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# Les modèles d'étude de la digestion : de l'in vitro à l'in silico en passant par l'animal et l'homme

Didier Dupont

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Submitted on 5 Jun 2020

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Les modèles d'étude de la digestion :  
de l'*in vitro* à l'*in silico* en passant par l'animal et  
l'homme

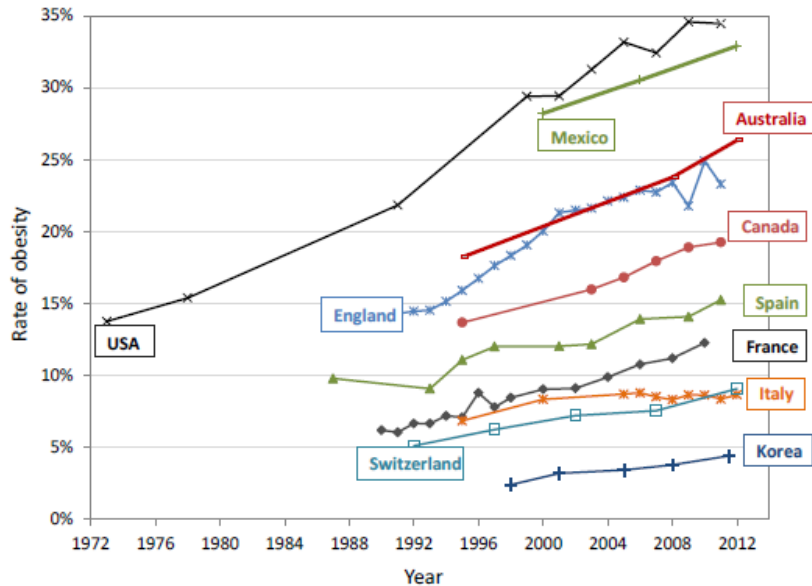


**Dr Didier DUPONT**

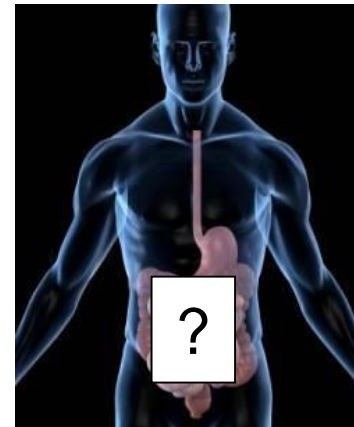
INRA, STLO, Rennes, France



# Pourquoi s'intéresser au processus digestif?



Pathologies liées à l'aliment ↑  
▷ Prévenir ces pathologies plutôt que de les traiter



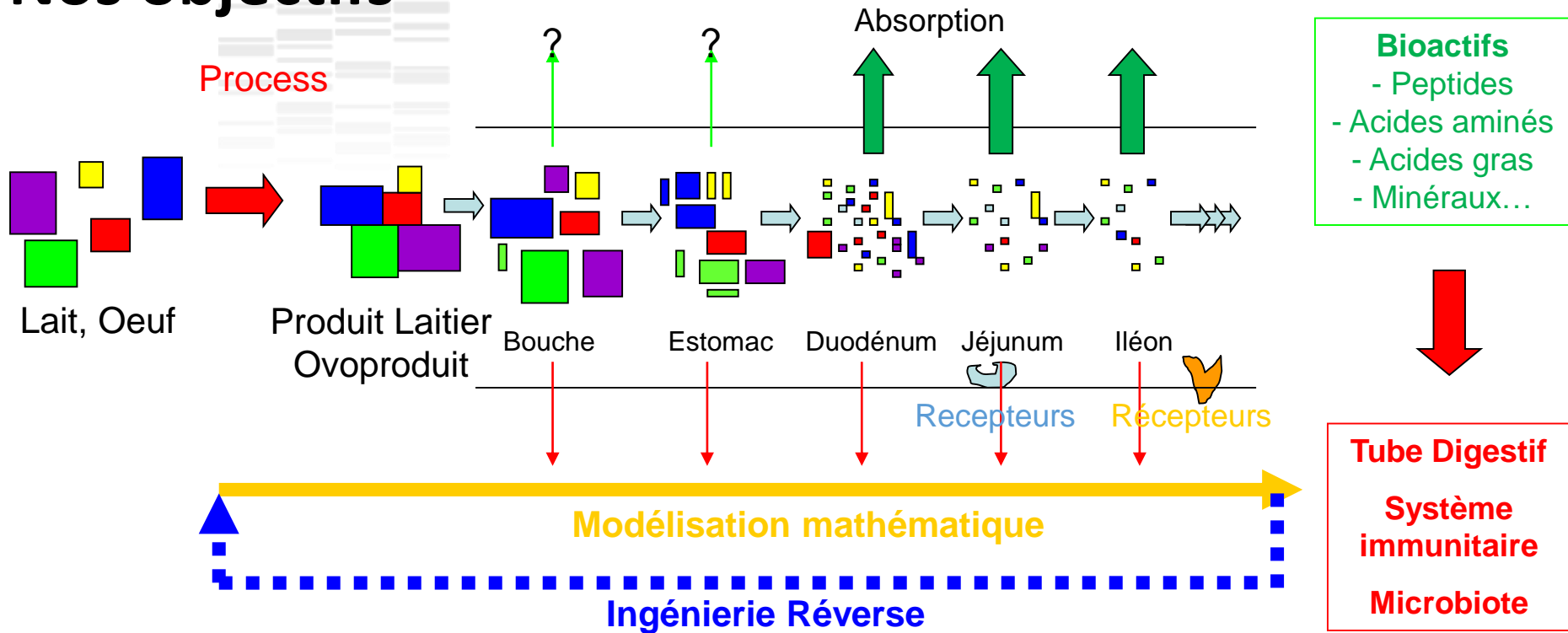
**Tube digestif = interface entre l'aliment et l'organisme**

**Digestion libère des constituants qui peuvent avoir un effet bénéfiques ou délétères sur la santé de l'homme**

... mais les mécanismes de déconstruction de l'aliment dans le TD restent méconnus et le tractus gastrointestinal a été souvent considéré comme une boîte noire

**En accroissant notre connaissance de la digestion, nous pourrions étayer les relations entre les aliments et la santé de l'homme**

# Nos objectifs



- ☞ Comprendre les mécanismes de dégradation des produits laitiers et ovoproduits dans le tube digestif et identifier les composés bénéfiques/délétères libérés lors de la digestion
- ☞ Déterminer l'impact de la structure des produits laitiers et ovoproduits sur la biodisponibilité des nutriments
- ☞ Modéliser ces phénomènes pour concevoir des aliments adaptés aux besoins nutritionnels de populations spécifiques par une démarche d'ingénierie reverse

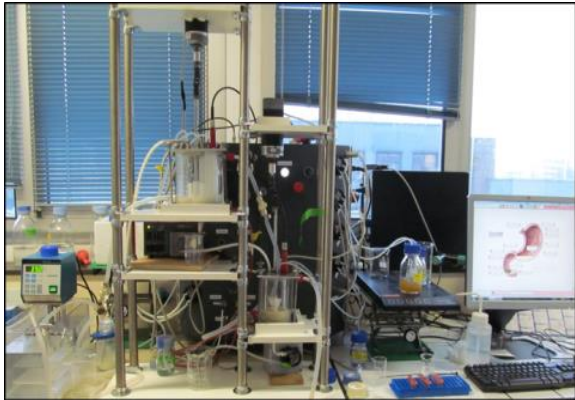
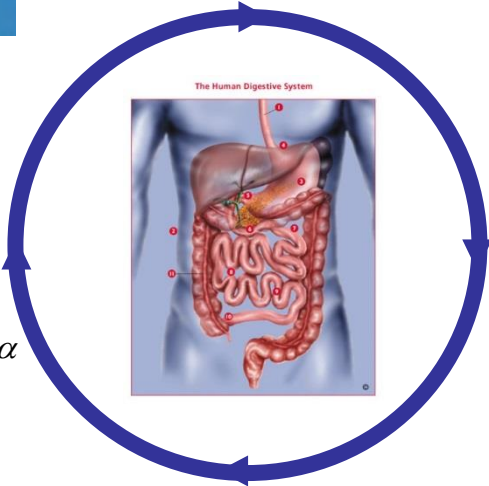
# Models for simulating digestion

*In vitro static models*

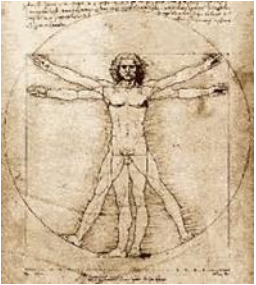


*In silico models*

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



*In vitro dynamic models*

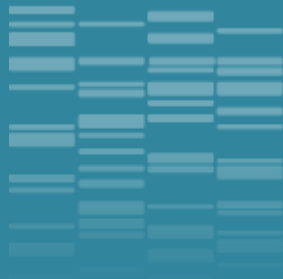


*Human models*



*Animal models*

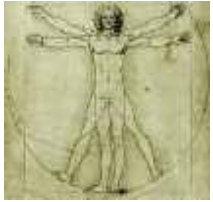




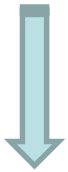
# Les modèles *in vitro*



# Static *in vitro* digestion models: pro's & con's



*In vivo*



*In vitro*

## Main Reasons :

Ethical - Technical – Financial

## Advantages:

Standardisation of the experimental conditions  
Good reproducibility and repeatability  
Easy sampling, possibility to follow kinetics

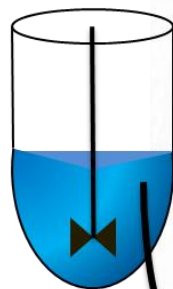
## Disadvantages:

Impossible to mimic the complexity of the GI tract in a test tube!!!  
Needs harmonization

