols.

Financial support: Biotechnology and Biological Sciences Research Council (BB/F008953/1 and BB/G005702/1). Mars Inc. supplied the intervention and control drinks.

P212
Effects of a spearmint extract on cognitive function, sleep, and mood in men and women with age-associated memory impairment

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Cognitive decline associated with brain aging may result from a number of factors including increased oxidative stress or inflammation. Botanical ingredients, containing phenolic compounds with antioxidant properties, have been investigated for their neurocognitive benefits. The purpose of this study was to investigate the effects of supplementation with a proprietary spearmint (Mentha spicata L.) extract, containing high levels of phenolic compounds (i.e., rosmarinic acid), on cognitive performance, sleep, and mood in individuals with age-associated memory impairmen (AAMI).

Participants (N = 90; 67% female; age =59.4 ± 0.6 y) were randomly assigned (n =30/group) to consume 900, 600, or 0 (placebo) mg/day spearmint extract for 90 days, in this double-blind, parallel-designed trial. Computerized cognitive function tests were completed on days 0, 45, and 90.Leeds Sleep Evaluation Questionnaire(LSEQ) and Profile of Mood States (POMS) were completed on days 0 and 90.

Spearmint extract supplementation (900 mg/day) improved quality of working memory and spatial working memory accuracy by 15% (P = 0.0469) and 9% (P = 0.0456), respectively, versus placebo. Participants consuming 900 mg/day spearmint extract reported improvement on LSEQ in their ability to fall asleep, relative to participants consuming placebo (P = 0.0046). Trends were observed in POMS vigor-activity ratings (P = 0.0646) and total mood disturbance (P = 0.0832) following consumption of 900 mg/day spearmint extract versus placebo.

These results suggest that chronic supplementation with a spearmint extract from a selectively bred spearmint variety may be a beneficial nutritional intervention for cognitive health in older subjects with AAMI.

Financial support: Kemin Foods, L.C.

P213

An investigation into the effects of wild blueberry supplementation on the cognitive behaviour and development of seven to ten year old children.

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Background: Acute consumption of a wild blueberry (WB) drink, rich in anthocyanins, has recently been shown to improve executive function (EF) and short-term memory 2-6 hours post-consumption in 7-10 year old children. However, detailed examination of these effects is still required to more precisely identify the specific aspects of EF and memory susceptible to acute anthocyanin ingestion. Furthermore, the effects of chronic WB ingestion on cognition remain relatively unexplored in children.

Methods: Healthy children, aged 7-10 years, consumed a 200ml WB drink (253mg anthocyanins) or an energy and vitamin matched placebo, according to a randomised participant-blind, parallel groups, acute-on-chronic design. Verbal memory, executive function, and mood were assessed at baseline and 2 hours post consumption. Subsequently, participants consumed their assigned drink once daily for 4 weeks, with cognition assessed after 2 and 4 weeks of treatment, and following a 4 week washout.

Results: To date, only preliminary results from our acute analysis are available. Significant improvements in executive function (accuracy and reaction time for incongruent trials from the flanker task) and verbal memory (total word recall and reduced proactive interference) were observed two hours post consumption relative t baseline for both the WB and placebo drinks. No changes in mood were observed. Chronic data collection is ongoing.

Conclusions: Consumption of a WB anthocyanin-rich drink or a sugar, energy and vitamin matched placebo is associated with improved cognitive function in healthy 7-10 year old children. There is potential for flavonoid-rich blueberry foods and drinks to provide not just acute, but also chronic cognitive benefits in children. Such findings would support the accumulating body of evidence that flavonoid-rich products are beneficial for maintaining and enhancing cognitive health, particularly during critical periods in the lifespan such as childhood.

Financial support: Wild Blueberry Association of North America (WBANA)

P214

The association between dietary intake of Flavonoids and cognitive function in the healthy elderly: A longitudinal analysis of cognitive ageing, initial outcomes.

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Background: Many studies have shown an association between the flavonoids that exist in fruits and vegetables and maintenance of health and cognitive function.

Objective: To examine the association between habitual dietary flavonoid intake and cognitive decline over the past 10 years in healthy older adults.

Method: Seventy six healthy older adults (29 male and 47 women; mean 74 years) were recruited. All participants had performed at least one cognitive assessment in the past 2-10 years. Upon recruitment, participants performed a further cognitive assessment the Mini–Mental State Examination, Digit Symbol Substitution Test (DSST), immediate and delayed word recall, Block Design, Stroop, Trails A and B, and Verbal Fluency. MMSE scores<24, history of stroke, myocardial infarction, type 1 o 2 diabetes, mental health illness, and smoking were exclusion criteria. Flavonoid intake was assessed with a single dietary assessment upon recruitment using a food frequency questionnaire, 24 hour recall and a food diary. Flavonoid intake was calculated using the FLAVIOLA Food Composition Database.

Results: Participants were initially grouped into quartiles of flavonoid intake per day (lowest = 87-210mg; highest 371-580mg). A cross sectional analysis (ANCOVA) using the most recent cognitive assessment showed no difference in cognitive performance between the four flavonoid quartiles after including various covariates (age, gender, education level, energy intake, IQ, supplement intake, physical activity, and health problem and alcohol consumption). Similarly, the ANCOVA analysis using cognitive change scores (initial assessment – final assessment) showed no associations between flavonoid intake and cognitive function.

Conclusion: Overall, there were no associations between flavonoid intake at follow-up and cognitive outcomes over the past 10 years after considering important covariates in this sample of healthy older adults, free of disease. Future research should examine how longitudinal changes in habitual flavonoid intake are associated with incidence of neuropsychological disease in ageing such as mild cognitive impairment and dementia.

Financial support: University of Reading

P215

Flavonoid-rich orange juice is associated with acute improvements in cognitive function in healthy middle-aged males.

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PURPOSE: Epidemiological evidence suggests that chronic consumption of fruit-based flavonoids is associated with cognitive benefits; however, the acute effects of flavonoid-rich (FR) drinks on cognitive function in the immediate postprandial period require examination. The objective was to investigate whether consumption of FR orange juice is associated with acute cognitive benefits over 6 h in healthy middle-aged adults.

METHODS: Males aged 30-65 consumed a 240-ml FR orange juice (272 mg) and a calorie-matched placebo in a randomized, double-blind, counterbalanced order on 2 days separated by a 2-week washout. Cognitive function and subjective mood were assessed at baseline (prior to drink consumption) and 2 and 6 h post consumption. The cognitive battery included eight individual cognitive tests. A standardized breakfast was consumed prior to the baseline measures, and a standardized lunch was consumed 3 h post-drink consumption.

RESULTS: Change from baseline analysis revealed that performance on tests of executive function and psychomotor speed was significantly better following the FR drink compared to the placebo. The effects of objective cognitive function were supported by significant benefits for subjective alertness following the FR drink relative to the placebo.

CONCLUSIONS: These data demonstrate that consumption of FR orange juice can acutely enhance objective and subjective cognition over the course of 6 h in healthy middle-aged adults.

Financial support: University of Reading

Poster Session D: Polyphenols and Cancer prevention

P300

Novel biopolymer with anticancer efficacy against prostate cancer cells

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The high-molecular fractions from species of two genera (Symphytum and Anchusa) of Boraginaceae family Symphytum asperum, S.caucasicum, S.officinale and Anchusa italica were isolated. The structure elucidation of these high-molecular preparations was carried out according to IR, 13C and 1H NMR, 2D heteronuclear 1H/13C HSQC spectral data and 2D DOSY experiment. Besides, anticancer efficacy of S. caucasicum preparation against prostate cancer cells was investigated as well.

The main structural element of these preparations was found to be a regularly substituted polyoxyethylene, namely poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA) or poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl)ethylene]. Most of the carboxylic groups of PDPGA from A. italica unlike the polymer of S.asperum, S.caucasicum and S.officinale are methylated. S.caucasicum PDPGA inhibited the growth of androgen-dependent and –independent prostate cancer (PCA) cells both in vitro and in vivo. Consistent with in vitro results, in vivo study showed that PDPGA feeding of mice strongly inhibited 22Rv1 tumors growth by 88% at 5 mg/kg body weight doses for 5 weeks, without any toxicity, together with a strong decrease in androgen receptor and prostate specific antigen expression but increase in apoptosis in tumor tissues from PDPGA-fed mice.

Such caffeic acid-derived biopolymer to our knowledge has not been known and has been identified for the first time. This compound represents a new class of natural polyethers with a residue of 3-(3,4-dihydroxyphenyl)glyceric acid as the repeating unit. Overall, present study identifies PDPGA as a potent agent against PCA without any toxicity, and supports its clinical application.

Financial support: Georgian Research and Development Foundation (GRDF), USA Civilian Research & Development Foundation (CRDF), Grant N GEB2-3344-TB-06

P301

Anthocyanin-rich Blackcurrant juice induces a redox-sensitive caspase 3-related pro-apoptotic effect in leukemia Jurkat cells: role of delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside

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Blackcurrant, a rich source of anthocyanins, has been reported to exhibit an antiproliferative effect on several types of solid tumor cancer cells, but the active molecules and the precise mechanism of action remain unclear. The aim of the present study was to investigate the anticancer effect of black currant-derived products (juice (BCJ), extract (BCE) and anthocyanins) in Jurkat cells, and, if so, to characterize the underlying mechanism. Cell cycle and apoptosis were assessed by flow cytometry, the formation of reactive oxygen species by dihydroethidine and protein expression by Western blotting. BCJ and BCE inhibited the proliferation and induced G2/M phase cell cycle arrest and apoptosis in Jurkat cells associated with an increased expression of p73 and caspase 3, dephosphorylation of Akt and Bad, and down-regulation of UHRF1 and Bcl-2. A pro-apoptotic response was also observed in response to two major black currant anthocyanins, delphinidin-3-O-rutinoside and delphinidin-3-O-glucoside. The BCJ- and BCE-induced activation of caspase 3 was markedly inhibited by pretreatment with the antioxidant N-acetyl cysteine. BCE and the two active anthocyanins induced the formation of reactive oxygen species.

BCJ and its' major anthocyanins induced a redox-sensitive caspase 3-dependent apoptosis in Jurkat cells, involving a dysregulation of the Akt/Bad/Bcl-2 pathway.

Financial support: AJLG was supported by a postdoctoral fellowship by Alfonso Martín Escudero Foundation. TS was supported by a fellowship from the Higher Education Commission of Pakistan.

P302

Curcumin coloaded nano-bioenhancers: a synergistic approach exploiting polyphenols as bioenhancers for cancer chemotherapy

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Introduction: Polyphenols has a significant role in modulating cancer incidence and mortality. Polyphenols rich medicinal plants like Piper longum (P), Piper nigrum (N) Zingiber officinale (Z) exploited as bioenhancers. Curcumin, a diphenolic compound exhibited growth inhibitory effect in various types of cancers including prostate.

Objective: The purpose of this study is to formulate nanobioenhancers coloaded with curcumin and P, N, Z and to investigate the bioenhancer effect of P, N and Z on well known anticancer agent curcumin (CUR).

Methodology: The nanobioenhancers coloaded with curcumin and P, N, Z were formulated using PLGA polymeric system (drug/polymer weight ratio of 1:10) by the nanoprecipitation method. The characterization of the formulation was done using Zeta sizer for particle size, zeta potential, Transmission Electron Microscopy for morphology and evaluated for anticancer activity.

Result: The selected nano-bioenhancer formulation CP1 with particle size 216.2±4.6 nm and zeta potential of -20.6±1.6 mV was capable of lowering viability in PC3 cell line 2.5 times than CUR. Further, mechanistic studies in PC-3 cells indicated that the apoptosis caused by CP1 was mediated both through intrinsic and extrinsic pathways by increasing bax (protein levels); and decreasing bcl-2, phosphor-AKT in PC-3 cell Our findings suggest that CP1 also increase Reactive Oxygen Species and Reactive Nitrogen Species in cancerous cells. The CP1 nano-bioenhancer showed PGP inhibitory effect as investigated by Hoechst assay. A major highlight of study showed CP1 augmented oral bioavailability of CUR by 2.2 folds and had a longer half-life than CUR.

Conclusion: Through this study, we propose that nanobioenhancers loaded with polyphenols can be developed as potential chemo-preventive or therapeutic agents with enhanced cellular uptake and bioavailability.

Financial support: DST-INSPIRE

P303

Polyphenols and breast cancer survival – results from the European Prospective Investigation into Cancer (EPIC) cohort

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Background/objectives: Intake of dietary fiber and soy has been related to better survival among breast cancer survivors, but the evidence is limited. Dietary fiber and soy are rich in polyphenols, which may have biological effects that are relevant for breast cancer progression including estrogen and antioxidant activity. Our objective was to investigate the association between pre-diagnostic intakes of total polyphenol and polyphenol classes (flavonoids, lignans, phenolic acids, and stilbenes) in relation to all-cause and breast cancer-specific mortality among women diagnosed with breast cancer.

Methods: We used data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Diet was assessed from food frequency questionnaires, and polyphenol intakes were estimated using the Phenol-Explorer database. We followed 11,784 breast cancer cases from time of diagnosis until death, end of follow-up or last day of contact. During a median of 6 years, 1,483 women died (753 from breast cancer). We related polyphenol intake to all-cause and breast cancer-specific mortality using Cox Proportional Hazard Models with follow-time as underlying time.

Results: Among postmenopausal women, an intake of lignans in the highest quartile was related to a 36% lower risk of dying from breast cancer compared with participants in the lowest intake quartile (adjusted model: HR, quartile 4 vs. quartile 1, 0.64, 95% CI: 0.47;0.87). We found no association for other polyphenol classes. For premenopausal women no associations were found.

Conclusion: Lignans may be related to improved survival among postmenopausal women with breast cancer.

Financial support: The Danish Council for Strategic Research (ELIN research project) and Danish Cancer Society.

P304
Comparison of anti-inflammatory effects of Mango (Mangifera Indica L.) and Pomegranate (Punica Granatum L.) in rat colitis model

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Ulcerative colitis, a chronic inflammation of the large intestine, may increase the risk of human colorectal cancer. Several studies have focused on developing natural anti-inflammatory products, and have reported that different classes of polyphenolics could have different anti-inflammatory mechanisms, due to structural variations or different cellular targets. Polyphenolics from mango (mainly gallic acids and gallotannins) and pomegranate (mainly ellagic acids and ellagitannins) have previously been shown to have potent anti-inflammatory properties.

To compare the anti-inflammatory effects and possible mechanisms of mango and pomegranate polyphenolics in DSS-induced colitis, SD rats were administered the experimental beverages for ten weeks with three cycles of 3% DSS. Further investigation of the effects of chemical structures of the different polyphenols were performed with ICM docking score and in vitro study.

Both mango and pomegranate beverages decreased the level of pro-inflammatory cytokines including hs-CRP, TNF-, IL-1, and IL-6 in mucosa and IL-1 and IL-6 in serum. In the multiplex and mRNA quantitation, mango beverage suppressed the ratio of the phosphorylated/total protein of IGF-1R-AKT/mTOR axis and down-regulated the expression of *Igf1*, *Insr*, and *pik3cv* mRNA. Pomegranate decreased the ratio of the phosphorylated/total protein of p70S6K and RPS6, as well as the mRNA of *Rps6ka2, Map2k2*, and *Mapk1*. The structure of gallic acid showed higher affinity into the inhibited conformation of IGF-1R catalytic domain, whereas the ellagic acid structure showed higher affinity into the inhibited conformation of EGFR catalytic domain. Mango and gallic acid only decreased the level of IGF-1R, whereas pomegranate and ellagic acid reduced the levels of both IGF-1R and EGFR in LPS treated CCD-18Co cells.

These results suggest that polyphenolics of the different predominant structure may differentially regulate inflammation-involved pathways while attenuating DSS-induced colitis.

Financial support: National Mango Board, USA

P305
Bone targeted nanoparticles mediated metastatic tumor growth inhibition co-formulated with Paclitaxel and Kaempferol

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Background: Metastasis of breast cancer to bone is one of the most serious complications where tumor cells destroy the bone by altering the function of normal osteoblast and osteoclast. On this basis a delivery systems was designed which not only have inhibitory action on metastatic tumor cells but also can suppress the osteoclastogenesis induced by tumor cells and thus can reduce the induced osteolysis of bone. Paclitaxel (PTX) was selected to kill the metastatic breast cancer cells and Kaempferol (Polyphenol,KMP) to inhibit the cancer cell induced osteolysis by reducing the number of TRAcP-positive osteoclast. The study was designed to estimate the potential of KMP as novel antiosteoclastogenic agents for preventing and treating cancer related bone loss.

Methods: Lipidic nanoparticles (NPs) co-formulated with PTX and KMP was developed with Alendronate (ALD) as bone guided targeting ligand. The delivery system was characterized for particle size, morphology and in-vitro release. The synergistic efficacy between two drugs on cancer cells (MDA-MB 231) was established by performing cytotoxicity, apoptosis and cell cycle arrest studies.

Efficacy of KMP in reducing the osteoclast number after co-culture with MDA-MB was performed by TRAcP test.

Results: Dual drug loaded NPs in particle size range 100-200nm were developed wit spherical morphology. On MDA-MB231 cells, KMP significantly enhanced and synergies the toxicity of Paclitaxel and thereby reduce the IC50 of PTX by 3 fold. Bone binding ability of NPs was investigated by hydroxyapatite binding assay where ALD-NPs showed much higher binding as compared to plain unmodified NPs.

Osteoclastogenesis induced by co-culture of MDA-MB231 and osteoclast cells (RAW267.4) was significantly prevented in the present of KMP in dose range of (1µM to 10µM). The effect was correlated when Bone marrow derived macrophages (BMM) were used in place of RAW267.4 and co-cultured with MDA-MB 231 cells. The results suggest delivery system co-formulated with PTX and KMP can be highly potential against metastatic breast cancer.

Financial support: CSIR-CDRI

P306

Effects of selected polyphenol compounds on the nitrite induced formation of nitroso compounds in vitro.

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Background and objectives: The formation of potentially carcinogenic N-nitroso compounds are a potential explanation for the observed positive association between an increased risk of colorectal cancer (CRC) and intake of red and processed meat. Previously, we could show a close relationship between red meat and ATNC (apparent total nitroso compounds) formation. While it is known that ascorbic acid can inhibit the formation of these compounds, there is a paucity of information about the potential beneficial effect of polyphenols. In this study, we have investigated the effect of different polyphenols in a simulated stomach system.

Methods: Equal amounts of 10 mg/ml bovine serum albumin and 0.1 mg/ml aqueous solution of (–)-epicatechin, chlorogenic acid, quercetin, rutin, rosmarinic acid and taxifolin were incubated with 10 M $NaNO_2$ for 15 to 60 minutes. Samples were take at different time intervals to compare the amount of ATNC formed with those incubated with water only. ATNC were determined by selective reductive reaction to nitric oxide by iodine/iodide system followed by chemiluminescence detection. All experiments were carried out at room temperature in dark test tubes, at pH 1.2.

Results and Conclusion: ATNC levels were below the quantification limit in all reagents and components of the stomach juice. In all experiments ATNC was formed with and without the presence of polyphenols. (–)- epicatechin showed the strongest inhibition on ATNC formation with a reduction of 55% ± 3.35% at 35 minute reaction time and 78% ± 4.94% (n=2) at 50 minute reaction time. Our results demonstrate the ability of certain polyphenols to inhibit the nitrite induced formation of ATNC.

Financial Support: This work was supported by an FP7 grant of the European Union, PHYTOME.

P307

Resveratrol, a polyphenol of wine, modulates immune system and inflammation to counteract cancer progression

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Numerous epidemiological studies show that various polyphenols may protect again various diseases such as vascular diseases, cancers and inflammatory pathologies. One of the best known is the polyphenol resveratrol which is a trans-3,4',5-trihydroxystilbene and seems appear to a great interest in the prevention of these pathologies. We have recently shown that resveratrol could prevent carcinogenesis via an anti-inflammatory action through a modulation of miRNA. This inflammation could also promote tumoral progression through a modulation of the immune response. Indeed, innate immune cells and adaptive immune cells communicate through direct contact or through cytokine secretion and it is the expression of those mediators and of the pro-inflammatory status that will tip the balance towards tumor progression or anti-tumor immunity. Thus, the objectives of this study were to evaluate the potential effect of resveratrol in the immune response in mice with melanoma.

Our results highlight that resveratrol modulates differentiation of lymphocytes Th17 which exerts protumoral activity via the interleukin-17 (IL-17) production. Indeed, naïve CD4 T cells derived from C57blc/6 mice displayed less capability to differentiate into Th17 cells in vitro after resveratrol treatment. Moreover in mouse model of melanoma (B16F10), resveratrol prevented tumor growth and angiogenesis in an IL-17-dependent manner. This phenomenon could be due to a strong inhibition of the expression of the Th17-specific transcription factor Rorc at the mRNA level, which is dependent of the sirtuin-1.

This work brings a new mechanism by which resveratrol prevents cancer related inflammation through a modulation of the immune cells and interleukin production.

Financial Support: Regional Council of Burgundy.

P308

Polyphenol inhibition of benzo[a]pyrene-induced mitochondrial dysfunction and oxidative stress in an in vitro model of carcinogenesis

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Benzo[a]pyrene (BaP), one of the most potent carcinogens, causes gene mutations and cancer. The metabolites of BaP react with both nDNA and mtDNA to form bulk DNA adducts which result in DNA mutations and cancer initiation. We hypothesized that BaP may cause mutations to mitochondria which decrease mitochondrial function and increase generation of reactive oxygen species (ROS), and that certain dietary polyphenols protect against this action.

In an in vitro model of carcinogenesis, Bhas 42 mouse fibroblast cells were pre-treated with quercetin, berberine, catechin, resveratrol, and cyanidin (1, 5 and 10 µM) for 2 hours then treated with 4 µM BaP for 12 and 24 hours. Total intracellular ROS and mitochondrial superoxide were measured with 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) and MitoSOX Red, respectively Expression of mRNAs (nDNA-encoded) for UCP2 and markers of mitochondrial biogenesis (NRF1), matrix enzymes (citrate synthase and SOD2), and respiratory complexes (subunits of complexes I, III, IV and V) was measured with qPCR.

BaP significantly increased intracellular ROS (25%) mitochondrial superoxide (119%), and UCP2 (70%) compared to control cells. Almost all of the polyphenols prevented or strongly inhibited the increase in intracellular ROS and expression of UCP2. Only anthocyanins (cyanidin, kuromanin, and at 10 µM malvidin) significantly inhibited (24-39%) the increase in mitochondrial superoxide. BaP significantly decreased expression of mRNA for some mitochondrial proteins (NDUFS8, 30%; ATP5a, 35%), and mildly but non-significantly all others. The presence of one or more polyphenols reversed these changes, and in many cases increased the expression of mRNA for mitochondrial proteins, and NRF1, to levels significantly higher than in control cells.

Together the data indicate that exposure of cells to BaP increases mitochondrial and intracellular ROS and suppresses expression of nuclear genes for mitochondrial proteins. Polyphenols have potential to inhibit many of these effects of BaP.

Financial Support: Saskatchewan Health Research Foundation (SHRF)

P309

Kaempferol modulates expression of phase I and II drug-metabolizing enzymes

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Expression levels of drug-metabolizing enzymes are deeply involved in chemical-induced cancer progression and prevention. An aryl hydrocarbon receptor (AhR) induces expression of phase I and phase II drug-metabolizing enzymes directly after binding of its ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and indirectly through expression of nuclear factor-erythroid-2-related factor 2 (Nrf2). W previously demonstrated that kaempferol inhibited AhR transformation, but expression of drug-metabolizing enzymes through AhR and Nrf2 pathways is unclear yet. In this study, we examined the modulation effect of kaempferol on expression of drug-metabolizing enzymes in Human hepatoma HepG2 cells after treatment with TCDD or tert-butylhydroquinone (t-BHQ). Kaempferol was treated to HepG2 cells and then TCDD or t-BHQ was treated to the cells. CH-223191 and siRNA for Nrf2 were introduced for inhibition of AhR pathway and knock down of Nrf2 pathway, respectively. The gene and protein expression levels of phase I and phase II enzymes and Nrf2 were measured by RT-PCR and western blotting, respectively. It is confirmed that AhR modulated expression of phase I and phase II enzymes.

Kaempferol does-dependently inhibited TCDD-induced expression of cytchrome P450 1A1 (CYP1A1) as the phase I enzyme and NAD(P)H:quinone oxidoreductase- (NQO1) and glutathione-S-transferase P1 (GSTP1) as the phase II enzymes.

CH-223191 suppressed not only TCDD-induced CYP1A1 but also NQO1 and GSTP expressions. Moreover, Kaempferol dose-dependently inhibited t-BHQ -induced NQO1 and GSTP1 expressions. Thus, kaempferol inhibits not only TCDD-induced expression of phase I and phase II enzymes but also t-BHQ-induced phase II enzyme by modulating the AhR and Nrf2 pathways.

Financial Support: JSPS KAKENHI Grant Number 2529207and Coordination Funds for Promoting Science and Technology, Creation of Innovation Centers for Advanced Interdisciplinary Research Areas (Innovative Bioproduction Kobe), MEXT, Japan.

P310

Resveratrol chemosensitizes colon cancer cells to apoptosis induced by rosiglitazone a role for peroxisome proliferator-activated receptor gamma

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Resveratrol (trans-3,4',5-trihydroxystilbene), a main wine microcomponent, has been widely described as a chemopreventive agent. Researches performed since the last decades in vitro, in animal models and in (pre)clinical studies have pointed out its pleiotropic health benefits by acting on multiple signaling pathways which go beyond its originally described direct antioxidant activity. One of its potential intracellular targets, as suggested in a neuronal model, is peroxisome proliferators activated receptors (PPARs). We sought to determine in colorectal cancer cells whether PPAR could be involved in resveratrol-induced cell death.

In our cell lines, we showed that PPARs agonists strongly decreased tumoral cell proliferation and contributed to resveratrol-induced cell cycle arrest. In addition, we found that PPARgamma agonist rosiglitazone enhanced resveratrol-induced cell death. Inhibition of PPARgamma with its specific antagonist GW9662 or by transient transfection of cancer cells with a dominant-negative PPARgamma mutant abrogated resveratrol-induced effects. Tumor cells death was also potentiated by combining resveratrol with rosiglitazone. Altogether our data show that PPARgamma contributes to resveratrol-induced colon cancer cells death and suggest that the combination of this polyphenol with PPARg agonists could be relevant as a new therapeutic approach to treat digestive cancers.

Financial Support: "Ligue Inter-Régionale Grand-Est contre le Cancer"

P311

Short-term effects of food rich in polyphenols on DNA-Integrity: results of two intervention studies

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DNA damage is associated with the pathogenesis of many degenerative diseases. Intake of foods rich in polyphenols may contribute to reduce the risk of these diseases, by virtue of supporting or improving physiological defence. Intervention studies have indicated long-term consumption of coffee and/or fruit juice to relate to improved DNA integrity in healthy volunteers [1,2]. However, short-term kinetics of such effects haven't been reported yet. We report on two independent intervention studies, with coffee and with anthocyanin-rich juices, to study short-term effects on DNA integrity.

In the first human intervention study 13 healthy male volunteers consumed coffee brew during eight hours (four times 200 ml every second hour). Blood samples were taken at baseline, immediately before the first coffee intake, and subsequently every two hours. The results showed a significant ($p<0.05$) reduction of background DNA strand breaks already two hours after the first coffee consumption in comparison to baseline. Further coffee intake was associated with incremental decrease of background DNA damage ($p<0.01$ and $p<0.001$, respectively).

In the second intervention study, five healthy male volunteers consumed a bolus (700ml) of four different anthocyanin-rich drinks. Blood samples were drawn at the beginning, two and eight hours after intake. In this study, only one was found to significantly decrease total DNA strand breaks ($p<0.01$) in white blood cells eight hours after juice consumption.

Taken together, the studies clearly showed that short-term consumption of coffee and anthocyanin rich juice has the potential to reduce DNA damage.

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Financial Support: German Ministry of Economics and Technology (via AiF), cluster project no. 18068 N and by Tchibo GmbH.

P312

Volatile and phenolic compounds, cupric ion reducing antioxidant capacity and cytotoxic activity of organically and conventionally grown Brassica vegetables

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Phenolic and sulfur compounds of Brassica vegetables are tough to be the key molecules responsible for their antioxidant and cancer chemopreventive role. Cropping conditions can affect content of these compounds.

The objective of this study was to determine volatile profiles, total phenolic (TPC) and flavonoid content (TFC), cupric ion reducing antioxidant capacity (CUPRAC) and cytotoxic activity of three Brassica varieties (broccoli, cauliflower and kohlrabi) organically-O and conventionally-C grown. The main volatiles of fresh vegetable tissues were detected by headspace GC-MS. TPC, TFC and CUPRAC of vegetable juices were determined by colorimetric methods [1, 2, 3]. Cytotoxic activity was determined by sulforhodamine B assay on human cervical cancer (HeLa), human colon adenocarcinoma (HT-29), human breast adenocarcinoma (MCF-7), human Burkitt` lymphoma (Raji) and one healthy cell line –human fetal lung fibroblast (MRC-5).

Degradation products of linoleic acid, S-methyl-L-cysteine sulfoxide and aliphatic glucosinolate sinigrin, were the main volatiles identified by headspace GC-MS. TPC ranged from 338.2 (Kohlrabi-C) to 743.9 (Cauliflower-C) mg GAE/L, TFC from (Cauliflower-O) to 373.7 (Broccoli-C) mg QE/L and CUPRAC from 1.3 (Kohlrabi-C) to 4.0 (Broccoli-C) mM TE. Significantly higher TFC of vegetables-C (broccoli, cauliflower, $p< 0.01$; kohlrabi, $p<0.05$) and cupric ion reducing antioxidant capacity of broccoli-C and cauliflower-C ($p<0.01$) were recorded. Investigated juices showed cytotoxic effect against all cell lines. Broccoli-C and kohlrabi-O were the most active agents against HeLa cells (5.82 and 6.82% of cell viability, respectively) Cropping conditions affected content of health–promoting compounds and biological activity of Brassica plants. The most promising among selected varieties was broccoli-C.

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Financial Support: National Grant of the Republic o Serbia: No 172058 and Secretary for Sciences and Technological development of the Province Vojvodina, RS.

P313

Elucidating polypharmacological anti-cancer mechanisms of the xanthone polyphenol mangiferin

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The identification of biologically active and potentially therapeutically useful polypharmacological natural products has been a long-term focus in cancer research. Drugs designed to act against individual molecular targets usually cannot combat multigenic diseases such as cancer. Combination therapies or natural compounds that impact multiple targets simultaneously are better at controlling complex disease systems and are less prone to drug resistance.

The xanthone polyphenol mangiferin purified from Mangifera Indica holds much promise as a potential novel cancer chemopreventive or therapeutic compound. The in vivo antitumor activity of mangiferin was tested in in three syngeneic murine colon cancer models with CT26.WT cells. In ectopic /subcutaneous allograft and induced lung metastasis models, a direct relationship between the significant reductions of tumor volume, the formation of pulmonary metastatic nodules surface and survival was observed with increasing oral dose of mangiferin. In the tumor angiogenesis model, macroscopic observation showed a reduction of blood supply and tumor size. Histological analysis revealed a decreased number of blood vessels with increased concentration of mangiferin. To further elucidate the polypharmacological anti-cancer mechanisms of mangiferin, we further analyzed corresponding tumor gene expression profiles following in vitro/in vivo treatment with mangiferin.

Subsequent transcriptome based pathway and drug target enrichment analysis revealed that the medicinal effects of mangiferin extend far beyond its well-known antioxidant activities by regulating various antimetastatic and antiangiogenic networks.

Financial Support: VLIR UOS programme nr. ZEIN2011PR383 Belgium-Cuba and Project of Cuban Ministry of Health nr. 1401049.

P314
Soy Foods and Biomarkers of Breast Cancer Risk: Is the Relation Different in Asians?

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Background/Objectives. Based on observational studies, soy food consumption may provide protection against breast cancer in Asian but not in Western populations. We examined if concentrations and effects of soy foods on biomarkers of breast cancer risk differed between Asian and non-Asian women in two soy intervention studies.

Methods. The Breast, Estrogen, and Nutrition study (BEAN1) randomized 220 women to a 2-year clinical trial to examine the effects of 2 daily soy servings on sex steroids and mammographic densities. BEAN2, a randomized, crossover design wit 96 women, examined the effects of 2 daily soy servings on sex steroids and nipple aspirate fluid (NAF). The following biomarkers were assessed using standard methods: NAF volume, estrogens in serum, urine, insulin-like growth factor-1(IGF-1), inflammatory markers in serum, and mammographic densities. Mixed linear models were applied to assess ethnic differences in biomarker levels and on intervention effects related to soy foods.

Results. CRP and leptin, NAF volume and estrone sulfate were lower, while urinary isoflavones were higher in Asian than non-Asian women. The high soy diet was significantly associated with higher urinary isoflavone excretion in all women ($p<0.0001$) before and after stratification by Asian ethnicity ($p<0.0001$ for both). A significant interaction ($p=0.01$) between ethnicity and high soy diet was observed for IGF-1 levels but not for other biomarkers.

Conclusions. The current findings in a small number of premenopausal women suggest possible differences in biomarker levels by ethnicity but only a modest influence, if any, of soy intake.

Financial Support: grant R01CA 80843 from the US National Cancer Institute

P315
Resveratrol alters lipid profile and plasma membrane microdomains of MCF-7 cancer cells

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Resveratrol produced by grapes exhibit chemopreventive properties against breast cancer. It suggests that resveratrol-induced apoptosis involves its endocytosis by lipid rafts. Rafts are membrane microdomains rich in sphingolipids and cholesterol. The aim of this study was to investigate the effect of resveratrol on the lipid profile and plasma membrane microdomains of MCF-7 cancer cells.

MCF-7 cells were cultured in DMEM medium complemented with 10% (v/v) fetal bovine serum. After 24 hours of resveratrol (120µM DMSO (0,5%,control) and ethanol (0,1%, control)) treatment the membrane microdomains were purified with the non-ionic detergent treatment (Triton X-100 1% at 4°C), followed by sucrose gradient ultracentrifugation for 22 hours at 4 °C. Different fractions (1 mL/fraction) were collected from the top of the gradient (fraction 1) to the bottom (fraction 12). The lipid composition of membrane was determined by High Performance Thin-laye Chromatography (HPTLC) and the content of flotillin-1 was analyzed by Dot-Blotting in each fraction.

The ultracentrifugation buoyant material was observed in 5-30% sucrose gradient interface. The HPTLC shows that resveratrol changes neutral lipid contents by increasing the distribution of lipids along the fractions. Lipids were identified as CHOE, TG, FA, CHO+DG e MG. Dot-Blotting analyzes show the presence of flotillin-1, typical protein of lipid rafts. Results indicate a tendency of resveratrol move the rafts to the right. Our results suggest that resveratrol treatment modified lipid profile and lipid rafts, however, is necessary the use of techniques such as gas chromatography MS/MS and fluorescence microscopy for higher accuracy of results

Financial Support: CNPq / FAPERJ / CAPES / Fundação do Câncer

P316

Dietary anthocyanins from purple corn ameliorates doxorubicin-induced cardiotoxicity in vivo and in vitro

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Doxorubicin (DOX) is an anthracycline antibiotic widely used as chemotherapeutic agent for many solid and haematological cancers. Its clinical use is restricted by a cumulative dose-dependent and irreversible cardiotoxicity that can cause progressive cardiomyopathy and congestive heart failure. Doxorubicin has been postulated to induce cardiotoxicity mostly through redox cycling and ROS generation. A cardioprotective therapy that can decrease heart damage without reducing the anticancer efficacy during DOX therapy is of outmost importance. Here, we validate the protective role of an anthocyanin-rich diet against DOX-induced cardiotoxicity by comparing corn isogenic lines with high levels of anthocyanins (purple corn, PC diet or without anthocyanins (yellow corn, YC diet) incorporated in standard rodent diets In mice fed PC diet, dietary anthocyanins from purple corn attenuated DOX-induced mortality and prevented cardiac histopathological alterations, which were instead observed in mice fed YC diet, such as disorganized myofibrils, fragmentation and degradation of mitochondria, defects in sarcolemma junctions. We then verified *in vitro* whether pretreatment with anthocyanins was able to repress the DOX-induced cytotoxicity in HL-1 cardiomyocytes and to preserve the antitumoral activity of DOX in breast cancer cells. Indeed, our results indicated that pretreatment with both yellow and purple corn extracts repressed the DOX-induced cytotoxicity without affecting the antitumoral effect of DOX, suggesting that not only antocyanins, but also other flavonoids present in yellow corn have a cytoprotective effect. Nonetheless, both yellow and purple corn extracts contributed to block the DOX-induced transcriptional activation of the RAS system genes in HL-1 cardiomyocytes *in vitro*.

Financial Support: Fondazione Umberto Veronesi Grant 2012 and EU FP7 ATHENA (Grant Agreemen245121)

P317

Vitis cell culture as source of new polyphenols with potential anti-tumoral properties

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For years, new strategies of anticancer treatments developed with the aim of looking for new molecules increasing the sensibility of the tumoral cells in the conventional therapies. Resveratrol, a powerful antioxidant, participates, in particular, to delay the ageing opposes the neurodegenerative diseases but also acts on the several phases of the carcinogenesis which are initiation, promotion, and tumor progression.

An innovative method of production allowed the synthesis of various polyphenols directly in the culture medium. Grapevine-cell suspensions of Vitis labrusca (Concord) performed in flasks and bioreactors, were used for the production of resveratrol and derivatives in response to various types of inducing elicitors. Thus w could study, in vitro, the action of these various molecules on a model of cutaneous cancer: the melanoma which is the most serious skin tumors. Chemotherapy is not very effective in this kind of cancer and the use of new therapeutic molecules is therefore necessary to fight against metastatic dissemination.

In this work, we studied the action of our bio-produced molecules on 2 human melanoma cell lines, the SK-Mel 28 and HT144. Firstly, the antiproliferative activities of polyphenols were determined to evaluate the treatments concentrations. Secondly, we have studied the molecular mechanisms of these bioactive products, where we observed i) a disruption on cell cycle progression, ii) a modulation of the migratory properties, and, iii) a modulation of the regulation of the expression of proteases.

These results highlight effectiveness of bioactives polyphenols synthetized by an innovative method of production on melanoma cells proliferation and invasiveness.

Financial Support: Champagne-Ardennes region

P318
Quercetin derivatives induced p53 independent apoptosis in HCT116 human colon carcinoma.

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Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is one of the most abundant flavonol-type flavonoids. It has been reported to exhibit antioxidative, anticarcinogenic, anti-inflammatory, anti-aggregatory, and vasodilating effects. Therefore, quercetin is an attractive natural compound for cancer prevention. The hydroxyl groups of flavonoids are important for their bioactive functions and also prone to be oxidated to quinones. To directly demonstrate the role of hydroxyl groups, we have synthesized acetyl and benzyl derivatives of quercetin, 3,7,3',4'-*O* -tetraacetylquercetin (4Ac-Q) and 3,7,3',4'-*O*-tetrabenzylquercetin (4Bn-Q), which substituted the hydroxyl groups of quercetin with acetyl or benzyl groups at the3,7,3',4' positions. In this study, we investigated the anti-proliferative effects and molecular mechanisms of these quercetin derivatives in wild-type p53-carrying (p53+/+) and p53 null (p53-/-) HCT116 human colon carcinoma cells, which can be used to test p53-dependence for apoptosis.

4Ac-Q and 4Bn-Q were synthesized and determined by NMR, IR spectral data. HCT116(p53+/+) and HCT116(p53-/-) cells were cultured in DMEM supplemented with 10% FBS and used for the examination. Cell viability was determined by MTT assay. Apoptosis induction was characterized by DNA fragmentation, caspase-3 activation, and poly (ADP-ribose) polymerase (PARP) cleavage and quantitated by Annexin V/PI assay. Reactive oxygen species (ROS) were detected by flow cytometric analysis of MitoSOX red fluorescence.

Exposure of HCT116 cells to quercetin and 4Ac-Q resulted in apoptosis induction but not 4Bn-Q. Quercetin revealed a p53-independenct apoptosis induction. Moreover, 4Ac-Q revealed stronger apoptotic activity than parent quercetin. Cell viability inhibition and apoptosis induction resulting from quercetin and 4Ac-Q exposure were markedly diminished in the presence of antioxidants, MnTBAP (a superoxide scavenger), in HCT116(p53+/+) cells but less impact on 4Ac-Q-induced apoptosis in HCT116(p53-/-) cells.

These findings provide a valuable strategy to increase the sensitivity of HCT116 human colon carcinoma cells toward apoptosis by modifying quercetin structure.

Financial Support: JSPS KAKENHI #26850084 National Grant

P319
Improved anticancer activity of a phenolic-enriched ITC extract

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In recent years, the recovery of bioactive ingredients from natural sources has received considerable attention for their potential health benefits. Watercress is a species of Brassicaceae family, which is known for its high concentration in phytochemicals such as flavonoids, phenolic acids, carotenoids, glucosinolates and their hydrolysis products, isothiocyanates (ITCs).

In this work, high pressure extraction was explored in order to recover ITCs and phenolic compounds, with anticancer potential, from watercress. Extractions were performed using supercritical CO_2 and a mixture of CO_2:ethanol(80:20, % w/w) on freeze-dried plants (humidified with water to promote glucosinolate hydrolysis) at 35^{o}C and 25 MPa for 2 hours. The resulting extracts were characterized in terms of ITCs and phenolic content using HPLC-DAD and HPLC-MS/MS, respectively, and antioxidant capacity was assessed by ORAC, HORAC and HOSC assays. Watercress extracts were tested in a human colorectal adenocarcinoma cell line (HT-29), in order to evaluate their potential as cancer cell proliferation inhibitors.

By using supercritical CO_2as solvent it was possible to obtain highly ITC-rich extracts (27.0±2.4µmol ITC/g dry water cress) with promising anticancer activity. Additionally, when a CO_2:ethanol mixture was used as solvent, an extract with a higher phenolic content and consequently higher antioxidant capacity was obtained. These CO_2-expanded ethanol extract revealed to be the most promising cancer cell-growth inhibitor. This result might be related with the chemoprotective effect of flavonol and hydroxycinnamic acid derivatives present in watercress extracts and the positive synergistic effects between these phytochemicals.

Financial Support: Portuguese Fundaçãopara a Ciência e Tecnologia (FCT) through the PTDC/AGR-TEC/3790/2012 project.

P320

Rosemary (Rosmarinus officinalis) extract modulates NRF2 pathway in human colon cancer cell lines and decreases xenograft tumor growth in animal model

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Carnosic acid, carnosol and rosmarinic acid are the main compounds present in *Rosmarinus officinalis* extract (RE) and have shown anti-proliferative effects on various tumor cell lines. The objective of this work was to study the anticancer effect of rosemary polyphenols on different human colon cancer cell lines (HGUE, HT29 and SW480) and in nude mice to explore its putative mechanism.

For this purpose, intracellular free radical generation and modulation of nuclear facto erythroid 2 [NF-E2]-related factor 2 (NRF2) pathway was explored. Cells were treated with RE (10-40 μg/L) for 24h at 37°C and cell viability was determined by MTT assay. Reactive oxygen species (ROS) production was measured with DCF-DA In addition, cells were transfected with Nrf2-targeting siRNA to determine the potential role of this transcription factor. To test the antitumor effects of RE, the evolution of colon cancer xenografts was followed into several groups of nude mice (untreated, pretreated for two weeks and treated) during four weeks receiving 200 mg/kg rosemary polyphenols, either intra peritoneally or by oral gavage.

The inhibition of cell proliferation was correlated to an increase of intracellular ROS content by RE. Furthermore, NRF2 silencing in siRNA experiments decreased cell viability in SW480 cells, then the molecular mechanism seems to involve activation Nrf2/ARE pathway as a response for cell survival at least in this cell line. The results of the animal trial showed a significant inhibition of the tumor growth in the group pre-treated with rosemary polyphenols compared to the untreated group.

In conclusion, the antiproliferative effects of a rosemary extract obtained by CO_2 -supercritical fluid extraction may be related to their pro-oxidative capability by increasing the intracellular generation of ROS, which leads to the activation of NRF2 as a response for cancer cell survival. Oral treatment with rosemary polyphenols can inhibit the growth of colorectal cancer in nude mice model.

Financial Support: AGL2011-29857-C03-03 (Spanish Ministry of Science and Innovation),PROMETEO/2012/007, ACOMP/2013/093 and VALi+D fellowshipsACIF/2010/162 and ACIF/2013/064 (Generalitat Valenciana) and CIBER (CB12/03/30038, CIBERobn, Instituto de Salud Carlos III).

P321

Study of the synergic behavior of the antiproliferative effect of Rosmarinus officinal compounds in a colon cancer cell model

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We have previously reported the antiproliferative effect of a rosemary extract (*Rosmarinus officinalis*) obtained by using supercritical fluids extraction in several colon cancer cell models. Four major compounds of the extract, betulinic acid, ursoli acid, carnosic acid and carnosol have previously shown antitumor effect in isolated form in cell models under different mechanisms, but the potential additive/synergistic interactions between them remain unknown.

In this study, triterpenes exhibited a higher antiproliferative effect than diterpenes, being ursolic acid the most active one with an IC_{50}value close to10μM. Then, pairwise combinations of these compounds were evaluated in order to determinate their putative pharmacological interactions (synergy, additivity or antagonism). FICI values were calculated and the isobolole graphical method was used to determine the interaction between the compounds. Furthermore, the specific software Compusyn, specially designed to study pharmacological interactions, was utilized and the Combination Index (CI) at different percentage of the antiproliferative effect was determined.

The results showed that, with the exception of the betulinic acid and ursolic acid combination that showed antagonism, all the different combinations exhibited either additive or weak synergistic behaviour. These results could be explained by the capacity of these two compounds to modulate the NF-B pathway in a different way.

Finally, our results confirm the convenience of the use of the supercritical fluid rosemary whole extract compared to pure compounds for further research.

Financial Support: AGL2011-29857-C03-03 (Spanish Ministry of Science and Innovation),PROMETEO/2012/007, ACOMP/2013/093 and VALi+D fellowshipsACIF/2010/162 and ACIF/2013/064 (Generalitat Valenciana) and CIBER (CB12/03/30038, CIBERobn, Instituto de Salud Carlos III).

P322
An Investigation into the Effect of Quercetin on Human Colorectal Adenocarcinoma Cells

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Quercetin and its conjugates are widely distributed in plants and are considered to have therapeutic effects on the cardiovascular system, as well anti-inflammatory and anti-allergic properties. Furthermore, quercetin inhibits growth of certain cancer cell-types. Interest exists as to the role flavonoids in cancer prevention in the gut.

Interestingly, faecal water concentrations of quercetin (aglycone) are in the range of 0.16–1.30μM.Faecalwatercontainssubstancesthatcanmodulateenzymeactivityi Caco-2 cells, albeit the effects studied are more closely related with the gut flora catabolites of quercetin than the quercetin itself. The aim of this project is to investigate the impact of quercetin on cell death pathways in human colorectal adenocarcinoma cells.

The specific objectives of this study are (1) to identify the effect of quercetin on the growth of Caco-2 cells, (2) to investigate whether quercetin induces growth inhibition of Caco-2 cells through one of the cell death pathways, and (3) to measure role of quercetin in modulating intracellular reactive oxygen species (ROS) generation.

Quercetin reduced the viability of Caco-2 cells and the IC_{50} was 32±1.2μM after 48 h. At low quercetin concentrations (10 M) apoptosis was the major route of cell death. Yet, at higher concentrations (150 M) the necrotic cell death pathway became predominant. *Tert*-butyl hydroperoxide (*t*-BHP, 3000 M) reduced Caco-2 ceviability by ~45 % but quercetin (0.2-20 M) was not protective. Measurement of RO generation revealed that *t*-BHP increased ROS by 65-fold compared to control and quercetin (10 M) had a protective effect as ROS production was reduced to only 7-fold that of control.

Quercetin may have potential therapeutic properties at concentrations achievable in the gut. Future investigations will involve the improvement of an *in vitro* model and study the synergistic interactions between different polyphenols, including quercetin, to delineate this potential.

Financial Support: University of Surrey

P323
Cytotoxic effect of phenolic acids in human colon adenocarcinoma cells

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Colorectal cancer is a major cause of morbidity and mortality throughout the world Issues related to the role of diet in the prevention and treatment of cancer are feature each year. In this context, several studies have observed an inverse association between consumption of phenolic compounds and the risk of development of chronic diseases including cancer. This study aimed to evaluate the effects of phenolic acids on cell viability, cell cycle and apoptosis in human colon adenocarcinoma cells (HT-29).

HT-29 cells were incubated with different concentrations (0.1 to 100 M) of caffeic, p-coumaric, ferulic, vanillic and 3,4-dihydroxyphenylacetic acids for 24 h. Cell viability was measured using MTT method and cell cycle and apoptosis by flow cytometry. A potent inhibitory effect on HT-29 cell viability was observed after 24 h of treatment. The higher inhibitory growth was observed for 3,4-dihydroxyphenylacetic acid (78%) followed by ferulic acid (62%), caffeic acid (43%), p-coumaric acid (39%) and vanillic acid (16%). Moreover, p-coumaric acid promoted a cell cycle arrest in G2/M phase. In contrast, 3,4-dihydroxyphenylacetic acid promoted a cell cycle arrest in G0/G1 phase as well an increase in apoptotic cells.

Our results showed that phenolic acids presented cytotoxic effect in human colon adenocarcinoma cells, being 3,4-dihydroxyphenylacetic acid more highlighted. Considering that 3,4-dihydroxyphenylacetic acid is an important colonic metabolite of phenolic compounds, it may be a viable effective constituent for colon cancer prevention and treatment.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), ConselhoNacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação deAmparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

P324
An Investigation into the Effect of Quercetin on Human Colorectal AdenocarcinomaCells

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Quercetin and its conjugates are widely distributed in plants and are considered to have therapeutic effects on the cardiovascular system, as well anti-inflammatory and anti-allergic properties. Furthermore, quercetin inhibits growth of certain cancer cell-types. Interest exists as to the role flavonoids in cancer prevention in the gut. Interestingly, faecal water concentrations of quercetin (aglycone) are in the range of 0.16–1.30μM. Faecal water contains substances that can modulate enzyme activity in Caco-2 cells, albeit the effects studied are more closely related with the gut flora catabolites of quercetin than the quercetin itself. The aim of this project is to investigate the impact of quercetin on cell death pathways in human colorectal adenocarcinoma cells.

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Quercetin may have potential therapeutic properties at concentrations achievable in the gut. Future investigations will involve the improvement of an in vitro model and study the synergistic interactions between different polyphenols, including quercetin, to delineate this potential.

Financial Support: University of Surrey

P325
Polymeric nanoparticles for controlled delivery of bioactive RSV for prostate cancer chemoprevention/chemotherapy

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Nanoencapsulation of antiproliferative and chemopreventive trans-resveratrol (RSV) is likely to provide protection against degradation, enhancement of bioavailability, improvement in intracellular penetration and control delivery. In this study, polymeric nanoparticles (NPs) encapsulating RSV (nano-RSV) as novel prototypes for prostate cancer (PCa) treatment were designed and evaluated using PCa cells.

Nanosystems, composed of a biocompatible blend of poly(epsilon-caprolactone) (PCL) and poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol) conjugate (PLGA-PEG-COOH), were prepared by a nanoprecipitation method, and fully characterized. Cellular uptake of NPs was evaluated in PCa cell lines DU-145, PC-3, and LNCaP by confocal fluorescence microscopy, and antiproliferative efficacy was assessed using MTT assay.

With encapsulation efficiencies of 74%-98%, RSV was successfully loaded in PCL:PLGA-PEG-COOH NPs, which showed an average diameter of 150 nm. NPs were able to control the RSV release at pH 6.5 and 7.4, mimicking the acidic tumora microenvironment and physiological conditions, respectively, with only 55% of RSV released within 7 h. In gastrointestinal simulated fluids, NPs released 55% of RSV in the first 2 h in acidic medium, and their total RSV content within the subsequent 5 h at pH 7.4. Confocal fluorescence microscopy revealed that NPs were efficiently take up by PCa cell lines. Furthermore, nano-RSV significantly improved the cytotoxicity compared to that of free RSV toward all cell lines, at all tested concentrations (from 10 M to 40 M), proving a consistent sensitivity toward both the androgen-independent DU-145 and hormone-sensitive LNCaP cells.

Our findings support the potential use of developed nanoprototypes for the controlled delivery of RSV for PCa chemoprevention/chemotherapy.

Financial Support: M.S. was supported by Grant CRP 25920 of “Legge regionale n. 7/2007, promoziondella ricerca scientifica e dell’innovazione tecnologica in Sardegna, - Annualita2010”. I.A.S. was supported by ACS Grant 120038-MRSG-11-019-01-CNE.

Poster Session E: Polyphenols in Cardiovascular Diseases

P400

Orange juice flavanones reduce the transient decline in postprandial endothelial function in humans elicited by high fat consumption

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Flavonoid-rich foods and beverages have been avidly investigated in regards to their positive effects on vascular function, with recent evidence suggesting that they might be protective through their actions in affecting postprandial metabolic and vascular disturbances.

In the present study we investigated the postprandial time-dependent effects of increasing flavanone levels, delivered in orange beverages, on endothelial function.

A four-arm acute, randomized, controlled, double-blind postprandial crossover trial was conducted in 28 middle aged healthy men (30-65 yrs) when they consumed a high-fat double meal (delivering 81 g of fat). The impact of orange-derived flavanon intake on postprandial endothelial function was assessed with flow-mediated dilatation (FMD) at 0, 2, 5 and 7 h post-consumption of beverages containing either

a) 128.88 mg (Orange Juice), b) 272.14 mg (Advantaged Orange Juice), c) 452.80 m (Whole blended Orange) of total flavanones or d) a control drink matched for macro and micronutrients (0 mg flavanones). Circulating plasma levels of flavanones, naringenin and hesperetin, and Nitric Oxide (NO) species were also measured.

We showed that flavanone rich-beverages containing levels of flavanones ranging from 128 mg to 452 mg were effective at reducing the transient impairment in FMD induced by the double meal challenge at 7 hours post-consumption. Such effects coincided with the absorption into circulation of the main flavanones, naringenin and hesperetin, and was also accompanied by higher levels of plasma Nitrite in comparison to control.

Orange-derived flavanones are effective at counteracting the negative effects of a high-fat double meal on endothelial function, possibly by the actions of circulating metabolites on NO levels.

Financial support: This work was supported by PepsiCo Inc. (PEP-1122).

P401

Association between urinary total polyphenols excretion and clinical cardiovascular risk factors after 5 years of follow up, in a Mediterranean population at high cardiovascular risk

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Several epidemiological studies have shown an inverse association between the consumption of polyphenol-rich foods and the risk of cardiovascular disease. Urinar total polyphenols excretion was considered as a reliable biomarker for polyphenols intake.

