



Unravelling the mechanisms of food digestion to improve our knowledge of the effect of food on human health

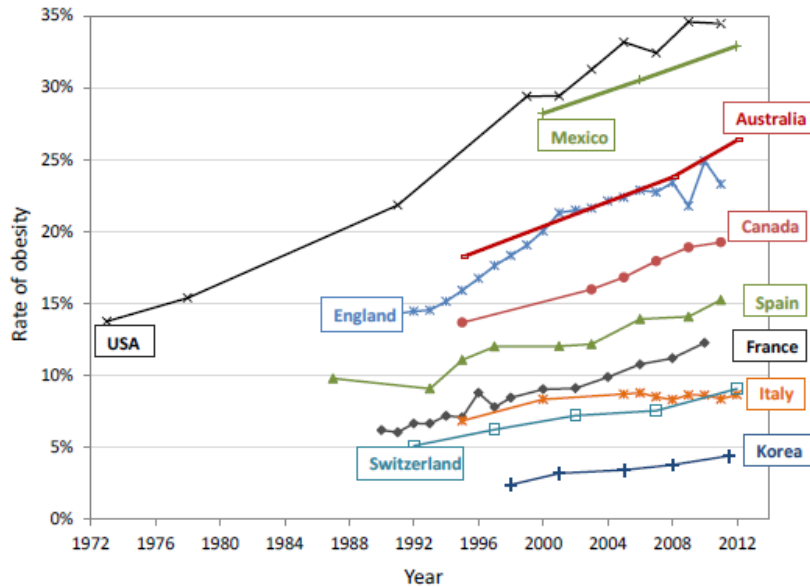


Dr Didier DUPONT

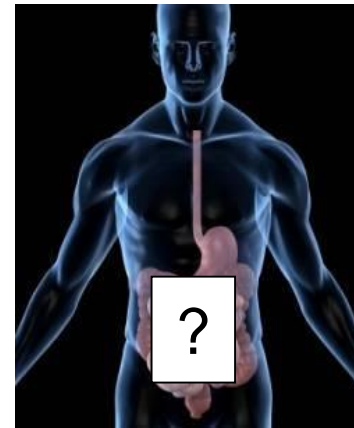
INRA, Rennes, France



Food and human health: the key role of digestion



Diet-related diseases ↑
Prevent these pathologies rather than
cure them



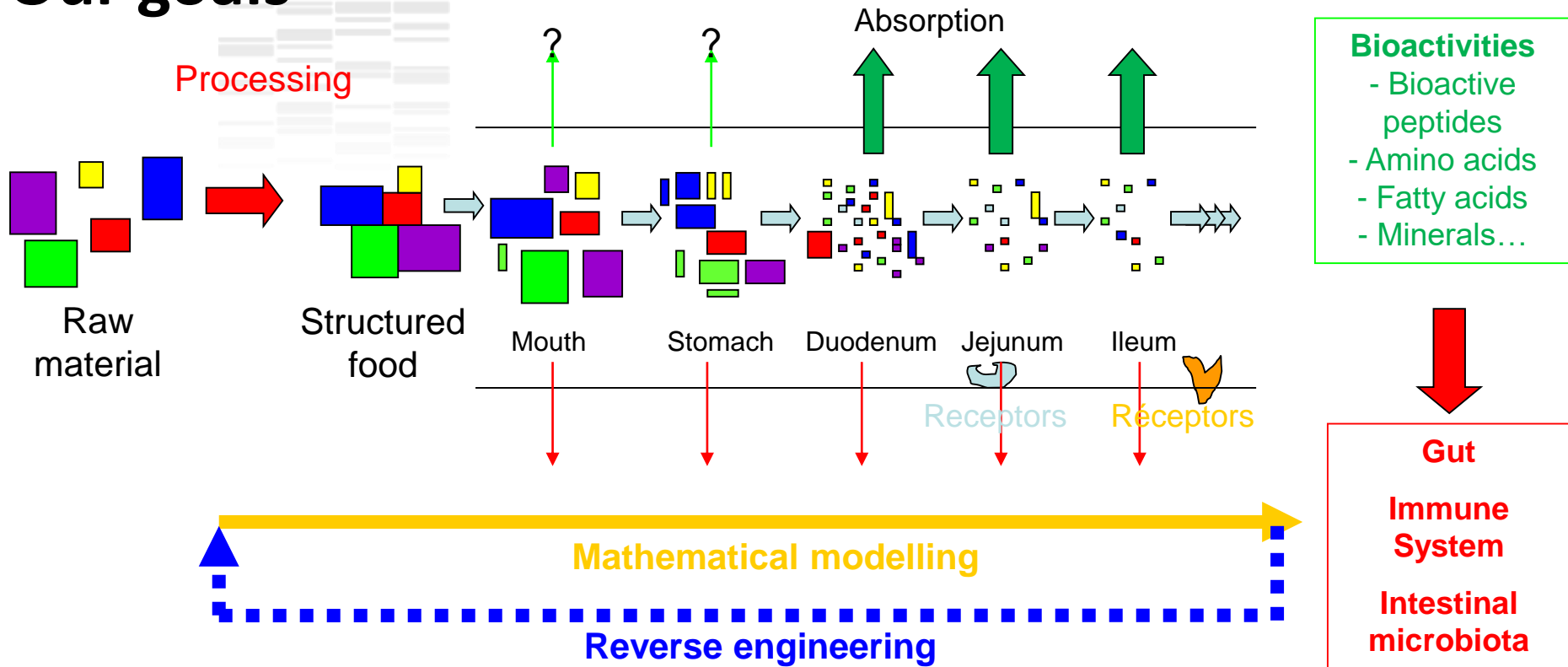
Gut = interface between food and human body

Digestion releases food components that can have a beneficial or a deleterious effect on human health

... but the mechanisms of food disintegration in the gastrointestinal tract remain unclear and the digestive process has been considered as a black box so far

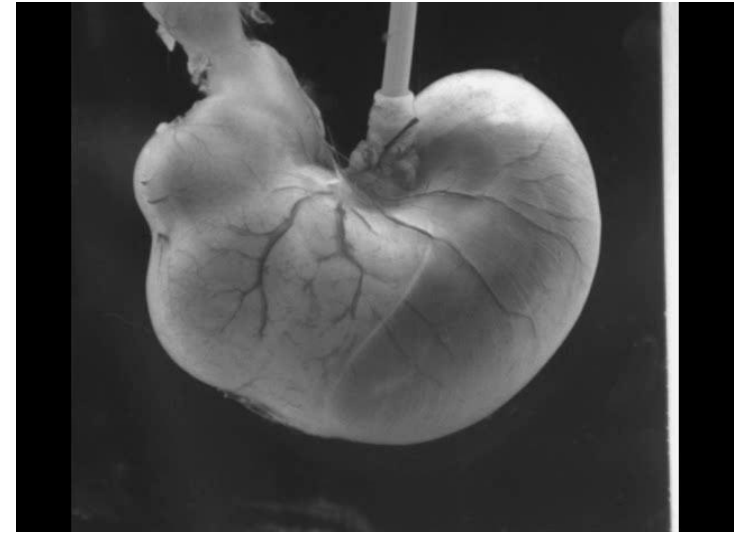
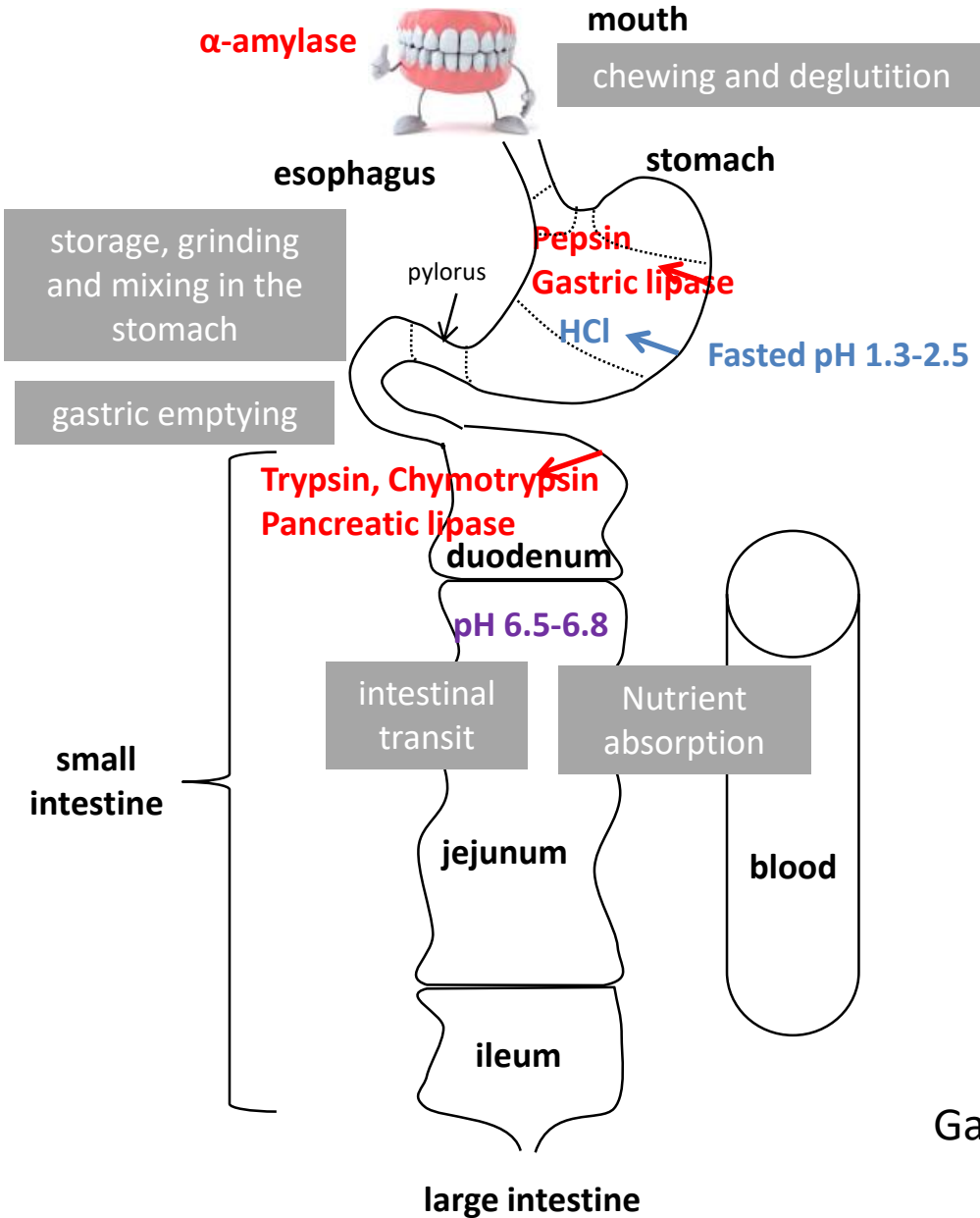
By increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health

Our goals

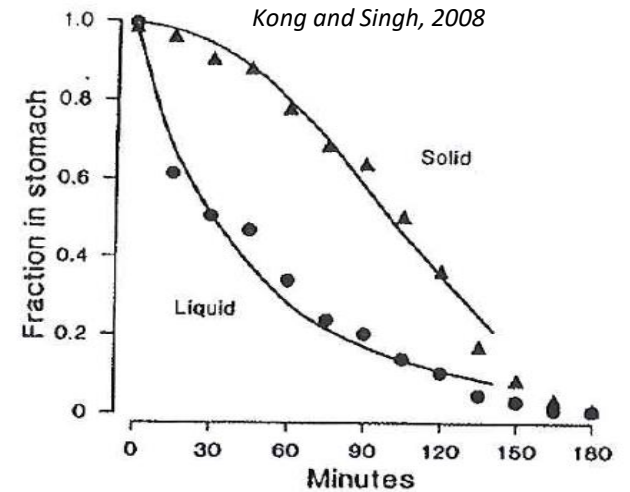


- ☞ To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion
- ☞ To determine the impact of the structure of food matrices on nutrient bioavailability
- ☞ To model these phenomena in order to develop a reverse engineering approach

The digestive process



From Roger Lentle, Massey Univ. NZ



Gastric phase = a very complex but crucial step for the whole digestion process

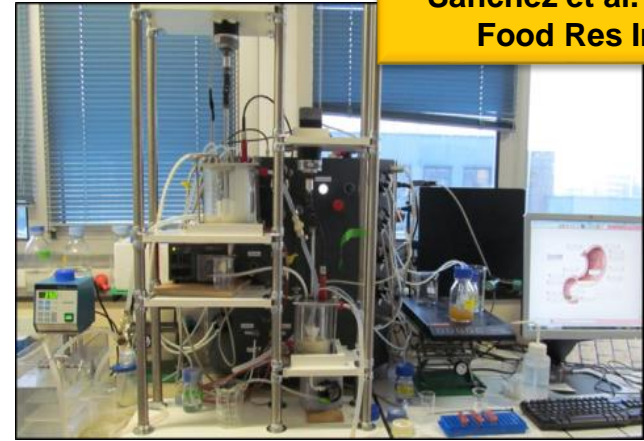
Models available at INRA for simulating digestion

Dupont et al. 2010ab,
Mol Nutr Food Res
Minekus et al. 2014
Food Funct



*In vitro static models
(infant, adult, elderly)*

Menard et al. 2014,
Food Chem
Sanchez et al. 2015
Food Res Int



*In vitro dynamic models
(infant, adult, elderly)*

Le Feunteun et al. 2014
Food Bioprocess Tech

*In silico
models*

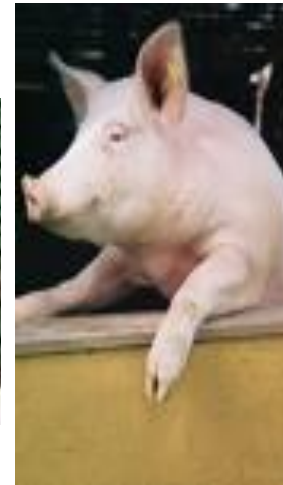
$$\Phi_{12} = k_{12\text{whey}} \times (V_1 - m_{\text{caswpd1}} \times \alpha) + k_{12\text{aggr}} \times m_{\text{caswpd1}} \times \alpha$$



