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**Fermented dairy products in the context of digestive cancer : the case of [*Propionibacterium freudenreichii*], a cheese starter bacterium with pro-apoptotic properties**

Fabien Cousin, Catherine Brenner, Sandrine Jouan-Lanhouet, Dominique Lagadic-Gossmann, Laurent Corcos, Sylvie Rabet, Marie-Thérèse Dimanche-Boitrel, Gwénaél Jan

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DOSSIER PARTICIPANT



INTERNATIONAL SYMPOSIUM

# Nutrition and cancer:

## Hot topics from biology to public health issues

JUNE 20<sup>TH</sup> 2013 | MAISON DE L'UNESCO | PARIS

## COMITÉ SCIENTIFIQUE

- Marie-Paule VASSON (Centre Jean-Perrin, Université Auvergne, Clermont-Ferrand)
- Pierre SENESSE (CRLC Val d'Aurelle, Montpellier)
- Isabelle ROMIEU (CIRC, Lyon)
- Denis CORPET (École Nationale Vétérinaire, Toulouse)
- Matthieu DUBOYS de LABARRE (Centre Émile Durkheim, Bordeaux)
- Serge HERCBERG (UREN- Université Paris 13, Bobigny)
- Christian BOITARD (ITMO Circulation, Métabolisme, Nutrition, Paris)
- Gérard LASFARGUES (ANSES, Maisons-Alfort)
- Paule LATINO-MARTEL (UREN, Réseau NACRe, Jouy-en-Josas)
- Carla ESTAQUIO (INCa, Boulogne-Billancourt)
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- Alexandra GOLAY (INCa, Boulogne-Billancourt)
- Myriam BILLACOIS (INCa, Boulogne-Billancourt)

# PROGRAM

8.30 am Registration and welcome coffee

## 9.00 am Symposium opening

- ▶ **PR AGNÈS BUZYN**  
President of the french National Cancer Institute

## SESSION 1 Dairy products

**Moderator:** **DR GÉRARD LASFARGUES**, ANSES, Maisons Alfort, France

- ▶ **DR MARIE-CHRISTINE BOUTRON-RUAULT**  
“Dairy products and cancer risk : fears and evidence”  
Equipe Nutrition, hormones et santé des femmes-UMR 1018, IGR, Villejuif, France
- ▶ **DR GWÉNAËL JAN**  
“Fermented dairy products in the context of digestive cancer: the case of *Propionibacterium freudenreichii*, a cheese starter bacterium with pro-apoptotic properties”  
INRA, UMR1253 Science et Technologie du Lait et de l'Œuf, Rennes, France.
- ▶ **OCÉANE MARTIN**  
“Calcium and  $\alpha$ -Tocopherol Suppress Cured Meat Promotion of Chemically-Induced Colon Carcinogenesis in Rats and Reduce Associated Biomarkers in Human Volunteers”  
INRA-Toxalim-E9, ENVT,Toulouse, France
- ▶ **DR TERESA NORAT**  
” Intake of dairy products calcium and prostate cancer risk”  
Department of Epidemiology and Biostatistics, School of Public Health, Imperial College, Londres, Grande-Bretagne

## SESSION 2 Obesity

**Moderator:** **DR ISABELLE ROMIEU**, International Agency for Research on Cancer (IARC),Lyon, France

- ▶ **PR ELIO RIBOLI**  
Director of the School of Public Health Imperial College, Londres, Grande-Bretagne
- ▶ **PR YVES JEAN BIGNON**  
“Long term improved weight control by a 2-week group physical and educational intervention shortly after breast cancer chemotherapy completion. Results of the “PACThe” randomized clinical trial of 251 patients”  
Comprehensive Anticancer Centre Jean Perrin, Clermont-Ferrand, France
- ▶ **DR VÉRONIQUE CHAJES**  
“Association between plasma phospholipid fatty acid profile and body mass index: results from the EPIC-PANACEA study”  
International Agency for Research on Cancer (IARC), Nutrition and Metabolism Section (NME), Nutritional Epidemiology Group (NEP), Lyon, France
- ▶ **PR JEAN-MICHEL OPPERT**  
“ACTI-Cités: Physical activity, active transport and urban environment”  
Human Nutrition Research Center Ile-de-France (CRNH-IdF), University Pierre et Marie Curie-Paris 6, Paris, France

## POSTERS SESSION

Lunch

## SESSION 3 Chemotherapy and metabolism

**Moderator:** **PR PIERRE SENESSE**, Institut du Cancer de Montpellier, Montpellier, France

- ▶ **PR VICKIE BARACOS**  
“Using nutritional assessment and nutrition therapy to optimize the success of systemic antineoplastic therapy”  
Palliative Care Medicine - Department of Oncology University of Alberta, Edmonton, Canada
- ▶ **DR LI XIAO-MEI**  
“Cancer inhibition through circadian programming of liver and tumor metabolism with meal timing”  
INSERM UMRS 776 " Rythmes biologiques et cancers" et Université Paris XI, Villejuif, France
- ▶ **WANNOUS RAMEZ**  
“PPAR $\beta$  mRNA expression, reduced by n-3 PUFA diet in mammary tumor, controls breast cancer cell proliferation”  
INSERM UMR1069 (N2C), Nutrition, Croissance et Cancer, Faculté de Médecine, Tours, France

## SESSION 4 Alcohol (societal aspects)

**Moderator:** **DR MATTHIEU DUBOYS DE LABARRE**, Centre Emile Durkheim, Bordeaux, France

- ▶ **DR PIERRE ARWIDSON**  
“Alcohol consumption and public policy in Europe”  
INPES, Saint-Denis, France
- ▶ **DR LOÏC LE MINOR**  
“Parenting style effect on alcohol and cannabis consumption among university students”  
GRESKO, Université de Poitiers, Poitiers, France
- ▶ **DR ANNE STOEBNER-DELBARRE**  
“Predicting health related behaviour change lessons from theories and future direction for research and practice”  
Institut du cancer de Montpellier, Pôle Prévention Epidaure, Montpellier, France
- ▶ **PR DAVID FOXCROFT**  
“ Developmental Epidemiology and Prevention”  
Department of Psychology, Social Work and Public Health Oxford Brookes University, Oxford Grande-Bretagne

## Symposium closure

- ▶ **PR FABIEN CALVO**  
Deputy General Director of the french National Cancer Institute  
Director of the Cancer Multi-Organization Institute of the french National Alliance for Life Sciences and Health (Aviesan)

## Pr Agnès BUZYN

President of the french National Cancer Institute



Agnès Buzyn, MD, PhD, is the President of the french National Cancer Institute. Previously, she was vice chair of the board of INCa.

Resident of the Paris Hospital, she spent most of her career as an hematologist at the Paris University R. Descartes – Necker Hospital, where she was Head of unit of the adult hematology and bone marrow graft intensive care from 2004 to 2011.

For the past 15 years she conducted research on tumors immunology at the Cochin Institute – Paris Descartes, where she had her own INSERM research group, and on cancer immunotherapy and anti –angiogenic therapies at the European hospital Georges Pompidou research centre. She has published more than 100 original articles, and has written more than 30 articles in books. She teaches haematology, tumor and transplant immunology.

She has also been member of various cancer societies, boards of directors and scientific boards.

# Dr Marie-Christine BOUTRON-RUAULT

Marie-Christine Boutron-Ruault is a Research Director at Inserm CESP in the team entitled “Nutrition, hormones and women’s health”. As a MD, she specialized in Internal Medicine and Gastroenterology. She was trained in Epidemiology at the London School of Hygiene and Tropical Medicine. Her career in epidemiology started as early as 1980 as a medical fellow at the Dijon University Hospital, where she has been working at a cancer registry on epidemiology of digestive cancers. She has been PI of a large case-control study on dietary factors associated with colorectal tumors, investigating the adenoma-carcinoma sequence i.e. the sequence from very benign lesions up to cancer. Later, she was the coordinator of a European intervention study for the dietary prevention of adenoma recurrence. She also investigated intermediate biological endpoints in colorectal carcinogenesis and their potential modification by pre and probiotics. She joined Françoise Clavel-Chapelon’s team in 2005, and she has been mostly working on the E3N prospective cohort of c.a. 100,000 women, and the European EPIC cohort study that includes a large part of E3N women as the French component of it. Her main focus of interest is the relationship between diet and severe chronic conditions, especially cancer, but also IBD and conditions related to ageing. She is a vice-president of the nutrition specialized expert committee at ANSES (the French food safety agency). She also still has clinical charges in the field of nutrition and obesity.

## Dairy products and cancer risk : fears and evidence

Milk and dairy products are an important part of the diet, although levels of consumption widely vary between countries. They are a major source of calcium, and they also constitute an important source of proteins. Current recommendations, especially in France, are of 2 to 3 portions per day although a higher consumption may be advised at certain periods of life because of the importance of calcium intake at those ages. Since dairy products also contain growth factors (GF), similarly to all animal tissues and fluids, and since GF are involved in several physiological mechanisms, including cell proliferation, the issue of the potential involvement of dairy products in carcinogenesis has been raised. A working group has been established at ANSES to summarize available evidence regarding this issue. Meta-analyses and recent prospective studies led us to conclude to a positive association between blood concentrations of Insulin-like growth factor 1 (IGF-1) and the incidence of three common cancers - prostate, breast (ER+ tumors) and colorectal cancers. Evidence from technological treatment of milk and dairies, from the maturation of the large bowel and from intervention studies is in favor of a low if any contribution of exogenous IGF1 from dairies to the IGF1 blood concentration. However, there is much evidence that nutritional factors, including, but not exclusively so, milk (rather than other dairies), influence the endogenous synthesis of IGF1, through mechanisms that are not fully understood, but could involve energy or some specific types of proteins. Finally, meta-analyses conclude in three opposite directions regarding the association between dairy intake and the three cancer types associated with IGF1 concentrations. Dairies seem to be positively associated with prostate cancer risk, inversely with colorectal cancer risk, and neutrally so with breast cancer risk. Thus, the involvement of exogenous IGF-1 from dairy sources in cancer risk, if any, is likely to be low. While dairy but also other nutritional factors can be associated with increased IGF1 concentrations, mostly through stimulation of endogenous IGF1 synthesis, the direct relationship between these aspects and cancer risk is spurious, and if any, would depend on the cancer site.

As a conclusion, to date, there is no evidence for modifying current recommendations of 2 to 3 dairy products per day, and in case of individual low tolerance, the alternative would be to optimize calcium intake through other sources as well as through correction of vitamin D deficiency.

## Dr Gwénaél JAN



**CURRENT RESEARCH:** Assessment of dairy propionibacteria probiotic potential  
1 - Adaptation to digestive stresses. Propionibacteria ability to adapt, survive and keep an active metabolism within the digestive tract is investigated in vitro and in vivo using an integrated approach (physiology, transcriptomics, proteomics)  
2 - Beneficial impact on the gut mucosa. The impact of propionibacteria is studied with a particular interest in colon cancer cells (modulation of proliferation/apoptosis balance) and in immune system (immunomodulation, anti-inflammatory properties).

### Professional experience

1995-1997: Post-doctoral Fellowship, molecular and cell biology, Institute for Medical Sciences, University of Aberdeen, U.K. Since 1997: Senior Scientist, INRA UMR STLO. 2011- 2014: Leader of a national research programme, National Agency for Research(ANR): Starter Surface against inflammation of the gut (SURFING)

### Fermented dairy products in the context of digestive cancer : the case of *Propionibacterium freudenreichii*, a cheese starter bacterium with pro-apoptotic properties

Fabien J. COUSIN<sup>a,b</sup>, Catherine BRENNER<sup>c,d</sup>, Sandrine JOUAN-LANHOUE<sup>e,f</sup>, Dominique LAGADIC-GOSSMANN<sup>e,f</sup>, Laurent CORCOS<sup>g</sup>, S. RABOTH, Marie-Thérèse DIMANCHE-BOITREL<sup>e,f</sup>, Gwénaél JAN<sup>a,b</sup> ; <sup>a</sup> INRA, UMR1253 Science et Technologie du Lait et de l'Œuf, F-35042 Rennes, France ; <sup>b</sup> AGROCAMPUS OUEST, UMR1253 Science et Technologie du Lait et de l'Œuf, F-35042 Rennes, France ; <sup>c</sup> INSERM, UMR-S 769, LabEx LERMIT, F- 92296 Châtenay Malabry, France ; <sup>d</sup> Université de Paris-Sud, F- 92296 Châtenay Malabry, France ; <sup>e</sup> INSERM, UMR-S 1085, IRSET, Rennes F-35043, France ; <sup>f</sup> Faculty of Pharmacy, University of Rennes 1, SFR BIOSIT, Rennes F-35043, France ; <sup>g</sup> Inserm U1078-ECLA Team, Faculty of Medicine, 22 Avenue Camille Desmoulins, 29238 Brest Cedex 3, France ; <sup>h</sup> INRA, UMR1319 Micalis, Jouy-en-Josas, France

**Background and objectives.** Dairy propionibacteria are isolated from various ecological niches, including soil, grass, silage, rumen, raw milk and dairy plants, showing great adaptability and robustness. Used as ripening starters in Swiss type cheeses, these food grade bacteria are described as nutraceutical producers and they release into the external medium short chain fatty acids (SCFA), folic acid, cobalamin and the bifidogenic 1,4-dihydroxy-2-naphthoic acid (DHNA). These compounds are known to play a pivotal role in the modulation of intestinal physiology through diet. Considering the pro-apoptotic potential of some of these metabolites, we investigated the potential of dairy products fermented by the main species, *Propionibacterium freudenreichii*, to prevent colon carcinogenesis.

**Methods.** We developed a new fermented milk. Taking advantage of milk fractions, high and stable populations of *P. freudenreichii* were obtained in this product. It was tested *in vitro* on gastric HGT-1 and colic HT-29 cells with respect to the typical hallmarks of apoptosis. *In vivo*, the fermented milk was evaluated in piglets with respect to general health parameters and in human microbiota-associated rats, treated or not with dimethylhydrazine (DMH), with respect to intestinal proliferation and apoptosis.

**Results.** Fermented milk induced cell death in both gastric and colon cancer cells. Cellular and molecular characterization of cells dying in response to SCFA treatment revealed cell cycle arrest, drop in intracellular ATP, depolarization of mitochondria, translocation of key apoptosis proteins, processing of caspases and fragmentation of the nucleus. Interestingly, the new fermented milk was shown to potentiate the cytotoxic effect of molecules used in cancer chemotherapy. In accordance, it modulated expression of key apoptotic proteins. *In vivo*, the fermented milk was evaluated in piglets. Daily oral gavage led to high colic *P. freudenreichii* populations, to modulation of the intestinal microbiota and modulation of intestinal cytokines. Promotion of piglets' growth also revealed general probiotic effects of fermented milk. Considering that propionibacteria were metabolically active in the intestine, we further investigated their impact on colon carcinogenesis (induced by dimethylhydrazine, DMH) in human microbiota-associated rats. Consumption of selected strains of propionibacteria resulted in enhanced apoptotic depletion of DMH damaged epithelial cells, yet had no effect on neither proliferation nor apoptosis in healthy rats.