Our aim was to assess the association between urinary total polyphenols excretion (TPE) and clinical cardiovascular risk factors (glucose, cholesterol, HDL-cholesterol LDL-cholesterol, triglycerides, systolic blood pressure, and diastolic blood pressure) in a population at high cardiovascular risk.

A baseline cross-sectional study was performed with 573 men and women (aged 67.3±5.9) from the PREDIMED trial. TPE was measured using Folin-Ciocalteu method with assistance of solid phase extraction (SPE) performed in 96-well plate cartridges (Oasis MAX). Anthropometric and clinical measurements were collected yearly during follow-up. Participants were categorized into three groups according to changes in TPE. Multiple linear regression models were used to assess relationships between TPE and clinical cardiovascular risk factors, adjusting for potential confounders.

After 5 years of follow-up, significant positive correlations were observed between changes in TPE, a biomarker

of total polyphenols intake, and triglycerides (=-8.563; P=0.007), glucose (=-4.164; P=0.036), and diastolic blood pressure (=-1.316; P=0.013). Our results suggest that higher polyphenol intakes, measured as TPE in urine, exert a protective effect on cardiovascular risk factors.

Financial support: CICYT (AGL2013-49083-C3-1-R) from the Spanish Ministry of Science and Innovation (MICINN), the Generalitat de Catalunya (GC) 2014 SGR 773 and the Instituto de Salud Carlos III, ISCIII (CIBEROBN-CB06/03). CSC (201306990001) from China Scholarship Council.

P402

Are really flavonoids able to revert catecholamine myocardial injury?

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Many epidemiological and in vitro studies have suggested potentially positive effects of flavonoids on cardiovascular diseases. But could flavonoids influence one of the most severe consequences of cardiovascular disorders, acute myocardial infarction (AMI)? Outcomes from coronary artery ligation studies are not in full agreement, but interestingly, one group has repeatedly shown that oral administration of different flavonoids was fully protective in a catecholamine model of AMI. We have recently questioned such results because of lack of clear mechanism(s) of such full protection and improbable high linear relationship between the dose and the effect. Additionally our experiments performed with the same experimental design have shown no protection against cardiac injury but have demonstrated a positive effect on the vascular system.

Since pharmacokinetics of flavonoids is very complicated and, in general, orally administered flavonoids result only in low concentration of parent compounds in plasma, we decided to test intravenous administration of rutin in the mentioned mode and to screen if metabolites formed from quercetin by the gut microflora or human enzymes might have some effects on the cardiovascular system.

Surprisingly, rutin deteriorated the injury caused by catecholamine isoprenaline likely due to its pro-oxidative properties. On the other hand, at least two bacterial metabolites of quercetin dose-dependently relaxed isolated arteries and decreased the blood pressure in rats while the most abundant metabolite of quercetin with preserve flavonoid structure quercetin-3-glucuronide was inactive.

In conclusion, flavonoids do not appear to fully revert severe cardiac injury but their bacterial metabolites seem to have positive vascular effects.

Financial support: Charles University in Prague grant No. 253115

P403

Beneficial effect of a fermented bean powder on ox-LDL induced endothelial dysfunctions

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Many clinical and experimental studies support a direct role of microcirculatory dysfunction in the genesis of target organ damage during oxidative stress and dyslipidemia. Due to a chronically enhanced oxidative stress, diabetes and hypertension are characterized by high levels of oxidized-LDL (ox-LDL) and microcirculatory dysfunction.

The aim of this study was to characterize and evaluate the protective effect of a fermented bean powder (Lys LJ) on endothelial dysfunctions induced by ox-LDL treatment.

Compared to the unfermented powder, Lys LJ contained a higher polyphenols and flavonoids content and showed a better antioxidant activity ($p<0.01$). Human microvascular endothelial cells (HMEC-1) were pre-treated 1hour with 0.7mg/ml Lys LJ extract, then exposed 24hours to 200µg/ml ox-LDL. The oxidation degree, induced by ox-LDL treatment, was assessed by malondialdehyde production. ICAM-1, ET-1 and IL-6 medium levels were measured by ELISA, while LOX-1 and CHOP gene expression were analyzed using quantitative Real-Time PCR (qRT-PCR). Oxidized-LDL treatment induced a significantly higher oxidative stress ($p<0.01$), which was completely reduced by Lys LJ pre-treatment. Besides, compared to basal condition, a significant increment of ICAM-1, IL-6 and ET-1 levels ($p<0.05$ for all) was found after ox-LDL exposure. Otherwise, Lys LJ pre-treatment exerted an inhibitory effect on ox-LDL-induced endothelial dysfunctions with ICAM-1 levels comparable to control; however, IL-6 and ET-1, although reduced, resulted still higher than control ($p<0.05$). Moreover, qRT-PCR analysis showed a significant rais of both LOX-1 and CHOP gene expression after ox-LDL treatment ($p<0.05$), which completely abolished by Lys LJ pre-treatment. Finally, Lys LJ alone did not induce any differences in ICAM-1, IL-6 and ET-1 concentration, as well as LOX-1 and CHOP gene expression, compared to control.

Our data suggest an improved endothelial functions in the oxidative stress-exposed cells after Lys LJ pre-treatment, which was able to inhibit the oxidation process, but poorly active against inflammation and ET-1 production.

P404

Nutraceutical properties of whole bergamot extract against LPS-induced endothelial activation and dysfunction

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Inflammation and oxidative stress are key factors of vascular endothelial dysfunction underlying many chronic diseases, such as diabetes, obesity and cardiovascular disease.

The aim of this study was to characterize and evaluate the antioxidant capacity of a lyophilized powder from whole-bergamot fruit. Moreover, we investigated on human microvascular endothelial cells (HMEC-1) its protective effect against LPS-induced endothelial activation and dysfunction.

Bergamot extract (BE) was analyzed for total polyphenols, flavonoids, flavonols and ascorbic acid content. We also investigated its antioxidant property by in vitro assay (ORAC, DPPH and ABTS) and ex vivo human erythrocytes (CAA-RBC and hemolysis test). HMEC-1 were 1hour pre-treated with 50µg/ml BE, then exposed 6hours to 0.1ng/ml LPS. Specifically, we evaluated IL-6, ICAM-1, ET-1 and CHOP gene expression using quantitative Real-Time PCR. Furthermore, we assessed the cellular ROS production and quantified the NO levels in the medium by a colorimetric assay.

Our results showed high levels of bioactive compounds and in vitro antioxidant activity; besides, a better cellular antioxidant activity and anti-hemolytic effect was detected following BE pre-treatment ($p<0.01$). Moreover, LPS-exposed HMEC-1 resulted in a significant up-regulation of IL-6, ICAM-1, ET-1 and CHOP gene expression compared to control ($p<0.001$). Otherwise, BE pre-treatment exerted a significant inhibitory effect on LPS-induced gene expression reducing IL-6, ET-1 and CHOP induction ($p<0.05$). However, after BE pre-treatment, ICAM-1 expression was significantly reduced compared to LPS-treated cells ($p<0.01$) but still higher than control ($p<0.05$). Furthermore, compared to unexposed cells, we found a higher ROS production and a reduced NO level following LPS exposure, which is completely reversed by BE pre-treatment.

This preliminary data suggest that BE pre-treatment is able to prevent and protect HMEC-1 from LPS-induced endothelial activation and dysfunction. This protective effect is probably linked to the high content of bioactive compounds that exhibit goo radical scavenging and anti-inflammatory activities.

P405

Higher fruit intake, especially citrus, is associated with lower blood pressure within adults in the United Kingdom's National Diet & Nutrition Survey Rolling Program (2008/09 – 2011/12)

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High flavonoid intake is associated with lower risk of cardiovascular disease (CVD). Intervention studies report inverse associations specifically between hesperidin intake and blood pressure, a major risk factor for CVD. Fruits and vegetables (F&V) are rich in polyphenols, and types of F&V are rarely explored in epidemiological studies. The current study aimed to explore F&V intakes by polyphenol content and blood pressure in the UK National Diet & Nutrition Survey.

Total F&V (g/day) were derived from 3-4 day food diaries from 1,002 adults (age range 19 to 91 years) after exclusions were applied. F&V intakes were sub-categorised by polyphenol profiles from Phenol Explorer, including berries, citrus, drupes, pomes and tropical fruits; and Allium, Brassicaceae, fruit, pod and root vegetables. The measurement outcome was systolic (SBP) and diastolic blood pressure (DBP) (mmHg). Multivariate regression was conducted to calculate change in SBP and DBP per unit increase in F&V.

For each 80 g/day total F&V consumed, SBP and DBP were -0.58 mmHg (95% CI -1.00, -0.15) and -0.43 (95% CI -0.74, -0.12) mmHg lower respectively. DBP was -0.82 (95% CI -1.34, -0.29), -0.96 (99% CI -1.83, -0.09) mmHg and -1.14 (99% CI -2.27, -0.01) mmHg lower respectively for 80g/day intake of total fruit, citrus and pomes respectively. High intake of total fruits, citrus and pomes in particular, was associated with a lower DBP. Vegetable consumption was not associated with lower SBP or DBP in this cohort. The lowering of DBP by higher consumers of citrus could be due to the hesperidin content.

P406

Chlorogenic acid improves ex vivo vessel function and protects endothelial cells against HOCl-induced oxidative damage, via increased production of nitric oxide an induction of Hmox-1

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Background: Coffee consumption may be associated with reduced risk of cardiovascular disease (CVD). The mechanisms by which coffee can exert potential protective effects remain unclear. Dietary polyphenols are potential contributors for improved cardiovascular health. Coffee is one of the richest sources of dietary polyphenols in a coffee drinking population, the most abundant form being chlorogenic acid (CGA). Endothelial dysfunction (ED) is an early and major risk factor for CVD, and oxidative stress plays a critical role in the development of ED. Nitric Oxide (NO) is a key factor in regulation of endothelial function. Heme oxygenase-1 (Hmox-1) is an inducible isoform of heme oxygenase that is produced i response to stressors such as oxidative stress and may play a key role in vascular protection

Methods: Aortic rings from 10-week-old C57BL mice were collected and incubated with or without CGA, before exposure to HOCl. Endothelium-dependant relaxation was determined using myography. Confluent human aortic endothelial cells (HAECs were serum starved before pre-treatment with CGA and exposure to HOCl. MTS formation was used to determine cell viability and western blot to determine eNOS dimerization and Hmox-1 induction. NO production was measured by gas phase chemiluminescence.

Results: Pre-treatment of isolated aortic rings with 10M CGA protected vessels against HOCl-induced endothelial dysfunction ($P<0.05$). Pre-treatment of cultured HAECs with 10M CGA increased endothelial cell viability following exposure to HOCl ($P<0.05$). Moreover, CGA increased NO production in HAECs culture media in a dose-dependent manner, peaking at 6h ($P<0.05$). In addition, CGA increased eNOS dimerization and induced Hmox-1 protein expression in HAECs.

Conclusion: Our results are consistent with a cardiovascular protective effect of coffee polyphenols and demonstrate that CGA can protect vessels and cultured endothelial cells against oxidant-induced damage. The mechanism behind the beneficial effect of CGA appears to be in part via increased production of NO and induction of Hmox-1.

Financial support: NHMRC Grant

P407

Effects of a natural food colourant on plasma enzymes and peripheral blood cells injury

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G8000™ is a natural food colorant obtained by the nanofiltration of grape juice. Previously, our research group has observed several physiological effects of short consumption of the colorant due to its high polyphenolic content. The aim of this study was to investigate the effects of 28-day consumption of G8000™, by 16 health individuals at dietary dose (70g), on plasma enzymes aspartate transaminase (AST), alanine transaminase (ALT), C-reactive protein and against peripheral blood cells H2O2-induced injury. Parameters of total cholesterol and its fractions, triglycerides were also measured. LDL and total cholesterol levels significantly decreased (140±3 mg/dl to 53,7±35,4 mg/dl and 195,6±35,2 mg/dl to 102,3±65,8 mg/dl, respectively,$p<0.001$). No significant statistically differences were observed for HDL triglyceride and VLDL ($p>0.05$),. After G8000 intake no significant differences were observed for AST, ALT and C-reative protein levels. G8000™ was not able to decrease oxidative DNA damage induced by H2O2 in peripheral blood cells. In conclusion, G8000 showed no effects on plasma enzymes related to hepatic metabolism or oxidative DNA damage. The results, however, demonstrate G8000´s remarkable ability to lower plasma LDL-cholesterol, confirming our previous data.

P408

Punicalagin inhibits angiotensin converting enzyme and induces endothelial nitric oxide (NO) production in the EA.hy926 endothelial cell line

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Background: Accumulating evidence indicates that some fruit species have antioxidant and antihypertensive properties. In particular, punicalagin extracted from pomegranate showed potential antihypertensive activity in human derived EA.hy926 endothelial cell model; however, its molecular mechanisms are still poorly understood. In this study, activities of punicalagin were investigated via two mechanisms related to hypertension, in human endothelial cell EA.hy926 cells; firstly the increase of nitric oxide production, and secondly the mechanism related to the inhibition of angiotensin converting enzyme (ACE).

Methods: The cell viability and nitric oxide production of EA.hy926 cell line were identified. The activity and expression of endothelial nitric oxide synthase enzyme (eNOS) were investigated by spectrophotometry and Western blotting. Moreover, cellular calcium concentration by fluorescence method was examined. In addition, reactive oxygen species produced in the presence of punicalagin were evaluated by flow cytometry and fluorometry. The effect of punicalagin treatment on ACE activity was also studied by fluorometry.

Results: Punicalagin (1-60 µM) clearly exhibited the potential for reducing hypertensive activity by a dual mechanism of nitric oxide synthase induction by increasing calcium levels, and ACE inhibition.

Conclusion: Results obtained from all of the above experiments indicated that punicalagin increased nitric oxide production in endothelial cells (EA.hy926) through decreased ROS levels and increased eNOS activation. Activation of eNOS enzyme was achieved by an increase in cellular calcium concentration. Punicalagin concentrations (1-60 mM) were tested. The activity of ACE was significantly inhibited at (1-60 mM) concentrations. The results indicate that punicalagin may have potential as an antihypertensive agent.

Financial support: King Abdulaziz University, Jeddah, Saudi Arabia

P409

The Crataegus extract WS1442® retards the replicative senescence in endothelial cells by preventing eNOS down-regulation: Role of the redox-sensitive expression o p53/p21 and p16

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Endothelial cell senescence has been suggested to contribute to the development of age-related vascular disorders. Endothelial senescence is characterized by an irreversible cell cycle arrest, oxidative stress and down-regulation of endothelial nitric oxide synthase (eNOS). The present study has evaluated whether the Crataegus special extract WS1442®, a rich source of polyphenols and a potent activator of eNOS, prevents replicative senescence in cultured porcine coronary artery endothelia cells, and, if so, to elucidate the underlying mechanism.

Replicative senescence was induced by sequential passaging of primary cultures of endothelial cells up to the fourth passage (P4). Senescence was assessed using senescence-associated -galactosidase (SA--gal) activity, the formation of reactive oxygen species (ROS) using dihydroethidine (DHE) by flow cytometry, and the level of protein expression by Western blot analysis.

Passaging of endothelial cells was associated with a gradual increase in the SA--gal activity, DHE signal and expression level of p53, p21 and p16, and the down-regulation of eNOS from P1 to P4. The increased DHE signal was associated with increased expression of NADPH oxidase subunits ($gp91^{phox}$, $p47^{phox}$, $p22^{phox}$) COX-1 and COX-2. The Crataegus extract, apocynin and indomethacin prevented the increase in SA--gal activity, oxidative stress, and up-regulation of $gp91^{phox}$, $p47^{phox}$ $p22^{phox}$, COX-1, COX-2, p53, p21 and p16, and the down-regulation of eNOS in P3 cells.

In conclusion, the Crataegus extract WS1442® have delayed endothelial cell replicative senescence most likely by preventing oxidative stress, which leads to down-regulation of eNOS and cell cycle arrest due to the up-regulation of the p53/p21 and p16 pathways.

Financial support: Dr. Willmar Schwabe GmbH & Co KG

P410

The acute effect of quercetin-3-O-glucoside on blood pressure, endothelial function and NO status in healthy men and women

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Background: Epidemiological studies suggest an inverse association between flavonoid consumption and cardiovascular disease. Results from randomised, controlled trials indicate that this protection could occur through effects on blood pressure (BP) and endothelial function. Few human trials, however, have been performed with isolated flavonoids. As quercetin is thought to be a key player in the cardioprotective effects of flavonoids, the aim of this study was to assess a dose-related effect of dietary derived quercetin-3-O-glucoside (Q3G) on blood vessel function, BP and plasma nitric oxide production in healthy human volunteers.

Methods: In a double blind, randomised, placebo-controlled, cross-over trial the acute effects of Q3G (0, 50, 100, 200 and 400mg) were compared in healthy men and women (60.8 ± 9.3 years, n=15). Endpoints included plasma nitric oxide production (determined by measuring S-nitrosothiols and other nitroso species (RXNO), nitrate and nitrite), BP, flow-mediated dilatation (FMD) and plasma quercetin metabolites (measured as total quercetin/isorhamnetin by LC/MS/MS). These were measured at baseline and one hour post intervention.

Results: A linear dose-response to Q3G intake was observed with the quercetin metabolites, in plasma ($p<0.001$) 1 hour post intervention. No Q3G was detected in plasma pre or post intervention. Q3G intake did not alter BP, FMD or plasma nitric oxide production 1 hour post intervention.

Conclusion: Despite observed increases in plasma quercetin metabolites, no changes in FMD, BP or plasma nitrate, nitrite or nitric oxide were observed for any dose of Q3G one hour after ingestion. These results support a recent randomized intervention trial with 160mg/day Q3G for 4 weeks which found no significant change in FMD o BP. The results with pure Q3G contrast with that of whole fruit extract which have shown significant changes in FMD after 1 hour.

Financial support: NHMRC

P411

Are habitual fruit consumption or hydration status modulators of baseline endothelia function and is this important for polyphenol intervention studies?

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A fruit/polyphenol-rich diet and hydration status are associated with reduced cardiovascular disease (CVD) risk and vascular reactivity respectively. Fruits have high water content however there is limited evidence on whether habitual fruit consumption or acute hydration status modulates microvascular/endothelial function. This study investigates potential baseline vascular modulators in overweight/obese, but otherwise healthy individuals.

Data were collected from 80 participants (45.39±9.29y; BMI=29.33±2.61 kg/m^2).Fruit consumption (0-8 portions/day) was measured using the 'FACET' validated questionnaire. Urine osmolality (single urine sample) was used to determine hydration status. Vascular endothelium function was assessed by Laser Doppler Iontophoresis (LDI) used acetylcholine (ACh) and sodium nitroprusside (SNP), delivered to dermal forearm vessels at 100Ax60secs. Data were analysed using linear regression.

Compared with no fruit consumption, one portion of fruit was positively associated with ACh ($p=0.038$) and remained statistically significant after controlling for hydration ($p=0.024$). However, there was no evidence for an association between increasing fruit consumption and SNP. There was no evidence of interaction between hydration and fruit consumption on either measure of LDI.

LDI results with ACh are due to endothelium-dependent capillaries. Hydration status did not significantly modulate LDI, confirming tight physiological control. Associations between lower-end fruit consumption and endothelial function are not replicated with increasing fruit consumption, possibly due to imprecision of self-reported estimates using 'FACET'. Given relatively small samples per fruit category and potential misreporting, these results are unlikely to reflect endothelial function plateauing with fruit consumption. Larger studies (particularly for hydration status), using more precise estimates of fruit consumption are now required.

Financial support: MRC and NSA LLC (USA)

P412
Dose-dependent effects of pure (-)-epicatechin on flow-mediated dilatation in health males.

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Evidence suggests that flavanol-containing foods/beverages and pure (-)-epicatechin (1 and 2 mg/kg BW) are capable of inducing significant improvements in vascular function. However, full dose-dependency is yet to be established, particularly at doses below 1 mg/kg BW. The current study was designed to address this by examining the vascular effects of pure (-)-epicatechin at doses below 1 mg/kg BW.

An acute, double-blind, placebo-controlled, crossover intervention trial was conducted using 20 healthy males subjected to 4 treatment arms: control, 0.1, 0.5, an 1 mg/kg BW pure, food-grade (-)-epicatechin. Vascular function was measured by flow-mediated dilatation (FMD), laser Doppler imaging with iontophoresis (LDI) and blood pressure (BP) at baseline, 1, 2, 4 and 6 hours post-intervention. Plasma analysis of epicatechin metabolites was conducted using LC-MS and circulating plasma NOx using an HPLC-based approach (ENO-30).

We show for the first time that significant and physiologically relevant increases in FMD occur at 1 and 2 h following intake of the 1 mg/kg BW dose of (-)-epicatechin, and at 2 h for the 0.5 mg/kg BW intake. Although no significant changes in LDI or BP, a trend for an increase in microvascular blood flow (LDI) was apparent at the 1 mg/kg BW dose at 2 h post-intervention. Increases in FMD over the 6 h timeframe were closely paralleled by the appearance of total plasma EC metabolites and circulating NOx.

Our data adds further evidence that (-)-epicatechin (and structurally related metabolites) is/are the likely vasoactive component(s) of flavanol-containing foods/beverages over acute timeframes. We also show that in its pure form, doses of 0.5 mg/kg BW of (-)-epicatechin are capable of inducing acute improvements in vascular function (FMD) in healthy men.

Financial support: BBSRC industrial CASE Studentship

P413
Does proanthocyanidin chain length effect anti-hypercholesterolemia?

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Proanthocyanidins (PAs), a class of polyphenols, are oligomers or polymers of flavon-3-ols. The PAs are abundant in grape seeds and have been demonstrated with cardioprotective functions. However the protective properties depends upon the bioavailability of the PAs, which in turn depends on degree of polymerization (DP). Hence to understand the effect of DP of PAs, the grape seeds extracts were fractionated into monomers, oligomers and polymers by chromatography and precipitation methods. The fractions were tested for their anti-hypercholesterolomic activity in experimental rodent model (Male Wistar rats) over a period of 60 days. Rats were fed with 0.5% cholesterol diet (HCD) and the ameliorative effects of various fractions were studied in comparison with statin drug (simvastatin).

Supplementation of fractions individually did not induce any toxicity in animals. Groups fed with either monomers or oligomers exhibited a 21% decrease in serum cholesterol, while polymers revelled only 7% decrease, compared to HCD. In case of triglycerides, oligomers resulted in 10% decrease compared to either monomers (6% or polymers (7%). Further, supplementation of PA fractions reduced the incidence of fatty liver as indicated by histopathology and body weight index. The results suggest that short chain PAs (monomers and oligomers) have a better ameliorative influence compared to polymers probably due to enhanced absorption. These results were contrary to in vitro radical scavenging assay, which indicated that polymers had least IC50 value with 26.59 µg compared to either oligomer (32.91 µg) or monomers (29.88 µg) indicating better scavenging properties of polymers, which supports the view that therapeutic properties is based on the bio-availability. Further, the mechanism of PA action towards anti-hypercholesterolemic activity will be discussed.

Financial support: Research supported by 'Indian Council of Medical Research (ICMR), India'

P414

Impact of a polyphenol – rich hibiscus drink consumption on postprandial blood pressure and vascular reactivity measured by flow mediated dilatation

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Background: Hypertension is the major risk factor for cardiovascular diseases (CVD and can be modified by the diet. Chronic consumption of hibiscus has been reported to significantly lower blood pressure (BP). However, there is limited information on the acute impact of hibiscus on blood pressure (BP) and endothelial function, an earl predictor of CVD risk.

Objectives: The objective of this study was to investigate the acute impact of hibiscus drink (HD) consumption on BP and endothelial function measured by flow mediated dilatation of the brachial artery (FMD) in men with < 10 % risk of CVD.

Methods: A randomised, controlled, single – blinded, cross over study involving 25 men (mean age of 49 years and BMI 26.9 kg/m^2) was conducted. Volunteers consumed 250 ml of HD containing 150 mg anthocyanins or water with breakfast, followed by a lunch at 2 hours, on two visits separated by 2 weeks. BP was measured at baseline and hourly for 4 hours. FMD was measured at baseline, 2 and 4 hours post intervention drink consumption.

Results: HD had no significant effect on systolic and diastolic BP. Interestingly, consumption of HD was found, for the first time, to significantly increase % FMD ($P<0.001$) compared with water.

Conclusion: HD consumption can acutely improve endothelial function measured by FMD and may be a useful dietary strategy to reduce endothelial dysfunction, although this requires confirmation in further studies.

Financial support: Nigeria Tertiary Education Trust Fund and Bayero University Kano sponsored S. Abubakar.

P415

Effects of cyanidin-3-glucoside, pelargonidin-3-glucoside and its degradants on LPS-stimulated cytokine production in whole blood cultures of healthy volunteers

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Anthocyanins are abundant in berry fruits and their consumption has been associated with lowered cardiovascular disease risk. This effect is thought to be mediated through anti-inflammatory effects and this experiment was designed to investigate the effect of two selected anthocyanins and their degradants on the cytokine secretion by LPS-induced whole blood cultures of healthy human volunteers.

The poster will present results from an ex vivo experiment investigating blood samples from 10 volunteers aged 40 to 65 years. For the experiment, blood samples were diluted with RPMI medium, stimulated with LPS and spiked with cyanidin-3-glucoside (C3G), pelargonidin-3-glucoside (P3G) or their degradation products (protocatechuic acid, PCA; 4-hydroxybenzoic acid, 4-HBA; and 2,4,6-trihydroxybenzaldehyde, Ald) to provide final concentrations of 0.08, 0.31, 1.2 and 5mM. Samples were incubated at 37 °C in a 5 % CO2 atmosphere for 24 h.

Concentrations of TNF-, IL-1ß, IL-6, IL-8 and IL-10 were analysed in supernatants using a cytometric bead array kit. Stimulation with LPS increased the concentration of all cytokines tested. P3G and C3G significantly down-regulated IL-8 production at the highest dose tested (5 M; $P<0.05$ and $P<0.01$ respectively) and there appears to be a dose-response relationship. Low concentrations of P3G and Ald (0.08 M) increased IL-10 secretion but the effect was not significant at higher concentrations. TNF-, IL-1ß and IL-6 concentration in LPS-stimulated cultures were not significantly affected by C3G, P3G, PCA, 4-HBA or Ald. C3G, P3G and Ald exhibit moderate anti-inflammatory effects and these can, at least in part, contribute to the cardioprotective effects observed in epidemiological studies.

Financial support: BBSRC CASE studentship with GlaxoSmithKline as industrial sponsor.

P416
The effects of acai consumption on vascular function

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Introduction: Evidence suggests that polyphenol rich foods can improve vascular function. In preliminary studies we identified a very high polyphenol content for acai pulps and therefore set out to assess whether there are any cardio-protective benefits associated with acai consumption. Methods: Using flow mediated dilatation (FMD) of the brachial artery as our primary endpoint, we performed a randomized, double blind crossover study in overweight males (n=23). Volunteers consumed either an acai smoothie (AS) or placebo alongside a high fat challenge in a single sitting; FMD responses were recorded over the subsequent six hours. Results: We observed significant increases in FMD as a measure of vascular endothelial function at 2 hours ($p<0.001$), 4 hours ($p<0.05$) and 6 hours ($p<0.01$) in volunteers on the acai arm relative to the placebo arm. There were no significant effects of consuming the acai smoothie on blood pressure (Systolic or Diastolic) or heart rate compared with baseline or with consumption of the placebo. Conclusion: In this acute study, consuming acai as a whole food in the form of a smoothie (equivalent to 700 mg of total polyphenols) improved vascular function in overweight men. Acai is marketed with implied general health benefits despite a lack of human intervention studies showing health effects; the findings of this double-blinded cross over study are therefore timely and important

Financial support: Saudi Arabia Ministry of Higher Education (Saudi Arabia)

P417
Positional isomers of prenylquercetin differently induce hemeoxygenase-1 expression in vascular endothelial cells

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Positional isomers of prenylquercetin are present in some medicinal plants and food ingredients. However, beneficial effects of these flavonoids on vascular functions have never been demonstrated. We prepared three prenylquercetin isomers by chemical synthesis for clarifying the effect on the position of prenyl group attached to quercetin. Then we investigated effect of each prenylquercetin on the expression of hemeoxygenase-1 (HO-1), a key player in protecting vascular endothelial cells from stress.

Human umbilical vein endothelial cells (HUVEC) were treated with quercetin (Q) or its prenylated derivatives, 5'-prenylquercetin (5'PQ), 6-prenylquercetin (6-PQ), or 8-prenylquercetin (8-PQ). Compared with Q, all prenyl derivatives showed higher accumulation in the cell and higher HO-1 expression. In particular, prenylation of 6-position (6-PQ) was the most effective in cellular uptake and HO-1 induction. It is suggested that mitogen-activated protein kinase (MAPK) pathway is involved in HO-1 expression. In our study, inhibitor of p38 and ERK, but not JNK inhibitor, suppressed 6-PQ-induced HO-1 expression. The expression is known to be regulated by nuclear factor E2-related factor-2 (Nrf2), a major transcriptional factor for HO-1, and BTB and CNC homology (Bach1), a competitor for Nrf2. We found that not only 6-PQ but also Q promoted nuclear translocation of Nrf2. On the other hand, Bach1 in the nuclei was reduced by 6-PQ, but not by Q.

These results suggest that higher accumulation of 6PQ in HUVEC is responsible for its superiority of the induction of HO-1 to other isomers. Phosphorylation of ERK and p38 is likely to be involved in the induction. 6-PQ may promote the translocation of Bach1 from the nucleus to the cytosol, resulting in the effective induction of HO-1 through Nrf2.

Financial support: Grant-in-Aid for Research activity Start-Up, Grant-in-Aid for Scientific Research B

P418
Gene-regulatory effects of citrus flavonoids in human endothelial cells

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Cardiovascular disease (CVD) represents one of the leading causes of mortality worldwide. Epidemiological studies have indicated that the consumption of citrus fruit is inversely associated with the risk of CVD. Although clinical studies suggest that the intake of citrus flavonoids might improve endothelial cell function and reduce blood pressure, the molecular mechanisms by which different citrus flavonoids contribute to cardiovascular health have not been fully elucidated. The aim of this study therefore was to investigate the potential of selected citrus flavonoids to modulate gene expression relevant to nitric oxide (NO) production and antioxidant status in endothelial cells.

Confluent human endothelial cells (Ea.hy926 hybrid cell line) were incubated with increasing concentrations of hesperetin, tangeritin and nobiletin for different time points. Cell viability was measured using neutral red assay. Expression of endothelia NO synthase (eNOS) as well as expression of genes associated with antioxidant and cytoprotective function (such as gamma glutamyl cysteinyl synthethase, GCLC and heme oxygenase 1, HO-1) were determined using quantitative real time PCR.

The results demonstrate that incubation of Ea.hy926 cells with nobiletin and tangeretin (25 µM, 18 h) lead to a marked increase of eNOS mRNA levels, whereas only nobiletin was able to induce HO-1 and GCLC expression significantly.

Present data indicate specific effects of different citrus flavonoids on gene expression in endothelial cells could partially contribute to the health effects associated with citrus fruit product consumption.

Financial support: China Scholarship Council-University of Leeds Scholarship

P419
Arginase inhibition by several polyphenolic compounds

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It is well established that polyphenols such as flavonoids and phenolic acids from foods and beverages provide health benefits and that a high polyphenol intake is associated with decreased cardiovascular risk. Arginase, a trimeric metalloenzyme, produces L-ornithine and urea by catalyzing the hydrolysis of L-arginine. By consuming L-arginine, arginase competes with nitric oxide synthase (NOS) for the production of NO, the main endothelium-derived relaxing factor. Previous experimental studies showing that inhibition of arginase increased NO bioavailability improved endothelial function, reduced oxidative stress and reduced hypertension gave the rationale for discovering efficient arginase inhibitors. The aim of the present study was to determine the arginase inhibitory capacity of several polyphenolic compounds.

Ten polyphenolic compounds were tested on a new *in vitro* screening test developed in our unit research on purified bovine liver arginase (b-AGR 1). The results showed that chlorogenic acid and piceatannol exhibited the highest inhibitory activities (IC_{50} = 10.7; 12.1 µM, respectively), close to those of commercially-available arginase inhibitors: (*S*)-(2-Boronoethyl)-L-cysteine (BEC, IC_{50} = 5.5 µM) and N^{ω}-hydroxy-nor-L-arginine (nor-NOHA, IC_{50} = 1.7 µM). Moreover, the experimental results were supported by docking studies, from which piceatannol and chlorogenic acid showed good predictive affinity with enzyme (docking scores = -21.1; -15.6 KJ/mol, respectively) and could interact with several crucial amino acids of the active side of enzyme such as Ala125, His126, Asp232.

Our data suggests the use of chlorogenic acid and piceatannol as two potential core structures for the design of new arginase inhibitors.

Financial support: The French Ministry of Higher Education and Research

P420
Effects of dark chocolate 72% cacao intake in healthy Japanese (Part1)

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Cacao beans are used as an ingredient in cocoa and chocolate and are known to be rich in polyphenols. The meta-analyses of randomized, controlled trials have been performed to examine the effect of the high-polyphenol chocolate on cardiovascular risk and hypertension. These reports showed the results of clinical study carried out i Europe and the United States, but not in Asian countries. This study aimed to evaluate the association of dark chocolate intake with blood pressure, lipid profile in serum, oxidative stress and inflammation in Japanese.

Methods: Intervention clinical trial includes 385 healthy Japanese taking no medicine regularly suffering from no allergy of cacao, aged 45 to 69 years. All subjects took 25 g dark chocolate 72% cacao per day for 4 weeks. The study protocol was approved by the Human Ethics Committee of the Gamagori City Hospital (Aichi Japan).

Results: Three hundred forty seven subjects completed the study. Dark chocolate 72% cacao effective in decrease systolic and diastolic blood pressure. HDL-cholesterol concentration at 4 weeks was significantly higher than at baseline. The concentration of 8-OHdG and hs-CRP are not compared different at baseline and at 4 week. Their stratified analysis of subjects of 8-OHdG concentrations 10.52 ng/mg creatinine showed that 8-OHdG concentrations at 4 weeks were significantly lower than at baseline And their stratified analysis of subjects of hs-CRP concentrations 0.062 mg/dL showed that hs-CRP concentrations at 4 weeks were significantly lower than at baseline.

Conclusion: The present study indicates the reduction of cardiovascular disease and atherosclerosis.

The authors have no financial conflicts of interest to disclose concerning the presentation.

P421
Ellagitannin metabolites exert antiatherogenic effects in vitro, through modulation of endothelial activation and macrophage cholesterol metabolism

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Consumption of ellagitannins, a subclass of hydrolysable tannins has been associated with cardiovascular protective effects. Ellagitannin extracts have been reported to reduce cholesterol accumulation and to improve vascular function. However, these compounds are poorly absorbed after ingestion and are metabolized by the colonic microbiota to form a series of catabolites called urolithins. This study aimed to evaluate whether urolithins and their precursor ellagic acid could have an impact in atherogenesis in vitro, in particular affecting endothelial activation and macrophage cholesterol metabolism.

Calcein-labelled monocytes attached to TNF- pre-treated human endothelial cells (HUVEC) were quantified fluorimetrically. sVCAM and IL-6 secretion from HUVE was quantified by ELISA. Macrophage cholesterol uptake was evaluated fluorometrically in differentiated THP-1 cells. Macrophage cholesterol efflux was quantified in the same model using a radioisotope-based method. HUVEC or THP-1 weretreatedwithurolithins1-10µMfordifferenttimes.

EllagicacidandassociationofurolithinAandB10µMsignificantlylimitedmonocyte adhesiveness to HUVEC (-29.3%, -39.8%). Ellagic acid and to a lesser extent urolithin C reduced sVCAM secretion(-25,6%, -17,5%) and IL-6 secretion (-36.2%,-39.7%).UrolithinC10-5µMandellagicacid10µMreducedcholesterolaccumulation induced by hypercholesterolemic serum (-21.3%, -18.6%, -16.9%). Association of urolithins A and B 10µM significantly limited cholesterol accumulation promoted by acetylated LDL (-29.3%). None treatments affected cholesterol efflux from macrophages.

These data demonstrated that urolithins might affect different stages of atherogenesis. Although further insights are necessary to better characterize these effects, the current study provides possible explanations of the in vivo atheroprotective role of ellagitannin-containing products.

Financial support: National Processed Raspberry Council

P422

Acute effects of ginger drink consumption on blood pressure and vascular function in healthy men

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Background: Various intervention studies have shown an inverse relationship between the consumption of polyphenol-rich foods and cardiovascular diseases (CVD). Hypertension and endothelial dysfunction are among the major risk factors o CVD. Ginger is known to possess health effects but there is paucity of information on the anti-hypertensive and modulatory effect of ginger on vascular function in human

Objectives: The objective was to assess whether the consumption of ginger drink acutely affects blood pressure and vascular function in men at low risk of CVD.

Methods: A randomized, placebo-controlled, single-blinded, crossover trial involving 22 apparently healthy men (mean BMI 27.0 kg/m^2) was conducted. Subjects consumed 300 ml of freeze-dried ginger extract containing 5.35mg 6-gingerol, 1.7m8-gingerol and 0.5mg 6-shogaol with high fat meal as breakfast and two hours after moderate fat meal was given as lunch on the study visit. There was two weeks wash out period and water was given followed by the same meals. Blood pressure measurement was done at baseline and hourly for four hours while flow mediated dilatation (FMD) was measured at baseline and two more times at 2 and 4 hours.

Results: The administration of ginger drink significantly ($P<0.05$) reduced systolic and diastolic blood pressure but did not significantly ($P>0.05$) increase %FMD compared to placebo.

Conclusion: Our results suggest that crude ginger intake may improve cardiovascular health by lowering blood pressure.

Financial support: This research was sponsored by the Tertiary Education Trust Fund of Nigeria and University of Agriculture, Makurdi, Nigeria. Tertiary Education Trust Fund of Nigeria and University of Agriculture, Makurdi,Nigeria

P423

Bioactivity of a resveratrol-arginine conjugate compared to trans-resveratrol on vascular function in postmenopausal women

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Resveratrol (resv) supplementation may improve markers of vascular function, but poor bioavailability and bioactivity limits efficacy. The effects of a novel resveratrol-arginine conjugate (ResArg) were compared to responses from trans-resveratrol (t-Res), the standard form used in dietary supplements, on measures of vascular function 1h and 2h after intake. Postmenopausal women were randomly assigned to consume 90 mg of ResArg or t-Res, in a crossover design, at least one week apart, after an overnight fast. Microvascular function was assessed by peripheral arterial tonometry at 1h and 2h, and calculated as the reactive hyperemia index (RHI) and the Framingham RHI (fRHI); at 1h, platelet reactivity, plasma resveratrol and nitrate/nitrite levels were also measured. At 1h, significant increases in both RHI and fRHI were observed after intake of ResArg ($p=0.035$ and $p=0.009$, respectively), with no differences noted for t-Res. Significant reductions in platelet reactivity under certain test conditions were noted at 1h after ResArg intake, but not for t-Res. Plasma resv levels were significantly increased 30 and 60 min after the consumption of both ResArg and t-Res, but the levels were only correlated with RHI and fRHI in the ResArg group ($r_s=0.435$; $p=0.002$ and $r_s=0.527$; $p<0.0001$,respectively). Plasma nitrate/nitrite levels trended lower in the ResArg group at 1hand were significantly decreased at 2h ($p= 0.110$ and $p = 0.016$, respectively), while no significant changes were observed in the t-Res group. The results suggest a more rapid and pronounced response from ResArg than t-Res on markers of vascular function.

Financial support: unrestricted gift from Gateway Health Alliances (Fairfield,CA, USA), by USDA NIFA National Needs Graduate Fellowship to D. Djurica, and by the Agriculture and Food Research Initiative Competitive Grant #2012-01370 to R. Holt.

P424

Effect of acute consumption of Passiflora setacea juice on the phagocytic capacity and production of free radicals by monocytes in overweight individuals

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Passiflora setacea (PS) is a wild species of passion fruit from Brazilian Savanna, which recently was found to be rich in polyphenols. Recent studies have shown that PS pulp also presents anti-stress effects and improves night time sleep. There are some associations between a diet rich in phenolic compounds (PC) and reduction in mortality from cardiovascular disease (CVD). Despite these associations are still under study, it can be suggested that these effects occur because PC decreases overproduction of reactive oxygen and nitrogen by activated monocytes and macrophages. Therefore, the aim of the present study was to investigate the antioxidant and anti-inflammatory effects of PS juice consumption by overweight volunteers.

Seven middle-aged, moderately overweight men were included in a randomized, controlled, double-blinded, cross-sectional study to compare the effect of 250mL PS juice and placebo with a 4-week-washout-period. Fasting blood samplings were performed before (t0h) and 3h after the consumption (t3h) and the human peripheral blood mononuclear cells (PBMC) were isolated to assess nitric oxide (NO) and hydrogen peroxide (H2O2) analysis and phagocytic capacity of monocytes.

In preliminary results, PS juice consumption affected H2O2 production compared with placebo. H2O2 production increased by 18.9% with placebo drink and 10.6% with PS juice ($p=0.08$) - Welch's t test. Our data showed no phagocytosis or NO production modifications.

Hydrogen peroxide is an oxygen free radical that in individuals with high Body Mas Index, may contribute as risk factor for acute myocardial infarction when in high concentrations. They are molecules that in a long-term, can facilitate cholesterol oxidation and deposition of atheromatous plaques in blood vessels. So modulation of H2O2 by PS juice ingestion may be a promising strategy to prevent CVD. However, larger n would be necessary to prove that the passiflora negatively modulates the production of free radicals.

Financial support: National Grant (CNPq - National Counsel of Technological and Scientific Development)

P425

Effect of acute consumption Passiflora setacea juice on the monocyte adhesion to endothelial cells: a validation ex vivo study

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The pulp of Passiflora setacea (PS), a wild species of Brazilian Savanna, has been recently shown to have higher levels of phenolic compounds, when compared to its commercial species. The leading cause of CVD is atherosclerosis and a diet rich in phenolic compounds may have some effects on monocyte adhesion. Therefore, the aim of the present study was to investigate effect of consumption of PS juice by healthy volunteers on the endothelial cell function, particularly on monocyte adhesion on TNF-alfa-activated endothelial cells.

Three middle-aged volunteers were included in a cross-sectional study to analyse the effect of 250mL PS juice consumption. A pull of fasting serum samplings were performed before (t0h) and 3h after the consumption (t3h). Primary human umbilical vein endothelial cells (HUVEC) were cultivated in an endothelial growth medium (EGM) with 25% of volunteers human serum (HS) for 7h with a 4-hour-stimulation of 1 ng/mL of TNF-alfa. Monocytes (THP-1) were added and incubated for 15min with HUVEC. The number of THP1 per HUVEC was counted in microscopic fields.

In preliminary results, the treatment with TNF-alfa significantly increased cell adhesion compared with unstimulated control cells (a 3.93-fold increase) (Fig. 2). Pre-exposure of HUVEC to human serum 3 hours after the consumption of PS juice significantly reduced monocyte adherence to TNF-alfa-activated endothelial cells from - 59 to - 43% ($P<0,05$). The decrease of monocytes adhesion to endothelial cells may indicate an anti-inflammatory and atheroprotective effects of PS juice consumption in healthy volunteers.

Financial support: National Grant (CNPq - National Counsel of Technological and ScientificDevelopment)

P426

Vascular and Nutrigenomic effects of grapefruit flavanones consumption: A RCT in post-menopausal women

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The consumption of citrus fruits is associated with health benefits. However, clinical data regarding the effects of grapefruit flavanone consumption on vascular function are lacking. The present clinical study aimed at 1) characterizing the specific role of naringin in the effect of a long-term consumption of grapefruit juice on vascular function in humans and2) analyzing changes in PBMCs transcriptome profile induced by grapefruit naringin.

52 healthy post-menopausal women were enrolled in randomized, controlled, crossover trial. For two periods of 6 months, subjects consumed 340ml/d of grapefruit juice (212mg naringin) or an iso-energetic control beverage mimicking the composition of the juice but without flavanones. The effects on endothelial function (FMD), arterial stiffness (PWV) and blood pressure as well as on conventional systemic biomarkers of CV risk factors were evaluated. Furthermore, a global gene expression profiles in peripheral blood mononuclear cells was performed using microarrays.

PWV was significantly lower after consumption of grapefruit juice than after consumption of the matched control drink without flavanones. Endothelial function, blood pressure, anthropometric measures, glucose metabolism, and biomarkers of inflammation and oxidative stress were not affected by the intervention. The PBMCs nutrigenomic analyses showed that the intake of naringin through grapefruit consumption regulated the expression of genes involved in inflammatory processes and in interactions between vascular and circulating immune cells.

This study showed that a regular consumption of grapefruit juice by middle-aged, healthy postmenopausal women is beneficial for central aortic stiffness and that this effect may be related to flavanones. The molecular mechanisms showed as modulated by flavanones may be potentially involved in the beneficial effect of grapefruit juice consumption on arterial stiffness.

Financial supports: This study was supported by a grant from the Florida Department of Citrus (FDOC), USA.

P427

Identification of polyphenol metabolites in Dahl salt-sensitive hypertensive rat tissue after a sustained daily intake of a cardioprotective berries mixture

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Epidemiological studies have shown a consistent beneficial relationship between the consumption of fruits and vegetables, rich sources of polyphenols, and a reduced risk of Cardiovascular Diseases (CVD). Abundant dietary polyphenols are not necessarily the most bioavailable and effective in tissues. Thus, knowledge on the specific bioactive polyphenol metabolites and their molecular targets is a key point.

Our goal was to identify the polyphenols metabolites present in plasma, urine, faeces heart, liver, and kidney in an in vivo model of hypertension (Dahl-salt sensitive rats). A berries mixture (blueberries, blackberries, raspberries, Portuguese crowberry and strawberry tree fruit) was supplemented to the Dahl-salt sensitive rats diet (2g berries/day). Rats divided into 4 groups have been fed for 9 weeks with: Low Salt (LS), Low Salt and Berries (LSB), High Salt (HS) or High Salt and Berries (HSB) diet. Tail-cuff pressure was measured, and heart and kidney mass index estimated. Plasma, urine, faeces, heart, liver, and kidney were collected and processed for phenolic extraction, and analysed by UPLC MS/MS.

Five rats in HS group died from stroke before the end of the trial, while the remaining groups survived. Remarkably

HSB diet prevented the cardiac damage independently of changes in systolic pressure. It was detected an increase in kidney weight index in HS rats that was attenuated in HSB rats, moreover some polyphenol metabolites exclusively present in the HSB were identified in this organ. Furthermore, differentia urinary and faeces phenolic profile is also observed for HBS rats that are in agreement with kidney results.

In conclusion, phenolic metabolites putatively responsible for the effects observed were identified. Further investigations will be important to fully elucidate the mechanisms of biological activity of these metabolites in CVD cell models.

Financial support: Fundação para a Ciência e Tecnologia (FCT-ANR/BEX-BCM/0001/2013); Agence Nationale de la Recherche (ANR-13-ISV1-0001-01) ; and individual PhD fellowship of Andreia Gomes (SFRH/BD/103155/2014).

P428
Establishing an endothelial cell model to study the effects of polyphenols on glucose uptake

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Background: Endothelial cells are critical components of the vascular system as a primary recipient and transmitter of nutrients and metabolites. We have characterize Human Umbilical Vein Endothelial Cell (HUVEC) glucose transporter expression as a model to study the effects of polyphenols on endothelial cell glucose uptake and metabolism.

Methods: Digital PCR complemented by automated capillary Western protein detection was used to establish the expression of glucose transporters in HUVECs and other primary endothelial cells. Sugar transport was examined using ^{14}C-labelled monosaccharides and was monitored after acute or chronic exposure to physiologically relevant concentrations (<5 mM) of quercetin and its conjugates.

Results: Glut expression in HUVECs was comparable to other endothelial cells, with Glut1 and Glut3 being the most prominent, but several other transporters could also participate in glucose uptake. Acute presence of quercetin or its conjugates did not affect glucose and deoxyglucose uptake. However, 18 hour incubation with quercetin significantly increased glucose uptake while conjugates had no effect. Uptake of deoxyglucose was unchanged indicating no direct effect on transport.

Conclusions: HUVECs present a suitable system for studying effects of polyphenols on endothelial glucose uptake. Quercetin conjugates do not exert a significant effect but longer term exposure to quercetin apparently affected intracellular glucose metabolism. Quercetin is not normally present in plasma but this effect could be relevant during inflammation when locally released glucuronidases generate the free aglycone from, for example, quercetin-3-O-glucuronide.

Financial support: ERC grant POLYTRUE? (project number 322467)

P429
Changing lifestyle by polyphenol ingestion and exercise practice prevents pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is disease with scarce and expensive therapeutic options with a poor prognosis.

The aim of the present study is to evaluate if regular consumption of a polyphenol-enriched beer or physical exercise, or both simultaneously, can improve the prognostic of PAH.

Adult male Wistar rats (180-200g) were divided by two groups: monocrotaline (MCT) (sc, 60mg/kg) and Control (sc, equal volume of vehicle). Each group of animals were then randomly divided in another 4 groups: i) sedentary drinking 5% ethanol (SED+SHAM), ii) sedentary drinking xanthohumol (XN)-enriched beer (SED+EB), iii) exercise

training drinking 5% ethanol (EX+SHAM) and iv) exercise training drinking XN-enriched beer (EX+EB). At days D25-27 animals were submitted to cardiac stress test, echocardiography and hemodynamics followed by sample collection.

MCT+SED+SHAM animals revealed an increase in right ventricle (RV) weight, RV cardiomyocyte cross sectional and fibrosis and RV maximal pressure (RVPmax). Ingestion of XN resulted in an attenuation of all the MCT effects. Isolated exercise training also ameliorated these parameters but induced also RV hypertrophy. At pulmonary level, the reduction in medial hypertrophy was mainly due to XN. XN ingestion combined with exercise seems not to have cumulative beneficial.

The present work demonstrates that a lifestyle based on the consumption of polyphenol-rich beverages and in the practice of regular physical activity can positively modulate the natural history of the PAH.

Financial support: Fundação para a Ciência e Tecnologia, Portugal(FCT) (PEst-OE/SAU/UI0038/2014; UID/BIM/04293/2013) and The EuropeanFoundation for Alcohol Research (ERAB) (EA 14 23).

P430

Long term consumption of Aronia juice decreases serum zonulin levels and the expression of CD11b on monocytes in subjects at high CVD risk

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It has been suggested that impairment of intestinal wall integrity may contribute to the low-grade inflammation in subjects with metabolic disturbances and foster progression of cardiovascular, liver or neurological diseases. Recent studies provide evidence that dietary polyphenols may play important role in gut health and homeostasis, as one of the proposed mechanisms of their beneficial systemic effects.

We investigated the impact of long-term consumption of anthocyanin-rich Aronia juice on intestinal wall integrity and cellular markers of low-grade inflammation by the assessment of serum levels of zonulin, a tight-junction protein of gastro-intestina epithelium, and the expression of CD11b on monocytes, in basal state and after *ex-vivo* activation.

Twenty subjects with metabolic syndrome, randomly selected from a larger cohort o a randomized, placebo-controlled, double-blind, cross-over intervention trial, were included in the study. Defined parameters were assessed before and after 4 weeks of consumption of either 100 ml of pure Aronia juice (appx. 1000 mg of GAE) or polyphenol-free, nutrient-matched placebo drink.

Aronia juice consumption significantly decreased serum zonulin levels (p=0.0114), while placebo consumption did not show significant effects. Expression of CD11b o resting monocytes determined by flow cytometry was not significantly influenced by the consumption of either Aronia juice or placebo. However, CD11b expression on monocytes after *ex vivo* treatment of the whole blood with adenosine-diphosphate was significantly decreased after Aronia juice (p=0.047), but not after placebo consumption.

Shown effects of Aronia juice on intestinal integrity accompanied with the effects on monocyte reactivity may indicate a decrease in intestinal permeability for pro-inflammatory gut constituents as a contributing indirect factor in anti-inflammatory effects of Aronia juice polyphenols.

Financial support: EU 7th Framework Programme(FP7/2007–2013) under agreement no 312090 (BACCHUS) and Nutrikad.o.o.,Belgrade, Serbia.

P431

Genetic determinants of coffee intake in a Mediterranean population. Mendelian randomization analysis of their association with cardiovascular risk factors and disease incidence

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Coffee intake is the main dietary source of polyphenols in our high cardiovascular (CVD) risk Mediterranean Spanish, being the 5-caffeoylquinic acid the most abundantly ingested individual polyphenol. However, the association between coffee intake and CVD risk factors and disease may be confounded by reverse causation another related factors. Our aims were: 1) to study whether relevant genetic variants near the candidate CYP1A1 (Cytochrome P450, family 1, subfamily A, polypeptide 1), CYP1A2 and AHR (Aryl hydrocarbon receptor) genes are associated with coffee consumption in a high CVD risk Mediterranean population and 2) To estimate the association between these genetic variants (as proxies of coffee intake) and cardiovascular risk factors and CVD incidence using a mendelian randomization approach.

We analyzed the participants in the PREDIMED-Valencia Study (n=1094; aged 67+/-7 y). Coffee intake was measured by a validated FFQ. Prospectively, we identified 44 new CVD cases after a median 5 y follow-up. Blood pressure, fasting glucose and lipids were analyzed at baseline. 52 SNPs in the CYP1A1 (n=16), CYP1A2 (n=12) and AHR (n=24) genes were determined by the Human OmniExpress Illumina array. Multivariable Cox regression models were fitted to estimate the risks.

Coffee consumption was 7.4+/-5.7 cups/week, being lower for caffeinated than for decaffeinated coffee (3.5+/-5.7 vs 3.9+/-5.4 cups/w). Hypertensive patients showed consumption (P<0001). Only 17 SNPs were polymorphic. We detected several associations with intake. AHR-rs17137566 was associated with caffeinated coffee (P=0.024), whereasAHR-rs2106728 and CYP1A1-rs2606345 were linked with total coffee intake (P=0.010 and P=0.031, respectively). However, these SNPs were not consistently associated with CVD risk factors or disease. In men, the CYP1A1-rs2606345 variant allele, associated with lower coffee intake, was associated with lower CVD incidence, but this association was not observed in women. In conclusion, despite some AHR and CYP1A1 SNPs were associated with coffee intake, no consistent associations with CVD were found.