*Human
models*



Animal models



Barbé et al. 2013, 2014
Food Chem
Le Huerou-Luron et al.
2016 Eur J Nutr

De Oliveira et al. 2016
Am J Clin Nutr
De Oliveira et al. 2017
Clin Nutr



Food structure as modified by processing affects the kinetics of food digestion

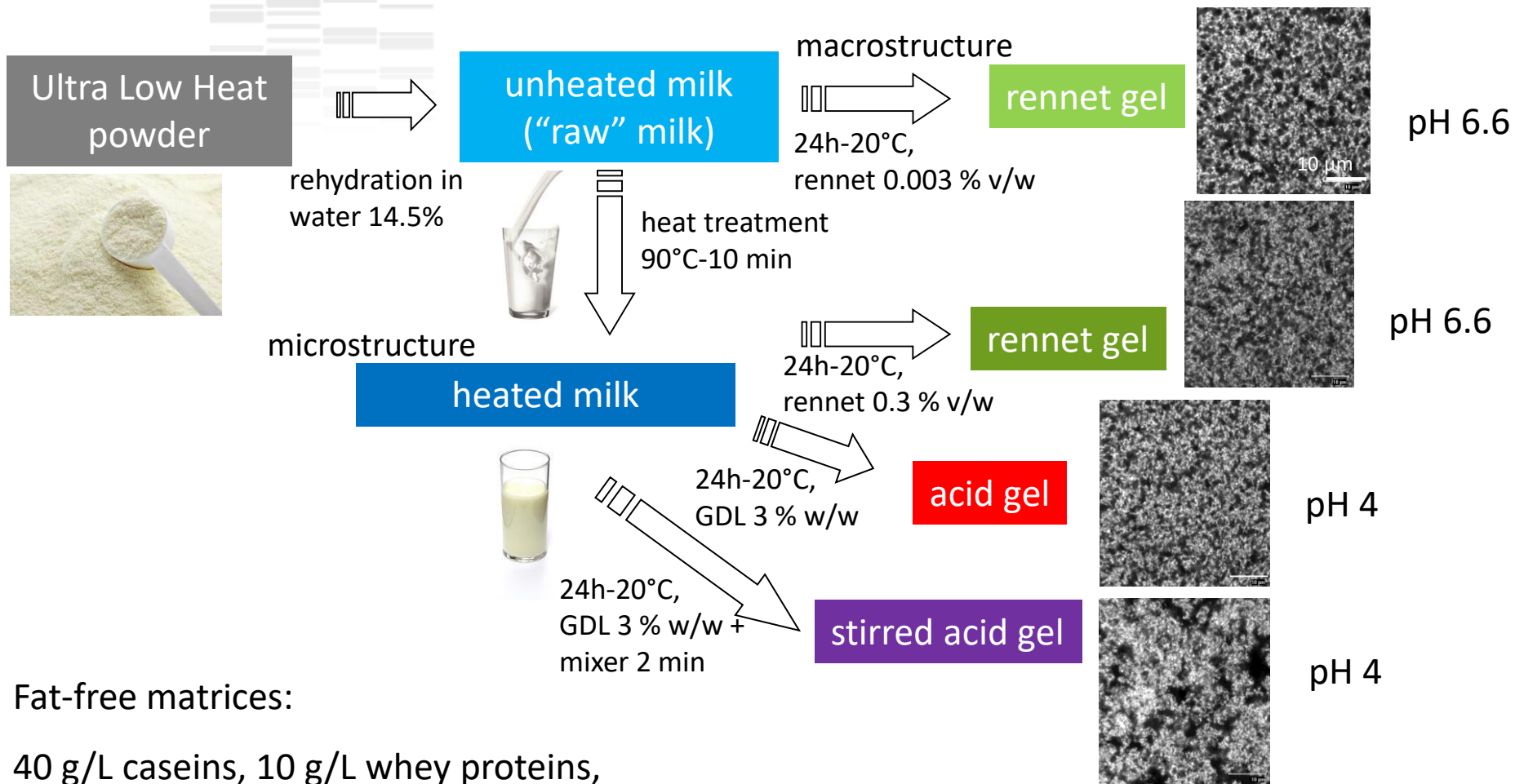


Dupont D.

INRA, Rennes, France



Comparison of 6 dairy products of identical composition but different structure



Fat-free matrices:

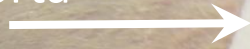
40 g/L caseins, 10 g/L whey proteins,
95 g/L lactose and minerals

+ marker of the meal transit (Cr^{2+} -EDTA) → Gastric emptying half-time

The multi-cannulated mini-pigs

6 minipigs (20 ± 1kg)

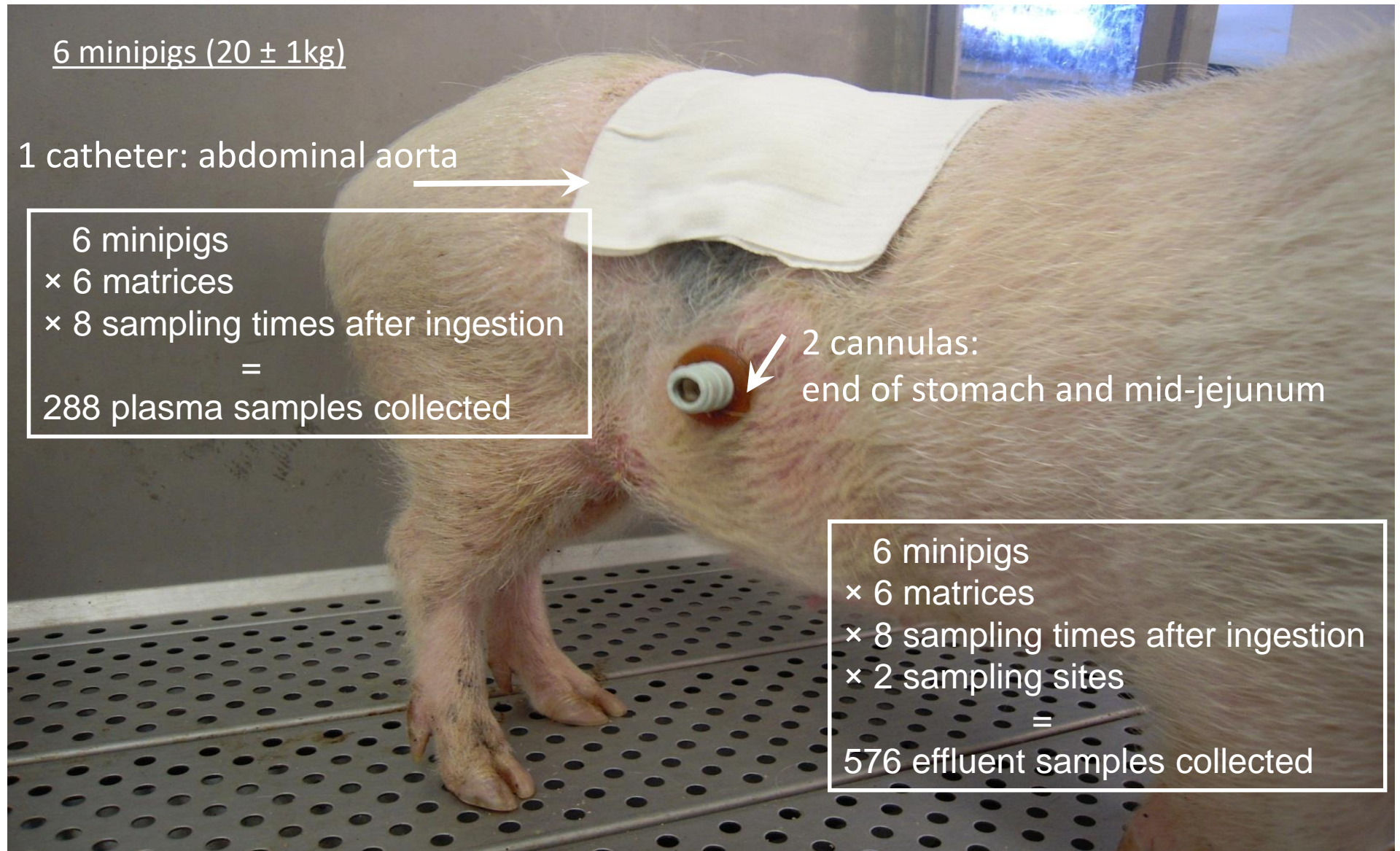
1 catheter: abdominal aorta



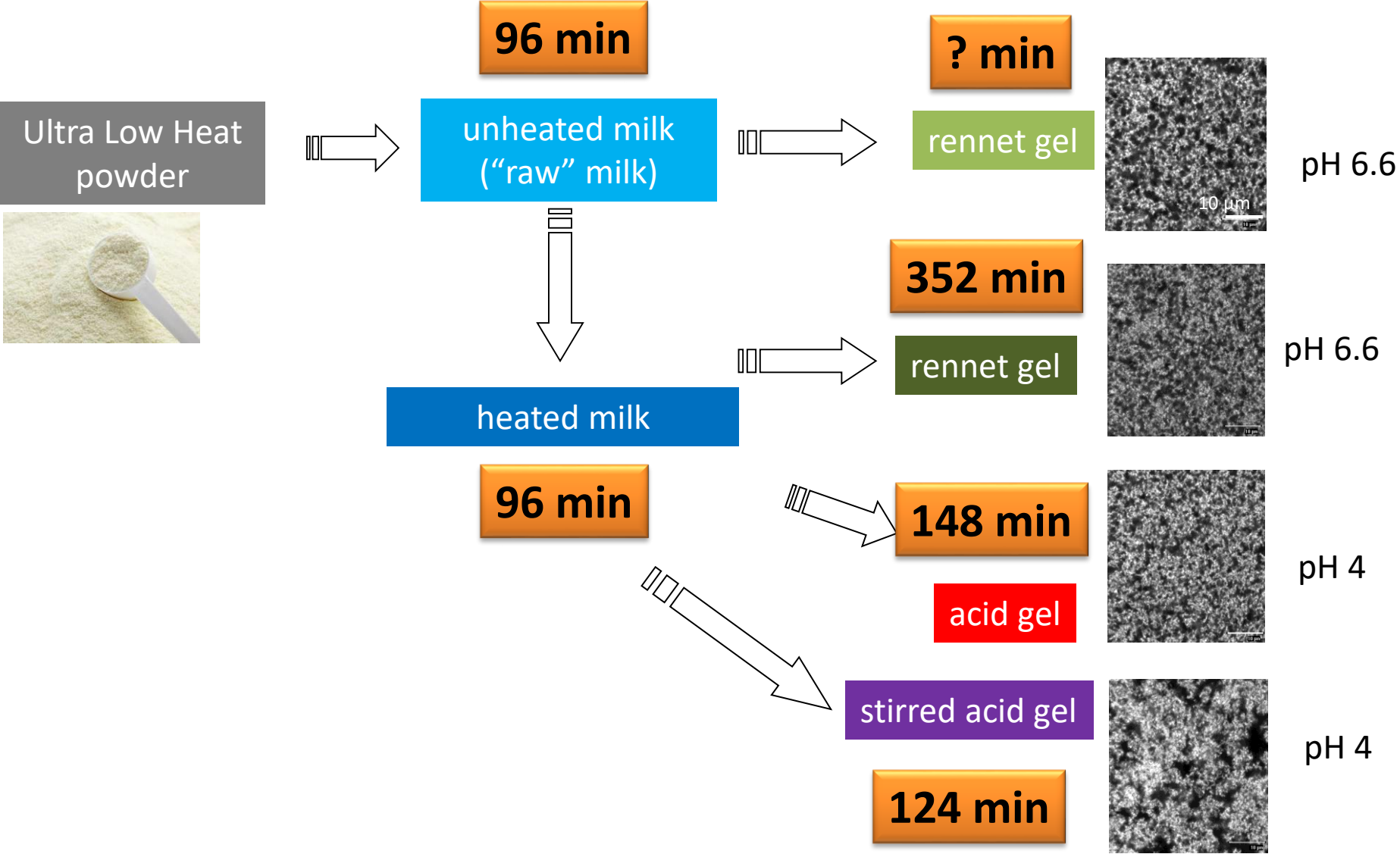
6 minipigs
× 6 matrices
× 8 sampling times after ingestion
=
288 plasma samples collected

2 cannulas:
end of stomach and mid-jejunum

6 minipigs
× 6 matrices
× 8 sampling times after ingestion
× 2 sampling sites
=
576 effluent samples collected

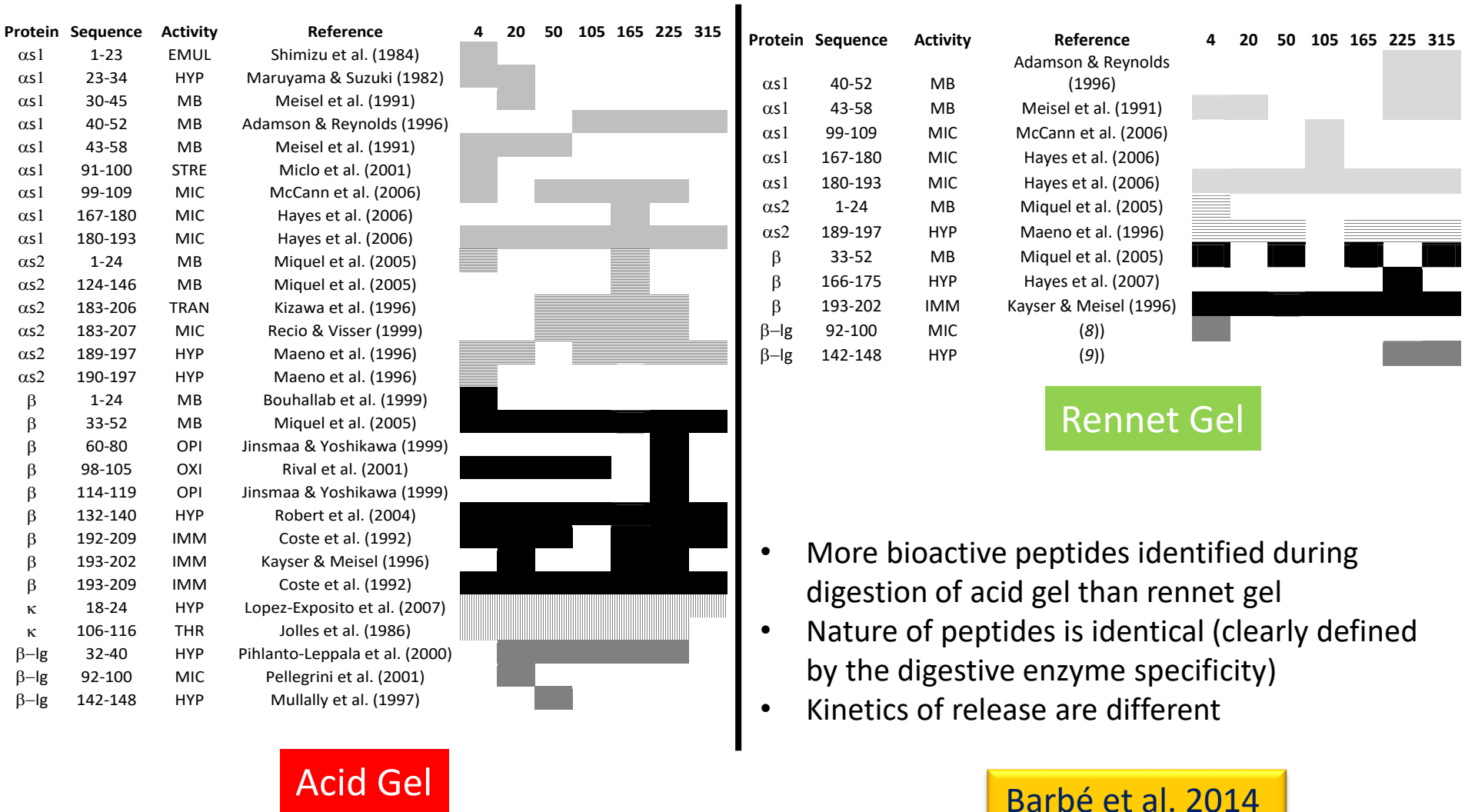


Gastric emptying half time



Bioactive peptides released during digestion differ from one matrix to another

More than 16000 peptides identified by LC-MS-MS in the jejunum



Rennet Gel

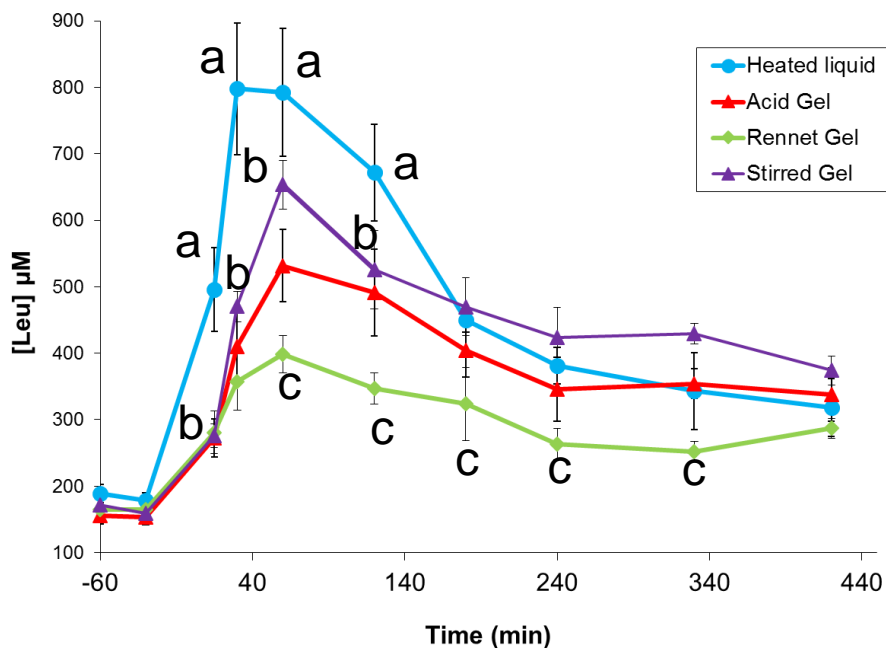
Acid Gel

Barbé et al. 2014
Food Res Int

- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
- Kinetics of release are different