**Conclusions and perspectives.** These results open new perspectives in the field of colon cancer cells prevention and/or treatment. The synergy with pro-apoptotic chemotherapy molecules suggests that such a fermented product may be proposed as a food supplement to enhance the effects of anticancer treatments.



# Océane MARTIN



PhD student on preventive strategies of colorectal carcinogenesis in production and processing of meat in the team E9 (Promotion and Prevention of Carcinogenesis by Food) in INRA ToxAlim laboratory.

## Calcium and $\alpha$ -Tocopherol Suppress Cured Meat Promotion of Chemically-Induced Colon Carcinogenesis in Rats and Reduce Associated Biomarkers in Human Volunteers

Pierre FHF, Martin O.C.B., Santarelli RL, Taché S, Naud N, Guéraud F, Meunier N, Attaix D, Venduvre JL, Mirvish SS, Kuhne GCG, Cano N, Corpet DE

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<sup>4</sup> CHU Clermont-Ferrand, Service de Nutrition, F-63000 Clermont-Ferrand, France

<sup>5</sup> Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska, 68198, USA

<sup>6</sup> Department of Food & Nutritional Sciences, University of Reading, Reading RG6 6AP, United Kingdom

**Financial support:** ANR, INRA

**Background:** Processed meat intake is associated with colorectal cancer risk. We have shown that a cured meat promotes carcinogen-induced preneoplastic lesions and increases specific biomarkers in the colon of rats.

**Objectives:** To test if this cured meat modulates these biomarkers in human volunteers, and if specific agents can suppress cured meat-promotion in rats, and associated biomarkers in rats and in humans.

**Design:** Six additives (calcium carbonate, inulin, rutin, carnosol,  $\alpha$ -tocopherol and trisodium pyrophosphate) were added to cured meat or to diet given to groups of rats for fourteen days, then fecal biomarkers were measured. Results led us to select calcium and tocopherol for next step: the cured meat, added or not with calcium or tocopherol, was given to dimethylhydrazine-initiated rats (47% meat diet for 100d), and to human volunteers in a cross-over study (180 g/d for 4d). Rat colons were scored for mucin depleted foci, putative precancer lesions. Biomarkers of nitrosation, lipoperoxidation, and cytotoxicity were measured in rats and volunteers' urine and feces.

**Results:** Cured meat increased nitroso-compounds and lipoperoxidation in humans stools (both  $P < 0.05$ ). Calcium normalized both biomarkers in rats and humans' feces, while tocopherol only decreased nitro-compounds in rats and lipoperoxidation in volunteers' feces (all  $p < 0.05$ ). Lastly, calcium and tocopherol reduced the number of mucin depleted foci per colon in rats compared with non supplemented cured meat ( $P = 0.01$ ).

**Conclusion:** The data suggest that addition of dairy foods rich in calcium to the diet, or of  $\alpha$ -tocopherol to cured meat, may suppress colorectal cancer risk associated with cured meat intake.

# Dr Teresa NORAT

Teresa Norat is Epidemiologist in the Department of Epidemiology and Biostatistics, School of Public Health, Faculty of Medicine, Imperial College London.

Her main research interest is on the relationship of nutrition and metabolic factors with the risk of chronic diseases, in particular cancer. One of her research areas is summarizing the epidemiologic evidence in order to support lifestyle recommendations for cancer prevention. In this area, she coordinates at Imperial College the project Continuous update of the scientific evidence on the relationship of diet, physical activity, obesity and cancer funded by WCRF/AICR. Teresa Norat also collaborates in the European Prospective Investigation into Nutrition and Cancer, a large prospective study in ten European countries.

## **Intake of dairy products, calcium and prostate cancer risk**

T. Norat, D. Aune, D.S.M. Chan, A.R. Vieira, D.A. Navarro Rosenblatt  
WCRF-Continuous Update Project  
School of Public Health, Imperial College London

Prostate cancer is the second most common cancer among men worldwide. To date few modifiable risk factors for prostate cancer have been firmly established. In the World Cancer Research Fund/American Institute for Cancer Research report "Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective" from 2007 it was stated that there was probable evidence that diets high in calcium increase prostate cancer risk, and limited suggestive evidence that milk and dairy products increases risk. However, no recommendation was provided for calcium and dairy intake because the evidence for prostate cancer conflicted with a decreased risk of colorectal cancer with high milk intake.

Relatively high IGF-I concentrations have been found consistently associated with an increased risk for prostate cancer and this association is evident for both localized and advanced disease. This finding supports a role for IGF-I in the development of prostate cancer, conceivably via established mitogenic and antiapoptotic mechanisms. It is also known that circulating IGF-1 concentrations can change in response to nutritional changes including energy and protein restriction, and some studies suggest that, even within well-nourished western populations, men and women with relatively high intakes of protein from dairy products have higher blood levels of IGF-1. These observations have led to the hypothesis that high intakes of dairy products might increase the risk for prostate cancer by increasing the endogenous production of IGF-1.

We summarized the evidence from 29 prospective studies on the association of milk and dairy products and prostate cancer. Overall, the relative risk of prostate cancer for the highest compared to the lowest intakes was 1.13 (95% CI: 1.03-1.24) and there was a 3% risk increase per every 200g/d increase in milk intake. The relative risk for the highest compared to the lowest intakes of cheese was 1.07 (95% CI: 1.00-1.13). Total calcium and dairy calcium intakes were also associated with increased prostate cancer risk, but no association with nondairy calcium intakes was observed. The relative risk for the highest vs the lowest intake of calcium from dairy was 1.16 (95% CI: 1.02-1.30) and the relative risk estimate for nondairy calcium was 0.91 (95% CI: 0.79-1.05).

Our results support that high intake of dairy products, milk and cheese may increase prostate cancer risk, whereas no association was observed for nondairy calcium

## Pr Elio RIBOLI



Elio Riboli received his medical degree from the State University of Milan, an MPH degree from the Postgraduate School of Hygiene and Public Health, Milan, and his Master of Science in Epidemiology from the Harvard School of Public Health, Boston.

He is currently Director of the School of Public Health of Imperial College London and Professor in Cancer Epidemiology and Prevention.

He worked from 1983 to 2005 at the International Agency for Research on Cancer where he was the Head of the Nutrition, Hormone and cancer Unit.

He started in 1988 to work at the design and implementation of large prospective cohort studies in which detailed personal data and blood samples are collected from healthy population volunteers that are subsequently followed up for years. This work led to the European Prospective Investigation on Cancer that enrolled half a million study participants in 10 European countries.

During recent years ER developed large collaborative projects to investigate the role of behavioral, metabolic, anthropometric and genetic characteristics in cancer aetiology.

Dr. Riboli has co-authored over 600 peer-reviewed publications and over 100 book chapters and books and serves on editorial boards of major journals on nutrition, cancer and epidemiology.

## Pr Yves Jean BIGNON



M.D. in medical oncology in 1984 and Ph.D. in molecular biology in 1991 at the University of Auvergne (France), geneticist at the faculty of medicine at the Comprehensive Cancer Center Jean Perrin in Clermont-Ferrand (France). Post-doctoral position at University of California of San Diego and at the Salk Institute of La Jolla (California USA). I pioneered oncogenetics in France (hereditary predisposition to cancers) in 1988. 240 papers published in peer-review journals (6,000 citations), H index at 36.

### Long term improved weight control by a 2-week group physical and educational intervention shortly after breast cancer chemotherapy completion. Results of the “PACThe” randomized clinical trial of 251 patients

BIGNON YJ<sup>1F</sup>, KWIATKOWSKI F<sup>1A,1F</sup>, MOURET-REYNIER MA<sup>1B</sup>, DUCLOS M<sup>2</sup>, LEGER-ENREILLE A<sup>1C</sup>, BRIDON F<sup>3</sup>, HAHN T<sup>4</sup>, VAN PRAAGH-DOREAU I<sup>1B</sup>, GIRONDE M<sup>1D</sup>, LECADET J<sup>5</sup>, VASSON MP<sup>1E</sup>, JOUVENCY S<sup>1E</sup>, CARDINAUD S<sup>1A</sup>, ROQUES CF<sup>6</sup>

<sup>1</sup> Comprehensive Anticancer Centre Jean Perrin, 58, rue Montalembert, 63011 Clermont-Ferrand, France (departments: 1a-clinical research, 1b-oncology, 1c-biology, 1d-psycho-oncology, 1e-nutrition, 1f-oncogenetics)

<sup>2</sup> Gabriel Montpied University Hospital, Department of Sport Medicine and Functional Explorations, Place Henri Dunant, 63000 Clermont-Ferrand, France

<sup>3</sup> Institut de Formation des Masseurs-Kinésithérapeutes, Centre Hospitalier Lacarin, 3 Boulevard Denière, 03200 Vichy, France

<sup>4</sup> Centre Nutritionnel de Vichy, 111 Boulevard des États Unis, 03200 Vichy, France

<sup>5</sup> Direction Régionale du Service Médical, 48-50 boulevard Lafayette, BP 48, 63002 Clermont-Ferrand, France

<sup>6</sup> University of Toulouse, Physical Medicine and Reeducation, 31059 Toulouse (France)

**Financial support:** AFRETH, Auvergne Regional Council, Clermont-communauté, Ligue contre le Cancer

**Background:** After cancer treatment mainly when including chemotherapy, metabolic changes induce weight gain of 2.5 to 5 kg in about half of treated women (sarcopenic weight gain): only 10% of these women are able to recover later their initial weight. This weight gain is significantly related to worse prognosis (breast cancer recurrence, chemoresistance), increased overall mortality, increased breast cancer mortality, compared to women with stable weight. Complementary interventions are therefore needed. Several strategies including group behavioral-educational interventions, dietary education and/or physical training, have been tested with moderate success on the long run.

**Methods:** 251 non metastatic women were accrued immediately after completion of radio/chemotherapy (maximum 6 months) in a prospective randomized multicenter trial between 2008 and 2010, testing a two-week intervention in SPA centers (SPA group), versus standard advices every 6 months (control group). Intervention comprised group physical training, dietary education and physiotherapy. Anthropometric measures and quality of life evaluations (SF36 questionnaire, anxiety and depression with the HAD one) were obtained before randomization and every 6 months during 3 years.

**Results:** 220 patients were evaluable at one year, 116 at two years. 60% of women were post-menopausal, 54% are overweight when entering the trial (medium BMI at 26). In control group thanks to repeated advices, weight gain is only 1% at one year and two years; on the other hand SPA group losses weight. Difference between SPA and control group curves of weight evolution is highly significant ( $p < 10^{-7}$ ) with a weight difference of 4.7% at one year and 4% at 2 years. Considering overweight women only, difference between curves is about the same ( $p = 22 \times 10^{-6}$ ). Physical activity (but not sedentary score) is also significantly increased in SPA group at one and 2 years ( $p=0.004$ ). Intervention increased SF36 score by 9.5 points ( $p=0.000006$ ), 4.6 ( $p=0.032$ ) and 6.2 ( $p=0.028$ ) respectively at 6, 12 and 24 months.

**Conclusion:** this 2-week group intervention seemed to durably influence weight control of breast cancer patients treated by chemotherapy. Differences, smaller at 24 months than at 12, suggest that a second but shorter intervention could help maintaining the 1-year benefits

## Dr Véronique CHAJES

Dr Véronique Chajès, Ph.D, Scientist in the Nutrition and Metabolism section at the International Agency for Research on Cancer, Lyon, France. Dr Chajès is a molecular epidemiologist with extensive experience with biomarkers relating to dietary fatty acids, fatty acid metabolism, and cancer development.

### **Association between plasma phospholipid fatty acid profile and body mass index: results from the EPIC-PANACEA study**

The epidemiological evidence linking estimated dietary fatty acid intakes to obesity remains controversial. The measurement of blood phospholipid fatty acids represent an alternative approach to estimate some exogenous and endogenous fatty acids. The objective of this study was to determine the association between biomarkers of fatty acids and body mass index (BMI), as an indicator of adiposity, within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Physical Activity, Nutrition, Alcohol, Cessation of Smoking, Eating Out of Home and Obesity (PANACEA) Study. The EPIC study is an on-going multi-centre study designed to investigate the relationship between diet or dietary habits, nutrition, lifestyle and environmental factors and the incidence of cancer of various sites. The total EPIC cohort includes 519,978 participants from 23 centres in 10 European countries. 373,803 subjects were recruited in 10 countries (1992-2000) in the PANACEA project of the EPIC cohort. A cross-sectional study design nested within the EPIC-PANACEA cohort was used to determine plasma fatty acid profiles in 3,003 subjects from 16 centres, for whom 24-hour dietary recalls and food frequency questionnaires were collected at baseline, and for whom blood samples were available. Plasma phospholipid fatty acid concentrations were determined through gas chromatography at IARC. Mean fatty acids (% of total fatty acids) were categorized in tertiles of body mass index (BMI) [ $<25$ ;  $25-30$ ;  $>30$  kg/m<sup>2</sup>]. A logistic regression model was used to estimate the relationship between plasma phospholipid fatty acids and BMI. Analyses were adjusted for age, alcohol, smoking, education, physical activity, and energy. A positive association was found between BMI and palmitate and stearate derived from both diet and endogenous synthesis ( $p=0.021$ ;  $p<.0001$ ; respectively), while a negative association was found with pentadecanoate and heptadecanoate derived exclusively from dairy products ( $p<.0001$ ). When considering ratios of fatty acids as putative indexes of hepatic desaturase activities, a positive association was found between BMI and the ratio of palmitoleate to palmitate [desaturation index  $\Delta-9_{-16}$ ] ( $p<.0001$ ), while a negative association was found with the ratio of arachidonate to di-homo- $\gamma$ -linolenate [desaturation index  $\Delta 5$ ] ( $p<.0001$ ). Results were similar for both men and women. Finally, similar associations were found with waist to hip ratio as an indicator of abdominal obesity. We concluded that some specific dietary fatty acids, along with an alteration of hepatic fatty acid metabolism, are associated with overall obesity. Further research is needed to explore the causality of the associations and the underlying biological plausibility.