Financial support: CIBER 06/03, CNIC-06, PI11/02505, SAF2009-12304, AGL2010-22319-C03-03,PRX14/00527, P1-1B2013-54 and BEST/2015/087

P432

Sustained effects of wild blueberry consumption on vascular function in healthy individuals

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Recent studies have shown that blueberries can improve endothelial function in healthy individuals acutely, but little is known on the chronic effects of daily consumption of blueberries. In a double-blind randomized controlled parallel study we investigated the effects of daily intake of a wild blueberry (the equivalent to 100 grams of fresh blueberry twice per day) or a placebo beverage for 1 month on vascular function (n=40). The results showed a significant increase in flow-mediated dilation (FMD) after 1 month supplementation (-0.28% vs 2.11%, p<0.0001) and acutely after 2 h post-consumption (0.02% vs 1.48%, p<0.005) when the intervention group was compared to the placebo. Polyphenols in urine and plasma were quantified using micro-elution SPE and targeted metabolomics by UPLC-Q-TOF MS. A multivariate regression analysis showed that the plasma concentrations of hippuric acid (r=0.57, p<0.01), catechol-O-sulfate (r=0.56, p<0.01), 4-methylcatechol-O-sulfate (r=0.42, p<0.05), 3-hydroxyphenylacetic acid (r=0.46, p<0.05), 4-hydroxyphenylacetic acid (r=0.40, p<0.05), 3-(4-hydroxy-3-methoxyphenyl)propionic acid (r=0.47, p<0.05), 1-methylpyrogallol-O-sulfate (r=0.50, p<0.01), dihydro caffeic acid 3-O-sulfate (r=0.63, p<0.001), homovanillic acid (r=0.52, p<0.05), ferulic acid 4-O-sulfate (r=0.43, p<0.05), isoferulic acid 3-O-ß-D-glucuronide (r=0.48, p<0.05) and the sum of total phenolics (r=0.40, p<0.05) were significantly correlated with changes in FM detected after 1 month, while other compounds as dihydro ferulic acid 4-O-ß-D-glucuronide (r=0.42, p<0.05) and chlorogenic acid (r=0.44, p<0.05) were correlated with acute FMD increases 2h post consumption.

These results suggest that blueberry polyphenol metabolites positively affect vascular function and may be, at least in part, involved in mediating the observed increases in FMD after 1 month blueberry supplementation.

Financial support: Wild Blueberry Association of North America and by the Research Committee of the Medical Faculty of Heinrich-Heine University Dusseldorf (grant number 9772574).

P433
Intake and time-dependent effects of cranberry (poly)phenol consumption on vascular function in healthy individuals

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Accumulating evidence from epidemiological and human intervention studies indicates that the cardiovascular health benefits of diets rich in berries are in part related to their (poly)phenol content. Cranberries, in particular, are rich in procyanidins, anthocyanins, and phenolic acids. Whether cranberry (poly)phenol consumption can improve vascular function and health when given over relevant time periods and in relevant amounts is not known. This may be due to an incomplete understanding of the intake and time-dependence of vascular effects. In the present project, a randomized, controlled crossover study was conducted in order to investigate the time-course and the intake-dependence of acute cranberry (poly)phenol-related improvements in vascular function in healthy individuals.

Plasma and urinary levels of cranberry (poly)phenols were assessed to correlate vascular effects with cranberry-derived (poly)phenols and excretion, respectively. Our current results show a dose and total (poly)phenol amount dependent increase in flow-mediated dilation. This increase was significant at amounts of 787 mg and occurred as early as 1 h, a maximum at 4 h, and lasted up to 6 h. We observed a trend towards inverse responses with regards to central systolic blood pressure and aortic augmentation index. Furthermore, we provide a basic pharmacokinetic characterization. We determined the time course of approximately 60 phenolic compounds and their metabolites in plasma and urine. Concentrations varied between the low nM and high μM range depending on the compounds and the cranberry drink amounts that each individual consumed. Taken together our results demonstrate that cranberry (poly)phenols can acutely and dose-dependently increase endothelial function.

Poster Session F: Innovative Approaches in polyphenol research

P500
Transcriptional biomarkers of calorie restriction in adipose tissue and modulation by dietary interventions

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Calorie restriction (CR) is the only dietary intervention shown to increase maximum lifespan by slowing the aging process in diverse species. Studies using whole-genome transcriptional profiling have identified thousands of genes that are changed in expression in response to a CR diet, but many of these changes are specific to the genetic background of the organism being studied. Thus, there is great interest in identifying robust biomarkers of CR that are conserved across species and are applicable to humans.

We used gene expression profiling to identify transcripts that were consistently changed in expression in response to short-term CR in white adipose tissue from seven strains of mice. Of the 20,789 genes represented on the microarray, 414 genes were changed in 6/7 strains ($p<0.01$). The expression of 20 genes was confirmed by RTqPCR and a subset of 11 genes was selected as the final panel of CR biomarkers. We then used this panel of genes to assess the ability of selected dietary intervention to mimic the effect of CR in mice. Pioglitazone (a known insulin sensitizing drug) had the greatest ability to mimic the effect of CR (significant mimicry for 8 of 11 biomarkers). Interestingly, consumption of a low dose of either resveratrol or quercetin alone only modestly mimicked the effect of CR, whereas a diet containing both compounds had a greater effect than either compound alone.

In summary, we have identified robust transcriptional biomarkers of CR in white adipose tissue and have successfully used these biomarkers to screen for compounds that have the ability to mimic CR at the transcriptional level.

Financial support: NIH/NIA Grant Number R43 AG034833 to JLB.

P501
Microencapsulated polyphenols from cocoa hulls: new antioxidant-rich ingredients for functional food and nutraceuticals

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Theobroma cacao L. beans and cocoa products are rich in polyphenols which contribute significantly to the bitter flavour, leading also astringency in the fermented/roasted cocoa beans. This bitter feature can trigger sometime a negative sensorial impact, when cocoa is used as ingredient, so requiring the masking of its typical aroma.

Cocoa polyphenols are largely studied because of their antioxidant in vitro and in vivo properties. Various methods have been developed over the years to extract polyphenols from cocoa beans, primarily the flavan-3-ols monomers: (-)-epicatechin (+)-catechin and oligomeric procyanidins. Their use can be strategically used to improve antioxidant capacity in functional ingredients. Anyway, few scientific papers report the recovery of polyphenols from hulls (an interesting waste, separated during the roasting of the whole beans, rich in fiber (1) and polyphenols with antioxidant capacity (2-3)) and the use of the spray-dr technique to obtain functional ingredients.

Aims of this work were:

- the extraction and characterization of water/ethanol-soluble polyphenols from coco hulls;
- the spray-drying of the extract, using maltodextrins and fibre from different food sources.

The powders were chemically and morphologically characterized; the thermo-protection was evaluated in oven, simulating a baking process. Finally, the microencapsulation permitted to stabilize phenolic powders allowing new functional ingredients with antioxidant capacity.

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P502

Satiating effect of microencapsulated bitter secoridoids from gentian root: a new food ingredient to control energy intake

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Recent studies demonstrated that tasting compounds may modulate appetite and eating behaviour. The sensing of bitter tasting compounds was suggested to trigger satiety by linking bitter receptors along the gastro-intestinal tract. Few evidence are known in animals administered with bitter compounds by gastric gavage.

The aim of this study was to evaluate the influence on human appetite and energy intakes of microencapsulated bitter compounds consumed in a food.

A microencapsulated food ingredient (EBI) with a core of bitter Gentiana lutea extract and a coating of ethylcellulose-stearate was developed. EBI allowed to mask the bitterness in the mouth and to deliver bitter secoridoids in the gastrointestinal tract. Two vanilla puddings, one including EBI (EBIP) in a dose providing 100 mg o secoridoids per portion and one without (CP) were developed.

Twenty healthy subjects participated in a cross-over randomized study. They had a breakfast with EBIP or CP in two different occasions. Blood samples, to measure 9 gastrointestinal hormones, glucose and appetite ratings were collected at baseline, 30 min, 1, 2, and 3 h after breakfast. Energy intakes were measured at an ad libitum lunch offered 3h after breakfast and over the remaining pa of the experimental day (post lunch) through food diaries.

No difference of glucose and appetite response between EBIP and CP over 3h post-breakfasts was found. A slight increase of GLP-1 was found and could explain the 10% reduction of energy intakes at lunch after EBIP vs CP ($p>0.05$). EBIP significantly reduced energy intakes over post-lunch period vs CP (-30% kcal after EBIP vs CP, $p<0.05$).

In conclusion, it was demonstrated that microencapsulated bitter secoridoids from gentian root are effective to reduce daily energy intakes in humans. New studies may tested the anorexigenic effect of other bitter compounds and investigate in depth the biochemical mechanisms behind this effect.

Financial support: PON01_ 02863 Incapsulazione di principi attivi per il miglioramento di qualità e sicurezza degli alimenti funded by the Italian Ministry of University and Scientific Research (MIUR).

P503

Mining polyphenol diversity in 50 different berry species using untargeted metabolomics methods

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Polyphenols comprise a wide range of compound classes ranging from phenolic acid (cinnamic acid and benzoic acid derivatives), flavonoids (such as anthocyanins, flavonols, flavanols, flavones, flavanones and isoflavones), stilbenes (such as resveratrol) and lignans. More than 8000 polyphenolic compounds have been described in several plants species and within these a number of compounds have been found to possess potential bioactive properties.

Soft fruit in particular is renowned for their rich polyphenol composition and a number of studies have focused in the testing of polyphenols present in these types of biological material. As part of an EU-funded project (www.bachberry.eu) an untargeted metabolomics method was developed which allowed the characterization of over 112 different cultivars/subspecies that spanned a total number of 50 different species. The germplasm collection analysed included samples from varied regions of the world (Chile, China, Portugal, Russia and UK) and it is so far the largest soft fruit germplasm collection analysed. Samples were analysed using an Agilent LC-ToF-M system in both positive and negative modes which allows the coverage of a wide range of polyphenols (including phenolic acids, anthocyanins, flavonols, flavones and stilbenes). Extraction of approximate 1800 molecular features and subsequent statistical analysis has

allowed the determination of the most phytochemically divers species. It is expected that by restricting the number of species to the most diverse will significantly increase the efficiency in novel bioactive compound discovery.

Financial support: BacHberry project which is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-613793)

P504
C. elegans as a model to elucidate the mechanisms of action of flavonoids: a transcriptomic approach

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Dietary polyphenols have been related with the reduction of chronic diseases, although the precise mechanisms involved in their in vivo biological activity are not fully understood. Different approaches have been designed to overcome this situation such as the use of murine or cell culture models. However, murine models are expensive and have bioethical limitations and it is not always easy to extrapolate the conclusions obtained using cell cultures to humans.

In recent years, the nematode *Caenorhabditis elegans* has become an important model to study molecular mechanisms of ageing, stress resistance or degenerative diseases. This is a multicellular organism with a complete system of tissues and organs, which raises the possibility to consider the metabolism of compounds, it has well-defined cell lineages and development stages, and their genome is fully sequenced which facilitates global transcriptomic analysis.

In this study, Illumina RNA-Seq analysis was used to screen the global transcription patterns of *C. elegans* treated with different flavonoids (quercetin, epicatechin and 3' *O*-methylepicatechin) compared with control nematodes, both under normal cultivation and thermal stress conditions. Raw data were processed using Cuffdiff algorithm to compare expression patterns and identify differentially expressed genes that were significantly up- or down-regulated in response to the treatment. Data meta-analysis using bioinformatics tools, such as Venn diagrams, gene ontology (GO or Kyoto encyclopaedia of genes and genomes (KEGG), allowed obtaining a list of candidate genes that might contribute to explain the mechanisms involved in the biological effects of the dietary flavonoids.

Financial support: Spanish Government through the project BFU2012-35228.

P505
Bioprospection of a berry germplasm for the identification of novel therapeutic (poly)phenols using a humanized yeast platform

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The chemodiversity of plant secondary metabolites provides a pristine pool of high-value compounds with potential therapeutic application. Among these compounds stand out the (poly)phenols, particularly present in berry fruits at high contents, great diversity and unique profiles. In the framework of BacHBerry, an EU-funded project (www.bachberry.eu), a germplasm comprising 112 different cultivars/subspecies from 50 distinct berry species from Chile, China, Portugal, Russia and UK were chemically characterized regarding their (poly)phenol profile using metabolomics approaches. These analyses identified the 26 most chemically diverse candidates spanning the *Rubus, Ribes, Vaccinium, Corema, Lycium, Berberis Aristotella* and *Ugni* genus. Bioprospection of (poly)phenol-enriched extracts protective activities towards relevant molecular aspects of chronic diseases were carried out using well established 'humanized' *Saccharomyces cerevisiae* models of neurodegenerative disorders (Amyotrophic lateral sclerosis and Parkinson's, Alzheimer's and Huntington's diseases), cancer and inflammation. Candidate extract from *Rubus genevieri, Rubus idaeus* (var. Prestige), *Rubus idaeus* (var. Octavia), *Rubus sp.* (var. B13), *Rubus loganobaccus* (var. Sunberry), *Rubus armeniacus* (var. Himalayan giant), *Ribes holosericeum* (XR2), *Corema album* and *Lycium chinense* emerged as those displaying protective activities for at least two diseases. Some insights into the molecular mechanisms underlying protection of candidate extracts from the *Rubus* genus will be discussed.

Financial support: BacHBerry project, which is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-613793)

P506
Protein targets of curcumin and its autoxidative metabolites- The NF-B pathway

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Curcumin (turmeric) has been used in traditional Asian medicine as an anti-inflammatory agent for centuries. One of the targets for anti-inflammatory activity of curcumin is the NF-B pathway. Increased levels of NF-B activity have been associated with over 90 % of cancers. The increased activation keeps the cell in a state of sustained inflammation. We are investigating the hypothesis that inhibition is exerted by oxidative metabolites of curcumin rather than the parent molecule.

Oxidative transformation of curcumin is a spontaneous transformation at physiological pH that leads to the formation of a series of electrophilic intermediates (quinone methide, spiroepoxide) in addition to dioxygenated bicyclopentadione as the final product. These electrophilic intermediates have the ability to covalently adduct with regulatory cysteines in target proteins. We have synthesized novel curcumin analogs that do or do not undergo oxidative transformation as a tool to investigate if autoxidation is required for inhibition of NF-B activity. The autoxidation rates of curcumin and the synthetic analogs were determined using UV/Vis spectrophotometry in ammonium acetate buffer, pH 7.5. RAW267.4 cells were stable transfected with a pNFkB-TA-MetLuc with TB vector for expression of secreted luciferase in order to quantify LPS-induced NF-B activity using a Luciferase Reported assay. We observed a correlation between oxidizability of curcumin and its synthetic analogs and inhibition of NF-B activation. As a mechanistic explanation it has been shown that curcumin inhibits phosphorylation of Cys-179 of the upstream kinase IKK. We have developed an LC-MS strategy to identify IKK peptide adducts with curcumin and/or its oxidative intermediates. Results suggest the oxidative metabolite of curcumin rather than curcumin itself may be a factor in the suppression of NF-B activity, i.e., its anti-inflammatory properties.

Financial support: NIH awards CA159382 and AT006896

P507
Vitamin E – quercetin coloaded curcumin nanoemulsion a cross talk between antioxidant and immunomodulatory potential for therapeutic intervention of chronic inflammation induced sepsis.

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Introduction: Dietary polyphenols has emerged as a new concept in modulating human health. These includes common component of food plants like curcumin (CUR) with antioxidative, antitumorigenic, anti-inflammatory, antibacterial and antifungal properties and quercetin, a potent antioxidant.

Objective: The objective of this study is to develop a nanostructured delivery system for co-delivery of vitamin E-quercetin with curcumin for the therapeutic intervention of sepsis and associated pathologies as a prospective cross-talk between antioxidant and immunomodulatory activity.

Methodology: To improve the pharmaceutical credentials of CUR it was co-delivered with quercetin in vit E nano delivery systems, overcoming limitations including intrinsically low aqueous solubility, bioavailability and cell uptake by macrophages for chronic inflammation. CUR was incorporated in vitamin E nanoemulsion preloaded with quercetin using high pressure homogenization. The study was extended to pharmacodynamic and pharmacokinetic studies on a rat model of lipopolysaccharide (LPS)-induced sepsis

Results: The vitro release studies of optimized nanoemulsion (particle size 256 ± 08 nm, polydispersity index (PDI) of 0.120, zeta potential of -41.1 ± 1.2 mV) demonstrated that nanoemulsion was able to sustain the release of CUR for up to 24h. Minimal hemolysis and cellular toxicity demonstrated its suitability for intravenous administration. Significant increase in antioxidant property and reduction of inflammatory mediator levels was mediated through enhanced uptake by in RAW264.7 and THP-1 in absence/ presence of LPS. Nanoemulsion resulted in an improvement of plasma concentration (AUCF5/AUCCUR= 8.80) of CUR in rats leading to a reduction in LPS-induced lung and liver injury due to less neutrophil migration, reduced TNF-a levels and oxidative stress (demonstrated by levels of lipid peroxides as well as carbonylated proteins).

Conclusion: The findings suggest that the therapeutic performance (i.e., reduction in oxidative damage in tissues) of CUR with quercetin incorporated in vit E nanoemulsion improved the pharmacokinetics of CUR and served as a platform for drug delivery in sepsis-induced organ injury.

Financial support: ICMR, INDIA

P508

A metabolomic approach to elucidate beer polyphenol effects in a human cross over intervention trail

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Introduction: Moderate alcohol consumption is associated with a decrease in cardiovascular risk, but fermented beverages seem to confer greater cardiovascular protection due to their polyphenolic content. Beer contain a wide range of polyphenols coming from the malt or the hops used during brewing, including simple phenolic acids, hydroxycinnamoylquinics, flavanols, flavonols, flavones, alkylmethoxyphenols, alpha- and iso-alpha-acids, hydroxyphenylacetic acids and prenylflavanoids.

Objective: To compare the effects of moderate consumption of beer, non-alcoholic beer and gin on the overall urine metabolome.

Methods: A crossover trial with 33 men at high cardiovascular risk were randomized to receive beer (30g alcohol/d), the equivalent amount of polyphenols in the form of non-alcoholic beer, or gin (30g alcohol/d) for 4 weeks. Diet and physical exercise were carefully monitored. Urine samples were analyzed by LC- High resolution mass spectrometry (LTQ-Orbitrap). A combination of univariate statistical analysis, multivariate analysis (PLS-DA), data-dependent MS/MS scan and accurate mass database matching was used to measure the effect of beer, non-alcoholic beer and gin intake in the urinary metabolome.

Results and conclusions: Metabolomic analysis allowed to identify metabolites, mostly related to alcohol metabolism and beer constituents in urine samples. These metabolites could be used as a novel biomarkers of beer and nonalcoholic beer consumption.

Financial support: The European Foundation for Alcohol Research (ERAB) EA 1117 and EA 1324 and CIBEROBN.

P509

Differential isotope labelling of 38 dietary polyphenols and their quantification in urine by liquid chromatography electrospray ionization tandem mass spectrometry

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A large number of studies support a role of polyphenols in the prevention of chronic diseases such as cardiovascular diseases, diabetes or cancers, however epidemiological evidence is still limited. Robust methods are needed to reliably assess exposure to a large variety of dietary polyphenols which can be easily applied to epidemiological studies.

We report here the development of a method to quantify dietary polyphenols in urine based on differential ^{12}C-/^{13}C-isotope labelling of polyphenols through derivatization with isotopic dansyl chloride reagents and on the analysis of the labelled polyphenol by tandem mass spectrometry.

Urine samples are first hydrolysed with a -glucuronidase/sulfatase enzyme mixture and the resulting polyphenol aglycones extracted twice with ethyl acetate. Samples containing the polyphenols tagged with ^{13}C-dansyl groups are mixed with a reference urine sample containing polyphenols tagged with ^{12}C-dansyl groups and analyzed by tandem mass spectrometry. Polyphenol concentrations are estimated through the calculation of ratios of labelled over non-labelled polyphenols

The method is set up for the measurement of 38 different polyphenols and metabolites representative of the main classes and subclasses found in the diet, It provides a simple and robust mean for measuring polyphenols in urine, and overcomes the need of having expensive labelled standards for each compound.

The method was applied to the analysis of 475 urine samples of the European Prospective Investigation on Cancer and nutrition (EPIC) calibration study. Results will also be presented on the analysis of the same polyphenols in plasma.

Financial support: Institut National du Cancer (INCa 2011-105) and Wereld Kanker Onderzoek Fonds (WCRF NL 2012/604).

P510
A novel tag-free probe for targeting molecules interacted with flavonoid catabolites

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**Equally contributed to this work*

Quercetin is one of the most widely distributed flavonoids in vegetables and fruits and has attracted much attention because of its beneficial biological effect. Quercetin-4'- glycoside (Q4'G) is one of the major quercetin conjugates in onion. Q4'G reaching the colon is subjected to hydrolysis by intestinal bacteria into 3,4-dihydroxyphenylacetic acid (DOPAC), and then DOPAC is converted into 3-hydroxyphenylacetic acid (OPAC) or protocatechuic acid (PCA) in the large intestine. We have reported that DOPAC has the strongest ability to induce expression of phase 2 enzyme genes compared to other metabolites. However, the underlying mechanism in which DOPAC exerts the antioxidant effect via inducing phase 2 enzymes is not clarified. Therefore, developing a new probe of DOPAC give an important clue to reveal the mechanism of its biological activity.

In the present study, to detect directly binding target of DOPAC, we designed a novel DOPAC probe using the copper (I)-catalyzed alken azide 1,3-dipolar cycloaddition (CuAAC) reaction. First, we introduced an alkyne group into DOPAC by esterification with propalgyl alcohol, thereby yielded DOPAC propargyl ester (DPE). To examine its efficacy, DPE was incubated with a model protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and followed by CuAAC click reaction with azide-labelled biotin. Finally we detected the DOPAC-modified protein by a labelled streptavidin biotinylated antibody (LSAB) method. The formation of 1,2,3-triazole by the Huisgen cycloaddition was also confirmed by both mass spectrometry and nuclear magnetic resonance spectroscopy. These results provide an alternative approach to understand how polyphenol catabolites modify cellular proteins.

Financial support: MEXT KAKENHI Grant Number 25292073.

P511
Solid/liquid extraction as key step for quality assessment of commercial cranberry products using HPTLC-densitometry.

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Increasing number of dietary supplement containing cranberry polyphenols are commercialized every year. The composition of cranberry ingredients varies from whole fruit powder to cranberry extract or mixture with blueberry. In order to analyze the quality of cranberry based commercial products, researchers proceed to quality control based on BL-DMAC analysis after one step extraction. In our previous work, we demonstrated that BL-DMAC analysis is not sufficient alone to assess the quality of cranberry ingredients. BL-DMAC as quality control couple to High Performance Thin Layer Chromatography -densitometry bring complementary information, especially rate of epicatechin, PAC-$A_2$and PAC-B_2.

In this work, different extraction solvents were screened for extraction of PACs from a range of commercial dietary supplements. Thus, solid / liquid extraction step was repeated until total extraction of polyphenols was achieved, i.e no coloration of the residue in contact with DMAC reagent. If the solvent nature was found to have a minor impact, the number of required extraction steps varied significantly between commercial products. This step was then demonstrated to be a key factor to control for meaningful comparison between various food supplements.

Quality assessment of cranberry extracts was then conducted using both BL-DMAC and HPTLC-densitometry protocols, in order to obtain global PACs content and metabolic profile. A large diversity of PACs global content and profile was observed

This work highlighted the need of new standardization protocols to control and asses the quality of cranberry ingredients, to guarantee biological effect.

P512
Polyphenolic extracts from fruit pomace by microwave assisted extraction

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Fruit pomace is a low-cost raw material with industrial potential. Phenolic compounds, the pigmented molecules responsible for protecting organisms against oxidative stress, are trapped in the pomace (skins, pulp and seeds) after juice is pressed out. Fruit pomace presents a great challenge in recovery and purification of specialty chemicals because the desired molecules are usually low in concentration, with tons of biomass to be processed. New advances in extraction technology have led to alternative solutions for the valorization of pomace with Microwave-Assisted Processing(MAP™), regarded as a simple, low cost, and robust alternative to conventional extraction methods.

Our research program aims atoptimizing MAP™extraction variables(microwavepower / energy density, material pre-processing, solvent composition, solvent ratio)towards a goal of economically converting pomace residues into high-value ingredients. We propose a model that provides insights into an automated, continuous and rapid extraction process of polyphenols from three pomace biomasses (cranberries, blueberries and apples). Proanthocyanidins, anthocyanins and flavonoids, as target molecular groups, have been quantified by colorimetric method The antioxidant activity of each extract was measured by the oxygen radical absorbance capacity assay (ORAC). Further profile determination and quantification of phenolic compounds have been performed by Ultra Performance Liquid Chromatography (UPLC-MS) using a database of 200 natural polyphenols found in fruits and over 50 known standards.

The optimization of a microwave-assisted extraction and validation of the biomass model for scale-up contribute to knowledge and know-how for the bio-based chemical industry and should lead to industrial bioprocesses with applications in multiple sectors.

Financial support: Fonds de recherche du Québec - Nature et technologies (FRQNT), Natural Sciences and Engineering Research Council of Canada (NSERC), Institute of Nutrition and Functional Foods (INAF).

P513
Biomarkers of cocoa exposure in nutrition research: from clinical trials to epidemiological studies for uncovering disease-risk biomarkers through an HPLC-Q-ToF-MS metabolomic approach

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Background: There is a growing body of evidence on the beneficial cardiovascular effects of cocoa consumption. Untargeted metabolomics is used as a hypothesis-generating tool.

Objectives/Methods: The main aim of this work was to contribute to the identification of biomarkers related to food ingestion (biomarkers of intake), as well as their potential association with health (biomarkers of effect) in a high-risk of cardiovascular disease population using an untargeted HPLC-Q-ToF-MS metabolomics strategy in acute and short-term clinical trials, as well as in observational studies.

Results: Dietary cocoa fingerprinting was characterized by a complex metabolic pattern linked to cocoa phytochemicals (alkaloids and polyphenols) and processing-derived compounds, as well as endogenous metabolites. A large proportion of metabolites were characteristic of cocoa exposure independently of the study design. They belong both to theobromine metabolism and to microbial-derived metabolism of polyphenols.

With respect to the endogenous metabolome, methylglutarylcarnitine showed reduce levels associated with cocoa consumption, both in the short-term clinical trial and in the observational study. Because of the potential role of acylcarnitines in insulin resistance, this observation could provide a mechanistic insight into the beneficial effects of cocoa consumption on insulin sensitivity previously described in epidemiological studies.

Conclusion: Discriminating metabolites of cocoa exposure were replicated among three studies with different designs, increasing the level of evidence from observed associations. Since some of the discriminating compounds are produced by gut microbiota, it reinforces the hypothesis that the microbial food metabolome is an important source of dietary biomarkers.

Financial support: MINECO and co-founded by FEDER: AGL2009-13906-C02-01; CONSOLIDER-INGENIO 2010 Program, FUN-C-FOOD (CSD2007-063); PCIN-2014-133; and PI13/01172 Project (Plan Nacional de I+D+i

2013-2016) by the "ISCIII-Subdirección General de Evaluación y Fomento de la Investigación". We also thank the award of 2014SGR1566 from AGAUR. MG-A thanks the AGAUR for the predoctoral FI-DGR 2011 fellowship. RV-F thanks the FPI fellowship, RLl and MU-S thank the "Ramón y Cajal" program (RYC-201007334 and RYC-2011-09677, respectively), all from the MINECO and Fondo Social Europeo.

P514

Nontargeted Profiling of Strawberry Metabolites: Accumulation Patterns in 15Cultivars Grown in Northern Europe

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Large-scale metabolomic approaches enable phenotyping the biochemistry of food plant samples grown in different environmental conditions and/or having different genetic backgrounds. Strawberry metabolism is to some extent regulated in response to environmental stimuli. However, the strawberry metabolite profile is often fluctuating in cultivar's genetic framework. As the high content of bioactive compounds is becoming a general aim of strawberry breeding and strawberry product development, we wanted to survey the nontargeted metabolite profiles of 15 strawberry cultivars grown in commercial farms under northern European environments (Finland or Estonia).

Metabolite profiling (3 biological replicates/sample type) was conducted with LC-qTOF-ESI-MS. The final dataset comprised 1569 metabolic features that were detected in all sample replicates of at least one sample type, with significant ($p < 0.05$) variation between all samples. *k*-Means and hierarchical clustering analyses were applied to analyze and display the compound accumulation patterns in strawberry samples. 206 major molecular features were tentatively identified on the basis of the fragmentation patterns in the data-dependent MS/MS acquisition.

Ancestry is the most important factor affecting the metabolism of strawberries: based on the metabolite profiles and cluster analyses, strawberries grown in Finland or in Estonia were distinguished mainly according to genetic background. As expected, the most abundant compound groups of strawberries included phenolic acids, flavonoids flavan-3-ol derivatives, hydrolyzable tannins, and terpenoids. As these major bioactive compounds demonstrated significantly different concentrations between samples it can be concluded that careful cultivar selection is essential for the successful development of optimal high-value strawberry raw materials and products for health-conscious consumers.

Financial support: the Finnish Cultural Foundation, the Maiju and Yrjo Rikala Horticultural Foundationthe Northern Savo ELY Centre

P515

Application of phenolic profiling and principal component analysis to selection of blueberry germplasm for biomedical and nutritional research.

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The diversity of phenolic profiles represented in the blueberry germplasm presents a challenge in selection of materials both for research related to human health benefits and eventual translation to dietary recommendation or consumer products. We have developed method for selecting blueberry cultivars that represent the broader phenolic diversity available to researchers and consumers. Concentrations of 17 marker anthocyanin (ANC) derivatives were quantified across 267 blueberry genotypes over two crop years (2011 and 2012) representing highbush (V. corymbosum), rabbiteye (V. virgatum) and lowbush (V. angustifolium) cultivars. Main ANCs included glucosides, arabinosides and galactosides of malvidin, delphinidin and cyanidin derivatives. Mean content of total ANCs across all genotypes was 17.3±6.0 (range 1.7-37.7) mg/100g dw. Highly skewed distributions of individual ANCs contents were log transformed and resulting variables were analyzed using principal components analysis (PCA). The first two components, each of which is a linear combination of the 17 ANC concentrations, explained 62% of the variation in the 26 genotypes over the two crop years. Principal component scores were standardized and the location of 5 principal points were identified at (0.0, 0.0), (-1.39, 0.0), (+1.39 0.0), (0.0, -1.39) and (0.0, +1.39).Genotypes near these points included both commercial cultivars (Ozarkblue) as well as breeding lines (NC 4263; SA06-2:53; SA06-7:46) and experimental crosses (Reveille x NC 3511; NC 4295 x Arlen) that capture the broad diversity of phenolic profiles and provide a logical path for prioritization of relevant blueberry materials for preclinical/clinical evaluation.

Financial support: NIH Grant 1R01AT008754-01 Berries and Bones, C. Weaver PI

P516
Effect of novel extraction technology on phenolics of commercially processed potato-peel

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The agro-food-processing industries generate significant amount of phenolic rich by-products that could be valuable natural sources of antioxidants. Potato processing industries in Ireland alone annually generate 16,500 tonnes of potato-peels as low value by-products. Industrially generated potato-peel of two different varieties, Lady-Rosetta (LR) and Lady-Clare (LC), were examined for their total phenolic contents (TPC), antioxidant properties and phenolic compositions. Due to limited reports on application of novel techniques, such as ultrasound-assisted-extraction (UAE), on phenolics of potato-peel, the extraction was carried out using ultrasonic-water-bath (USB) for various treatment times. The TPC were determined by the Folin-Ciocalteu procedure and expressed as gallic-acid-equivalents (GAE). The antioxidant properties were ascertained using DPPH and FRAP assays and expressed as trolox-equivalents (TE). Moreover, ultra-performance liquid-chromatography coupled with tandem mass-spectroscopy (UPLC-MS/MS) was developed to characterize and quantify the phenolics.

Amongst the three solvent compositions, 80% aqueous methanol exhibited the best results and selected as extraction medium for further UAE experiments. The conventional solid-liquid-extraction (SLE) of LR and LC varieties for 15 hours has been used as control. It was observed that TPC, DPPH and FRAP values for both the varieties after 30 minutes of extraction in USB were higher than the control.

UPLC-MS/MS data reveal that the major phenolic acid present in potato-peel of LR is chlorogenic acid, whereas caffeic acid is the highest in LC. The results demonstrated that the USB is superior to SLE and the LR variety is a better candidate for sourcing the natural antioxidants for food preservation or functional food ingredients.

Financial support: Irish Department of Agriculture, Food and the Marine under Food InstitutionalResearch Measure (FIRM 11/F/050, NovTechIng project).

P517
Lemon balm extract and their major polyphenolic compounds reduces UVB-induce doxidative stress and DNA damage in human keratinocytes

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Solar radiation exposure is the main cause of a variety of cutaneous disorders, including photoageing and skin cancers. Ultraviolet radiation (UV), especially UVB (290 nm-320 nm) component, causes DNA damage, pyrimidine dimmers, 8-hydroxy-2´-deoxyguanosine (8-OHdG), p53 induction, protein oxidation and generation of reactive oxygen species (ROS). In recent years, an increase in the use of botanicals with antioxidant and anti-inflammatory properties as skin photoprotective agents is emerging. Phytochemicals with polyphenolic structure show potential benefits to protect the skin from the adverse effects of UVB radiation, reducing the risk of skin cancers. This study evaluated the protective action of lemon balm extract (LBE) (*Melissa Officinalis*, L) and its main phenolic compound, rosmarinic acid (RA), against UVB-induced damage in human keratinocytes.

The composition of a polyphenolic LB extract was determined by HPLC analysis coupled to photodiode array detector and ion trap mass spectrometry with electrospray ionization (HPLC-DAD-ESI-IT-MS/MS). RA derivatives were the major compounds and some flavones, isoflavones, bisresorcinol, naphthodianthrone and anthocyanin structures were also found. Both, LB and RA inhibited significantly the UVB-induced decrease of human keratinocyte (HaCaT) viability. However LB showed a higher potency than RA. In addition, treatment of keratinocytes with LB suppressed the UVB-induced intracellular ROS production and also reduced DNA damage by Comet assay. Nevertheless, the putative molecular mechanism deserves further attention.

In conclusion, our results suggest that LB extract show potential photoprotective advantages compared to pure RA in human skin cell model against UVB-induced damage. The contribution of flavones to this protective effect, most probably through a synergistic action, needs to be further explored.

Financial support: AGL2011-29857-C03-03 (Spanish Ministry of Science and Innovation);PROMETEO/2012/007, ACOMP/2013/093 and VALi+D fellowships(ACIF/2010/162 and ACIF/2013/064) from Generalitat Valenciana (GV); Nutrafur, S.A., Monteloeder, S.L.

P518
The insoluble fraction –Is it possible to improve phenolic compounds quantification in common beans?

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Common beans nourish millions throughout the world. Rich in nutritional, but also is bioactive compounds (e.g. phenolic compounds - PCs), it shows potential health beneficial effects preventing chronic diseases (e.g. colon cancer and cardiovascular diseases). Until now, most of the common bean studies focused on soluble fractions, but PCs are also bound to starch, protein and fibre (insoluble compounds) only accessed by complex hydrolysis assays.

In order to increase the accuracy in determining common beans phenolic composition, the present study analysed by HPLC (High Performance Liquid Chromatography), different varieties of raw Portuguese traditional landraces (including seed coats and cotyledons), considering the soluble and insoluble fractions after hydrolysis. In addition, the soluble and insoluble fractions of raw and cooked beans were compared using a commercial Portuguese variety ("Moleiro").

Results were treated using Chromquest Software. Calibration curves of standards (gallic acid, ferulic and p-coumaric acids) were used in PCs', hydroxycinnamic acids and flavonoids' quantification. Statistical analysis was performed by IBM® SPSS ® Statistics.

Soluble free phenolic acids were mostly linked to macromolecules. After chemical hydrolysis, hydroxycinnamic acids such as ferulic and p-coumaric acids' isomers were identified. Despite the reduce weight (12%), coats were mostly responsible for the flavonoid composition. Cotyledons contribute mostly to hydroxycinnamic acids composition. Raw and cooked common beans' insoluble fraction showed both phenolic acids and flavonoids.

This study highlighted the richness of the common beans' fraction with highest interest for human consumption in PCs applying hydrolysis assays followed by HPLC.

Financial support: FCT for: BEGEQA - Exploiting Bean Genetics for food Quality and Attractiveness Innovation, Ref. PTDC/AGR-TEC/3555/2012; PhD Grant: SFRH/BD/89287/2012

P519
High-pressure CO2 assisted extraction: preparation of strawberry-tree (Arbutusunedo) fruits extracts

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The strawberry tree (Arbutus unedo) is a small (4 m) evergreen plant belonging to the Ericaceae family and an endemic specie of the Mediterranean basin.

Strawberry tree fruits have been used in traditional medicine as antiseptic, laxative and diuretic mainly due to the presence of phytochemicals with recognized health promoting activity such as phenolic compounds. Amongst the phenolic compounds i the fruits, the main compounds are gallic and quinic acid derivatives, anthocyanin's, tannins but also other compounds as vitamins as C and E and carotenoids have been referred.

Arbutus unedo fruits were dried and extracted using a mixture of ethanol: water (50:50) and high-pressure CO_2. Different pressure (100, 175 and 250 bar), temperature (40ºC, 55ºC and 70ºC) and solid: liquid (S: L)/ CO_2ratios were chosen by experimental design. Experiments were carried out for 15 minutes in a supercritical fluid extractor (Thar Technology, Pittsburgh, PA, USA, model SFE-500F-2-C50) comprising a 500 mL cylinder extraction cell (extraction vessel). Extracts were analyzed for total phenolic content (TPC) by Folin-Ciocalteau method and antioxidant activity by the oxygen radical absorbance capacity (ORAC) method. Highest phenolic content and antioxidant activity was achieved with the highest pressure (250 bar) and temperature (70ºC), and lowest S:L/CO_2ratio (20%). The analysis by LC-MS/MS showed there were differences in the content of galloylquinic acid and 5-O-galloylquinic acid, which may be responsible for the increase in the antioxidant activity of these extracts.

Financial support: FCT, PhD grant SFRH/BD/52532/2014

P520
FoodComEx, a new chemical library useful for the polyphenol community

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The lack of commercial standards for food-derived metabolites is a major limitation in nutritional metabolomics as well as for the study of health effects of polyphenol metabolites in cell models. FoodComEx (Food Compound Exchange) is a new chemical library initiated in the FoodBAll (Food Biomarkers Alliance) project (http://foodmetabolome.org) to facilitate the sharing of not easily accessible standards for food compounds and their human metabolites. FoodComEX will be a virtual library, with compounds stored in the laboratory where they have been isolated or synthesized. Version 1.0 will be an online catalog of pure compounds and reference materials (food extracts, biofluids from animals fed pure compounds, incubation media from in vitro systems to produce metabolites...) made available by academic laboratories. Each compound will appear with data such as physico-chemical properties, origin, purity, storage conditions, links to existing databases, type of spectral data available and contact details of the laboratory offering to share the standard. In the final version, which should be available at the end of 2016, spectral data (GC-MS, LC-MS, NMR, UV, IR) will be made searchable online. Anyone interested in one compound will directly contact the provider. A bilateral negotiation will define the terms of collaboration, within the rules defined in a charter of good practices. For example, the acquirer will have to share the spectral analyses acquired on his own analytical platform. This will continuously enrich FoodComEx contents. FoodComEx is a collaborative initiative widely open to new contributors and users. Anyone interested to contribute can contact us.

Financial supports: Funding: JPI HDHL FoodBAll project (2015-2017)

P521
PhytoHub: An online database to facilitate nutritional metabolomics studies focusing on polyphenols and other food phytochemicals

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PhytoHub (www.phytohub.eu) in an online database dedicated to all phytochemicals commonly ingested with the diet. It is the first database to collate information on phytochemical metabolites for metabolomics studies, and should facilitate identification of unknowns in non-targeted profiling. More than 1140 food phytochemicals have been inventoried. PhytoHub V1.0 contains the 240 most consumed terpenes and their known metabolites. Every metabocard provides a downloadable chemical structure as well as identifiers, synonyms and physico-chemical properties. It also include the dietary sources with references and a link to the FooDB foodcard. The known metabolites extracted from the literature by experts are given with biofluid location and literature references. Since the metabolism of many phytochemicals has not been studied yet, in silico prediction of metabolism will be added in the near future. Mass spectral data come from online libraries of spectra such as RIKEN ReSpect and MassBank, and will be complemented by experimental and literature data. For a queried monoisotopic mass or molecular formula, PhytoHub returns a downloadable list of metabolites or phytochemical precursors, along with their spectral data and possible dietary and metabolic origins. For a queried food, it returns a list of its phytochemicals and their metabolites likely to be present in biofluids after consumption. Custom advanced searches are also possible. PhytoHub will be updated continuously. Collaborators from the COST POSITIVe Action will help to accelerate data entry and curation regarding polyphenols in the following months. PhytoHub is open to new contributors. Anyone interested to collaborate can contact us.

Financial supports: ANR Project ALIA-007-PhenoMeNEp

Poster Session G: Mechanisms of action of Polyphenols

P600

Resveratrol-dependent changes of miRNA levels in mitochondrial fatty acid oxidation-deficient human fibroblasts harboring Carnitine-Palmitoyl Transferase 2 (CPT2) mutations.

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Resveratrol is a well known phytophenol produced in huge amount by grape plant in response to biotic or a-biotic stresses. Related to its anti-oxidant properties, numerous papers established that the main targets of resveratrol are the transcription factors AP-1, NFkB, STAT-3 and COX gene. Interestingly, the effects of resveratrol depend on at least in part upon miRNAs whether they are anti- inflammatory (ex. miR-663), pro-inflammatory (ex. miR-155), tumor suppressors (ex. miR-663) or oncogenic (ex.miR-21). In addition to the above-mentioned functions, changes in miRNA levels are observed in mitochondrial fatty acid oxidation deficient-human fibroblasts harbouring mutations in Carnitine-Palmitoyl Transferase 2 (CPT2) gene. Indeed, we reported the resveratrol is able to boost residual CPT2 activities in human fibroblasts derived from patients having CTP2 deficiencies and can restore normal fatty acids oxidation rates (V. Aires et al, J Orphanet Rare Dis, 2014).Changes are observed in mi-RNA expression in human fibroblast from individuals having (CPT2-deficient patient) or not (Control) a mitochondrial CPT2 deficiency. CPT2 deficiency either increases or decreases the expression of miRNAs. For instance, miR-301 was down-regulated by resveratrol in control cells, while miR-10b level was not modified either in control o in CPT2-deficient cells. In addition, resveratrol differently affected the levels of 193 miRNAs in CPT2-deficient cells as compared with control (WT) cells. For instance, following resveratrol treatment, the levels of miR-110a, miR-110b and miR-495were respectively 2.9, 3.6 and 2.3 lower in CPT2-deficient fibroblasts as compared with control fibroblasts. Based on this study, miRNA changes depend on the cell genotype and are associated to the resveratrol-dependent stimulation of mitochondrial fatty acid oxidation in CPT2-deficient cells.

Financial support: ANR (grant ANR-09-GENO-024-01) and by the UNESCO Chair, Culture and tradition of wine.

P601

Loss of cellular antioxidant activity during the gastro-duodenal phase of digestion of honey is due to hydrogen peroxide formation.

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Honey is a complex mixture of antioxidant polyphenolic acids and flavonoids that *in vitro* have been shown to have cellular antioxidant activity (CAA). The aim of this study was to determine and elucidate the effect of each phase of gastrointestinal (GIT) digestion on an antioxidant content and activity as well as CAA of honey.

Four honey samples (undigested (u)) were subjected to simulated GIT digestion and the total polyphenolic content (TPC) (F-C assay), antioxidant activity (ORAC assay) and CAA (Caco-2 cell line, DCFH-DA assay) of gastric (g) and gastro-duodenal (g-d digests was determined. Phenolic acids (caffeic and gallic acid) and flavonoids (catechin and quercetin) were also subjected to digestion. Formation of H_2O_2 was determined with the FOX assay.

With digestion of all honey samples the TPC and antioxidant activity was unchanged or slightly increased for g and g-d compared to u. For u and g, CAA reflected TPC and antioxidant activity. In contrast CAA was significantly reduced following g-d indicating a strong pro-oxidant effect. This was due to the formation of H_2O_2 by polyphenols at neutral pH in the presence of $NaHCO_3$. This effect was confirmed following the digestion of pure polyphenolics. Formation of H_2O_2 was the greatest for phenolic acids, while the oxidation products formed by flavonoids retained CAA possibly masking the effect of H_2O_2.

In conclusion, the g-d phase of GIT digestion of polyphenolic rich mixtures yields H_2O_2 which is not necessarily disadvantageous because *in vivo* H_2O_2 has been shown to have antibacterial and anticancer effects in the digestive and urinary systems.

Financial support: Research Committee of the Faculty of Health Sciences, University of Pretoria.

P602
Interactions of flavonoids and their metabolites with transition metals iron and copper

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Iron and copper play essential roles in the human organism. It has been known that interactions with transition metals contribute to effects of flavonoids, however, comprehensive data have been missing. Considering the common presence of flavonoids in human diet and the link between transition metals and various pathological states, this issue might be clinically important. The present study was aimed at detailed *in vitro* assessment of iron and copper interactions with a set of flavonoids including their known metabolites.

Metal chelation and reduction were assessed by spectrophotometric approaches base on ferrozine, hematoxylin and bathocuproinedisulphonate under pH conditions corresponding to different physiological and pathological states.

Some flavonoids were shown to be equally or even more active under some conditions when compared to the clinically used chelators deferoxamine (iron) and trientine (copper), namely baicalein as both iron and copper chelator and kaempferol and 3-hydroxyflavone as copper chelators. There were not marked differences between structural features necessary for iron and copper chelation. These include dihydroxyl group in ring B or A in the *ortho*-position and the 3- or 5-hydroxyl with the 4-keto group. However, some structural features, in particular catechol ring B, were associated with both metal chelation and reduction and some of these flavonoid were apparently pro-oxidative due to iron reduction in Fenton chemistry.

Flavonoids show complex interactions with transition metals. While some of them are potent iron/copper chelators even in comparison with the clinically used drugs, attention must be paid to their possible pro-oxidative properties based on reduction o transition metals.

Financial support: Czech Science Foundation (project No. P303/12/G163) and by Charles University in Prague (grant No. 1220314B and proje No. SVV 260 185).

P603
The impact of metabolism on anthocyanin bioactivity: effects on IL-6 and VCAM-1 expression

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Studies suggest that anthocyanins modulate cardiovascular disease risk; however, given anthocyanins extensive metabolism, it's likely that their metabolites are responsible for their reported activity. We explored the anti-inflammatory activity of 15 recently identified metabolites of cyanidin-3-glucoside (namely: protocatechuic acid (PCA), homoPCA, PCA-4-glucuronide, PCA-3-glucuronide, PCA-4-sulfate, PCA-3-sulfate, phloroglucinaldehyde, 3,4-dihydroxybenzaldehyde, vanillic acid, isovanillic acid, homovanillic acid, vanillin, vanillic acid-4-sulfate, isovanillic acid-3-sulfate, and ferulic acid) at concentrations of 0.1, 1 and 10 μM on human vascular endothelial cells (HUVEC), following stimulation with oxidized LDL (oxLDL), CD40 or TNF-. Interleukin-6 (IL-6) and vascular cell adhesion molecule-1 (VCAM-1) protein and gene expression was quantified by ELISA and RT-qPCR. In oxLDL-stimulated cells the parent anthocyanin had no effect on IL-6, whereas nine of its metabolites displayed significant activity. Following CD40L-stimulation cyanidin-3-glucoside and eleven of its metabolites reduced IL-6, while following TNF- stimulation six metabolites were active. Cyanidin-3-glucoside and ten of its metabolites reduced VCAM-1 production following CD40L-stimulation, while three metabolites were active following TNF- stimulation. In oxLDL-stimulated cells, PC produced the greatest reduction in IL-6 (>75% reduction, p0.05), while PCA-4-sulfat was most active following CD40L-stimulation (>96% reduction, p0.03). Conversely, ferulic acid was most active on CD40L-stimulated VCAM-1 protein production (>65% reduction, p0.04), indicating differential bioactivity following different pro-inflammatory stimuli. Interestingly, some metabolites displayed positive, negative, u-shaped or inverse u-shaped responses, suggesting bimodal activity. This evidence suggests that the metabolism of anthocyanins does not reduce their potency and that metabolites modulate the expression of inflammatory mediators at physiologically relevant concentrations.

Financial support: UK Biotechnology and Biological Sciences Research Council (BBSRC), including BB/H532059/1, BB/H004963/1, BB/I006028/1, BB/I005943/1 and BB/J004545/1.

P604
A novel approach for the investigation of key lipid intermediates and the effect of quercetin

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Hepatic and extra-hepatic lipids have become the centre of attention in the progress o metabolic dysfunctions. Fatty acids present from the diet or de novo synthesised are processed by the cell only in their activated form, conjugated to Coenzyme A. The levels of acyl-CoAs in tissues reflect the activities of both acyl-CoA synthetases and enzymes in the -oxidation cycle, whilst at the same time control the levels and composition of intracellular lipids in the anabolic pathway, affecting membrane function and fluidity. Acyl-CoAs constitute signalling molecules of critical significance in the rise of inflammatory responses and activation of protein kinases involved in insulin resistance.

Polyphenols like quercetin are known to influence cellular lipid metabolism by interacting with key enzymes thus potentially altering the acyl-CoA profile in cells. Analysis of acyl-CoAs in biological samples remains a challenging analytical task due to the molecular features, instability and diversity of acyl-CoAs. A novel quantitative HPLC-ESI-MS/MS method was developed to incorporate 12 species spanning from short (C4) to long chain members (C20). Acyl-CoA levels in HepG2 cells subjected to various treatments were measured and the effect of quercetin was studied at various time points both under "normal" (5.5 mM) and "high" (25 mM) glucose conditions. The effect of AICAR, a well-known AMPK activator clinically used for treatment of lipid related cardiovascular dysfunctions was also explored in the same setting. This approach provides new insights into potential mechanisms of action of polyphenols on interactions with key players of lipid metabolism.

Financial support: European Research Council Advance Grant (POLYTRUE? 322467).

P605
Delphinidin and calcium signaling in human T lymphocytes: a basis to elucidate pathways leading to proliferation

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Delphinidin, an anthocyanin present in red wine, has been reported to preserve the integrity of endothelium. However, the effect of delphinidin on cells involves in inflammation in cardiovascular diseases remains unknown. We investigated the effect of delphinidin on the proliferation of T lymphocytes with respect to calcium signaling. Human T cells were isolated from blood of healthy patients. Proliferation was assessed using CyQUANT® NF Assay Kit. Calcium signaling was analyzed using Fluo-4 probe and the mechanisms were depicted using appropriate drugs.

Delphinidin did not modify basal proliferation but it was able to decrease the phytohemagglutinin (PHA)-induced proliferation. Inhibitors of capacitative entry an estrogen receptor (ER) antagonist, fulvestrant, or deletion of ERa but not the nitric oxide (NO)-synthase inhibitor prevented the effect of delphinidin. Interestingly, delphinidin decreased the ability of thapsigargin to increase both calcium release and entry. The effect of delphinidin was sensitive to capacitative entry inhibitors and partially prevented by fulvestrant but not by the NO synthase inhibitor. Delphinidin reduced membrane translocation of STIM-1, a principal step in capacitative entry activation through "calcium release activated channels" CRAC. Interestingly, delphinidin activate the HDAC pathways downstream of calcium signaling for its anti-proliferative effect. Together, these data suggest that delphinidin attenuates T lymphocyte activation via multiple cellular targets and subtle regulation of calcium signaling and HDAC pathway mostly via ERa-dependent mechanism. The consequence of which results in inhibition of proliferation. These effects concur to the protective effect of delphinidin on processes leading to inflammation associated with T lymphocyte activation.

Financial support: OSEO (Bpifrance).

P606
A mechanistic study on Rottlerin induced autophagic death in MCF-7 breast cancer cells

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Background. Emergence of chemotherapeutic resistance to anticancer drugs still remains a major obstacle in successful management of human tumor. One of the several mechanisms that contribute to cellular resistance includes defects in the apoptotic machinery and therefore the ability of cancer cells to evade apoptosis. For this reason, the challenge of the modern anticancer therapy, in the treatment of apoptosis resistant cancer cells, is to induce cell death through a different mechanism such as autophagy. In our previous study, we found that Rottlerin, a natural polyphenolic compound, inhibits proliferation and, most importantly, kills caspase-3 deficient breast adenocarcinoma MCF-7 cells, through a non-canonical Bcl 2-Beclin 1-Akt-Erk- independent autophagic death.

Aim. We focused our study on understanding the mechanistic action in autophagic response of MCF-7 cells to Rottlerin and we determined the involvement of other upstream signaling molecules, such as AMPK, in the axis that leads to mTOR inhibition.

Methods. Several molecular pathways were evaluated after cell treatments with Rottlerin alone or in combination with enzyme inhibitors followed by Western blotting analysis. Pull down assay and RNA silencing techniques were used to confirm the direct involvement of mTOR.

Results. We found that the Rottlerin-triggered autophagy is mediated by inhibition o mTORC1 activity, but surprisingly, through a novel AMPK and mTORC1 phosphorylation-independent mechanism. Our results suggest that autophagy induction is due to a direct, inhibitory interaction between Rottlerin and mTOR.

Financial support: National Grant

P607
Key role of mitochondria in the paradoxical effects of red wine polyphenols on angiogenesis

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Red wine polyphenol (RWPC) extracts and delphinidin display paradoxical effect on angiogenesis. Low-dose of RWPC is pro-angiogenic whereas high dose is anti-angiogenic in a model of postischemic neovascularization. Delphinidin is anti-angiogenic whatever the concentration used. RWPC and delphinidin induce nitric oxide (NO) release through the activation of the estrogen receptor alpha (ERa). NO and ERa are key regulators of mitochondrial biogenesis and the proangiogenic factor vascular endothelial growth factor (VEGF) induces mitochondrial biogenesis through the activation of Akt pathway. The aim of the present study was to assess the effect of RWPC and delphinidin on the mitochondrial function in endothelial cells with respect to angiogenesis.

Endothelial cells were stimulated with RWCP at low (10^{-4} g/l, LCP) or high (10^{-2} g/ HCP) concentration, delphinidin (10^{-2} g/l), VEGF (50 ng/ml) or both. Mitochondrial respiration and DNA content, and mRNA expression of mitochondrial biogenesis factors were assessed using oxygraphy, qPCR and RT-qPCR respectively.

LCP and VEGF increased mitochondrial respiration, mitochondrial DNA content an mRNA expression of mitochondrial biogenesis factors (NRF-1, ERRa, Tfam and PolG). HCP and delphinidin did not affect mitochondrial respiration and DNA content. LCP-induced increase of mitochondrial function was prevented by the inhibition or ERs and NO synthase. Delphinidin prevented VEGF-induced mitochondrial biogenesis and Akt pathway.

The present study highlights the key role of mitochondria in the regulation of angiogenesis by RWPC. The pro-angiogenic effect of LCP and VEGF is associated with an increase of mitochondrial biogenesis, whereas the antiangiogenic effect of delphinidin involves the inhibition of VEGF-induced mitochondrial biogenesis.

Financial support: INSERM and the University of Angers.