The liquid-gel transition

Effect on absorption



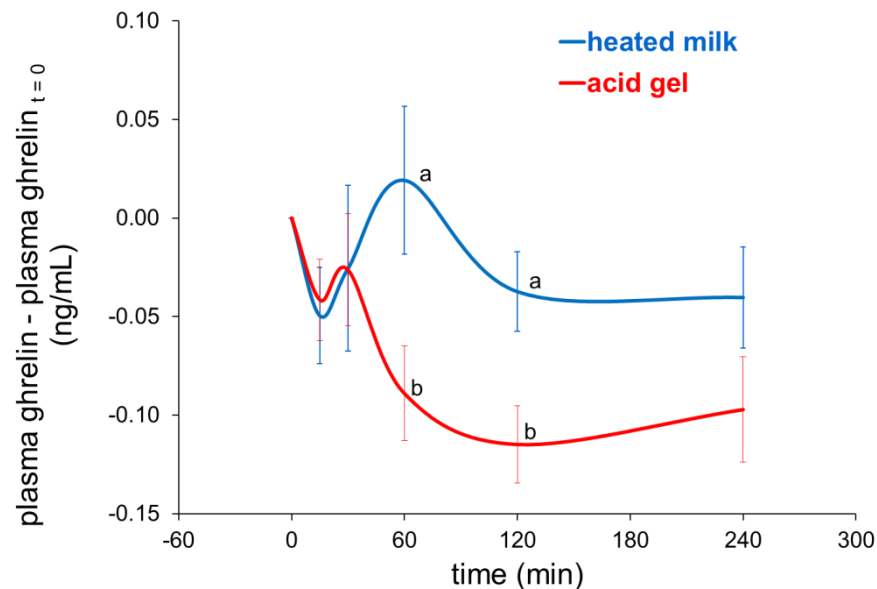
milk gelation:

→ delayed proteins transit → delayed AA absorption

↘ maximal AA concentration in the plasma

Potential effect on satiety

ghrelin (gastrointestinal hormone → appetite stimulation)



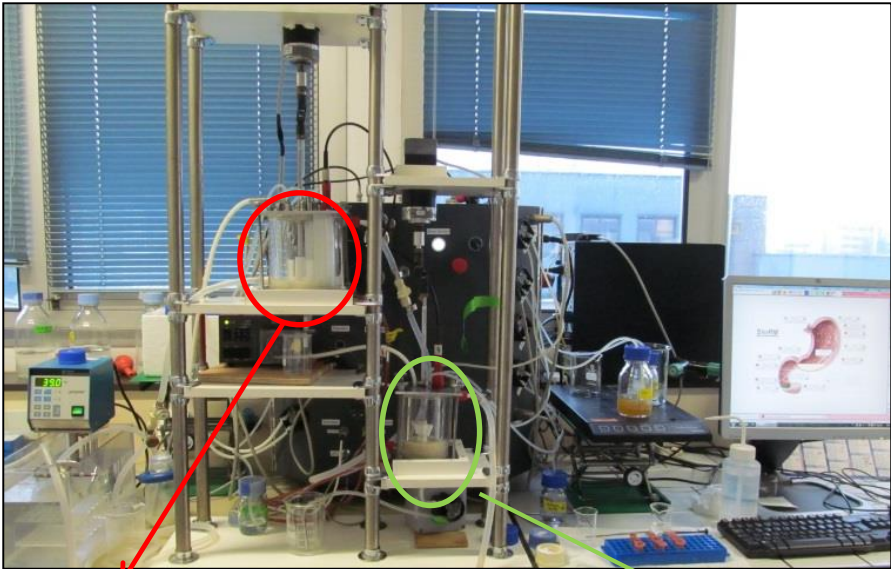
milk gelation:

↘ postprandial ghrelin concentration =
↑ satiety ?

Differential behaviour of acid/rennet gels in gastric conditions

- ☞ Acid/Rennet gel: identical composition, similar rheological properties and pore size
- ☞ ≠ Time of residence in the stomach (Acid 148 min /Rennet 352 min)
 - ☞ How can we explain this difference? Dynamic *in vitro* digestion of the 2 gels

Ménard *et al.*
Food Chem 2014



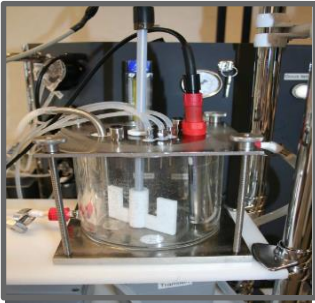
DIDGI®

StoRM® software

Stomach

Small intestine

- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl



Emptying :
Elashoff's model



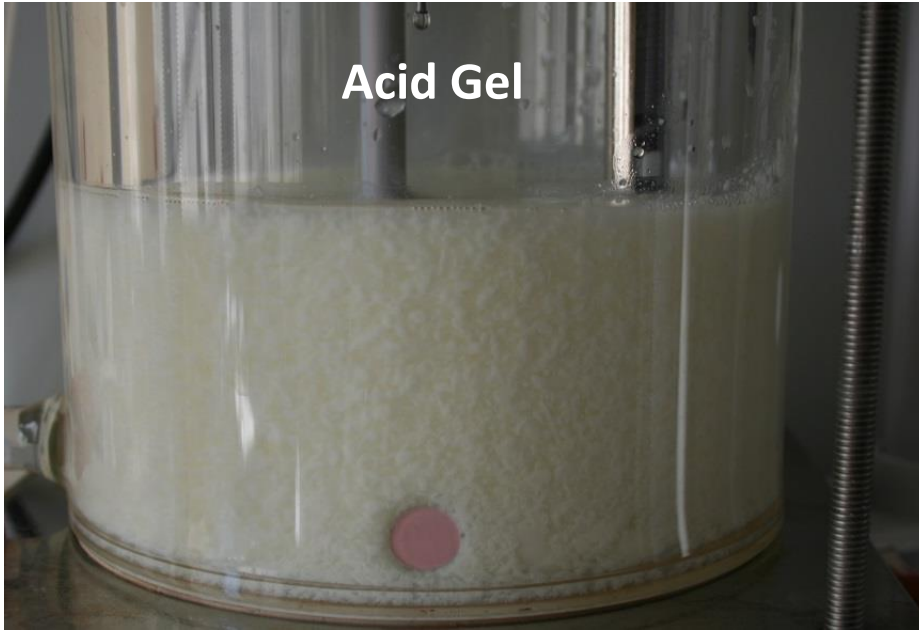
- Pancreatin
- Bile
- Simulated intestinal fluid
- NaHCO₃

Emptying :
Elashoff's model

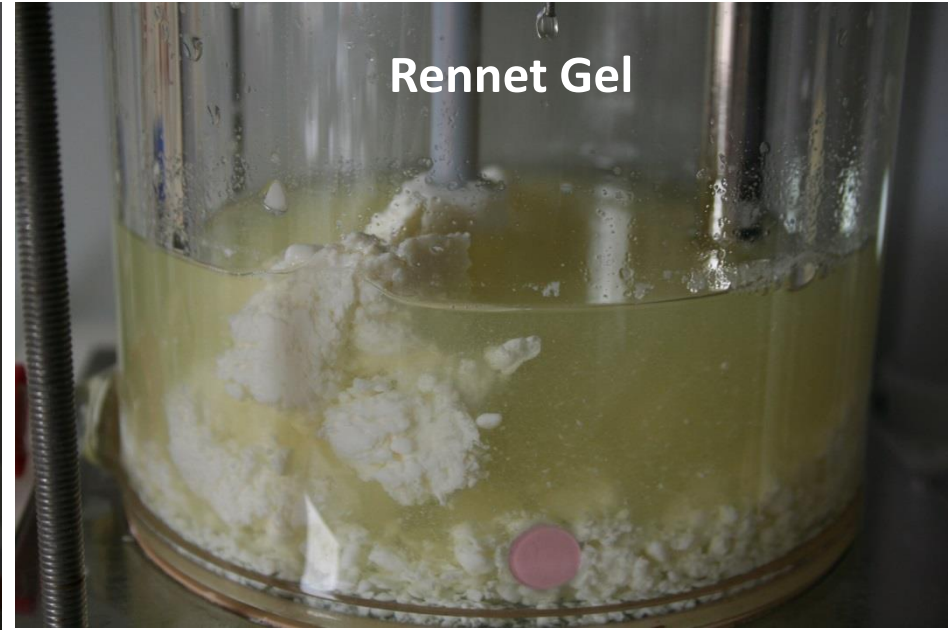
Behaviour of acid and rennet gels in the stomach during *in vitro* dynamic digestion

Barbé et al.
Food Chem. 2014

Acid Gel



Rennet Gel



Formation of a strong coagulum with rennet gel → slow down the gastric emptying of caseins

The structure that a food adopts in the stomach is essential to understand its digestion

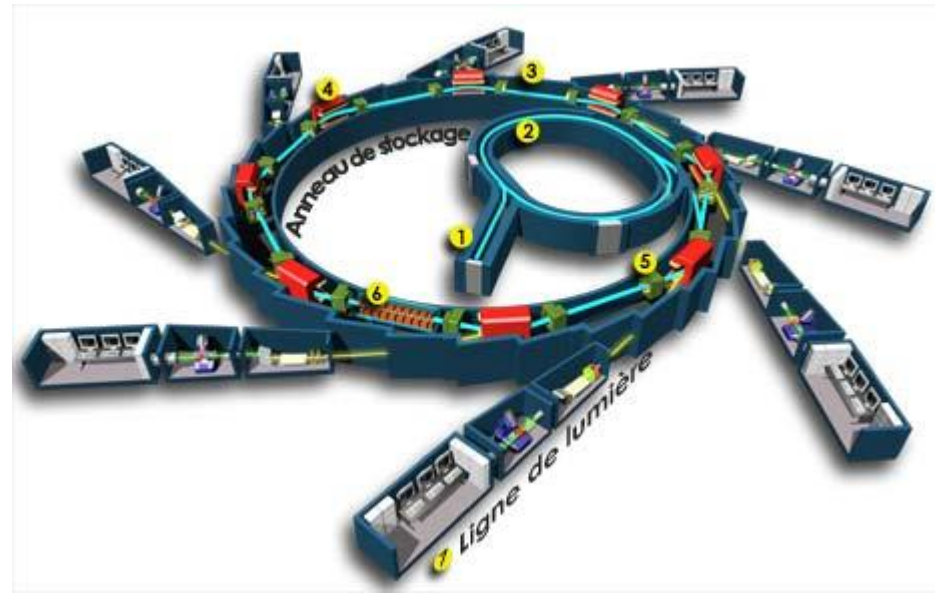


Soleil is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful source of light that permits exploration of inert or living matter