# *In vitro* gastro-intestinal digestion Consensus INFOGEST protocol

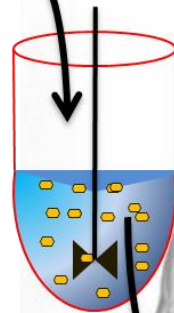
## Oral phase

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



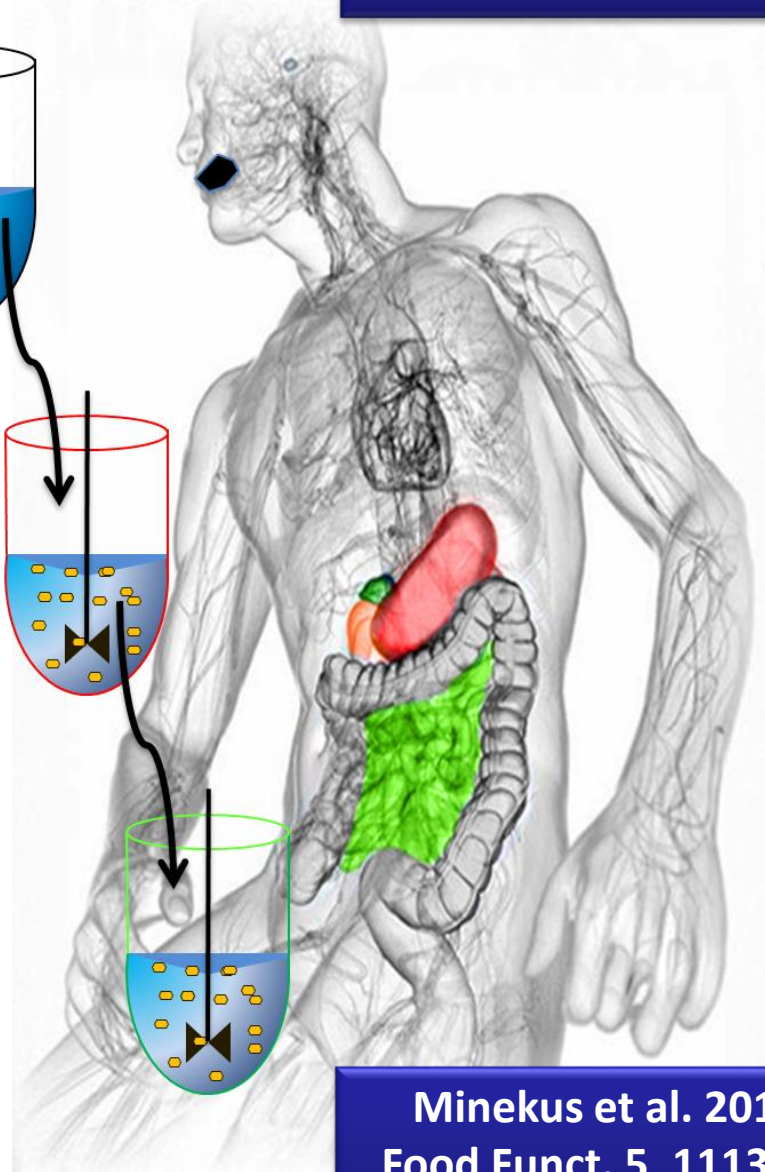
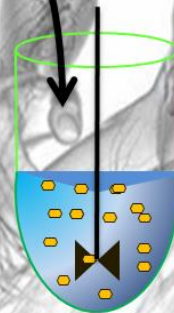
## Gastric Phase

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





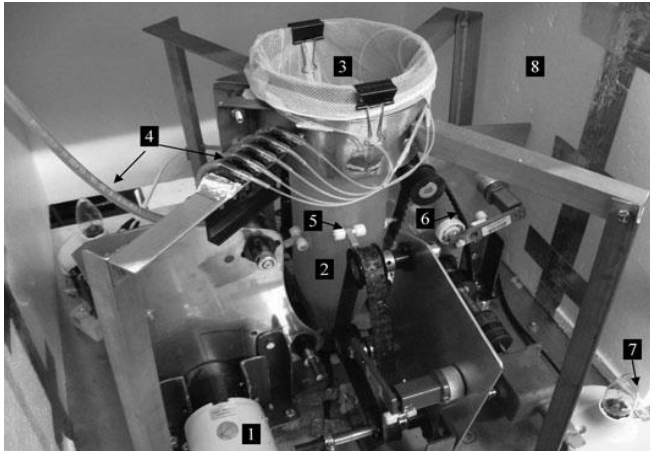
# The consensus model can be learned with videos on YouTube

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-NP:9kTDGyH\_kZCgpQWg'. The YouTube interface includes a search bar with 'cost infogest' entered, a navigation menu on the left with categories like 'Populaire sur YouTube', 'Musique', and 'Sport', and a main content area with video thumbnails. The first video is titled 'Alpha Amylase Activity Assay for In Vitro Food Digestion' with a duration of 5:12. The second video is 'Static In Vitro Digestion Method for Food' with a duration of 8:55. A sidebar on the right lists 'Chânes populaires sur YouTube' with channels like 'Seb la Frite' and 'MissCreatives'. The bottom of the browser shows the Windows taskbar with various application icons and the system clock at 10:38.

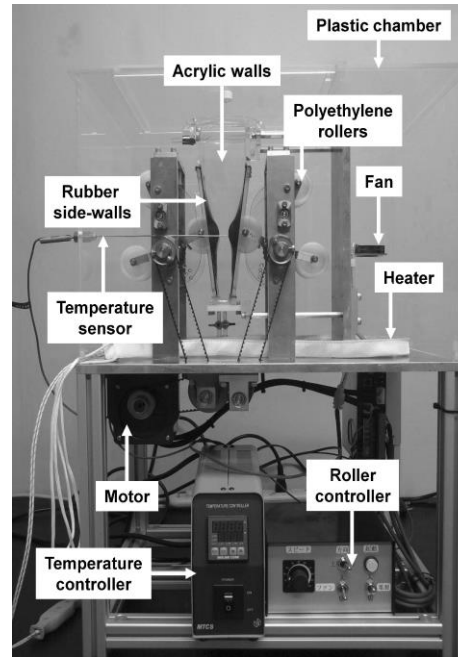
https://www.youtube.com

The Windows taskbar at the bottom of the screen displays several system tray icons, including network, volume, and power, along with the current time of 10:38.

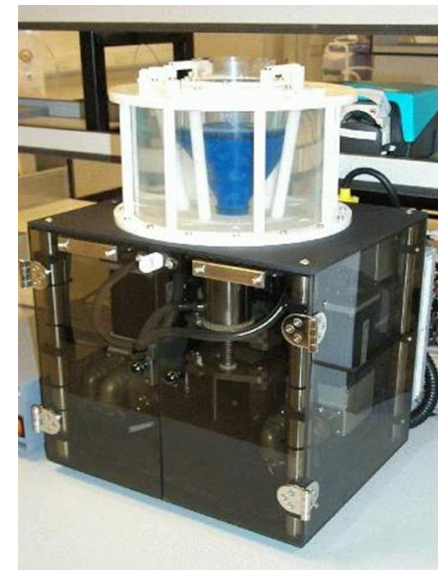
# Mono-compartmental dynamic systems



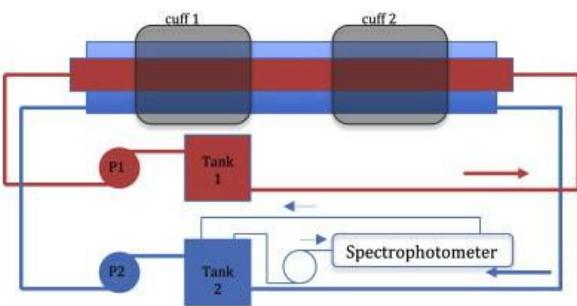
Human Gastric Simulator  
(Univ California Davis, USA)



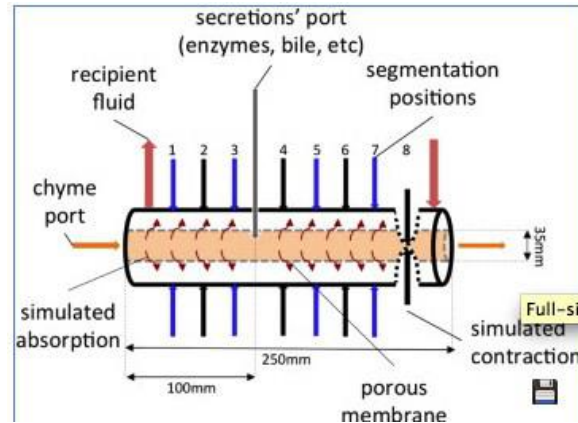
Gastric Digestion Simulator (GDS, Japan)



Dynamic gastric model  
(IFR/Bioneer)



Small intestinal Model  
(SIM, Birmingham)



Dynamic Duodenal Model  
(Dduo, Birmingham)

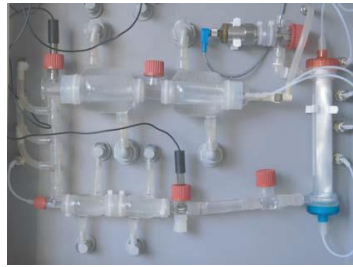


Artificial colon  
(Univ Clermont)

# Multi-compartmental dynamic model



TIM (TNO Intestinal Model)



Tiny-TIM



SHIME (Simulated Human Intestinal Microbial Ecosystem)



ESIN (Univ Clermont)



Digestor (AINIA)



SIMGI® (CSIC)