# Pr Jean-Michel OPPERT

Prof. Jean-Michel Oppert (MD, PhD) is professor of Nutrition at Université Pierre et Marie Curie (UPMC-Paris 6), head of the Department of Nutrition at Pitié-Salpêtrière university hospital and a research associate with the UREN Nutrition Epidemiology Unit (INSERM U 557/INRA/Cnam/UP13). His current research interests include the measurement of physical activity and sedentary behaviour in health and disease and the investigation of environmental determinants of physical activity and eating habits. At national level, he was the coordinator of the multidisciplinary ELIANE (Environmental links to physical activity, nutrition and health) project (funded by ANR 2007-11). He is currently coordinator of the ACTI-Cités project, focused on active transport, urban mobility and health (funded by INCa 2011-14). He has been or is involved in EU projects centred on obesity and/or physical activity such as Nugenob (FP5), Hope (FP6), Alpha (DG Sanco), Spotlight (FP7), Metacardis (FP7). He is immediate past president of the European Association for the Study of Obesity (EASO).

## **ACTI-Cités : Physical activity, active transport and urban environment**

Jean-Michel Oppert<sup>1,2</sup>, Hélène Charreire<sup>2,3</sup>, Christophe Enaux<sup>4</sup>, Christiane Weber<sup>4</sup>, Stéphane Blanc<sup>5</sup>, Chantal Simon<sup>6,7</sup>

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**Background.** The protective role of physical activity for cancer risk is now well recognized. Promoting active transport or active mobility (walking, cycling) is a priority in physical activity promotion policies, since these activities can usually be performed all day long and at a low cost. Active mobility depends on a variety of factors including objective (degree of urbanization, land use, density of destinations...) as well as perceived attributes of the environment. There is a need to analyse relationships between active mobility and characteristics of the local environment (built, social and perceived) in a variety of geographic and cultural contexts.

**Objectives.** ACTI-Cités is a multidisciplinary project funded by INCa (2011-1-PL-SHS-10 ) with the aim : 1) to propose new tools, adapted to the French context and to web-use, to better assess active transport behaviors and urban mobility as well as the perception that individuals have of their local environment; 2) to analyse relationships between active transport, urban mobility and characteristics of the local environment (built, social, perceived), taking into account other individual and neighborhood characteristics, using data that will be collected in an ongoing French web-based cohort.

**Methods and first results.** 1) Movement recordings combining accelerometry and GPS were tested in subjects in various geographical contexts (French cities including Lyon, Paris and Strasbourg). Comparisons were performed with activity diaries and algorithms were tested to identify active transport modalities and intensity of physical activity from the combined recordings. 2) An existing physical activity questionnaire (Recent Physical Activity Questionnaire, RPAQ, Besson et al. Am J Clin Nutr 2010) was adapted to include relevant questions on active transport and urban mobility that would correspond to the French situation. A web version was designed and is currently being implemented in the NutriNet-Santé study, an ongoing web-based cohort (Hercberg et al. BMC Public Health. 2010). 3) A new questionnaire to assess perceptions of characteristics of the environment (built, social) associated with urban mobility and a new web tool to describe the perceived limits of neighborhoods were developed and are currently being implemented in the NutriNet-Santé study.

**Conclusions and perspectives.** Data obtained in this project will inform policy and decision making when planning interventions at individual (eg interventions focused on physical activity and active transport) and/or environmental (equipments, facilities, transport networks...) levels. Such data will be of interest not only to public health professionals but to community leaders, architects, urban planners... who are all facing the challenge of physical activity promotion for improving health, and in particular for preventing cancers.

## Pr Vickie BARACOS



Vickie Baracos is currently professor in the Department of Oncology at the University of Alberta and the Alberta Cancer Foundation Chair in Palliative Medicine. In 2012, the European Association of Enteral and Parenteral Nutrition accorded the achievement award [Sir David Cuthbertson award] to Dr. Baracos.

### **Using nutritional assessment and nutrition therapy to optimize the success of systemic antineoplastic therapy**

The premise of research in nutrition and oncology is to deploy nutritional assessment and nutrition therapy to optimize the success of the cancer therapy in both the short term and longer-term. Cancer treatment with chemotherapy is always a delicate balance between the efficacy and toxicity of the treatment. The objective of both the oncologist and of the nutritionist is to increase the therapeutic index of treatment [ie increase the efficacy and /or reduce the toxicity]. Cancer treatment toxicity is expected and in some individuals it is unaccountably, unusually severe. This toxicity is largely gastro intestinal and hematologic and may be severe, potentially life-threatening or even fatal. Some aspects of nutritional status including weight loss and depletion of the lean body mass [skeletal muscle] are predictive of such high risk toxicity. *The term sarcopenia was coined by Rosenberg in 1988 to denote a reduced quantity of skeletal muscle.* The generally accepted definition of sarcopenia is an absolute muscle mass >2 standard deviations below that typical of healthy adults. Sarcopenia is not restricted to people who appear thin or wasted. Aging is often paralleled by decreased muscle and increased fat, which may culminate in *sarcopenic obesity*. Our research group has developed evidence related to sarcopenia in cancer patients. Patients with sarcopenia behave as if overdosed and had toxicity of sufficient magnitude to require dose reductions, treatment delays or definitive termination of treatment. We obtained consistent evidence that patients with sarcopenia experienced more severe toxicity when treated 5-fluorouracil (5FU), capecitabine, a single agent tyrosine kinase inhibitor (TKI) (sorafenib), or chemotherapy regimen (adjuvant FEC: 5FU, epirubicin; cyclophosphamide). Mechanisms underlying drug toxicity in patients with severe muscle wasting are not well understood. One speculation is altered drug pharmacokinetics. One measure of drug exposure [area under the concentration time curve, AUC] in patients with hepatocellular carcinoma treated with sorafenib, was nearly double in patients with sarcopenia compared to that of patients without this feature (102.4 vs. 53.7 ng/mL.h). Prado *et al.* found epirubicin clearance to be linearly related to skeletal muscle mass ( $r = 0.43$ ,  $P = 0.04$ ).

A current series of findings suggest that patients with cancer may be affected by low level or deficiency of omega-3 polyunsaturated fatty acids. Some preliminary findings suggest that under conditions of dietary supplementation with fish oils or with purified to docosahexanoic acid, tumor response to chemotherapy may be enhanced providing longer disease-free and overall survival. In metastatic breast cancer patients supplemented with DHA, those patients who showed robust incorporation of this fatty acid into plasma phospholipids showed increased time to progression and overall survival compared with those patients who showed weak or total incorporation. Those promising results have led to a large randomized phase 3 and placebo-controlled clinical trial [DHALYA trial], which is ongoing. Likewise in patients with advanced stages of non-small cell lung cancer, supplementation with 2 g per day of long chain omega-3 polyunsaturated fatty acids as fish oil, was associated with a higher rate [80%] of objective response of tumor to cisplatin-based chemotherapy, compared with patients receiving standard of care [20%].

Further studies are required to accurately define how nutritional assessment can contribute to the early identification of patients at risk for severe treatment toxicity. Further understanding of the interaction between nutritional status and drug pharmacokinetics could lead to more appropriate dosing scales for malnourished individuals. The positive interaction between omega-3 fatty acids and chemotherapy to enhance treatment efficacy suggested by preliminary results, is worthy of further investigation.

## Dr Li XIAO-MEI



Xiao-Mei Li, obtained his MD in 1983 at the Faculty of Medicine, Shanghai Fudan University, and his Ph.D. in Experimental and Clinical Pharmacology in 1997 at the Paris-Sud University. It shows the possibility of pharmacological modulation of the period and amplitude of the rhythms of tolerance to anticancer agents in mice. She continues her research in the "Biological rhythms and cancers" laboratory where she specifically studies experimental chronopharmacology of several chemokines and anticancer agents *in vivo*. It highlights the value of a continuous telemetric recording of rest-activity and body temperature rhythms to identify the optimal dosing time of anticancer drugs. She shows that both circadian biomarkers also enable to characterize and quantify the effects of cancer and its treatment on the circadian system. His current research focuses on slowing down of experimental cancer progression and improving the efficacy of anticancer treatment by reinforcing the physiological and molecular organization of the circadian timing system.

### Cancer inhibition through circadian programming of liver and tumor metabolism with meal timing

Xiao-Mei Li<sup>\*1,2</sup>, Sandrine Dulong<sup>1,2</sup>, Franck Delaunay<sup>3</sup>, Michelle Teboul<sup>3</sup>, Jacques Beau<sup>1,2</sup>, Ming-Wei Wu<sup>1,2</sup>, Francis Lévi<sup>1,2</sup>  
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**Background.** Circadian disruption accelerates experimental and clinical cancer progression. Meal timing (MT) is a potential synchronizer of clock genes in mouse liver and gastro-intestinal tract and slows down tumor progression in mice on chronic jet-lag.

**Purpose.** To inhibit cancer progression through the reinforcement of host circadian and tumor molecular clocks with MT.

**Methods.** Mice bearing Glasgow osteosarcoma (GOS) or pancreatic adenocarcinoma (P03) were synchronized with LD12:12. They were fed *ad libitum* or with MT for 4 or 6h during light or darkness with normal diet. The circadian timing system was assessed through 1/ telemetered rest-activity and body temperature and 2/ tumor gene expression profiling was determined with DNA microarrays at endogenous Circadian Time (CT) 4 and CT16 and circadian liver and tumor mRNA expression was investigated using qPCR for clock genes (*Rev-erb $\alpha$* , *Per2* and *Bmal1*) and two clock-controlled temperature-sensitive stress genes (*Hspa8* and *Cirbp*), regulators of cell cycle and apoptosis.

**Results.** In GOS-bearing mice, MT during darkness doubled the circadian amplitude in host body temperature and reduced tumor growth by ~30% as compared to *ad libitum*. MT during light tripled circadian amplitude of temperature and reduced tumor growth by 62%. In P03-bearing mice, MT during light nearly doubled the circadian amplitude of body temperature and halved tumor growth as compared to *ad libitum*. While MT during light phase-advanced the rhythms in clock gene transcription by 8-12h in liver, the tumor clock gene patterns remained arrhythmic both on *ad libitum* and on MT. A circadian transcriptome study revealed that MT up- or down-regulated the expression of 423 tumor genes, according to CT. Moreover, 36 genes involved in cellular stress, cell cycle and metabolism were up-regulated at one CT and down-regulated 12-h apart. MT induced strong rhythmic transcription of *Hspa8* and *Cirbp* both in liver and tumor. The MT-induced peaks of *Hspa8* and *Cirbp* expressions respectively corresponded to peak and trough body temperature.

**Conclusion and perspective.** The reinforcement of the host circadian timing system with MT induced 24-h rhythmic expression of regulators of cell cycle and apoptosis genes in clock-deficient tumors, which translated into cancer inhibition. Such novel circadian-based supportive care deserves clinical testing for cancer prevention and therapeutics.



## Wannous RAMEZ



Ramez Wannous obtained his diploma of pharmacist at the University of Damas in 1999) with specialties in Pharmacology and pharmaceutical chemistry. He started biological studies in France in 2008 and he is currently finishing his PhD training at the Inserm UMR1069 “Nutrition, Growth and Cancer” at the university of Tours.

### **PPAR $\beta$ mRNA expression, reduced by n-3 PUFA diet in mammary tumor, controls breast cancer cell proliferation**

Ramez Wannous<sup>1</sup>, Emeline Bon<sup>1</sup>, Karine Mahéo<sup>1</sup>, Caroline Goupille<sup>1,2</sup>, Julie Chamouton<sup>1</sup>, Philippe Bougnoux<sup>1,2</sup>, Sébastien Roger<sup>1</sup>, Pierre Besson<sup>1</sup>, Stephan Chevalier<sup>1,3,4</sup>  
INSERM UMR1069 (N2C) Nutrition, Croissance et Cancer,

**Financial support:** “Region Centre” et “Ligue Nationale Contre le Cancer”

The effect of numerous anticancer drugs on breast cancer cell lines and rodent mammary tumors can be enhanced by a treatment with long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) such as docosahexaenoic acid (DHA, 22:6n-3) which is a natural ligand of peroxisome proliferator-activated receptors (PPAR). In order to identify the PPAR regulating breast cancer cell proliferation, we tested the impact of siRNA, selected to suppress PPAR $\alpha$ , PPAR $\beta$  or PPAR $\gamma$  mRNA, in MDA-MB-231 and MCF-7 breast cancer cell lines. The siPPAR $\beta$  was the most effective to inhibit breast cancer cell proliferation in both cell lines. Using PPAR $\alpha$ , PPAR $\beta$  and PPAR $\gamma$  pharmacological antagonists, we showed that PPAR $\beta$  regulated DHA-induced inhibition of proliferation in MDA-MB-231 and MCF-7 cells. In addition, the expressions of all 3 PPAR mRNA were co-regulated in both cell lines, upon treatments with siRNA or PPAR antagonists. The expression of PPAR mRNA was also examined in the NitrosoMethylUrea (NMU)-induced rat mammary tumor model. The expressions of PPAR $\alpha$  and PPAR $\beta$  mRNA were correlated in the control group but not in the n-3 PUFA group in which the expression of PPAR $\beta$  mRNA was reduced. Although the PPAR $\alpha$  expression was also increased in the n-3 PUFA-enriched diet group under docetaxel treatment, it is only the expression of PPAR $\beta$  mRNA that correlated with the regression of mammary tumors: those that most regressed displayed the lowest PPAR $\beta$  mRNA expression. Altogether, these data support the idea that PPAR $\beta$  is an important player capable of modulating other PPAR mRNA expression, under DHA diet, for inhibiting breast cancer cell proliferation and mammary tumor growth.

## Dr Pierre ARWIDSON

After studying educational innovation called problem-based learning for medical students at the University of Southern Illinois in the United States (Professor Barrows) and the University of Health Sciences Linnköping Sweden (Pr Denneberg), Pierre Arwidson has a PhD in medical education at the Faculty of Medicine of Tours in 1986. He was then in charge of educational mission and set up educational experiments with medical students in Tours.

From 1990 to 1997 he was Chief Medical Officer at the departmental committee for education of Indre-et-Loire health and the Regional Association of Cardiology Centre. In 1997, he became Deputy Head of French Studies Committee of Health Education. Since 2002 he is Director of Scientific Affairs of the National Institute for Prevention and Health Education.

### **Alcohol consumption and public policy in Europe**

To briefly introduce the session, we will examine the main findings of ECAS (European Comparative Alcohol Study, Österberg & Karlsson 2002) and Amphora (Alcohol Measures for Public Health Research Alliance Allamani 2011). This work, funded by the European Commission, was design to look the evolution of alcohol consumption in different countries and public policies. In ECAS, it was difficult to find a simple correlation between the indices trading public policies and changes in consumption. In Amphora, other factors of changes in socio-economic contexts of each country have been introduced in the models to better understand the observed phenomena. Finally, some elements of the recommendations based on evidence (Babor, 2010, Anderson 2009) public policy will be discussed.