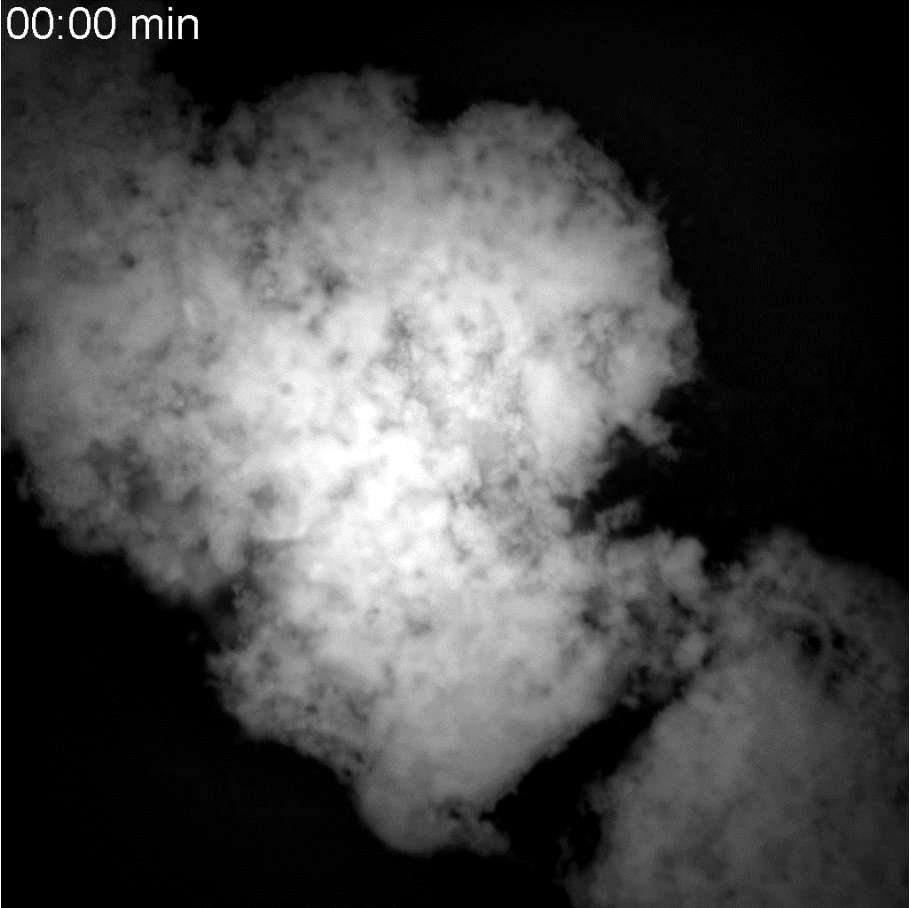
DISCO

DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light

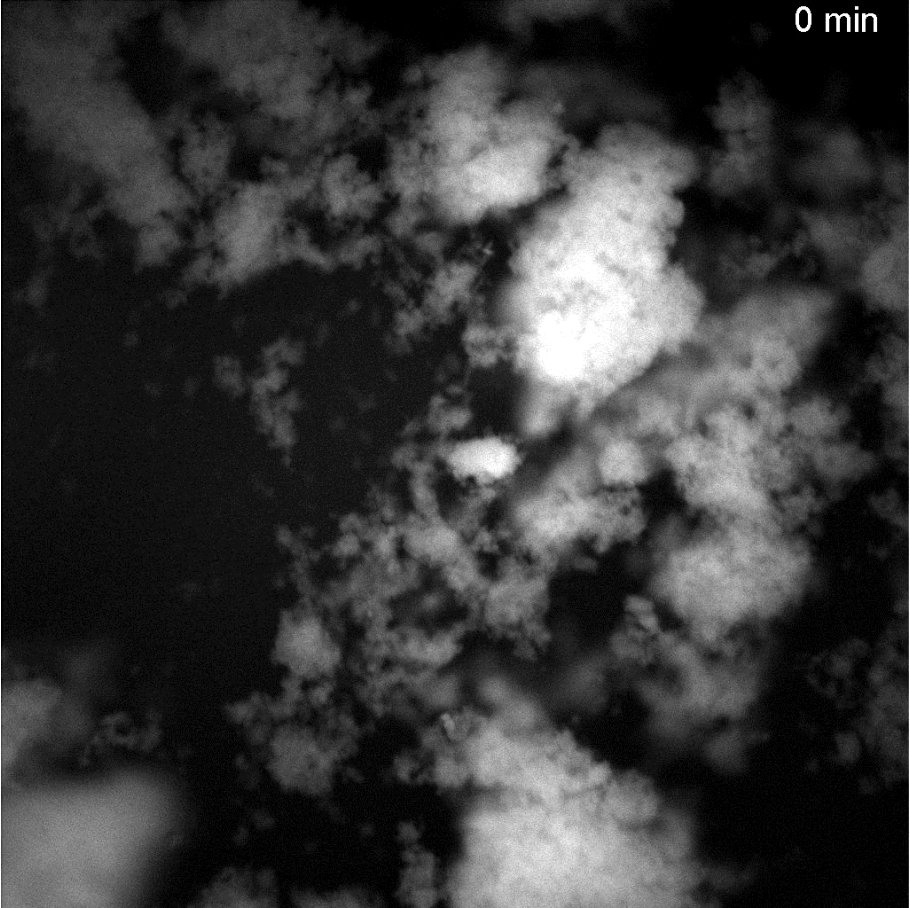
☞ Allow the imaging of protein intrinsic fluorescence with a UV microscope



Kinetics of gel particles disintegration

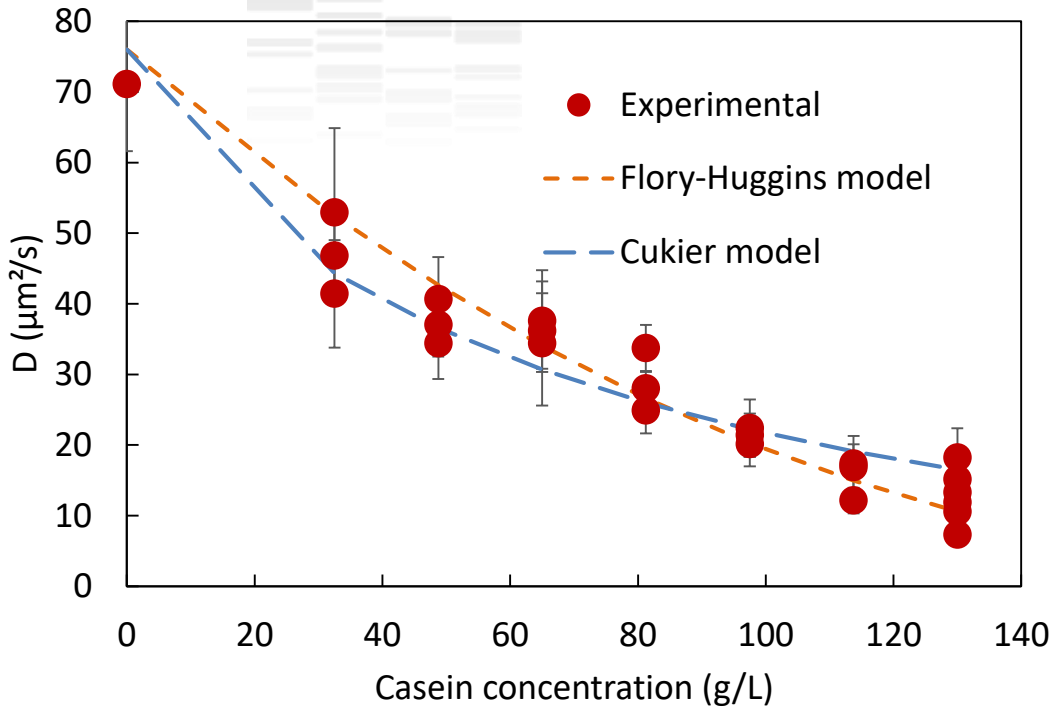


Rennet Gel



Acid Gel

Diffusion of FITC-pepsin in Rennet gel of increasing casein concentration (37°C)

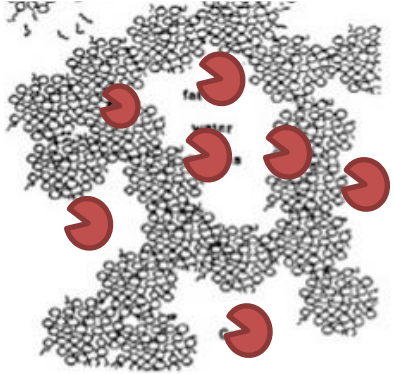


Thevenot et al. Food Chem 2017

Pepsin diffusion of enzyme through the protein network is possible



Food proteins can effectively be hydrolyzed by pepsin from the interior of the gel pores.



What happens when food have the same macrostructure but different microstructures? The case of egg white gels



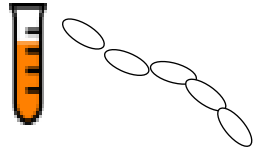
Dupont D.

INRA, Rennes, France

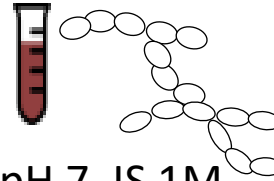


The microstructure of egg-white gels made from different types of aggregates affects the kinetics of proteolysis

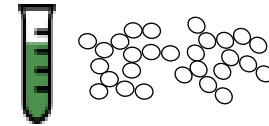
80°C/6h



pH 9, IS 1M

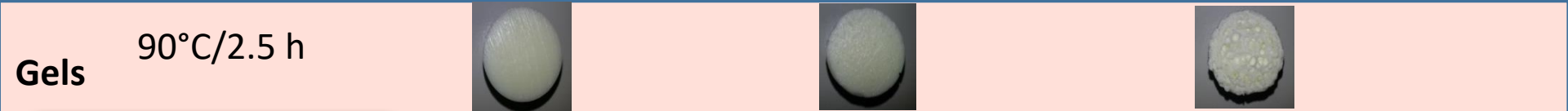


pH 7, IS 1M



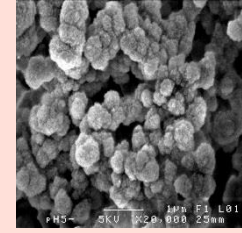
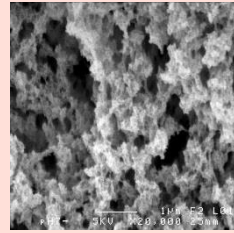
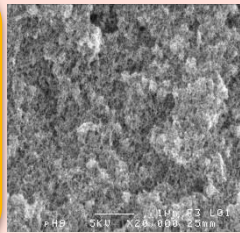
pH 5, IS 1M

Aggregates	linear	branched	spherical
Rate of <i>in vitro</i> digestion	+++	++	+

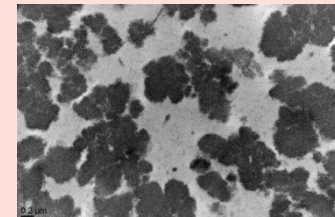
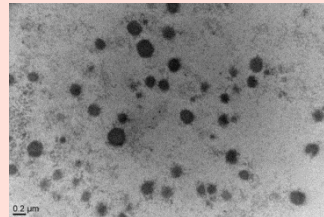
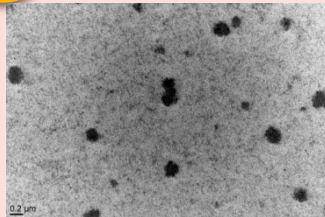


Nyemb et al., 2016. Food Hydrocolloid, 54, 315-27

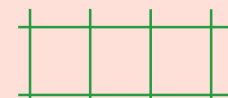
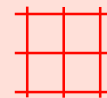
Nyemb et al., 2016. Food Res. Int., 88, 302-9



SEM



CRYO-TEM



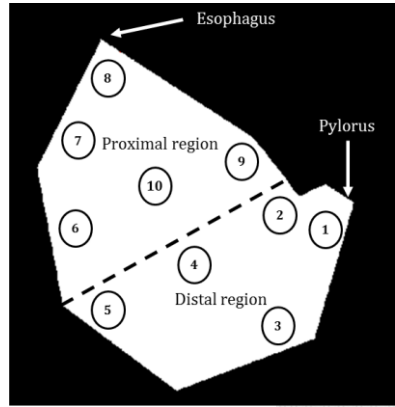
Rate of *in vitro* digestion

+

++

+++

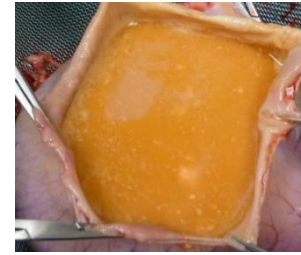
Spatio-temporal evolution of pH during an *in vivo* digestion



20 min



60 min

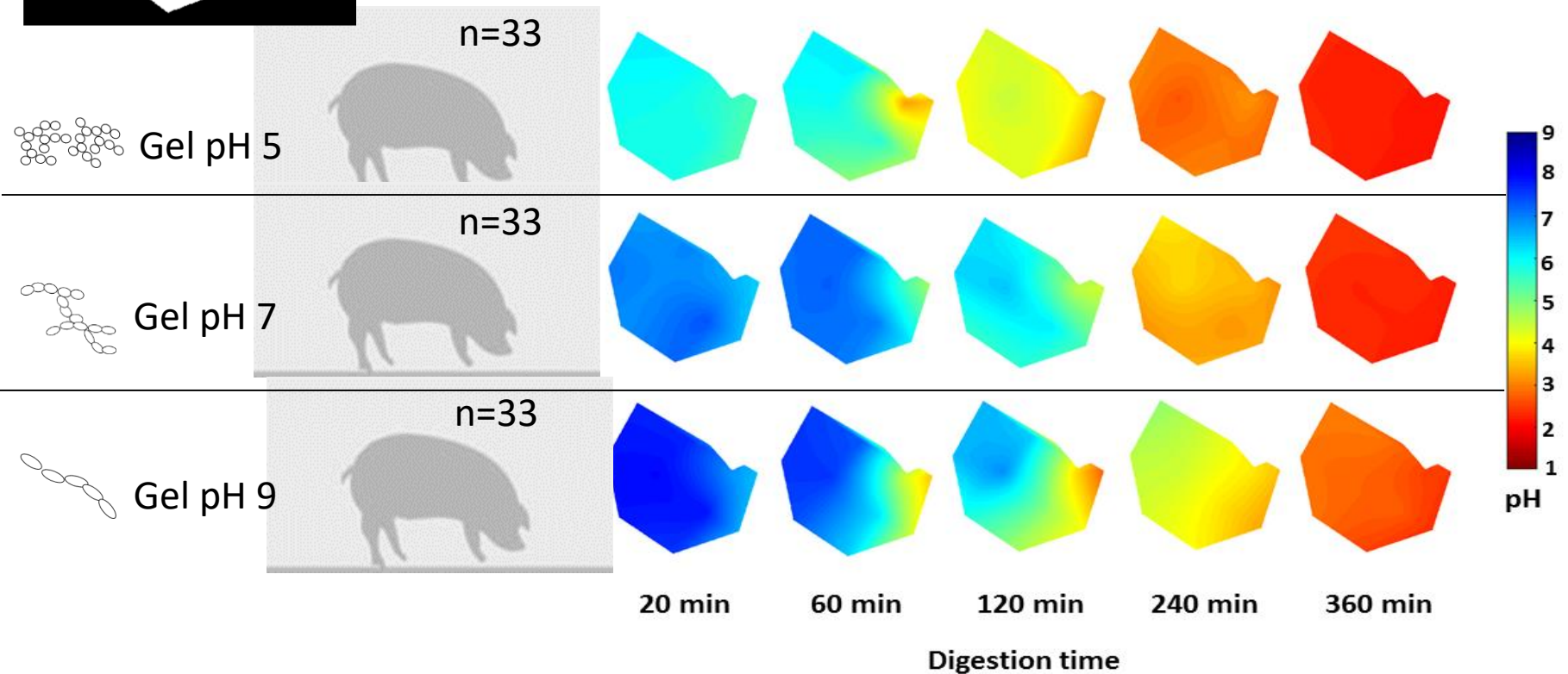


120 min

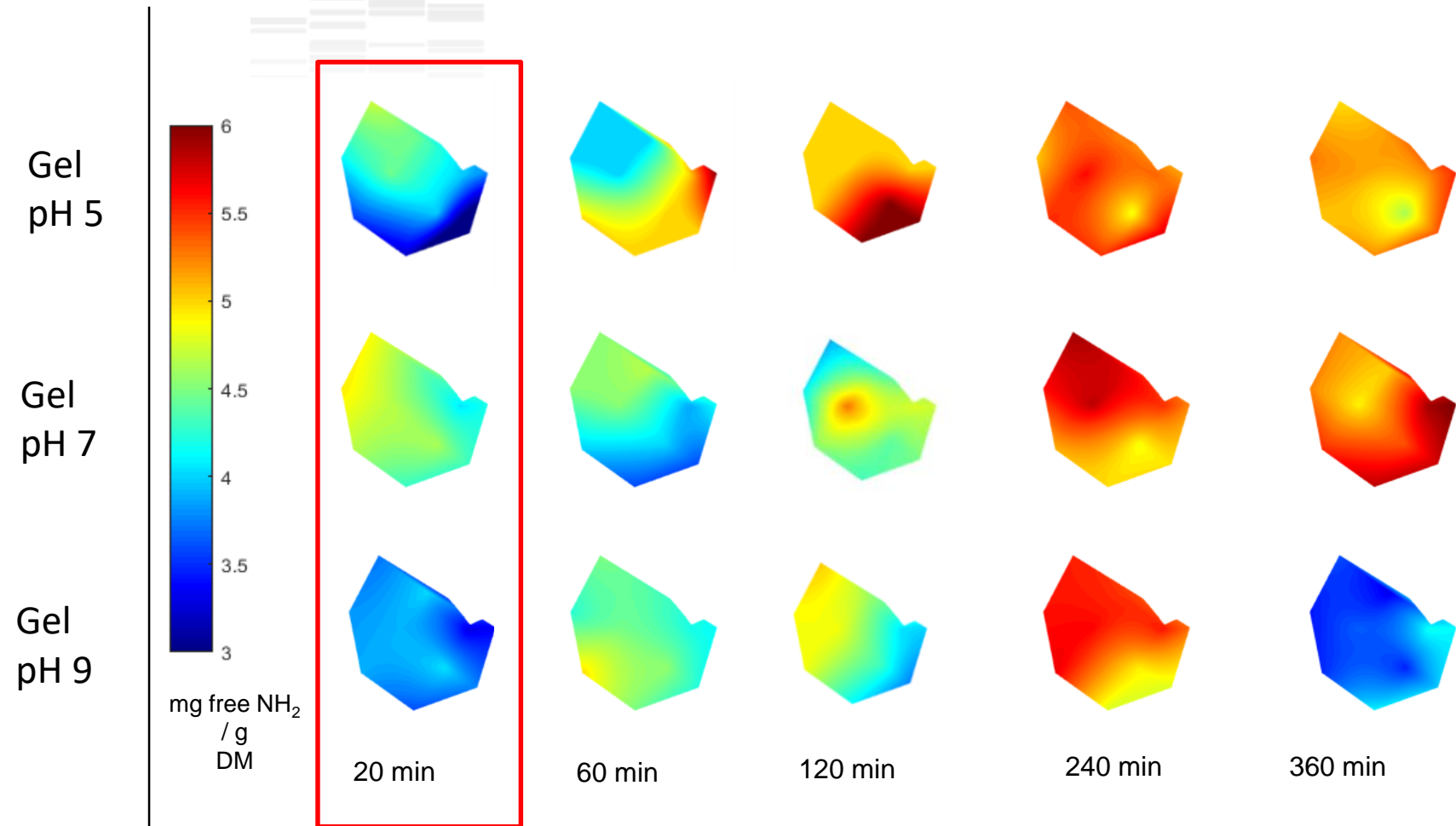


240 min


pH 7.0



Kinetics of proteolysis



Proteolytic activity even at pH 7 and 9: probably driven by local pH



Understanding human milk digestion to design new infant formulas that will have the same behaviour in the GI tract