P608

Grape pomace extract (Vitis vinifera L.) from Brazilian wine industry decreases glucose utilization and affects mitochondrial bioenergetics in human hepatocarcinom HepG2 cells

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Wine industry produces large quantities of by-products rich in bioactive compounds. Grape pomace (GP) represents an environmental problem due to its high quantity an pollutant characteristics. In Brazil, the majority of GP is discarded and research is required to decrease its environmental burden and to add value to this residue. We evaluated GP extract (GPE) bioactivity from Brazilian wine industry in human hepatocarcinoma cells.

Pinot Noir pomace derived from white wine vinification was used to produce hydro-alcoholic extracts. GPE was characterized regarding its bioactive compounds by HPLC analysis. Bioactivity was assessed on HepG2 cells as a function of viability 13C NMR glycolytic flux analysis and cellular respiration by high-resolution respirometry.

GPE polyphenols' content was high and the major anthocyanins found were cyanidin-3-O-rutinoside and cyanidin-3-O-glucoside. Glycolytic flux and cellular respiration were measured under short-term incubation, to evaluate GPE effect on HepG2 bioenergetics irrespective of effects on cell viability. Lactate production was decreased in treated cells, indicating that GPE decreased glucose utilization. Additionally, GPE promoted a 33% increase in oxygen consumption rate. The fraction of oxygen consumption utilized for ATP synthesis increased from 16% in control to 27% in treated cells, suggesting that GPE increased HepG2 requirement for ATP.

Increased respiration coupled to ATP synthesis in GPE-treated HepG2 are early sign of bioenergetics alterations. The mechanisms underlying these effects are to be determined and might be a compensation to decreased glucose utilization by cells. We reasoned that pomace from Brazilian winemaking presents pharmacological properties related to potential anticancer effects on HepG2 cells.

Financial support: CAPES, CNPq and FAPERJ (Brazil)

P609

Intracellular flavonoid accumulation as a cellular stress response in valinomycin and angiotensin-II-treated epithelial and endothelial cells

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Flavonoids have been widely investigated for their role in health promotion. In this study, we assessed the ability of flavonoids, quercetin and baicalin, to alleviate cellular dysfunction caused by exposure to physiological stressors, valinomycin and angiotensin-II. Valinomycin is an antibiotic that causes mitochondrial stress in intestinal cells, whereas angiotensin-II is a vasoconstrictive peptide that causes endothelial dysfunction via a myriad of mechanisms. To test the effect of flavonoid co-administration on cellular stress induction, quercetin aglycone was used for intestinal cells, while baicalin, a phase II metabolite of baicalein (glucuronide), was used for endothelial cells to mimic the in vivo conditions. Valinomycin (25-100 µM) and angiotensin-II (0.001µM-100µM) caused a dose dependent decrease in cellular viability in epithelial (75% at 50µM) and endothelial cells (70% at 50µM), respectively, as measured by MTT and SRB assays. Meanwhile, co-administrating the stressors with quercetin and baicalin resulted to a recovery in cellular viability of epithelial (100% at 50µM:50µM [valinomycin:quercetin]) and endothelial cells (98% at 50µM:10µM [angiotensin-II:baicalin]). Moreover, flavonoid intracellular accumulation was monitored for the first time using flow cytometry. Stress induction in cells caused an increase in intracellular flavonoid concentration compared to unstressed cells. Therefore, cellular stress triggers an increase in the in situ concentration of flavonoids as a means of cellular stress response. This accumulation aids in the recovery of the cells, especially via the sequestration of reactive oxygen/nitrogen species that causes further cellular damage. This study thus suggest new mechanisms by which flavonoids (aglycone or glucuronide) ameliorate cellular stress.

Financial support: Ghent University Special Research Fund (BOF) Hercules Project (AUGE028 and AUGE014)

P610
Hancornia speciosa polyphenols prevent the senescence-associated pro-thrombotic and pro-coagulant responses in endothelial cells

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The endothelial cells senescence may be involved in age-related vascular disorders. This study investigated the pro-thrombotic and pro-coagulant properties of replicative senescent endothelial cells (EC), and determined the underlying mechanism and the protective effect of a polyphenol-rich extract of Hancornia speciosa leaf (SFH), a tree whose leaves are popularly used to treat hypertension in Brazil.

Replicative senescence was induced by serial passaging of primary porcine coronary artery EC up to passage 4 (P4). Senescence was assessed by the senescence-associated--galactosidase (SA--gal) activity, oxidative stress using dihydroethidium, protein levels by Western blot, nitric oxide (NO) formation by electron spin resonance, and platelet aggregation by nephelometry.

Serial passaging induced senescence in EC and the expression of cell cycle regulator proteins p53, p21 and p16. Senescent P3 cells had a reduced ability to inhibit platelet aggregation and an increased tissue factor (TF) expression compared with young P1 cells, associated with reduced NO formation and eNOS protein levels, and increased oxidative stress and expression of NADPH oxidase subunits $gp91^{phox}$ and $p47^{phox}$, and COX-1. Selective inhibition of NADPH oxidase and COX reduced SA--gal activity and oxidative stress in P3 cells. SFH and its major polyphenols reduced SA--gal activity, oxidative stress, and the expression of p53, p21, p16, $gp91^{phox}$, $p47^{phox}$, COX-1 and TF, and restored NO formation in P3 cells leading to inhibition of platelet aggregation.

Replicative senescent EC have a reduced ability to inhibit platelet aggregation and an increased expression of TF. These effects are initiated by an imbalance between eNOS-derived NO and NADPH oxidase- and COX-1-mediated oxidative stress, and prevented by polyphenol-rich SFH and its major polyphenols.

Financial support: CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and G. C. Silva received a fellowship from Capes/PNPD (Programa Nacional de Pós Doutorado da CAPES).

P611
Green coffee and yerba mate protect against oxidative stress in HepG2 cells

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Green coffee (GC) beans and yerba mate (YM) leaves are two foodstuffs sharing a similar phenolic profile, characterized by a high content of hydroxycinnamic acids (mono- and dicaffeoylquinic acids). The loss of bioactive compounds during coffee roasting has prompted an increase on GC beans consumption, either as nutraceutical or blended with roasted coffee, since GC has been associated with a lower risk of oxidative diseases. Similarly, YM is a beverage extensively consumed in South America for its stimulating properties, also having beneficial health properties.

The aim of this study was to compare the capacity of YM and GC phenolic extract to protect HepG2 cells against an oxidative damage induced by t-butylhydroperoxide (t-BOOH). Direct effect of the extracts on HepG2 cell integrity and redox status was evaluated after incubation with 1, 10 y 50 µg/mL for 20h. To test the protective effect cells were pre-treated with the extracts for 20h and then incubated with 400 µM t-BOOH for 3h. Cell viability and cytotoxicity, ROS generation, antioxidant defense and macromolecular oxidative stress biomarkers were evaluated. The phenolic content was determined by Folin-Ciocalteau method.

Pre-treatment of cells with both extracts significantly prevented the increased protein damage caused by t-BOOH. Moreover, decreased levels of reduced glutathione and increased ROS, lipid oxidation and antioxidant enzymes activity induced by t-BOOH were dose-dependently recovered.

In conclusion, both extracts confer a significant protection against oxidative stress as an initial mechanism of action that may lay on the basis of their beneficial effects against oxidative stress-related diseases.

Financial support: AGL2010-18269 from Spanish Ministry of Science and Innovation. Projects CSD2007-00063, AGL2014-58205-REDC, and grant BES-2011-047476 are also acknowledged.

P612
Interaction of flavonoids with lipid monolayers and multilamellar vesicles formed with Dioleoyl phosphatidylcholine (DOPC) – Novel approaches to study interactions

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Flavonoids are a general class of polyphenol substances that are believed to have health benefits to the body in terms of their antioxidant capacity and various other properties. Elucidation of potential health benefits of flavonoids depends on their ability to interact and permeate the cell membrane. Although there are a number of techniques for investigating the interactions between flavonoids and model membrane systems, the molecular mechanisms of the interaction are not yet fully understood and little is known in detail about how flavonoids interact with such membranes. In order to gain more knowledge in this area, a novel electrochemical approach was reported: a dioleoyl phosphatidylcholine (DOPC) monolayer coated mercury (Hg) film electrode was studied via rapid cyclic voltammetry (RCV). In conjunction with the RCV studies, flavonoid interaction with DOPC Langmuir monolayers at the air-water interface was also studied. Among the eight (8) flavonoids studied, quercetin, kaempferol, tiliroside, naringenin, hesperetin and catechin showed strong interaction with DOPC, whereas other flavonoids, such as rutin and naringin, exhibited limited interactions. The results suggest that binding or penetration of different flavonoids into the monolayer was sensitively dependent upon the number and orientation of the hydroxyl groups. The work is currently being extended to study of multilamellar vesicles of DOPC using small-angle X-ray scattering (SAXS) to understand the preferential localization and membrane fluidity characteristics of flavonoids in the model membranes.

Financial support: Turkish Ministry of Education

P613
Mitochondrial targets of nobiletin

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Nobiletin is an O-methylated flavonoid found in citrus peels that has anticancer, antiviral, neuroprotective, anti-inflammatory activities and depending on the cell types exhibits both pro- or anti-apoptotic properties. We have found that nobiletin decreases oxygen consumption in the presence of glutamate and malate and increase in the presence of succinate in both resting state and state 3 of electron transport chain. In parallel, nobiletin increases NADH oxidation, accelerate NADH:coenzyme Q10 reductase and a-KGD activities and through matrix substrate-level phosphorylation elevates a-KG-dependent production of ATP in brain mitochondria. In addition, nobiletin reduces the production of peroxides in the presence of complex 1 substrates and does not change succinate-driven H_2O_2 formation. Besides, nobiletin induces transient elevation of membrane potential followed by mild depolarization. Affinity purified nobiletin binding proteins revealed one anti-NDUFV1 positive protein with 55 kD and NADH: ubiquinone oxidoreductase activity. We propose that nobiletin may act as a “mild” uncoupler, which through activation of a-KGDH-complex and acceleration of matrix substrate-level phosphorylation maintains membrane potential in normal level. This switch in mitochondrial metabolism could elevate succinate-driven oxygen consumption that may underlay in both pro- and anti-apoptotic effects of nobiletin. Nobiletin is an O-methylated flavonoid found in citrus peels that has anticancer, antiviral, neuroprotective, anti-inflammatory activities and depending on the cell types exhibit both pro- or anti-apoptotic properties. We have found that nobiletin decreases oxygen consumption in the presence of glutamate and malate and increases in the presence of succinate in both resting state and state 3 of electron transport chain. In parallel, nobiletin increases NADH oxidation, accelerate NADH:coenzyme Q10 reductase and a-KGD activities and through matrix substrate-level phosphorylation elevates a-KG-dependent production of ATP in brain mitochondria. In addition, nobiletin reduces the production of peroxides in the presence of complex 1 substrates and does not change succinate-driven H_2O_2 formation. Besides, nobiletin induces transient elevation of membrane potential followed by mild depolarization. Affinity purified nobiletin binding proteins revealed one anti-NDUFV1 positive protein with 55 kD and NADH: ubiquinone oxidoreductase activity. We propose that nobiletin may act as a mild ‘uncoupler, which through activation of a-KGDH-complex and acceleration of matrix substrate-level phosphorylation maintaines membrane potential in normal level. This switch in mitochondrial metabolism could elevate succinate-driven oxygen consumption that may underlay in both pro- and anti-apoptotic effects of nobiletin.

Financial support: Basic Science Research Program of Ilia State University

P614

Elucidating the mechanism of action of quercetin on chronic high glucose-induced mitochondrial stress in a hepatic cell model.

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Liver mitochondrial dysfunction plays a pivotal role in the onset of type II diabetes and quercetin protects against this through regular dietary exposure. Quercetin primarily accumulates in mitochondria and affects cellular metabolism including decreased lactate production, lipid storage, ATP demand and reactive oxygen species (ROS) generation, as well as regulating energy metabolism. High glucose treatment of human hepatic HepG2 cells results in ROS amplification, modified lipid metabolism and mitochondrial damage. We determined whether quercetin could dose-dependently reverse high glucose-induced mitochondrial dysfunction.

HepG2 cells were cultured in low (5.5 mM) or high (25 mM) glucose for 4 days and treated with quercetin for the final 24 hours. High glucose treatment significantly decreased electron transfer system complex I (ETS1) specific activity but led to higher citrate synthase (CS) activity, indicating increased number of lowered-efficiency mitochondria; quercetin attenuated these deleterious effects (ETS mean recovery of 99%, $p<0.001$; CS mean decrease of 10.5%, $p=0.03$). Routine respiration and membrane proton leak, as determined by high-resolution respirometry, was increased in high glucose-treated cells and changes again suppressed by quercetin (with quercetin routine mean change of -18%, $p=0.01$; leak mean change of -29%, $p=0.02$). Cells treated with quercetin displayed higher viability (Trypan Blue), in both low (mean +3%, $p=0.02$) and high (mean +4%, $p=0.001$) glucose-treated cells.

These results suggest that quercetin may restrict high-glucose induced mitochondrial stress by maintaining ETS1 efficiency. The mechanism may be due to a direct and/or indirect effect on oxidative status, metabolic status and/or cell signalling, and the detailed mechanism will be presented.

Financial support: Research Council Advanced Grant POLYTRUE? (322467)

P615

Effect of anthocyanins and their microbiota metabolites on monocyte adhesion to endothelial cells at phyiologically-relevant concentration

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Epidemiological studies suggest a protective role of dietary anthocyanins, micronutrients present mainly in berries, against cardiovascular diseases. Clinical and pre-clinical studies showed effects on improvements in endothelial function, modulation of inflammation or decreased atherosclerosis development. However, the underlying cellular and molecular mechanisms still remain unknown. The aim of the present study was to investigate the effect of anthocyanins and their gut metabolites identified in plasma on monocyte adhesion to TNF-stimulated endothelial cells, the initial step of atherosclerosis development.

Human umbilical vein endothelial cells (HUVECs) were exposed to cyanidin-3-O-glucoside, cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, delphinidin-3-O-glucoside, peonidin-3-O-glucoside and 4-hydroxybenzaldehyde for hours and to protocatechuic, ferulic, hippuric and vanillic acid for 18h at physiologically-relevant concentrations, all at 0.1µM, 0.2µM, 0.5µM, 1µM and 2µM. Subsequently, HUVECs were stimulated with TNF (1ng/ml) for 4 hours followed by 15 minutes incubation with monocytes. Monocytes adhesion to HUVECs was determined using the flow cytometry.

This study showed that exposure of HUVECs to cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, delphinidin-3-O-glucoside and peonidin-3-O-glucoside significantly attenuated monocyte adhesion at 0.1µM by 27.4% in average. Ferulic acid decreased adhesion at 1µM and 2µM by 28.5%, hippuric at 2µM by 18.1% and protocatechuic by 36.5% at all concentrations. Exposure of HUVECs to mixture of compounds used in 3-hour exposure period, resulted in adhesion decrease by 28.3%.

In conclusion, this study showed the biological potency of plasma anthocyanins and their gut metabolites to modulate the adhesion of monocyte to endothelial cells at physiologically-relevant concentrations. The further investigation using nutrigenomics approaches is needed to decipher underlying molecular mechanisms o action.

Financial support: INRA ; Campus France

P616

Pro-oxidant activity of resveratrol in the induction of DNA damage, apoptosis and senescence in colon cancer models

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Various studies have shown that trans-resveratrol can induce cell death of various tumour cells through apoptosis. Restoring a normal apoptotic homeostasis or inducing cell death or other cytostatic mechanisms like senescence in cancer cells might be interesting for preventive and therapeutic interventions. Resveratrol has also been shown to induce DNA damage-related responses (DDR). The initiation of the DDR is mediated by ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia-Rad3-related kinase (ATR) and DNA-dependent protein kinase (DNA-PK), subsequent activations of the downstream checkpoint kinases Chk1/2 an p53. Activation of these pathways could explain the pleiotropic effects of resveratrol since they have been widely described to regulate the balance between cell survival and cell death of cancer cells.

The aim of this study was to precisely characterize the effects of resveratrol on the rat PROb (DHD-K12-TRb, ECACC) and human SW620 colon cancer cells in vitro. We studied the involvement of the DDR in resveratrol anticancer properties and also made a parallel with the response of these models in vivo.

Our results highlight that the transient anticancer activity of resveratrol towards these models of colon cancer is related to its ability to promote the production of Reactive Oxygen Species responsible for: i) an induction of the DDR; ii) an early induction of a cell cycle delay in S phase, correlated with a replicative stress; iii) transient apoptosis and senescence phenomena. The activation of these pathways and consequences were further confirmed in vivo and highlight the pro-oxidant activity o resveratrol as a key of its anticancer properties.

Financial support: "Ligue contre le Cancer", comités de Côte d'Or et du Jura.

P617

Naringenin Improves Antioxidative Capacity and Membrane Lipid Compositions in the Liver of Old-Aged Wistar Rats

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Naringenin is a flavanone most abandon in grapefruit. It was identified as the promising therapeutic agent due to its putative anti-atherosclerotic and anti-oxidative properties. Age-related decline in liver functioning is associated with increase in lipi accumulation or increased prevalence of nonalcoholic fatty liver disease, which is partly due to increased free radical generation. The aim of this study was to test whether naringenin triggers liver antioxidant enzyme gene and protein expressions, phospholipid fatty acid profile, liver structure and biochemical parameters of its activity in sera.

Twenty-four-month-old rats were per os supplemented with naringenin, at a dose of 15 mg/kg, once daily for 4 weeks. Controls received the same amount of vehicle alone (sunflower oil).

After naringenin supplementation, the protein expressions of catalase, glutathione peroxidase and glutathione reductase were significantly ($p<0.05$) increased, while the superoxide dismutase enzyme level remained unchanged. Unlike protein expression of antioxidative enzymes, their mRNA expressions did not change. Liver histoarchitecture remained unchanged, with no signs of hepathocellular alterations, fibrosis, steatosis or inflammation. Phospholipid profile in liver was altered ($p<0.05$): monounsaturated fatty acids and n-3 polyunsaturated fatty acids (PUFAs) increased, while n-6 PUFA content and n-6/n-3 ratio decreased. Serum levels of alanine aminotransferase and aspartate aminotransferase decreased, while gamma-glutamyl transpeptidase level remained unchanged. All the changes pertinent to

phospholipid profile in liver and serum enzyme levels were statistically ($p<0.05$) significant.

It can be concluded that naringenin treatment improved antioxidative capacity and membrane phospholipid content in the liver of old-aged rats. Liver structure and excretory function were preserved.

Financial support: Ministry of Science, Education and Technological Development of the Republic of Serbia, Grant number 173009.

P618

Transcription Factor EB is a crucial transducer of the biomedical action of Pterostilbene and Resveratrol

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Although the beneficial properties of resveratrol and pterostilbene are well recognized, much remains to be discovered about their molecular mechanisms of action. Autophagy is a catabolic process activated by cells in response to stress conditions. It involves lysosomes whose biogenesis and function are under the control of TFEB (Transcription Factor EB), activated under starvation. The induction of autophagy by polyphenols is well supported. In particular, a pro-autophagic role is ascribed to Resveratrol. Thus, we wanted to verify: Whether Resveratrol and Pterostilbene could induce TFEB translocation into the nucleus and consequently autophagy; The efficacy in our system of some metabolites in case of a positive answer to the 1st point, how these molecules trigger TFEB translocation HeLa cells overexpressing TFEB-GFP were treated with different concentrations of Resveratrol and Pterostilbene and with two types of metabolites and monitored by confocal microscopy. To verify the implication of AMPK, cells were treated also with a known AMPK activator.

Both Resveratrol and Pterostilbene, in our system, induce TFEB translocation into the nucleus of HeLa cells already at low, physiologically meaningful concentrations, but to a lesser extent than nutrient deprivation. While sulfates are almost ineffective, reduced forms show an activity similar to that of the parent compounds. Treatment with an AMPK activator results in a less pronounced TFEB translocation into the nucleus than the one induced by Resveratrol and Pterostilbene. These observations lead us to speculate that our compounds might induce TFEB nuclear translocation also by modulating other signaling pathways.

Financial support: Research project of National Interest (PRIN, Research Italy)

P619

Brazilian Berry Modulates Mitochondrial Function And Reduces Oxidative Stress In Human Fibroblasts Cells (MRC-5)

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Jaboticaba (*Plinia* sp.), termed the Brazilian berry, has emerged as a new functional food with potential health benefits, mainly because of their significant levels of phenolic compounds, primarily anthocyanins and flavonols. *Plinia trunciflora* (O. Berg) Kausel is one of the main species of jaboticaba that is naturally occurring and cultivated in Brazil. Thus, the aim of this study was to evaluate the *in vitro* antioxidant activity of jaboticaba peel extract (JPE) and its capacity to modulate oxidative stress as well as mitochondrial function in human lung fibroblast cells (MRC-5). Additionally, the macronutrient and phenolic compositions of P. trunciflor pulp and peel were determined. JPE was able to reduce the decreases in complex I activity and the ATP levels induced by H_2O_2 and thereby decreased the oxidative stress in MRC-5 cells. Besides, the macronutrient found to have the highest level in both the peel and pulp was carbohydrates, followed by the high fiber content of the peel. The total phenolic compounds and anthocyanin levels were higher in the peel than in the pulp. High resolution mass spectrometry (HRMS) showed the presence of cyanidin-3 O-glucoside and kaempferol. These findings show a new role for jaboticaba, a berry rich in phenolic compounds, as a mitochondrial protectant in pathological conditions where mitochondrial dysfunction is involved.

Financial support: CAPES, CNPq, FAPERGS, UCS

P620
Epicatechin reduces oxidative stress induced by amiodarone on human lung fibroblasts cells (MRC-5)

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Amiodarone is among the most widely used drugs for the treatment of cardiac arrhythmias. This drug has adverse effects that include pulmonar toxicity, which can lead to treatment interruption. The mitochondrial changes and oxidative stress may play a role in the mechanism of pulmonar toxicity. Polyphenols are able to restore mitochondrial dysfunction and decrease oxidative stress. However, there has been no study regarding polyphenols decreasing pulmonary cytotoxicity induced by amiodarone until now. The aim of this study was to evaluate the ability of the polyphenol epicatechin in reversing mitochondrial dysfunction and oxidative damage caused by amiodarone on cultured human lung fibroblast cells MRC-5. The cells were treated with different epicatechin concentrations, and then treated with amiodarone. Mitochondrial function (activity of complex I of the electron transport chain and ATP biosynthesis), cell viability, oxidative damage, and production of nitric oxide were evaluated. Epicatechin totally reversed the mitochondrial dysfunction caused by amiodarone. The polyphenol also was able to significantly reduce oxidative damage and cell death in the amiodarone-treated fibroblasts. This study shows that the toxicity of amiodarone may be associated with mitochondrial dysfunction and the polyphenol epicatechin could prevent mitochondrial damage induced by this drug, reducing the cytotoxic effect caused by amiodorane in lung cells.

Financial support: CAPES, CNPq; FAPERGS; UCS

P621
Polyphenols-rich extract from Araucaria angustifolia induces pro-oxidative effects o human larynx HEp-2 cancer cells

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Polyphenols are known for their ability to exert anti-carcinogenic actions. *Araucaria angustifolia* (Araucariaceae) is one of the main conifer species found in South America. Its female strobili consist of seeds (edible part) and bracts (sterile seeds). These bracts present high levels of flavonoids, including flavan-3-ols and flavonols. The aim of this study was to examine the antiproliferative effect of a polyphenols-ric extract (PE) from *Araucaria angustifolia* on laryngeal carcinoma HEp-2 cells and its mechanisms of action. The results demonstrated that PE induced a significant cytotoxicity in HEp-2 cells compared to non-tumor human epithelial (HEK-293) cells, indicating a selective activity of this extract for cancer cells. PE was able to increase production of nitric oxide, oxidative damage to lipids and proteins, along with depletion of enzymatic antioxidant defenses (superoxide dismutase and catalase in the tumor cell line. Moreover, it was able to induce DNA damage, nuclear fragmentation and chromatin condensation. A significant increase in apoptosis inducing factor (AIF), Bax and cleaved caspase-3 expression was also found, suggesting that PE presents distinct pathways to induce changes in cell dynamics in order to cause programmed cell death. The phytochemical analysis of the extract, performed using High Resolution Mass Spectrometry (HRMS) in MS and MS/MS mode, indicated presence of polyphenols which may be associated with the antitumor effect observed in this study

Financial support: CAPES, CNPq, FAPERGS, UCS

P622

Mechanisms of protective effect of epigallocatechin gallate against oxidative stress induced by UVB in model systems and cells

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Background/objectives: UVB photons penetrate the capillary plexus in the dermis and induced oxidative damage in the formed elements and other components of blood reinforced by the advent of the hemoglobin and its photo-oxidized products as a result of erythrocyte hemolysis. We investigated the protective effect of epigallocatechin gallate (EGCG) against oxidative damage in swine erythrocytes and bovine serum albumin (BSA) induced by UVB.

Methods: SH groups in BSA, GSH, MDA, metHb levels in erythrocytes were determined using spectrophotometric method. The level of ROS, fluidity of erythrocytes membrane, BSA tryptophan fluorescence were measured spectrofluorimetrically.

Results: It was shown that UVB induced SH-groups formation as result of breaking the S-S bonds. This effect correlated with photo-quenching of tryptophan fluorescence suggesting their participation in photo-destruction of albumin. EGCG in a concentration dependent way decreased the SH groups content in albumin formed under UVB irradiation. EGCG also strongly reduced the amount of generated reactive oxygen species and inhibited lipid peroxidation but however, much less inhibited the oxidation of glutathione and hemoglobin. As was determined by DPH fluorescent probe, EGCG significantly decreases fluidity of erythrocytes membrane. EGCG also strongly reduced DPPH radicals.

Conclusion: The results indicate that the antioxidant activities of EGCG play a potential role in protection against oxidative deleterious responses to UVB in erythrocytes and BSA. But the strength of the effect depends on the molecular target In the case of the erythrocyte membrane protective effect is associated not only with antiradical activity of EGCG but also with the impact on the membranes structure.

P623

Free Radical Scavengers and Anticancer activity of flavonoid extracts from Lepidiu sativum (Brassicaceae) leaves

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Abstract- Lepidium sativum known as Garden cress belongs to Brassicaceae family, has been known centuries ago, this plant is widely distributed in Algeria, Lepidium sativum seeds is used in Algeria in folk medicine. In Europe the leaves of garden cress are consumed in salad.

In this study three flavonoid extracts were obtained from Lepidium Sativum leaves: O-glycosides, C-glycosides and flavones/flavonols. The O-glycosides and C-glycosides were obtained by separation with ethyl acetate and butanol respectively whereas the Aglycones (flavones/ flavonols) were generated by acid hydrolysis. The antiradical activity of aglycones and glycosides flavonoids has been determined using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and expressed as half inhibitory concentration (IC_{50}).

Our results showed that ethyl acetate fraction exhibit stronger scavenger effect (IC_{50} 99±0,003µg/mL) than ether ethylique and butanolic extracts with respectively IC_{50}= 145±0,004µg/mL, 300±0,005 µg/ mL. Antitumoral activity has been tested towards HEp2 cells (Human Laryngeal Carcinoma Cells). All extracts obtained from L. sativum leaves exhibited a strong inhibition of cell proliferation but it has not provoked apoptosis. Significant inhibition of cells proliferation more than 78% was observed at 57 g/ml of extract concentration for ethyl acetate, butanolic and ether ethylique fractions.

We report the first study on flavonoids of L. sativum leaves, we can conclude that all extracts possess a good cytotoxic activity.

P624
Influence of Humulus lupulus L. flavonoids on stress resistance and the ageing process in C. elegans

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Introduction: Xanthohumol (XH) and isoxanthohumol (IXH) are the main prenylated flavonoids in hop (*Humulus lupulus* L.) Naringenin (N) is a related flavanone found in high concentrations in Citrus fruits. These compounds are known to possess interesting biological pro-health activities which give the possibility of medical applications or use as food nutraceuticals. In order to contribute to elucidate the mechanisms of their *in vivo* activity, the effects of these compounds on lifespan and resistance against thermal stress have been evaluated using *C. elegans* as a mode organism.

Materials and methods: Studies were carried out using the wild type N2 strain of *C elegans* grown on NGM plates containing the studied compounds (200 µM).

Results: XH, IXH and N showed a positive impact on the survival of worms subjected to thermally-induced oxidative stress and increased the survival rate by 9, 22 and 11%, respectively compared to untreated worms. Maximum lifespan, determined as the longest living 10% individuals of each population, was increased by the tested compounds by 2-6%. Treatment with XH and IXH reduced protein-carbonyl levels by 29 and 25%, respectively, and also decreased ROS levels (44 and 23%, respectively), whereas N did not show any significant effect on these markers.

Conclusions: XH, IXH and N increased life duration and thermal stress resistance in *C. elegans*. XH and IXH reduced the levels of oxidation biomarkers, suggesting that they could be efficient to protect the animals against the oxidative stress.

Financial support: Spanish Government through the project BFU2012-35228.

P625
Effect of isoflavonoids on platelet aggregation

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Many studies suggested that consumption of isoflavonoids from soy has beneficial effects on the human health, in particular the lower risk of the coronary artery diseases was reported. The aim of this study was to test anti-platelet activities of a row of chemically related isoflavonoids and to find the exact mechanisms of their action.

Different approaches using human platelets were used for the assessment of anti-platelet activity on arachidonic acid (AA)-based pathway /thromboxane receptors, cyclooxygenase-1 (COX-1), and thromboxane synthase/ in 17 isoflavonoids including their metabolite equol.

The screening in full blood has shown that 12 isoflavonoids blocked platelet aggregation induced by AA. Structure-activity relationship revealed that 7,4´-dihydroxyl group represents the most efficient functional group. Substitution of these positions by a methoxyl group led to a reduction of the effect and substitution by glucose in position C-7 was associated with almost complete loss of the activity. Presence of a 5-hydroxyl group seemed to be beneficial, as well as, the co-presence of a 6-methoxy group. The latter was associated with higher effect than the standard anti-platelet drug - acetylsalicylic acid (ASA). A mechanistic study has shown that the active isoflavonoids acted as antagonists on thromboxane A_2receptors and inhibitors of COX-1. Their effect on thromboxane synthase are being tested.

Isoflavonoids with appropriate chemical structure possess even higher antiplatelet activity in comparison with ASA and their advantage over this standard drug might be associated with simultaneous influence on two steps on the platelet aggregation.

Financial support: Zentiva – Sanofi Company (Travel ScholarshipProgram) and Charles University in Prague (253115 C).

P626
Soy isoflavone daidzein increases the expression of NaPi 2a cotransporter in a rat model of the andropause

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Sodium phosphate cotransporter type 2a (NaPi 2a) is specifically expressed in the brush border membrane of renal proximal tubules and mediates the phosphate reabsorption from the primary urine. One of the main soy isoflavones, daidzein, has been widely studied for its therapeutic potential and already recognized as the bone protective substance. Aim of this study was to investigate the effects of daidzein application on NaPi 2a expression, phosphate and parathormone concentration in a rat model of the andropause.

Middle-aged Wistar rats were divided in three groups: sham operated (SO), orchidectomized (Orx) and daidzein treated orchidectomized group (Orx+D). Daidzein (30 mg/kg b.w.) was subcutaneously applicated, while the control groups received the vehicle alone. Expression of NaPi 2a was determined by RT-PCR and Western blot, while the serum and urine parameters were analyzed biochemically.

The abundance of NaPi 2a cotransporter expression was decreased in Orx animals in comparison with the SO group, while daidzein treatment enhanced the cotransporter expression when compared to the Orx rats. Serum phosphate concentration was decreased in Orx rats by 13% ($p<0.05$) in comparison with the SO group. Daidzein treatment elevated the serum phosphate by 10% ($p<0.05$) when compared to the Orx animals. Serum PTH concentration was increased after Orx in comparison with SO by 24% ($p<0.05$), while daidzein application decreased the same parameter by 18% ($p<0.05$) when compared to the Orx animals.

Our results demonstrated that daidzein treatment increases NaPi 2a cotransporter expression in the kidney of andropausal rats, which suggests some benefit of daidzei application when it comes to the regulation of mineral metabolism in advanced age.

Financial support: Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 173009.

P627
Generation and identification of quercetin phase-II metabolites to study drug transporter interactions

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Polyphenols are a large class of natural compounds which can interact with intestinal membrane drug transporters and thus possibly alter drug disposition. Polyphenols also undergo extensive intestinal phase-II metabolism, and these metabolites themselves can also be active towards drug transporters. This project aims to study the interactions of polyphenols and their metabolites with the major intestinal drug transporters P-gp, BCRP and MRPs. Quercetin was chosen as a model compound to develop assays and analytical methods to produce and identify phase-II metabolites, which will also be used for transporter interactions studies.

Quercetin and isorhamnetin (3'-O-methylquercetin) were incubated with HT29 cells for 24 h with samples taken at various time points. The chemical stability of quercetin in the medium alone was also investigated. The samples were analysed using UPLC-MS and Ion-Mobility Spectrometry (IMS).

Quercetin was completely metabolized after 4 hours, while showing a good chemical stability for the same period of time. Quercetin produced 7 detectable phase-II metabolites, glucuronides and methyl glucuronides, which were tentatively attributed based on retention times, MS data and previously published material. Isorhamnetin produced as expected only 2 metabolites, which confirmed the tentative attribution. IMS showed a significant difference in drift time between the different glucuronides, and thus could be used as an additional identification tool.

The developed strategy will be applied to other polyphenols selected through structural cluster analysis. These compounds and their metabolites will then be tested in transporter assays in order to build a predictive computational model for drug interactions.

Financial support: ARIADME, a European FP7 ITN Community's Seventh Framework Programme under Grant Agreement No. 607517.

P628

Grape-seed proanthocyanidin extract induces Glucagon-like Peptide-1 release in a Rat ex Vivo Intestinal Model.

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Certain phenolic extracts, including grape seed proanthocyanidin extract (GSPE), have been reported to increase GLP-1 levels in *in vivo* models, though their effects oenterohormone secretion remain unclear. Nonetheless, monomeric flavan-3-ols have been described to act at different levels of the enteroendocrine cell metabolism, activating bitter receptors (hTAS2R14 and hTAS2R39) and inducing an increase of intracellular Ca^{2+}concentration. The aim of our study was to evaluate if GSPE and its digested metabolites are effective stimulators of GLP-1 release in an *ex vivo* intestinal model.

The GLP-1 secretion was evaluated in rat intestine (distal ileum and proximal colon) sections (0.75cm^2). Tissue viability was checked by measuring LDH. Ileum sections were incubated with 200 mg/L of GSPE and colon sections were incubated with GSPE metabolites during 1 h at 37 °C and 5% (v/v) CO_2. In order to obtain the digested GSPE metabolites, rats (n=7) were administered a dose of 500mg/kg BW intragastric gavage 1h before sacrifice, and then the caecum content was extracted with ethylacetate.

The GSPE treatment on ileum induced an increase of active GLP-1 secretion. In the colon, the digested metabolites obtained from rats treated with GSPE induced a significant increase of active GLP-1 release compared with the digested metabolites obtained from non-treated rats.

In conclusion, the *ex vivo* results show that GSPE and its digested metabolites modulate entero endocrine hormone secretion, inducing an increase of active GLP-1 secretion at various locations of the intestine.

Financial support: AGL 2014–55347 from MINECO (Spanish Government)

P629

Common dietary flavonoids differentially regulate hemeoxygenase-1 relative to their bacterial catabolites and phase II conjugates in human endothelial cells

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Introduction. Flavonoid consumption has been implicated in the prevention of cardiovascular disease, although their mechanisms of action have yet to be elucidate Previous studies have suggested that flavonoids act through the modulation of hemeoxygenase-1 (HO-1), however, studies frequently utilize supraphysiological concentrations of unmetabolized flavonoids, while major circulating forms are methylated, glucuronidated, and sulfated derivatives.

Objective. Establish whether common dietary flavonoids have differential bioactivitrelative to their bacterial B-ring catabolites and related phase II conjugates at physiologically relevant concentrations.

Method. HO-1 protein expression was quantified by ELISA following treatment of endothelial cells with 6 dietary flavonoids and 14 conjugated and unconjugated phenolic derivatives at 1 M. Active metabolites were further studied for concentration response (0.1-50 M), structure-activity relationships (SAR), and effect on HO-1 mRNA expression, quantified by RT-qPCR.

Results & Discussion. HO-1 protein expression was increased by >20% in response to 4-hydroxybenzoic acid (4HBA; p0.01) and protocatechuic acid-4-sulfate (PCA4S; p0.05), while quercetin had modest effects (12%, p=0.07). Increasing concentrations above 1 M did not increase response, signifying activity at lower, physiologically achievable concentrations. SAR analysis suggests catabolism to phenolic acids increases HO-1 protein expression, while phase II metabolism has differential effects as sulfate conjugation preserved activity while glucuronide conjugation neutralized activity. HO-1 mRNA was upregulated in response to quercetin (2 fold, p0.01) and 4HBA (1.5 fold, p0.05) but not PCA4S (p=0.67).

Conclusions. Certain flavonoids may require metabolism to exert their maximal effects and metabolites may possess differential bioactivities as they are systematically metabolized and eliminated from the circulation.

Financial support: UK Biotechnology and Biological Sciences Research Council Diet and Health Research Industry Club (BBSRC-DRINC) (BB/I006028/1).

P630

Exploring the involvement of the insulin/IGF-1 pathway in the enhancing effects of quercetin and epicatechin on oxidative resistance. Assays in C. elegans mutant strains.

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Introduction. In previous studies, epicatechin and quercetin were shown to induce positive effects on life span extension and to improve the response against thermal and oxidative stress in the wild type N2 strain of C. elegans. In the present work the molecular mechanisms involved in these effects are explored by checking the ability of those flavonoids to enhance the stress resistance in knock-out worm mutants in different genes of the insulin/IGF-1 pathway.

Experimental. Different mutant strains of C. elegans (age-1, akt-1, akt-1;sgk-1, daf-2 daf-16, and hsf-1) were grown on NGM plates in the presence and absence of quercetin or epicatechin (200 M). At days 1 and 8 of adulthood, about 160 nematode per assay were submitted to thermally-induced oxidative stress at 35 ºC for a variable period up to 8h according to the strain resistance. Worms were scored as dead when they failed to respond to the touch with a platinum wire.

Results. A significant increase in the survival of worms submitted to stress was observed in the case of the daf-16 mutant treated with quercetin (but not with epicatechin), suggesting that the effect of quercetin on improving resistance to oxidative stress did not rely on that transcription factor. However, neither quercetin nor epicatechin produced significant enhancement in the stress resistance in any of the other mutant strains checked, i.e., age-1, akt-1, akt-1;sgk-1, daf-2, or hsf-1, suggesting that those genes are required to explain the mechanisms involved in the effects of the studied flavonoids on improving the resistance against thermal/oxidative stress in C. elegans.

Conclusion. The positive effects of quercetin and epicatechin on improving stress resistance might be related with their ability to modulate the activity of transcription factors in the insulin/IGF-1 pathway.

Financial support: Spanish Government through the projectBFU2012-35228.

P631

Interaction of iron and copper with small phenolic acids, human colonic metabolites of quercetin

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The metabolism of flavonoids is very extensive and includes conjugation and methylation by human enzymes and cleavage into small phenolic acids by human microflora. Due to the large metabolism of the parent flavonoids and the absence or markedly diminished pharmacodynamics effects of their conjugates, small phenolic acid might be partly responsible for positive effect of flavonoids on human being.

In this study, we tested a series of 10 colonic metabolites of quercetin on iron/copper chelating and reducing properties by spectrophotometric approach using iron and copper indicators ferrozine, hematoxylin and bathocuproine. Metabolites with catecholic ring /3-(3,4-dihydroxyphenyl)propionic acid, 4-methylcatechol and 3,4-dihydroxyphenylacetic acid/ were effective iron chelators in neutral conditions however they were as well very efficient iron reductants. Other compounds did not evoke significant interaction with iron with exception of homovanilic acid, which showed moderate ferric reducing properties. Contrarily, although initial screening of copper chelating properties by hematoxylin suggested chelation, none of the metabolites appeared to be a powerful copper chelator in a competitive assay with bathocuproine. In addition, all metabolites reduced cupric ion but in various extent.

Behaviour of quercetin´s colonic metabolites toward transition metal is complicated. Additional experiments on their effect on Fe/Cu based hydroxyl radical production, which are in progress, might help better understand their effects.

Financial support: Zentiva – Sanofi Company (Travel Scholarship Program) and Charles University in Prague (253115 C).

P632

Physiologically relevant olive oil phenolics are neuroprotective in SH-S5Y5 cells through modulation of intracellular signaling pathways.

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Background: Polyphenol-containing foods/beverages have received much attention with regard to their neuroprotective effects, including a potential to protect neurons against neurotoxin-induced injury, suppress neuroinflammation and promote healthy cognitive function. Olive oil derived phenolic compounds are known to exert protective effects in a variety of cell types and tissues, through the down-regulation o inflammatory pathways, the enhancement of antioxidant defense, and the modulation of intracellular signaling pathways. Thus there is a lack of understanding with regard to the mechanisms of action of olive oil derived phenolic compounds in the brain.

Objectives: To address this, we investigated the potential neuroprotective effect of hydroxytyrosol and tyrosol and homovanillic alcohol, 3 olive oil derived phenolic compounds proven to be bioavailable, in SHSY-5Y neuronal cells.

Methods: Their potential to counteract H_2O_2-induced cell injury was determined after exposing the cells to a range of low and high concentrations expected to be physiologically relevant (0.1-3μM) and by measuring cell viability (MTT assay) and the modulation of signal transduction pathways (Path scan membrane antibody array).

Results: Our data revealed that all tested compounds were able to exert a significant protective effect at all concentrations tested. The antibody array analysis demonstrated the ability of the compounds to modulate some intracellular pathways, significantly altering the expression of signaling proteins such ERK1/2, mTOR, Akt and JNK.

Conclusion: This research provides a preliminary basis for further study of the molecular mechanism of these polyphenols as potential neuroprotective compounds.

Financial support: University of Roehampton lectureship startup funds.

P633

Extra virgin olive oil phenolic extracts counteract the pro-oxidant and pro-inflammatory effect of dietary cholesterol oxidation products in human intestinal cells

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The intestinal tract is constantly exposed to endogenously generated as well dietary oxidants, like oxysterols, the cholesterol oxidation products. They are able to interfere with the homeostasis of the mucosal intestinal epithelium, by promoting and sustaining irreversible damage.

The phenolic fraction of extra virgin olive oil (EVOO) concentrates before absorption in the intestinal lumen, where it may contribute to the modulation of the oxidative stress and inflammatory response to toxic agents.

The aim of this study was to investigate the ability of two EVOOs phenolic extracts, to exert an antioxidant and anti-inflammatory action against oxysterols harmful effects in differentiated Caco-2 monolayers as a model system of the human intestine

Changes in the cellular redox state were evaluated determining reactive oxygen species (ROS) generation, variation of intracellular glutathione (GSH) concentration and glutathione peroxidase (GPX) activity; malondialdehyde (MDA) production was then evaluated as marker of oxidative damage. Caco-2 were also used in order to investigate the production of pro-inflammatory cytokines, specifically IL-6 and IL-8 already known to be crucial in chronic intestinal inflammation and sustaining intestinal barrier disruption.

Pre-incubation of cells with the phenolic extracts (PE) significantly attenuated oxidative modifications induced by oxysterols: increase of ROS production, GSH depletion, increase of GPX activity and MDA production; in addition the PE inhibite the increase of both pro-inflammatory cytokines production. These results suggest a possible preventive effect of olive oil phenolic fraction on intestinal diseases related to oxidative stress and inflammation.

Financial support: Regione Autonoma della Sardegna

P634
Procyanidins modulate the lipid raft-associated epidermal growth factor receptor signaling

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The consumption of procyanidins (PACs)-rich fruit and vegetables is inversely related to the risk of colorectal cancer (CRC). Previous studies showed that hexameric PACs (Hex), oligomers of the flavan-3-ols (-)-epicatechin and (+)-catechin, interact with intestinal epithelial cell membranes modulating cell signaling. These interactions occur in a specialized area of membrane (lipid rafts) where proteins involved in signal transduction co-localize, including the epidermal growth factor receptor (EGFR). Using Caco-2 cells as an in vitro model of CRC, we investigated the capacity of Hex to inhibit CRC cell growth via the modulation of the EGFR signaling cascade.

We investigated the capacity of Hex to inhibit the epidermal growth factor (EGF)-triggered activation of the EGFR and its downstream signaling. After incubating cells with and without EGF and in the presence of Hex (2.5-10 M), both total cell fractions and lipid rafts (using a detergent free method) were isolated. EGF activation (dimerization and phosphorylation at Tyr1068), and the downstream activation of Raf/MEK/ERK1/2 and PI3K/Akt cascades were investigated. Hex did not affect EGFR localization at lipid rafts, but inhibited EGFR phosphorylation (2.5-10 M) and EGFR dimerization (10 M). Hex also inhibited EGF-induced activation of both Raf/MEK/ERK1/2 and PI3K/Akt. We evaluated the capacity of Hex to affect the internalization of EGFR using ELISA and immunofluorescence assays. Both methods showed that Hex promoted EGFR internalization.

Our findings suggest that the anti CRC actions of Hex can be in part associated to its capacity to interact with lipid rafts and inhibit the EGFR signaling cascade at different steps.

Financial support: NIFA-USDA (CA-D*-xxx-7244-H) and UC Davis. E.D. was a fellow from Italian National Grant

P635
Olive oil polyphenols prevent pro-inflammatory responses induced by oxysterols inhuman blood mononuclear cells: insight on the cellular mechanism of action

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Oxysterols, cholesterol oxidation products, of both endogenous and dietary origin have shown cytotoxicity, pro-oxidant and pro-inflammatory effects, and have been linked with chronic diseases. Absorbed from the gut to the circulatory system, they may activate an inflammatory response.

The intake of dietary antioxidants, as those present in extra virgin olive oil (EVOO) could counteract the harmful effect of toxic agents, not only in the gastrointestinal tract but even at systemic level where can be found after absorption.

We investigated the interaction of EVOO polyphenols and oxysterols on the release, by peripheral blood mononuclear cells (PBMCs), of pro-inflammatory cytokines involved in the initial phase and amplification of the inflammatory process.

PBMCs, separated from the whole blood of healthy volunteers, were cultured and stimulated with a biologically compatible oxysterols mixture, in the presence of physiologically relevant concentrations of the EVOO polyphenols, hydroxytyrosol, tyrosol and homovanillic alcohol.

Oxysterols significantly increased the production of the pro-inflammatory cytokines MCP-1, IL-1, RANTES and MIF in ex-vivo cultured PBMCs. These effects were associated with an increased reactive oxygen species (ROS) production and, a net increment of phosphorylation of the mitogen activated protein kinase (MAPK) p38 and JNK was observed.

All phenolic compounds significantly reduced the increase of cytokines secretion induced by the oxysterols; such an effect was accompanied by an inhibition of ROS production and MAPK phosphorylation.

These results suggest that extra virgin olive oil polyphenols are able to modulate the immune response induced by dietary cholesterol oxidation products in human blood cells.

Financial support: Regione Autonoma della Sardegna

P636
Grape pomace extract from Brazilian viticulture and isolated anthocyanins present different biological activities in human carcinoma cell lines.

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Anthocyanins represent the majority of polyphenol compounds presented in grapes, which have powerful chemo-preventive and chemotherapeutic effects. This study aimed to evaluate grape pomace extract (GPE) bioactivity of Brazilian viticulture an isolated anthocyanins Cyanidin-3-O-glucoside (C3G) and Delphinidin (Del), in human carcinoma cell lines HepG2 (hepatocarcinoma) and MCF-7 and MDA-MB-231 (mammary). Grape pomace was provided by Embrapa Uva e Vinho/RS, Brazil. Hydroalcoholic extract was concentrated by nanofiltration. GPE was characterized according to its anthocyanins profile and antioxidant capacity. GP cytotoxicity was evaluated in HepG2 and MDA-MB-231 cells and C3G and Del cytotoxicity was evaluated in MCF-7 and MDA-MB-231 cells. C3G and Del effects on cellular oxygen consumption was performed by high-resolution respirometry.

GPE antioxidant capacity was(mean ± SE; mmolEq Trolox / g.):101.4 ± 0.1 and 16.7 ± 1.8, by TEAC and ORA-Cassays, respectively. C3G and Del corresponded to 0.1 and 0.14 g % dry matter, respectively. GPE showed time-dependent cytotoxic effects on HepG2 and MDA-MB-231, with a 75% and 30% reduction in viability, respectively, after 24h. Del cytotoxicity was only observed in MCF-7 cells, with a 7 decrease in viability after 48h. Cellular respiration in MDA-MB-231 and MCF-7 wa not affected by Del. In contrast, C3G promoted a 5% decrease in MDA-MB-231 respiration and a 46% increase in MCF-7 respiration. The different outcomes among these carcinoma cell lines might be related to their distinct energy metabolism and proliferative profiles. The synergic effects of phenolic compounds in GPE and isolated anthocyanins indicate their potential chemotherapeutic and nutraceutical properties.

Financial support: CNPq and FAPERJ (Brazil)

P637
Inhibition of lipid raft-mediated signaling by curcumin in Jurkat T cells

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Background: Curcumin, a major component of turmeric (Curcuma longa), exhibits anti-inflammatory, anti-oxidant, and anti-proliferative properties. However, the effect of curcumin on the lipid raft-mediated immunological responses remains largely unknown. Here we investigated the effect of curcumin on the lipid raft-mediated signaling process after T-cell receptor (TCR) stimulation.

Methods: Jurkat cells (human leukemia T cells) were pretreated with or without curcumin (10μM for 10min). For TCR stimulation, the cells were stimulated by anti-CD3 and anti-CD28 antibodies or magnetic beads conjugated with these antibodies. Gene expressions of IL-2 and HPRT were measured by real-time PCR. Detergent-insoluble fractions including lipid rafts were collected by sucrose gradient fractionation. The dynamics of actin after stimulation were visualized by confocal microscopy.

Results: Curcumin suppressed gene and protein expression of IL-2 and inhibited the lipid raft localization of ZAP-70, SLP-76, and Vav as well as the tyrosine phosphorylation of Lck, CD3 and LAT after TCR stimulation. Curcumin directly inhibited autophosphorylation of Lck. F-actin polymerization at the anti-CD3/CD28 antibodies-conjugated bead-cell contact site was upregulated, but the pretreatment of curcumin suppressed the polymerization.

Conclusion: In this study, we demonstrated that curcumin inhibited TCR/lipid raft-mediated activation of signaling molecules, thereby decreasing IL-2 production. Curcumin suppressed co-localization of raft-associated proteins and down-regulated the subsequent activation of phosphorylation cascades. Lck was found to be a direct target of curcumin. We identified a new function of curcumin in modulating the response of TCR stimulation at the cell surface level.

Financial support: Chubu University Grants A (22IM22A)

P638
Structure and activity relationship of stilbene monomers on the inhibition of synuclein aggregation

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The interest for the beneficial impact of polyphenols on health rose decades ago. Their properties are nowadays studied for the design of new therapeutic agents against various diseases. Among polyphenols, stilbenes are a family of molecules that displayed their ability to prevent neurodegeneration by modulating different pathway either through the inhibition of toxic aggregate formation or through the modulation of inflammation signaling pathways.

To date, an increasing number of polyphenols and stilbenes have been tested against the aggregation of the synuclein protein. Synuclein aggregation is thought to be a key phenomenon that leads to the neuron degeneration, causing the motor symptoms of the Parkinson disease. In our study, we screened several stilbenes monomers for their ability to inhibit synuclein aggregation using thioflavin T fluorescence assay. Thioflavin T dye fluorescence is used regularly to quantify the formation of ß-sheet fibrils. We desire to establish a relationship between the structure of stilbenes monomers and their activities.

Our preliminary results point out that the number and the position of hydroxyl and glycoside groups on stilbene may be an important parameter to prevent synuclein aggregation. Our study provides new data that would help in the design of therapeuti molecules.

Financial support: University of Bordeaux

P639
Integrated analysis of the nutrigenomic effects of wild blueberry consumption in healthy volunteers

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Recent epidemiological and clinical studies suggest that blueberries may improve cardiovascular function in humans. Investigation of nutrigenomic effects of blueberry holds promises to unravel their anti-inflammatory and cardio-protective molecular mechanisms.

A randomized, placebo controlled, double-blind parallel dietary intervention trial was conducted in 40 healthy volunteers. Peripheral blood mononuclear cells (PBMC) were taken at baseline and after one month supplementation of a freeze-dried wild blueberry powder (equivalent to 100 grams fresh blueberries, twice daily) containing a total of 297 mg polyphenols. Agilent Microarray chips were used for mRNA and miRNA expression analysis in a subset of the study population. Bioinformatic analyses were performed using Metacore, Pathway studio and KEGG. For monocyte to endothelial cell adhesion assay in-vitro, THP-1 cells were exposed to plasma-derived blueberry phenolic acids and co-incubated with TNF-stimulated HUVECs.

Microarray analysis identified 2085 differentially expressed genes upon consumption of wild blueberries. Pathway analysis revealed strong enrichment in cell adhesion, cell migration, platelet aggregation, phagocytosis and anti-inflammatory pathways. An increased expression of FoxP1, IL-10 genes was observed as well as a decreased expression was found for Vav1, Cfl1, Capn1, Nf-kB and EGFR genes. In vitro experiments revealed a reduced adhesion of monocytes, incubated with plasma-derived phenolic acids, to endothelial cells.

Our transcriptomic approach suggests that wild blueberry consumption improves cardiovascular health by promoting anti-inflammatory gene expression and by reducing expression of genes related to monocyte-endothelium interaction and inflammation in PBMCs. These findings are in line with parallel results of cardio-protective effects on endothelial function and blood pressure in the same volunteers. The impact on microRNA (post-transcriptional regulators of gene expression) is currently under investigation.

Financial support: Research Committee of the Medical Faculty of the University of Duesseldorf (GrantNumber 9772574)

P640
Moracin M, a stilbene from Morus alba, attenuate oxidative and inflammatory responses in microglial cells

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Microglia are macrophage-like immune cells of the central nervous system. They are implicated in the pathology of neurodegenerative diseases. Microglia insults, such as lipopolysaccharide (LPS) treatment, trigger their activation and in consequence the production of proinflammatory factors such as tumor necrosis factor-alpha (TNF-a), interleukin-1 beta (IL-1b), nitric oxide (NO), and the accumulation of reactive oxygen species (ROS). In excess, these mediators can exacerbate brain injury. Polyphenols are effective compounds in reducing LPS-induced microglial activation. Based on a screening of 25 polyphenols previously performed in our lab, moracin M, a stilbene from Morus alba, was selected for its promising anti-inflammatory activities. In the present study, we attempted to understand how moracin M would reduce pro-inflammatory mediators in LPS-stimulated murine BV2 microglial cells. For this purpose, we followed, by RT-qPCR, the expression of genes encoding TNF-a, inducible NO synthase (iNOS), IL-1b, cyclooxygenase 2 (COX2), and Toll-like *receptor*4 (TLR4). We also assayed the production of NO. Furthermore, we measure the transcript level of genes encoding anti-oxidative enzymes as catalase (CAT), glutathione S-transferase (GST) and heme-oxygenase 1 (HO-1). Moracin M, in a dose-dependent manner, can reduce both the production of NO in LPS-activated microglia and the accumulation of iNOS messenger RNA. The levels of TNF-a, IL-1b, COX2 and TLR4 transcripts were also diminished after moracin M/LPS co-treatment compared to LPS alone. The expression of CAT, GST and HO-1 genes was up-regulated following moracin M addition to the cells. These results suggested that moracin M may exert beneficial effects against oxidative stress and/or inflammation.