# Dr Loïc LE MINOR

Associated Researcher, laboratory GRESCO (Group of Researches and Sociological Studies of the Center the West), University of Poitiers. Sociology PhD. Head of mission : Mission of Support Drugs and Drug addiction Poitou-Charentes and National Center of Ressources Rare Handicap - Deafblindness.

## Parenting Style Effect on Alcohol and Cannabis Consumption among University Students

Loïc Le Minor, Ludovic Gausso, Nicolas Palierne, Nathalie Gallais  
GRESCO, Poitiers University

**Financial support:** MILDT/INCa, IREB

**Background and objectives.** the question of young people's consumption of psychotropic drugs has become a burning question and hence is difficult to comprehend 'serenely' as it is trapped into another set of issues. Among the most studied ones are the issues of public health and prevention but also business, cultural, political and media issues (Peretti-Watel, Beck, Legleye, 2007). Whereas the 'public problem' would be proven, seen as new and alarming, the answer would already be known and simple: young people must drink less and especially less quickly. If there are a few voices trying to make it understood that the phenomenon does not affect the majority (far from it) of young people, or that it is not so new nor so Anglo-Saxon, a relative consensus seems however to have established itself around this question. On the issue of prevention, there is an agreement on the necessity to inform, to alert and even alarm young people, and more generally, to educate them. What is clear is that the adults, press and public health actors have developed some new sensibility and awareness towards young people's consumption: is this an altruistic concern towards others, towards the youth, or the tightening of social control? Or is it both: for their own good, do they need to be under tougher control? This is the context which surrounds this research on young people's (students) consumption and on risk and protective family factors. The key objective of this study is to deepen our initial results (Gausso and al. 2011a, 2011b) on the influence of parenting style (PS) on young people's consumption patterns, with an age span of 13-24 years.

**Methods.** PS is measured by the API test (Authoritative Parenting Index, Jackson and al. 1998) which combines the dimensions of *demandingness* and *responsiveness* and leads to the construction of four parenting styles: authoritative, authoritarian, indulgent and neglectful. The research focuses on students from Poitiers and goes beyond the use of alcohol (monitored with the AUDIT-C) to study the use of cannabis (monitored with the CAST, Cannabis Abuse Screening Test) and other psychotropic substances. Between May 2011 and May 2012, a questionnaire sent out to the students of Poitiers by email generated a sample of 2364 people, that is to say, more than 10% of the registered students of Poitiers University at the time. In order to have a more representative sample of the population, we used the variables of gender and course to adjust the sample. In addition, we conducted 60 semi-directive interviews from a 'purposive sample' created out of the questionnaire survey.

**Results.** As we have taken into account several consumed psychotropic substances, we can focus our thought on the study of both poly-consumption and its associated risks: 80% of heavy cannabis users or users at elevated risk for dependence are heavy drinkers (chronic or occasional). Conversely, nearly 20% of heavy chronic drinkers are heavy cannabis users or are at elevated risk for dependence. Very significant statistics ( $p < 0.1\%$ ) on each substance consumption - alcohol, cannabis and other drugs - prove that over-consumption affects men. The results of these tests cross-checked with that of the PS show a very strong relation. The "authoritative" and "indulgent" styles have the less marked high-risk consumption of alcohol (chronic and occasional). This result coincides with the results of the CAST test which show that the "authoritative" and "indulgent" parenting styles display the highest number of cannabis 'non-triers' and non-users, and the smallest number of heavy users or users at elevated risk for dependence. In the same way, the lowest proportions of other drugs experimenters tally the "authoritative" PS and, to a lesser extent, the "indulgent" one. The PS dimension of responsiveness seems to moderate consumption of alcohol and cannabis, and to prevent from cannabis and others drugs first trying.

**Conclusions and perspectives.** These preliminary results remind us of the importance of a behavioural approach compared to a 'substance' approach in preventive actions. They also underline gender differences. The analysis highlights the effects of gender both on the PS variations associated with the father and the mother and applied to girls and boys, and on the modalities of psychotropic substances use and their evolution. To refine these results, we will cross-check them with other variables and with an approach which will not anymore be only in terms of PS, but in terms of "responsiveness" and "demandingness" scores. The qualitative approach will shed light on the learning processes and modalities of onset of drug use, and sometimes, it will also be able to criticise some indicators of the quantitative approach. As psychotropic drugs consumption can be associated with the development of cancers (Hill, 2003) (and especially those of the upper aero-digestive tract) and can consequently even strengthen the particularly strong social health inequalities in France, a better understanding of the processes involved to maintain or reject the consumption practices can only prove beneficial when it comes to public health concerns

## Dr Anne STOEBNER-DELBARRE



MD, Addiction specialist, MSc, Epidemiologist. Anne has specialised in methods and the science of health education. Since 1988, she has been working as a researcher and project manager at Epidaure; a centre devoted to cancer prevention. She is the author of more than 120 articles, books, professionals guidelines, health education tools for youth and educators, and 2 health websites. She also works for French National Agencies (ANSM, DGS, HAS, Inca, Inserm, Inpes). She has trained more than 20 000 health or educational professionals and initiated a network with more than 113 partners to implement tobacco free schools in south of France. Her fields of interest are the use of the behavioural change theories in health education programs, and the evaluation of the impact of prevention programs on professionals and population behaviour.

### **Predicting health related behaviour change: Lessons from theories and future direction for research and practice**

Perhaps never before have there been so many demands to facilitate behaviour changes, or so many potential strategies from which to choose. Over the past two decades, research programs have been established to identify and test the most effective methods to achieve health behaviour change.

A growing body of empirical literature suggests that patient perceptions of health and threat of disease, as well as barriers in a patient's social or cultural environment, appear to influence the likelihood that a patient will engage in health-promoting or treatment behaviours, such as proper diet or nutrition, engaging in alcohol or tobacco cessation or participating in cancer screening.

Health behaviour researches have proposed different appropriate means of generic and specific intervention to support attitude and behaviour change at individual or population and community levels. Systematic reviews have shown that using theory in crafting interventions can lead to more powerful effects than interventions developed without theory. We will describe some of the main theories and give examples for cancer prevention.

These main theories could be applied in different settings. Seven major settings are usually recognised to implement contemporary health education: schools, communities, worksites, health care settings, homes, the consumer marketplace, and the communications environment. We will give examples to illustrate 3 of them.

In conclusion, the science and art of health behaviour and health education are eclectic, rapidly evolving and have experienced great progress, but mixed findings raise new questions and pose new challenges. We will discuss the importance of developing new successful strategies to improve health Behaviour taking into account an amalgamation of different strategies from social and health sciences and several practice tools of disciplines such as psychology, sociology, anthropology, communications, nursing, economics, marketing, epidemiology, statistics, and medicine.

## Pr David FOXCROFT



As Professor of Community Psychology and Public Health, my programme of work is focused on understanding (and improving) behaviour in context, especially how social structures (e.g. families, schools, communities, employers, regulation, government) can support improved health and wellbeing in communities and populations. A focus is the prevention of problem behaviours that contribute to poor health outcomes, for example alcohol misuse. I'm involved in research projects which identify promising prevention programmes; develop or adapt them for different settings and cultures; and then evaluate their effectiveness. Research methods include systematic reviews, participatory and qualitative research, and randomised controlled field trials. Underpinning this applied research, other more theoretically oriented work is exploring the relationship between individual's and their environment to better understand why people develop problem behaviours. Ideas from Ecological and Environmental Psychology are influential. The aim is to provide a more robust theoretical rationale for prevention programmes and systems.

### **Developmental Epidemiology and Prevention**

Alcohol is a major risk behaviour for non-communicable diseases (NCDs), which now account for most death and disability worldwide. Early risk and protective factors are significant determinants of problem drinking and other risk behaviours. In this talk I will discuss some of the latest research into alcohol education and prevention in schools and families. Community-oriented developmental prevention can have a significant impact on risk factors associated with drinking, but we need a better understanding of how prevention effects can vary according to moderators such as gender.

## Pr Fabien CALVO

Deputy General Director of the french National Cancer Institute

Director of the Cancer Multi-Organization Institute of the french National Alliance for Life Sciences and Health (Aviesan)



Fabien Calvo, MD PhD, Deputy General Director of the National Cancer Institute, France, has been in charge of the Research and Innovation Programmes since 2007. Fabien CALVO is also the director of the Cancer Multi-Organization Institute of the French National Alliance for Life Sciences and Health (Aviesan), which includes INSERM, CNRS, CEA, INRA, INRIA, IRD, Pasteur Institute, Universities and University hospitals.

Previously resident and senior registrar of Paris Hospitals, research associate of the National Cancer Institute in Bethesda (NIH / NCI / DCT, USA), he specialised in oncology and haematology. He is currently professor of pharmacology at the Denis Diderot Medical University in Paris.

He has been the director of the Saint-Louis Hospital CIC (clinical investigation center) in Paris and he was the director of INSERM unit 716 on the identification of new molecular targets for the treatment of cancer from 1995 to 2008. He is a board member of the post-graduate school “oncogenesis fundamental basis”. He also headed the clinical research programmes in cancer in the Paris area. He has published more than 200 original and review articles.

He also worked as a coordinator of the cancer mission for the Director of the Research and Innovation department, Ministry of Research and Higher Education in 2006 and 2007.

His spheres of activity and interest are the biology of metastatic processes, especially proteases, translational research, preclinical pharmacology and early clinical trials in haematology and oncology.

# POSTERS SESSION PROGRAM



# “Fatty acids”

## Polyunsaturated fatty acid composition of subcutaneous adipose tissue and risk of colorectal cancer: results from the AGARIC case-control study

Cottet Vanessa, Vaysse Carole, Bourredjem Abderrahmane, Ortega-Deballon Pablo, Combe Nicole, Bonithon-Kopp Claire for the AGARIC study group.

Inserm UMR 866, ITeRG, Inserm CIE 01, CHU de Dijon, Université de Bourgogne

**Financial support:** INCa, Fondation de France, Ligue contre le cancer, Conseil régional de Bourgogne

**Background and objectives.** It is admitted that 30 to 70% of sporadic forms of colorectal cancer (CRC) could be directly associated with food and nutrition. Contrasting with experimental studies, epidemiological studies regarding the relationships between dietary intake in n-3 and n-6 polyunsaturated fatty acids (PUFA) and the risk of colorectal cancer did not provide any convincing results. These discrepancies are mainly due to difficulties in the evaluation of actual dietary intake in fatty acids from dietary questionnaires. Thus, the use of biomarkers of dietary fatty acid intake and fatty acid metabolism may be an interesting approach for exploring the associations between exogenous and endogenously produced fatty acids and the cancer risk. In this respect, fatty acid composition of subcutaneous adipose tissue may be a promising alternative due to the slow turnover time so that it is generally considered as reflecting dietary intake over 1-3 years. The objective of the study was thus to investigate the associations between n-3 and n-6 PUFA content of subcutaneous adipose tissue and the risk of CRC

**Methods.** The AGARIC (Acides Gras, métabolisme du tissu Adipeux et Risque de Cancer colorectal) case-control study was carried out in 5 departments of digestive surgery from University hospitals of Eastern France. Cases were patients with newly diagnosed CRC and planned surgery with curative intent without any preoperative cancer treatment. Controls were patients with planned abdominal surgery for benign disease and no history of CRC. Both cases and controls were aged more than 45 and free of inflammatory bowel disease. From September 2008 to June 2011, we recruited 224 patients with CRC and 252 controls. During surgery, two samples (50 mg) of abdominal subcutaneous adipose tissue could be collected at the incision site among 203 cases and 223 controls and stored at  $-80^{\circ}$  until centralized analysis. The fatty acid composition of adipose tissue lipids was determined by high performance gas chromatography (on 100m-CP Sil 88 column). ORs and 95% confidence intervals for CRC in relation to individual PUFAs were estimated by multivariate logistic regression stratified on centre. PUFAs were divided into tertiles based on the distribution among controls.

**Results.** Compared to controls, CRC patients were significantly older (median age: 66.4 versus 69.6 years,  $p=0.04$ ), more frequently diabetic (13% versus 21%,  $p=0.03$ ) and had a lower body mass index (BMI) (median BMI: 25.9 versus 26.9 Kg/m<sup>2</sup>,  $p=0.05$ ). There was no gender difference between groups (57% and 61% males in controls and cases). There was a trend for more frequent family history of CRC and higher alcohol consumption in CRC patients. In both cases and controls, saturated fatty acids represented around 31% of all adipose tissue fatty acids, monounsaturated fatty acids around 55% and PUFA around 13%. PUFA were mainly represented by n-6 PUFA (around 12% of all fatty acids versus 1% for n-3 PUFA). After adjustment for age, gender, BMI, diabetes, family history of CRC and alcohol consumption, significant positive associations with CRC risk were observed in the highest versus the lowest tertiles of C20:3 (n-6) (OR:1.83, 95% CI: 1.10-3.02,  $p$  for trend=0.019), C22:4 (n-6) (OR:1.85, 95% CI: 1.13-3.05,  $p$  for trend=0.017), C22:5 (n-3) (OR:1.78, 95% CI: 1.10-2.90,  $p$  for trend=0.014) and for the ratio n-6/n-3 PUFA (OR:1.63, 95% CI: 1.02-2.58,  $p$  for trend=0.049). Significant inverse associations were observed for C18:3(n-3) (OR:0.55, 95% CI: 0.34-0.87,  $p$  for trend=0.009). No significant associations were found between CRC risk and adipose tissue content in EPA (eicosapentaenoic acid, C20:5 (n-3)), DHA (docosahexaenoic acid, C22:6 (n-3)), or CLA (conjugated linoleic acid, C18:2 9c-11t).

**Conclusions and perspectives.** These results indicate that high adipose tissue content in some n-6 PUFAs and a high ratio of n-6/n-3 PUFA are associated with a high risk of CRC whereas high levels of  $\alpha$ -linolenic acid, an essential n-3 fatty acid may be protective. This fatty acid profile may reflect both dietary pattern and altered fatty acid metabolism. Associations between adipose tissue content in SFA and MUFA, including *trans*-fatty acids remain to be thoroughly investigated.

## Association of plasma phospholipid *trans* fatty acids with risk of breast cancer in the E3N-EPIC Stud

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**Background and objectives.** The epidemiological evidence linking estimated dietary fatty acid intakes to breast cancer risk remains controversial. Among fatty acids, little is yet known about the effects of unnatural industrial *trans* fatty acids (ITFAs) and natural *trans* fatty acids from ruminant sources (RTFAs) on breast cancer risk. Epidemiological studies are limited by the assessment of dietary fat through food-frequency questionnaires, methods shown to be prone to measurement error. The measurement of blood phospholipid fatty acids represent an alternative approach to estimate exposure to fatty acids. The objective of this study was to determine the association between biomarkers of TFAs and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort .

**Methods.** We designed a case-control study nested within the French arm of the EPIC study, the E3N study, including 1,038 breast cancer cases. For each breast cancer case subject, one matched control was chosen randomly among cohort women without breast cancer. Control subjects were matched to case subjects on age, menopausal status at recruitment, recruitment center, and recruitment date. Plasma phospholipid fatty acid levels were determined through gas chromatography at the Gustave Roussy Institute and IARC. Hazard ratios (HR) and their 95 % confidence intervals (CI) were estimated using the Cox proportional regression model. Analyses were adjusted for body mass index, height, parity, education, menopausal hormone use, energy intake, physical activity, smoking, alcohol intake, family history of breast cancer and history of benign breast disease. Analyses were conducted separately on pre- and post-menopausal women. All statistical tests were two tailed.

**Results.** An increased risk of breast cancer was associated with increasing levels of ITFA isomers, as validated biomarkers of industrially processed foods, 18:1n-9*trans* (highest versus lowest tertile, OR=1.61, 95%CI=0.99-3.25, *ptrend*=0.04), and 18:2n-6*cis-trans,trans-cis,trans-trans* (OR=3.38, 95%CI=1.08-10.60, *ptrend*=0.04) in pre-menopausal women, while no significant association was found in post-menopausal women. No significant association was found between breast cancer risk and plasma phospholipid RTFA isomers from natural animal sources in pre-and post-menopausal women.

**Conclusion and perspectives.** These data suggested that a high intake of ITFAs may increase the risk of pre-menopausal breast cancer, while RTFAs had no effect on breast cancer risk. The extension of the French study to the the large EPIC cohort with a wide range of fat intake will greatly enhance scientific awareness of the link between ITFAs and breast cancer risk.

## Erythrocyte membrane phospholipid fatty acid concentrations and risk of colorectal adenomas in the French E3N-EPIC cohort study

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**Background and objectives.** Although dietary fatty acids may influence colorectal carcinogenesis, few studies have examined the association between fatty acids and colorectal adenoma risk. We assessed the association between biomarkers of dietary fatty acids, metabolism of fatty acids, and the risk of colorectal adenomas in women in a nested case-control study from the French E3N-EPIC cohort.

**Methods.** Among 19,934 women who completed a diet history questionnaire and provided blood samples, we identified 328 cases of adenomatous polyp during an average of 6.6-year follow-up. We randomly matched to cases, 619 polyp-free colonoscopy controls according to center, date, and age at blood collection. Erythrocyte membrane phospholipid fatty acid concentrations were determined by gas chromatography. Adjusted odds ratios for risk of colorectal adenomas with increasing concentrations of fatty acids were calculated using conditional logistic regressions, separately for advanced and non-advanced adenomas.

**Results.** Associations were stronger with advanced than non-advanced adenomas. High concentration of pentadecanoate+heptadecanoate acids were inversely associated with risk of advanced adenomas (highest vs. lowest tertile: OR<sub>T3vsT1</sub>=0.40 [95% Confidence Interval 0.20-0.79] p-trend=0.009). Oleic acid was associated with an increased risk of advanced adenomas (OR<sub>T3vsT1</sub>=2.32 [1.16-4.64] p-trend=0.018). Some polyunsaturated fatty acids were significantly associated with the risk of advanced adenomas, positively for di-homo- $\gamma$ -linolenate, (OR<sub>T3vsT1</sub>=2.07 [1.15-3.72] p-trend=0.013), and negatively for docosapentaenoic and docosahexaenoic acids (OR<sub>T3vsT1</sub>=0.50 [0.27-0.93] p-trend=0.044, and OR<sub>T3vsT1</sub>=0.50 [0.26-0.96] p-trend=0.028, respectively).

**Conclusion and perspectives.** Our results suggest that a specific prediagnostic erythrocyte membrane phospholipid fatty acid profile, presumably reflecting both a complex dietary pattern and altered fatty acid metabolism, is associated with advanced colorectal adenoma risk.

## Adipose microenvironment modifies breast cancer cell proliferation and angiogenesis, particularly in case of obesity

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**Background and objectives.** In postmenopausal women, obesity is a recognized breast cancer risk factor. In addition, tumor microenvironment and particularly adipose tissue seems to be involved in mammary carcinogenesis. Indeed, breast cancer cells are surrounded by stromal cells, such as fibroblasts, endothelial, inflammatory cells and adipose tissue. This tissue is able to secrete numbers of molecules, e.g. adipokines, including leptin and adiponectin, those circulating levels are modified in obese patients. So, our objective was to characterize the impact of adipocyte secretions on tumor growth and angiogenesis, particularly in overweight situation.

**Methods and Results.** First, to characterize the *role of the tumor microenvironment and its secretions*, we developed an innovative *tridimensional model culture* consisting of a reconstructed fatty skin equivalent permitted to mimic the interface between breast tumor cells and their environment, particularly adipose tissue. In this model, fibroblasts and preadipocytes were deposited on a dermal substrate. After 3 weeks of culture, a fatty equivalent dermis was obtained and MCF7 mammary cancer cells were then deposited on the dermis in order to obtain the reconstructed fatty skin equivalent. At the end of the experimentation, MCF7 cells (n=3) were separated from the dermis with a thermolysin treatment to extract RNA. The expression of 31 key genes was investigated by qRT-PCR (StepOne™, Applied Biosystems) and compared to the expression of MCF7 cells cultured in normal conditions. Tumor microenvironment induced several transcriptional programs involved in promoting tumorigenesis compared to cells cultured alone. Concerning cell proliferation, the induction of Cyclin D1 (9-fold) and MAPK (11-fold) was observed. The anti-apoptotic Bcl2 gene was importantly upregulated (68-fold) in the same way that invasive potential (MMP9: 3-fold; VEGF: 15-fold). Key changes also involved the induction of tumor suppressor genes such as BRCA1 (7-fold), CDH1 (13-fold), and oncogenes (MYC: 7-fold). The expression of genes involved in hormonal pathways was modified with an upregulation of estrogen receptor (16-fold) and progesterone receptor (2.5-fold). Second, we wanted to characterize the *impact of adipocyte secretions from thin or obese women on tumor growth and angiogenesis*. For that, mature adipocytes (MA) obtained from lean (MA20) or obese women (MA30) were cultured and the effects of MA supernatants (Sn, Sn20 and Sn30 respectively) were evaluated *in vitro* on the proliferation of MCF-7 breast cancer cells (fluorescent resazurin test) and on different steps of angiogenesis (proliferation, migration and endothelial tube formation by endothelial HUVEC cells). The influence of Sn obtained from co-cultures between MA20 or MA30 and breast cancer cells MCF-7 (respectively Sn7/20 and Sn7/30) was also investigated on angiogenic process. Statistical analyses were realized using paired t-test (Statview). We found an increased MCF-7 cell proliferation which was more pronounced in the presence of Sn30 (50% vs Control (T), p <0.001) compared to Sn20. Angiogenesis was also stimulated, in case of obesity, since Sn30 increased proliferation (53% vs T, p<0.001), migration (63% vs T, p<0.001) of HUVEC and their endothelial tube formation. The effect of Sn7/20 and Sn7/30 was even more pronounced on all experimentations.

**Conclusions and perspectives.** Our results highlighted the influence of the microenvironment-secreted factors on 1) the proliferation of mammary cancer cells and on endothelial HUVEC cells, and 2) on different steps of the angiogenesis events. By developing a new tridimensional model, we found that the mammary microenvironment was able to completely modified the transcriptional programs of mammary cancer cells by increasing the expression of genes involved in cell proliferation (*cyclinD1*, *MAPK*), angiogenesis (*MMP9*, *VEGF*) and hormonal pathways. We also demonstrated that the adipose microenvironment could promote increased angiogenic and proliferative capacities, and that these processes were more pronounced in case of obesity. Our major perspective is now to identify the major components involved in the induction of cell proliferation and to evaluate the role of the menopausal status.

## Paracrine role of adipose tissue in tumor progression : a link between cancer and obesity

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Overweight and obesity are now established risk factors for cancer and cancer-related mortality. Noteworthy, obesity is not only associated to increased incidence of breast cancer, but is also an independent negative prognosis factor independently of menopause status. Multiple factors might contribute to the poorer survival rates in obese patients with breast cancer including a higher likelihood of co-morbid conditions and other “non-biological” effects. However, there is now clear evidences that host factors contribute to the occurrence in obese women of tumors exhibiting at diagnosis an aggressive biology defined by an increase in lymph nodes involvement and a higher propensity to distant metastasis. Cells immediately adjacent to a tumor are not only passive structural elements but also active actors in tumor progression. Among the many different cell types surrounding breast cancer cells, the most abundant are those that compose mammary adipose tissue (MAT), mainly mature adipocytes and progenitors (preadipocytes and Adipose-Derived Stem cells [ADSCs]). A paracrine role of adipocytes in stimulating tumour progression is an attractive hypothesis. In fact, aside from its energy-storing function, adipocyte is also an active endocrine cell that secretes a large variety of molecules (termed adipokines), including hormones, growth factors, chemokines or pro-inflammatory molecules. Adipocytes are therefore excellent candidates to influence tumor behavior through heterotypic signaling processes and might prove to be critical for tumor survival, growth and metastasis. The crosstalk between the adipose and epithelial tumour components might be positively affected in obesity where the normal balance of these adipose tissue secretory proteins is perturbed. The goal of our study was to determine the role of mature adipocytes in breast cancer progression and the molecular mechanisms involved.

**Methods.** To answer this question, we set up an original 2D coculture system where breast cancer cells were cocultivated either with murine or human mammary adipocytes. The tumor cell behavior was compared between non cocultivated and cocultivated cells. Results were confirmed *in vivo* using murine models of metastasis and in human breast tumor samples.

**Results.** Using this 2D coculture model, we extensively described tumor-induced changes in adipocytes that are reproducibly displayed by both mouse and human mammary adipocytes. Adipocytes cocultivated with various human and murine breast cancer cell lines generally exhibit a loss of lipid content, a decrease in late adipose markers expression, and over-expression of inflammatory cytokines (such as IL-6 and IL-1 $\beta$ ) and proteases (such as MMP-11 and PAI-1). We named these tumor-surrounding adipocytes, “Cancer-Associated Adipocytes” (CAAs). Equally important, using immunohistochemistry and quantitative PCR, we confirmed the presence of these modified adipocytes at the invasive front human breast tumors. What are the effects of these CAAs on tumor progression? All murine and human tumor cells cocultivated with mature adipocytes exhibited increased invasive, but not proliferative, capacities *in vitro* and *in vivo*. In the case of IL-6, we further showed that it plays a key role in the acquired pro-invasive effect, and associated EMT, by tumor cells. Interestingly, the human breast tumors of larger size and/or with lymph nodes involvement exhibit the higher levels of IL-6 in tumor surrounding adipocytes. Additional and unpublished results obtained in our laboratories demonstrate that cancer cells may “dedifferentiate” mature adipocytes into fibroblast-like cells that in turns participate to the desmoplastic reaction of breast cancers. Finally, we demonstrated that a lipid transfer exists between cancer cells and tumor-surrounding adipocytes enabling a metabolic crosstalk between these two populations.

**Conclusions and Perspectives.** Taken together, all these data strongly support the concept that an intimate crosstalk is established between cancer cells and mature adipocytes, whose relevance has been clearly demonstrated in human tumors. In breast human tumors, the association of high levels of IL-6 in CAAs with high tumor size and enhanced local invasion reflect those traits present in obese patients. Therefore, it is tempting to speculate that CAAs within a context of obesity should be more prone to amplify the negative crosstalk with tumor cells. Experiments are ongoing in our laboratories to demonstrate this hypothesis using original 3D coculture system with primary isolated adipocytes from lean and obese subjects, xenografts in mouse models of obesity and human breast cancer annotated collections.

# “Obesity”

## Prevalence of metabolic syndrome in women diagnosed with breast cancer

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**Background and objectives.** The relationship between obesity and cancer mortality is actually debated. The link between body-mass and cancer could be dependent to insulin resistance associated with central obesity, possible factors included in so-called metabolic-syndrome. For breast cancer, obesity is associated with an increased incidence of mortality in post-menopausal women. Therefore, it is important to evaluate the prevalence of metabolic syndrome in women diagnosed with breast cancer.

**Methods.** The prevalence of metabolic syndrome was performed in 70 caucasian women newly diagnosed for hormone dependent non-metastatic breast cancer, before surgery. Metabolic syndrome was diagnosed according the criteria set out by the International Diabetes Federation (IDF) and also according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III).

**Results.** In the studied population, the mean age was 60,6 years [range: 39,6-85,3 years]. Performance Status 0 was found for 84 % patients (16 % PS =1). More than half women were postmenopausal (55,3 %).

Studied carcinoma were invasive ductal (82,6 %) and invasive lobular carcinoma (17,4 %) with histological grading mainly II (71 % vs 25 % grade I). The TNM stages were T0N0 (n=2), T1N1 (n=34), T2N0 (n=23), T2N1 (n=4), T3N0 (n=3) à T4N0 (n=1). Hormone receptor status was confirmed for each tumor, without any HER2 overexpression.

The mean Body Mass Index (BMI) was 25,6 kg/m<sup>2</sup> [range: 17,7-39,6 kg/m<sup>2</sup>], with 27,5% and 21,7 % of patients overweighted and obese, respectively. The mean waist circumference was 88,1 cm [range: 52-121 cm], with 65,7 % and 53,7 % of women, more than 80 and 88 cm, respectively. The mean Systolic blood Pressure and Diastolic blood Pressure were 131 et 79 mmHg, respectively [range: 101-174 and 58-104 mmHg] ; with 53,7 % of patients SBP≥ 130 and/or DBP≥ 85 mmHg. 43,3% of women were treated with antihypertensive drugs. The mean values of fasting triglycerides were 0,96 g/L [range: 0,28-3,4 g/L], with 16,4 % of patients presenting TG>1,5 g/L. The mean values of HDL-C were 0,66 g/L [min-max 0,32-1,52 g/L]. HDL-C<0,5 g/L was measured in 19,4 % of patients, and 22,4 % were treated with normolipidemic drugs. The mean fasting glycemia was 0,97 g/L [range: 0,68-1,74 g/L]. More than 34% of women had glycemia> 1 g/L, with only 4,5 % under hypoglycemic treatment. In the studied population, the metabolic syndrome prevalence was 35,6 % according to NCEP ATP III criteria, and 44,8 % according to IDF definition.