Deglaire A., Menard O., De Oliveira S., Bourlieu C.
& Dupont D.

INRA, Rennes, France



Human/ bovine milk / Infant Formula

Protein structures

Human milk



Bovine milk



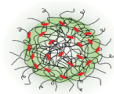
Infant Formula



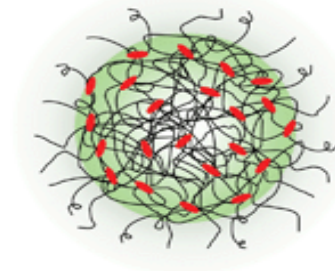
(Turck *et al*, 2010)

$\varnothing = 64 \text{ nm}$
(β , κ -casein)

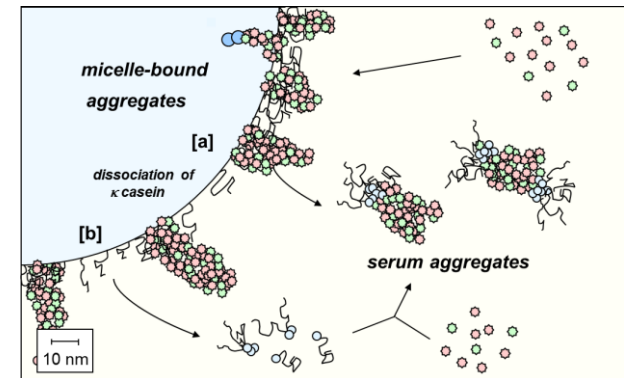
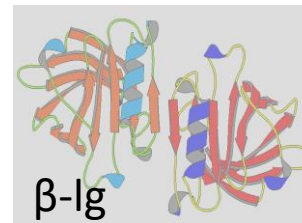
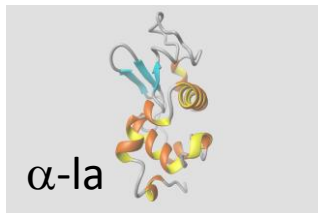
Casein
micelle



$\varnothing = 182 \text{ nm}$
(α 1, β -casein)



Whey
Proteins



Human/ bovine milk / Infant Formula

Lipid globule structure

Human milk

Bovine milk

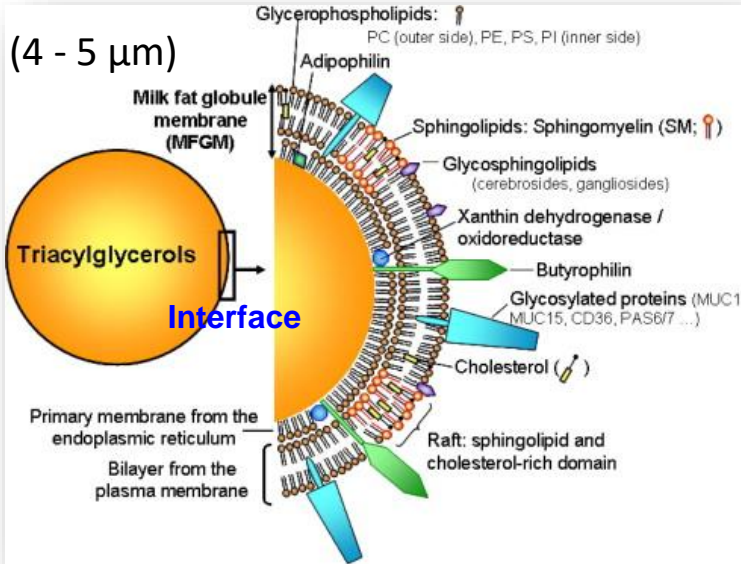
Infant Formula



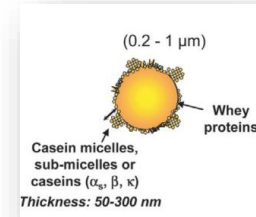
Native milk fat globule

Lipid droplets

Triacylglycerols




(Lopez, 2010)



(0,2 - 1 μm)

(Lopez and Briard-Bion, 2007)



Preterm hospitalized infants
Feeding nasogastric tube
Fed every 3 hours

Aim: to compare meals with similar composition but different structure

Human milk from their **own mother**

Human milk from **anonymous donor**

GROUP A

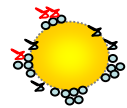
GROUP B



Raw human milk

Pasteurized human milk

Pasteurized human milk



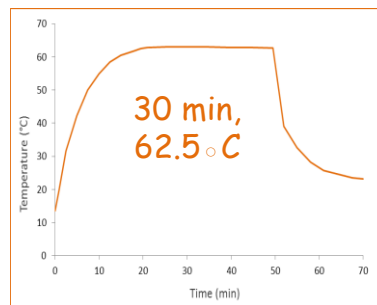
Pasteurized-homogenized human milk

24h before feeding (4°C)

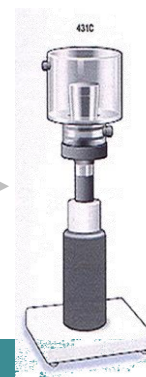
Stored at -20°C
(bank milk)

Stored at -20°C (bank milk)

Bank milk



Homogenization
by sonication

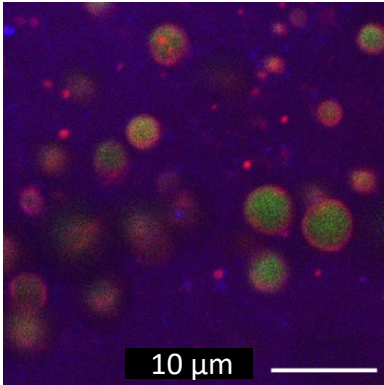


Pasteurization affected the initial structure and the emulsion disintegration of HM

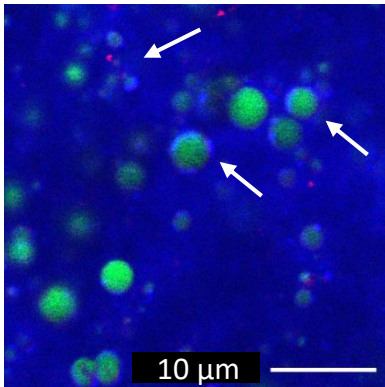
(n = 6 infants)

Initial structure

Raw HM



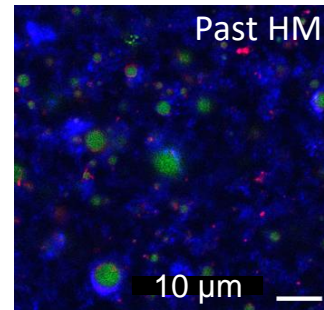
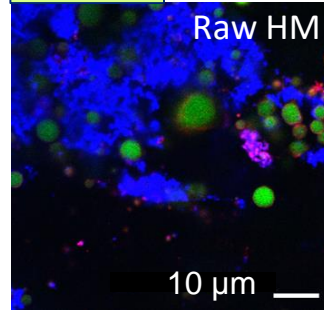
Past HM



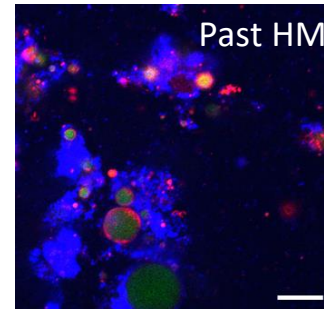
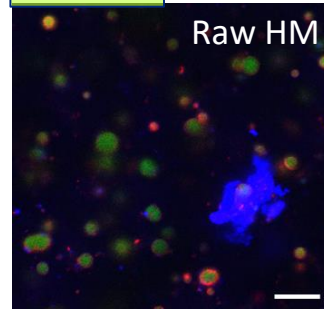
→ Protein heat-induced aggregation

Gastric disintegration

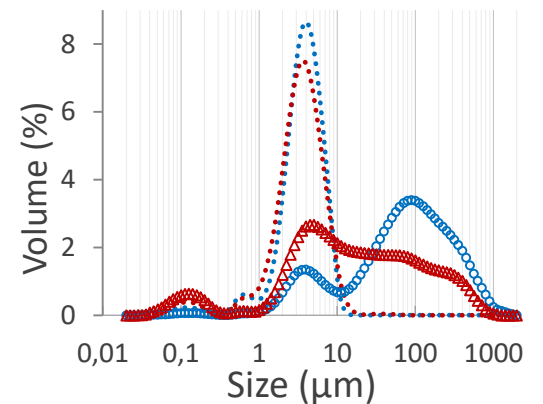
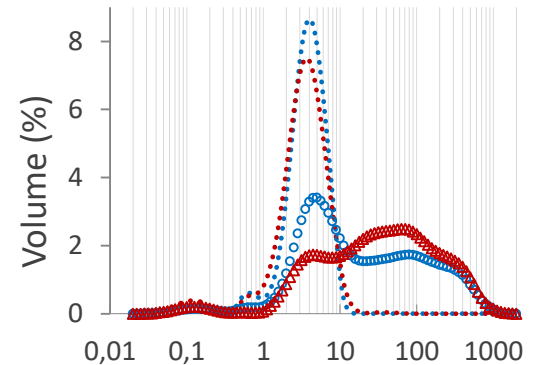
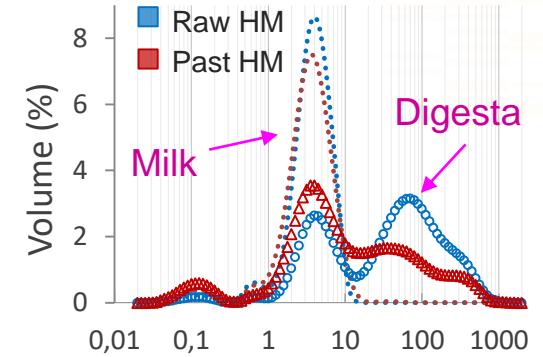
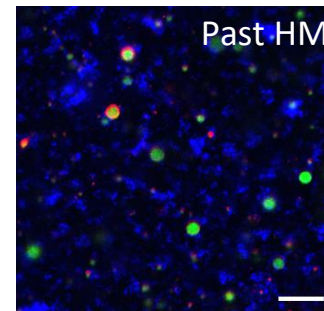
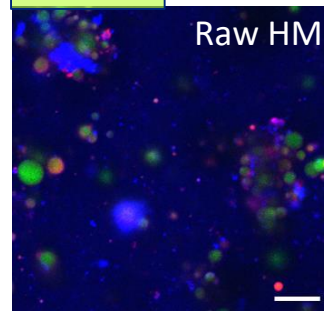
35 min



60 min



90 min

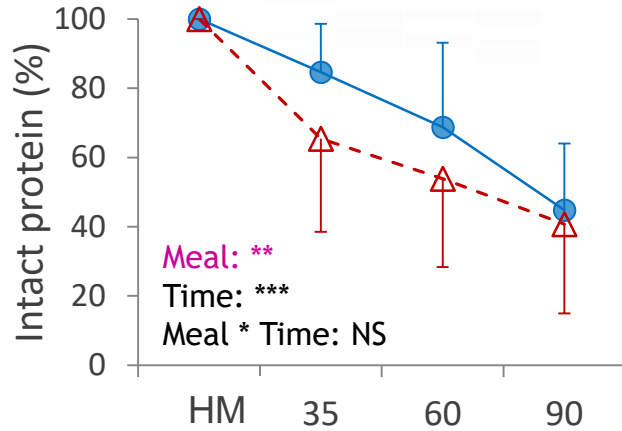


■ Apolar lipids ■ Amphiphiles ■ Proteins

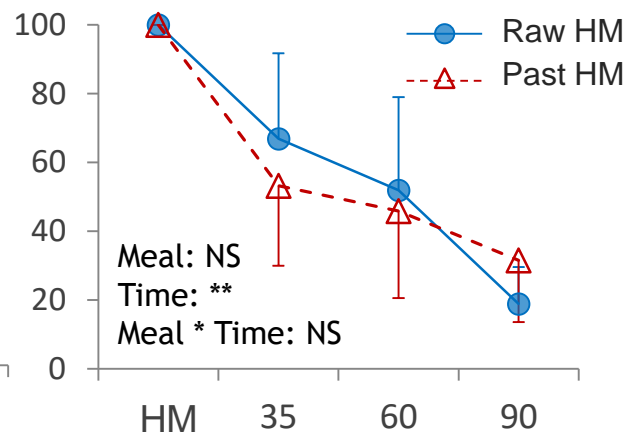
Pasteurization selectively impacted gastric proteolysis