Financial support: University of Bordeaux

Poster Session H: Polyphenols and gut Microbiota Interactions

P700

Human gut microbiota metabolism of ellagic acid. Interindividual variability for urolithins production is correlated with health status: Cause or consequence?

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Urolithins are metabolites produced by the gut microbiota from dietary ellagitannins and ellagic acid. This metabolism has been conserved through phylogeny. *Gordonibacter genus* from *Actinobacteria phylum* is critically involved in urolithin production. Urolithins mainly exert anti-inflammatory and cancer chemopreventive activities in preclinical research. Interestingly, urolithins target malignant and normal colonic mucosa from colorectal cancer (CRC) patients. Not all individuals produce the same amount and type of urolithins. Human subjects can be stratified into three urolithin-producing groups. Individuals belonging to the so-called 'Phenotype A' produced only urolithin A conjugates (around 67% of the subjects, range 25-80%). 'Phenotype B' produced isourolithin A and/or urolithin B plus urolithin A (24%, range 10-50%). In 'Phenotype 0' (13%, range 5-25), urolithins were not detected.

These phenotypes were observed independently of the subjects' health status and characteristics (age, gender, BMI), and of the ellagitannin food source ingested (waltnus, pomegranates, strawberries, raspberries, etc.). Individuals belonging to Phenotype B usually present higher blood lipid levels than those from Phenotype A. Interestingly, a higher percentage of Phenotype B was observed in those subjects with chronic illness (metabolic syndrome or CRC) associated to gut microbial imbalance (dysbiosis) but also in overweight-obese individuals which was correlated to changes in gut microbiota. These urolithin phenotypes could show differences in the gut microbioma associated to the health status and should be considered in intervention trials dealing with health benefits of ellagitannins or ellagic acid. Whether this phenotypic variation could be a biomarker related to differential health benefits or illness predisposition deserves further research.

Financial support: AGL2011-22447 (MINECO, Spain), FP7-KBBE-2012-6-single stage (BACCHU Project, European Commission Grant Agreement 312090) and 201370E068 (CSIC, Spain).

P701

The GIDM-colon a new in vitro method to study the availability and colonic metabolisation of polyphenolic compounds

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The biological effects of polyphenols depend on their mechanism of action in the body. This is affected by bioconversion by colon microbiota and absorption of colonic metabolites. We developed and validated an in vitro continuous flow dialysis model with colon phase (GIDM-colon) to study the gastrointestinal metabolism and absorption of phenolic food constituents. Chlorogenic acid was used as model compound. The physiological conditions during gastrointestinal digestion were mimicked. A continuous flow dialysis system simulated the one-way absorption by passive diffusion from lumen to mucosa. The colon phase was developed using pooled faecal suspensions. Several methodological aspects including implementation of an anaerobic environment, adapted Wilkins Chalgren Broth medium, 1.10^8 CFU/ml bacteria suspension as inoculum, pH adaptation to 5.8 and implementation o the dialysis system were conducted. Validation of the GIDM-colon system showed a good recovery and precision (CV < 16%). Availability of chlorogenic acid in the small intestinal phase (37 ± 3%) of the GIDM-colon is comparable with in vivo studies on ileostomy patients. In the colon phase, the human faecal microbiota deconjugated chlorogenic acid to caffeic acid, 3,4-dihydroxyphenyl propionic acid, 4-hydroxybenzoic acid, 3- or 4-hydroxyphenyl acetic acid, 2- methoxy-4-methylphenol and 3-phenylpropionic acid. The GIDM-colon is a new, reliable gastrointestinal simulation system. It permits a fast and easy way to predict the availability of complex secondary metabolites, and to detect metabolites in an early stage after digestion. Isolation and identification of these metabolites may be used as references for in vivo bioavailability experiments and for investigating their bioactivity in in vitro experiments.

P702
Antibiotic treatment reduces enterolactone levels - a model experiment with pigs

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High levels of enterolactone are known to be protective on several chronic diseases such as cancer. Enterolactone is produced from plant lignans by the colonic microflora. The final step is carried out by a penicillin-sensitive member of the microflora.

The aim of this model study was to investigate the impact of antibiotic treatment on enterolactone levels in pigs to gain knowledge for use in an epidemiological study in humans relating use of antibiotics to enterolactone levels.

A total of 20 intact pigs were served a lignan-rich diet for 14 days. Antibiotic treatments were administered daily to a group of 10 animals on day 8-14. Fasting blood samples were collected from the jugular vein in the morning of day 7 and again at day 14. At slaughtering urine samples were taken directly from the bladder. Enterolactone concentrations in plasma and urine samples were analyzed by LC-MS. Animals treated with antibiotics showed significantly reduced levels of enterolactone in plasma and urine compared to untreated animals with reductions of 36% and 64%, respectively in treated animals. All animals responded equally to the lignan-rich diet on day 1-7, thus we suggest that the lower enterolactone level is caused by the antibiotic treatment most likely due to compromised enterolactone production by the colonic microflora.

Antibiotic treatments of pigs ingesting a lignan-rich diet significantly reduce plasma and urinary levels of enterolactone. Future experiments will study enterolactone levels measured in 3914 human blood samples with antibiotic intake 12 months prior to the blood sampling.

Financial support: The Danish Council for Strategic Research

P703
Microbial metabolites from phenol and tryptophan metabolism in humans consuming oolong tea

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While the specific effects of the polyphenols found in tea are largely unknown, it is thought that these compounds play a significant role in the health benefits seen in tea consumers. These polyphenols are not typically well absorbed by humans; and therefore, pass into the colon where they can be metabolized by microbes. Microbia metabolites in individuals consuming oolong tea have not been described. As part of a larger, double-blind crossover study, blood samples were collected from men (n=19; mean age=51 y; mean BMI=30 kg/m2) who consumed as part of a highly-controlled diet 1.4 L/d of water (control) or oolong tea for 5 days. On the fifty day, blood samples were collected following a 12 h fast (baseline) and again 4 h after they consumed 240 ml of beverage. Samples were analyzed by metabolomic profiling (GC/MS and LC/MS/MS) with 338 compounds identified. Hippurate, 1-hydroxyhippurate, catechol sulfate, and 4-methylcatechole sulfate are all metabolites of plant phenolics derived from microbial metabolism, and were significantly increased at baseline and 4 h following tea consumption (q-value <0.05 compared to the control. Other microbially-derived phenolics that were increased at baseline and 4 h following tea consumption were from phenylalanine (p-cresol sulfat and phenol sulfate) and tryptophan (indolepropionate and indoxyl sulfate). Despite short plasma half-lives of tea phenolics, microbial metabolites remain elevated in plasma 12 h after oolong tea consumption. Their elevated concentration following consumption may be one mechanism by which oolong tea can impact health outcomes.

Financial support: US Department of Agriculture and Suntory Limited. Tea was provided by Suntory Limited.

P704

The impact of change in diet on the gut microbial ecology in a crossover human trial – AJWA Study

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Current research has been directed towards the potential of fruit and vegetables, to influence the gut microbiota (Eid et al, 2013). Variations in host microbial ecology, influence changes in bacterial numbers, for example, populations exhibiting high baseline counts of potentially beneficial groups, (e.g bifidobacteria) encounter few changes in bacterial numbers in response to a diet intervention, in comparison to populations with lower bifidobacteria groups, such as those in high risk groups, like the elderly, malnourished populations, obese and with inflammatory conditions. The impact of date fruits, rich in both polyphenols and fibres (Eid et al, 2013), were tested in vitro using faecal batch cultures and FISH analysis, leading to bifidogenic effects (Eid et al, 2014). On the other hand, a human intervention trial in healthy young volunteers consuming (7 dates, approx. 50g) for 3 weeks, did not lead to significant alterations in the gut ecology. Further analysis of the baseline diet of the volunteers (day diet diaries) revealed that different modulation of gut microbiota in response to date supplementation. As such, volunteers with high fibre intake, consuming in average 18.5g/day, had no significant changes in their gut microbiota, whilst volunteers consuming low fibre levels, average of 6g/day, experienced significant increases in the beneficial bacteria, including Bifidobacterium, Lactobacillus, Collinsella, Bacterdoides, Rosburia, and Ruminococcus bacterial groups. Our result suggest that volunteers' baseline diet, particularly in regards to fibre content, influences the prebiotic potential of dietary interventions. Future clinical trials addressing prebiotic potential of foods, must take into consideration baseline fibre and also polyphenol intake of the study population.

Financial support: Ministry of Education in Saudi Arabia

P705

Effect of phenolic compounds of extra virgin olive oil on the adhesion of potentially probiotic Lactobacillus on pathogens to enterocyte like cells

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Polyphenols present in extra virgin olive oil have been demonstrated to exert many potentially beneficial biological effects. The phenolic fraction of extra virgin olive oil (EVOOph) concentrates before absorption in the intestinal lumen, where may selectively modulate the gut microbiota by its antimicrobial and prebiotic-like properties. Previous researches suggested that dietary polyphenols may stimulate the growth of beneficial bacteria such as Lactobacillus spp.

The aim of the present study was to evaluate the effect of EVOOph on the growth of potentially probiotic Lactobacillus plantarum 62LP39 (LB) and E. coli O157:H7 ATCC 35150 (EC) and the ability to interfere with the adhesion of probiotic or pathogenic strains to Caco-2 cells.

The tested EVOOph was extracted from a monovarietal oil obtained from olives selected from the most typical and widespread variety grown in Sardinia, Bosana, particularly rich in phenolic compounds; LB was isolated from artisanal ewes cheese.

Antimicrobial or stimulatory activity of EVOOph on LB and EC growth was determined on culture liquid media by plate count method. The effects on adhesion t Caco-2 cells were measured by adhesion and competitive inhibition assay.

EVOOph did not exert any inhibitory effect on LB growth and adhesion, while significantly inhibited the growth and adhesion of EC.

Competition studies demonstrated an antagonistic activity of probiotic LB against EC, enhanced in presence of EVOOph, suggesting a synergic effect between polyphenols and L. plantarum 62LP39 against gastrointestinal pathogens.

Financial support: RAS (Regione Autonoma della Sardegna), LR7

P706
Berry rich in polyphenols increases production of short chain fatty acids and lipids excretion

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Dietary polyphenols could be metabolized by the intestinal microbiota and their metabolites can act as anti-inflammatory compounds. They can modulate intestinal microbiota and stimulate short chain fatty acids (SCFA) production, which is also linked to hypocholesterolemic effects. This work aimed to investigate the effects of Syzygium malaccense berry (SMB) as a polyphenols source, in the production of SCFA and lipid profile of high-fat diet-fed mice.

The polyphenols of the freeze-dried SMB were analyzed using HPLC-DAD. Adult Swiss mice were divided in groups according to their diet compositions: normal-fat (N), N+5% SMB (NS), high-fat with 31% lard (HF), and HF+5% SMB (HS), during 10 experimental weeks. The SCFA production in the large intestine content was assessed using GC-FID. Serum LPS, circulant lipids, lipid contents in feces, liver and serum were analyzed using enzymatic commercial kits.

Catechin, epicatechin and quercetin were the polyphenols found in the hydrolyzed extract of SMB. Propionate content decreased in the HF group in relation to the N group. Acetate, propionate and butyrate contents increased in both groups NS and HS, representing together 69.93% (NxNS) and 81.75% (HxHS) of increment in the total SCFA production ($P<0.01$), respectively. Serum LPS levels were higher in HF animals, when compared to N and NS, and might indicate higher inflammation in this group. The HS animals showed reduced levels of serum cholesterol, hepatic triglycerides, and increased both triglycerides and cholesterol excretion.

The addition of SMB to HF diets decreased serum and hepatic cholesterol and triglycerides. The higher production of SCFA could be related with such results and also suggests other health benefits.

Financial support: Swedish Research Council (C0618901), Brazilian foundations: CNPq (206092/2014 and 300533/20136) and CAPES.

P707
Yoghurts and lignans work synergistically decreasing cholesterol LDL

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Lignans are interesting from a public health point of view as they constitute, along with isoflavonoids, the major phytoestrogen intake in western population and they play a role in the prevention of cardiovascular and other chronic diseases. Probiotics are live microorganisms that confer a health benefit on the host when they administered in adequate amounts. Nowadays, most of the probiotics consumed by humans come from fermented dairy products as yoghurt (produced using a culture of Streptococcus thermophilus and Lactobacillus bulgaricus).

We hypothesized that yoghurts may improve lignans bioavailability, increasing absorption by colonic mucosa and/or facilitating the bioactivation of polyphenols, lowering cardiovascular risk factors.

We used data from the PREDIMED study (ISRCTN 35739639), a cohort that includes 7447 elderly participants at high cardiovascular risk. Intake of lignans was calculated by matching food consumption data from baseline food frequency questionnaires with the Phenol explorer database on the polyphenol content of each reported food. General lineal models were used to assess the association between lignans and yoghurt and different cardiovascular risk parameters.

Participants with higher consumption of lignans and yoghurt had significant lower total cholesterol and LDL cholesterol. However, no differences were observed when separated analyses of lignans and yoghurt were performed.

Financial support: Instituto Danone, and to the CICYT (AGL2013-49083-C3-1-R) from the Spanish Ministry of Economy and Competitivel the Generalitat de Catalunya (GC) 2014 SGR 773, and to the CIBEROBN from the Instituto de Salud Carlos III, ISCIII (CIBEROBN-)

P708

Comparison and proteomic analysis of anti-inflammatory activity and quercetin responsivity among strains of Bifidobacterium adolescentis

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We have demonstrated that quercetin and several polyphenols enhance in vitro anti-inflammatory activity of *Bifidobacterium adolescentis* JCM1275^T. The aims of this study were to investigate the strain variation in the anti-inflammatory activity and the quercetin responsivity and to identify proteins involved in these mechanisms by proteomic analysis.

Fivestrains of *B. adolescentis* (1275^T, 1251, 7044, 7045, and 7046) were incubated with either DMSO as a control or quercetin in serum- and antibiotic-free DMEM at 37 °C for 3 h under static and anaerobic conditions. The supernatants of the culture media were added to macrophages to evaluate the suppression of LPS-induced nitric oxide(NO) production in the cells. Bifidobacterial proteins (1275^{T}and 1251, treated with either DMSO or quercetin) were separated by two-dimensional gel electrophoresis with an agarose IEF gel (pH3-8) and a SDS-polyacrylamide gel (5-20%). Image analysis was performed using PDQuest Advanced software.

The supernatants from the media incubated with either DMSO or quercetin as well as from the strain cultures with DMSO hardly affected the NO production in LPS-stimulated macrophages (0-10%), while the combination of the strains (JCM7044, JCM7045, and JCM7046) with quercetin markedly increased the NO inhibitory activity to a similar or lesser extent than that of JCM1275^{T}with quercetin (16-42%), indicating that the anti-inflammatory activity and the quercetin responsivity may be fairly common in *B. adolescentis*. Interestingly, however, JCM1251 completely failed to suppress the NO production regardless of the culture condition. Additionally, we detected two different spots with statistical significance ($P < 0.05$) by proteomic comparison between JCM1275^{T}and JCM1251. Quercetin treatment reduced the size of same spots in both strains, while, in the DMSO group, spot of JCM1251 protein was much lower than that of JCM1275^T, suggesting that JCM1251 may be the anti-inflammatory system-deficient stain, albeit with the quercetin responsivity.

Financial support: Grant-in-Aid for Young Scientists (B) (No. 26850085 to K. K.) from MEXT, Japan

P709

Lipid modification in Lactobacillus cells in response to oxygen and green tea extract exposure

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Exposure of oxygen-sensitive probiotic strains to dissolved oxygen generally results in a decrease of their growth rates or in a complete inhibition of their growth. Nevertheless, a study in our laboratory demonstrated that a Lactobacillus helveticus strain with oxygen sensitivity was able to grow under aerobic conditions to the same extent than under anaerobic conditions in a culture medium enriched with green tea extract (GTE). To get more insight of the mechanism through which GTE stimulated the growth of that strain in the presence of oxygen, the objective of this study was to evaluate the impacts of oxygen exposure and of GTE in the growth medium on the cell's lipids.

The effects of GTE (0 to 2000 µg/mL) in a MRS medium and oxygen exposure on Lactobacillus helveticus R0052 populations and lipids were evaluated using viable counts and gas chromatography analyses.

In unsupplemented medium, significantly lower populations were obtained in the presence of oxygen than under anaerobic conditions. Maximal populations reached in MRS + 2000 µg/mL GTE were not significantly different for cells grown under aerobic and anaerobic conditions and important changes in fatty acid composition of the bacterial lipids occurred. Cells have adapted to the presence of a high concentration of GTE in the growth medium by changing their lipid composition. At our knowledge, this is the first study showing a link between the modifications of bacterial lipids following exposure to 2000 µg/mL GTE and a stimulation of growth, in the presence of oxygen, of an oxygen-sensitive probiotic strain.

Financial support: Natural Sciences and Engineering Research Council of Canada (NSERC) grant

P710

Digested and fermented seaweed phlorotannins reduce DNA damage and inhibit the growth of HT-29 colon cancer cells.

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Background: Brown seaweeds are a rich source of phlorotannins, and unlike other classes of polyphenols, there is a lack of knowledge regarding phlorotannins and their bioactivity, particularly after their metabolism by the gut microbiota.

Objectives and Methods: We investigated the effects of a phlorotannin-rich Food Grade Extract (FGE) from the brown seaweed Ascophyllum nodosum and its High Molecular Weight (HMW) fraction and Low Molecular Weight (LMW) fraction on colon cancer cells after being subject to in vitro gastointestinal digestion (GID) and colonic fermentation (CF). We tested the ability of the three extracts to reduce H_2O_2induced cellular DNA damage (COMET assay) and inhibit cancer cell growth (SRBassay).

Results: The pre-treatment of cells with GID extracts did not significantly reduce DNA damage compared to H2O2 alone, however the CF extract HMW was significantly effective ($P<0.05$), whereas the CF extracts LMW and FGE did not induce any significant protective effect. All GID extracts significantly inhibited ($P<0.05$) HT-29 cell growth (at 250 and 500 µg/ml), with HMW being the most effective. When the cells were treated with the CF extracts, only HMW was able to significantly inhibit ($P<0.05$) the growth of the cells (at 250 and 500 µg/ml).

Conclusion: Gut microbiota metabolism of the seaweed extracts increased their ability to counteract the H_2O_2 induced DNA damage, whereas it reduced the ability of the extracts to inhibit the cell growth. In both cases, HMW was the most effective, indicating that the high molecular weight phlorotannins potentially exert a stronger beneficial effect in the colon.

Financial support: EU FP7/2007-2013 under grant agreement No 262519.

P711

The antimicrobial activity of citrus leaves flavonoid compounds collected in the region of Mitidja (Algeria).

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This study is the first contribution of the citrus leaves flavonoids chosen for their abundance in Algeria, economic and therapeutic interest. The phytochemical study and antimicrobial activity of flavonoids, has allows us to obtain the following contents: (2,477mg / g ± 0.361) flavones-flavonols (3,331mg / g ± 0.296) C-glycosides (0,337mg / g ± 0.046) and of Anthocyanins (3,77mg / g ± 0.204) and a variable antimicrobial activity against microbial organisms: S. aureus S. epdermidis, E. coli, A. tumefaciens, Bacillus subtilis, Pseudomonas aeruginosa, A. tumefaciens (I and II), C. albicans and S. cervisai. The MCI on solid agar medium ranging from 40µg / ml to 1680µg / ml. Flavones, flavonols shows better antimicrobial activity against E. coli (Ø = 27 mm), P. aeruginosa (Ø = 27mm), S. aureus (24,8mm) and S. cerevisras (22,4mm), with MIC = 382µg / ml of A. tumefaciens (27.8 to 29 mm) with MIC = 40µg / ml, of S. epidermidis (28,5mm) with MIC = 150µg / ml. The free aglycones is active against A. tumefaciens I and II (Ø = 23-21,5mm) with MIC = 50µg / ml, S. epedermidis (21mm) and E. coli (21mm) with MIC = 250µg / ml. Anthocyanins and C-glycosides are active only against A. tumefaciens (31.4 to 23.6) mm with MIC = 40µg / ml. The method in liquid medium dilution is applied only to extract flavones, flavonols, for their high antimicrobial activity demonstrated in solid medium, and to S. aureus strain to its pathogenicity to humans, the MIC (238µg / ml) is better than that obtained by the disk method and the BMC determined graphically is of 1845µg / ml.

P712

Influence of ferulic acid on gut microbiota growth during 24h in vitro fermentation

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The gut microbiota is recognised as having a large impact on the human health. While consumption of whole grains beneficially affects the gut function partly due to their fibre content. These include reduction in transit time and faecal pH and increase in faecal bulk. However wholegrain is also a rich source of phenolic acids, in particular ferulic acid may have additional beneficial effects. We aimed to examine if ferulic acid capable of positively modulating the gut microbiota composition.

Faecal Batch-Culture Fermentation experiment were performed with faecal samples from three healthy volunteers. Vessels were inoculated with faecal slurry (10%), and 5mM (high dose), 0.77mM (low dose) ferulic acid or positive control (i. prebiotic FOS). A further vessel was prepared under the same conditions but without the additions of any compound (negative control). Fermentation run under anaerobic conditions for 24h, samples were collected 0, 10, 24h.

Bacterial group were enumerated using fluorescent in situ hybridisation (FISH) combined with flow cytometry (FLOW) The faecal bacterial population were assessed such as Bifidobacterium spp., Bacteriodes spp., Lactobacillus/ Enterococcus spp., Clostridium coccoides –Eubacterium rectale group, C. histolyticum group. 5mM and 0.77mM ferulic acid did not significantly change the main bacterial groups after 10h or 24h fermentation compared to the negative control. However, both concentration of ferulic acid and negative control statistically increased the growth of Lactobacilli at 24h compared to their baseline.

Data suggest that gut media complexity can overlap the changes in gut microbiota composition. Future research needs to test potential synergetic effects of wholegrain fibre and phenolic acids on the gut microbiota.

Poster Session I: Polyphenols in other diseases

P800

Glycyrrhiza glabra L. as a promisor candidacidal in biofilms and planktonic cells: comparison between phenolic extract and isolated compounds

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Opportunistic fungal infections, particularly involving *Candida* species (candidiasis) have become a serious problem of public health. Current antifungal agents have been loosing the effectiveness, in part due to their overuse, and appearance of resistant *Candida* species. Plants have been used over years and recommended by natural physicians for multitude of health conditions. The aim of the present study was to evaluate the anti-*Candida* potential of a hydromethanolic extract of rhizomes and roots of *Glycyrrhiza glabra* L. (licorice), in planktonic cells and biofilms, as well as to compare its effect with individual phenolic compounds identified in the extract.

Licorice extract evidenced an antifungal activity against all of the nineteen tested *Candida* strains, including *C. albicans, C. glabrata, C. parapsilosis* and *C. tropicalis* species. The inhibition zones varied between 10-13 mm; MIC and MFC values varied, between 0.375-1.5 and 0.75-3 mg/mL for planktonic cells and biofilms, respectively. Considering the pronounced antifungal activity, a chemical characterization of the extract was conducted, and the main phenolic compounds identified were tested. Flavones (mainly apigenin derivatives), flavanones (mainly liquiritin derivatives), an isoflavone and a chalcone, were the most abundant compounds. However, these compounds were not active neither individually nor combined. Thus, probably synergistic effects among all components in the phenolic pool and/or other compounds present in the extract should be responsible for the observed antifungal activity.

Detailed *in vivo* studies should be performed, not only to evaluate the actual effects in a complete organism, but also the safety of the preparation and bioavailability in systemic infection models.

Financial support: FCT (Portugal) for financial support CIMO PEst-OE/AGUI0690/2014, N. Martins grant SFRH/BD/87658/2012 and L. Barros research contract.

P801

Tea of jaboticaba peel has positive effects in model of induced-colitis.

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Polyphenols have antioxidant and anti-inflammatory properties, which can positively act in inflammatory bowel diseases (IBD). A Brazilian source of these compounds is the jaboticaba (Myrciaria jaboticaba), mainly its peel, which contains anthocyanins and other phenolic compounds.

The aim of this work was to evaluate different concentrations (15, 25 and 50 g L^{-1} – T15, T25, and T50, respectively) of aqueous extract (Tea) of jaboticaba peel in oxidative status and macroscopic parameters of inflammation of adult rats with induced colitis using TNBS (2,4,6-trinitrobenzenesulfonic acid).

Diet intake and weight gain were similar when compared to control groups (Healthy- H and Colitis - C), as well as disease score. Tea intake was not altered in T50 in relation to water intake by C group. T50 and T15 showed tendency to increase the weight gain after induction, and they were statistically similar to the H group, which could be explained by the fasting hours before instillation. The ratio between weight and length of colon was 15% lower in T15 related to C, and equivalent to H group. Treatment in T50 reduced 15% lipid peroxidation in colon compared to C group (such as in H group), according to TBARS assay. However, changes in antioxidant status on colon of animals were not found by FRAP test.

These initial results showed that aqueous extract of jaboticaba peel could be a potential adjuvant in IBD management, mainly in concentration of 50 g L^{-1} (125 mg polyphenols kg^{-1} day^{-1}).

Financial support: CNPq (142493/2013-9; 300533/2013-6) and CAPES

P802

Grape juice influence during the periods of pregnancy and lactation on biochemical parameters and oxidative stress in serum and liver of rats wistar

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This study aimed to evaluate biochemical parameters and oxidative stress in serum and liver of Wistar rats, in the period after pregnancy and lactation, treated chronically with red grape juice variety Bordo (Vitis labrusca). Forty Wistar rats were placed for mating, and the confirmation of pregnancy was through vaginal swab.

After these animals were randomly divided into 4 groups with and without manipulation. We observed that red grape juice reduced the levels of aspartate aminotransferase liver enzyme in the serum of the manipulated animals tested.

Regarding the parameters of oxidative stress, red grape juice was able to reduce the levels of protein oxidation in liver and serum of the animals as well as to modulate the enzymatic activity of SOD in the liver and the enzymatic activity of CAT in the serum of the same, consequently promoting changes in the relationship of these enzymes. Based on the obtained results, we can see that red grape juice V. labrusca benefits presented before the evaluated biochemical and oxidative stress parameters, suggesting it as an important ally in pregnant women diet.

Financial support: CAPES, CNPq, FAPERGS, IBRAVIN

P803

Black tea and flavonoid intake predict lower risk of osteoporotic fracture in elderly women

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Observational studies have linked tea drinking, a major source of dietary flavonoids, with higher bone density. However, there is a paucity of prospective studies examining the association of tea drinking and flavonoid intake with fracture risk. The objective of this study was to examine the associations of black tea and flavonoid intake with serious fracture leading to hospitalisation in a prospective cohort of women aged over 75 years.

A total of 1,188 women were assessed for habitual dietary intake with a food frequency and beverage questionnaire. Incidence of verified osteoporotic fracture over 10 years was determined through the Western Australian Hospital Morbidity Data system. Multivariate adjusted Cox regression was used to examine the hazard ratios (HR) for incident fracture.

Over 10 years of follow up, osteoporotic fractures were identified in 288 (24.2%) women, with 212 (17.8%) of women experiencing a major osteoporotic fracture, and 129 (10.9%) of women experiencing a hip fracture. Compared to the lowest tea intake category (1 cup/week), consumption of 3 cups/day was associated with a decreased risk of osteoporotic fracture (HR: 0.70; 95% confidence interval (CI): 0.50, 0.96; P=0.029; actual risk 27.9% versus 22.5%). Compared to the lowest tertile of flavonoid intake, women in the highest tertile of flavonoid intake had a lower risk of osteoporotic fracture (HR: 0.65; 95% CI: 0.47, 0.88; P=0.006; actual risk 28.3% versus 21.0%), major osteoporotic fracture (HR: 0.66; 95% CI: 0.45, 0.95; P=0.026; actual risk 19.7% versus 15.4%), and hip fracture (HR: 0.58; 95% CI: 0.36, 0.95; P=0.030; actual risk 12.4% versus 8.3%).

These findings support the hypothesis that tea and dietary flavonoids may be protective against fracture risk in older women.

Financial support: Healthway Health Promotion Foundation of Western Australia; Sir Charles Gairdner Hospital Research Advisory Committee; National Health and Medical Research Council of Australia.

P804

Polyphenol intake biomarker is positively associated with geriatric conditions, frailty and mortality within the InCHIANTI cohort. Relevance to dietary polyphenol recommendations

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There is growing evidence of the health-protective role of dietary polyphenols intake on aging. In nutritional epidemiology, the use of a valid polyphenol biomarker provides a more accurate and objective measure of polyphenol intake than dietary questionnaires. Therefore, we examined the association between dietary polyphenols and its biomarker, urinary polyphenols, and cognitive and physical decline, frailty phenotype, and all-cause mortality in older adults from the InCHIANTI study.

The InCHIANTI ("Invecchiare in Chianti", i.e., Aging in the Chianti Area) is an ongoing prospective community-based study designed to evaluate risk factors affecting the loss of mobility and related conditions in the older population. The stud population (n=1155 participants aged 65-102 years) underwent multidimensional evaluation. Urinary polyphenols were determined with a rapid and validated colorimetric and a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodologies after a solid-phase extraction of urine. Dietary polyphenols were estimated using a validated Food Frequency Questionnaire and our own food composition polyphenol database based on three USDA and phenol-Explorer databases. Cognition was assessed using the Mini-Mental State Examination (MMSE). Physical performance was objectively measured using the Short Physical Performance Battery (SPPB). The frailty phenotype was defined as the presence of a least three of the following five criteria: shrinking, exhaustion, sedentariness, slowness and weakness.

We observed that participants who consume a diet rich in polyphenols reduce their risk of cognitive and physical decline, frailty phenotype and all-cause mortality, compared to those who consume diets low in polyphenols. Association was also observed between habitual dietary resveratrol exposure and frailty syndrome. Additionally, we conclude that urinary polyphenols expressed by 24-hour volume is better biomarker of dietary polyphenols than by urinary creatinine normalization.

A polyphenol-rich diet may protect against cognitive and physical performance impairment, frailty and all-cause mortality in older persons.

Financial support: CONSOLIDER INGENIO 2010 Programme, FUN-C-FOOD (CSD2007-063), and the JPI HDHL FOODBALL (PCIN-2014-133).

P805

Digested (poly)phenols from Arbutus unedo confer cytoprotection against oxidative stress in Saccharomyces cerevisiae

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The increase of population lifespan is closely associated to the rising of age-related diseases such as diabetes, cancer, cardiovascular and neurodegenerative disorders. These diseases constitute the major causes of mortality and morbidity in Europe and Western societies and are closely related to disturbance of cell redox homeostasis. The lack of effective therapeutic solutions for these diseases prompts the identification of novel compounds and bio-products

with therapeutic activity.

Nowadays, several studies supports the health potentialities of (poly)phenols for age-related diseases. However, the metabolic alteration of (poly)phenols in the gastro-intestinal tract must be considered for a physiological evaluation of the effects of their intake from diet, since it affects their bioaccessibility and bioactivity.

The aim of this study was to evaluate the cytoprotective activity of strawberry tree (*Arbutus unedo*) fruit and leave (poly)phenols after an *in vitro* digestion procedure mimicking the chemical transformations in the gastrointestinal tract. For that, we took advantage of *Saccharomyces cerevisiae* as a model organism. The protective activity of bioaccessible (poly)phenol metabolites was evaluated in wild type (WT) and *YAP* -deleted *S. cerevisiae* cells exposed to H_2O_2-mediated oxidative stress. *YAP1* plays a central role in the regulation of oxidative stress responses in yeast and its deletion increases susceptibility to oxidative stress. Metabolic capacity, ROS production, redox homeostasis and transcriptional analyses of genes involved in oxidative stress responses and protein clearance pathways were compared when yeast cells were treated with (poly)phenol metabolites.

Interestingly the results obtained indicate that after *in vitro* digestion, *A. unedo* bioaccessible (poly)phenols confer protection against oxidative injury in both WT an *YAP1*-deleted strain by mechanisms that do not simply rely on the activation of cell antioxidant defences. Besides, *A. unedo* bioaccessible (poly)phenols also demonstrate to be able of ameliorate the *YAP1*-deleted strain phenotype for WT levels in normal growth conditions.

Financial support: BacHberry project which is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-613793) and by the FCT Project (PTDC/BIA-BCM/111617/2009). Acknowledgements also to FCT for the individual fellowship of C.N. Santos (IF/01097/2013).

P806

Beneficial effects of combined therapy with Dry olive leaf extract (DOLE) and methotrexate in early rheumatoid arthritis patients

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Objectives: Impaired oxidative metabolism and dysregulation of proinflammatory cytokines play pivotal role in the pathogenesis of rheumatoid arthritis (RA). Aim was to evaluate the effects of six week supplementation with dry olive leaf extract (DOLE) in combination with standard methotrexate (MTX) therapy on reduction of cell damage and improvement in disease activity in early RA patients.

Methods: Patients newly diagnosed with RA were assigned to one of two groups: G1group (n=8) received only MTX while the G2 group (n=8) received DOLE 390 m twice per day, for a period of six weeks in addition to MTX. Lipid peroxidation, DN damage by comet assay and IL-6 levels were determined at the beginning of the stud and after 3 and 6 weeks.

Results: Significant decrease in disease activity score DAS 28-CRP was observed in both groups after 3 and 6 weeks compared to the beginning of therapy. Baseline concentrations of IL-6 of both G1 and G2 were reduced after 3 week and continued to decrease until 6 week in the G2 but not in G1. Same trend was exhibited in relation to the lipid peroxidation and the percentage of lymphocytes with DNA damage where G2 had significantly lower damage after 3 and 6 weeks while G1 that received only MTX showed reduction after 3 weeks but an increase after 6 weeks, compared to the initiation of treatment.

Conclusions: Combined therapy of DOLE and MTX demonstrated increased anti-arthritic efficacy compared to standard MTX therapy after six weeks in the early phase of RA.

P807
Anti-hyperuricemia polyphenols from medicinal plants used by local tribes in Indonesia

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Xanthine oxidase inhibitor has the potential to be a therapeutic agent for hyperuricemia and ROS-induced diseases. Allopurinol is a xanthine oxidase inhibitor that has been developed commercially and used clinically for several decades. However, the use of the drug led to some negative effects such as hepatitis, allergies and toxicity of 6-mercaptopurine. In our search into biological activities from Indonesian plant having ethnopharmacological information, extracts of 30 medicinal plants from 19 families were investigated for their anti-hyperuricemia activities.

Anti-hyperuricemia activity was evaluated by the xanthine oxidase inhibitory assay. The compounds were identified by NMR and MS spectroscopy. Of the 30 extracts assayed, *Sonneratia caseolaris* (Sonneratiaceae) and *Clausena excavata* (Rutaceae) leaves extracts displayed potential to inhibit the xanthine oxidase activity with activity greater than 50% at 50 mg/ml. Bioassay-guided fractionation of the leaves extract of S. caseolaris, led to the isolation of compound 1, identified as luteolin-7-O-glucoside. Compound 1 inhibited the activity of xanthine oxidase with the IC_{50} values of 12 mg/ml. Isolation and identification of flavonoid glycosides having xanthine oxidase inhibitory activity from Clausena excavata, a tropical perennial shrub will also be discussed. Allopurinol showed IC_{50} value of 6 mg/ml in our xanthine oxidase inhibitory activity assay. These findings indicate that the flavonoid isolates from *S. caseolaris* and *C. excavata* extracts are potential to be acted as natural anti-hyperuricemia agents.

Financial support: DGHE-Ministry of Research, Technology and Higher Education, Republic of Indonesia, under an International Collaboration and Scientifi Publication Research Grant (Grant no. 140/SP2H/PL/Dit.Litabmas/II/2015).

P808
Cranberry-derived Phenolic Metabolites and Urinary Tract infections

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The beneficial effects of cranberry products against urinary tract infections (UTIs) have been attributed, at least partly, to their A-type proanthocyanidin (PAC) content. A-type PACs have shown uropathogenic Escherichia coli (UPEC)-anti-adhesive activity, although they are unlikely to appear in urine at relevant concentrations as they are poorly absorbed. One leading hypothesis is that PAC-derived metabolites present in urine would operate in the phase of UPEC adherence to uroepithelial cells, preventing bacterial colonization. In addition to this, and as it is becoming evident that the intestine is a reservoir for uropathogenic bacteria, other hypothesis is that

A-type proanthocyanidins specifically decrease the transient intestinal colonisation b UPEC, consequently reducing the risk of UTI incidence. In any case, gut microbiota (and its inter-individual variability) seems to be an important factor to be considered.

In this communication, we summarize our results from different approaches aimed to look into the mechanisms that are behind the protective action of cranberry polyphenols against ITUs: 1)in vitro fermentations of cranberry polyphenols with colonic microbiota, that were performed to access the microbial-derived metabolic profile of cranberry polyphenols as well as their effect on gut microbiota survival, 2) an in vivo trial with model mouse intraurethral-inoculated with UPEC, that evaluated the effectiveness of cranberry supplementation in bacterial infection as well as its impact on faecal phenolic metabolism and faecal microbiota, 3) testing the UPEC-antiadhesive capacity of cranberry phenolic compounds and their metabolites in bladder epithelial cell cultures, and 4) ex vivo studies of UPEC-antiadhesive capacity of mice urines collected after cranberry supplementation.

Financial support: Ministry of Economy and Competitiveness (MINECO) (Projects AGL-2010-17499 and AGL2012-40172-C02-01) and the Comunidad Autónoma de Madrid (Project ALIBIRD S2013/ABI-2728), Spain.

P809

Resvega, a new preparation of resveratrol and omega-3 fatty acids, to fight age-related macular degeneration (AMD) progression: a role in VEGF-R pathway

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Numerous epidemiological studies have suggested that bioactive compounds as vitamin E, fatty acids, especially polyunsaturated acids, could be used as supplementation to protect against various diseases such as ocular diseases through their anti-oxidant power. Recently, another compound, resveratrol which is a trans-3,4',5-trihydroxystilbene (RSV), seems to have a great interest in the prevention of ocular diseases especially of age-related macular degeneration (AMD). Indeed, the polyphenol could counteract VEGF production in sick retinal pigment epithelium cells, ARPE-19. In our study, we have determined the potential synergy between omega-3 fatty acids and resveratrol to counteract VEGF-R pathway. We observed that the combination omega-3/RSV preparation (Resvega®) leads a stronger inhibition of the VEGF-R pathway activation that free preparation of RSV or RSV alone in sick retinal cells. Very surprisingly, Resvega® maintains and increases the functional VEGF-R pathway in normal retinal cells. This work brings a new mechanism by which omega-3/RSV could counteract AMD and could protect the second eye when AMD is already present.

Financial support: Théa laboratory.

P810

Soy phytoestrogen daidzein upregulates ER and p53 in the uterus of middle-aged rat

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Daidzein (DAI) is bioactive, non-nutritive food ingredient of the traditional East Asian diet and appears to be thoroughly discussed as an alternative to classical hormone replacement therapies. Currently, there is still a lack of information on the potential risks of its' therapeutic usage. In order to obtain further data useful for estimation of the safety profile of DAI, we investigated the potential of DAI for improvement of uteri function in middle-aged female rats. Also, we investigated the expression of steroid receptors (estrogen - ER, ER and progesterone - PR) as well as tumor suppressor p53 expression in the uterus of the same animal model.

Middle-aged (13-months-old) female Wistar rats subcutaneously received 35 mg/kg of DAI or the vehicle (olive oil and ethanol, 9:1), daily for 4 weeks. Changes in the structure and function of uteri were analyzed using the histological, immunofluorescence, Western blot and qPCR methods.

DAI did not change uterine weight or histomorphometric characteristics of uterus. Furtherly, the expression of ER and PR mRNA was not affected, while the expression of ER mRNA was increased. The expression level of phospho-p53 was decreased, while p53 was increased after DAI treatment, compared to the control group.

These results suggest that changes in ER and p53 expression/activity in the response to DAI may be an important mechanism to protect endometrial cells from proliferation. Therefore, DAI exhibited beneficial effects on the uterus of middle-aged female rats in the conducted study, figuring as the compound with a promising safety profile, therefore justifying the further investigations pertinent to its efficacy in alleviation of postmenopausal discomforts.

Financial support: Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 173009.

P811

Synthesis and evaluations of lipo-phenolic derivatives as anti-carbonyl stressors in retina pathologies

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Purpose: Carbonyl and oxidative stress play a substantial role in various neurodegenerative diseases such as Alzheimer's Disease, Parkinsonismor Age-relate Macular Degeneration (AMD). In retinal pathologies, both mechanisms are involved in the transformation of all-trans-retinal (AtR, reactive aldehyde) to bis-retinoid A2E Since accumulation of trans-retinal and A2E contribute to photoreceptor apoptosis, we designed and synthesized a resorcinol derivatives (phloroglucinol and resveratrol featuring enhanced anti-carbonyl stress properties.

Methods: Structural modifications include alkylation to increase nucleophilic and carbonyl trap properties. To improve their bioavailability and to induce a vectorization process to retinal tissue, more lipophilic derivatives have been considered. Regarding the high level of polyunsaturated fatty acids (PUFAs) present in the membrane of photoreceptors, the phenolic moity was linked by chemical process to polyunsaturated lipid moieties such as docosahexaenoic acid (DHA, C22: n-3), sn2-Lyso-PC-DHA, eicosapentaenoic acid (EPA, C20:5 n-3), alpha linolenic acid (ALA, C18:3, n-3) and linoleic acid (LA, C18:2, n-6).

Results and Conclusion: Exposure of RPE cells to carbonyl stressors (trans-retinal) caused dose-dependent decreases in cell viability, whereas pretreatment with Phloroglucinol-isopropyl-DHA and LA significantly reduced the decline. Biological evaluations pointed out the benefit of the isopropyl and PUFA substituents for cell survival in the presence of a carbonyl stressor.

Financial support: Université de Montpellier

P812

Anthocyanin, cyanidin 3-sophoroside, has suppressive effects on inflammatory responses

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Inflammation is one of protective biological reactions against tissue damages and infectious diseases. However, long-term inflammation is known to be associated with chronic disease or syndromes such as diabetes, arteriosclerosis, and cancers. Intake o phytochemicals in vegetables and fruits could prevent excess inflammation.

Berries are one of promising health foods and contain cyanidin glycosides as major anthocyanins, which are responsible for their colour. In this study, we investigated anti-inflammatory effects of anthocyanins such as cyanidin and cyanidin glycosides. Among the cyanidin glycosides used, we focused on cyanidin 3-sophoroside (C3S), which is included in some berries but not well researched.

First, an inhibitory effect on inflammatory enzyme, myeloperoxidase (MPO), was confirmed by HPLC methods (Kato et al., Biosci. Biotechnol. Biochem., 2003). The extract of berry and authentic cyanidin glycosides had an inhibitory effect on MPO activity. Among the glycosides, C3S showed the strongest inhibitory effect.

Next, we examined the influence of berry extract on secretion of interleukin-6 (IL-6) from lipopolysaccharide-stimulated RAW264.7 cells by measuring secreted IL-6 with an ELISA kit. As a result, berries suppressed the IL-6 secretion from RAW264.7 cells, and especially boysenberry tended to have a slightly high inhibitory effect among the berries examined. Since C3S is abundantly present in boysenberry, C3S might predominantly contribute to the suppressive effect.

Investigation of the effect of gene expression related to inflammation by adding anthocyanins to stimulated RAW264.7 cells is now in progress.

P813
Natural table olives phenolic extracts from Sardinian cultivars protect Caco-2 cells against oxidative damage

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Table olives are one of the most important and popular fermented products of the Mediterranean diet, greatly appreciated not only for his sensorial characteristics, but also for the beneficial health effects associated with its consumption. The health benefits reported for table olives have been associated with the levels of tocopherols and phenolic compounds, as hydroxytyrosol and oleuropein, known for their strong antioxidant and anti-inflammatory activity. Olives phenolic composition is influenced by several factors, mainly the cultivar, the degree of ripeness and the processing methods.

In this study we analyzed the antioxidant activity of phenolic extracts obtained from three cultivars belonging to the Sardinian germplasm: Nera di Villacidro, Pizz'e Carroga and Tonda di Cagliari. The extracts were obtained from olives processed through natural fermentation.

The activity of the extracts was tested on human intestinal cells (Caco-2) monolayers where oxidative stress was induced by tert-butil hydroperoxide (TBH). TBH treatment induced a significant production of intracellular ROS and malonyldialdehyde (MDA), with respect to the control samples.

All the extracts, tested at not toxic concentrations, were able to protect Caco-2 cells against the oxidizing action of TBH. The extract obtained from "Tonda di Cagliari" was the most active, due to the higher content in phenolic compounds, mainly hydroxytyrosol, compared to the extracts of the other cultivars.

Financial support: FBS (fondazione Banco di Sardegna) Prot. U915.2014/AI.798.MGB, and C.R.P. Regione Sardegna, L.R.7, Project "Sartol".

P814
Effect of quercetin on iron absorption

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Balancing systemic iron levels within narrow limits is critical for human health, as both iron deficiency and overload lead to serious disorders. There are no known physiologically controlled pathways to eliminate excess iron from the body and therefore iron homeostasis is maintained by modifying dietary iron absorption. Several dietary factors, such as polyphenols, are known to greatly affect iron absorption. Furthermore, it is proposed that polyphenols can affect iron status by regulating expression and activity of proteins involved in iron absorption.

To reveal how polyphenols affect iron absorption *in vivo*, experiments which include longer-term and chronic forced feeding (gavage) of Sprague Dawley rats with quercetin – the most abundant dietary polyphenol – were performed. Longer-term exposure comprised of iron uptake studies which were performed five hours after single and five and eighteen hours after double quercetin gavage. After both treatments, quercetin decreased iron absorption by increasing apical iron uptake but decreasing basolateral efflux into the circulation.

In chronic studies, quercetin treatment was applied once per day during 10 days. Subsequently, serum measurements concerning serum iron and transferrin saturation were done, duodenal, liver and spleen iron were determined and duodenal iron-relate gene expression levels estimated. After chronic treatment animals became iron-deficient, as both spleen and liver iron pools decreased. At the same time mRNA levels of the duodenal iron transporters decreased (DMT1, Dcytb and FPN), while duodenal iron increased.

In conclusion, oral quercetin administration caused iron deficiency in rats by two mechanisms. The main mechanism seems to be increase in mucosal iron uptake and inhibition of iron efflux from duodenal mucosa due to chelation of iron by quercetin. Secondly, quercetin treatment decreased mRNA levels of duodenal iron transporters and thus inhibited iron absorption. Bearing all mentioned, diet rich in polyphenols might be greatly undesirable for patients affected with anaemia.

Financial support: Biotechnology and Biological Sciences ResearchCouncil (BBSRC) (Grant No. BB/H003576/1) and Ministry of Education, Sciencesand Technological Development Republic of Serbia (Grant No. 172058). Marija Lesjak was supported by Dr. Willmar Schwabe Research Scholarship for Young Scientists awarded by Society for Medicinal Plant and Natural Product Research.

P815
Studies toward the synthesis of viniferifuran and analogues

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Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens [1]. Stilbenes are a class of plant polyphenols, and have attracted intense interest for their intricate structures and diverse biological activities. These compounds and their derivatives and/or analogues are of significant interest for drug research and development because of their potential in therapeutic or preventive applications, exemplified by resveratrol and combretastatin A-4 [2]. Recently our lab has discovered (-) hopeaphenol and other oligostilbenoids as inhibitors of type III secretion system in gram-negative bacteria [3, 4]. These compounds have potential to be developed as new generation of antibiotics that targets the virulence in contrast to conventional drugs that all target the bacterial growth [5, 6]. (-) Hopeaphenol belongs to a very large polyphenolic natural products formed by oligomerization of resveratrol and to sub-class structure containing (dihydro)benzofuran ring [7]. The biochemical pathway to form (-) hopeaphenol is believed to pass by dimerization of (+) -viniferin itself is formed by dimerization of resveratrol. Up to date, there is no total synthesis of (+) -viniferin and (-) hopeaphenol reported. Our lab has recently completed the total synthesis of rac--viniferin [8]. We are also interested in the total synthesis of viniferifuran - a didehydro analogue of -viniferin, which contains the unsaturated benzofuran ring. Viniferifuran has showed anti-inflammatory effects, including alleviate the asthmatic inflammation in asthmatic mice and decrease cigarette smoke-induced autophagy in human bronchial epithelial cells and very recently has beendiscovered as a potent ATP-competitive inhibitor of Sykkinase in vitro (IC_{50}=62 nM) [9]. A few approaches were reported to the synthesis of pentamethylprotected viniferifuran [10, 11, 12]. However, the final step of demethylation of this intermediate to obtain viniferifuran is not evident when we have tried to reproduce the result [10]. Here we present our effort toward the synthesis of viniferifuran and analogues (such as gnetuhainin B [13]). The synthetic routes developed will be used not only to prepare viniferifuran and its analogues to study structure-activity relationship but also to use in chemical biology such as the preparation of conjugates of viniferifuran and/ or analogues with biotin or fluorescent prove for target identification.

Financial support: Vetenskapsradet (Swedish Research Council), Umea Centre for Microbial Research(UCMR), Molecular Infection Medecine Sweden (MIMS)

P816
Vitis vinifera L. procyanidins as strong anti-inflammatory polyphenols at gastrointestinal level

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Human gastrointestinal mucosal inflammation can be generated mostly by a disregulated interaction between human host and microbial population leading to gastritis, ulcer, IBD besides representing an important risk factor for gastric cancer development. Helicobacter pylori has been identified as one of the most relevant agent for the development of gastric inflammation. During Helicobacter pylori infection, gastrointestinal epithelial cells activate different pro-inflammatory processes through NF-kB mediated over-expression of genes, including IL-8. Several studies have already reported a potential role of dietary polyphenols in modulating inflammatory processes at gastrointestinal level.

The present work has been focused on the in vitro investigation of the potential anti-inflammatory effect of a highly standardized grape seed extract from Vitis vinifera L. (Enovita®, Indena Tours, Tours, France) containing a well-characterized amount of procyanidins with different molecular weight. For this purpose, human gastric (AGS) and intestinal (CaCo-2) epithelial cells have been stimulated in vitro with pro-inflammatory cytokines (TNFα or IL-1β) and treated with the grape seed highly standardized extract at increasing concentrations (0.05-1 µg/mL) for 6 hours. IL-8 release was assessed by ELISA kit.

The results obtained demonstrated a strong capacity of the extract to reduce IL-8 release, with an IC_{50} 0.070 ± 0.013 µg/mL (mean ± s.d.) in AGS cells.

These preliminary results reveal a peculiar anti-inflammatory effect of procyanidins higher than other classes of polyphenols, which seem weaker modulator of inflammatory responses when tested in comparable experimental conditions. The data suggest a possible clinical testing in subjects with inflammatory related disorders of the gastrointestinal tract.

Financial support: AR and PM are employees of Indena

P817

Innovative bioguided fractionation of cranberry juice for Candida biofilm treatment and infected-wound healing.

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Cranberry polyphenols are intensively studied for their anti-infectious and antioxidant properties. Nevertheless, in biofilm and infected-wound healing, the identity of active polyphenols still remains unclear. In order to identify active polyphenols, an innovative fractionation scheme based on Centrifugal Partition Chromatography (CPC) was developed. CPC is a support free liquid/liquid chromatographic technology used here for the first time to fractionate cranberry juice. The fractionation protocol involved 3 mains steps: firstly polyphenol and sugars separation; then polyphenols fractionation based of molecular weight and polarity, and finally CPC or on LH-20 resin.

Following the second step, the 6 main fractions obtained were screened on Candida biofilm and toward infected wound. Fractions containing low molecular weight proanthocyanidins (Fr III and IV) and containing high molecular weight PAC (Fr V ad VI) exhibited the most interesting activities. Concerning Candida albicans biofilm a prophylactic effect was observed by prevention of adhesion. On infected wound, a wound-healing potential related to anti-inflammatory effect but not to antibacterial activity against Staphylococcus aureus was highlighted.

These four active fractions were further purified using either CPC or LH-20 resin, to lead to almost 40 sub-fractions. Again fractions containing PACs of medium and high molecular weight exhibited the highest activity against adhesion step of C. albicans strains and infected wound healing.

This work is a step further to understand mechanisms of action of cranberry polyphenols on *C. albicans* biofilm prevention and in infected wound healing.

Financial support: ARC Tours Poitiers 2014 grant, FERI Région Centre 2013 grant

P818

Chemical fingerprints and biochemical activities of edible congolese hibiscus leaves

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Hibiscus acetosella, Hibiscus cannabinus and *Hibiscus sabdariffa* leaves are widely consumed as vegetables in the form of sauces by Congolese people (Democratic Republic of Congo).

The aim of this study was to compare the chemical fingerprints and the correlations between anti-inflammatory (myeloperoxidase (MPO) activity) and antioxidant properties of polar and non polar extracts of these plants. Antioxidant properties were evaluated by using 2',7'-dichlorofluorescein-diacetate (DCFH-DA) and lucigenin on neutrophils and HL-60 cells. Chemical fingerprints were performed by Thin Layer Chromatography and High Performance Liquid Chromatography.

Chemical fingerprintings revealed that *H. acetosella* has a fingerprint different to *H. cannabinus* and *H. sabdariffa* that have the same fingerprint. Major compounds are phenolic acids represented by caffeoyhydroxicitric acid for H. acetosella and neochlorogenic acid for the others. All extracts displayed high ABTS and DPPH radical-scavenging activities at the concentration range of 10 – 80µg mL^{-1} and antioxidant activity assays at 0.05 – 10 µg mL^{-1}, according to the following order: *H.acetosella* > *H.cannabinus* > *H.sabdariffa*. Non polar extracts exhibited a strongest superoxide

anion scavenging effect than polar extracts.

By specific immunological extraction followed by enzymatic detection (SIEFED) technique, the selected extracts exhibited a strong dose dependent inhibitory effect on MPO activity at 0.1 – 10 µg mL^{-1}.