**Conclusions and perspectives.** The prevalence of metabolic syndrome is higher in breast cancer patients compared to control population. The prevalence of metabolic syndrome range, according the chosen definition and the studied population, between 12% to 40% (Crichton *et al.*, 2011). In the French DESIR study (Balkau *et al.*, 2003) the prevalence of metabolic syndrome aged 60 to 64 years was around 12 %. The waist circumference criterion seems to be the more significant : 24,4 % of women had waist circumference >88 cm in control vs 53,7% in our study. Few clinical studies have been done to evaluate the prevalence of metabolic syndrome in women with breast cancer; it ranges between 16 and 39 %. Our data are in agreement with those described in post-menopausal breast cancer patients (39% according to IDF criteria - Healy *et al.*, 2010).

## Independently of cell neoplastic status, leptin modulates the pro/anti oxidant balance in human mammary epithelial cells

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**Background and objectives.** Nutritional status and hormonal factors, such as leptin, an adipokine up-regulated in obesity, induce cellular signalling pathways, some of which involving reactive oxygen species (ROS) as intracellular messengers. High levels of ROS contribute to oxidative stress, cellular damages and pathogenesis. Therefore ROS production associated to obesity could be a major risk factor of mammary carcinogenesis. Thus this study aimed to determine leptin effects on ROS production in 3 human epithelial mammary cell models which present different neoplastic status (healthy primary HP (HMEC) cells, MCF-7 and MDA-MB-231).

**Methods.** ROS production was measured by fluorescence in presence of two leptin concentrations (10 ng/ml close to physiological values, 100 ng/ml as obesity level) with 4 probes at 2 $\mu$ M (Dichlorofluorescein (DCF) for total cellular ROS, Diaminofluorescein (DAF) for NO, Dihydrorhodamine (DHR) for mitochondrial ROS and Dihydroethidine (DHE) for cytosolic superoxyde anion (O<sub>2</sub><sup>•-</sup>). Gene expression and catalytic activities of anti-oxidant enzymes (heme oxygenase, glutathione -reductase, -peroxidase or -S-transferase) were performed.

**Results.** Whatever the cell model and the leptin concentration, a slight increase of total cellular ROS production was observed. This increase was independent of mitochondrial production as DHR signal remained stable for HP cells (5.51  $\pm$  0.40 RFU) and decreased for MCF7 and MDA-MB-231 cells. Inversely, this ROS increase was dependent of cytosolic O<sub>2</sub><sup>•-</sup> production as shown by DHE signal enhanced for HP cells (0.66  $\pm$  0.01 to 0.81  $\pm$  0.01 RFU), for MCF7 (0.79  $\pm$  0.02 to 0.89  $\pm$  0.03 RFU) and for MDA-MB-231 (0.82  $\pm$  0.01 to 0.89  $\pm$  0.02 RFU). Interestingly, this ROS production contributed to a different antioxidative response in regard to the neoplastic cell status. Leptin stimulated, only in HP cells, the antioxidative enzymes expression and activities such as heme oxygenase (0.8  $\pm$  0.2 to 2  $\pm$  0.5 UI/l) and glutathione peroxydase (35  $\pm$  10 to 52  $\pm$  15 UI/l). In neoplastic cells, these enzyme activities did not change whatever the leptin concentration used.

**Conclusions and perspectives.** Whereas leptin induces a similar ROS production in the 3 cell models, the antioxidant response is different *i.e.* induced in HP cells and unchanged in MCF-7 and MDA-MB-231 cells. These data suggest that leptin could modulate the oxidative status of epithelial mammary cells in different ways according to the neoplastic cell status. This is a possible explanation for why obesity increases the risk of carcinogenesis and breast cancer recurrence.

## Associations between breast cancer survival and SNPs identified through GWAS meta-analyses for metabolic and cardiovascular diseases/traits

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Early detection and improvement of treatments have resulted in an increasing population of cancer survivors in Western countries. However, if factors associated with cancer incidence have been extensively studied, those related to overall and disease-free survival are still largely unknown. Among lifestyle factors that could influence breast cancer survival, excess weight and obesity have been the most studied and were associated with poor prognosis among women with breast cancer. However, physiological mechanisms that could explain these associations are not yet completely identified.

**Objective.** To examine the association between genetic polymorphisms in obesity-related pathways and breast cancer disease-free survival within the French prospective E3N cohort.

**Methods.** The E3N cohort is an ongoing prospective study investigating risk factors for cancer and other chronic diseases. Samples from 1,605 women with diagnosed incident breast cancer were genotyped for 196,725 genetic polymorphisms identified in published GWAS on metabolic and cardiovascular diseases/traits ("Metabochip", Illumina iSelect). Only 126,777 SNPs passed quality control (missing < 5%, MAF ≥ 0.01, p<sub>HW</sub> ≥ 0.001) and were kept for analyses. To date, 231 breast cancer cases are have reported a recurrence, metastases or a second primary tumor. The risk of disease-free survival by genotype was estimated by Cox proportional hazard models adjusted for age at diagnosis.

**Results.** We identified 15 distinct loci with a  $P_{\text{trend}} < 10^{-4}$  in analyses of disease-free survival adjusted for age at diagnosis, with  $P_{\text{trend}}$  ranging from  $8.64 \times 10^{-5}$  to  $5.05 \times 10^{-6}$ . These findings will be replicated in 1500 additional breast cancer cases from the E3N cohort. Subsequent pathway analyses and gene-environment interactions will also be performed. The area under the curve (AUC) of a prediction model moved from 0.63 for a model including only clinical data to 0.83 for a model including clinical data plus a selection of top SNPs.

**Conclusions and perspectives.** The results of the study will allow identification of genetic polymorphisms associated with excess weight, obesity and weight gain and related to breast cancer recurrence and survival. The results will also permit a better understanding of mechanisms underlying the association between obesity and breast cancer prognosis. The polymorphisms identified can be used in clinical testing to identify women at greater risk of disease progression among breast cancer patients. When identified, the genes involved are potential candidates for targeted therapies.



## Anthropometric measures, body shape silhouette, body shape trajectory throughout life and risk of Breast cancer among Mexican women

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**Background.** Obesity has been associated with breast cancer (BC) risk in the Caucasian population but the association remains unclear in the Hispanics. Previous studies conducted among Hispanics in the US have shown inconsistent results. The association between anthropometry, body shape evolution across lifetime and the risk of breast cancer was assessed using a multi-center population-based case–control study conducted in Mexico.

**Methods.** One thousand incident cases and 1,074 matched control women aged 35 to 69 years were recruited between 2004 and 2007. Conditional logistic regression models were used to compute BC odd ratio (OR). SAS Proc Traj (Automatic procedure) was used to estimate the different groups of body shape evolution throughout life on the basis of six body shapes at different ages.

**Results.** Height was related to an increased BC risk in both premenopausal ( $P$ -trend = 0.03) and postmenopausal women ( $P$ -trend = 0.002). In premenopausal women, increase in body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist hip ratio (WHR) were inversely associated with breast cancer risk ( $P$ -trends < 0.001 for BMI and WC, 0.003 for HC and 0.016 for WHR). Similar inverse relationships were observed in postmenopausal women. However, among women who had menopause more than 10 years since enrolment, no association with anthropometry was observed (with the exception of height). Further analysis of body shape evolution throughout life showed strong and significant increase in BC risk among women with increasing silhouettes size over time compared to women with no or limited increase.

**Conclusion:** These findings suggest that anthropometric factors may have different associations with BC risk in Hispanic women than in Caucasian women. This study also shows the importance of considering the evolution of body shape throughout life.

## Insulin like growth factor (IGF)-I, IGF-I binding protein 3 (IGFBP-3) and body size in premenopausal Mexican women: the EsMAESTRAS cohort

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**Background and objectives.** Circulating levels of insulin-like growth factor I (IGF-I) and its major binding protein (IGFBP-3) have been associated with the risk of different cancers and other chronic diseases, and particularly in breast cancer in young women. The relation between circulating concentrations of IGF-I and IGFBP-3 with body weight and body fat distribution is still not well understood. Most of the published studies have been conducted among Caucasian women, and little is known about this association in other ethnic groups. The aim of the current study is to investigate the association between circulating levels of IGF-I and IGFBP-3 with body size (height, BMI, waist circumference – WC-, hip circumference –HC- and waist to hip ratio -WHR-) in a Mexican population of premenopausal women.

**Methods.** We conducted a cross-sectional analysis among 593 premenopausal women who are part of the large Esmaestras cohort study (a prospective study including more than 100,000 women in Mexico). For these women, measured anthropometric variables and blood samples were available. Measurements of serum IGF-I and IGFBP-3 were performed by commercially available immunoassays. Geometric means of IGF-I and IGFBP-3 were compared across deciles of height, BMI, WC, HC and WHR based on the population distribution.

**Results.** Mean (SD) age was 43.1 (SD=3.7) years, mean (SD) anthropometric measurements were height 155.9 cm (5.9), BMI 28.5 (5.3), waist circumference 90.8 (13.3) hip circumference 105.6 (10.8) and WHR 0.9 (0.1). Mean levels of IGF-I and IGFBP-3 were respectively 118 (SD=42.5) ng/ml and 3284.5 (SD=609.7) ng/ml. After adjustment for age, region and batch of analyses, IGF-I and IGFBP-3 concentrations increased significantly with increasing deciles of height ( $p$  trend<0.01 for both parameters), and decreased significantly with increasing deciles of BMI ( $p$  trend<0.01 for both parameters), with the highest IGF-I and IGFBP-3 concentrations in women with  $22.5 < \text{BMI} < 23.7$  (136.3 ng/ml and 3,383.0 ng/ml, respectively), and the lowest in women with  $\text{BMI} > 36.3$  (81.9 ng/ml and 2,968 ng/ml respectively). IGF-I concentrations decreased significantly with increasing deciles of waist ( $p$  trend<0.001), while no association was observed for IGFBP-3. No associations were observed between IGF-I and IGFBP-3 concentrations and WHR.

**Conclusion and perspectives.** Our data confirm the relationships of circulating IGF-I and IGFBP-3 with body size in this subsample of young Mexican women, which however may differ from those observed in Caucasian women. Further research is needed to better understand the association between circulating growth factors and body size, especially in young women, and in different ethnic groups.

## Physical activity level and nutritional modifications in French breast cancer patients starting chemotherapy

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**Background and objectives.** In breast cancer patients, a lack of physical activity is a probable factor of weight gain during adjuvant treatment. A moderate-intensity physical activity could reduce recurrence and improve survival. A randomized controlled trial is ongoing since June 2011 to study the feasibility and benefits of a program for a healthy diet and supervised adapted physical activity in breast cancer patients starting adjuvant chemotherapy (ClinicalTrials.gov identifier: NCT01331772). Baseline anthropometrics and physical activity level of women are presented.

**Methods.** At inclusion (first day of chemotherapy), weight (kg), height (cm) and waist circumference (cm) were measured. Body mass index (BMI, kg/m<sup>2</sup>) was calculated. Body fat percentage was assessed by bioelectrical impedancemetry (Bodystat® Quadscan 4000). Aerobic capacity (estimated VO<sub>2max</sub>), usual average daily energy expenditure (DEE), time spent in moderate-intensity physical activity (≥ 4 METs) and screen time were estimated by the interviewed-administered questionnaire PAQAP® during the month prior to chemotherapy and retrospectively during the pre-diagnostic period. Descriptive statistics were performed. Medians were compared using the Wilcoxon signed-rank test.

**Results.** Among the first 37 patients enrolled (median age, 53 years), median BMI was 23.4 kg/m<sup>2</sup>. While 16 (43 %) women were overweight or obese (BMI > 25 kg/m<sup>2</sup>), 33 (89 %) presented excess body fat (median body fat, 35 %). Median waist circumference was 85 cm and 23 (62 %) patients had excess visceral adiposity (waist circumference > 80 cm). Between the pre-diagnosis and pre-chemotherapy periods, the usual daily energy expenditure (− 591 kJ/d, *p* < .001), the estimated VO<sub>2max</sub> (− 1.7 ml/min/kg, *p* < .001) and the moderate-intensity physical activity (− 8 min/d, *p* < .001) decreased and the screen time increased (+ 42 min/d, *p* < .001).

**Conclusions and perspectives.** While the proportion of overweight breast cancer patients (43 %) starting chemotherapy was similar to that of their French counterparts of same age, more than half of patients had a waist circumference reflecting metabolic risks and 89% had excess body fat. Moreover, a significant decrease in physical activity level early after diagnosis supports the necessity of engaging patients in supervised physical activity programs soon after breast cancer diagnosis to prevent physical deconditioning, risk of weight gain and co-morbidities.

# “Alcohol (societal aspects)”

## Clustering of cancer-risk factors associated with alcohol consumption

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**Background and objectives.** Alcohol is a cancer-risk factor per se but few studies examined its association with a broad range of other risk factors. The objectives were 1) to assess which sociodemographic, economic, behavioural and dietary factors were independently associated with alcohol use; 2) to identify potential clustering of cancer-risk factors associated with alcohol consumption; 3) to assess how many cancer-risk factors were cumulated by the same individuals according to alcohol use.

**Methods.** Alcohol and dietary intakes were assessed by six 24h web-based records among 29566 subjects of the NutriNet-Santé cohort. Factors independently associated with alcohol use (<vs.  $\geq 10$  g/d) were assessed by multivariate logistic regression. Multiple correspondence analysis was used to examine clustering of factors.

**Results.** Several factors were independently associated with alcohol use  $\geq 10$ g/d: older age ( $P_{\text{men}}=0.001$ ,  $P_{\text{women}}<0.0001$ ), smoking ( $P_{\text{men\&women}}<0.0001$ ), overweight in men ( $P<0.0001$ ), unemployment in women ( $P=0.0003$ ), higher socioprofessional category among employed people ( $P_{\text{men}}=0.0001$ ,  $P_{\text{women}}<0.0001$ ), higher education in women ( $P=0.0004$ ), lower education in men ( $P=0.03$ ), higher income ( $P_{\text{men}}=0.003$ ,  $P_{\text{women}}<0.0001$ ), no dietary supplement use ( $P_{\text{men}}=0.0002$ ,  $P_{\text{women}}=0.0005$ ), low intakes of fruits/vegetables, dairy products and dietary fiber and high intakes of meat, processed meat and salt (compared to the recommendations,  $P_{\text{men\&women}}<0.0001$  for all dietary factors). Multiple correspondence analyses identified a cluster of dietary and behavioural cancer-risk factors associated with alcohol use. The number of dietary and behavioural cancer-risk factors (except alcohol itself) cumulated by the same individuals was higher in subjects who drank  $\geq 10$ g/d compared to lower drinkers (median number = 5 and 4 respectively).