Lactoferrin



Serum albumin

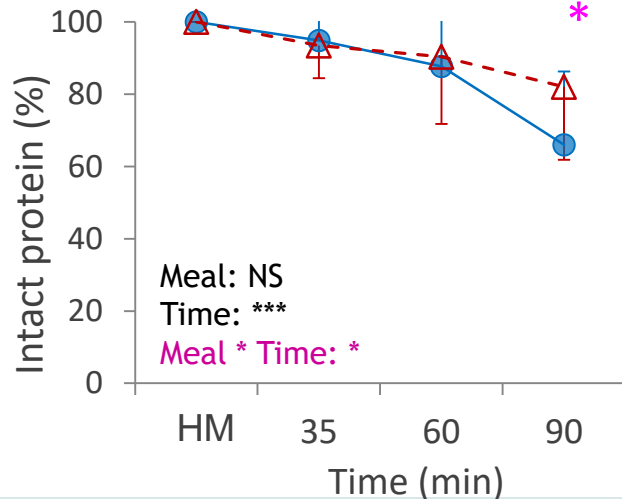


→ Faster decrease of lactoferrin in Past HM

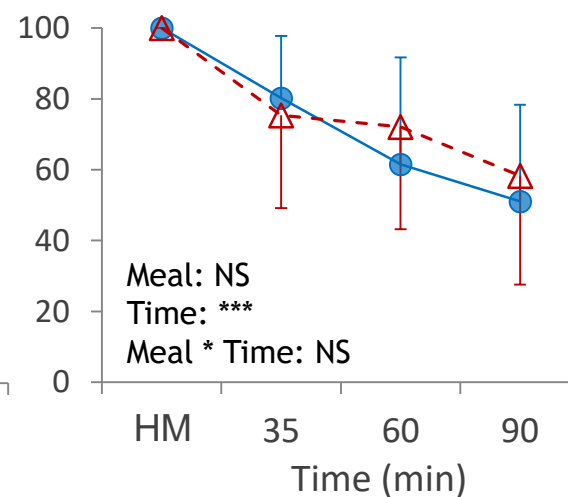
→ Slower decrease of α -lactalbumin in Past HM (only at 90 min)

→ α -lactalbumin was the most resistant to gastric digestion

α -lactalbumin



β -casein



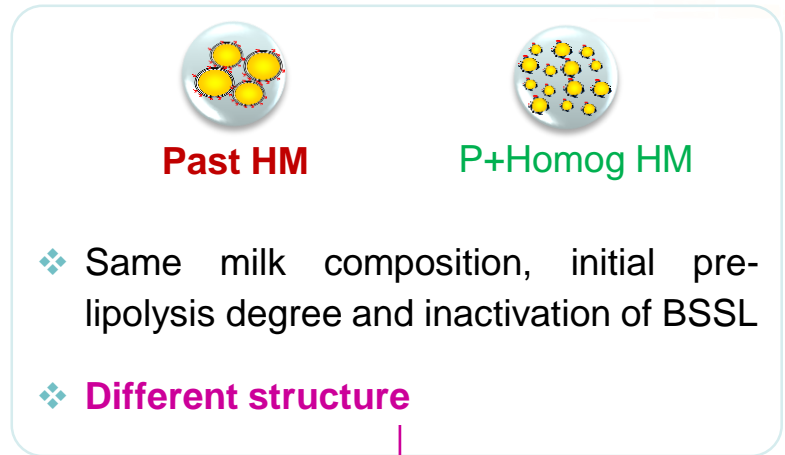
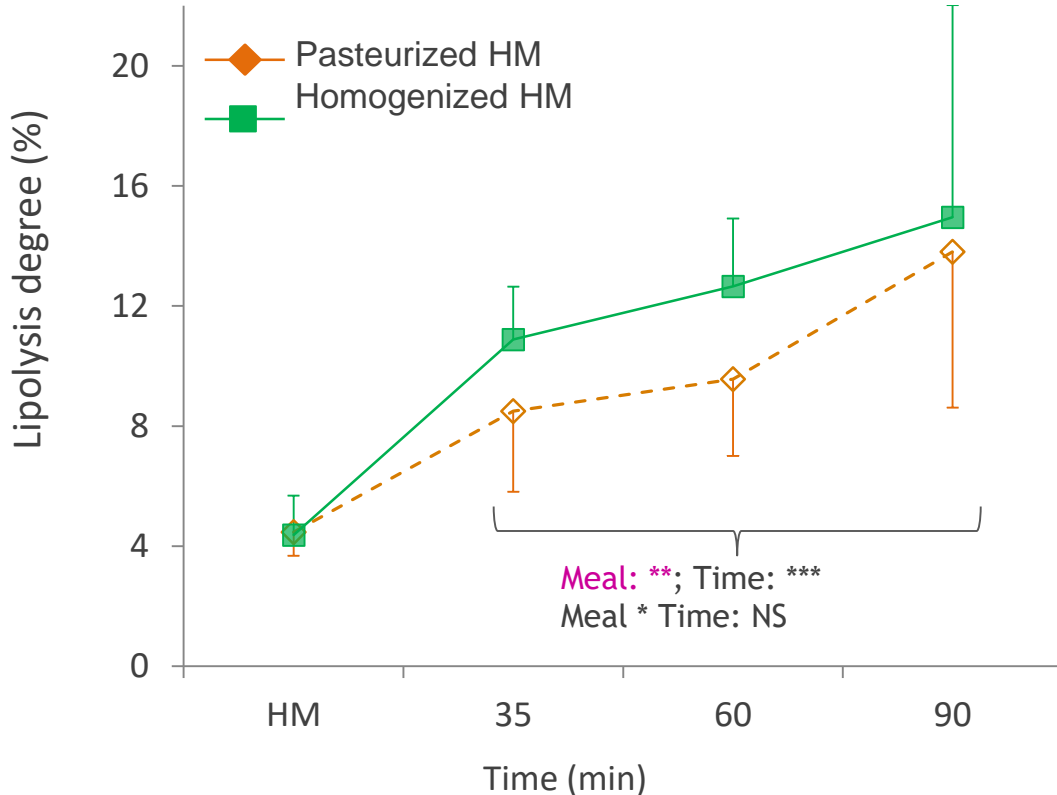
Impact on protective qualities of the native lactoferrin?

De Oliveira et al.,
Am J Clin Nutr 2017

Homogenization accelerates gastric lipolysis



Instantaneous lipolysis level

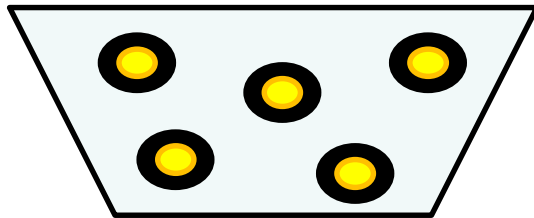


➔ Increase of specific surface of droplets facilitating HGL adsorption

De Oliveira et al.,
Clin Nutr 2017

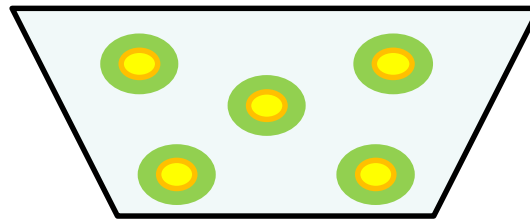
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula
T1



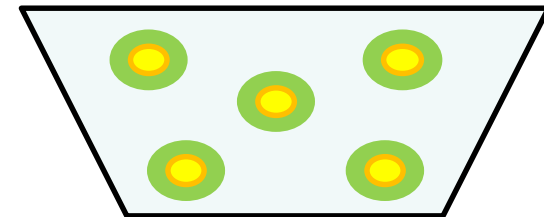
Interface 100 % Proteins
100% vegetable oil

Formula
T2

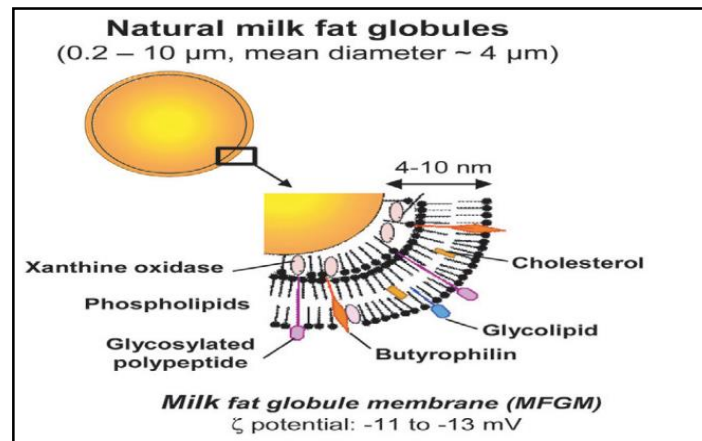


Interface 100 % phospholipids
100% vegetable oil

Formula
T3

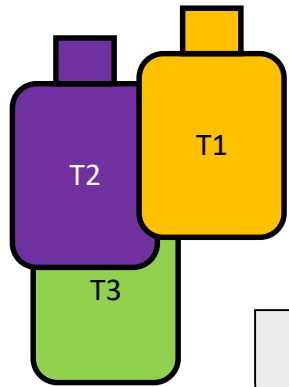


Interface 100 % phospholipids
40% vegetable oil + 60% milk fat



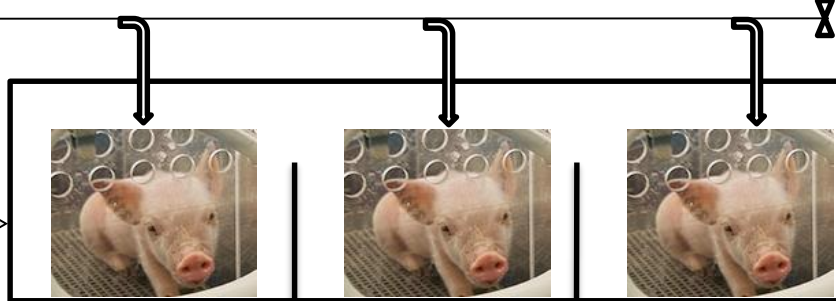
Lopez, (2007)

Can the composition of infant formula modulate the physiological response of the neonate?



- Veg
- Veg + PL
- Dairy Fat + PL

Automatic meal delivery (10 meals/ day)



+
Mother-fed piglets
(MF = + control)



Slaughtering after

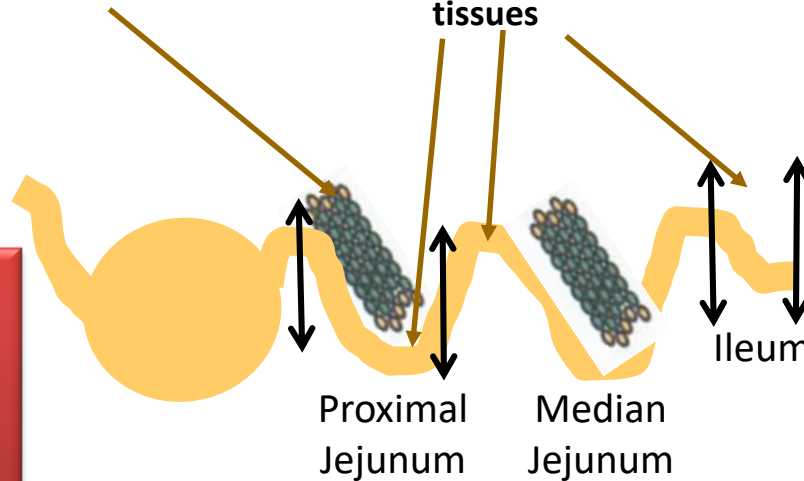
7 days

28 days

(90 min postprandial)

Mesenteric Lymph Nodes (MLN)

Collect of effluents and tissues



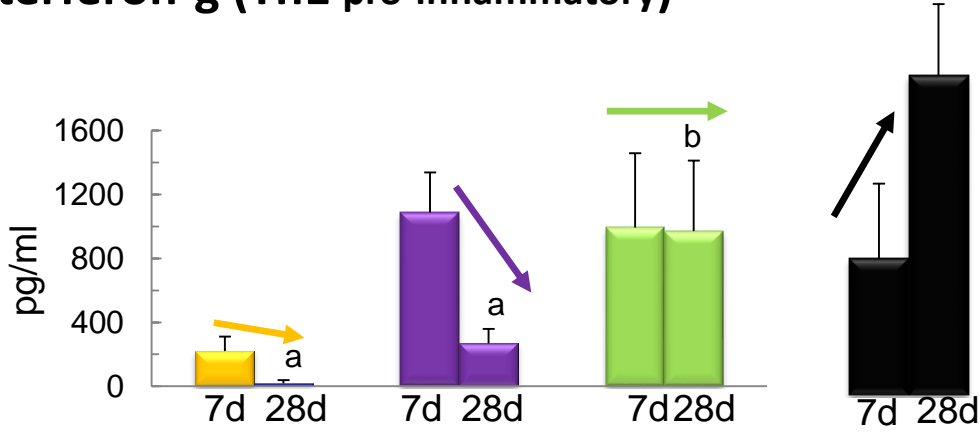
Effluents:
-SDS-PAGE
-Elisa

Tissues:

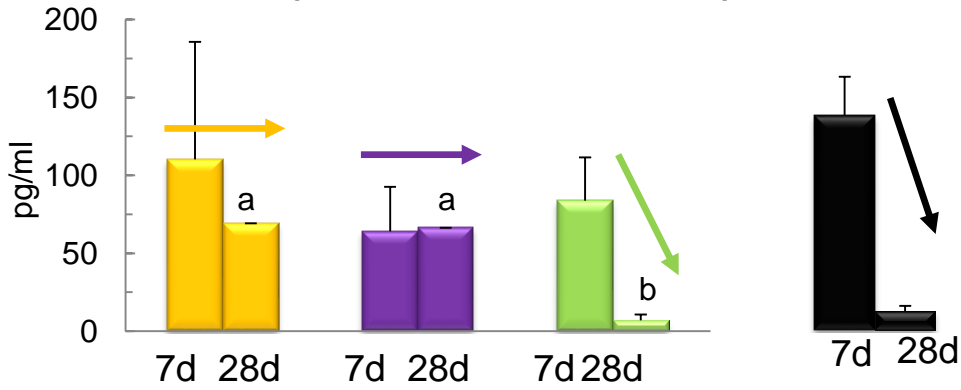
- Morphometry
- Enzyme Activities
- Intestinal Permeability
- Local immune response
- Microbiota

Secretory activity of MLN

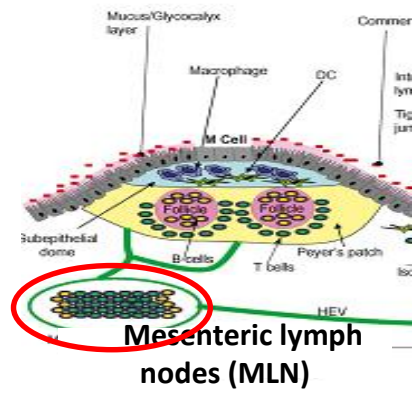
Interferon-g (Th1 pro-inflammatory)



Interleukine-10 (Th2 anti-inflammatory)



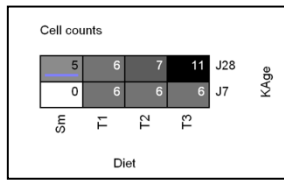
- Veg
- Veg + PL
- Dairy Fat + PL
- Porcelets SM