This study suggests that leaves of these Hibiscus species have the potential to be used as nutraceuticals for protection against oxidative damage and inflammatory processes under different conditions including konzo

Financial support: Fogarty International Center and NIEHS (Grant R01ES019841, NIH, and Bethesda,USA) and University of Liege

P819
Inhibitory effect of caffeic acid phenethyl ester on osteoclast differentiation through interrupting NADPH oxidase assembly

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Oxidation of biological components in our body by reactive oxygen species and free radicals have been thought to be major or minor factors in various life-style related diseases, such as cardiovascular diseases, diseases of nervous system, diabetes, cancer and aging. Osteoclasts have been shown to produce reactive oxygen species (ROS) that can stimulate osteoclast differentiation and bone resorption. Caffeic acid phenethyl ester (CAPE), a biologically active component of the honeybee propolis, has been reported to have several interesting biological regulatory properties such as antioxidant and inflammation. In the present study, we evaluated the suppressive effects of CAPE on RANKL-induced osteoclast differentiation of murine osteoclast progenitor RAW 264.7 cells through regulation of ROS production. We found that CAPEdose-dependently decreased O_2^- production, which was confirmed by a fluorescence measurement. In addition, CAPE dose-dependently inhibited RANKL-induced osteoclast differentiation and bone resorption activity according to tartrate resistant acid phosphatase (TRAP) staining and bone resorption assay.

Furthermore, the treatment of CAPE suppressed GTP-Rac1 activity and expression o NADPHoxidase1 (Nox1),p-$22^{phox}$and $p47^{phox}$translocation in a dose-dependent manner. Consequently, we propose that the direct inhibitory effect of CAPE on osteoclastogenesis may be mediated via inhibition of ROS generation through Nox1 signaling pathway.

Financial support: BK21 Plus project of the National Research Foundation of Korea Grant

P820
Biological activities of cyanidin-3-O--D-glucoside and protocatechuic acid

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Cyanidin-3-O--D-glucoside (C3G) is a predominant anthocyanin found in purple-black rice and black soybean. The compound has been reported to show high antioxidative and anti-allergic activities. However, we previously reported that large amounts of C3G were degraded and protocatechuic acid (PCA) was produced during the thermal processing of foods. To elucidate which of the two forms has higher biological activity, the antioxidative and anti-allergic activities of purified C3G and PCA were examined *in vitro* and *in vivo*.

Antioxidative activity was compared using the hydrophilic oxygen radical absorbance capacity (H-ORAC) assay. Moreover, the levels of thiobarbituric acid reactive species (TBARS) in the plasma and renal homogenates were evaluated in mice orally administered with C3G and PCA before the induction of oxidative stress by iron. For the anti-allergic activity assay, inhibitory effects of the compounds on-hexosaminidase release were examined using a degranulation assay with a combination of anti-dinitrophenyl IgE antibody and dinitrophenylated albumin in rat basophilic RBL-2H3 cells. Finally, the effects of orally administered C3G and PCA on passive cutaneous anaphylaxis reaction on the ears of mice were evaluated.

The H-ORAC value of PCA was higher than that of C3G. Moreover, the TBARS levels of plasma and the homogenate were lower when PCA was administered to mice before the induction of oxidative stress compared to those of mice administered C3G. On the other hand, C3G and PCA showed the same inhibitory levels to allergic reaction. These findings indicate that PCA has higher antioxidative activity rather than C3G, but there is no difference in anti-allergic activity between the two forms.

Financial support: JSPS KAKENHI Grant Number 253500114.

P821

Ferulic acid ethyl ester attenuates LPS-induced inflammation by interrupting NF-B/AP-1 signaling pathway through down-regulation of superoxide anion production in RAW 264.7 macrophages

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Ferulic acid ethyl ester (FAEE) is an ester derivative of ferulic acid and the latter is known for its anti-inflammatory property. Previous studies have shown that ferulic acid is a phenolic compound and its treatment of disorders is linked to control of oxidative stress and inflammation. FAEE is lipophilic and is able to easily penetrate into lipid bilayer. Therefore, the present study was designed to investigate the anti-inflammatory effects of FAEE and its underlying mechanism in LPS-induced RAW 264.7 macrophages. FAEE attenuated the productions of NO and the protein expression of iNOS. FAEE also reduced pro-inflammation cytokines such as TNF, IL-1 , and IL-6 in LPS-induced RAW 264.7 macrophages. In addition, FAEE suppressed LPS-induced superoxide anion generation in RAW 264.7 macrophage through its inhibitory effects on NADPH oxidase activation. Further study demonstrated that FAEE abrogated LPS-induced the activation IB, IKK and mitogen-activated protein kinases (MAPK) including JNK, ERK and p38 by phosphorylation. Finally, we confirmed that FAEE suppressed the activation of AP-1 in the nucleus and the translocation of NF-B into the nucleus. These findings imply that FAEE attenuates LPS-induced inflammatory responses in macrophages by interrupting the MAPKs/IKK/IB signaling pathway and downstream transcription factors, AP-1 and NF-B, which may result from down-regulation of NADPH oxidase-dependent superoxide anion production.

Financial support: BK21 Plus project of the National Research Foundation of Korea Grant

P822

Effects of Aronia melanocarpa constituents on biofilm formation of Escherichia coliand Bacillus cereus.

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Many bacteria growing on surfaces form biofilms. Adaptive and genetic changes of the microorganisms in this structure make them resistant to antimicrobial agents. Biofilms formed by pathogenic Gram-negative Escherichia coli strains and Gram-positive Bacillus cereus can pose serious problems to human health, such as prostatitis, biliary tract infections, anthrax and urinary catheter cystitis. Deleterious biofilms are also problematic in industry since they can cause fouling and corrosion in systems such as heat exchangers, oil pipelines, and water systems. Bacteria in a biofilm are often responsible for reoccurring symptoms and medical treatment failure. Thus, there is a need for novel prevention and treatment strategies. The aronia plant (Aronia melanocarpa (Michx.) has gained popularity in recent years due to its berries with a high content of polyphenols with antioxidant activity. Aronia berries contain high levels of flavonoids, mostly proanthocyanidins and anthocyanin.

The aim of this study was to screen extracts, anthocyanins and proanthocyanidins from A. melanocarpa for their abilities to prevent biofilm formation and to inhibit bacterial growth of E. coli and B. cereus in vitro.

Biofilm screening was performed by the microtiter plate biofilm formation assay. The disk susceptibility test was employed to study bacterial growth inhibition. It was found that exposure of biofilm-forming E.coli and B.cereus strains to several aronia constituents reduced biofilm production, however, they were not toxic to the bacteria. This non-toxic inhibition may confer a lower potential for resistance development compared to conventional antimicrobials. Whether our results can be used in future development of biofilm inhibitors requires further investigation.

Financial support: The Research Council of Norway and Tine SA under project number 19040402/110and Jahre Foundation

P823
Polyphenols in skins of red-kerneled rice and their inhibition of advanced glycation end products (AGEs) formation

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Red-kerneled rice is recently noted in a functional food because of its high content of polyphenols. The occurrence of proanthocyanidin in skins of red-kerneled rice has been reported. However, the details of the chemical structure of proanthocyanidin still remain unsolved. Herein, we investigated the separation and characterization of proanthocyanidin from skins, and evaluated inhibitory effects of the separated ingredients on advanced glycation end products (AGEs) formation. The rice skins were homogenized with 70% aqueous acetone. The extract was chromatographed over Diaion HP-20 with H_2O and MeOH. The MeOH eluent showed a content of proanthocyanidin by the formation of cyanidins on heating with *n*-BuOH-HCl. A number average molecular weight of the proanthocyanidin was estimated to be 2086 corresponding to an octamer structure based on gel permeation chromatography analysis. The ^{13}C-NMR spectral pattern of the oligomer was closed to that of catechidimer (procyanidin B-3). The monomeric units of the oligomer were revealed by acid-catalyzed degradation with phloroglucinol. The HPLC analyses of the reaction mixture exhibited the formation of catechin-(42)-phloroglucinol and catechin as major products. These data indicated that the procyanidin from skins of red-kerneled rice was assigned to involve an octamer composed of a catechin-(48)-catechin unit. The procyanidin oligomer from skins of red-kerneled rice was assessed for inhibitory activities towards the formation of AGEs by the glycation reaction of human serum albumin with glucose and fructose. The oligomer exhibited more potent inhibitory activity than that of aminoguanidine as a positive control.

Financial support: Grants-in-Aid for Scientific Research / The Tojuro Iijima Foundation for Food Science and Technology

P824
Renal cortex inflammation in LPS-treated rats: effects of dietary ()-epicatechin

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This study evaluated the effect of ()-epicatechin on renal inflammatory and functional manifestations in rats subjected to treatment with bacterial lipopolysaccharide (LPS) Sprague-Dawley rats were divided into two groups and treated during 4 d with control diet (C) or ()-epicatechin supplemented diet (100 mg ()-epicatechin /kg body weight/ day) (E). On the fourth day, animals were administered ip with saline solution (S) or LPS (L) (4 mg/kg body weight) obtaining 4 experimental groups: CS; CL; ES; and EL, and euthanized 6 h later. Creatinine and urea plasma levels were higher in CL respect to CS (CS=0.45±0.04 and CL=0.58±0.03* mg/dl of creatinine; CS=28±3 and CL=56±7* mg/dl of urea; *$p<0.05$). Activation of NF-kappaB-mediated inflammatory pathway in renal cortex was studied determining p65 phosphorylation in total homogenates and the ratio nuclear/cytosolic p65 protein. Both parameters were higher in CL respect to CS (37%, $p<0.05$, and 86%, $p<0.04$, respectively). In addition, phosphorylation and degradation of the NF-kappaB inhibitory peptide IkappaBalpha were significantly higher in renal cortex of CL respect to CS. Inducible nitric oxide synthase expression was higher (38%, $p<0.05$) in the renal cortex of rats in group CL with respect to CS. Rats receiving the diet supplemented with ()-epicatechin prior to LPS administration (EL) did not show alterations in renal function and NF-kappaB-associated parameters in renal cortex were similar to non-LPS treated animals (ES).

These results suggest that dietary administration of the ()-epicatechin was associated with an attenuation in the LPS-mediated inflammatory response in renal cortex resulting in kidney protection.

Financial support: UBACyT 20020120100177 and 20020130100760BA, PIP0612, PICT-2012-0765

P825
Polyphenols from flowers of Magnolia coco and their inhibitory effects on the formation of advanced glycation end products (AGEs)

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Magnolia coco is a shrub, and its flower gives strong fragrance. The flowers open in evening and the petals fall until morning. In a survey of glucosidase inhibitors from natural sources, we investigated inhibitory effects of several plant extracts using *in vitro* glucosidase inhibition assay. Among tested samples, the flower extract of *M.coco* showed inhibition on -glucosidase. We investigated the separation and characterization of glucosidase inhibitors from the flowers. In addition, we evaluate inhibitory effects of the isolated compounds on advanced glycation end products (AGEs) formation. The 70% aqueous acetone homogenate was extracted with Et_2O, EtOAc, and *n*-BuOH, to afford the respective extract. The extract of*n*-BuOH was separated by repeated column chromatographies and purified by preparative TLC, to give chlorogenic acid, epicatechin, rutin, kobusin, (+)-sesamin, and proanthocyanidi The chemical structural feature of proanthocyanidin was indicated by the formation of cyanidins on heating with *n*-BuOH-HCl, the analyses of ^{13}C-NMR and gel permeation chromatography, and acid-catalyzed degradation with phloroglucinol.

These results revealed that the proanthocyanidin from flowers of *M. coco* was determined to involve a 22-mer composed of an epicatechin-(48)-epicatechin unit. The isolated compounds were assessed for inhibitory activities towards -glucosidase and the formation of AGEs by the glycation reaction of human serum albumin with glucose and fructose. Proanthocyanidin showed significant inhibitory effect towards AGEs formation.

Financial support: National Grant

P826
Membrane-related effects underlying the antibacterial capacity of Cistus salviifolius polyphenols

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Polyphenolic extracts obtained from Cistus plants have shown antimicrobial effect against Gram-positive and Gram-negative bacteria but the mechanism of this action remains elusive. In this work, the interaction between polyphenols and bacterial membranes has been studied as a putative mechanism for the antimicrobial activity o these extracts.

In this study, the interaction between the four main polyphenols of a *C. Salviifolius* extract (ellagic acid, punicalagin, quercetin and myricetin) and model membranes simulating both Gram-positive or Gram-negative bacterial membranes have been studied using differential scanning calorimetry (DSC), membrane leakage and membrane affinity studies.

DSC experiments showed that some of the main polyphenols of *C. Salviifolius* significantly perturbed bacterial membranes either by decreasing the transition temperature or the enthalpy transition of the phospholipid membrane. This effect was more relevant for myricetin on the Gram-positive model and for ellagic acid on the Gram-negative model. Leakage experiments confirmed that these polyphenols, especially ellagic acid and punicalagin affected the integrity of bacterial membrane models, especially in the Gram-negative model. Finally, membrane affinity studies pointed out that highest polyphenol hydrophobicity mostly correlated with strongest membrane affinity but this did not match the strongest effect.

According to our results, the antimicrobial activity of *C. salviifolius* polyphenols and therefore, that one of the whole extract, may be due in some extent to the capability of these polyphenols to destabilize the bacterial membrane compromising bacterial cell viability.

Financial support: AGL2011-29857-C03-03 (Spanish Ministry of Science and Innovation),PROMETEO/2012/007, ACOMP/2013/093 (Generalitat Valenciana) and CIBERCB12/03/30038, CIBERobn (Instituto de Salud Carlos III).

P827

Polyphenol characterization, free radical scavenging capacity and antimicrobial activity of extracts derived from Cistus salviifolius and Thymus moroderi.

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Plant polyphenols are a source of antioxidant and antimicrobial compounds that may improve the therapeutic arsenal against oxidative stress-related pathologies and microbial infections. Previous research has reported the polyphenolic composition and the antioxidant capacity of extracts derived from species well adapted to the Mediterranean area such as *Cistus salviifolius* (*Cistus* genus) and *Thymus moroderi* (*Thymus* genus). In the present study, the extraction procedure of the aerial part of these two plants has been optimized (type of solvent, extraction time and plant/solvent ratio) to maximize their polyphenolic content and the antioxidant capacity through a bioguided multistep protocol. The composition of the extracts was characterized by using high performance liquid chromatography coupled to ion trap mass spectrometry with electrospray ionization (HPLC-ESI-IT-MS/MS). The antioxidant and the antibacterial activity was also tested using previously established methods.

Acetone, 1:10 plant/solvent ratio and 4 h were selected as the most convenient extraction conditions to achieve extracts with the highest polyphenolic content and antioxidant capacity. Extracts derived from *Cistus salviifolius* mostly contained flavonoids (flavonols such as quercetin and kaempferol derivatives and small amount of flavanols) and punicalagins. Extracts derived from *Thymus moroderi* were composed of flavonols, anthocyanins and phenolic acids. *C. salviifolius* extract exhibited a higher antimicrobial activity against *E. coli* that *T. moroderi* extract. The results obtained in the present study suggest the suitability of both extracts as ingredients for dermocosmetic applications that needs to be further explored.

Financial support: AGL2011-29857-C03-03 (Spanish Ministry of Science and Innovation),PROMETEO/2012/007, ACOMP/2013/093 (Generalitat Valenciana) and CIBERCB12/03/30038, CIBERobn (Instituto de Salud Carlos III).

P828

Anthocyanidin-rich extracts in the prevention of intestinal permeabilization

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An increased permeability of the intestinal barrier is proposed as a major event in the pathophysiology of inflammatory bowel diseases, and as one underlying factor in the development of obesity-associated pathologies. Chronic inflammation is usually a common factor in conditions with increased intestinal permeability. In this scenario, tumor necrosis alpha (TNF) plays a central role in part by promoting tight function barrier dysfunction. This study studied the capacity of extracts, containing different types and amounts of anthocyanidins (AC), to inhibit TNF-induced permeabilization of Caco-2 cell monolayers, investigating the association of their protective actions to the relative content of individual AC. The composition of the extracts in terms of AC was measured by LC-MS. Caco-2 cells differentiated in permeable inserts into intestinal epithelial cell monolayers, were initially incubated for 24 h with interpheron to upregulate the TNF receptor. Monolayers were subsequently incubated in the absence/presence of TNF (lower chamber), with or without the addition of 1-10 g extract/ml for 6 h (upper chamber). The transepithelial electrical resistance (TEER) was measured as a parameter of monolayer permeability. The protective actions of the extracts on TNF-induced monolayer permeabilization were concentration-dependent. Within the range of concentrations studied the protective actions of the extracts on TNF-induced TEER increase was not significantly correlated to the total AC, malvidin, petunidin, or peonidin content of the extracts.

However, a positive and significant correlation was found for TEER and cyanidin (r^2=0.73,$p<0.04$) and delphinidin (r^2=0.73, $p<0.04$) extract content. These results suggest that consumption of foods/supplements containing cyanidin and delphinidin could mitigate the adverse effects of inflammation at the gastrointestinal tract.

Financial support: NIFA-USDA (CA-D*-XXX-7244-H) (PIO); Nu Skin Enterprises, Provo, UT, USA.

P829

In vitro antioxidant, cytotoxic and anti-inflammatory activity of uncommon xanthones from branches of Garcinia achachairu Rusby

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Background/Objectives. Medicinal plants represent a huge source for new drugs development. The genus Garcinia is a rich natural source of xanthones, biflavones, and benzophenones, which show a variety of biological properties. We were interested in evaluating the in vitro antioxidant, cytotoxic and anti-inflammatory activity of two uncommon xanthones isolated from Garcinia achachairu, in order to determinate their potential therapeutic applications in inflammatory pathologies or cancer.

Methods. The two compounds (GAG 45-47 and GAG 51-65) were investigated for antioxidant properties by using ABTS assay. Cytotoxic activity was evaluated on HT-29 cells by sulforhodamine B assay after 48 and 72h. Anti-inflammatory activity was performed on THP-1-macrophages, which were treated with the xanthones (5, 1 and 20 M) for 30 min, followed by stimulation with LPS for 24h. Pro-inflammatory cytokines levels (TNF-, IL-1, IL-8 and IL-6) were monitored by ELISA and COX-2 and iNOS protein expression was measured by Western blotting.

Results. The xanthone GAG 51-65 exhibited strong radical-scavenging activity, with an antioxidant capacity slightly superior to that of the Trolox standard. The two xanthones showed moderate cytotoxicity activity in HT-29, being GAG 45-47 the most active compound (IC50=14,4 ±0,6 μM, 72h). Pre-treatment with these compounds reduced LPS-stimulated cytokines production in THP-1 cells as well as attenuated LPS-induced COX-2 and iNOS protein expression after 24 h.

Conclusion. The two xanthones have a moderate antioxidant and cytotoxic activity as well as a potent anti-inflammatory effect. This kind of polyphenols may represent an interesting alternative for the treatment of inflammation-related diseases or cancer.

Financial Support: Rede Iberoamericana de Investigação em Câncer. Da Genômica aControle (RIBECANCER/CYTED/CNPq)

P830

Melatonin and hydroxytyrosol-rich wines influence the generation of lipoperoxidation markers based-lipidomics after the dietary intake of three types of wine by healthy female volunteers

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IsoPs have been established as general and reliable "in vivo" oxidative stress biomarker. Prostaglandins (PGs) and IsoPs are considered as lipid mediators, involved in the physiopathology of all organs. Feeding is essential for the correct maintenance of health, and Mediterranean Diet has been proved to exert several benefits in order to the maintenance of the oxidative organism balance. Wine has been demonstrated their antiradical scavenges capacity, mainly attributed to polyphenols but their bioavailability is quite scarce. So that other compounds as melatonin or hydroxytyrosol could be taken into account. Both melatonin and hydroxytyrosol act as a radical scavenger, regulating the oxidation status of the organism, they have demonstrated endogenous enzymatic activation of the antioxidant defense system. The present research aims to clarify the state of the art about whole body lipid peroxidation and enzymatic lipid oxidation related to wine intake. OHTyr/Mel in particular, performing an in vivo cross-study with 18 female volunteers during five weeks. Red wines treatments exhibited the highest oxidative stress protection attributed to hydroxytyrosol content ($p<0.05$), and with minor relevance to melatonin content ($p>0.05$). The red wine intake decreased the isopros-

tane generation in higher extent than must and white alcohol intake (rum). Concerning to the 29 prostanoids analyzed, the red wine intake was able to reduce the urinary excretion of pro-inflammatory prostaglandins (platelet aggregation and vasoconstriction) such as 2,3-dinor-11-β-PGF2α, U-44619 y U-44069 while increased the production of the cardioprotective prostaglandin E1 (vasodilation). These actions were related to hydroxytyrosol content of red wines rather than melatonin or alcohol content.

P831

Melatonin and hydroxytyrosol-rich wines exert protection of the central nervous system against oxidative stress after the intake of three types of wine by healthy volunteers

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Lipid peroxidation products have been investigated in order to determine their use as biomarkers of oxidative status in the human body. Adrenic acid (AdA), and docosahexaenoic acid (DHA) results in F2-dihomo-Isoprostanes (F2-dihomo-IsoPs) that have been related to early brain damage and white matter injury, and F4-neuroprostanes (F4-NeuroPs) which affords the dimension of neuronal oxidative damage, respectively. Wine polyphenols, especially from red wine, have demonstrated in vivo and in vitro antioxidant capacity. Besides polyphenols, other compounds as melatonin (MEL) and hydroxytyrosol (OHTyr) could be part-responsible of the antioxidant benefits of red wine among other known or unknown compounds. To elucidate if these compounds take part into the protective antioxidant effects of red wine a double-blind-crossover-placebo controlled study by the intake of red wine and their native musts was accomplished. Main results indicate that urinary F4-NeuroPs and F2-dihomo-IsoPs decreased upon administration of red wine in higher extent than those found after the intake of their corresponding musts. Hydroxytyrosol and homovanillic alcohol seems to be responsible of the decrease of F2-dihomo-IsoPs. Remarkable effects were highlighted concerning to melatonin and homovanillic acid, a colonic metabolite from hydroxytyrosol which were inversely correlated with the excretion of F4-NeuroPs. To the best of our knowledge, this is the first report comparing musts and wines in base on their content in melatonin and hydroxytyrosol metabolites and their effects on markers of oxidative stress of the central nervous system.

P832

In vitro antioxidant, cytotoxic and anti-inflammatory activity of uncommon xanthones from branches of Garcinia achachairu Rusby

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Background/Objectives. Medicinal plants represent a huge source for new drugs development. The genus Garcinia is a rich natural source of xanthones, biflavones, and benzophenones, which show a variety of biological properties. We were interested in evaluating the in vitro antioxidant, cytotoxic and anti-inflammatory activity of two uncommon xanthones isolated from Garcinia achachairu, in order to determinate their potential therapeutic applications in inflammatory pathologies or cancer. Methods. The two compounds (GAG 45-47 and GAG 51-65) were investigated for antioxidant properties by using ABTS assay. Cytotoxic activity was evaluated on HT-29 cells by sulforhodamine B assay after 48 and 72h. Anti-inflammatory activity was performed on THP-1-macrophages, which were treated with the xanthones (5, 10 and 20 μM) for 30 min, followed by stimulation with LPS for 24h. Pro-inflammatory cytokines levels (TNF-α, IL-1β, IL-8 and IL-6) were monitored by ELISA and COX-2 and iNOS protein expression was measured by Western blotting. Results. The xanthone GAG 51-65 exhibited strong radical-scavenging activity, with an antioxidant capacity slightly superior to that of the Trolox standard. The two xanthones showed moderate cytotoxicity activity in HT-29, being GAG 45-47 the most active compound (IC50=14,4 ± 0,6 uM, 72h). Pre-treatment with these compounds reduced LPS-stimulated cytokines production in THP-1 cells as well as attenuated LPS-induced COX-2 and iNOS protein expression after 24 h. Conclusion. The two xanthones have a moderate antioxidant and cytotoxic activity as well as a potent anti-inflammatory effect. This kind of polyphenols may represent an interesting alternative for the treatment of inflammation-related diseases or cancer.

Poster Session J: Polyphenols and Food process

P900

Alternative bioprocess to obtain phenolic compounds with high biological potential using orange juice industry co-products.

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In recent years, attention has turned to phenolic compounds with biological activity due to their ability to promote benefits for human health, as well as their antioxidant and anti-mutagenic activities. Colorectal cancer constitutes one of the most frequent malignancies worldwide and is one of the prevalent causes of cancer-related mortality in the western world. Numerous studies have evaluated Citrus phenolic compounds a promoters of cancer cell growth inhibition and apoptosis, and with potential to reduce invasion, angiogenesis and metastasis. Currently, phenolic compounds are obtained by chemical synthesis or extraction, which may have low efficiency on the obtainment of most bioactive forms of the molecules. Biotransformation reaction technology is deemed economically and ecologically competitive in the search for phenolic compounds. The objective of this study was to evaluate a microbial biotransformation process to obtain bioactive phenolics from Citrus residues. The development of the microbial biotransformation of Citrus residue extracts increased the hesperetin, naringenin and ellagic acid content by 900, 1400 and 1330%, respectively, after 48 hours of fermentation with Paecilomyces variotii strain. The antiproliferative activity of the produced extracts against HT29 cells, achieved 42% of tumoral cellular inhibition. These results suggest that biotransformation process may be a viable alternative for rich bioactive phenolic extract production using residues.

Financial support: CAPES, FAPESP, CNPq

P901

Biotransformation of teas phenolics by immobilized tannase and the effect on antioxidant activity

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The aim of this work is to compare antioxidant activities and phenolics profiles in different types of tea before and after the biotransformation by immobilized tannase from Paecilomyces variotii. A total of 300 ml of the green tea (infusion in 20 mM phosphate buffer, pH5,5) was biotransformed with 1U of immobilized tannase at 60°C, 2h. The enzymatic reaction was also carried out in black, mate and white tea. At the end of the reaction, the immobilized enzyme was collected and the product was freeze dried. High Performance Liquid Chromatography was used for the phenolic identification of gallic acid, epigallocatechin gallate (EGCG), epicatechin gallate (ECG), catechin, epigallocatechin (EGC), epicatechin (EC), quercetin and rutin. DPPH free radical scavenging activity, ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) were analyzed. In this study, the enzyme catalyzes the biotransformation of compounds in green tea, releasing the aglycones such as epigallocatechin (EG), epicatechin (EC), galic acid and lower molecular weight phenolics such as quercetin. The biotransformation significantly increased antioxidant capacities of green tea ($p<0,05$). In green tea extracts there was an increase by 32% in potential to capture of the peroxyl radical (ORAC), 95% in potential to reduce the ferric salt (FRAP) and 26% in DPPH free radical scavenging. The use of immobilized tannase proves to be an interesting resource for enhancing nutraceutical power in teas. Taken together, these results indicate that immobilized tannase biotransformation change not only phenolics profile but also potential health-promoting effects of green tea.

Financial support: Capes, CNpq

P902
Beneficial effects of biotransformation of tea on adipogenesis: 3T3-L1 cell model

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Camellia sinensis and Ilex paraguariensis are rich in polyphenolic compounds which can contribute to the health benefits of tea. The aim of this study was to evaluate the cytotoxicity and adipogenic effects of green, mate and white tea extracts after the biotransformation catalysed by immobilized tannase on adipocytes cell line (3T3-L1 A total of 300 ml of the white tea, mate or Green tea (infusion in 20 mm phosphate buffer, ph 5,5) was biotransformed with 1 U immobilized tannase from Paecilomyce variotii at 60°C, 2h. At the end of the reaction, the immobilized enzyme was collected and the product was freezed dried. The tea extracts cytotoxicity before and after biotransformation was evaluated by cell viability test (MTT) and adipogenesis was evaluated by Oil Red O assay staining in mature 3T3-L1 cells culture.

Biotransformation of white tea decreased its toxicity – maximum 5% of cell viability losses. The results showed that during adipogenesis the biotransformed white tea reduced the lipids incorporation in the cells compared to spontaneous adipogenesis and cells treated with tea without biotransformation. The inhibitory effect of lipid absorption was dose-dependent. The biotransformation led to 26% to 59% inhibition of lipids absorption while the tea without hydrolysis inhibited only 47% compared to spontaneous adipogenesis. In conclusion, the results showed that biotransformation modulated the incorporation of lipids, without offering harmful effect on cells. The present study suggest that biotrasnformation by Paecilomyces variotii tannase is beneficial for the suppression of adipocyte hyperplasia which is responsible for obesity, and it may reduce the risk of associated diseases including diabetes and coronary heart disease.

Financial support: Capes, CNPq

P903
Improvement of the antioxidant potential of grape pomace polyphenols by enzymatic biotransformation

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Grape pomace derived from wine making has been considered a potential source of bioactive phenolic compounds and large amounts of agro-industrial residues are generated and wasted every year. Recently, there is an increased appreciation of these renewable raw materials, because they are abundant and inexpensive for promising applications. Although they are abundant in our diet, the phenolic compounds have a chemical structure that can compromise their properties and bioavailability.

Therefore, the aim of this study was to determine if a bioprocess like enzymatic hydrolysis may release the compounds from their conjugates and consequently improve the antioxidant activity of red and white grape pomace. Enzymatic treatments using semipurified tannase extract, pectinase and celullase and a combination of these enzymes were applied. The effects on the release of total polyphenols, modification of individual phenolic compounds and the antioxidant activity of grape pomaces were investigated. It was demonstrated that the tannase treatment was able to enhance total polyphenols release, in particular gallic acid. Significative improvements in the antioxidant activity were also detected in all of the samples analyzed. Besides, the tannase action was observed by the hydrolysis of the glycoside rutin, producing its aglycone form, quercetin. These results demonstrate the benefits of the biotechnological modification of natural products and renewable raw materials in order to enrich bioavailable and bioactive polyphenols in foods.

Financial support: Capes and CNPq

P904
Citrus pulp incorporation into diary cattle feed leads to incorporation of citrus flavanones in milk

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Regular intake of flavonoids may benefit human health, although there remains a challenge to incorporate adequate levels of such compounds into the diet. The aim of this study was to explore the feasibility of enriching cows' milk with flavanones and other polyphenols by supplementing a cow diet with citrus pulp. Eight mid-lactation Holstein-Friesian cows were fed either a standard farm diet, or the standard diet with added citrus pulp and soya meal. Milk samples were collected twice daily, and analysed for macronutrient composition, including lactose, somatic cell count and the presence of polyphenols/flavonoids. After four weeks of feeding, significant levels of hesperetin and daidzein were detected in milk harvested from the citrus/soya fed cows. Our data suggest that incorporation of polyphenols and flavonoids into ruminant milk through animal feeding may represent a novel means by which to increase regular consumption of these beneficial compounds in humans.

P905
Evaluation of the antioxidant potential of extra virgin olive oils produced in Brazil

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Extra virgin olive oil is considered a functional food with important biological properties. The region where the olive trees are grown have direct influence on oil composition, especially with regard to minority compounds. This study aimed to evaluate antioxidant potential of two monovarietal olive oils produced in two different areas in Brazil (ArbequinaRS and ArbequinaMG) and a third brazilian oil produced from a variety developed specially for brazilian culture (Maria da Fé). A Control oil sample was chosen on the market, a monovarietal Arbequina oil from Spain (ArbequinaMed), traditional in the Brazilian market. The sample "Maria da Fé stood out in terms of oleic acid, measured by GC-FID method. ArbequinaRS and Maria da Fé showed significant concentration of tocopherols by UHPLC (AOCS, 2004). The determination of total phenolic content by Folin-Ciocalteu assay highlighted ArbequinaRS and ArbequinaMed. The antioxidant capacity was evaluated by ORAC and DPPH assays, and showed significant differences between the samples tested only for DPPH assay. Among the samples of Arbequina variety, stood out the oil produced in southern Brazil (Arbequina RS), statistically similar to the trade of Mediterranean origin. A positive correlation between total phenolic content and antioxidant activity by DPPH assay was observed. The biological potential was evaluated by measuring the activity of superoxide dismutase and catalase (HepG2 cell line). In general, the endogenous antioxidant cellular system of the hepatocytes were highly activated by the Brazilians oils, with emphasis on the Arbequina variety produced in the state Rio Grande do Sul, ArbequinaRS.

Financial support: CNPq/FAEPEX/FAPESP

P906
Biological activity of Amazon oils and structured lipids formed by enzymatic interesterification

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In Amazon, there is a variety of underexplored vegetable oils, with bioactive compounds. These oils have a great potential to apply in cosmetics, drugs and functional foods, attracting the interest of the food industry in obtainment of structured lipids, with better physical chemical and nutritional properties. Enzymatic interesterification, involving the rearrangement of fatty acids among glycerol backbones is proving to be a good alternative. The aim of this work was to evaluate the biological potential, by measuring the minor compounds and the antioxidant capacity, of the selected Amazon oils and the structured lipids generated by enzymatic interesterification in three different enzymatic systems: with non-commercial lipase from Rhizopus sp; with commercial lipase TL-IM of Novozymes® and with the use of this two lipases concurrent. The blends were composed with buriti oil and murumuru fat (7:3 w/w) with 2,5% (w/w) of lipases. The reaction occurred at 40ºC, 150 rpm, for 24 hours. The results showed burity oil as an important source of carotenoids and tocopherols without cytotoxic effects and wit good antioxidant capacity, exceeding murumuru fat in all of these requirements. The enzymatic interesterification originated structured lipids with these same biological potential characteristics of original oil (burity oil), however with improved technological characteristics. The antioxidant capacity of structured lipids was preserved or improved according to the lipase applied. In vitro assays showed the structured lipids as substances capable of modulating the activity of antioxidant enzymes and capable to operate in combating oxidative stress.

Financial support: CNPq/FAEPEX/FAPESP

P907
Culinary heat treatments increase polyphenols in selected vegetables of Mediterranean diet

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The World Health Organization promotes higher consumption of vegetables, rich in antioxidants, mainly phenolic compounds, to contribute to the prevention of several chronic diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases. However, antioxidant capacity and phenolic compounds could be influenced by many factors, such as culinary processing. The aim of this work was to study the impact of cooking heat treatments on the antioxidant capacity and bioactive compounds of onions, green peppers and cardoon, commonly consumed as crude in salads and cooked in several ways in Mediterranean diet.

Ethanolic (80%) extracts were prepared from raw and cooked vegetables (griddled, fried in olive and sunflower oils). Antioxidant capacity was measured by DPPH and ABTS assays. Total phenolic and flavonoid compounds were determined by spectrophotometric assays. Polyphenol compounds were identified and quantified using a UPLC-PDA-HESI-MS. Principal Component Analysis (PCA), based on Pearson's correlation matrix, was applied in order to study the effect of heat treatment.

Flavonoid compounds were the main polyphenols in onion (quercetin and isorhamnetin derivates) and green pepper (luteolin and quercetin derivates). In contrast, the most abundant polyphenols of cardoon were chlorogenic acids (caffeoylquinic, diccaffeoylquinic and succninyl dicaffeoylquinic acids).

All heat treatments increased polyphenols content in vegetables. This increase, especially that observed for chlorogenic acids, was correlated with an increase in the antioxidant capacity measured by DPPH (r=0.70). The griddled vegetables showed the highest amounts of phenolic compounds with increments of 55.5%, 246% and 203% compared to raw onion, pepper and cardoon, respectively. The higher temperature during griddling than that applied during frying might favor the release of these polyphenol compounds from the food matrix.

Financial support: PIUNA (Plan de Investigación de la Universidad de Navarra) for their contribution to the financial support of this work. I.Juániz is grateful to ''Asociación de Amigos de la Universidad de Navarra'' for the grant received.

P908
Polyphenol composition of Australian apple varieties: effect of variety and harvest year

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Background/Objectives: Polyphenols have been highlighted as a potential candidate for reduction in risk of chronic disease observed with a diet high in fruit and vegetables. Apples, rich in polyphenols and widely consumed, are a major contributors to total polyphenol intake. Breeding and selecting for high polyphenol apples, therefore, could translate into a natural and low cost method of improving population health. The aim of this study was to examine the genetic and seasonal variation of four polyphenols with potential health benefits in Australian apple identities.

Methods: Total quercetin glycosides, (-)-epicatechin, chlorogenic acid and phloridzin levels were quantified by HPLC in skin and flesh of 21 Australian apple identities (9 breeding accessions; 12 commercial varieties) harvested between December 2011 and February 2012. These polyphenols were again assessed in a subset of 7 Australian apple identities harvested between December 2012 and February 2013.

Results: Quercetin glycosides (mean, 8.8 mg/100g fresh weight, range, 0.9 - 15.5 mg/100g) were the predominant polyphenol and were concentrated in the skin. Chlorogenic acid (mean, 3.1 mg/100g fresh weight, range, 0-10.5 mg/100g) was found predominantly in the flesh. (-)-Epicatechin (mean, 2.1 mg/100g fresh weight, range, 0.3-8 mg/100g) and phloridzin (mean, 0.5 mg/100g fresh weight, range, 0.2-1.4 mg/100g) also concentrated in the skin were found at lower concentrations. Polyphenol content and concentration decreased in 2013 compared to 2012.

Conclusions: Several apple varieties (with skin) had very high polyphenol content. There was, however, large genetic influence on polyphenol content, while seasonal variation was less marked.

Financial support: ARC and ANABP

P909
Search of non-genetic methods for designing Health-Promoting Food enriched with Bioactive Polyphenols

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Currently, technology of functional foods production is being actively developed in a number of ways. On the one hand, genetically modified foods are being created; on the other hand, ways for effective management of organic (sustainable) farming are being searched for in order to obtain not only high productivity of plants, but also to increase the proportion of bioactive components in their contents.

The paper explores the ways of increasing plant productivity through the use of non-genetic methods. Involvement of exogenous secondary metabolites, and the heterogeneous group of phenolic compounds in particular, into the metabolism, proves to be an effective way of plant growth processes activation, especially at low concentrations of active solutions (100 uM and 200 μM). These effects can be considered in two ways. Firstly, this is the change in the redox balance of the cell, leading to an increase in the pool of antioxidants. Secondly, this is involvement of them, and salicylic acid in particular, into the general phenolic exchange, which stimulates the cell lignification, and thereby increases the resistance of plants to exposure to unfavourable environmental factors.

Thus, low concentrations of exogenous substances, being intermediate metabolites o plant biochemical pathways, prove to be effective non-toxic agents that are able not only to affect anatomical and morphological features of plants (biomass set, shoot and root length) in a positive way, but also to change the biochemical status of the plant, in particular to increase the proportion of antioxidants.

P910

Microencapsulation of polyphenols from cocoa and rice: evaluation of thermo-oxidative stability, in vitro gastric-intestinal digestion in a cookie model and prebiotic activity

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Increasing interest towards the health benefits of polyphenols has led the request to use them as functional ingredients, improving the nutritional and healthy properties o foods. Polyphenols are unstable molecules, suffering for light, oxygen and high temperature. Microencapsulation can overcome these problems, being a useful technique to enclose phenols in an inert shell able to protect them from external environment, permitting i) gastro-protection ii) controlled release during digestion, iii) masking their bitterness/astringency. The bioavailability strictly depends on the food matrix's composition; therefore the evaluation of the bioaccessibility of these encapsulated molecules in the gastro-intestinal tract is a key topic. Recent highlights confirm that polyphenols can work as prebiotics, modulating microbiota.

This study aimed to evaluate the microencapsulation of polyphenolic extracts with different coatings on:
- the stability of polyphenols during a baking process of cookies;
- the release of polyphenols by cookies during digestion (in vitro);
- the capacity to work as prebiotics (in vitro).

The polyphenols were extracted in Soxhlet apparatus from cocoa hulls and pigmented dark rice using Ethanol/Water (50:50, V:V), then characterized by HPLC-DAD, spray-dried and powdered using maltodextrin and arabic gum, added as ingredients i model biscuit, cooked in oven. The biscuits were digested using gastro-intestinal enzymes; digested material was used to assess prebiotic activity using different probiotic strains.

An improved thermal stability of coated polyphenols towards oxidation was showed; coating materials permitted different release of polyphenols. Concluding, the microencapsulation improves stability, still permitting the prebiotic activity, confirming its usefulness to protect functional ingredients.

Financial support: Fondazione Comunità Novarese (Novara, Italy), Project: "TecSalus rice" and by European Regional Development Fund Project PON01_02863 "Incapsulazione di principi attivi per il miglioramento di qualità e sicurezza degli alimenti".

P911

Effect of thermal processing and simulated in vitro digestion on bioactive compound of a spiced risotto

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Thermal processing which foods are submitted during cooking, associated with the digestion, might affect the content of bioactive compounds. The objective of this study was to make a spiced risotto containing turmeric, black pepper and hot paprika and to evaluate the effect of both cooking and in vitro digestion on the content of tot polyphenols and bioactive compounds.

The risotto contained turmeric (1g), black pepper (0.1g), hot paprika (0.1g), Arborio rice (100g) and followed a basic recipe. Spices were added and cooked for 5 minutes. Simulated in vitro digestion was performed and the contents of total polyphenols, curcuminoids, piperine and capsaicin were determined.

The thermal processing and the in vitro digestion resulted in loss of bioactive compounds. It was found that five minutes cooking led to a decrease of 25% in the content of curcuminoids, 31% of piperine and no decrease was noticed in the capsaicin content. The in vitro digestion resulted in a decrease of 95% in the content of curcuminoids, 31% of piperine and 100% decrease of capsaicin. It was concluded that both the thermal processing and the digestive process are determinant for the content of bioactive compounds in this recipe. Thus it is suggested that new studies should be done to evaluate the possibility of formation of metabolites of these compounds after digestion.

Financial support: PNPD/CAPES, CNPq and FAPERJ

P912

The effect of rosehip (Rosa canina) phenolic compounds on lipid and protein stability in smoked pork sausages, compared to classic additives

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The aim of this study was to evaluate the effect of rosehip phenolic compounds on lipid peroxidation and protein stability in smoked pork sausages subjected to refrigeration and moreover, to evaluate if rosehip phenolic compounds could be used as natural additives for the preservation of smoked pork sausages.

Pork sausages added with rosehip polyphenols, sodium nitrite, butylated hydroxyanisole and tetrasodium pyrophosphate in different concentrations and combinations were prepared, smoked and refrigerated for 20 days. To evaluate lipid oxidative processes, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) value were assayed. Stability of proteins was evaluated by assessing electrophoretic patterns of proteins by SDS-PAGE.

After 20 days of refrigeration, the lowest PV was determined for the samples treated with the mixture of classic additives (sodium nitrite 0.01%, tetrasodium pyrophosphate 0.3% and butylated hydroxyanisole 0.005%), but no significant differences were recorded compared with samples treated with rosehip phenolic compounds (0.005%) in combination with sodium nitrite (0.01%). The lowest TBARS values were obtained for samples of pork sausages treated with rosehip phenolic compounds (0.005%) and sodium nitrite (0.01%). Protein pattern of sausages treated with sodium nitrite and rosehip phenolic compounds was similar to that obtained for sausages treated with the mixture of classic additives with the difference that the intensity of actin band was lower by 33%.

The results showed that rosehip phenolic compounds in combination with sodium nitrite are able to protect smoked pork sausages against lipid peroxidation and protein degradation as effective as the combination of classic additives.

Financial support: this work was carried out through Partnerships in priority areas Program – PN II, implemented with the support of MEN – UEFISCDI (Romania), project nr. 149/201

P913

Protective effects of hawthorn (Crataegus monogyna) polyphenols against oxidative processes in minced pork

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Synthetic antioxidants added in food for preventing oxidative reactions are suspected to negatively affect the health of consumers; thus, replacing synthetic antioxidants with natural antioxidants from plant materials has greatly increased. In this study we tested the antioxidant activity of hawthorn fruits polyphenols on minced pork compared to buylated hydroxyanisole (BHA) synthetic antioxidant.

Minced pork was treated with different concentrations of hawthorn fruits polyphenol and BHA and then refrigerated at 4°C for one week. To assess the protective effect o polyphenols on lipids, peroxide value (PV), conjugated dienes (CD), conjugated trienes (CT), and thiobarbituric acid reactive substances (TBARS) values were determined by spectrophotometric methods. Oxidative processes of soluble proteins were evaluated by assessing protein electrophoretic patterns (by SDS-PAGE) and relative concentration of myoglobin (Mb) and metmyoglobin (MMb), by spectrophotometric methods.

After 7 days, the lowest PV and TBARS values were found in samples of minced meat treated with polyphenols (200 ppm); CD and CT concentrations decreased during storage, in the 7th day the lowest values being observed in meat samples treated with BHA (100 ppm). The highest concentration of myoglobin and the lowest metmyoglobin concentration were found in meat samples treated with polyphenols (200 and 100 ppm, respectively). Electrophoretic pattern of soluble proteins in pork samples treated with polyphenols was similar to the one found for samples treated with BHA.

The results showed that polyphenols extracted from hawthorn fruits are able to protect lipids and myoglobin against oxidation as effective as BHA synthetic antioxidant.

Financial support: This work was carried out through Partnerships in priority areas Program – PN II, implemented with the support of MEN – UEFISCDI (Romania), project nr. 149/201

P914
Stability of polyphenols during drying and storage of aromatic plants of Lamiaceae

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There are several research directions on the production of plant foods with a high proportion of natural polyphenols. Recently, however, studies of polyphenols stability in plants during technological processing and storage have become actual. The aim o this work is to study stability of polyphenols during drying and storage of aromatic plants of Lamiaceae.

The proportion of polyphenols was determined in salvia (Salvia officinalis L.), hyssop (Hyssopus officinalis L.), lavender angustifolia (Lavandula angustifolia Mill. salvia nemorosa (Salvia nemorosa L.), summer savory (Satureia hortensis L.), monarda (Monarda didyma L.), rosemary officinalis (Rosmarinus officinalis L.).

Determination of polyphenol proportion was performed directly after collection and drying at 60 °C for 24 hours. The total proportion of polyphenols was determined after five, fifteen and thirty weeks in order to identify stability of antioxidants during storage.

It was shown that the total proportion of polyphenols in aromatic plants varied from 8.05 mg/g in rosemary to 23.1 mg/g in monarda. Meanwhile, drying led to reduction in polyphenols proportion. The maximum loss was observed in lavender angustifolia and was equal to 17.5 %, the least loss in rosemary, which was equal to 3.3%. Storage of plants also resulted in polyphenols proportion reduction. After thirty weeks of storage, the maximum loss of polyphenols was observed in salvia nemorosa and summer savory and was equal to 74.9 %. The minimum loss was observed in rosemary –22.4 %.

Thus, the research showed that stability of polyphenols depends on its initial proportion, plant species and storage period. The data obtained can be used in pharmaceutical preparation and storage of plant raw materials. Particular attention in the development of functional foods should be paid to rosemary, in which loss of polyphenols has been minimal during drying and storage.

P915
The Content of Phenolic Compounds in Medicinal Plants of Baltic Sea Region

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Medicinal plants contain a complex set of chemical substances that influence the human organism in different and versatile ways. The researchers are particularly interested in plants containing phenolic compounds and polyphenols, because these substances are valuable sources of broad spectrum agents in medicine and pharmacology. Many natural phenolic oxidants that are found in medicinal plants provide for their antioxidant, antiinflammatory, antimicrobic, spasmolytic, and neuroprotective action. The aim of this work was to study the total content of phenolic compounds in medicinal plants and to estimate their antioxidant activity.

The total content of phenolic compounds in the plants was determined using the spectrophotometric method. The total antioxidant content was determined by the amperometric method.

The total content of phenolic compounds and antioxidants were determined in medicinal plants (66 species from 31 families). The highest number of phenolic compounds was found (in descending order) in the leaves of Jacob's ladder Polemonium caeruleum, bloody cranesbill Geranium sanguineum, lemon balm Melissa officinalis, houndstongue Cynoglossum officinale, glutinous sage Salvia glutinosa, Baikal skullcap Scutellaria baicalensis, Chinese rhubarb (Rheum palmatun), and sage Salvia officinalis. The total content of phenols in these plants was 47.7–166.3 mg/g. The content of phenols was rather high in the leaves of golden root, oregano, crimson beebalm, bigflow ered foxglove, lungwort, Japanese spikenard, nodding onion, lesser meadowrue, narrowleaved lavender, common chicory, redstem wormwood, great burnet, motherwort, Himalayan mayapple, great masterwort, and great stonecrop. These plants turned out to accumulate phenolic compounds in their leaves (from 21.8 to 42.3 mg/g).

Based on these data, the plants can be used as a basis for making innovative functional food products with an increased antioxidant effect.

P916
Effect of different cooking methods on the polyphenol concentration and antioxidant capacity of selected vegetables

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The study evaluated the effect of different cooking methods on the polyphenol concentration and antioxidant capacity (AC) of selected vegetables. The edible parts of squash, broccoli, carrots, kale, cabbage, green beans and tomato were separated and divided into five portions. Four portions were submitted to different cooking methods (pan; microwave oven; pressure cooker; steam) and then compared with their fresh form.

The soluble and hydrolysable polyphenols composition was determined using the Folin-Ciocalteu method. Aliquots of 100 l of vegetable extracts were added to microtubes and combined with 500 l of extraction solutions. After treatment and centrifugation the resulting supernatant, called polyphenol extract, was used for the analyses. The results were expressed as milligrams of gallic acid equivalent per 100 grams of fresh weight.

The AC was determined by the DPPH method (1,1- Diphenyl- 2 picrylhydrazyl). A DPPH solution at 100 M was prepared using 80% methanol. The reading of the DPPH solution in the absence of the sample was carried out at 517 nm to obtain absorbance at approximately 1.1. The results were expressed as percentage of AC, and the AC was calculated in terms of percent inhibition relative to the control.

The results of the experiments were submitted to one-way analysis of variance (ANOVA), followed by the post hoc Tukey's test to examine the null hypothesis of no significant difference among samples of vegetables submitted to the different cooking methods and the raw sample.

Heat treatment was shown to have a significant effect on the AC and polyphenolic content of most vegetables, either increasing or decreasing the concentration of these compounds. The results suggest that the best cooking method for increasing the concentration of polyphenols and antioxidants was steaming, while microwave cooking was the least recommended method.

Financial support: Research Support Foundation of the State of Rio de Janeiro – FAPERJ

P917
Change correlation of saperavi (Vitis vinifera L.) stilbenoides to wine type.

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Georgian Saperavi is unique, red sort of vine. Stilbenoides are one of the main compounds of this sort. In Saperavi grape vine we identified cis- and trans-resveratrol, cis- and trans-piceid, epsilon-viniferin and hopeaphenol. Below we are presenting change correlation of Saperavi grape vine stilbenoides to wine type.

For our research we took dry European wine: (1) fermented with natural microflore and (2) with dry cultural yeast. We also took wine, made with Georgian traditional technology - fermented together with skin, seeds and stem of saperavi grape and fermented with natural microflore. Stilbenoides were extracted with ethyl acetate, implemented on the sephadex and analyzed by HPLC method.

Analyses showed that trans-resveratrol was not found in Saperavi grape juice, but there were found resveratrol glucosides, cis- and trans-piceid. In European type of wine stilbenoides concentration were: 1. trans-resveratrol 0,076mg/L, cis-resveratro 0,50mg/L, trans-piceid 4,9mg/L, cis-piceid 12,2mg/L. Wine fermented with dry yeast piceides were hydrolyzed more intensively producing there aglicones and localized (2) in wine: trans-resveratrol 0,176mg/L,cis-resveratrol 0,92mg/L. Georgian (Kakhetian) type of wine distinguished more rich stilbenoides profile. They transformed from all parts of grape and following items were found: trans-resveratrol, cis-resveratrol, trans-piceid, cis-piceid, viniferins, Hopeaphenol at al.

Difference of the profile of stilbenoids of the saperavi grape vine is in correlation to wine type. When comparing the European type of wine to Georgian, we can see that Georgian is much richer with stilbenoides.

P918

Effect of the irrigation, the cultivation method and the olive variety on the phenolic content of olive oils

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Background/Objectives: Olive oil is considered one of the healthiest dietary fats due to its phenolic composition. Nevertheless, the nutritional quality of the olive oils is affected by agronomic and technological factors, the variety of olive, the geographical area of production, the harvest period and the extraction process, as well as the climatic conditions prevailing in the production year. Irrigation positively influences the composition and organoleptic characteristics of olive oil. The aim of this work was to study the phenolic profile of oils produced with two different types of olives harvested in Andalucía, Spain, Hojiblanca and Picual, and to determine whether there are differences in the phenolic content of these oils according to the type of irrigation received and the cultivation method (conventional or organic).

Methods: A double liquid-liquid extraction (LLE) was used to isolate the phenolic fraction of the oils using hexane:methanol (1:2, v/v). Organic solvents were evaporated, then samples were reconstituted with 850µL of MeOH:H_2O(20:80)(v/v) and injected to an Acquity UHPLC coupled to an API 3000 triple-quadrupole mass spectrometer with a TurboIon spray source in negative mode for phenolic quantification. Samples were also injected to an UHPLC-LTQ-Orbitrap-MS to identify phenolic compound present in the olive oil samples.

Results and conclusion: Thirty-two phenolic compounds were identified in the two types of olive oils. According to the irrigation received, the Riego technique increases the phenolic content of the two types olives giving oils richest in phenolic compounds. Likewise, organic farming increases the phenolic content of the olive oils. Statistically differences were found mainly for: tyrosol, elenoic acid, ligstroid and derivatives, luteolin, hydroxyoleuropein aglycone and other oleuropein derivatives.

Financial support: CICYT(AGL2013-49083-C3-1-R), Generalitat de Catalunya FI-DGR 2013/FI-DGR 2014,the Instituto de Salud Carlos III and ISCIII (CIBERObn) from the Spanish Ministry of Science and Innovation (MICINN) and Manuel Heredia Halcón, owner of the farm organically grown Suerte Alta.

P919

Effect of a simulated gastrointestinal digestion on the (poly)phenolic fraction of raw and cooked vegetables (green pepper and cardoon)

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Plant foods are the main source of dietary bioactive compounds, such as (poly)phenols. Usually dietary vegetables are eaten after cooking in different ways, which can influence their antioxidant capacity and phenolic compounds. Moreover, after ingestion, (poly)phenols can be modified in the gastrointestinal tract by digestive enzymes and, consequently, their bioaccessibility might be affected. The aim of this work was to study the effect of in vitro gastrointestinal digestion on the (poly)phenolic compounds of green pepper and cardoon, both raw and cooked.

Samples of raw and cooked vegetables (griddled, fried in olive and sunflower oils) were submitted to in vitro gastrointestinal digestion and extracted with methanol/acidified water (0.1% formic acid) (80:20 v/v). (Poly)phenol compounds were identified and quantified using a UHPLC-ESI-MS^n.

In green pepper, a total of 19 (poly)phenolic compounds were quantified, being flavonoids (luteolin and quercetin derivates) the most abundant. In cardoon, a total of 16 (poly)phenols (mainly phenolic acids) were quantified. Total phenolic compounds decreased in raw samples of green pepper after gastrointestinal digestion. However, digested cooked pepper had higher content of phenolics than non digested samples. In cardoon, a significant decrease in phenolic compounds was observed for all the samples after digestion, although losses were lower in the cooked ones. Additionally, phenolic acids were affected by the in vitro gastrointestinal digestion to a greater extent (losses up to

99%) than flavonoids, which were even increased in some cooked samples. Thus, the higher bioaccesibility of phenolic compounds in cooked vegetables suggests that changes during cooking process, including Maillard Reaction Products formation, might have a protective effect against digestive enzyme activity.