# Development of a simple and robust dynamic model (DiDGi®): application to infant formula digestion

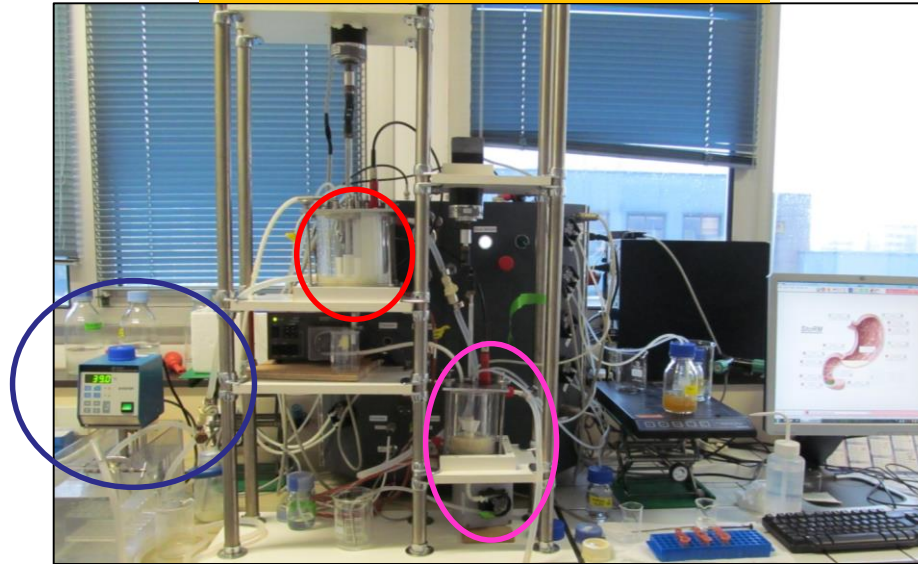
## Digestion model system

HM/IF



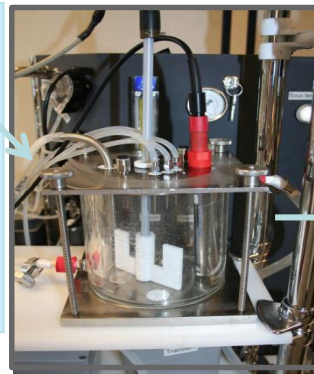
### Ingestion

10 ml/min -10 min  
→ 100 ml, 37° C

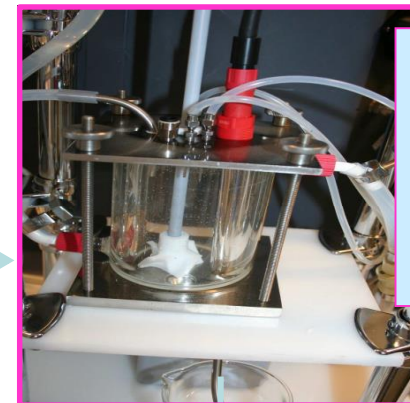


StoRM® software

## Stomach



## Intestine



Elashoff

Elashoff

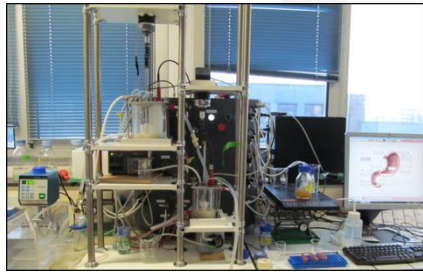
### Gastric fluids

- pepsine (260 U/mL)
- lipase (18,4 U/mL)
- simulated gastric fluid (NaCl, CaCl<sub>2</sub>, pH=6.5)
- HCl for pH regulation

### Intestinal fluids

- pancreatin
- bile
- NaHCO<sub>3</sub> (pH = 6.5)

# Validation of the dynamic *in vitro* digestion system

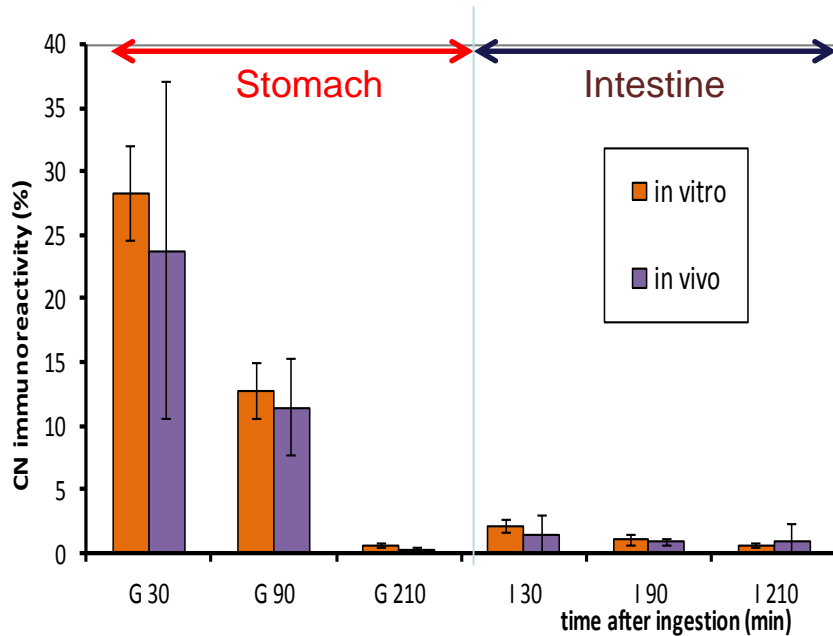


*In vitro*  
N=3

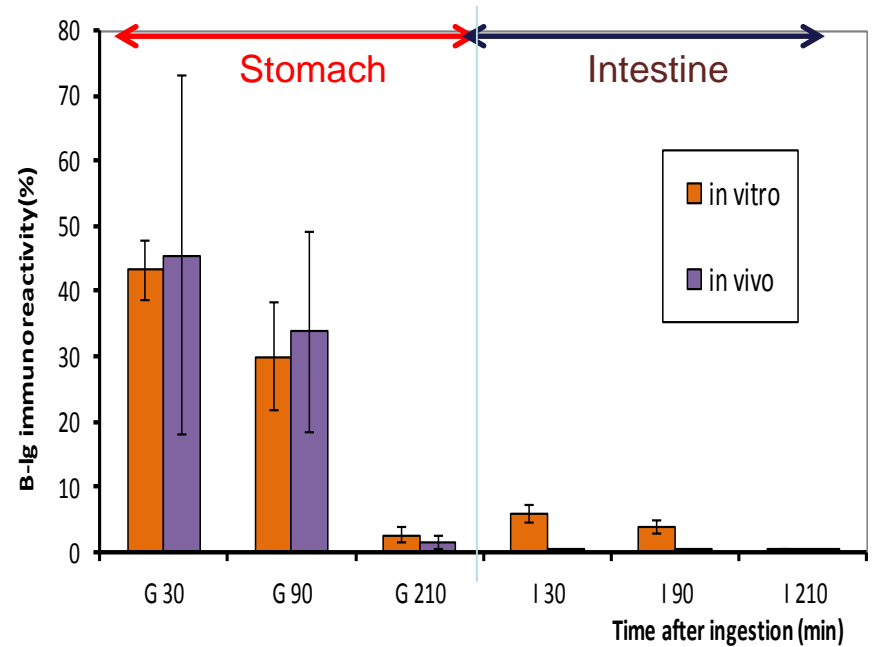
Infant  
Formula



*In vivo*  
18 piglets



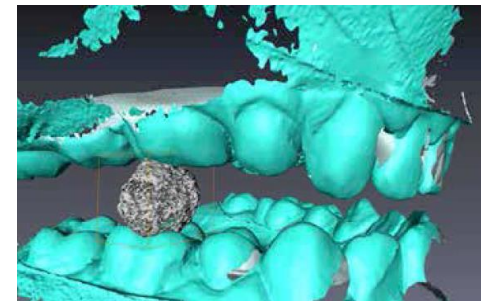
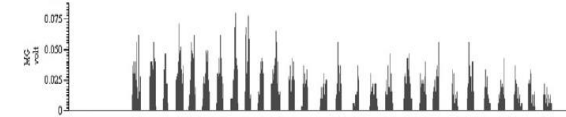
**Caseins**



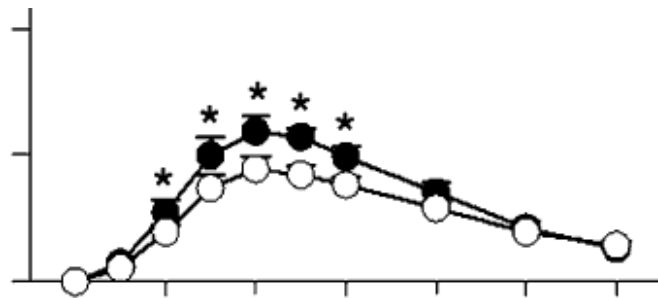
**beta-lactoglobulin**

# Limits of the *in vitro* models – The oral phase

- Destroys food structure and allows saliva to penetrate into the bolus
- Good knowledge, « easy » to study, to model
- Less known: consequences of oral processing on the whole digestive process and the on nutrient bioavailability

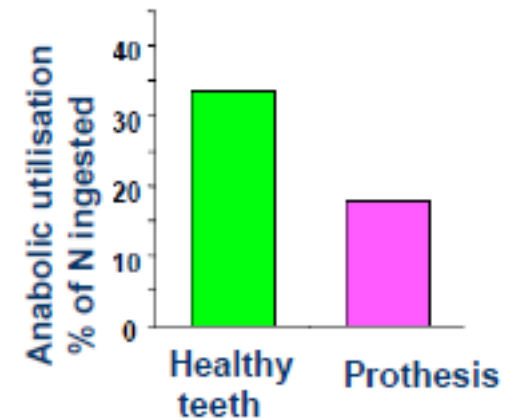


Pennings et al, 2013 Comparison between minced beef and beef steak in elderly people



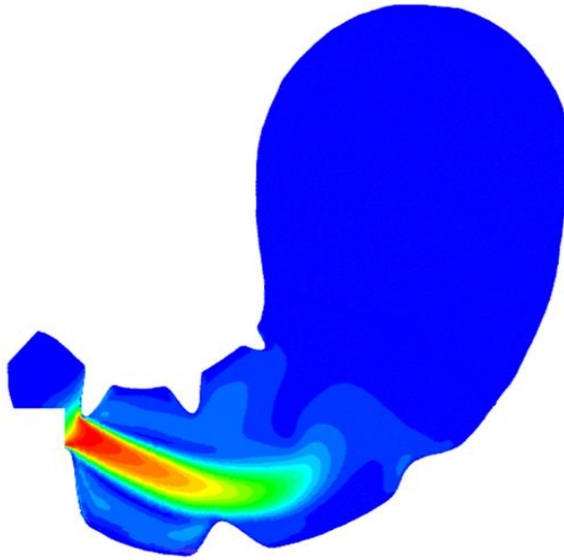
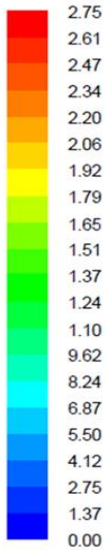
↗ Amino Acids peripheral bioavailability