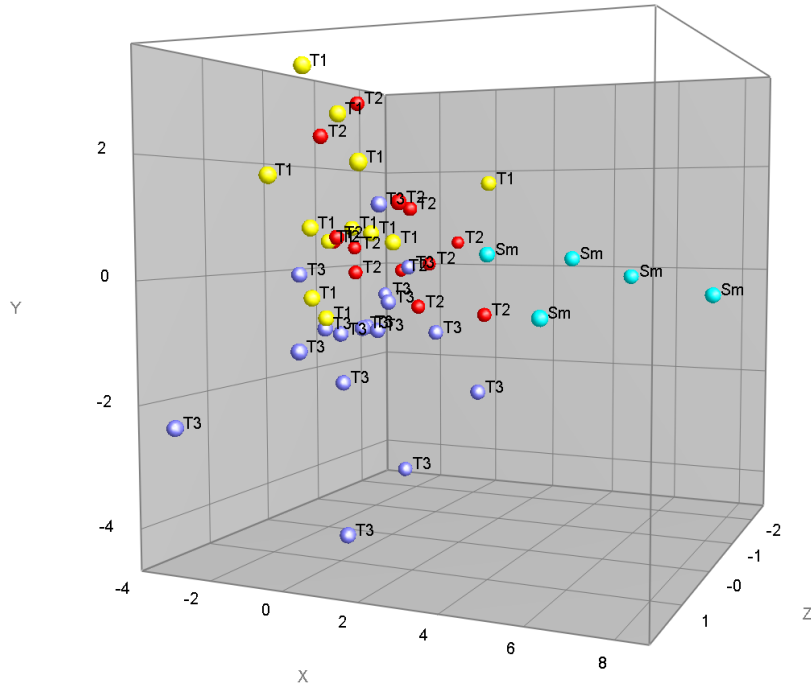
Milk lipids → maturation of the piglet's immune system more similar than with sow's milk

Le Huerou et al.
Eur J Nutr 2017

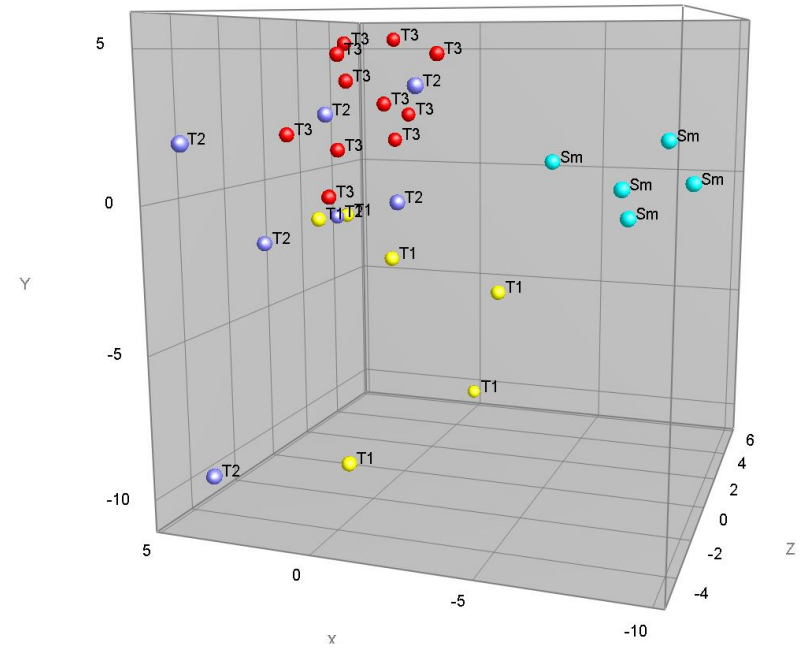
Microbiota by DHPLC



D7 & D28

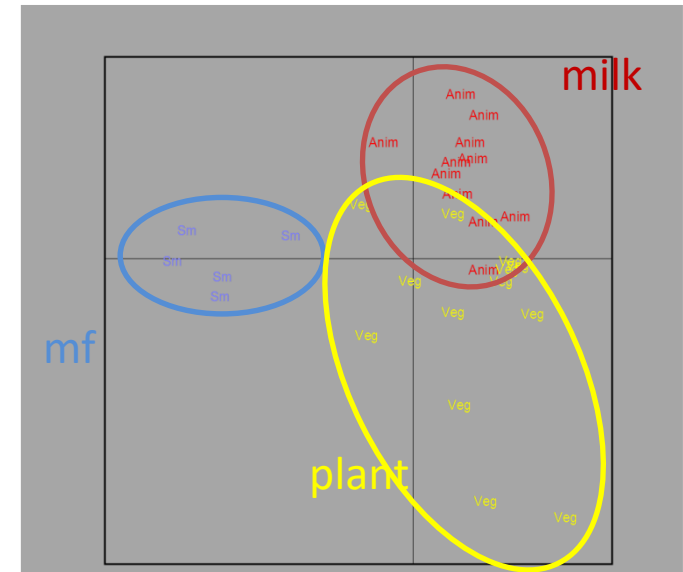


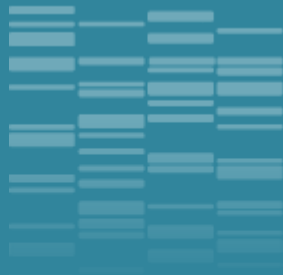
D28



The composition/structure of the infant formula « orientates » the microbiota

More Proteobacteria with milk fat /
More Firmicutes with plant oil



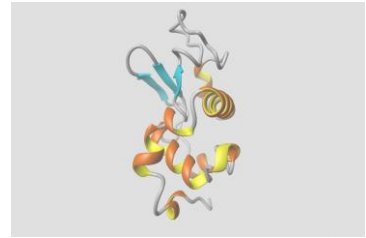


May bioactive peptides released
during digestion have an effect *in
vivo*?



In vivo digestion of milk proteins

- 16 subjects were fed with 30g of either:
 - Caseins
 - Whey proteins

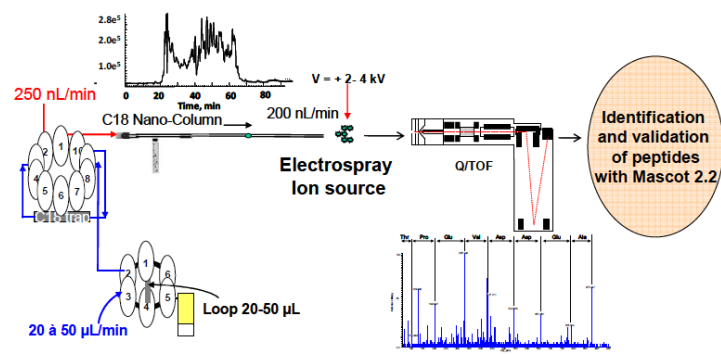


- Intestinal contents were collected through a double lumen nasogastric tube placed in their jejunum during 6 h after ingestion (location of the sampling site was controlled by radiography)



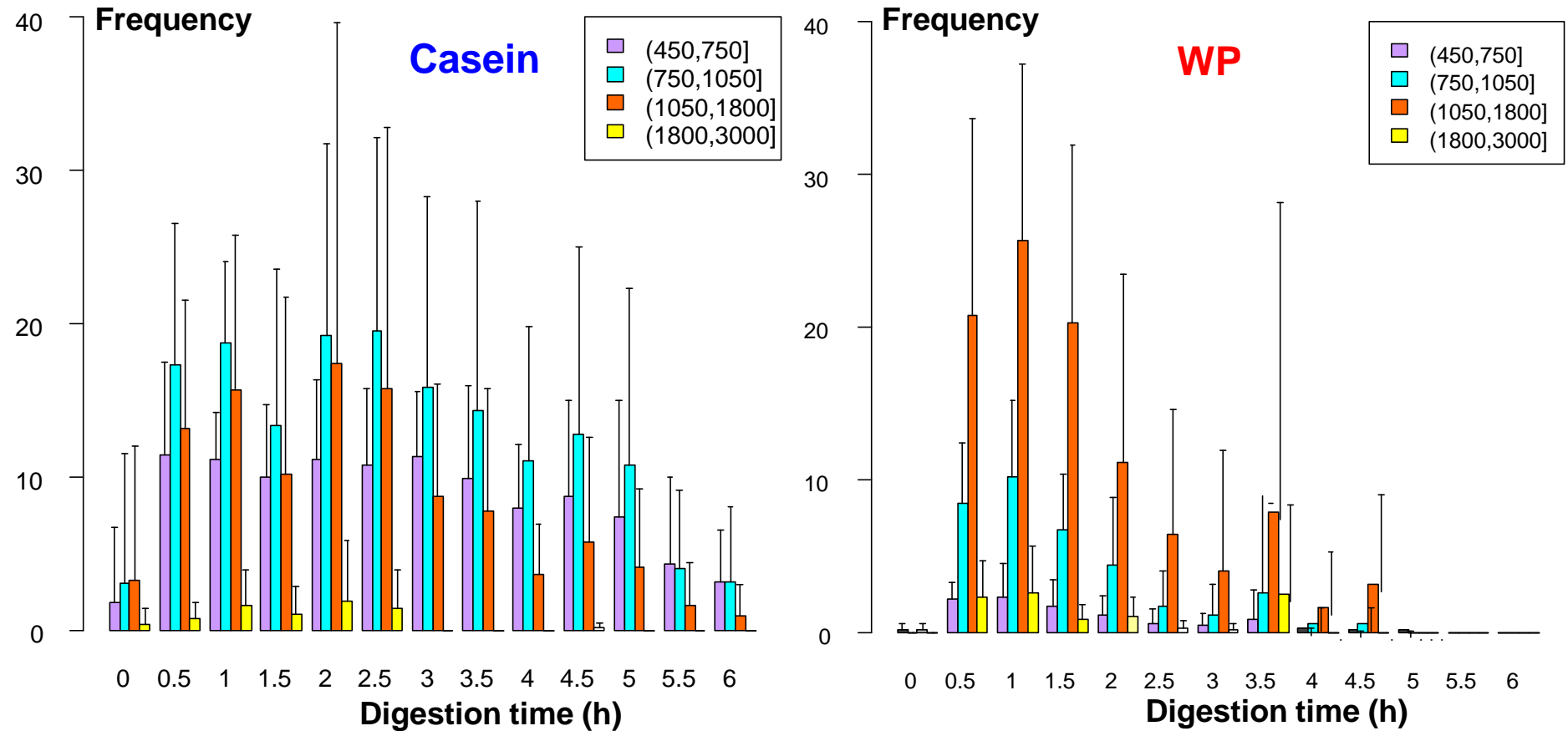
- Effluents were then freeze-dried until further use. Before analysis, effluents were rehydrated in 50 mM Tris HCl buffer pH 8.0 and 2M urea, centrifuged for 10 min a 2000 g

- Samples were characterized by ESI-MS-MS. Only peptides larger than 5 amino acids are considered



Kinetics of peptides release

↪ 4704 dietary peptides unambiguously identified



☞ Release of casein peptides throughout digestion

☞ Peptides generated from whey proteins digestion are larger

↪ Generation of a unique database of dietary peptides bioaccessible

A great variability in patterns but some peptides present in all subjects

31 peptides present throughout digestion

14 peptides found in all the subjects

1-6	RELEEL		
57-66	SLVYFPFGPI	57-66	SLVYFPFGPI
58-66	LVYFPFGPI		
58-68	LVYFPFGPIP		
59-66	VYFPFGPI	59-66	VYFPFGPI
59-67	VYFPFGPIP		
59-68	VYFPFGPIP	59-68	VYFPFGPIP
60-66	YFPFGPI	60-66	YFPFGPI
61-66	PFGPI	60-67	YFPFGPIP
73-79	NIPPLTQ		
73-80	NIPPLTQT		
73-82	NIPPLTQTPV		
80-91	TPVVVPPFLQPE		
81-87	PVVVPPF		
81-91	PVVVPPFLQPE	81-91	PVVVPPFLQPE
81-92	PVVVPPFLQPEV	81-92	PVVVPPFLQPEV
83-91	VPPFLQPE		
84-91	VPPFLQPE		
85-091	PPFLQPE		
107-113	KEMPFK		
108-113	EMPFK	108-113	EMPFK
114-119	YPVEPF	114-119	YPVEPF
134-139	HLPLPL		
135-139	LPLPL		
156-160	MFPPQ		
168-173	VAPFPQ	168-173	VAPFPQ
170-175	VLPVPQ	170-175	VLPVPQ
171-175	LPVPQ		
194-201	QEPVLGPV	177-182	AVPYPQ
195-201	EPVLGPV	194-201	QEPVLGPV
196-201	PVLGPV	195-201	EPVLGPV

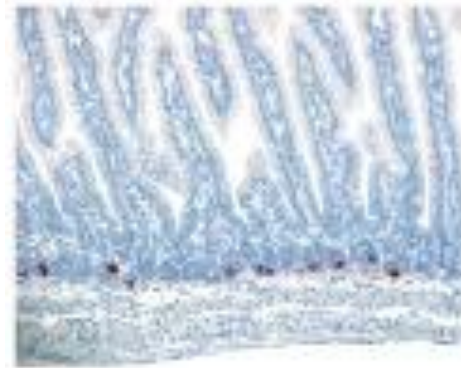
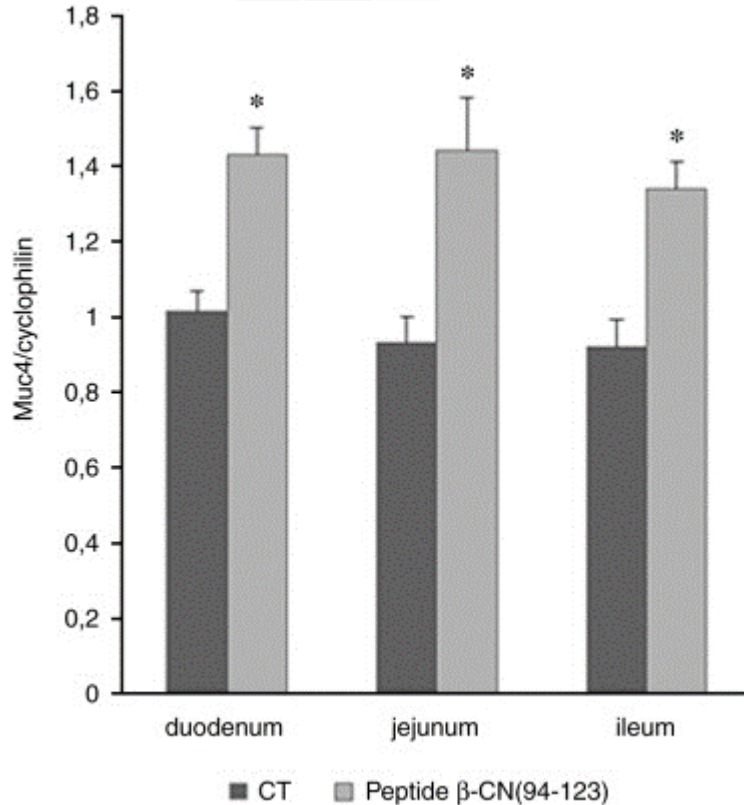
β-CN

Among those 5 bioactive peptides

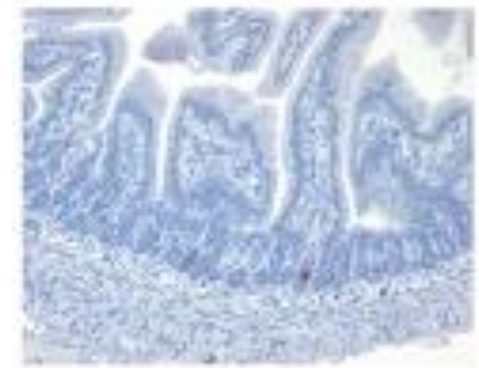
59-66	VYFPFGPI	} Anti-hypertensive Opioid
59-68	VYFPFGPIP	
60-66	YFPFGPI	
108-113	EMPFK	
114-119	YPVEPF	

Boutrou et al. 2013
Am J Clin Nutr

Bioactive peptides released during digestion can reinforce the defense of the intestinal epithelium



Before



After

Sections of duodenum after 10 d of administration of peptide β -CN (94-123)

β -CN(94-123) stimulates the mucus production *in vitro* (HT-29 MTX) and *in vivo* (rat)

Plaisancie et al. 2013, J Nutr Biochem
Plaisancie et al. 2015, J Dairy Res

Conclusion

The structure/composition of food regulate the kinetics of protein digestion and the release of amino acids in the bloodstream

Gastric emptying rate will highly depend on the structure that the product will adopt in the stomach cavity.