Financial support: Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P) and PIUN(Plan Investigación Universidad de Navarra). I.Juániz is grateful to ''Asociación de Amigos de la Universidad de Navarra'' for the grant received.

P920

Optimization of the methodology for identification and quantification of (poly)phenolic compounds in cactus cladodes (Opuntia ficus-indica)

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Consumption of vegetables is related with lower risks of chronic diseases associated with oxidative stress such as cancer, cardiovascular diseases or diabetes. The cactus *Opuntia ficus-indica* is one of the most consumed foods in Mexico and a vegetable rich in antioxidant compounds, like (poly)phenolics. Most (poly)phenolic compounds, particularly flavonoids, are in their glycosides forms, so that for their quantification with HPLC-DAD it is necessary to apply a previous hydrolysis to release their respective aglycones. The acid hydrolysis under continuous heating is the traditional method, but it should be standardized and optimized for each food because it is dependent on the binding sites of the glycosides in the flavonoid nucleus. The aim of this work was to optimize hydrolysis conditions for a better identification and quantification of (poly)phenolic aglycones. Additionally, antioxidant capacity was evaluated in the selected extract.

Cactus cladodes were successive extracted with methanol, acetone and water. Hydrolysis was carried out using HCl at 90ºC at different concentrations (0.6, 1.2, 1. and 1.7M) and times (2 and 3 hours). After hydrolysis, (poly)phenolic aglycones and phenolic acids were identified and quantified using HPLC–DAD. Antioxidant capacity was measured by DPPH and ABTS spectrophotometric assays.

Three flavonoids (isorhamnetin, quercetin and kaempferol) and two phenolic acids (ferulic and hidroxybenzoic acids) were identified and quantified. The 1.5M HCl hydrolisis during 2 hours showed the disappearance of glycosides peaks yielding the highest amount of (poly)phenols, especially flavonoids aglycones. Furthermore, there was an increase in the antioxidant capacity measured by DPPH after hydrolisis, which might release the flavonoids aglycones from their glycosides forms in the food matrix.

Financial support; Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P) and PIUN(Plan Investigación Universidad de Navarra). E. De Santiago and I. Juaniz thank to''Asociación de Amigos de la Universidad de Navarra'' for the grants.

P921
Characterization of jabuticaba (Myrciaria jaboticaba) and jussara (Euterpe edulis) powders produced by different drying technologies

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Jabuticaba and jussara are Brazilian berries rich in phenolics and show high antioxidant activity, underlining their potential for production of functional ingredients by the food industry. This study aimed to obtain and characterize powder from jabuticaba depulping residue and jussara pulp by different drying techniques.

Jabuticaba powders (JAP) were obtained by oven-drying the depulping residue (peel and seed) at 55ºC/14h and 75ºC/22h. Jussara powders (JUP) were produced by spray-drying using capsul®, inulin and maltodextrin as encapsulating agents (EA) ancore (pulp) to EA ratios of 0.5, 1.0 and 2.0. Phenolics by HPLC-DAD, antioxidant activity (FRAP and TEAC assays) and water activity (a_w) were determined in JAP and JUP. Water solubility, morphology, hygroscopicity and wettability were determined in JUP.

JAP produced at 55°C/14h showed higher values for FRAP (123 mmol Fe^{2+}/100g), TEAC (62 mmol trolox/100g) and anthocyanins (0.61 mg/100g) and similar totalphenolics (1.2 g/100g) when compared to JAP produced at 75°C/22h. JUP produced with capsul®and capsul/inulin/maltodextrin at 0.5 ratio showed the highest value of anthocyanins (2.3 g/100g, on average), FRAP (223mmol Fe^{2+}/100g) and TEAC (98mmol Trolox/100g). All JAP and JUP exhibited $a_w <$ 0.47, which limits microorganisms' growth. In general, JUP showed high water solubility (77% to 83% and wrinkled/spherical particles). JUP produced with capsul®at 0.5 ratio showed low hygroscopicity (10.7 g/100g) and high wettability (267s).

Oven-drying at lower binomial temperature/time and spray-drying at lower core to EA ratio were suitable for the production of Brazilian berries powders rich in bioactive compounds.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação deAmparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

P922
Characterization of (poly)phenolic fraction in peel and pulp of different Italian figs

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Background and Aims: Figs are widespread in Mediterranean countries. Besides sugars and fibers, raw figs provide phytochemicals, including polyphenols. The aim of this study is the characterization of the (poly)phenolic fraction in the peel and pulp of different Italian figs.

Methods: Twelve different figs, derived by different cultivars but harvested from the same area of Tuscany, have been analyzed for their polyphenol skin and pulp composition through UHPLC-MS^n. One cultivar was further analyzed for its polyphenol content after undergoing sun-drying process.

Results: Forty-two (poly)phenolic compounds were identified in figs, mostly flavonoids. Since the majority of polyphenols are located in the peel, they were only quantified in the peels, while only qualitative analysis was performed for pulps.

Quercetin-3-O-rutinoside was the most abundant flavonoid, while 5-caffeoylquinic acid was, by far, the most abundant phenolic acid. Four pigmented peels were also characterized for their anthocyanin content, showing how cyanidin-3-O-rutinoside was the most abundant one, but with huge differences amount among the sampled cultivars. Some differences emerged also for flavan-3-ols, which were particularly high in one cultivar, as well for apigenin-O-rutinoside, that was specifically found only in a few samples.

Conclusions: This work demonstrates that fig peel may be considered a good source of polyphenols, but with huge variability in their amount among cultivars. Ideally, polyphenol-rich cultivars might be used in dried fig production in order to improve polyphenol intake by consumption of the whole fig. Alternatively, fig peels could be also used as innovative functional ingredients targeted to increase the polyphenol content of food products.

P923
Effect of maize bread processing in phenolic composition

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Maize is one of the most consumed cereals worldwide. In Portugal, it is also used to produce "broa", a traditional maize bread. Phenolic compounds, mainly hydroxycinnamic acids, are among the potentially health beneficial components present in maize bread. These compounds are mainly bonded to arabinoxylans, important constituents of cellular walls, considered a major component of dietary fibres.

In this work, 11 maize (Zea mays) traditional open pollinated varieties and one commercial hybrid variety, as well as their corresponding maize breads, were milled and their phenolic compounds extracted and analysed by liquid chromatography (LC with diode array, mass spectrometry (and electrochemical detection. Maize flour extracts were prepared with ethanol:water (50:50 v/v). Total phenolic content (Folin-Ciocalteu assay) and antioxidant capacity (Oxygen Radical Absorbance Capacity assay) were also determined. Results are discussed considering the differences observed between the different varieties studied. Total free phenolic content ranged from 66.33±1.85 mg/100g dry weight (dw) to 101.74±15.67 mg/100g dw in maize flours and from 152.16 ±3.04 mg/100g dw to225.08 ± 28.10 mg/100gdw in maize breads. Maize breads showed a higher total phenolic content and antioxidant capacity than corresponding maize flours. The bread produced with the commercial variety presented a lower antioxidant capacity than the traditional ones. The analysis by LC showed that hydroxycinnamic acids derivatives, namely ferulic and p-coumaric acids bonded to arabinoxylans were present in both samples, but a higher content of free hydroxicinnamic acids was present in maize breads. Processing of maize bread made phenolic compounds more available for absorption.

Financial support: FCT for PTDC/AGR-ALI/099285/2008 "Exploiting antioxidants, flavours andaromas diversity on broa bread maize breeding"

P924
Development of soybean meal cookies for bioactivity and bioavailability studies in humans

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Soybean meal (SM), a co-product from soybean oil industry rich in bioactive compounds such as isoflavones, was used in this study to elaborate cookies to be use in bioactive compounds bioavailability and activity studies in humans.

The SM was processed by fermentation with baker's yeast and/or dry-heat, in order to modify its isoflavones profile. Non-processed SM was used to substitute 95 of wheat flour from original cookie's formulation. Samples were characterized for proximate composition, total phenolic compounds, total saponins, antioxidant activity by FRAP and TEAC assays and isoflavones profile by LC-DAD-MS. Cookies were subjected to sensorial analysis with untrained panelists (n = 105). Fermentation process increased aglycone contents from 13% to up to 82% of total isoflavones. These forms are believed to be more bioavailable in humans.

Dry-heating for 10 min increased acetylglucosilated forms from 7 to 29%. After 20 min, aglycone forms were increased from 13% to 36%. Cookies containing non-processed SM presented higher protein (3.7-fold) and bioactive compounds contents (up to 2.8-fold) and antioxidant capacity (3.5-fold) than control formulation Control and non-processed SM cookie formulation containing sucralose and vanilla flavor showed the highest scores in sensory evaluation (7.0 for global impression score, on average).

Fermentation and dry-heating processes were successfully employed to increase aglycone content in SM. Cookies produced with non-processed SM are rich in potentially bioavailable isoflavones and are well-accepted by consumers, and will be employed hereafter by our research group in bioavailability and bioactivity studied in humans.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação deAmparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

P925

Simultaneous determination of polyphenols by liquid chromatography triple quadrupole mass spectrometry in Arbequina table olives

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Table olives, commonly consumed in the Mediterranean diet, are a good source of bioactive compounds that contribute to the healthy properties of this food. Among them, hydroxytyrosol, oleuropein and tyrosol are associated with beneficial activities such as the prevention of cardiovascular disease, cancer or degenerative ailments.

Therefore, we have established an analytical LC-QqQ-MS method for their separation and identification in olives. Table olives were grinded with a Polytron homogenizer and polyphenols were extracted using ethanol-methanol (1:1, v/v) as solvent and 2,3-hydroxyphenylethanol as I.S. Chromatographic separation was carried out in a Zorbax Eclipse XDB column at 30°C using gradient elution with an aqueous phase formed by water with 0.025µM acetic acid and organic phase consisting of a mixture of acetonitrile:acetone (95:5, v/v) at a flow rate of 0.8 mL/min. Polyphenols were ionized in an electrospray source at 350°C in negative mode. The validation results gave a LOQ for hydroxytyrosol, tyrosol and oleuropein of 1, 6 and 0.2 nM, respectively. The method was linear with correlation coefficients exceeding 0.99 and precision and accuracy lower than 15%. Recovery of the polyphenols extracted from olives was 94.7± 4.2, 98.8± 2.0and 98.0 ±6.4% for hydroxytyrosol, tyrosol and oleuropein, respectively. The applicability of the validated method was assessed with the analysis of the polyphenols in table olives of Arbequina variety. Hydroxytyrosol was the major polyphenol with concentrations of465.5 ± 10.5 mg/kg of pulp whereas tyrosol and oleuropein were found in minor amounts of 12.2 ± 0.7and 17.8 ± 0.2mg/kg. In conclusion, the developed HPLC-QqQ-MS method allows the unambiguous quantification of the main polyphenols in Arbequina table olives and constitutes an excellent starting point for evaluating the beneficial effects of these bioactive compounds in human beings and their use as functional ingredients.

Financial support: Supported by grants AGL2013-41188 from MINECO and 2014SGR1221 from Generalitat de Catalunya.

P926

Melanoidins, melanoidin-bound phenolics and antioxidant capacity of coffee and bread: estimation of dietary intake in the Brazilian diet

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Melanoidins are end products generated in the Maillard Reaction between reducing sugars and proteins or amino acids during food thermal processing. The aim of this work was to estimate the intake of melanoidins, melanoidin-bound phenolics and antioxidant capacity from coffee and bread in the Brazilian diet.

Four coffee (regular, decaffeinated, soluble and decaffeinated soluble) and two bread samples (white and whole meal crusts) were analyzed. Samples were obtained from three different commercial brands, each from three different batches. Extraction of melanoidins was performed by percolation/dissolution in water for coffee and digestion with pronase E for bread crusts. Quantification of melanoidins contents was performed gravimetrically after ultrafiltration. Antioxidant capacity of melanoidins was measured by FRAP and TEAC assays. Contents of melanoidin-bound phenolics were determined by HPLC-DAD after alkaline hydrolysis.

The estimated mean intake of melanoidins from coffee and bread were 2.4 and 0.1 g/day, respectively. Coffee melanoidins showed bound caffeic (9.0 mg/g) and ferulic acids (2.4 mg/g), representing an estimated intake of 21.6 and 5.8 mg/day, respectively. Ferulic acid was the only bound phenolic compound in white (0.6 mg/g and whole meal bread melanoidins (1.4 mg/g), representing an estimated intake of only 0.1 mg/day. Soluble coffee melanoidins showed the highest antioxidant capacity values, followed by decaffeinated soluble coffee, regular coffee, decaffeinated coffee whole meal bread and white bread.

Our results suggest that coffee rather than bread is the main contributor for the dietary intake of melanoidins (96%) and melanoidin-bound phenolics (99%) in the Brazilian diet.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação deAmparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

P927
Nanoencapsulation of white tea extract for nutraceutical application

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Because of their attractive bioactive properties, the present study aims to formulate the white tea extract into polymeric nanoparticles (NPs) based on poly-caprolactone (PCL) and alginate for nutraceutical applications, in order to control the tea polyphenols release and to preserve the antioxidant activity. Moreover, the influence of encapsulation on the storage stability of extract was investigated.

NPs were prepared by nanoprecipitation method and characterized in terms of morphology and physicochemical properties. Total polyphenols (TPC) and catechins contents before and after encapsulation were determined. Moreover, in vitro release profiles of encapsulated polyphenols from NPs were investigated in simulated gastrointestinal fluids. The antioxidant activity and stability of encapsulated extract were further evaluated.

To optimize the NPs formulation conditions, different amounts of alginate (0.1%, 0.5%, and 1.0% w/v) were initially employed. Results demonstrated that the composition of NPs strongly influenced the size and distribution of NPs. The TPC was established as 24 GAE g/mg of NPs. The amount of EGCG and ECG into NPs correspond to encapsulation efficiency values of 31 and 33%, respectively. Interestingly, NPs released about 20% of the polyphenols in simulated gastric medium, and 80% after 5 h at pH 7.4, showing a good capacity to control the polyphenols delivery. Furthermore, DPPH•assay confirmed that white tea extract retains its antioxidant activity and NPs protect tea polyphenols, preventing the losses of TPC and catechins over 30 days of storage.

The application of this nanotechnology could offer interesting perspectives for the potential use of encapsulated white tea extract as innovative nutraceuticals.

Financial support: Grant CRP 25920 of "Legge regionale n. 7/2007, promoziondella ricerca scientifica e dell'innovazione tecnologica in Sardegna, - Annualita2010".

Poster Session K: Other topics

P1000
New antimicrobial polyphenols extracted from stumps of Eucalyptus globulus

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The increasing resistance of pathogens towards antibiotics presents a major threat to public health because it reduces the effectiveness of antibiotic treatment, which could lead to an increase in morbidity and mortality. Hence, there is an urgent need to find alternative antimicrobial agents to treat resistant pathogenic microorganisms. By other side, these past years have seen an enormous development of the area of natura antioxidants due to the increasing limitations on the use of synthetic antioxidants and enhanced public awareness of health issues. Thus, research has been focused on the identification of novel antioxidants from natural sources.

In the Portuguese context, Eucalyptus globulus is the third species in terms of forest area, representing nearly 31% of the E. globulus area planted worldwide. Stump is defined as the basal part of the tree. The purpose of this work was to study the phytochemical profile and to evaluate the antioxidant and antimicrobial properties of several crude extracts of E. globulus (stumps wood and stumps bark), comparing it with similar extracts of E. globulus wood (industrial chips).

The results obtained in this work showed that all the extracts of E. globulus stumps presented great antimicrobial properties against several bacteria and yeasts strains. It is emphasized that it was found antimicrobial activity against yeasts, namely Candid spp., and Gram-negative bacteria, which is not common in extracts from natural origin. Moreover, the presence of high concentrations in total phenolic compounds and flavonoids in extracts were also found. Generally, the stumps wood extracts stands out from the other ones, presenting the highest percentages of inhibition of linoleic acid oxidation.

Financial support: Ângelo Luís acknowledges the research fellowship within the scope of the project titled "The below-ground biomass of Eucalyptus globulus: the forgotten components of forest sustainability" (PTDC/AGR-FOR/3872/2012) funded by Fundação para a Ciência e a Tecnologia.

P1001
Antioxidant activity of a crude preparation rich in phenolic compounds from the olive stones of two Moroccan cultivars

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Olives and olive oil are rich in phenolic compounds whose biological activities are being uncovered. Yet, very few studies have focused upon the phenolic composition of olive pits (aka seeds, stones). Because there is a need to provide added value to by-products such as olive pits and to build the bases for their potential exploitation.

We assessed the antioxidant activities of extracts from two Moroccan varieties, i.e. Picholine and Haouzia. Quantitative analysis was achieved by Folin-Ciocalteu method using gallic acid as a standard compound. Qualitative analysis was performed by HPLC with diode array detection and ESI-Ion Trap Mass Spectrometry.

The obtained results showed that the total amount of phenolic compounds varied according to the olive variety. The qualitative analysis performed through HPLC-DAD-MS analysis showed that the analyzed extracts were predominated by nüzhenide and its derivatives. We then performed three different in vitro tests of antioxidant activity that showed how the radical scavenging and antioxidant activity of the Picholine extract is comparable with that of pure oleuropein and of vitamin C.

In summary, olive pits from the Moroccan Picholine variety appear to be an interesting by-product that might provide useful as sources of phenolic antioxidants, to be potentially exploited in the food or cosmetic sectors.

KEYWORDS: Olea Europaea, Antioxidants, By-products, Moroccan olives, olive stones, phenolic compounds.

P1002

Inhibitory Effects of polyphenols extract from Anchsa azurea on Xanthine Oxidase Activity and its Hypouricemic effects on mice.

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In the present study, Anchusa azurea were extracted with solvent of varying polarity allowed its separation into four subfraction: crude extract (CrE), chloroform extract (ChE), ethyl acetate extract (AcE), and aquous extracts (AqE). Total polyphenol and flavonoids contents of Anchusa azurea extracts were determined as gallic acid equivalent, AcE was the most rich in polyphenols and the ChE was the richest fraction in flavonoids. The inhibitory activity of the extracts on the XO was evaluated, the results obtained showed that the inhibition is dose-dependent. ChE an AcE have the best inhibitory effect (IC_{50}= 0.334 ± 0.006 and 0.263 ± 0.002 mg/ml, respectively). CrE and AqE weakly inhibit the XO activity. ChE and AcE inhibited XO noncompetitively, while the CrE and AqE are uncompetitive inhibitors.

Hyperuricemia is induced by intraperitoneally injection of potassium oxonate, the uric acid, urea and creatinine were measured in serum and supernatant of liver . AqE and CrE caused a decrease in serum uric acid (a decrease of 66%) followed by ChE with a percentage of 29.22 %. The AcE keep almost the same value of uric acid of "PO" group. For the supernatant, only CrE caused a significant decrease of liver uric acid (18.5 ± 4.83 mg/l). Comparing the urea level of "OP" group (0.48 g/m), only extracts CrE, AqE (0.41g/l, 0.9g/l) decreased the level of urea significantly ($P < 0.05$) to the normal values of urea (0.34 g/l), we can conclude that the rate of urea and creatinine after treatment with plant extracts are normal and that the results of this study indicate the absence of renal damage in mice

P1003

Dietary intake and major food sources of polyphenols in adults and elderly of Sao Paulo: population based study

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Background: Epidemiological studies have suggested an association between polyphenol intake and health. Therefore, a comprehensive estimation of polyphenol intake in nutritional studies is needed to gain a better understanding of this association.

Objective: To estimate the polyphenols intake and their major dietary sources in a general population of Sao Paulo.

Methods: Data from a population based cross-sectional survey in 2008 (ISA-Capital) among adults and elderly in Sao Paulo, Brazil was used. A total of 1,103 participants who were aged 20-90 years and who had completed one 24-hour dietary recall (24HR), participated in this study. Polyphenol intake was calculated by matching food consumption data from the 24HR with the Phenol-Explorer database on polyphenol content in foods.

Results: The mean total polyphenol intake was 377.49 (SE=15.34) mg/day. The main polyphenol groups were phenolic acids [284.77 (SE=15.88) mg/day] and flavonoids [54.64 (SE=3.46) mg/day].

Polyphenol intake was also calculated according to socio-demographic and lifestyle characteristics. Intakes were higher in elderly than in adults ($p<0.001$), 414.95 (SE=12.71) mg/day and 370.20 (SE=18.26) mg/day, respectively. Increased means o polyphenol intake were also found among individuals with lower education level ($p=0.006$) and smokers ($p=0.018$). The main dietary sources for the total polyphenols were coffee (70.5%) and fruits, especially citrus (4.6%) and tropical (3.4%), whereas vegetables accounted for a lower percentage of the total amount of polyphenols in diet (1.3%).

Conclusion: The current study provides the first estimation of dietary polyphenols in Sao Paulo and gives a description of the total polyphenol intake and main food contributors of dietary polyphenols intake in this population. These findings will be useful to assess potential beneficial role on health of specific polyphenol-rich food, such as coffee.

Financial support: The scholarship was granted by São Paulo Research Foundation (FAPESP – procedural 2014/04540-2).

P1004

Comparison of antioxidant activity and phenolic profiles of wild currant Ribes magellanicum Poir. from Argentinean and Chilean Patagonia

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The Patagonian wild currant (*Ribes magellanicum* Poir.) is a native species occurring in southern Chile and Argentina. There is an increasing international interest in South American native berries, mainly due to their potential health benefits. The aim of this work was to compare the chemical composition and antioxidant activity of *Ribes magellanicum* fruit samples collected in Chilean and Argentinean Patagonia.

The fruit collection was made in Chile and Argentina, two locations each. Ripe fruits were extracted with MeOH and an Amberlite XAD-7 resin was used to obtain a phenolic-enriched extract. Total phenolic (TP), total flavonoid (TF) and total anthocyanin (TA) content were determined. Antioxidant activity was evaluated against the free radicals (DPPH and ABTS) and by the ferric reducing antioxidant power (FRAP). The main constituents were identified by HPLC-DAD-MS/MS^n. The TP (154.8-189.7 g GAE kg $^{-1}$) and TF (40.4-76.0 g QE kg $^{-1}$) contents were higher for Chilean samples, while TA was higher for Argentina (2049.5-313.9 mg C kg^{-1}). Argentinian samples showed better activity in all antioxidant assays (DPPH S_{50}: 6.21-10.59 mg L^{-1}; FRAP: 4006.27-1616.33 mmol TE kg^{-1}; TEAC 2856.87 µmo L $^{-1}$ TE). The HPLC-ESI-MS/MS^n showed a complex set of anthocyanins in Argentinian samples, including cyanidin and delphinidin derivatives. The main phenolic compound in both samples was identified as caffeoyl quinic acid isomer.

The Argentinian *Ribes magellanicum* provided an interesting set of phenolic compounds, especially anthocyanins, which is reflected into a more efficient antioxidant activity. Further work, including quantification and complete identification of the main phenolics is in progress.

Financial support: FONDECYT Project N° 1120096, CONICYT-PCHA/Doctorado. Nacional/2013-21130048 (F. Jiménez-Aspee), and CONICYT-PCHA/Doctorado Nacional/2013-63130042 (S. Thomas-Valdés).

P1005

Salix daphnoides: A screening for oligomeric and polymeric flavan-3-ols

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Willow bark (Salicis cortex, *Salix* spec., Salicaceae) is an analgesic and anti-inflammatory herbal drug monographed in the European Pharmacopoeia. Typica components of *Salix* species are salicylic alcohol derivatives, flavonoids, and proanthocyanidins (PAs). In the present study, we are presenting a qualitative analysis of flavan-3-ols from an aqueous-methanolic extract of *Salix daphnoides* VILL. Purification of oligomeric procyanidins like procyanidin B1, B2 and B3 was achieved by Sephadex® LH-20 and MCI® CHP-20P column chromatography followed by preparative HPLC on RP-18 or a diol-column. Mass spectrometry, 1D- and 2D-NMR, circular dichroism and polarimetry were used for their structure elucidation.

Additionally, two fractions of very polar flavan-3-ols were compared: "regular" polymeric PAs with very low R_f-values on silica TLC, which were received by Sephadex® LH-20 chromatography with acetone 70% after salicylic alcohol derivatives, flavonoids, and oligomeric PAs were eluted with EtOH 70%, and "unusual" PAs with the same low R_f-value but already eluting together with flavonoids. These "unusual" PAs were enriched by CPC with BuOH/H_2O. ^{13}C-NMR polarimetry, thiolysis, acid hydrolysis, and phloroglucinol degradation were used to characterize both fractions. Differences in the composition of different flavan-3-ol units and the middle chain length were observed. The best of our knowledge, this is the first screening for oligomeric and polymeric PAs in *S. daphnoides*.

Financial support: Drug material was a gift from PhytoLab.

P1006

Optimization of extraction of polyphenolic antioxidants from Himalayan berries (Berberis asiatica) using Response Surface Methodology

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Antioxidants from wild edible fruits has been known to play a significant role in traditional medicine and used to prevent free radicles borne diseases. This study was designed to optimize the extraction of antioxidants from Berberis asiatica berries using response surface methodology (RSM). Solvent selection was done based on th preliminary experiments and a three-level-five-factor, Central Composite Design (CCD) consisted of 46 experiments was conducted to analyzed the effect of extraction temperature (X_1: 30 – 80°C), extraction time (X_2: 30 – 90 min), sample to solvent ratio (X_3: 1:10 – 1:50), pH of the solvent (X_4: 3 – 5) and solvent concentration (X_5: 20 – 80 %) on the extraction of total phenol content (TPC), total anthocyanin content (TAC), total tannin content (TTC), total flavonoid content (TFC), and antioxidant activity (ABTS, FRAP and DPPH). Extraction temperature (X_1), sample to solvent ratio (X_3) and solvent concentration (X_5) were found to produce greater significant effect on response variables, with linear, quadratic, and interaction effects of the independent variables. The regression coefficient (R^2) was found to be satisfactory for all the models except for DPPH response. The lack of fit was found to be non-significant ($p>0.05$) for TPC, TAC, TTC and TFC indicating that the models could adequately fit the experimental data. Response surface analysis showed that under optimal extraction conditions, the dried fruit sample was mixed with 80% methanol (X_5) having a pH of 3.0 (X_4) in a ratio of 1:50 (X_3) and the mixture was heated at 80°C (X_1) for 30 minutes (X_2), the phenolic antioxidant extraction maximized. The experimented values are in accordance with the predicted values, indicating the success of RSM in optimizing the extraction conditions for polyphenolic extraction from B. asiatica berries, and could further up-scaled for nutraceutical and pharmaceutical applications.

Financial support: State Biotech Department, Dehradun, Uttarakhand

P1007

Validation of traditional cosmetic utilization: In vitro and in vivo studies of Callicarpa albida leaf extract

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In searching new materials for cosmetic agents-from natural resources, we focused o the materials used traditionally by the Dayak tribe in East Kalimantan, Indonesia. Leaves of Callicarpa albida is one of the materials used by Dayak tribe as traditional cosmetic called "Bedak Dingin". Based on phytochemical test, methanol extract of Callicarpa albida leaves contains alkaloids, flavonoids, tannins, and saponins. Validation of the extract as a cosmetic agent was done by in vitro (tyrosinase enzyme and melanin biosynthesis assays) and in vivo (using volunteers) studies. The methanol extract of Callicarpa albida leaveas at concentrations of 500, 200, 50, and 5 mg/ml inhibited only 18, 12, 9, and 7% respectively on tyrosinase enzyme activity. Kojic acid was a positive control was 99% inhibited at 100 mg/ml. In melanin biosynthesis assay by using B16 melanoma cells, Callicarpa albida leaves extract at 200, 100, 50, and 5 mg/ml inhibited the formation of melanin by 48, 31, 11, and 4% respectively. Arbutin was a positive control (100 mg/ml), inhibited the formation of melanin 54%. In Vivo study using mexameter MX16 showed that ethanol extract of Callicarpa albida leaves (5000 mg/ml) reduced melanin content by 0.16% (N=32) while Arbutin (1000 mg/ml) reduced the melanin by 0.34% (N=29) after 4 weeks of treatment. The extract of Callicarpa albida leaves as traditional cosmetic of Dayak tribe in "Bedak Dingin" was in line with the scientific proven. Further investigation was needed to clarify the mechanism.

Financial support: Indonesia Ministry of Education Grant

P1008

Evaluation of nutraceutical properties of Tuscan bee-pollen: antioxidant, anti-hemolitic and antimicrobial activities

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Bee-gathered pollen (bee-pollen) is an apicultural product of great interest owing to its high nutritional and therapeutic properties, as well as an important source of energy, bioactive compounds and proteins for human nutrition. In a previous study we evaluated botanical origin, phytochemical profile and free-radical scavenging activity of a polyfloral organic Tuscan bee-pollen separated by colour into three botanical families, specifically Castanea, Rubus and Cistus. Among them, Castanea showed the highest phytochemical content. Moreover, a strong in vitro antioxidant activity has been highlighted.

Thus, the aim of this study was to evaluate the cellular antioxidant activity and anti-hemolytic effect of both polyfloral and separate bee-pollen fraction on human erythrocytes, using the CAA-RBC assay and the hemolysis test. Besides, we also evaluated the anti-microbial activity of each separate fraction on three Gram-negativ bacteria (Escherichia coli, Salmonella enterica ser. Typhimurium and Enterobacter aerogenes), and on two Gram-positive bacteria (Enterococcus faecalis and Staphylococcus aureus). The minimal inhibitory concentration (MIC) values was determined.

Our results showed a good biological activity of both polyfloral and separate bee-pollen samples. Polyfloral bee-pollen exhibited a hemolysis inhibition comparable to Castanea and Cistus sample, significantly higher than Rubus.

However, a significantly higher cellular antioxidant activity was detected in polyfloral bee-pollen pre-treated erythrocytes compared to each separate fraction suggesting a biological synergic activity.

Furthermore, Cistus and Castanea exhibited antibacterial action against all bacteria, higher than Rubus bee-pollen. For instance, the most sensitive Gram-negative microorganisms were Escherichia coli and Salmonella typhimurium, showing MIC values of 10 mg/ml. Whereas, both Gram-positive bacteria were inhibited at 10 and 5 mg/ml by Cistus and Castanea bee-pollen, respectively. Otherwise, Rubus was effective only against Gram-positive bacteria.

In conclusion, Tuscan bee-pollen represents a good natural antibacterial, as well as an excellent food supplement and potential nutraceutical product useful in the prevention of free radical associated diseases.

P1009

Comparison of chemical compositions and antioxidant activities of tannins from five different plant leaves

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Tannins, a specific class of plant polyphenols, are mixtures of oligomers and polymers consisting of different phenolic constitutive units. They are well-known for their antioxidant activities, but the activities could vary with their chemical compositions and plant sources. In the present work, the chemical compositions and antioxidant activities for five different leaf tannins were studied. The tannins from leaves of *Ficus microcarpa*, *Kandelia obovata*, *Aegiceras corniculatum*, *Myrica rubra* and *Sonneratia apetala*, depended on the identification by MALDI-TOF-MS and thiolysis-HPLC-ESI-MS analyses, were defined as propelargonidins (PP), procyanidins (PC), prodelphinidins (PD), prodelphinidin gallates (PDG) and Ellagitannins (ET), respectively. These five types of tannins were mainly composed of epi/afzelechin, epi/catechin, epi/gallocatechin, epi/gallocatechin gallate and ellagic/gallic acid, respectively. Oligomeric and polymeric fractions of each type of tannins were fractionated on Sephadex LH-20 column. The mean degree of polymerization (DP) of oligomeric and polymeric fractions ranged from 1.1 to 2.4 and from 6.8 to 9.1, respectively. Total phenolic content and three in vitro tests, namely FRAP, DPPH and ABTS, were investigated for antioxidant activities. Result showed that most of antioxidant activities of oligomers and polymers were in the similar order of ET > PDG > PD > PC > PP. Oligomers had lower activities than polymers except PP. It suggested that the antioxidant activity of tannins largely depend on their structure, particularly DP and constitutive units with different number and position of hydroxyl group. This study provided better understanding of the structure-activity of tannins, and for better utilization of tannins as natural antioxidants.

Financial support: National Grant

P1010
A recombinant fungal protein prevents tumor growth of colon cancer cells in vivo an in vitro

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Ostreolysin (Oly) is a protein found in the Pleurotus ostreatus mushroom (also called the oyster mushroom). It is a 15-kDa cytolytic protein expressed during fruiting bodies formation. This protein interacts with cholesterol-enriched raft-like membrane domains. Since cancer cells contain high levels of lipid rafts in their membranes, we tested what could be the functional changes, induced by Oly, in colon cancer cells.

We obtained a recombinant version of Oly (rOly) by expressing the protein in *E. coli* using compatible expression vectors. In cancer cells, we found that Oly is able to promote apoptotic death of HCT-116 colon cancer cells. A significant effect is observed when cancer cell viability is reduced to 50% after 8 hours only. Interestingly, Oly's effect on normal epithelial cells from the small intestine (FHs 74 Int) did not demonstrated antiproliferative effect as compared to colon cancer cells. Another interesting result obtained is that following treatment of cancer cells with Oly caveolin-1 (cav-1) reorganization in clusters in lipid rafts of the plasma membrane. We additionally tested the effect of rOly on HM-7 clones; HM-7 clone that expressed high levels of the protein cav-1 (#15) and HM-7 clone that does not expresses cav-1 (#1). The HM-7 clone expressing high cav-1 is significantly more sensitive to rOly and compared to HM-7 clone with no cav-1 expression. We conclude that the antiproliferative effect induced by rOly on colon cancer cells may be mediated by lipid raft associated proteins. *In vivo* rOly inhibited tumor development in C57Bl mice injected with MC38 cells and in xenografts induced in nude mice injected with HCT-116 human colon cancer cells.

In conclusion, this study represents a first effort to functionally characterize rOly as potential pro-apoptotic drug and provide an innovative scientific evidence for its contribution in promoting death of cancer cells via apoptosis. This novel recombinant version of Oly exerts unique qualities specifically against colon cancer cells and not against normal cells.

Financial support: Nofar grant from Ministry of Science, Israel

P1011
Total polyphenols and antioxidant capacity of Chenopodium botrys L.

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Total polyphenols and antioxidant capacity of methanol extract from aerial parts of Chenopodium botrys L. (Chenopodiaceae) collected from six different locations in Republic of Macedonia were investigated. Plant material was collected during summer in period 2012-2014. Total phenolic content (TPC) and total flavonoid content (TFC) were analyzed by Folin-Ciocalteau and aluminium chloride assay, respectively. Evaluation of antioxidant activity was based on different biochemical mechanisms using: 1) DPPH radical scavenging assay, 2) reducing power determination, 3) H_2O_2 inhibition effect, 4) non-site-specific hydroxyl radical-catalyzed 2-deoxy-d-ribose degradation (NSSOH) and 5) site-specific hydroxyl radical-catalyzed 2-deoxy-d-ribose degradation (SSOH).

TPC and TFC values ranged from 27.77-85.14 mg gallic acid equivalents per gram and from 7.35-16.33 mg quercetin equivalents per gram of dried plant material, respectively. IC_{50} values for methanol extract ranged from 5.3-62.15 mg/ml for DPPH, from 31.50 to 62.23 mg/ml for NSSOH and from 41.34 to 59.00 mg/ml for SSOH assay. The H_2O_2 inhibition activity of plant methanol extract ranged from 28.84 to 46.56% and ferric reducing power capacity from 9.20 to 15.27 mmolFe^{2+}/L Methanol extract of macedonian Chenopodium botrys could be considered as a potential source of polyphenol compounds. The data obtained *in vitro* clearly establish the antioxidant potency in concentration-dependent manner. However, further investigation to verify its effect *in vivo* and isolate the active principles is necessary.

P1012

Polyphenolic content and antioxidant activity of hawthorn (Crataegus monogyna) alcoholic extracts depending on solid-to-solvent ratio and extraction method

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Due to the beneficial effects and low toxicity, hawthorn (Crataegus monogyna) fruits are widely utilized in folk medicine. The objective of this study was to assess the influence of the extraction method and solid-to-solvent ratio on the composition in phenolic compounds and on the antioxidant activity of hawthorn ethanolic extracts.

The extractions were made by maceration, Soxhlet and ultrasound-assisted methods, in 60% ethanol. For each extraction method, two solid-to-solvent ratios were used: 1:5 and 1:10 (w/v). The total phenolics and flavonoid contents of the extracts were estimated by Folin-Ciocalteu and aluminium chloride assay, respectively. The antioxidant activity of the extracts (DPPH• scavenging activity, Fe^{3+} reducing power and Fe^{2+} chelation) was assessed by photocolorimetric methods.

Maceration and ultrasound-assisted methods provided the most effective extraction o hawthorn phenolics and flavonoids, for the 1:10 (w/v) solid-to-solvent ratio. The highest DPPH radical scavenging activity was found for the extracts obtained by maceration and 1:5 (w/v) solid-to-solvent ratio; also, the highest Fe^{3+} reducing power was registered for the extracts obtained in the same conditions. As for the Fe^{2+} chelating activity, the highest value was obtained for the extracts obtained by ultrasound-assisted method, for the 1:10 (w/v) solid-to-solvent ratio. These results suggest that the antioxidant activity of hawthorn extracts could be related not only to their concentration in phenolic compounds, but also to the phenolics chemical structure.

The results obtained in this study show that extraction method and solid-to-solvent ratio significantly influence the efficiency of phenolics extraction, as well as the antioxidant activity of hawthorn extracts.

Financial support: This work was carried out through Partnerships in priority areas Program – PN II, implemented with the support of MEN – UEFISCDI (Romania), project nr. 149/201

P1013

Determination of the polyphenol content and antioxidant capacity of a Calafate extract, a southern Chile super-fruit

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Introduction: The Calafate (Berberis microphylla) is a wild plant with an edible dark-blue fruit. This polyphenol-rich berry is native to southern Chile and Argentina The aim of this work was to generate and to characterize a purified Calafate extract. Methods: To prepare the extract, 163g of dry calafate fruit were used and extracted in 815 mL of solvent (methanol: water-50:50) for 24h with agitation. The mixture was then filtered and purified using an Amberlita XAD7HP column. Total polyphenol content for the purified extract was determined by Folin-Ciocalteu. Anthocyanin content was assayed by the differential pH method and the antioxidant activity was measured by FRAP. Results: The purified calafate extract presented a total polyphenol content of 2242.3 mg gallic acid equivalents/100g dry weight. The Anthocyanin content was of 66.1 mg cyanidin-3-glucoside equivalents/100g dry weight and the determined antioxidant capacity was 38.43 mmol Fe^{+2}/100g dry weight. Conclusion: The purified calafate extract showed a high antioxidant capacity which is related to the high content of total anthocyanins detected. The results obtained in this study suggest that calafate is a super-fruit which can be of great interest for treatment/prevention of several metabolic diseases.

Financial support: Grant 21120219/ Fondecyt 11110219/ Grant PUCV

P1014

Chilean grape cane: a promising source for bioactive (poly)phenols such as stilbenoids and procyanidins.

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Stilbenes and procyanidins are bioactive (poly)phenols found in Vitis vinifera. They prevent or inhibit the progression of chronic non-transmissible diseases like diabetes cancer, cardiovascular and neurodegenerative diseases. Viticulture generates big amounts of canes during the grapevine pruning season. These residues are burned or incorporated into the soil of the vineyard. The higher stilbene level found in grape canes (190-6533 mg kg^{-1}) compared to wine (0.1-14.3 mg L^{-1}) or grapes (0.06-33.5 mg kg^{-1}), makes these underutilized residues a promising source of stilbene. The post-pruning storage of grape canes at room temperature over some months has significative incidence on stilbenoid levels. The major stilbenes in grape canes are well characterized, but the minor ones are still unidentified, whereas in case of procyanidins, their identities and concentrations are unknown.

Stilbenoid levels in canes of different grape varieties were analyzed after three months of post-pruning storage under controlled temperature and moisture by HPLC-DAD–MS/MS. An enriched extract of grape canes collected in Chile were fractionated by CPC and analyzed by UHPLC-DAD-MS/MS. For procyanidin identity assignment, a new UHPLC-DAD-MS/MS method was developed.

Stilbenoids reach 4018 mg kg^{-1} at the third month of storage, increasing their levels up to hundredfold. Different dimers like resveratrol-piceatannol heterodimer and three trimers were detected. Vitisin B and ampelopsin A enriched fractions were obtained by CPC. Procyanidins such as (+)catechin, (-)epicatechin, B1 and B2 dimer and C1 trimer were found in grape canes.

Financial support: Beca Doctorado Nacional CONICYT/ FONDEF D10I1104/ FONDECYT 1110767/FONDECYT 1150721

P1015

Fruit fly larvae fed palm fruit juice (PFJ) showed delayed pupation via up-regulation of fat body heat shock protein gene expression

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Palm fruit juice (PFJ) is a phenolic acid-rich beverage obtained as a by-product from oil palm (Elaeis guineensis) fruit milling. This beverage contains three caffeoylshikimic acid isomers and demonstrated beneficial health properties in various biological models. We previously found that fruit fly (Drosophila melanogaster) larvae given PFJ showed delayed pupation. In this study, transcriptomic effects of PFJ on fruit fly larvae were examined to identify possible anti-aging properties. Fruit fly larvae genotype w^{1118} were fed a semi-purified diet with or without PFJ (4 mL per 100 mL, 22 g solids, 19,000 ppm GAE/kg). Total RNA was extracted from whole larvae and their fat bodies for microarray gene expression analysis using Affymetrix Gene 1.0 ST Array GeneChips. Genes with fold changes > |1.30| and p values < 0.05 were considered differentially expressed.

Compared to controls, 128 genes were up-regulated while 63 were down-regulated in PFJ supplemented larvae. Functional analysis showed that transport and metabolic processes were up-regulated, while development and morphogenesis processes were down-regulated. Microarray analysis on their fat bodies showed that 168 genes were up-regulated, while 103 genes were down-regulated following PFJ supplementation. Genes involved in defense response and determination of adult lifespan including various heat shock proteins were up-regulated, while cell cycle and growth genes were down-regulated. The results obtained correlated with the physiological changes previously observed. They also indicate that PFJ supplementation helped lengthen the active stage of the fruit fly larvae and hence delay their aging process via increments in fat body heat shock protein gene expression.

Financial support: Malaysian Palm Oil Board and Brandeis University (Foster Biomedical Research Laboratory)

P1016
Computer modelling in predicting food-drug interactions of dietary polyphenols through inhibition of Cytochrome P450 3A.

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Cytochrome P450 (CYP) enzymes play a critical role in the metabolism of phytochemicals, drugs and xenobiotics. CYP3A subfamily is particularly relevant in this respect, due to their abundance in human intestine and liver, and the broad structural diversity of their substrates. Many dietary polyphenolics, were shown to inhibit CYP3A4 activity and, as a result, e.g. to increase the actual doses of the drugs that reaches the blood circulation in its active form. The latter may lead to unfavourable interactions and possibly fatal toxicity. Continuous exposure to these compounds may lead, in a feedback fashion, to increased expression of CYP3A4 in the intestine, making the food–drug interaction even more complex. Hence, it is important to develop predictive tools to group and evaluate the potency of xenobiotic in inhibiting and/or inducing CYP. Here we report the use of computational tools, docking in particular, to predict the inhibition of CYP3A4 by resveratrol and its analogs, and to further predict interactions between consumed phytochemicals and prescribed drugs. Chemically modified stilbenes, cell culture assay and in-vitro reactions served to support the predictions. The biochemical findings correlate with the computational insights, where the IC_{50} of the tested compounds consist with their structural matching to the active site of CYP3A, represented by the CDOCKER energy scores. Our results suggest that the integration of results from several enzymatic models and computer approaches may promote comprehensive understanding of the polyphenolics-CYP3A4 interactions and their potential risk.

P1017
Effect of Torulaspora delbrueckii yeast on the anthocyanin and flavanol concentrations of Cabernet franc and Pinotage wines

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Pinotage and Cabernet franc grape musts were inoculated with *T. delbrueckii* isolate and compared to wines made with a commercial *S. cerevisiae* wine yeast strain (reference wine). The study was conducted over three consecutive vintages. The wines were analysed for anthocyanin and flavanol compound concentrations using a RP-HPLC-DAD technique. The results (data) were subjected to analysis of variance and principal component analysis. The Pinotage and Cabernet franc grape varieties reacted differently to the two yeast treatments. Anthocyanin and flavanol concentrations measured in the *T. delbrueckii* Pinotage wines were higher, compared to the reference Pinotage wines. Cabernet franc wines made with *T. delbrueckii* were lower in anthocyanin glycoside and flavanol compounds, compared to their reference wines. Significant differences in most acetylated and coumaroylated anthocyanins were evident between the two Cabernet franc wines and the two Pinotage wines, including the anthocyanin glucosides. Principal component analysis showed that epigallocatechin gallate, epicatechin 3-*O*-gallate, procyanidin B1, B2, peonidin 3-*O* - glucoside, cyanidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, petunidin 3-*O*-(6-*O*-*p* - coumaroyl) glucoside, delphinidin 3-*O*-(6-*O*-acetyl) glucoside, petunidin 3-*O*-(6-*O*- acetyl) glucoside, peonidin 3-*O*-(6-*O*-acetyl) glucoside, malvidin 3-*O*-(6-*O*-acetyl) glucoside and malvidin 3-*O*-glucoside compound concentrations were highest in Pinotage wines made with *T. delbrueckii*. In contrast, Cabernet franc wines made with *S. cerevisiae* yeasts were highest in peonidin 3-*O*-(6-*O*-acetyl) glucoside, malvidin 3 *O*-(6-*O*-*p*-coumaroyl) glucoside, petunidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside, petunidin 3-*O*-glucoside, delphinidin 3-*O*-(6-*O*-acetyl) glucoside, peonidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, malvidin 3-*O*-(6-*O*-acetyl) glucoside, cyanidin 3 *O*-glucoside, procyanidn B1, B2, epicatechin 3-*O*-gallate and epigallocatechin gallat concentrations. Total anthocyanins were highest in Cabernet franc wines made with *S. cerevisiae* and *Pinotage wines made with T. delbrueckii*. Total flavanol concentrations were also highest in Pinotage wines made with *T. delbrueckii* and Cabernet franc wines made with *S. cerevisiae*. The results show that *T. delbrueckii* is the preferred yeast for Pinotage grape must and *S. cerevisiae* the preferred yeast for Cabernet franc grape must in terms of anthocyanin and flavanol concentrations.

Financial support: National Research Foundation of South Africa, Winetech and the Agricultural Research Council for providing funds for the research.

P1018
Scutellaria radix extract as a natural UV protectant for human skin

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Ultraviolet (UV) radiation induces oxidative injury and inflammation in human skin. Scutellaria radix (SR, the root of Scutellaria baicalensis Georgi) contains flavonoids with high UV absorptivity and antioxidant properties. The purpose of this study was to examine the potential use of SR extract as an additive in cosmetic products for UV protection. SR extract, the butanol fraction (BuOH), and the two major components of SR extract, baicalein and baicalin, strongly absorbed UV radiation and displayed free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl radials and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radicals. They also attenuated the UV-induced apoptotic death of HaCaT cells as determined by cell viability, cleavage of procaspase 3, and the ratio of Bax to Bcl-2. Moreover, cellular lipid peroxidation was reduced by these materials as monitored by thiobarbituric acid-reactive substances. Sunscreen creams, with or without supplementation of SR extract, were tested in human trials to evaluate potential skin irritation and determine the sun protection factor (SPF). A sunscreen cream containing 24% ZnO showed an SPF value of 17.8 and it increased to 22.7 when supplemented with 5% SR extract (BuOH fraction). Both sunscreen creams induced no skin irritation. This study suggests that SR-derived materials are useful as safe cosmetic additives that provide UV protection.

Financial support: the Ministry of Science, ICT and Future Planning, Korea

P1019
Anti-melanogenic effects of resveratryl triglycolate, a novel hybrid compound between resveratrol and glycolic acid

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Resveratrol is known to inhibit cellular melanin synthesis by multiple mechanisms and resveratryl triacetate (RTA) is a good prodrug of resveratrol for cosmetic use. The purpose of this study was to examine the anti-melanogenic effects of resveratryl triglycolate (RTG) in comparison with resveratrol and RTA. The effects of RTG, resveratrol, glycolic acid, RTA, and arbutin on human TYR activity were determined in vitro. Their effects on cellular melanin synthesis were assessed in human epidermal melanocytes stimulated with L-tyrosine. Expressions of melanogenic enzymes such as TYR, tyrosinase related protein-1 (TRP-1) and DOPA chrome tautomerase (DCT) were analyzed at the mRNA and protein levels. Anti-melanogenic effects were additionally determined using a reconstituted skin model exposed to UV. RTG inhibited human TYR activity in vitro as potently as resveratrol and RTA. Its cytotoxicity was similar to resveratrol and RTA. RTG decreased both intracellular and extracellular melanin levels of L-tyrosine-stimulated human epidermal melanocytes most strongly among the tested compounds. It also suppressed mRNA and protein expressions of TYR, TRP-1 and DCT very strongly. Collectively, this study suggests that RTG has great potential as an anti-melanogenic agent.

Financial support: This research was supported by the Korea Health Technology R&D Project of the Ministry of Health & Welfare (Grant No. HN12C0051).

P1020

Effect of traffic pollution on phenolic profile and antioxidant activity of Rubusfruticosus L.

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Rubus fruticosus L. (blackberry) is a cosmopolitan plant considered as weed and invasive species in most of the countries. Also, it is a well known edible fruit and medicinal plant. It often grows near roads. Road traffic emits a cocktail of pollutants, such as sulphur and nitrogen oxides, ozone and heavy metals, which can influence the vegetation in neighbouring areas. The aim of this study was to investigate the effect of pollutants on phenolic profile and antioxidant activity of *R. fruticosus* extracts.

The plant samples were collected from three locations – two degraded sites near the high frequency roads and one preserved site isolated from traffic and direct urban impact. The content of heavy metals in plant samples and the soils was determined by ICP-MS technique. Forty four selected phenolic compounds were quantified in leaves extracts by LC-MS-MS. Total flavonoid content and antioxidant activity of the extracts was assessed by spectrophotometric assays (FRAP, DPPH$^{\bullet}$, NO, OH$^{\bullet}$and ability to inhibit lipid peroxidation).

Although the content of Pb, Sb, Mo, Se and Zn was double in the soil from polluted location, there was no significant difference in their content in plant material, indicating that *R. fruticosus* is well adapted on metal-polluted soils and accumulation of heavy metals in upper parts of the plant is controlled. The total flavonoid content was significantly higher in extracts obtained from plants from polluted locations (39. and 17.2 vs 10.1 mg quercetin eq per g dw). Among 44 investigated phenolic compounds, gallic acid, 5-*O*-caffeoil-quinic acid, kaempferol-3-*O*-glucoside, hyperoside and isoquercitrin were the most abundant, where the content of the three last mentioned flavonoids were significantly higher in extracts of plants from pollute sites. The investigated extracts expressed high antioxidant activity, but the correlation between the extent of activity and the level of pollution was not found.

Financial support: Ministry of Education, Science and Technological Development, Republic of Serbia, grant No. 172058; Provincial Secretariat for Science and Technological Development, Province of Vojvodina, Republic of Serbia, grant No. 114-451-1123/2014-03

P1021

Stereoselective synthesis of (poly)phenol human and microbial metabolites and related phase II conjugates

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Polyphenols and related compounds are a group of plant-derived secondary metabolites which epidemiology indicates play a role in the protective effects of a fruit and vegetable-based diet. Flavan3ols are a heterogeneous group of phenolics comprising from single monomers to oligomeric structures, present in cocoa, green tea, red wine and different nuts such as hazelnut, in which skin they are particularly concentrated. Their intake has been associated with a lower risk of fatal cardiovascular diseases and colorectal cancers. In the human body, through various metabolic pathways, these molecules generate many metabolites, including phenyl-γ-valerolactone derivatives. An asymmetric synthesis represents the only way to obtain these compounds in an enantio-pure form, considering the fact that γ-valerolactones are not commercially available.

We report here an efficient and enantioselective synthetic route toward these metabolites with the aim of subsequently testing their bioactivity. The stereodivergent plan leads through five steps to a series of differently substituted phenyl-γ-valerolactones in both enantiomeric forms (R and S configured). The key chemical manoeuver in this process is an asymmetric vinylogous Mukaiyama–Aldol reaction (VMAR) between the donor (TMSOF) and an (poly)hydroxylated benzaldehyde, which affords two different diastereoisomers. Appropriate transformations of each diastereoisomer allowed the entry to both enantiomeric forms of γvalerolactones, which were handled to obtain related phase II conjugates.

The selective synthesis of γvalerolactones represents an outstanding starting point for the study of their bioactivity, a key point to understand the health effects of diets rich in cocoa, green tea, and nut-derived foodstuffs, among other flavan-3-ol-containing products.

Financial supports : Università degli Studi di Parma and “Progetto AGER” (grant n° 2011-0283)

P1022
Antioxidative and wound healing properties of Crateva adansonii DC

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Leaves of Crateva adansonii DC, a small bush found in Togo, are widely used in traditional medicine to cure infectious abscess. Traditional healers harvest only young leaves and sprouts early in the morning in order to prepare their drugs. To validate this ancestral practice, we performed a phytochemical screening of various C. adansonii leaves samples collected in different places, at different time and at different ages.

Using antioxidant activity as selection criteria, optimal extracts were obtained with sprout leaves, collected at 5:00 am in Djidjolé. Wound healing potential was then investigated for several extracts in vitro on keratinocytes stimulated by Staphylococcus aureus proteins. Anti-inflammatory activity was highlighted with flavonoids-rich extracts, especially against TNFa. Those results validate the traditional practices and the potential of C. adansonii as wound healing drug.

Financial support: Grant from SCAC of French Embassy in Togo

P1023
Antioxidant activity, Bioactive Compounds Content and Identification of Phenolic from Cowpea (Vigna unguiculata L. Walp) biofortified

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Micronutrient deficiency affects people worldwide, especially poor families in developing countries. The biotechnology techniques are able to improve the micronutrient quality of staple crops and they are a new strategy to address micronutrient deficiencies. Legumes are an important source of nutrients and natural antioxidants. Cowpea (*Vigna unguiculata L.* Walp) is the most popular grain legume in Brazil and it is a very important subsistence crop in semi-arid regions. This study aimed to evaluate mineral content, antioxidant activity and identification of phenolic from cowpea cv. BRS – Xiquexique. The mineral content was determined by plasma emission spectrometry - ICP. Acetone 80% extracts (v/v) was made for measure the total fenolic content (TPC) and antioxidant activity (AA). The TPC was evaluated by Folin reagent and AA was tested using DPPH and ABTS assay. The phenolic content were identified and quantified by HPLC-DAD. In vitro colorimetric method was used to measure total flavonoids. The major minerals (mg.100 g-1): Calcium – 88; Potassium - 1034; Phosphorus - 505; Magnesium - 115; Zinc - 3.24; Iron – 7.6. The TPC obtained was 199.05 mg gallic acid equivalents (GAE) .100 g-1. The cowpea exhibited high AA by DPPH (575.4 TEAC µM.100 g-1) and ABTS (608.5 TEAC µM.100 g-1) assay. Four phenolic compounds were identified: catechin (2.07 mg.100g-1), gallic (67.19 mg.100 g-1), ferulic (32.07 mg.100 g-1) and clorogenic (3.08 mg.100 g-1). Moreover, it presented significant amounts of flavonoids (67.96 mg of quercetin.100 g-1). This study demonstrated that cowpea cv. BRS – Xiquexique could serve as potential source of antioxidants and minerals.