Beef steak	49 ± 3%
Minced beef	61 ± 3% (p < 0,01)



Chewing efficiency in elderly  
Rémond et al, 2007

# Limits of the *in vitro* models – The J-shape of the stomach



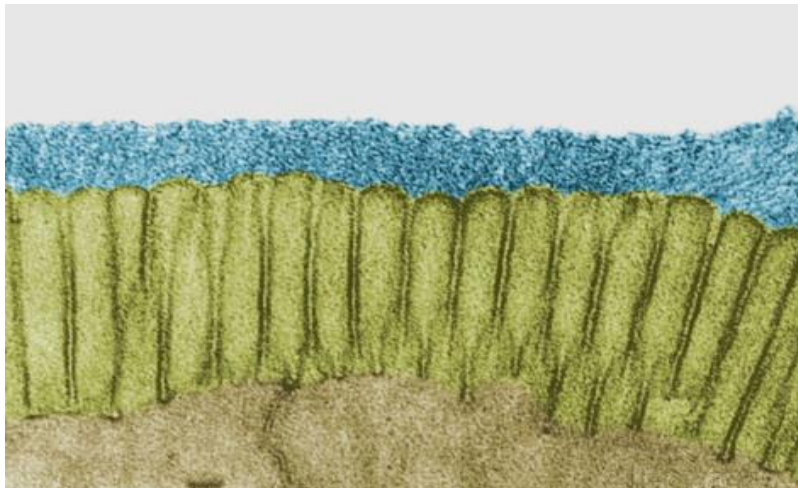
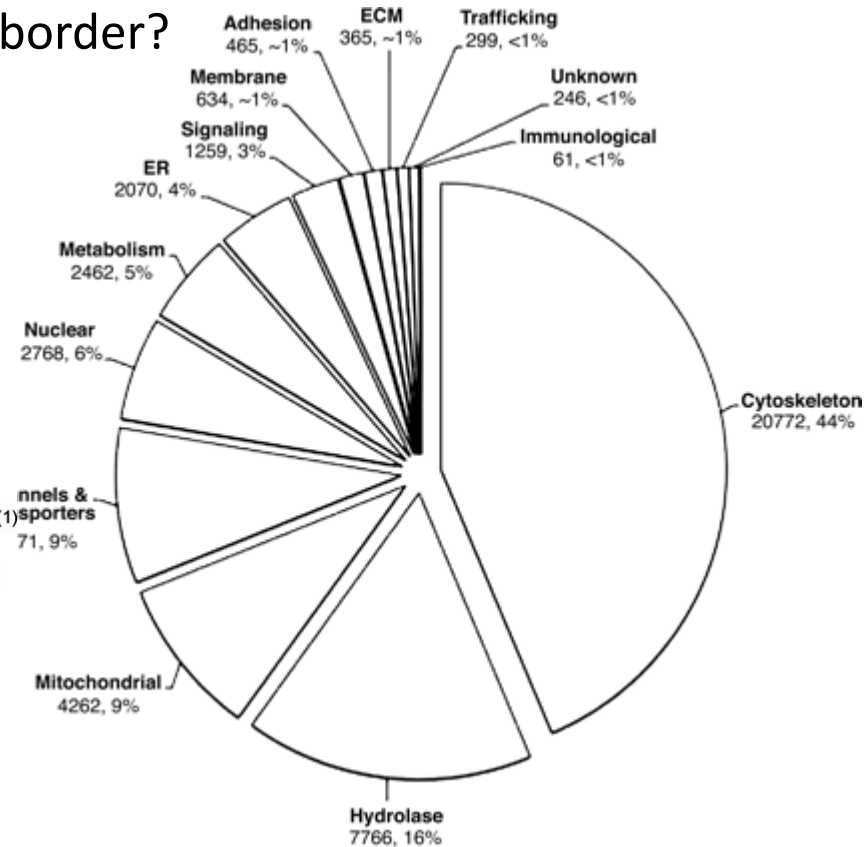
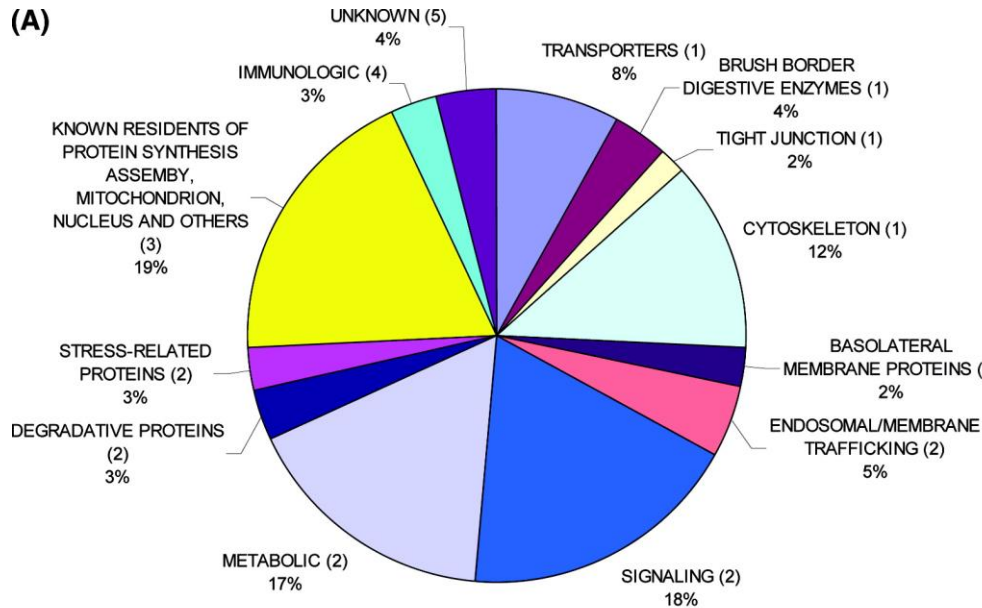
Ferrua and Singh, 2010



- 👉 Ongoing experiments at the Riddet institute (NZ) to design a 3D-printed stomach
- 👉 TIMagc developed by Triskelion

# Limits of the *in vitro* models – Small intestine

What is the role of the enzymes of the brush border?



Can we use brush border membrane vesicles to improve our models?  
How can we make it physiologically-relevant?



# Limits of the *in vitro* models – Absorption

## 1 Where does it occur and what are the mechanisms???

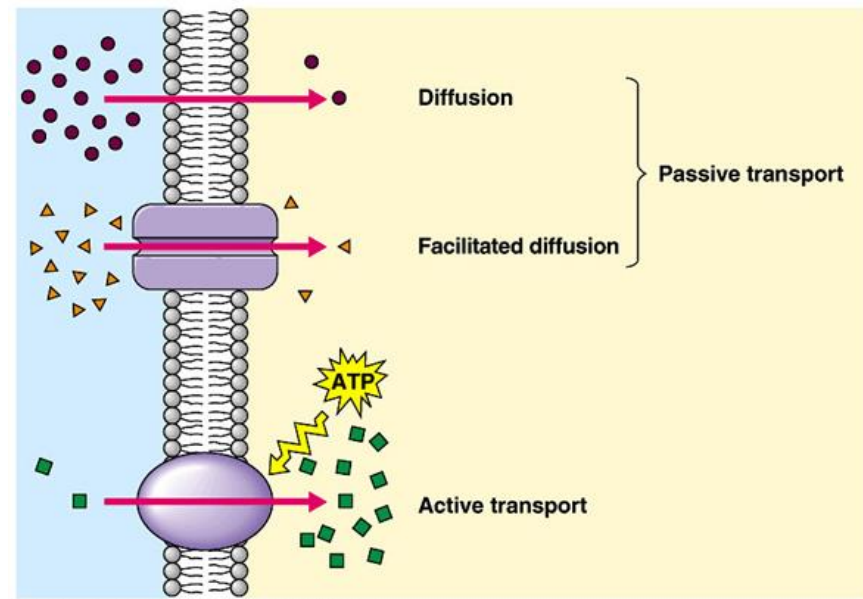
- ☞ Mouth? Yes (small molecules): important for drugs sensitive to pH or rapidly metabolized by the liver, allergy
- ☞ Stomach? Yes (alcohol)
- ☞ Small intestine? **YES** major site of absorption

## 2 What are the mechanisms of transport?

Facilitated diffusion

Pinocytosis

Active transport

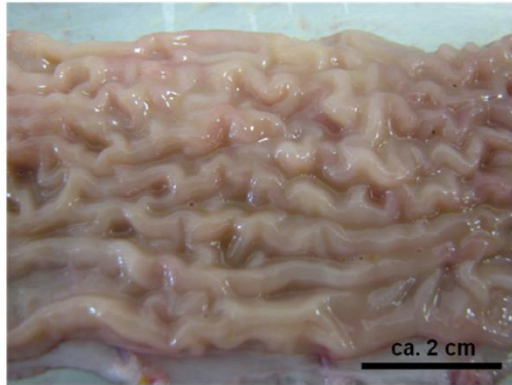


Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

Absorption is usually poorly mimicked in our models. But how can we do that?  
Caco2 cell monolayers, co-cultures with HT29-MTX, Ussing chambers...?

