

The structure of the food matrix at different length scales affects the mechanisms of digestion and the nutrient bioavailability

Didier Dupont

▶ To cite this version:

Didier Dupont. The structure of the food matrix at different length scales affects the mechanisms of digestion and the nutrient bioavailability. The University of Adelaide, School of Medicine and Human Health, Nov 2023, Adelaide, Australia. hal-04299538

HAL Id: hal-04299538

https://hal.inrae.fr/hal-04299538

Submitted on 22 Nov 2023

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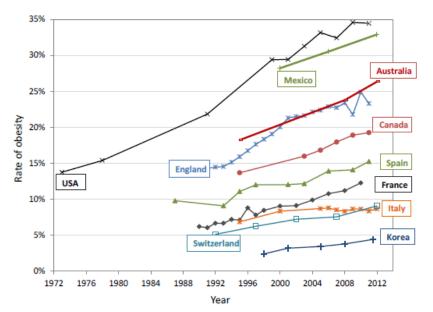
The structure of the food matrix at different length scales affects the mechanisms of digestion and the nutrient bioavailability



Dr Didier DUPONT, INRAE, STLO, Rennes, France

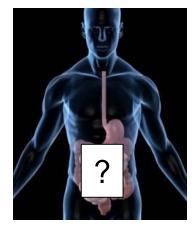


Food and human health: the key role of digestion



Diet-related diseases ↑

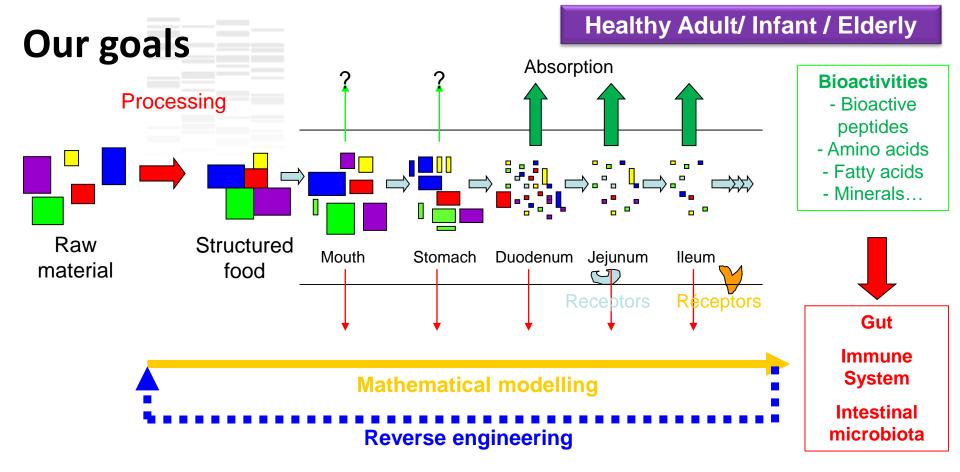
Prevent these pathologies rather than cure them



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

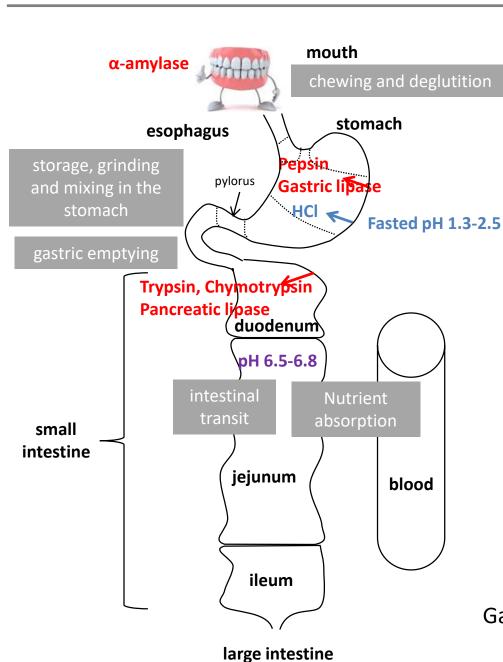


- To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion
- To determine the impact of the structure of food matrices on nutrient bioavailability
- To model these phenomena in order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



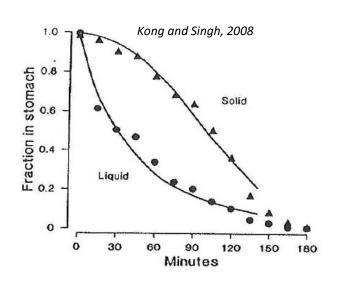


The digestive process





From Roger Lentle, Massey Univ. NZ



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

Models available at INRAE for simulating digestion

Menard et al. 2018, 2023 Wang et al. 2022

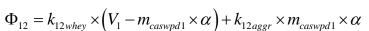


In vitro static models (infant, adult, elderly)

Le Feunteun et al.

2014, 2020

In silico models





Human

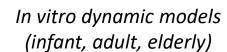






Animal models









De Oliveira et al. 2016 De Oliveira et al. 2017 Buffière et al. 2020 Boulier et al. 2023



Lemaire et al. 2021 Nau et al. 2022 Jimenez-Barrios et al. 2023 Charton et al. 2022, 2023

NERDT™: the NEar Real Digestive Tract



Xiao Dong Pro-Health Smart Digestion Suzhou University











STOMACH

Simulating the small intestine









The molecular structure of food protein affects the kinetics of digestion

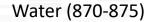


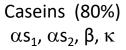
Boudry G,, Henry G & Dupont D. INRAE, Rennes, France

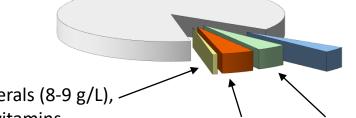














Minerals (8-9 g/L), vitamins, ...