Financial support: Cnpq - National Council for Scientific and Technological Development, Numberprocess 482292/2011-3. CAPES-CNPq, Number process 552239/2011-9.

P1024

Antioxidant activity, bioactive compounds content and identification of phenolic frocajuí (Anacardium humille St.) and castanhola (Terminalia catappa L.).

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Fruits are a good source of bioactive compounds, such as phenolics and vitamins that present antioxidant activity. Brazil has a large number of exotic fruit species, but most part is unexplored. Previous studies showed antimicrobial activity from leaves of cajuí (*Anacardium humille* St.) and castanhola (*Terminalia catappa* L.) which are present in the northeastern of Brazil. However, there is a lack of information regarding its fruits. The aim of this study was to determine bioactive compounds, antioxidant activity (AA) and identify phenolic compounds of 80% acetone (v/v) extract from cajuí and castanhola fruits. To do so, in vitro colorimetric methods were used to measure total carotenoids, proanthocyanidin and anthocyanidin. The total phenolic content (TPC) was evaluated by Folin reagent and AA was tested using DPPH and ABTS assays. The phenolic content were identified and quantified by HPLC-DAD. The cajuí showed significant amounts of carotenoids (83.22 mg β-caroten.kg-1) and the castanhola has significant amounts of proanthocyanidin (39.70 mg catechin equivalent.100 g-1) and anthocyanidin (4.94 mg cy-3-glu.100 g-1). The TPC ranged from 867.08 to 3071.49 mg gallic acid equivalents (GAE).100 g-1. Both fruits exhibited high AA by DPPH assay (18498.16; 37687.62 TEAC μM.100 g-1) and ABTS (12171.12; 37533.44 TEAC μM.100 g-1). Three phenolic compounds were identified in cajuí and castanhola, respectively: catechin (7.04; 34.11 mg.100 g-1), epicatechin (8.79; 8.24 mg.100 g-1) and gallic acid (144.46; 419.80 mg.100 g-1). This study suggested that cajuí and castanhola could serve as potential source of antioxidants.

Financial support: Cnpq - National Council for Scientific and Technological Development. CAPES-CNPq, Number process 552239/2011-9.

P1025

Analysis of phenolic compounds profile in common berries of Nordic diet by HPLC-CoulArray

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Berries are an essential part of a healthy Nordic diet. They are rich sources of bioactive phytochemicals such as Flavonols and phenolic acids with known beneficial health effects. Food composition data on these compounds in commercially available berries is scanty and hampered by analytical challenges. An HPLC-CoulArray method for simultaneous determination of flavonols and phenolic acids in berries consumed in healthy Nordic diet (bilberry, lingonberry, cloudberry, sea-buckthorn) were evaluated and applied.

Berry samples purchased from supermarkets were freeze dried, hydrolyzed and extracted with aqueous methanol (50%) containing TBHQ (80 mg) and HCL (1.2 M) for 2 hr in a shaking water-bath at 85°C. Analyte content was determined using coulometric array detection which is sensitive and selective with high resolving power for determination of phenolic compounds.

The accurate precision (RSD% max 10.6) and good recovery of all analytes (75.6-122.5 %) in investigated berries with current hydrolysis, extraction and separation methods validated the conducted methods. The phenolic profiles differed among berries. Highest concentration of quercetin was observed in lingonberry.

Myricetin and isorhamnetin were only detected in bilberry and sea-buckthorn respectively. Hydroxycinnamic acids were also detected in all berries although hydroxybenzoic acids were found in bilberry, lingonberry and cloudberry. The total concentration of phenolic compounds was highest in sea-buckthorn (270.5 mg/ 100 g DW) then bilberry (253.6 mg/ 100 g DW), lingonberry (219.7 mg/ 100 g DW) and cloudberry (121.7 mg/ 100 g DW). Knowledge on phenolic profiles of investigated berries could be useful in the assessment of their health-protective effects in human.

Financial support: Swedish University of Agricultural Sciences

P1026
Phytochemical analysis of flavonoids and antioxidant activity of the "Ghars" datepalm cultivar Phoenix dactylifera L

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The date palm Phoenix dactylifera is associated with the oasis and desert. Beyond the production of dates, there are culinary uses, medicinal and cosmetic from fruit and pinnae of some date palm cultivars. This study addressed the aspect of using pinnae of Ghars cultivar in a traditional pharmacopoeia. Qualitative analysis of secondary metabolites extracted from pinnae permit to demonstrate flavonoids after development in different mixtures of migration solvents and revelation with "Neu" then observation under UV at 254nm and 365nm before and after development, many bands appeared Each colored band corresponding to a chemical compound, The ethyl acetate extract has the great diversity of chemical compounds compared to the other extracts

Antioxidant activity has been positive in the ethyl acetate and methanolic extracts after spraying TLC plates with DPPH, there is immediate appearance of yellow spots in the ethyl acetate extracts, methanol and aqueous this indicates the presence of anti-radical activities, these active spots present constituents with antioxidant activity

Financial support: Plant Biochemistry laboratory, department of biology, university of Es-Senia, Oran, ALGERIE

P1027
Polyphenol profile and chemometrics as traceability tool for conventional and organic orange modelling discrimination

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Organic foods, fair trade products and protected designation of origin (PDO) are classified as added-value products. A recent survey showed that the demand for organic products by European consumers increased fourfold during the last decade for this reason traceability issues regarding organic production are an important issue.

A total of 660 orange fruit samples were collected in the south of Spain during 2014 and 2015 years. The polyphenol profiles of two orange cultivars 'Salustiana' and 'Navelina' produced under two management systems (conventional and organic) were obtained by UHPLC-DAD-HR-MS. Samples were divided in the different parts of hesperidium fruits: flesh, albedo and flavedo. Fifteen metabolites were determined in each part and their concentrations were affected by the management system. Furthermore, Partial Least Square Discriminant Analysis (PLS-DA), Soft Independent Modelling of class analogies (SIMCA) and k-Nearest Neighbors (k-NN), achieved an effective classification of samples based on the management system using their polyphenol profiles (external validation). A high classification accuracy model was obtained rounding 85 and 100 % corrected classification for both varieties. This work helped in understanding the knowledge of management effects on polyphenol profile and demonstrated the potential of these multivariate techniques for traceability modelling tool in authentication purposes.

References: Proposal for a Regulation of the European parliament and of the council on organic production and labelling of organic products. http://ec.europa.eu/agriculture/organic/documents/eu-policy/policy-development/report-and-annexes/proposal_en.p; 2014.

Financial support: IFAPA, the European Social (ESF) and European Rural Development Funds (ERDF) through the Project (PP.AVA.AVA201301.7), FJC is granted by a research contract funding and GPC and MJRM are granted by a postdoctoral research contract funding by IFAPA and ESF

P1028
Effect of organic and conventional crop management on the phenolic profile of eight plums Cultivars (Prunus salicina Lindl.)

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Plums (*Prunus salicina* Lindl.) are a rich source of (poly)phenols[1], especially chlorogenic acids (CQAs) and procyanidins, compounds with known benefits on human health[2,3]. In this study, we have investigated the phenolic profile of eight plum cultivars using UHPLC-HR-MS. The cultivars evaluated were "Black Amber", "Friar", "Angeleno", "Primetime", "Fortune", "Santa Rosa", "Showtime" and "Golden Japan", presenting a wide range of skin and fleshed colours from yellow to red/purple. The effect of organic and conventional management systems on their polyphenol composition was also evaluated. A total of 29 phenolic compounds were identified and quantified comprising 11 CQAs, especially 3-CQA and 4-CQA predominate along with lower amounts of feruloylquinic and *ρ*-coumaroylquinic acids; 7 flavonols, quercetin-3-*O*-glucoside and rutin principally, and 9 flavan-3-ols which comprised monomeric, dimeric and trimeric constituents. Almost all plum varieties contained two anthocyanins consisting of cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside, although significant differences between cultivars were also found. Anthocyanins and flavan-3-ols were presented in significant high quantities in "Black Amber", "Friar" and "Angeleno" (ranging from 122-375 µg/g FW and 336-541 µg/g FW, respectively), while "Fortune" and "Showtime" varieties showed the highest concentration of CQAs (from 187 to 253 µg/g FW). Flavonols were identified in significant quantities in "Black Amber", presenting the highest content (301 µg/g FW). Regarding the management system, organic plums had significantly higher polyphenol content (anthocyanins, flavan-3-ols, flavonols and CQAs) compared to the conventionally grown ones showing that the production system had a strong effect on the phenolic profile of plums. A deeper study on the mechanisms that could explain those results should be necessary.

Financial support: IFAPA, the European Social (ESF) and European Rural Development Funds (ERDF) through the Project (PP.AVA.AVA201301.7), FJC is granted by a research contract funding and GPC and MJRM are granted by a postdoctoral research contract funding by IFAPA and ESF.

P1029
Antioxidant activity and concentration of bioactive compounds in organic and conventional orange juice

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Conventional fruit cultivation is based on the intensive use of agrochemicals such as pesticides and fertilizers. Such cultivation system is characterized by high yields of products with reduced nutritional quality. Contrastively, organic cultivation yields fruits free of chemical inputs, making them a healthier choice for consumers, as demonstrated, for example, by studies indicating a smaller concentration of bioactive compounds in conventional fruits in comparison to their organic counterparts.

The present study analyzed the antioxidant capacity (AC), flavonoid content and concentration of soluble (SP) and hydrolysable (HP) polyphenols in the juice of organically (OG) and conventionally (CG) grown oranges. The results of the analysis were submitted to one-way analysis of variance (ANOVA) and Student's t-test to examine the null hypothesis of no significant difference between the groups. Results were expressed as the mean and standard deviation.

The use of the DPPH method showed a significant difference ($p<0.0001$) in the percentage of AC of OG (82.22 ± 0.3525)in comparison to CG (47.41 ± 0.7743). The analysis of the concentration of SP and HP also found a significant difference between the contents, expressed as milligrams of gallic acid equivalent per 100 gram of fresh weight (mg GAE/100g FW): ($p<0.0001$) on the concentration of SP – OG (0.04335 ± 0.002909) and CG (0.01817± 0.001606), and;($p=0.0055$) on the concentration of HP– OG (0.1527 ±0.003961) and CG (0.1274 ± 0.005982). Likewise, the analysis of the flavonoid content, expressed as mg of quercetin per mL showed a significant difference ($p<0,0001$) between the contents of the OG (3.940 ±0.00006195) and the CG (2.743 ± 0.0001223).

These results point to the conclusion that the orange cultivation system – organic or conventional –signifi-

cantly influences its chemical components, being the organic one that that generates the best nutritional output for this specific fruit. Nevertheless, it is clear that fruit consumption by the general population should be encouraged by public health policies no matter in what system they are grown.

Financial support: Proppi UFF; Research Support Foundation of the State of Rio de Janeiro – FAPERJ

P1030
Phenolic compounds and antioxidant activity in red wine and whole grape juice

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Phenolic compounds are known to have antioxidant capacity, decreasing and avoiding oxidative stress and hence, cellular damage. The main sources of polyphenols are dark coloured fruits, as grapes. Colour, taste, oxidative and chemical reactions in red wine, for example, result mainly from these compounds.

The aim of the study was to analyze the antioxidant capacity (through 2,2-Diphenil-1-picrilhidrazil – DPPH radicals sequestration), concentration of soluble (SP) and hydrolysable (HP) polyphenols, anthocyanins and flavonoids in red wine (RW) e whole grape juice (GJ). The results of the analysis were submitted to one-way analysis of variance (ANOVA) and Student's t-test to examine the null hypothesis of no significant difference between the groups. Results were expressed as the mean and standard deviation. Results with a $p < 0.05$ were considered significant. The following results were found for antioxidant capacity expressed as milligrams of gallic acid equivalent per 100 grams of fresh weight (mg GAE/100g FW) from RW: 85.53 ± 0.2594 and GJ: 88.41± 0.2594; solublepolyphenols concentration from RW: 0.1743 ±0.002886 and GJ:0.1788 ±0.002165; hydrolysable polyphenols concentration from RW0.1797 ± 0.01357 and GJ 0.3820 ±0.006838, with Allvalues); flavonoid concentration (expressed as mg quercetin/mL), RW: VT 12.54 ±0.0007496 and GJ: 2.417 ± 0.001290; anthocyanin antioxidant activity (expressed as mg/100g) were RW 4.965± 0.6067 andGJ 7.025 ±0.6515 . No significant differences were found regarding anthocyanin antioxidant activity ($p=0.08$), soluble polyphenols ($p=0.28$), when comparing RW and GJ.

These results point out that although red wine has presented higher phenolic compound concentrations, the whole grape juice presented higher antioxidant activity.

Financial support: Proppi UFF; Research Support Foundation of the State of Rio de Janeiro – FAPERJ

P1031

Effect of In Vitro Digestion on antioxidant activity of extracts recovered from grape by-products for application on nutricosmetic and nutraceutical products

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The winemaking process generates large amounts of by-products such as grape seeds and skins (grape marc) which are rich in polyphenols with strong antioxidant properties. The present work is part of a major on-going project which combines the efforts of industry and academia, to optimize and intensify polyphenols extraction from grape pomace using microwave technology, in particular the application of microwaves as a pretreatment followed by a shorter traditional extraction. In this context, three grape pomace-extracts, obtained applying different extraction conditions have been investigated to evaluate the impact of in vitro digestion (IVD) on polyphenols composition and antioxidant activity.

The extracts were characterized by HPLC-DAD and HPLC-MS methods and tested on human epithelial intestinal cells (Caco-2 cells) with the aim to evaluate the intracellular antioxidant activity and cytotoxicity profile before and after IVD. Among the three extracts (before IVD), the obtained by a combination of pressure and microwaves pretreatment followed by a conventional extraction at 60ºC presented the higher intracellular antioxidant activity (0.47 mol QC/mg GAE). None of the non-digested extracts were cytotoxic between the concentration range tested (0-1000 mgGAE/L).

Overall the IVD process led to a noticeable reduction of the polyphenols content, in particular after intestinal digestion step. Nevertheless, the digested extracts were evaluated in a physiological concentration range (0-180mgGAE/L) for their cytotoxicity and intracellular activity. Overall the digestion promoted a significant decrease on the cellular antioxidant activity but in this case, the extract obtained without MW pre-treatment showed the higher value (0.083 mol QC/mgGAE).

Financial support: Marie Curie Industry-Academia Partnerships and Pathways (European Commission) through the WineSense project (FP7-PEOPLE-2013- IAPP-612208).

P1032

Interactions of secondary metabolites of buckwheat and calcium

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Buckwheat have attracted much interest in recent years mainly because of its excellent nutrient profile. However, its secondary metabolites may influence mineral bioavailability reducing its absorption from the gut. The aim of the study was to evaluate and compare the interactions of calcium with rutin or quercetin in the experimental wheat-buckwheat bread and the model system.

Calcium content in the experimental bread and its hydrolysate was determined by the atomic absorption spectrometry; rutin and quercetin content were determined by high performance liquid chromatography coupled with a mass spectrometer equipped with a triple quadruple and ion trap. Confluent Caco-2 (colon adenocarcinoma) cells cultivated on inserts were used to study the interactions of secondary metabolites of buckwheat (rutin and quercetin) and calcium. Caco-2 cells were incubated with hydrolyzateof experimental bread or the model mixture of calcium ($CaCl_2$), rutinand/or quercetin. Then, amounts of calcium, rutin and quercetin absorbed into theCaco-2 cells and transported to the basolateral fluid were analysed.

Based on the results obtained it could be conclude that quercitin, and in the lower range rutin, stimulated the calcium uptake by Caco-2 cell. Calcium transport by Caco-2 cells was inhibited in the presence of rutin and quercitin. Compared to the model system containing calcium, rutin and quercitin, the increased calcium absorption from the experimental bread hydrolyzate might result from the presence of quercitin as well as other compounds present in the bread matrix. In the presence of calcium, the absorption and transport of quercitin increases.

Financial support: KNOW Consortium “Healthy Animal - Safe Food”, MS&HE Decision No. 05-1/KNOW2/2015.

P1033
Antioxidant properties of avocado leaf extract

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Background: Currently the agroindustrial activity has reached an important development, which has a negative impact on the environment due to the generation of by-products do not have a commercial use. These by-products could contain compounds with antioxidant properties to its use as functional foods ingredients (Kosinska et al., 2012). The 'Hass' avocado (*Persea americana* Mill.) is the most important avocado variety cultivated worldwide (García-Rojas et al., 2012) and has many nutritional qualities and high amount of bioactive compounds (Lee et al., 2004 However few studies have been focused in the characterization and antioxidant properties of by-products from avocado. The objective of this research was to determine and characterize the antioxidant activity and main polyphenols of hydroalcoholic leaves extract from avocado var Hass. Methodology: Hydroalcoholic leaves extract was performed with ethanol:water (50:50 v/v). Total polyphenol content (TPC) was determined by Folin-Ciocalteau method. Antioxidant activity (AA) was measured by DPPH and FRAP methods and polyphenol chromatographic profiles by LC-MS. Results shows a TPC of 47.4 mg CAE/g dry leaf and AA of 0.22±0.004mmol $Fe^{+}2$/g to FRAP and 0.055 mg/mL, expressed as $EC_{50}$to DPPH. The main compounds identified in this extract were B- trimer procyanidin (38.5%), quercetin-O-glucoronide or quercetin-O-hexoside (5.63%), quercetin-O-pentohexoside (4.03%) and B dimer procyanidin (4.03%), among others Conclusion: According to these results leaves of avocado are attractive sources of natural antioxidants. The enrichment of foods with these phenolic compounds may contribute to beneficial effects on human health, by preventing oxidative damage and reducing risks of chronic diseases.

Financial support: FONDECYT Grant 11130373 (CONICYT, Chile)

P1034
Antioxidant properties of avocado peels extract dried by spray drying

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Background: Currently the agroindustrial activity has reached an important development, which has a negative impact on the environment due to the generation of by-products do not have a commercial use. These by-products could contain compounds with antioxidant properties to its use as functional foods ingredients (Kosinska et al., 2012). In the context, peels of avocado (Persea americana Mill) contain bioactive compounds such as polyphenols (phenolic acids, flavonoids and procianidin, between others) (Rodriguez-Carpena, 2011). The advantages of the drie extract over conventional liquid forms are lower storage costs, higher concentration, and stability of active substances. The objective of this research was to determine and characterize the antioxidant activity and main polyphenols of hydroalcoholic peels extract dried by spray drying. Methodology: Hydroalcoholic peels extract was performed with ethanol:water (50:50 v/v). Total polyphenol content (TPC) was determined by Folin-Ciocalteau method. Antioxidant activity (AA) was measured by DPPH and FRAP methods and polyphenol chromatographic profiles by LC-MS. Results shows a TPC of 159.5±4,4 mg CAE/g powder and AA of 1.33±0.004 mmol $Fe^{+}2$/g powder to FRAP and 0.043±0005mg/mL, expressed as EC_{50} to DPPH. The main compounds identified in this extract were chlorogenic acid followed by B-dimeprocyanidin, catechin and B trimer procyanidin, among others. Conclusion: According to these results peels of avocado are attractive sources of natural antioxidants. The enrichment of foods with these phenolic compounds may contribute to beneficial effects on human health, by preventing oxidative damage and reducing risks of chronic diseases.

Financial support: FONDECYT Grant 11130373 (CONICYT, Chile)

P1035
Effect of arbuscular mycorrhizal fungi and fertilization on polyphenol profiles of marjoram, lemon balm and marigold

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The aim of this study was to examine the effect of arbuscular mycorrhizal fungi (AMF) inoculation and artificial fertilization (NPK) on polyphenol content of sweet marjoram (*Origanum majorana* L.), lemon balm (*Melissa officinalis* L.) and potmarigold (*Calendula officinalis*L). The soil of the experimental field without the addition of AMF and NPK served as control. In the 1st treatment NKP, in the 2nd treatment commercially available AMF inoculum (INOQ) and in the 3rdtreatment NKP and AMF mixture were added to the soil of the experimental field. The efficiency of the mycorrhization was verified by monitoring the fungal root colonization of the plants. Main compounds (based on HPLC-UV signal) were subjected for identification using HPLC-ESI-qTOFMS.

Five major polyphenols were tentatively identified from marjoram (apigenin-6,8-di-glucoside, luteolin-7'-*O*-glucuronide, rosmarinic acid, apigenin-glucuronide, lithospermic acid A isomer), three from lemon balm (rosmarinic acid and twolithospermic acid A isomers) and eight from marigold (chlorogenic acid, quercetin-3 *O*-rhamnosyl-rutinoside, isorhamnetin-3-*O*-rhamnosyl-rutinoside, rutin, calendoflavoside, narcissin, dicaffeoylquinic acid, isorhamnetin-malonyl-glucoside.

Treatments mostly affected lithospermic acid A isomers and rosmarinic acid content, which compounds are the most dominant polyphenol species in the studied samples. In lemon balm, AMF significantly increased these polyphenols and fertilization had the same stimulating effect on polyphenols. On the contrary, in marjoram increase in NKP supply dramatically reduced polyphenol levels, whereas AMF counteracted this effect.

Our results show that fungal root colonization (mycorrhization) should be considered as a significant environmental factor that can influence the synthesis of major polyphenols.

Financial support: OTKA (PD105750)

P1036
Phenolic and volatile composition of a dry spearmint (Mentha spicata L.) extract

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Spearmint (Mentha spicataL.) is an aromatic plant that can be used fresh, as dried leaves or powder, as a seasoning and flavouring herb, or as herbal tea. Based on the reported benefits of phenolic compounds (including rosmarinic acid), spearmint lines have been developed through selective-breeding techniques to contain higher levels of these compounds.

The present poster reports a complete mass spectrometric characterization of both the phenolic and volatile fractions of a dried spearmint extract. The spearmint extract was prepared by extraction of microwavedried leaves with acidified water.

The phenolic fraction of spearmint was characterised by means of UHPLC-ESI-MS^nA total of 66 different phenolic molecules were identified on the basis of their characteristic MS^nspectra. The analysis revealed that the extract was mainly composed of rosmarinic acid and its derivatives (230 ± 13 mg/g), but also contained measurable amounts of salvianolic acids, caffeoylquinic acids, hydroxybenzoic acids hydroxycinnamic acids, flavones, and flavanones. The volatile fraction of dried spearmint extract was characterised using head space solid-phase micro extraction coupled with GC-MS (HS-SPME/GC-MS). Ketones were the most representative compounds in the volatile fraction, constituting about 32%, followed by terpenoids (20%), aldehydes, esters, and furans (18-19%).

In conclusion, these analyses allowed a complete evaluation of the dried spearmint extract. These data indicate that the extract produced from the selectively-bred proprietary spearmint lines have high phenolic acid content. These sensitive techniques make it possible to test the product authenticity and assist in the evaluation of its biological properties.

Financial support: Kemin Foods, L.C.

P1037
Antioxidant, Anti-inflammatory and Antibacterial Activity of Herb and Root Extract of Wild Edible Plant - Rumex acetosella L. 1753 subsp. multifidus (L.) Arcangeli1882

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Rumex acetosella species belongs to Polygonoideae subfamily, whose members have long been used in traditional medicine, while some of them have been used in the die as well. That is why thorough investigation on biological activities and chemical composition of these plants is necessary. However, scientific data for this selected species are still very scarce.

In order to rate the biological potential of prepared herb and root extracts of collected sheeps sorrel plant material, a series of antioxidant (FRAP, DPPH, $OH^{\bullet}$, NO,$O_2^{-\bullet}$anLP), anti-inflammatory (LC/MS/MS monitoring of five metabolites of arachidonic acid) and antibacterial (microtiter assay with resazurin as an indicator of bacterial growth, on nine bacterial strains) in vitro assays were conducted. Also, our work included quantitative analysis of 51 secondary metabolites by LC/MS/MS. Among selected compounds, vitexin and hyperoside were dominant in the herb (10.47 mg/g DE; 10.47 mg/g DE), and epicatechin (57.8 mg/g DE) in root extracts.

Based on obtained results root extract showed better antioxidant activity than herb extract in 5 of 6 assays, and also better activity compared to BHT as a common antioxidant compound. Root extract showed selective inhibitory activity against COX-1 enzyme. Examined extracts prevented the growth of three *Echerichia coli* and two *Staphylococcus* strains, but were not active against *Bacillus subtilis* ATCC6633nor *Listeria innocua*. Significantly better antibacterial activity was shown by root extract.

In general, obtained results indicate a high potential for application of *R. acetosella* subsp. *multifidus*, especially root extract, as a source of natural, biologically active compounds.

Financial support: Ministry of Education, Science and Technological Development, Serbia, GrantNo.172058

P1038
Phenolic compounds profile of Brazilian wild passifloras' leaves

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Brazilian biodiversity presents several plants with potential for exploitation as functional food. Among them, wild passifloras are recognized by their antioxidant and anti-inflammatory properties, especially the leaves, possibly due to their composition regarding phenolic compounds. The objective of this study was to investigate the phenolic compounds profile of the leaves from two wild Brazilian *Passiflora* species.

Leaves of the wild *Passiflora alata* and *Passiflora setacea* were collected at Embrapa Cerrados, Brasilia, Brazil, and dried at 50°C for 5 days. The extraction of phenolic compounds was performed by infusion at 94°C for 10 minutes and filtration. Phenolic profile was assessed by using *quadrupole–time-of-flight* mass spectrometer (Q-TOF LC/MS) for the rapid identification of phenolics, and Waters Acquity UPLC BEH (150 x 2.1 mm, 1.7 µm) column at 40°C, flow of 0.4 mL/min and injection volume of 5 µL.

The presence of phenolic compounds was identified in *P. alata* and *P. setacea* leaves. Flavonoids such as isoorientin-2''-O-glucoside, luteolin-6-C-arabinosyl-8-C-glucoside, isovitexin-7-O-xyloside, apigenin-6-C-glucosyl-8-C-arabinose, orientin-4'-methyl ether were found in *P. setacea* leaves, as well as the phenolic acid dihydroxybenzoic acid pentoside. In the *P. alata* leaves, the flavonoids found were vitexin-2"-O-rhamnoside and the unknown diosmetin conjugate. The phenolic acid dihydroxybenzoic acid-O-dipentoside was also present in the phenolic composition of *P. alata* leaves. Isovitexin-2"-O-glucoside was present in both passifloras' leaves. Other studies from Passitec Network have already reported the presence of these phenolic compounds in wild passifloras. This is a first stage of the study of Brazilian wild passifloras' leaves to produce a functional infusion.

Financial support: National Grant (CNPq - National Counsel of Technological and ScientificDevelopment)

P1039
Phenolic compounds profile of Brazilian wild passifloras' leaves

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Brazilian biodiversity presents several plants with potential for exploitation as functional food. Among them, wild passifloras are recognized by their antioxidant and anti-inflamatory properties, specially the leaves, possibly due to their composition regarding phenolic compounds. The objective of this study was to investigate the phenolic compounds profile of the leaves from two wild Brazilian *Passiflora* species.

Leaves of the wild *Passiflora alata* and *Passiflora setacea* were collected at Embrapa Cerrados, Brasilia, Brazil, and dried at 50°C for 5 days. The extraction of phenolic compounds was performed by infusion at 94°C for 10 minutes and filtration. Phenolic profile was assessed by using *quadrupole–time-of-flight* mass spectrometer (Q-TOF LC/MS) for the rapid identification of phenolics, and Waters Acquity UPLC BEH (150 x 2.1 mm, 1.7 µm) column at 40°C, flow of 0.4 mL/min and injection volume of 5 µL.

The presence of phenolic compounds was identified in *P. alata* and *P. setacea* leaves. Flavonoids such as isoorientin-2''-O-glucoside, luteolin-6-C-arabinosyl-8-C-glucoside, isovitexin-7-O-xyloside, apigenin-6-C-glucosyl-8-C-arabinose, orientin-4'-methyl ether were found in *P. setacea* leaves, as well as the phenolic acid dihydroxybenzoic acid pentoside. In the *P. alata* leaves, the flavonoids found were vitexin-2"-O-rhamnoside and the unknown diosmetin conjugate. The phenolic acid dihydroxybenzoic acid-O-dipentoside was also present in the phenolic composition of *P. alata* leaves. Isovitexin-2"-O-glucoside was present in both passifloras' leaves. Other studies from Passitec Network have already reported the presence of these phenolic compounds in wild passifloras. This is a first stage of the study of Brazilian wild passifloras' leaves to produce a functional infusion.

Financial supports : National Grant (CNPq - National Counsel of Technological and Scientific Development)

P1039
Influence of boiling of frozen and fresh Brussels sprout on the content of biologically active nitriles released from glucosinolates

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Recently, more the increasing interest in phytochemicals having chemopreventive properties is recorded due to the alarming cancer prevalence. Phytochemicals include polyphenols, terpenoids as well as glucosinolates (GLS) and its derivatives. GLS are sulphur containing glycosides present in Brassica vegetables. During technological o culinary processing of Brassicas, GLS are enzymatically or thermally degraded to biologically active nitriles and isothiocyanates, having anticancer effect. The aim of this study was to identify breakdown products of aliphatic GLS and to compare the profiles of biologically active breakdown products released from GLS during boiling of fresh and frozen Brussels sprout.

Fresh and frozen Brussels sprouts were used as material for this study. Vegetables were boiled at 100 °C for 5, 15 or 30 minutes. The contents of parent GLS were analysed by high performance liquid chromatography (HPLC). Aliphatic breakdown products were determined with the use of gas chromatography (GC-MS).

In the examined boiled Brussels sprouts, isothiocyanates were not determined. In both, boiled fresh Brussels sprout as well as in boiled frozen Brussels sprout the presence of 3-butenylnitrile released from sinigrin and 2-phenylacetonitrile released from gluconasturtiin were determined. The presence of 4-methylsulfinylbutanenitrile released from glucoiberin were noted only in boiled fresh Brussels sprout. The contents of biologically active breakdown products of aliphatic GLS were higher in fresh boiled Brussels sprout as compared to the frozen vegetable, however the decrement of parent GLS was higher in frozen boiled Brussels sprout.

Financial support: National Science Centre, Poland (project N N312 342239) and grant of KNOWConsortium "Healthy Animal - Safe Food", MS&HE Decision No.
05-1/KNOW2/2015.

P1040
Resveratrol and its methoxy derivatives as modulators of DNA damage induced byionizing radiation

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Various naturally occurring stilbene-like compounds that are related to resveratrol have been reported to possess some of the beneficial effects of the parent molecule and provide even further benefits. Therefore a series of methoxylated analogs of resveratrol were prepared with the aim of increasing the antitumor and proapoptotic activity. In a previous paper we studied two methoxy-derivatives, pterostilbene and trimethoxystilbene, the first formed by the substitution of two hydroxyl groups with two methoxyl groups (trans-3,5-dimethoxy-4'-hydroxystilbene) and the second by the replacement of all three OH groups with methoxyl groups

(trans-3,5,4'-trimethoxystilbene), showing their stronger antioxidant activity when compared to resveratrol (RSV). In the present paper we focused on the analysis of the ability of RSV and its two methoxylated derivatives in protecting proliferating non tumoral cells from damage induced by Ionising Radiation (IR). First of all we showed that the methoxy derivatives contrary to their parental compound are unable to affect topoisomerase enzyme and consequently are not clastogenic per sè. Secondly both pterostilbene and trimethoxystilbene much efficiently reduce chromosome damage induced by ionizing radiation. Furthermore trimethoxystilbene but not pterostilbene causes a delay in cell proliferation particularly in mitosis progression increasing the number of cells in metaphase at the expense of prophases and ana/telophases.

Lastly we show confocal microscopic analysis of the action of trimethoxystilbene treatment on tubulin polymerization in CHO cells.

P1041
Identification and quantification of anthocyanins in fruits of Eugenia brasiliensis L.

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The species Eugenia brasiliensis L., popularly known as grumixama, belongs to the Myrtaceae family, and is native from Brazil. The fruits have a black peel, which increases the interest of scientists, once this dark colour is a characteristic of anthocyanins, substances with important properties such as antioxidant activity. This study aimed to identify and quantify the anthocyanins in the peel of Eugenia brasiliensis L fruit by High Performance Liquid Chromatography and mass spectrometry.

The fruits were collected in Barra da Tijuca neighbourhood in Rio de Janeiro city, being selected by the maturity stage. Their peels were separated, freeze-dried and weighed in triplicate to perform the analysis. The identification was performed by High Performance Liquid Chromatography and photodiode array detector with wavelength of 520 nm and quantified by external standardization. Confirmation of anthocyanins found in the fruit was performed by high resolution mass spectrometry with direct injection.

The majority anthocyanins identified were delphinidin-3-O-glucoside (949.1 mg/100g) and cyanidin-3-O-glucoside (3729.5 mg/100g). The same profile of anthocyanins was found in jabuticaba (Myrciaria cauliflora Berg) peel. It was observed that the E. brasiliensis L. peel has a higher content of total monomeric anthocyanins than the jabuticaba peel, which is considered rich in these compounds. Thus, E. brasiliensis L can be considered a new source of anthocyanins, adding value to this fruit not yet commercially exploited.

P1042
Anthocyanins content in fruits of Eugenia astringens Cambess

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Eugenia astringens Cambess, also known as *Eugenia rotundifolia* (previous scientifiname), is a Myrtaceae family species, which is an important Brazilian angiosperm family. It has purple fruits when ripe, characteristic of bioactive compounds, such as anthocyanins.

This study aimed to identify and quantify anthocyanins presents in the Eugenia astringens Cambess fruits. Fruits samples were collected at Restinga da Marambaia in Rio de Janeiro city, selected by the maturity, pulped and freeze dried. Anthocyanins were extracted with formic acid solution in methanol. The identification was performed by High Performance Liquid Chromatography analysis and photodiode array detector in 520 nm with quantification by external standardization.

The total monomeric anthocyanins was 1504 mg/100g. The anthocyanins profile was identified as delphinidin-3-O-glucoside (306 mg/100g), cyanidin-3-O-glucoside (66 mg/100g), petunidin-3-O-glucoside (245 mg/100g) peonidin-3-O-glucoside (157 mg/100g) and malvidin-3-O-glucoside (730 mg/100g). The majority anthocyanins were similar to the profile of red grape. Comparing the results of *E. astringens* andjabuticaba, it was observed that the fruit has a lower content of anthocyanins (1504 mg/100g) to that found in freeze dried jabuticaba peel (2894 mg/100g), which is also a Myrtaceae family specie considered rich in these substances. Even so, Eugenia astringens fruits presented a high content and a profile with five anthocyanins. This fruit is still unexplored scientifically and this work aimed to promote greater knowledge of the functional potential of the fruits of Brazilian biodiversity.

P1043
Phenolic profile and carbohydrase inhibition of three varieties of Nigerian Roselle (Hibiscus sabdariffa L)

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Some scientific reports give credence to the antidiabetic properties of *Hibiscussabdariffa* mentioned in folk medicine. This has been attributed to the activity of bioactive phenolic compounds present within the flowers. However, a common limitation of most of the research is that the geographical origin as well as variety used are not specified, thus making comparison between compositional data on polyphenols and possible antidiabetic properties difficult. Hence, the objective of this research was to determine the phenolic profiles of three different varieties (dark red, light red and white), of *H.sabdariffa* grown in a single geographical region and to assess their inhibitory potential on carbohydrate-digesting enzymes. Using HPLC-ESI-MS we showed that anthocyanins and chlorogenic acid were the main polyphenols in the dark and light red varieties with five isomers of chlorogenic acid tentatively identified in the white variety. Although extracts from all varieties were only very weak inhibitors of –amylase, a dose-dependent inhibition of –glucosidase (maltose) was observed, with the dark red variety exhibiting the lowest IC_{50} value (4.35 ± 0.07 mg/mL dried powder). Together delphinidin 3-O-sambubioside, cyanidi3-O-sambubioside and chlorogenic acid accounted for 65 % of this activity. This study shows the impact of *Hibiscus sabdariffa* varieties on phytochemical composition and their subsequent bioactivity on starch digestion.

Financial support: Educational Trust Fund, Nigeria and the Delta State University, Abraka, Nigeria forPhD funding.

P1044

The design and sensory testing of a placebo formulation for a polyphenol-rich Aronijuice with evaluation of potential impact on platelet function

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Randomised, controlled clinical trials are essential to demonstrate the potential beneficial effects of polyphenol-rich foods on cardiovascular health. But, the development of appropriate placebos that look and taste sufficiently similar to test foods is very challenging. We sought to develop and test a polyphenol-free formulation that could be used as a placebo in a clinical trial designed to assess the effects of Aronia juice on platelet function.

Three formulations with the same nutrient composition as Aronia juice (determined by HPLC) were prepared by mixing various nutrients, artificial colours and flavours with water. The similarity to Aronia juice in terms of taste, colour, smell and texture was assessed by six food panellists in a single-blinded manner using the Likert scale. The highest scoring placebo was further tested for its safety and impact on platelet function in a pilot study conducted on20 subjects (mean age 38.3 ± 6.9 years).

Subjects were pre-obese (body mass index: 25–30 kg/m^2) with systolic blood pressure 120-139 mmHg and high waist circumference (80cm for females and94cfor males). Platelet activation and aggregation markers were assessed by flow cytometer at baseline and after 4 weeks of regular 100 ml/day placebo consumption. Additionally, anthropometric measurements and analyses of blood pressure, blood glucose, lipid profile and liver enzymes were performed.

The final placebo formulation was chosen as the most similar to Aronia juice with regard taste, appearance, etc… Consumption of the placebo beverage did not cause any significant changes in platelet reactivity and aggregation, or in several cardiovascular and safety markers that were assessed.

In conclusion, a formulation that is suitable for use as a placebo for dietary intervention studies using Aronia juice has been developed and demonstrated to be well tolerated and have no significant effects on platelet function.

Financial support: This study was supported by funding from the EU 7th Framework Programme (FP7/2007–2013) under agreement no 2312090 (BACCHUS) and Nutrika d.o.o.,Belgrade, Serbia.

P1045

Chemistry of Red Wine Aging: Implications for Health and Biologic Activity

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Polyphenols are a large and complex group of bioactive compounds, responsible for characteristics that effect color and quality of red wines. These include proanthocyanidins, flavanols, anthocyanins, phenolic acids and stillbenes, such as resveratrol. Red wine is one of the richest sources of polyphenols in the human diet. Moderate drinkers will consume polyphenols at levels well above the population average. These compounds are potent radical scavengers and antioxidants and been associated with the prevention of age-related disorders such as cancers, neurodegenerative diseases, inflammation and cardiovascular disease.

The biochemical profile of red wines change with aging. It has been demonstrated that grape pigments degrade and reform into new and more stable compounds, a phenomenon occurring over weeks, months, and years.

We studied five red wines from vineyards in Napa, Sonoma, and Barolo, from 20 + years of age up to current barrel samples. Analysis was performed by an accredited wine laboratory (ETS Labs, Healdsburg, CA). Total anthocyanin content declined during aging including monomeric anthocyanins and even polymeric anthocyanins. Malvidin-3-glycoside, caftaric acid, quercetin significantly declined. Gallic acid, hydrolyzed from tannin over time, showed a non-significant increase with aging. Antioxidant capacity was unchanged by aging.

Slow oxidation of red wine, in the bottle, leads to polymerization of phenolics. The phenolics are not lost, but combine to form new compounds. As these polymers are derived compounds, it is not clear what biologic activity they might have that may be different from the "natural" compounds. Observed physiology may be effected by wine aging.

Financial supports : Wine specimens were donated by Fratelli Borgogno (Barolo), Kunde Family (Sonoma), Turley Wines (Napa), Biale Vineyards (Napa), Truchard Vineyards (Carneros). Chemical analysis donated by ETS Labs (Healdsburg). No other financial support reported.

P1046
Phytoprostanes fingerprinting is different depending on viticulture and enological process in must and wine

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Mediterranean diet is known as the gold-standard diet for health maintenance. Wine is a distinctive component of the Mediterranean diet, and one of the most consumed alcoholic beverages in Spain. Wine, especially the red wine, has demonstrated several benefits for health maintenance. In the last few years, many bioactive compounds have been supposed to exert these beneficial effects. One group of potential anti-inflammatory compounds is phytoprostanes (PhytoPs), that is to say, oxidative degradation products of linolenic acid. The aim of the present paper/trial/study was to measure, for the first time, PhytoPs content in wine and must. A UHPLC-QqQ-MS/MS method after SPE extraction was used for determining these compounds. Data showed two predominant PhytoPs classes, F_1-PhytoPs and D_1-PhytoPs series. In wines, total PhytoPs content ranged from 134.1±2.3 ng mL^{-1} to 216.2±3.06 ng mL^{-1}. Musts showed concentration between 21.4±0.8 ng mL^{-1} and 447.1±15.8 ng mL^{-1}. Both vinification and aging procedures for the production of wine seem to influence the final content of red wine and to modify the PhytoPs profile. Large concentration observed in wines and musts and previous reports on inflammatory effects of PhytoPs developed by in vitro cell models make interesting further research on the PhytoPs benefits.

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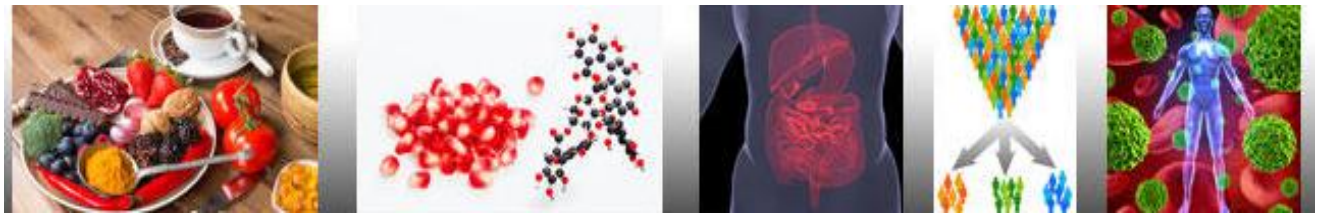
SATELLITE SYMPOSIUM OF THE 7th ICPH

1st POSITIVe SCIENTIFIC WORKSHOP

October 26th – 27th, 2015, Tours (FRANCE)



cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

POSITIVe

POSITIVe COST Action FA1403

INTRODUCTION

COST Action FA1403 (2014-2018)
Interindividual variation in response to consumption of plant food bioactives and determinants involved (POSITIVe)

POSITIVE is a European COST network in the field of Food & Agriculture that addresses inter-individual variation in bioavailability and physiological responses (bioactivity) to consumption of plant food bioactives in relation to cardio-metabolic outcomes. This Action is chaired by Christine Morand and co-chaired by Francisco Tomás Barberán.
Increasing the consumption of plant foods rich in a range of compounds with potential beneficial effects ('bioactive compounds') is an important strategy to fight chronic diseases, particularly for the prevention of cardiovascular and metabolic diseases. However, the translation of preclinical results into human clinical trials has so far proven challenging mostly due to the large heterogeneity in individuals' responsiveness to the intake of these compounds. POSITIVe specifically addresses this issue through a collaborative approach in which European top-level experts in human nutrition and plant food bioactives, in cutting-edge technologies (metagenomics, nutrigenetics, nutrigenomics, metabolomics) and in forefront research fields (personalised nutrition, human gut microbiota) together with experts from regulatory agencies and representatives from Agro-food industry join efforts to tackle: 1) the variability in human bioavailability and capacity to metabolize plant food bioactive compounds, in general, and of various groups of polyphenols, in particular (working group, WG1); 2) the variability in the response (bioactivity) of humans to the intake of these compounds (WG2) and 3) the best ways to address these points in future research projects and the potential applications for the scientific community, stakeholders and consumers (WG3). The Action also works and cooperates in the dissemination of results and information raised by POSITIVe for different end-users (Focus group, FG).
Detailed information about POSITIVe organization and activity can be found at http://www6.inra.fr/cost-positive

ORGANIZING COMMITTEE OF 1st POSITIVe WORKSHOP

- Dr. Christine MORAND - **Chair** (INRA - Clermont-Ferrand, France)
- Pr. Francisco TOMAS-BARBERAN - **Vice-Chair** (CSIC - Spain)
- Dr. Maria-Teresa GARCIA-CONESA (CEBAS-CSIC - Spain)
- Dr. Claudine MANACH (INRA - Clermont-Ferrand, France)
- Dr. Dragan MILENKOVIC (INRA - Clermont-Ferrand, France)
- Dr. Laurent-Emmanuel MONFOULET (INRA - Clermont-Ferrand, France)
- Vanessa BOISSERY (INRA - Clermont-Ferrand, France)

Acknowledgement
This booklet is based upon work from COST Action POSITIVe (FA1403) supported by COST (European Cooperation in Science and Technology).

COST (European Cooperation in Science and Technology) is a pan-European intergovernmental framework. Its mission is to enable break-through scientific and technological developments leading to new concepts and products and thereby contribute to strengthening Europe´s research and innovation capacities.
Detailed information about the COST office can be found at http://www.cost.eu/

COST is supported by the EU Framework Programme Horizon 2020

PROGRAM

Monday, 26th October 2015	
3:00 – 3:15 p.m.	Introduction of the COST Action POSITIVe **Dr. Christine Morand** (INRA-Clermont –Ferrand, France)
3:15 – 4:15 p.m.	Interindividual differences in polyphenols gut microbiota metabolism. Can they affect human health? **Prof. F.A. Tomás-Barberán** (CEBAS-CSIC, Murcia, Spain) Single nucleotide polymorphisms associated with carotenoid bioavailability **Dr. Patrick Borel** (INRA, Marseille, France)
4:15 – 4:30 p.m.	*Coffee Break*
4:30 – 6:00 p.m.	Differences in vascular response to cocoa flavanol intake according to age and sex **Dr. Ana Rodríguez-Mateos** (University of Dusseldorf, Germany*)* From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition? **Prof. Wim Vanden Berghe** (University of Antwerp, Belgium) Genetic polymorphisms and response to polyphenols/ Mediterranean diet **Prof. Dolores Corella** (University of Valencia, Spain)
Tuesday, 27th October 2015	
9:00 – 10:45 a.m.	Interindividual differences in metabolism of plant food bioactives: impact on dietary recommendations **Prof. Joanna Lampe** (University of Washington, Seattle, USA) From pathways to networks: A cofactor-protein interactome as a model for plant bioactives **Dr. Jim kaput** (Nestle Institute of Health Science, Switzerland) Lessons from Food4Me about opportunities and challenges for personalized nutrition **Dr. Eileen Gibney** (University College Dublin, Ireland)
10:45 – 11:00 a.m.	*Coffee Break*
11:00 – 12:00 a.m.	Round table with all speakers “The future for plant food bioactives in personalized nutrition” *Chairman*: **Prof. F. Tomás-Barberán**

LIST OF SPEAKERS

Prof. Francisco TOMÁS BARBERÁN - Co-Chair of the POSITIVe COST Action
Department of Food Science & Technology, CEBAS-CSIC, Murcia, SPAIN

Prof. Francisco Tomás-Barberán got his PhD in Pharmacy at the University of Valencia. He has carried out research in laboratories from England (Reading), Switzerland (Lausanne), France (Lyon), and the USA (Davis) and is co-author of more than 300 publications in scientific journals in the areas of Phytochemistry, Agricultural Chemistry, and Food Science and Nutrition. These articles have been cited over 12000 times. His main interest is deciphering the role of phenolic phytochemicals on food quality and health. His current research aims to the identification of those food constituents that provide health benefits, the mechanisms by which they exert their effects, their bioavailability and efficacy in humans and the role of gut microbiota on polyphenols metabolism and interindividual variability. His research has also been concerned with the transference of scientific results to industry and he has registered 6 patents of which 3 have been licensed and derived products are actually in the market.

Dr. Patrick BOREL - WG1 member of the POSITIVe COST Action
INRA, Faculty of Medicine de la Timone, Marseille, FRANCE

Dr. Patrick Borel obtained his PhD in Molecular Biology at Marseille University in 1988 and since he has published various highly cited papers on the metabolism of carotenoids and vitamin E in humans and on factors that modulate their bioavailability. He works for INRA in a join research unit with INSERM (the French national institute of health and medical research) and Aix-Marseille University. The research unit, NORT ("Nutrition, Obesity and Risk of Thrombosis") is located at the Faculty of Medicine de la Timone in Marseille (France). Since 2002, Dr Borel leads the research group "Bioavailability of fat soluble micronutrients" composed of various experts on lipid micronutrients, postprandial lipid metabolism, cellular and molecular biology and genetics. He is member of the scientist board of the nutrition department of INRA and vice-chairman of the SFVB (Société Francophone Vitamines & Biofacteurs ; French society on Vitamins). In 2002 he won the research award on vitamins research from the CEIV (Centre d'Etudes et d'Information sur les Vitamines; the French centre for information on vitamins). He is currently focused on the identification of intestinal proteins involved in the absorption of these compounds and has carried out a clinical trial showing the inter-individual variability in carotenoid and vitamin E bioavailability in association with combinations of single nucleotide polymorphisms.

Dr. Ana RODRÍGUEZ MATEOS – leader WG2 of the POSITIVe COST Action
Division of Cardiology, Pulmonology & Vascular Medicine, University of Dusseldorf, GERMANY

Dr. Ana Rodríguez Mateos received her PhD and conducted her postdoctoral studies at the Department of Food and Nutritional Sciences of the University of Reading in the UK, where she began to investigate the absorption, metabolism and excretion of dietary flavonoids and their impact on vascular function in Professor Jeremy Spencer's research group. Her research included development and validation of analytical methods for the identification and quantification of polyphenol metabolites in biological fluids using liquid-chromatography and mass spectrometry; design and undertaking of randomized controlled trials investigating the effects of polyphenol-rich foods on cardiovascular function and investigations on mechanisms of action using animal and cell models. Currently, she works as a research leader at the Univ. of Dusseldorf and her main research interests include investigating the factors affecting the bioavailability and bioactivity of dietary polyphenols, such as food matrix, processing, age or sex, and their mechanisms of action in the vascular system.

Prof. Wim VANDEN BERGHE - WG2 member of the POSITIVe COST Action
University of Antwerp & University of Ghent, BELGIUM

Prof. Wim Vanden Berghe (Univ. Antwerp, Belgium) holds a PhD degree in Chemistry-Biotechnology from the University of Ghent (LEGEST, UGent, Belgium). He received postdoctoral training at the Nuclear Signalling Lab of Prof. Mahadevan (Oxford, UK, 2000) and at the Department of Biochemistry lab of Prof. Hapgood & Prof. Louw (Stellenbosch, South Africa, 2001). Currently, he is professor of Epigenetic Signalling (PPES) and his research focuses on crosstalk of kinases and hormone signalling with epigenetic reprogramming in cancer-inflammation, CVDs and neuroplasticity in response to medicinal phytochemicals.

Prof. Dolores CORELLA - Invited speaker
Department of Preventive Medicine & Public Health, University of Valencia, SPAIN

Dr. Dolores Corella is a full Professor of Preventive Medicine and Public Health since 2009. She has also been Director of the Genetic and Molecular Epidemiology Laboratory since its creation in 1998. Since 2003, Dr. Corella has participated in the PREDIMED Study (PREvention with DIEt from the MEDiterranean sea) as principal investigator and from 2006, in the Physiopathology of Obesity and Nutrition department (CIBER). Her studies have generated more than 270 papers in peer-reviewed journals. Dr. Corella's interests are focused on the study of genetic and epigenetic determinants of cardiovascular diseases, diabetes, obesity and other cardiovascular risk factors. She has developed research methodology for analysing gene-environment interactions. Within the gene-environment interaction study, gene-diet interactions have constituted the main research line giving rise to the development of Nutritional Genomics. She has collaborated with Dr. Ordovás at the Human Nutrition Research Centre in Boston in various studies focused on the analyses of gene-diet interactions including the Framingham, GOLDN, PREDIMED and BPRHS studies. Currently, she is also studying how to integrate 'omic' technologies (genomics, epigenomics, transcriptomics, etc) into the field of the Mediterranean diet and obesity and cardiovascular-related diseases.

Prof. Joanna LAMPE - Invited speaker
University of Washington, Seattle, USA

Prof. Joanna Lampe is a Full Member and Associate Division Director in the Public Health Sciences Division at Fred Hutchinson Cancer Research Centre and a Research Professor in the Department of Epidemiology at the University of Washington in Seattle, USA. She received her PhD in nutritional sciences, with a minor in biochemistry, from the University of Minnesota and trained as a post-doctoral fellow in epidemiology at the University of Minnesota before joining the faculty at Fred Hutchinson Cancer Research Centre in 1994. Dr. Lampe's research focuses on the effect of diet constituents on cancer susceptibility in humans and the effects of genetic variation on response to diet. Her group uses controlled dietary interventions to evaluate cancer biomarker-response to diet and specific phytochemicals. In addition, her group studies the modifying effects of the gut microbiome on diet and disease risk. Dr. Lampe's research has been supported by the US National Cancer Institute for the past 15 years and she has published over 200 papers related to diet and human health. In 2014, Dr. Lampe received the American Society for Nutrition's Mary Swartz Rose Senior Investigator Award for research on the safety and efficacy of bioactive compounds for human health.

Dr. Jim KAPUT - WG2 member of the POSITIVe COST Action
Nestle Institute of health Sciences, Lausanne, SWITZERLAND

Dr. Jim Kaput currently works as the Head of the Clinical Translation Unit at Nestle. He's got his PhD at the University of Colorado in Biochemistry and Molecular Biology and is an expert in Translational Genomics and Personalized Nutrition for Health Care. His research focuses on human genetics molecular studies and association with the diet.

Dr. Eileen GIBNEY – Co-leader WG2 of the POSITIVe COST Action
Institute of Food and Health, Dublin, IRELAND

Dr. Eileen Gibney is a lecturer in Human Nutrition at the University College of Dublin. She is an active member of the UCD Institute of Food and Health, and a Registered Nutritionist. She graduated with a degree in human nutrition from the University of Ulster at Coleraine, she then obtained her PhD from the Dunn Nutrition Unit, University of Cambridge in 2001. Her current research interest lie in the molecular aspect of nutrition and disease, an area of nutrition research called Personalised Nutrition. Dr Gibney has successfully obtained funding for several research projects including 'Examination of the effect of genotype (PTC/PROP) on fruit and vegetable intake in children' (www.ucd.ie/foodandhealth/projects/geneticsofhealthyeating/) and more recently is involved in both the National Adult and Nutrition Survey (NANS) and National Phenotype Database (www.ucd.ie/JINGO) in Ireland. Eileen is a PI on the FP7 funded food4me project (www.food4me.org), which examined opportunities and barriers to personalised nutrition.