# Limits of the *in vitro* models – Role of mucus

Porcine Intestinal Mucus



>95% water  
>5% mucins  
+ cholesterol  
+ other proteins  
+ DNA

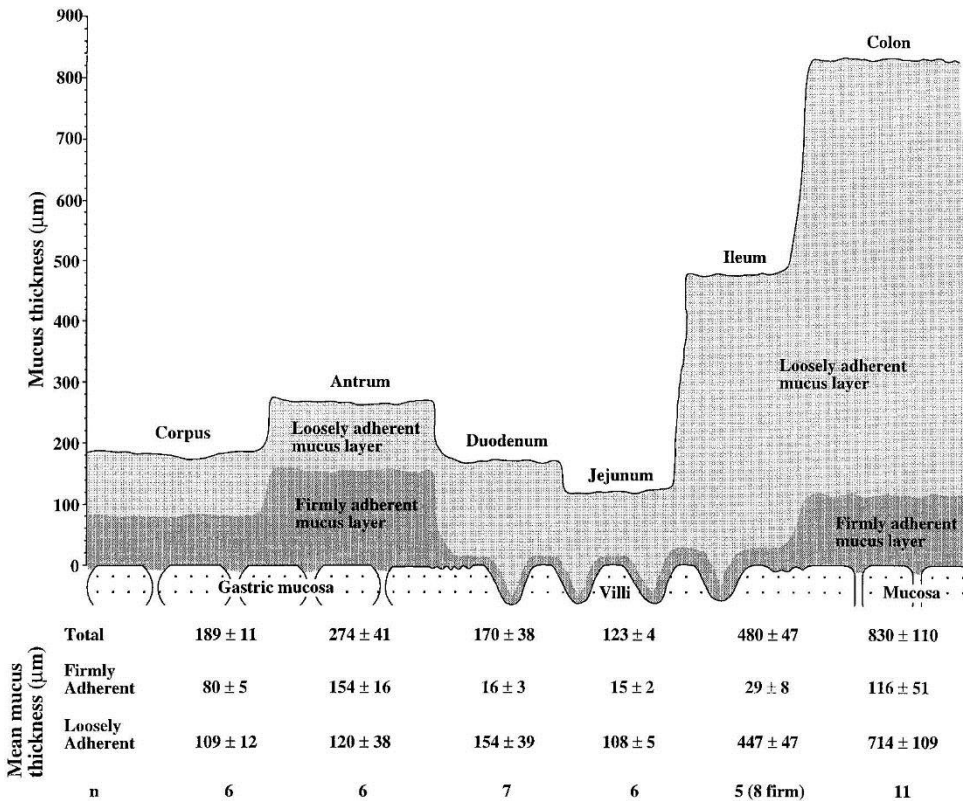


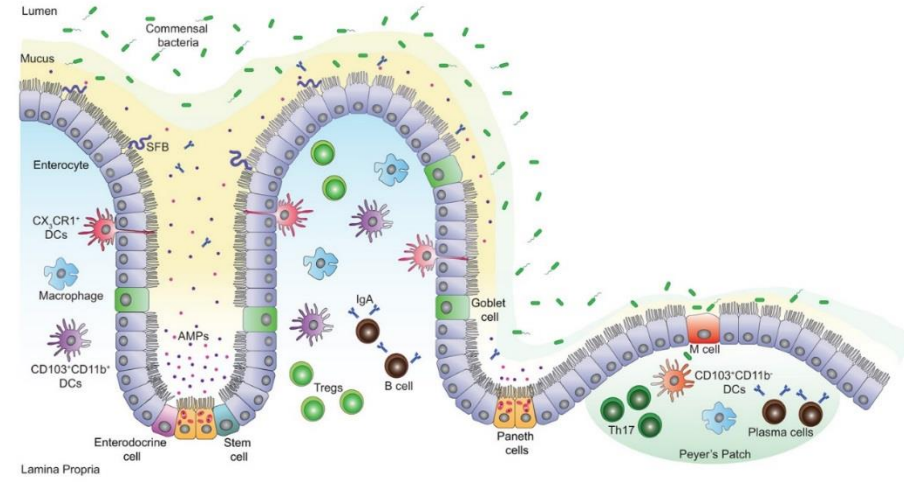
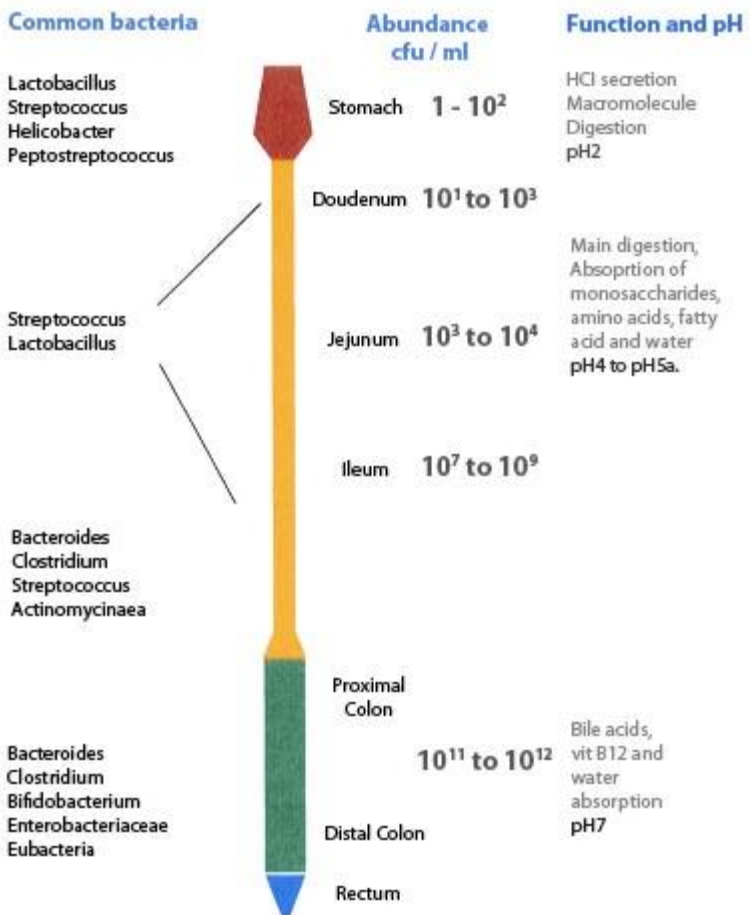
Table 1 Tissue distribution of mucins

Mucin	Distribution	References
<i>Secreted gel forming</i>		
MUC2	Small intestine, colon, respiratory tract, eye, middle ear epithelium	231–235
MUC5AC	Respiratory tract, stomach, cervix, eye, middle ear epithelium	235–239
MUC5B	Respiratory tract, salivary glands, cervix, gallbladder, seminal fluid, middle ear epithelium	235,236, 240–244
MUC6	Stomach, duodenum, gallbladder, pancreas, seminal fluid, cervix, middle ear epithelium	235,243, 245–247
MUC19	Sublingual gland, submandibular gland, respiratory tract, eye, middle ear epithelium	16,235,248
<i>Secreted non-gel forming (monomeric)</i>		
MUC7	Salivary glands, respiratory tract, middle ear epithelium	235,249,250
<i>Cell surface</i>		
MUC1	Stomach, breast, gallbladder, cervix, pancreas, respiratory tract, duodenum, colon, kidney, eye, B cells, T cells, dendritic cells, middle ear epithelium	235,251–256
MUC3A/B	Small intestine, colon, gall bladder, duodenum, middle ear epithelium	235,243, 257,258
MUC4	Respiratory tract, colon, stomach, cervix, eye, middle ear epithelium	235,255, 259–261
MUC12	Colon, small intestine, stomach, pancreas, lung, kidney, prostate, uterus	32,262
MUC13	Colon, small intestine, trachea, kidney, appendix, stomach, middle ear epithelium	35,235,262
MUC15	spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocyte, bone marrow, lymph node, tonsil, breast, fetal liver, lungs, middle ear epithelium	235,263
MUC16	Peritoneal mesothelium, reproductive tract, respiratory tract, eye, middle ear epithelium	235,264–267
MUC17	Small intestine, colon, duodenum, stomach, middle ear epithelium	235,268
MUC20	Kidney, placenta, colon, lung, prostate, liver, middle ear epithelium	235,269

# Colon and intestinal microbiota

Hot topic!!!

Connections between microbiota, diet and health status...



But little information on the impact of specific foods on intestinal microbiota

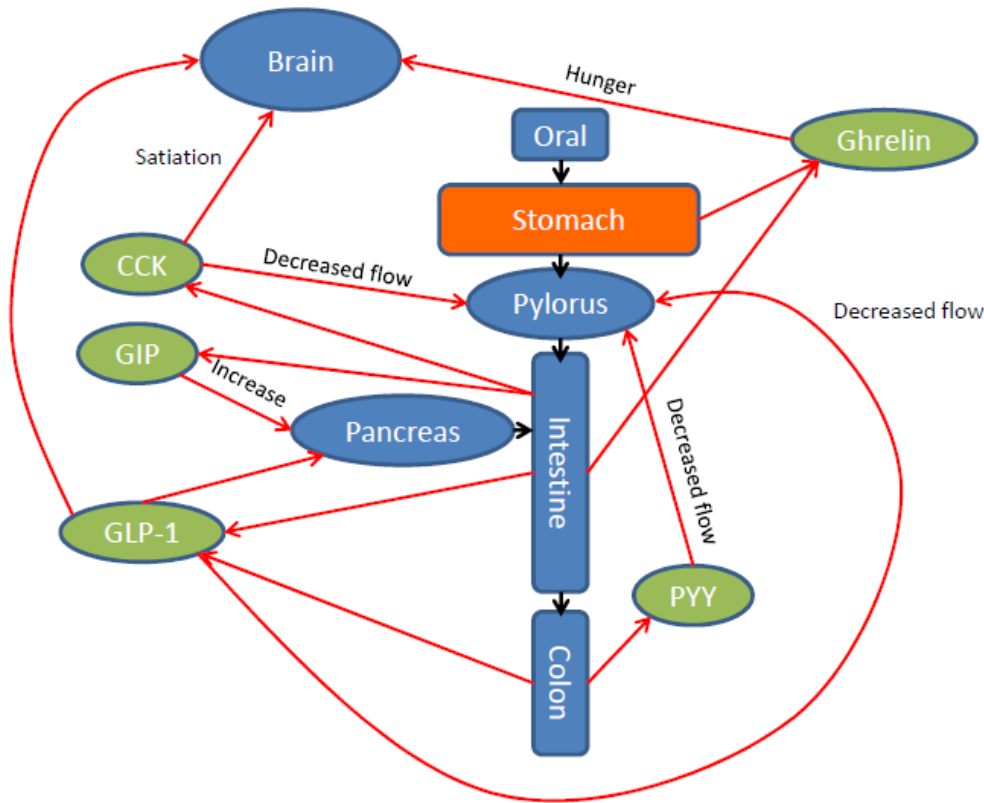
Are there periods in life when the intestinal microbiota is more able to be modulated by food?