**Conclusions and perspectives.** This study illustrates the complexity of socio-demographic and economic correlates of alcohol use: in women, unemployment but also high socio-professional categories among employed people were associated with higher alcohol use. Education level was differentially correlated with alcohol use in men and women. Our results also underline that alcohol use is associated with a cluster of other lifestyle and dietary cancer-risk factors. The multiplicity of deleterious behaviours associated with alcohol drinking must be taken into account to improve cancer prevention.

## Alcohol intake and breast cancer defined by receptors status in EPIC

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**Financial support:** Direction Générale de la Santé

**Background.** A consistent association has been observed between alcohol consumption and breast cancer among both pre and postmenopausal women. However some questions remained unanswered such as the strength of the association with specific BC hormonal status, age at start drinking, and types of drinks.

**Methods.** We used data from EPIC a large prospective cohort study conducted in 23 European centers. During 1.6 years of follow up 11,576 cases of incidence breast cancer were diagnosed with detailed information on receptor status (ER, PR and HER2), information on alcohol consumption, as well as age at start drinking and types of beverages. In addition information on anthropometry, reproductive, dietary intake, and other lifestyle factors were obtained. We used Cox proportional hazard model to estimate the association between alcohol consumption and specific BC hormonal status adjusting for age at baseline, recruitment center and other potential confounding factors.

**Results.** Alcohol consumption was related to an increased risk of BC in pre and postmenopausal women. An increase of 10g/day was associated with an overall HR of 1.042(95% CI =1.027-1.058; p for trend<0.001); for ER+/PR+ the HR was 1.039 (95% CI =1.013- 1.065 p for trend =0.003), for ER-/PR- HR was 1.053 (95% CI 1.01-1.10 p for trend= 0.03) and for TN HR was 1.12 (95% CI 1.026 -1.28 p for trend =0.012). Significant interactions were observed for ER- and PR- and ER-/PR- tumors with age at start drinking with a larger BC risk among women starting drinking prior to first full-term pregnancy. When considering the type of beverage, we considered only subject reporting drinking one type of alcoholic beverage, slightly stronger association was observed in subject reporting beer consumption compared to wine. Among postmenopausal women higher fiber intake was related to a lower risk of ER+/PR+ BC risk when compared to women with low fiber intake in particular with fiber from vegetables.

**Conclusion.** Alcohol consumption is associated to BC with ER+/PR+, ER-/PR- and TN tumors. Some factors appear to modify this association including age at start drinking prior to FFTP, type of drinks and fiber intake.

## Dual association between polyphenol intake and breast cancer risk according to alcohol consumption level: a prospective cohort study

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**Financial support:** This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM U557); the Institut National de la Recherche Agronomique (INRA U1125); and the Université Paris 13.

**Background and objectives.** Studies of the association between polyphenols dietary intake and breast cancer risk have been limited, due to the lack of detailed food composition tables. In addition, none has examined this association according to alcohol intake, despite the facts that alcohol is an established risk factor for breast cancer and that the contribution of alcoholic beverages to polyphenol intake varies according to the level of alcohol consumption. Our objectives were 1) to estimate the associations between breast cancer risk and a wide range of dietary polyphenols by using the recently published Phenol-Explorer database; and 2) to evaluate if/how alcohol intake modulates these relationships.

**Methods.** 4,141 women from the SU.VI.MAX prospective cohort were followed from 1994 to 2007 (median follow-up: 12.6 years); 152 developed a first incident invasive primary breast cancer. Dietary intakes were assessed by repeated 24h records. The Phenol-Explorer database was used to estimate polyphenol intake. Multivariable Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for quartiles of polyphenol intake. Analyses were stratified by median alcohol intake (< versus  $\geq$  6.5 g/d).

**Results.** In non-to-low alcohol drinkers, intakes of some classes of polyphenols were associated with decreased breast cancer risk: hydroxybenzoic acids (HR<sub>Q4vsQ1</sub>=0.38, 95%CI: 0.17-0.86, Ptrend=0.005), flavonoids (0.35, 0.17-0.75, Ptrend=0.02), flavonols (0.36, 0.18-0.74, Ptrend=0.002), catechins (0.48, 0.22-1.05, Ptrend=0.02), theaflavins (0.42, 0.19-0.93, Ptrend=0.02), and proanthocyanidins (0.39, 0.18-0.84, Ptrend=0.02). In contrast, in women with higher alcohol use, intakes of hydroxybenzoic acids (2.28, 1.16-4.49, Ptrend=0.04), flavonoids (2.46, 1.23-4.92, Ptrend=0.01), anthocyanins (2.94, 1.32-6.53, Ptrend=0.01), catechins (2.28, 1.19-4.36, Ptrend=0.02), and proanthocyanidins (2.98, 1.40-6.33, Ptrend=0.006) were associated with increased breast cancer risk.

**Conclusions and perspectives.** In conclusion, this prospective study suggests that several classes of polyphenols could potentially contribute to breast cancer prevention among non-to-low alcohol drinkers, but some may increase breast cancer risk among women with higher alcohol intake.

## The role of alcohol consumption on overall and cause-specific mortality in the European Prospective Investigation into Cancer (EPIC) study: a competing risks analysis

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**Financial support:** This work was supported by the “Direction Générale de la Santé” French Ministry of Health.

**Background.** In 2005, it was estimated that alcohol caused 3.2% of all deaths worldwide (WHO 2007). An exhaustive evaluation on the relationship between alcohol use and mortality was carried out in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, a large prospective cohort in 10 Western European countries, using a competing risks analysis.

**Methods.** Multivariable Cox regression models were used to quantify the association between baseline alcohol intake and risk of overall and cause-specific mortality, i.e. alcohol-related cancers, cardiovascular disease and deaths due to injuries/violence. Models were adjusted for relevant confounding factors. Participants with prevalent diseases at baseline (anyone of cancer, stroke, heart disease, diabetes, or hypertension) were excluded. The analyses used information on 288,406 study participants with over 3.7 million Person-years and 15,174 fatal events. An augmented dataset was used to evaluate the association categories of baseline alcohol (coded as never, former, (1-5] (reference category), (5-15], (15-30], (30-60], >60 g/day) and cause-specific mortality. The effect of alcohol across causes of death was compared.

**Results.** A total of 1 781 deaths due alcohol-related cancers (including cancers of upper aero-digestive tract, breast [women], liver and colorectum), 2 613 CVD deaths, and 694 injuries and violent death were used. In men, alcohol use was positively associated to death due to injuries, and to alcohol-related cancers. In women associations were overall less pronounced. Alcohol consumption showed an inverse relationship with CVD mortality, both in men and women. The alcohol/mortality relationships were heterogeneous ( $p < 0.001$ ) and across specific causes and non-linear, both in men and women. Based on a competing risks analysis, at the age of 60 years a heavy drinker woman had a chance of dying within the next 10 years from an alcohol-related cancer, CVD or injuries/violence equal to about 0.6%, 0.5%, and 0.3%, respectively. Corresponding figures in men were 2%, 2% and 1%.

**Conclusion.** Alcohol use was positively associated to death due to injuries/violence, and to mortality due to alcohol-related cancers in men. A non-linear J-shaped association was observed for CVD mortality, particularly in men.

# “Chemotherapy and metabolism”

## Cholesterol homeostasis actors and glioblastoma prognosis?

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**Background and objective.** Glioblastoma multiforme (GBM) is the most common and most aggressive malignant primary brain tumor in humans, involving glial cells, with poor clinical outcome. Postoperative radiotherapy (RT) with concomitant and adjuvant chemotherapy with temozolomide is the standard treatment; however the prognosis remains poor with a median survival in the range of 12 months. Identification and development of new markers could be beneficial for the diagnosis and prognosis of GBM patients.

It was previously demonstrated a relation between lipid composition and relation to the malignancy grade of glial human tumors. An increase in the malignancy is accompanied by a reduction of total lipids. It has been possible to demonstrate a correlation between a lower lipid content and an increase in the histological grading. In the other hand, profound modifications of membrane lipids can be responsible for functional variations connected with neoplastic growth. Liver X receptor (LXR) plays a key role in reverse cholesterol transport by inducing the expression of the ATP-binding cassette (ABC) transporters, such as ABCA1 and ABCG1, both involved in cholesterol efflux. LXR activation in cancer cells could deprive cells of cholesterol by stimulating its efflux, resulting in the inhibition of cell proliferation and stimulation of apoptosis. Therefore, it may be speculated that the expression level of ABCA1 and ABCG1 influences the proliferation / apoptosis balance of cancer cells. Glioblastoma (GB) is characterized by hypoxic and necrotic regions due to a poorly organized tumor vascularization, leading to hypoxic and necrotic areas. Hypoxia has been related to poor outcome of the patients since hypoxic/necrotic tumors are more resistant to chemotherapy and radiation therapies. Vascular endothelial growth factor (VEGF) an inducer of angiogenesis and neovascularization is also regulated by activation of liver X receptors (LXRs). In addition, under hypoxia GB primary cells increase the expression of stem cells markers as well as an increased expression of ApoE, ABCA1 and ABCG1. Inversely, a better vascularization contributes to reduce hypoxia and induces a decrease in ABCA1, ABCG1 and ApoE gene expressions. Then, it seemed interesting to evaluate some markers linked to this lipid metabolism and in relation with LXR (ABCA1, ABCG1, ApoE, VEGF, PON2) LXR- $\beta$  itself and FDPS (Farnesyl Diphosphate Synthase) a key intermediate in cholesterol and sterol biosynthesis. We looked for any correlation between these gene expressions and survival time as well as various markers of aggressiveness and dissemination (AKT, PDGFRB).

**Methods.** Expression of ABCA1, ABCG1, ApoE, VEGF, PON2, LXRb, FDPS was evaluated by quantitative PCR analysis with Gapdh, RPLPO and TATA box binding protein (TBP) as housekeeping selected genes. Non parametric estimates of the survivor function was computed with Kaplan-Meier method and log-rank test of the equality of survival distributions across median was performed. Then a Cox proportional hazards model was used to confirm results from log-rank test for the effect of gene expressions on survival rate and to calculate the hazard ratios (HRs) and their 95% confidence intervals with values above median as reference.

**Results.** We observed very significant correlations between selected biological parameters and survival time. For ABCG1 (HR= 0,51 [0,27-0,98] p=0,0418; log-rank test p=0,0191), ABCA1 (HR =0,45 [0,23-0,88] p=0,0202; log-rank test p=0,0071) and apo E (HR=0,36 [0,18-0,71] p=0,0030; log-rank test p=0,0005), low expression is associated with significant longer survival time. In contrast, VEGF (HR = 2,43 [1,25-4,73] p=0,0087; log-rank test p=0,0022), PON2 (HR= 2,12 [1,11-4,04] p=0,0224; log-rank test p=0,0084) and FDPS (HR=3,63 [1,82-7,23] p=0,0002; log-rank test p= <0,0001) high expression level is significantly associated with longer survival time. No significant association is found between LXRb expression and survival time (HR=1,74 [0,91-3,30] p=0,0911; log-rank test p=0,0528).

**Conclusions and perspectives.** Modulation of patient's survival with GBM is correlated with some lipid metabolism markers whose functions were described in different cancer cells either as involved in apoptosis/proliferation control balance or in cancer stem cells survival. The meaning of these changes on cellular functions remains to be explored in these tumors.



## Immunonutrition limits pro-inflammatory and pro-oxidant effects of radiochemotherapy in cancer patients

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**Background.** Peri-operative immunonutrition improves digestive surgical outcomes of cancer patients reducing complications and length of stay. Radiochemotherapy (RCT) generates deleterious effects on nutritional and immune status limiting functional capacities of patients. This study aimed to evaluate if immunonutrition enriched in arginine, EPA & DHA and nucleotides could limit these side effects in Head&Neck (HN), and oesophageal cancer patients.

**Methods.** A double-blind randomized clinical trial was driven with two groups receiving either an Immune Enteral Nutrition (IEN, n=13, Impact<sup>®</sup>, Nestlé) or a Standardized Enteral Nutrition (SEN, n=15) during all RCT (5-7 weeks). Nutritional status, plasma antioxidant capacity (TEAC, FRAP) and functional capacities (WHO score, Karnofsky index) were assessed at the beginning (Db) and at the end (De) of RCT. Immune functions were assessed by leukocytes phenotyping, *ex vivo* PMN ROS production, and PBMC cytokines & eicosanoids (PGE2) secretion and gene expression.

**Results.** At De vs Db, a significant improvement in total body weight (+2.1kg) was observed in IEN patients as well as albuminemia and NRI in IEN malnourished patients. TEAC and FRAP were increased (+100µM *EqTrolox*) in IEN patients and higher at De than for SEN patients. Functional capacities were maintained upper the autonomy threshold only in IEN patients.

The PMN density of chemo-attractant receptors (CD62L, CD15) and ROS production (+30%) were significantly increased in IEN patients at De vs Db. PBMC PGE2 secretion was unchanged at De in IEN patients and lesser than for SEN patients (65.9±56.8 vs 106.8±59.3 ng/ml, p<0,05). Folding ratios (De/Db) of PBMC mRNA expression were strongly increased for antioxidant response genes (*catalase*, *gpx1&4*, *sod1&2*) and for bactericidal NOX system (*cybA*, *rac1&2*).

**Conclusion.** Immunonutrition in adjunction to RCT contributes to maintain functional capacities of patients through improvement of nutritional, anti-oxidant and immune status. Immunonutrition could provide a higher bactericidal capacity and limit inflammatory and oxidative responses induced by RCT in immune cells of HN and oesophageal cancer patients.

## Fatigue and Weight-Loss Predict Survival On Circadian Chemotherapy For Metastatic Colorectal Cancer

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**Financial support:** Association Internationale pour la Recherche sur le Temps Biologique et la Chronothérapie (ARTBC International).

**Background and Objectives.** Chemotherapy-induced neutropenia was associated with prolonged survival selectively in patients on a conventional schedule (FOLFOX2) but not on a chronomodulated schedule (chronoFLO4), with drugs administered at specific circadian times. We hypothesized that the early occurrence of chemotherapy-induced symptoms correlated with circadian disruption would selectively hinder chronotherapy efficacy.

**Methods.** Fatigue and weight loss (FWL) were considered to be associated with circadian disruption, based on previous data. Patients with metastatic colorectal cancer (n=543) from an international phase III trial comparing FOLFOX2 with chronoFLO4 were categorized into 4 subgroups according to the occurrence of FWL or other clinically-relevant toxicities during the initial two courses of chemotherapy. Multivariate Cox models were used to assess the role of toxicity on time to progression (TTP) and overall survival (OS).