Understanding the mechanisms of food particle breakdown in the stomach is critical to control the structure a food will adopt in gastric conditions

Being able to design food structures for controlling the kinetics of hydrolysis of macronutrients will allow to obtain food particularly adapted to specific population



The Bioactivity & Nutrition group at INRA in Rennes



Head

Didier DUPONT - *Senior Scientist*

Scientists

Rachel BOUTROU – *Junior Scientist*

Amélie DEGLAIRE – *Lecturer*

Juliane FLOURY – *Lecturer*

Catherine GUERIN - *Lecturer*

Joëlle LEONIL – *Senior Scientist*

Steven LE FEUNTEN – *Junior Scientist*

Françoise NAU – *Professor*

Frédérique PEDRONO – *Lecturer*

Guilherme FURTADO – *Post-doc*

Xaoxi YU – *Post-Doc*

PhD students

Linda LEROUX (2016-2019)

Manon HIOLLE (2016-2019)

Yohan REYNAUD (2016-2019)

Amira HALABI (2017-2020)

Léa SALELLES (2018-2021)

Jun WANG (2018-2021)

2-3 Masters students

Technicians

Gwenaële HENRY

Yann LE GOUAR

Nathalie MONTHEAN

Engineers

Julien JARDIN

Olivia MENARD

Jordane OSSEMOND

Improving health properties of food by sharing our knowledge on the digestive process

International Network

Dr. Didier DUPONT, Senior Scientist, INRA, France

●
INFOGEST
●





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 Norwegian Univ Life Sci Chalmers Univ Tech VTT Nofima Riga Stradin Univ Univ Ljubljana
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 Univ Ghent Agroscope Posieux
 Inst Food Res Agrocampus Ouest
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 CNR
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 Ege Univ
 Univ California Davis
USA

USA

Japan



410 scientists - 150 institutes – 45 countries

Industry involvement

☞ ~ 50 private companies are following INFOGEST





INFOGEST



Chair

Didier Dupont - France

didier.dupont@inra.fr



www.cost-infogest.eu

Vice-chair

Alan Mackie - UK

**In vitro
models of
digestion
WG1**

**Food
interaction –
meal digestion
WG2**

**Absorption
models
WG3**

**Digestive
lipases and
lipid digestion
WG4**

**Digestive
amylases and
starch
digestion
WG5**

**In silico
models of
digestion
WG6**



Didier Dupont



Pasquale Ferranti



Milena
Corredig



Linda Giblin



Myriam Grundy



Nadja Siegert



Choi-Hong Lai



Alan Mackie



Uri Lesmes



Brigitte Graf



Frederic Carriere



Caroline Orfila



Steven Le Feunteun

Some outputs

In vitro gastrointestinal digestion
Consensus INFOGEST protocol

Minekus et al. 2014
Food & Function, 5,
1113-1124
700 citations

Oral phase

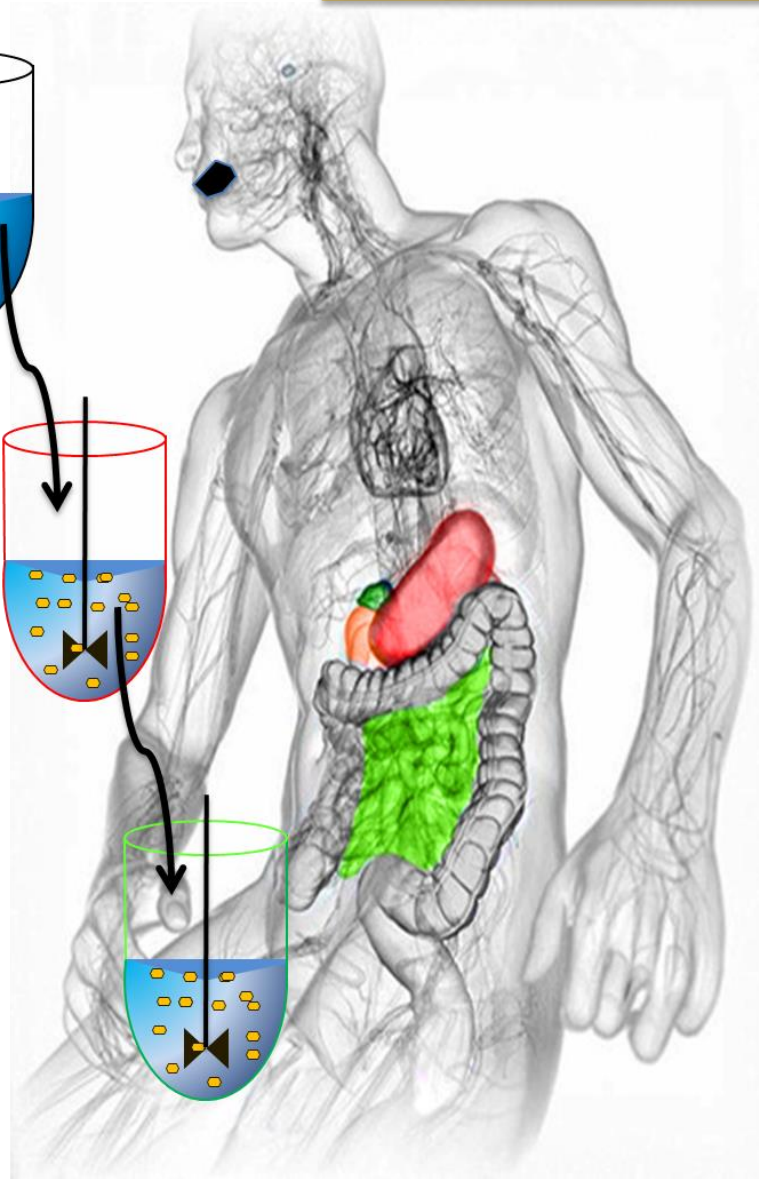
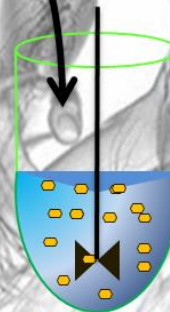
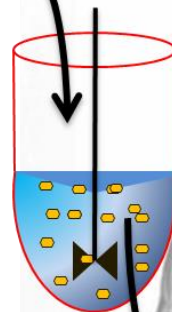
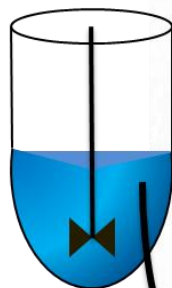
Mix 1:1 with Simulated Salivary Fluid (SSF)
salivary amylase (75 U/mL)
2 min, pH 7

Gastric Phase

Mix 1:1 with Simulated Gastric Fluid (SGF)
Pepsin (2000 U/mL)
2h, pH 3

Intestinal Phase

Mix 1:1 with Simulated Intestinal Fluid (SIF)
Enzymes
Pancreatin (based on trypsin 100 U/mL) or
Pure enzymes
Bile (10mM)
2h, pH 7



An updated version of the protocol published last week!

nature
protocols

PROTOCOL

<https://doi.org/10.1038/s41596-018-0119-1>

INFOGEST static in vitro simulation of gastrointestinal food digestion

André Brodkorb^{1*}, Lotti Egger², Marie Alminger³, Paula Alvito⁴, Ricardo Assunção⁴, Simon Ballance⁵, Torsten Bohn⁶, Claire Bourlieu-Lacanal⁷, Rachel Boutrou⁸, Frédéric Carrière⁹, Alfonso Clemente¹⁰, Milena Corredig¹¹, Didier Dupont⁸, Claire Dufour¹², Cathrina Edwards¹³, Matt Golding¹⁴, Sibel Karakaya¹⁵, Bente Kirkhus⁵, Steven Le Feunteun⁸, Uri Lesmes¹⁶, Adam Macierzanka¹⁷, Alan R. Mackie¹⁸, Carla Martins⁴, Sébastien Marze¹⁹, David Julian McClements²⁰, Olivia Ménard⁸, Mans Minekus²¹, Reto Portmann², Cláudia N. Santos^{22,23}, Isabelle Souchon²⁴, R. Paul Singh²⁵, Gerd E. Vegarud²⁶, Martin S. J. Wickham²⁷, Werner Weitschies²⁸ and Isidra Recio²⁹

5-year Impact Factor=15,269

The logo for INFOGEST, featuring the word "INFOGEST" in a bold, black, sans-serif font. Above the letter "O" is a solid orange circle, and below the letter "T" is another solid orange circle.

The consensus model can be learned with videos on YouTube

The screenshot shows a web browser window displaying the YouTube channel page for 'cost infogest'. The browser's address bar shows the URL 'https://www.youtube.com/channel/UCdc-NP9kTDGyH_kZCgpQWg'. The page features a search bar with 'cost infogest' entered, a navigation menu on the left, and a main content area with a video player and a list of recent uploads. The video player shows a video titled 'In vitro food digestion - COST action INFOGEST'. Below the video player, there are two video thumbnails with titles: 'Alpha Amylase Activity Assay for In Vitro Food Digestion' and 'Static In Vitro Digestion Method for Food'. The right sidebar contains a list of popular channels on YouTube, including 'Seb la Frite', 'MissCreatives', 'LanguedePub / M...', 'JunkFoodTasterD...', 'Madame Patachou', and 'halawiyat khadija...'. The bottom of the page shows a Windows taskbar with various application icons and the system clock.

Fichier Édition Affichage Historique Marque-pages Outils ?

Intranet UMR STLO - Accueil x GRR (Gestion et Réservatio... x YaZiba: 30/3 - 3/4 x In vitro food digestion - C... x +

https://www.youtube.com/channel/UCdc-NP9kTDGyH_kZCgpQWg

youtube

YouTube FR

cost infogest

Mettre en ligne Connexion

Vidéos à regarder

LE MEILLEUR DE YOUTUBE

- Populaire sur YouTube
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- Films
- Émissions TV
- Actualité

Parcourir les chaînes

Connectez-vous maintenant pour consulter vos chaînes et les recommandations.

Se connecter

Toutes les activités

In vitro food digestion - COST action INFOGEST a mis en ligne une vidéo. il y a 10 mois

Oral phase
Gastric Phase
Intestinal Phase

Alpha Amylase Activity Assay for In Vitro Food Digestion
de In vitro food digestion - COST action INFOGEST
il y a 10 mois • 846 vues
A step-by-step description of a static α -amylase activity assay for an in vitro digestion method presented suitable for food; recently published and available online (open access...)

5:12

In vitro food digestion - COST action INFOGEST a mis en ligne une vidéo. il y a 10 mois

Oral phase
Gastric Phase
Intestinal Phase

Static In Vitro Digestion Method for Food
de In vitro food digestion - COST action INFOGEST
il y a 10 mois • 723 vues
A step-by-step description of a static in vitro digestion method presented suitable for food (international consensus); recently published and available online (open access), see ...

8:55

Chaînes populaires sur YouTube

- Seb la Frite S'abonner
- MissCreatives S'abonner
- LanguedePub / M... S'abonner
- JFT S'abonner
- Madame Patachou S'abonner
- halawiyat khadija... S'abonner

FR

10:38

Kitty Verhoeckx · Paul Cotter
Iván López-Expósito
Charlotte Kleiveland · Tor Lea
Alan Mackie · Teresa Requena
Dominika Swiatecka
Harry Wichers *Editors*

The Impact of Food Bioactives on Health

In Vitro and Ex Vivo Models

 COST

 Springer Open

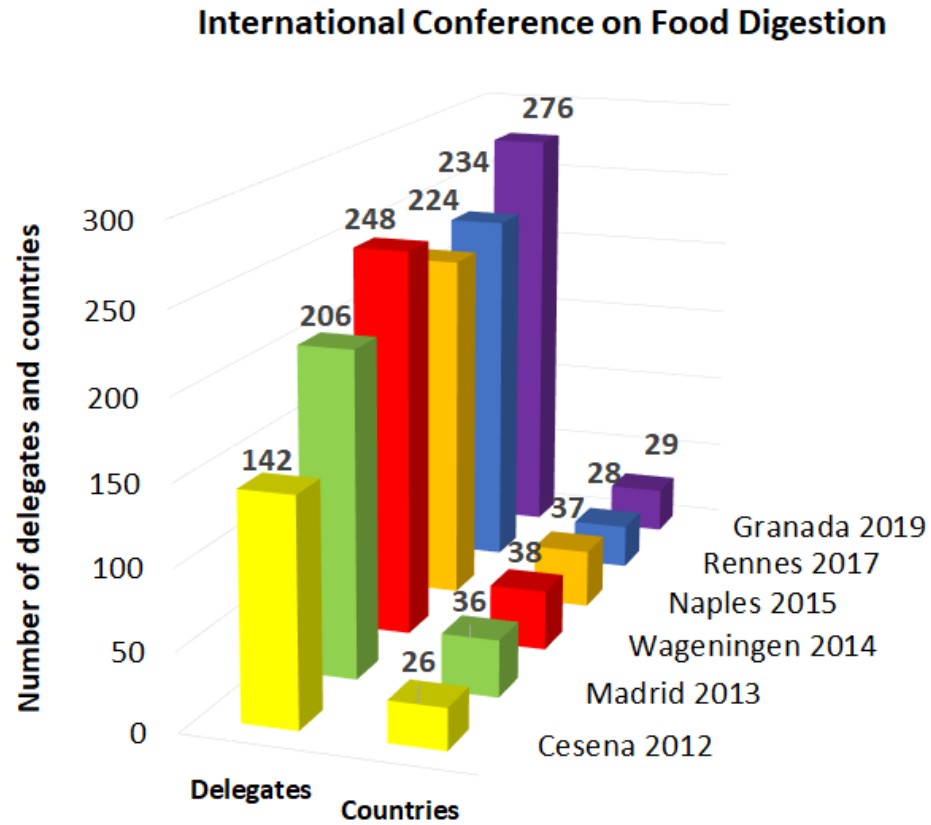
Some outputs

Release of a 341-page book on all the in vitro and ex vivo models to study the impact of food bioactives on health

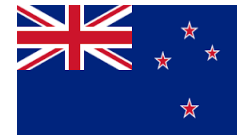
OPEN ACCESS

- * More than 620,000 chapter downloads
- * top 25% most downloaded eBooks in 2017

The International Conference on Food Digestion



The Conference has been created by INFOGEST and is now an event regularly followed by 200 scientists





We are pleased to announce the next
7th International Conference on Food Digestion



in Cork, Ireland, 2021