Need for colonic models connected to *in vitro* models

# What you cannot simulate *in vitro*!

Role of GI hormones highly complex!!!

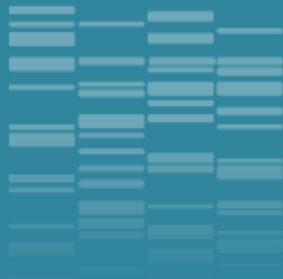
Ex: the Ileal break



When undigested nutrients (triglycerides) reach the ileum

- ↪ feed-back mechanism through ↗ GLP-1 and PYY
- ↪ Slow-down gut motility

Other receptors all along the GI tract?  
Mechanisms of action?  
Which nutrients can regulate motility?



# Les modèles *in vivo*



# ***In vivo* (animal) models**

## **Rodents**



Probably not the best model to mimic the upper part of the human GI tract  
Gnotobiotic animals (« humanized » mice) are of interest to study the effect of food on the microbiota

## **Pigs**



Piglets



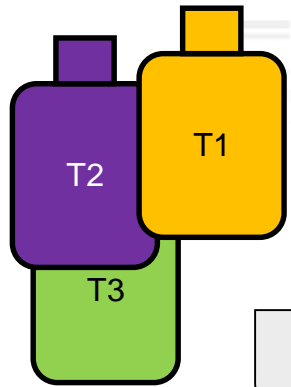
Mini-pigs



Multi-cannulated and catheterized pigs

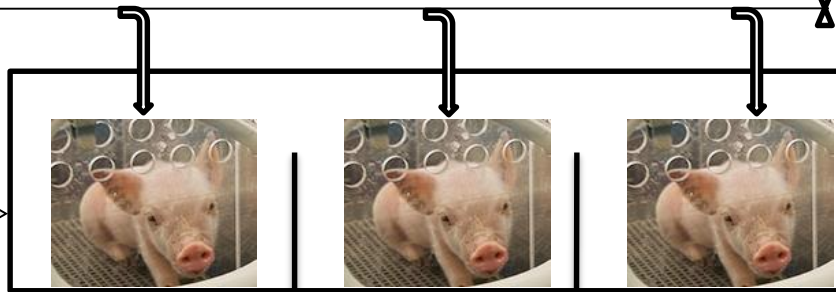
Pretty good model of the upper GI tract  
Cannulas and catheters allow to collect effluent and plasma throughout digestion (kinetics)

# The composition of Infant Formula affects the maturation of the immune system and the shape of the microbiota



- Veg
- Veg + PL
- Dairy Fat + PL

Automatic meal delivery (10 meals/ day)



+  
Mother-fed piglets  
(MF = + control)



Slaughtering after

7 days

28 days

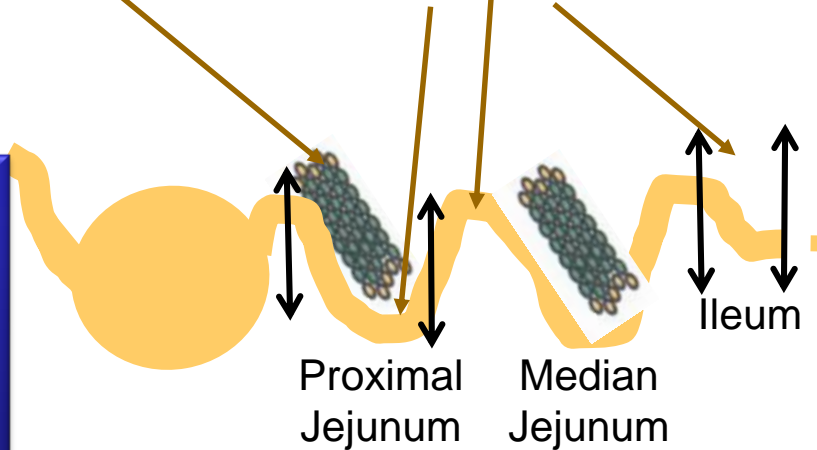
(90 min postprandial)

Mesenteric Lymph Nodes (MLN)

Collect of effluents and tissues

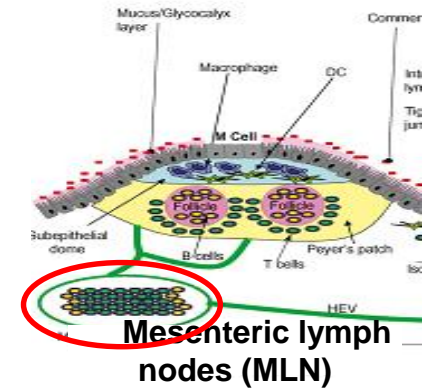
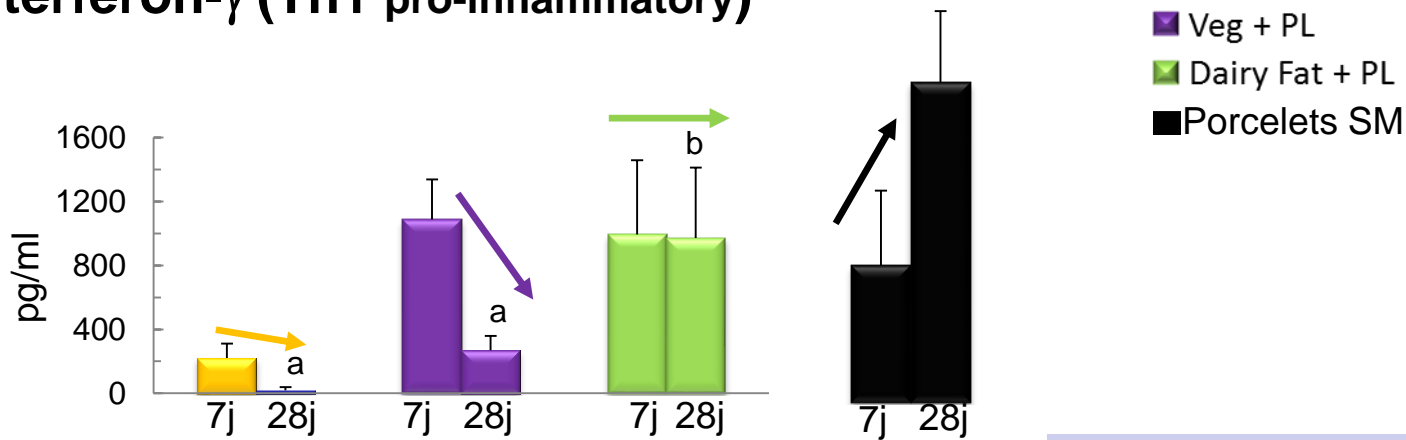
Effluents:  
-SDS-PAGE  
-Elisa

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

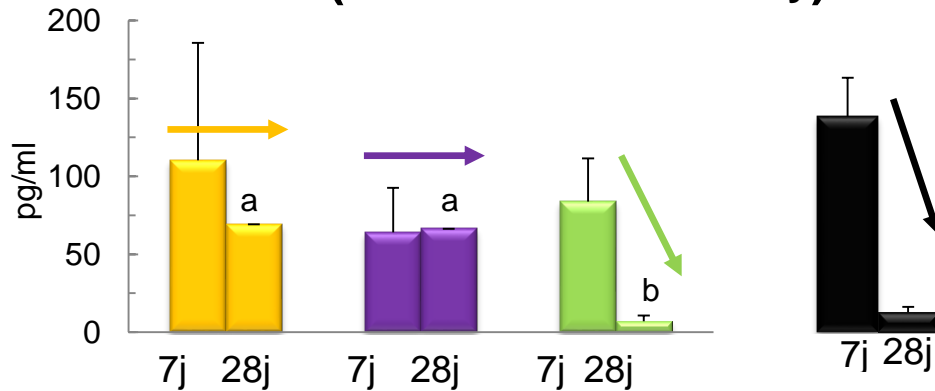


# Secretory activity of Mesenteric Lymph Nodes

## Interferon- $\gamma$ (Th1 pro-inflammatory)



## Interleukine-10 (Th2 anti-inflammatory)

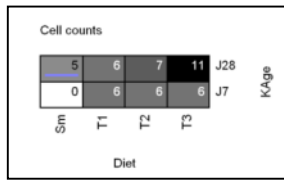


Milk lipids → maturation of the piglet's immune system more similar than with sow's milk

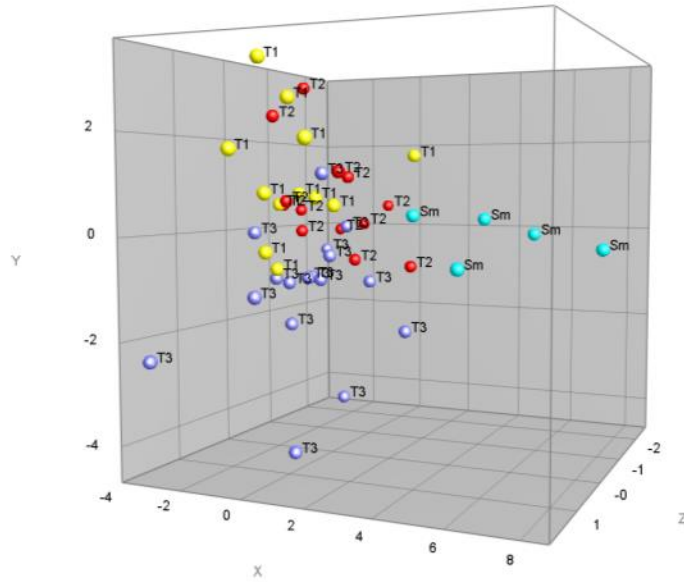
**Bourlieu et al. 2015**  
Eur J Lipid Sci Technol