**Results.** The proportion of patients in the 4 subgroups was comparable in both treatment arms (p=0.77). No toxicity was associated with TTP or OS on FOLFOX2. Median OS on FOLFOX2 ranged from 16.4 [95%CL, 7.2-25.6] to 19.8 months [17.7-22.0] according to toxicity subgroup (p=0.45). Conversely, FWL but no other toxicity, independently predicted for significantly shorter TTP (p<0.0001) and OS (p=0.001) on chronoFLO4. Median OS on chronoFLO4 was 13.8 [10.4 to 17.2] or 21.1 months [19.0 to 23.1] according to presence or absence of chemotherapy-induced FWL, respectively.

**Conclusions and Perspectives.** Early-onset chemotherapy-induced fatigue and/or weight loss was an independent predictor of poor TTP and OS only on chronotherapy. Dynamic monitoring detecting early chemotherapy-induced circadian disruption could allow rapid chronotherapy optimization and concomitant improvement of safety and efficacy.

# “Different Topics on nutrition”

## The systematic collection of new data on the effects of cooking on polyphenol content in foods to improve polyphenol intake measurement in cohort studies

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**Background and objectives.** A large number of experimental studies have shown the anti-carcinogenic properties of polyphenols. However, evidence from cohort studies for a role in the prevention of cancers is still limited. Progress has been hampered by a lack of adequate tools for the measurement of polyphenol intake. We present here an update of the Phenol-Explorer database, the first comprehensive source of information on polyphenol contents of foods, to incorporate new data on the effects of food processing and cooking upon food polyphenols. The database allows the retrieval of retention factors that allow the calculation of polyphenol content in processed foods from composition data on raw foods, information needed to refine the estimation of polyphenol intake in cohort studies.

**Methods.** Food composition data were systematically collected from peer-reviewed publications in which polyphenol content in raw and processed foods has been compared. Their quality was evaluated and data compiled and integrated into the Phenol-Explorer database. The new webpages were developed using the Ruby on Rails framework and the MySQL database management system.

**Results.** Over 4,000 retention factors for 148 polyphenols were collated from 143 publications on the polyphenol compositions of raw and processed foods. Retention factors were aggregated where possible. The new webpages in the Phenol-Explorer database allow querying of the database and the efficient retrieval of information on the degradation of various polyphenols from different food sources subjected to various cooking processes.

**Conclusions.** This novel information on the effects of cooking and processing on polyphenol content in foods will be applied to food items consumed in the European Prospective Investigation on Cancer and Nutrition (EPIC) study, and for which polyphenol contents before processing had already been calculated. The application of retention factors is expected to change the estimation of intake for some polyphenols, particularly those from commonly consumed vegetables that are often boiled, fried or baked. We speculate that the relation between polyphenol intake and cancer risk might be stronger than previously observed.

# Dairy Foods, Calcium, Vitamin D and Risk of Hepatocellular Carcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study

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**Background.** Dairy foods are an important food group whose intake may be associated with cancer. On the one hand, dairy foods are high in saturated fats and high dairy food consumption may increase circulating levels of IGF-1, a growth factor. Conversely, dairy foods contain calcium and vitamin D, which have been shown to be cancer protective at some sites. However, information on association of intake of dairy foods is limited or inconsistent for many cancer sites, such as hepatocellular carcinoma (HCC), the sixth most common malignancy and the third most common cause of cancer death worldwide.

**Objective.** To investigate the association between dairy products as well as calcium and vitamin D intake, with first incident HCC ( $N_{\text{cases}}=191$ ) in the EPIC cohort, including a nested case-control sub-set with assessment of hepatitis B/C virus infections status.

**Methods.** A total of 477,206 participants were followed up for an average of 11 years (PYs follow-up = 5,415,385). Diet was assessed by country-specific validated questionnaires. For cohort analyses, the Cox proportional hazard model was used to estimate hazard ratios (HRs) and 95% confidence intervals (95%CI). For case-control analyses, conditional logistic regression was used to calculate odds ratios (ORs) and 95%CI. All models were adjusted for relevant confounders.

**Results.** In the cohort study, a significant positive association for total dairy products (highest vs. lowest tertile, HR=1.65, 95% CI:1.13-2.43;  $P_{\text{trend}}=0.012$ ), milk (HR=1.51, 95% CI:1.01-2.24;  $P_{\text{trend}}=0.059$ ), cheese (HR=1.56, 95% CI:1.01-2.38;  $P_{\text{trend}}=0.101$ ), dairy calcium (HR=1.49, 95% CI:1.02-2.18;  $P_{\text{trend}}=0.046$ ) and dairy vitamin D (HR=1.90, 95% CI:1.18-3.05;  $P_{\text{trend}}=0.003$ ) were observed with HCC risk after multivariable adjustment. Non-significant associations were observed for yoghurt (highest vs. lowest tertile, HR=0.94, 95% CI:0.65-1.35), non-dairy calcium (HR=0.81, 95%CI:0.52-1.27;  $P_{\text{trend}}=0.360$ ) and non-dairy vitamin D (HR=0.80, 95%CI:0.51-1.25;  $P_{\text{trend}}=0.281$ ). The results were similar after excluding individuals diagnosed with HCC within 2 years after recruitment or with self-reported diabetes at baseline. In the nested case-control study, similar results were observed among hepatitis-free individuals.

**Conclusions.** Results from this large prospective cohort study suggest that higher consumption of total dairy products, calcium and vitamin D from dairy sources are associated with increased HCC risk, emphasizing the role of diet in liver cancer etiology. Potential biologic mechanisms that could explain the positive associations may include circulating IGF-I or vitamin D concentrations, and require further exploration in prospective settings.

## Association between pre-diagnostic biomarkers of inflammation, endothelial function and adiposity and cancer risk: a nested case-control study

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**Background and objectives.** Experimental and prevalent case-control studies suggest an association between biomarkers of inflammation, endothelial function and adiposity and cancer risk, but prospective studies are limited. The objective was to prospectively examine the relationships between these biomarkers and cancer risk.

**Methods.** A nested case-control study was designed within the SUplémentation en Vitamines et Minéraux Antioxydants cohort (SU.VI.MAX, France) to include all first primary incident cancers diagnosed between 1994 and 2007 (n=512). Cases were matched with randomly selected controls (n=1024, matching factors: sex, age by 2-year strata, body mass index < vs.  $\geq 25$  kg/m<sup>2</sup>, and intervention group of the initial SU.VI.MAX study). Conditional logistic regression was used to study the association between pre-diagnostic levels of highly sensitive C-reactive protein (hs-CRP), adiponectin, leptin, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble E-selectin (sE-selectin), monocyte chemoattractant protein-1 (MCP-1) and cancer risk. All statistical tests were two-sided.

**Results.** A total of 512 incident cancer cases were diagnosed during follow-up: 218 breast cancers, 156 prostate cancers, 50 colorectal cancers and 88 other cancers. Plasma sICAM-1 level was positively associated with breast cancer risk: multivariate odds ratio (OR) for Q4 vs. Q1 = 1.86 (95% confidence interval (CI): 1.06, 3.26), Ptrend=0.048. Plasma hs-CRP level was positively associated with prostate cancer risk: multivariate ORQ4vsQ1=3.04 (95% CI: 1.28, 7.23), Ptrend=0.03. Plasma adiponectin level was associated with decreased colorectal cancer risk (Ptrend =0.03). Quartiles of sVCAM-1 were associated with increased colorectal cancer risk (Ptrend=0.02).

**Conclusions and perspectives.** These results suggest that pre-diagnostic hs-CRP and sICAM-1 levels are associated with increased prostate and breast cancer risk, respectively. They also suggest that pre-diagnostic plasma adiponectin and sVCAM-1 levels are associated with decreased and increased colorectal cancer risk, respectively. These relationships must be confirmed in large validation studies.

## Metabolic switch in energetic metabolism of colon cancer cells by environmental pollutants.

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Persistent organic pollutants (POPs) are a major public health concern. Since humans are exposed to hundreds of pollutants it is one of the most challenging issues in current environmental toxicology to understand their effects. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and  $\alpha$ -endosulfan are common environmental POPs mainly produced by human activities. Both molecules are frequently found in the same environmental sources as TCDD is a by-product of  $\alpha$ -endosulfan. They have many toxic effects and the International Agency for Research on Cancer (IARC) has designated POPs as probably or yet well established carcinogenic to humans (ex: TCDD). Most studies of the effects of POPs are conducted on one compound at a time. There are few studies that report and characterize the interactions due to mixtures.

Our aim is to determine whether the alteration in cellular endogenous metabolism is implicated in the carcinogenic effects of the pollutants. It is well recognised that the alterations of cellular endogenous metabolism are critical during cancer development. That is characterized by an elevated uptake of glucose and an increased glycolytic rate with an increased lactate production (Warburg effect) that constitute a major feature of aggressive tumour.

**Methods.** The main contamination pathway of POPs for humans is the gastrointestinal tract. Thus we have chosen to work on a cell line model of colon cancer (Caco2 cells) under its proliferating state.

The techniques used involve the evaluation of metabolic flux using radioactive substrates such as  $^{14}\text{C}$ -Glucose, or  $^{14}\text{C}$ -Glutamine, both for oxidative/glycolytic pathway and for lipid synthesis. The levels of various metabolic intermediates such as lactate, glycogen, ATP or NADPH are measured. In order to evaluate the molecular basis of POPs the expression of genes encoding key proteins involved in glucose and or lipid metabolism are measured using real time RT-qPCR and western blot. The effect of the pollutants on cell growth is determined using BrdU incorporation.

**Results.** Proliferating colon cancer cells (Caco2) were treated with TCDD (25nM) or/and  $\alpha$ -endosulfan (10 $\mu\text{M}$ ), two environmental pollutants mainly produced by human activities and designated by the International Agency for Research on Cancer as probably or well-established carcinogenic to humans. A significant decrease of glucose and glutamine oxidation (60%) was observed after a treatment for 48 hours with the two pollutants while each pollutant alone had no significant effect. These observations are correlated with an increased lactate production by two fold. These effects are maintained in the presence of antioxidative NAC (10mM), suggesting that they are independent of the oxidative status of the cell. We also observed a decreased incorporation of glucose in total lipids (50%). The ATP production and the cell respiration level were significantly decreased by the mixture by about 50% and 80%, respectively. In the same conditions, the glycogen production and the NADPH/NADPH,H<sup>+</sup> ratio were unchanged.

**Conclusions and perspectives.** Taken together, these results suggest that POPs could get worse the metabolic phenotype of cancer cells. The molecular mechanisms underlying the POPs-induced metabolic reprogramming are under investigation and should provide a better understanding of the signalling pathways involved in POPs action on the regulation of the energetic metabolism balance and their consequence on cancer.

## Cooked beef meat, and cured pork meat promotion of tumors in Apc(Min) mice. Plant extracts added to meat show no protection

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**Background and Objectives.** Epidemiological studies show that red and cured meat intake increases colorectal cancer risk. This study had two aims: (i) to look for meat promotion in Min mice and (ii) to test plant-based additives that suppress preneoplastic lesions in carcinogen-injected meat-fed rats.


**Methods.** Four types of cured pork meat (high- or low-heme, and high-heme added with  $\alpha$ -tocopherol or pomegranate extract) and 4 types of fresh beef meat, (uncooked or cooked x with or without grape-olive marinade) were mixed with an AIN76-based powder. The meat/powder ratio was 4:1 (moist) and 40:60 (dry matter). These 8 meat-based diets and a no-meat control powder diet with matching fat and iron content, were given to 188 Apc(Min) mice for 50 d. Number of intestinal tumors, and their area, were measured double blinded after euthanasia.

**Results.** Cured meat: Compared with the no-meat control group, mice in the high-heme cured-meat group had more tumors ( $p=0.04$ ) and higher tumor load ( $p=0.005$ ). Pomegranate extract marginally reduced this tumor promotion ( $p=0.08$ ), but  $\alpha$ -tocopherol showed no protective effect. Mortality was higher in groups given cured meat than in controls ( $p=0.01$ ).

**Fresh red meat.** Meat, cooking and marinade effect was analyzed by a 3-factor ANOVA. Meat did not change the tumors number but increased the tumor load ( $p=0.002$ ). Cooking strikingly increased the tumors number ( $p=0.01$ ) and tumor load ( $p=0.001$ ). Marinade had no significant protective effect.

**Conclusions and Perspectives.** This is the first demonstration that a high meat intake promotes carcinogenesis in Min mice small intestine, and that cooking enhances tumor promotion. The three tested antioxidant plant-extracts showed little or no protection in this animal model, but other studies show that they can protect carcinogen-injected rats given cured meat.

## Red wine, pomegranate, curcumin and $\alpha$ -tocopherol suppress colon carcinogenesis promotion by cured meat in rats

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**Background and Objectives.** Processed meat intake is associated with colorectal cancer risk. In rats, the intake of cured pork meat (DCNO from a Dark muscle, cured with sodium Nitrite, Cooked and Oxidized by air), is associated with promotion of colon carcinogenesis (*Santarelli et al., Cancer Prevention Research, 2010*). We speculated that heme iron from meat would catalyze the formation of promoting nitroso-compounds and/or lipid peroxidation end-products, and that polyphenols would inhibit these effects.

**Methods.** Five polyphenol-rich plant extracts and  $\alpha$ -tocopherol were added to DCNO cured meat, and given to 6 groups of 10 azoxymethane-induced Fischer 344 rats for 100-days. A control group of 26 rats was given DCNO cured meat. Colons were then scored for precancerous lesions (mucin-depleted foci, MDF). Fat peroxidation markers and nitrosated compounds were measured in fecal and urine samples. Data are reported as mean $\pm$ SEM. P values were calculated by Dunnett's t test.

**Results.** DCNO cured meat with red wine extract (10.0 $\pm$ 1.1 MDF/colon), pomegranate extract (10.0 $\pm$ 2.2), curcumin (10.2 $\pm$ 1.6) or  $\alpha$ -tocopherol (10.3 $\pm$ 1.1) significantly decreased the number of MDF per colon, in comparison with control DCNO (16.0 $\pm$ 1.1, all P<0.01). White grape extract and carnosic acid afforded no protection. The additives that suppressed MDF promotion also suppressed fecal excretion of nitrosyl iron.

**Conclusions and Perspectives.** Protection against cured meat-induced promotion was associated with suppression of fecal excretion of nitrosyl iron, suggesting this type of nitroso-compound promotes carcinogenesis. Red wine, pomegranate, curcumin or  $\alpha$ -tocopherol might be used to reduce the risk of colorectal cancer in people, without losing the benefit of eating cured meat.



# NOTES



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