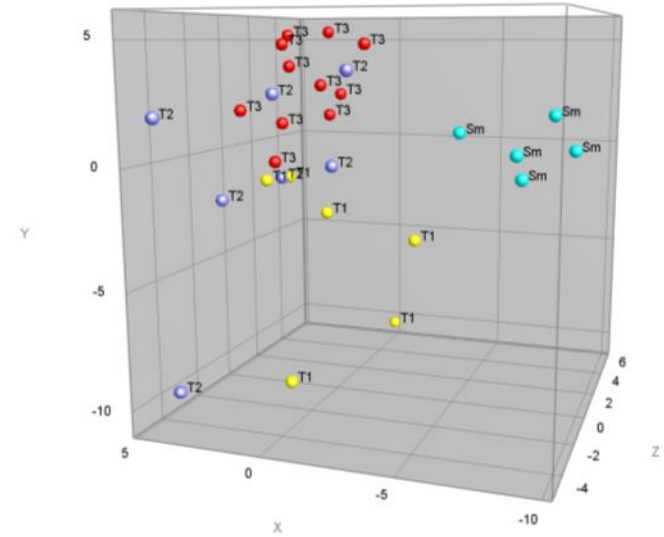
# Microbiota by DHPLC



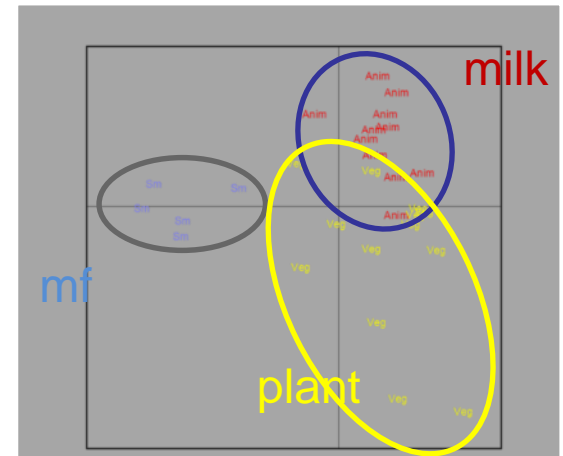
**D7 & D28**



**D28**



The composition/structure of the infant formula « orientates » the microbiota  
 More Proteobacteria with milk fat /  
 More Firmicutes with plant oil



Le Huêrou-Luron et al. 2016  
 Eur J Nutr

# Human Models

Patients with ileostomy

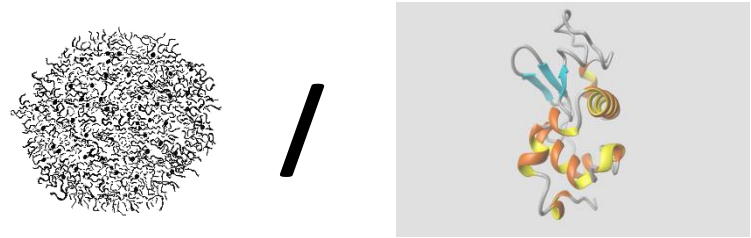


Naso-gastric or naso-jejunal probes



# Identification of bioactive peptides in the human GI tract

- 16 volunteers fed 30 g of:
  - Caseins
  - Whey Proteins
  - 50/50 Mix

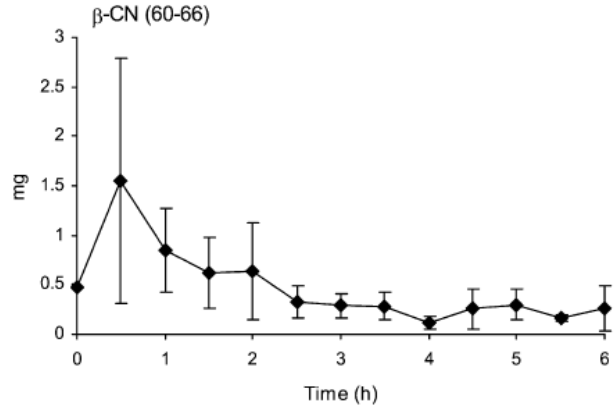


- Collect of effluents during 6h and analysis by MS-MS

4704 milk peptides identified = a unique database!

59-66	VYPFPGPI
59-68	VYPFPGPIP
60-66	YPFPGPI
108-113	EMPFPK
114-119	YPVEPF

Anti-hypertensive



Bioactive peptides concentrations are high enough in the lumen to generate a biological effect

**Boutrou et al.**  
**Am J Clin Nutr**  
**2013**

# Digestion gastrique du lait maternel chez le nouveau-né prématuré



- Enfants prématurés hospitalisés → nourris par sonde nasogastrique

ClinicalTrials.gov

NCT02112331

2014 - 2016

## A EFFET PASTEURISATION (n = 12)

Lait de la propre mère



Lait Cru



Lait Pasteurisé

## B EFFET HOMOGENEISATION (n = 8)

Pool de lait pasteurisé de donneuse anonyme



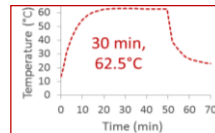
Lait Pasteurisé



Lait Homogénéisé

### Lactarium

Pasteurisation  
de Holder



Homogénéisation  
indirecte par  
ultrasons



→ 595 W

## Protocole expérimental

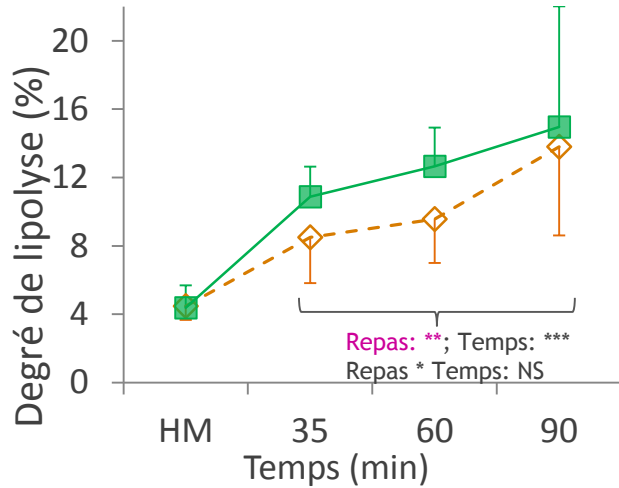
- **Essai croisé** : 2 repas tests par jour, pendant 6 jours
- 2 prélèvements gastriques par repas :
  - Avant administration du repas
  - **35, 60 ou 90 minutes** après le début du repas, en duplicat



# Homogénéisation et lipolyse gastrique

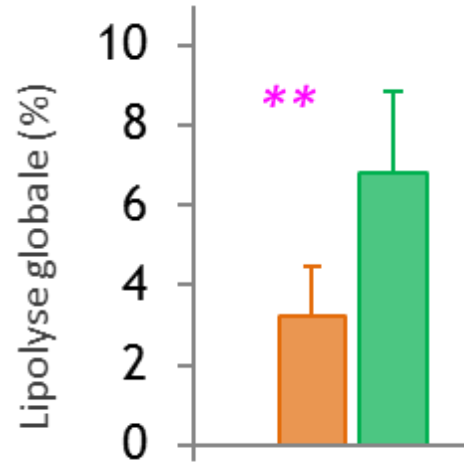
## Lipolyse instantanée

◆ Pasteurisé  
■ Homogénéisé



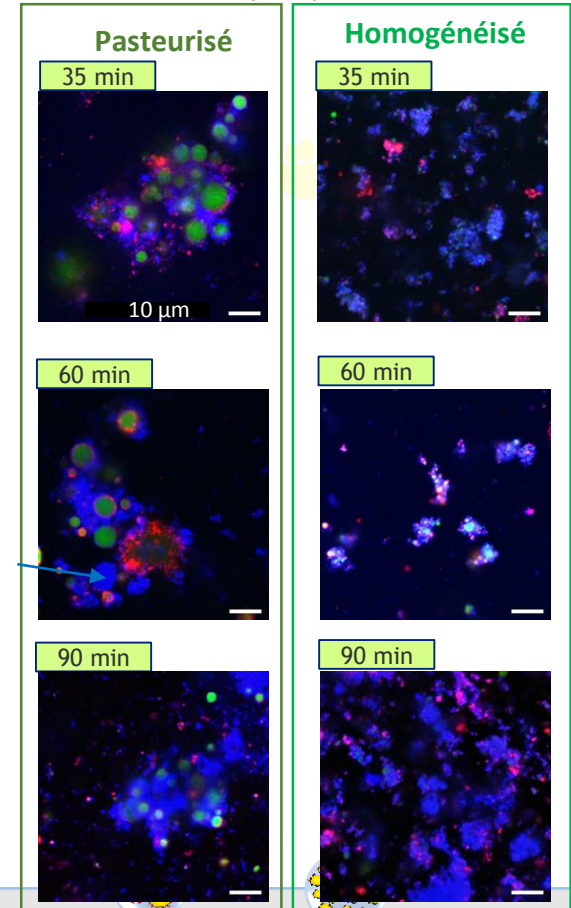
## Bilan postprandial

→ facilite l'adsorption de la lipase gastrique  
(Bourlieu et al., Food Chem, 2015)

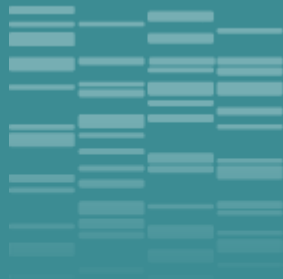


De Oliveira et al. 2017  
Clin Nutr

(n = 5)



- ❖ Homogénéisation → surface spécifique agmentée
- ❖ Plus de surface disponible pour l'adsorption et l'action de la lipase gastrique → lipolyse accélérée
- ❖ Moyen de favoriser la croissance des prématurés???

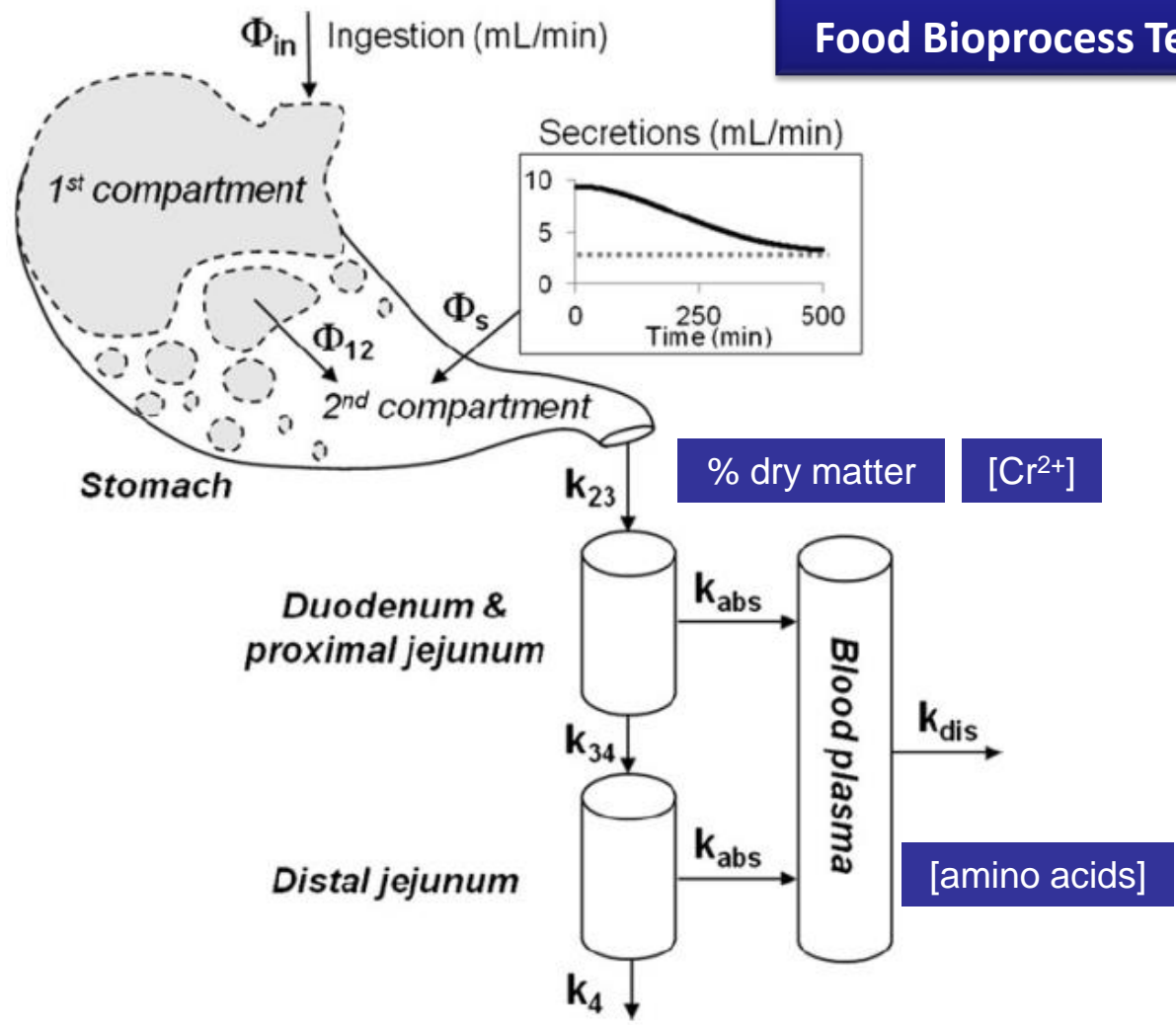
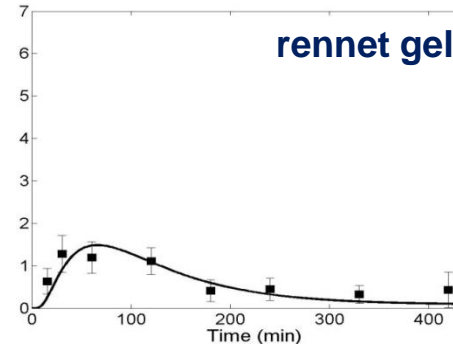
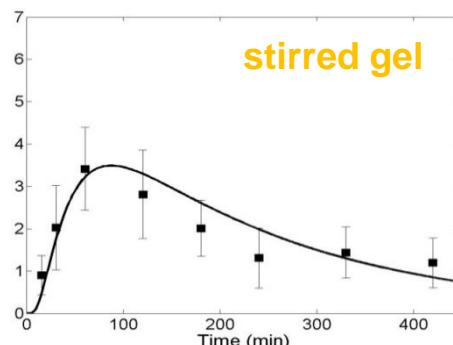
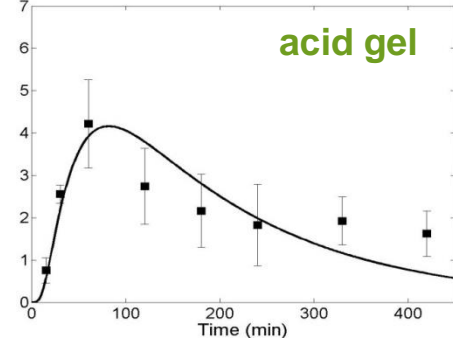
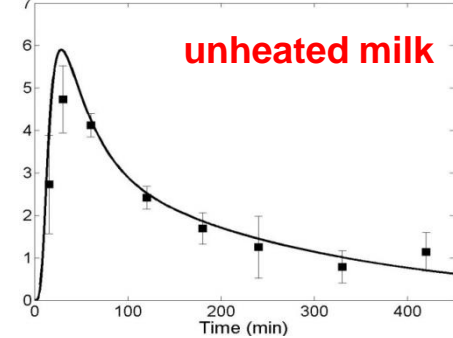


# Les modèles *in silico*



# Mathematical model of transit and absorption

Le Feunteun et al. 2014  
Food Bioprocess Tech



19 parameters including 3 to 6 describing the food matrix structure

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

## International Network

Dr. Didier DUPONT, Senior Scientist, INRA, France

●  
**INFOGEST**  
●







Tech Univ Denmark Univ Aarhus Univ Copenhagen MTT Univ Oulu Univ Eastern Finland  
 Norwegian Univ Life Sci Chalmers Univ Tech VTT Nofima Riga Stradin Univ Univ Ljubljana  
 Univ Zagreb  
 NIZO TNO Lund Univ

Wageningen UR Anabio  
 Teagasc Univ Reading James Hutton Inst  
 Univ College Cork Univ Birmingham  
 Cent Rech Lippmann Univ Greenwich  
 Univ Ghent Agroscope Posieux  
 Inst Food Res Agrocampus Ouest  
 Leatherhead Food Res ACW  
 FIBL Univ Greifswald  
 Univ Sevilla  
 IRD Max Rubner-Institut  
 INRA CNRS  
 AgroParisTech  
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 Univ Guelph  
 Czech Univ Prague  
 Inst Chem Technol  
 KTU Food Inst  
 Lithuanian Univ HS  
 Gdansk Univ Tech  
 Maize Res Inst  
 NGO  
 Polish Academy of Sci  
 Univ Belgrade  
 Univ Novi Sad  
 Aristote Univ Thessaloniki  
 Centr Food Res Inst  
 Ben Gurion Univ Technion

**Argentina**  
 CONICET  
 Univ Buenos Aires

**Australia**  
 Deakin Univ  
 Univ Queensland

**Albania**  
 Agric Univ Tirana

**Montenegro**



**New Zealand**  
 Riddett Inst  
 Plant Food Res

**USA**

360 scientists - 140 institutes – 39 countries

**China**  
 Soochow Univ

# Industry involvement

☞ ~ 40 European companies are involved in INFOGEST





Chair  
Didier Dupont - France



Vice-chair  
Alan Mackie - UK

# INFOGEST



[www.cost-infogest.eu](http://www.cost-infogest.eu)

In vitro/in vivo  
correlations  
**WG1**

In vitro semi-  
dynamic  
model of  
digestion  
**WG2**

Models for  
specific  
populations  
**WG3**

Digestive  
lipases and  
lipid digestion  
**WG4**

Digestive  
amylases and  
starch  
digestion  
**WG5**

In silico  
models of  
digestion  
**WG6**



Didier Dupont



Alan Mackie



Uri Lesmes



Myriam Grundy



Nadja Siegert



Choi-Hong Lai

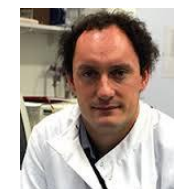


Guy Vergeres

The "Mind-  
the-Gap"  
group



Frederic Carriere



Fred Warren

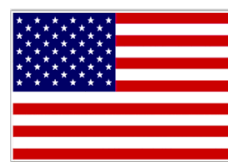
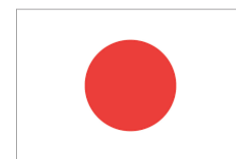
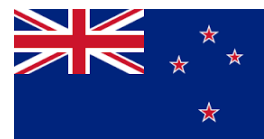


Steven Le Feunteun

# The International Conference on Food Digestion



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





**INFOGEST**  
International Conference  
Granada, Spain  
April 2019

We are pleased to announce the next  
**6<sup>th</sup> International Conference on Food Digestion**



**in Granada, Spain, 2-4 April 2019**