Lipids (34-44 g/L) Lactose (48-50 g/L)

Whey Proteins (20%) β -lg, α -la

ø~200 nm (Holt, 1994)





Proteins (32-35 g/L)

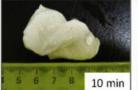
ø~11 nm ~15 casein molecules; (Thomar et al. 2013)

Casein organized into a supramolecular structure: the casein micelle (CM)

Casein can also be extracted after acidification followed by neutralization: the caseinate (CS)

Milk coagulates in the stomach









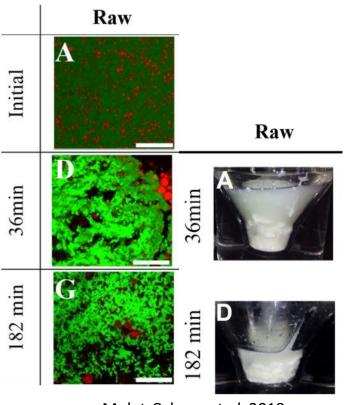
In vitro demonstration using the HGS
Ye et al. 2016





Unheated milk

Ye et al. 2019 In vivo evidence using a rat model



Mulet-Cabero *et al.* 2019 *In vitro* semi-dynamic model



Skim Milk Powder

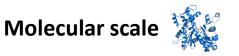


Sodium Caseinate

Casein micelles and sodium caseinate form different coagulums Wang et al. 2018



Objective



The objectives of the study were to:

1 Determine whether gastric emptying of an isoproteic solution of CM and CS are different or not (exp. 1)

2 Characterize the structure of the resulting chyme and determine if CM and CS are differently metabolized (exp. 2)

Experiment 1 – Determination of Gastric Emptying

96 g of CM or CS rehydrated in 800 ml of water

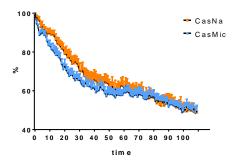
+12 g of glucose

+ ^{99m}Tc-colloidal (25Mbq)



9 pigs (20-25 kg)

 γ -scintigraphy over 120 min

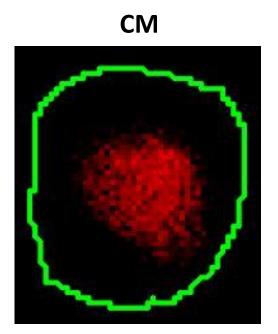


Gastric emptying halftime $(T_{1/2})$ and shape of the curve (β)

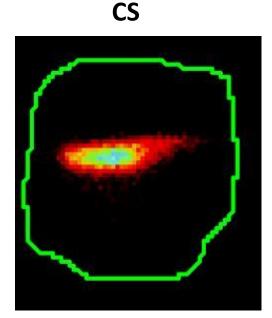


But a differential behaviour of CS and CM in the stomach

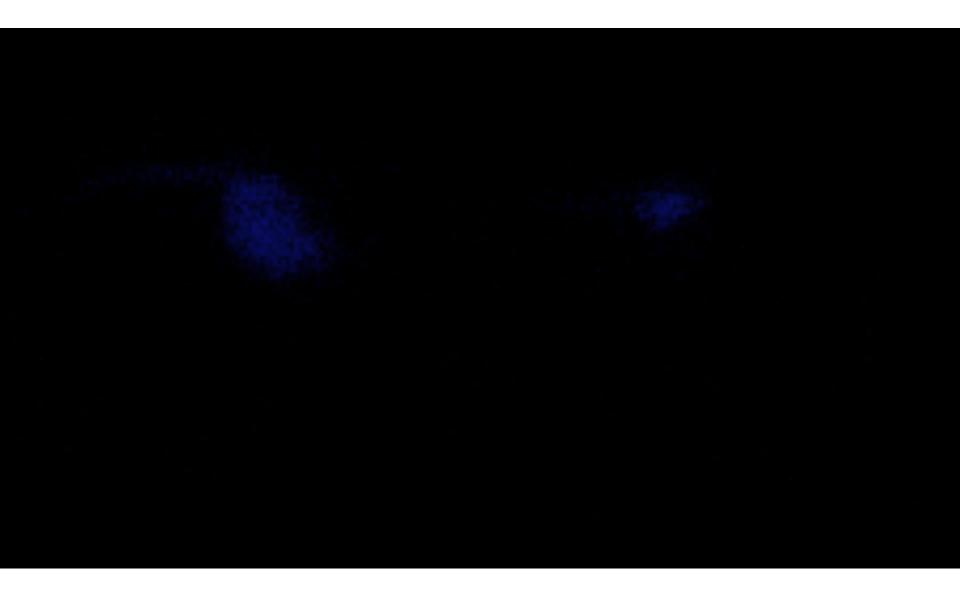
Exemple scintigraphic images at the beginning of gastric emptying (5-10 min after ingestion)



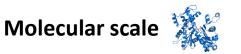
Radioactivity fully fills the stomach



Radioactivity is highly concentrated in the proximal part of the stomach



Objective



The objectives of the study were to:

- 1 Determine whether gastric emptying of an isoproteic solution of CM and CS are different or not (exp. 1)
- 2 Characterize the structure of the resulting chyme and determine if CM and CS are differently metabolized (exp. 2)

Experiment 2 – Chyme structure and protein metabolism

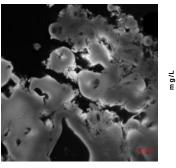
96 g of CM or CS rehydrated in 800 ml of water

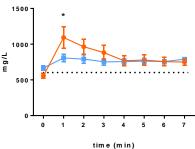
+12 g of glucose



10 catheterized pigs (20-25 kg)

Characterization of the chyme structure (slaughtering after 10 min, n=4)





Free plasma amino acids over 7h n=6



Collection of the stomach contents

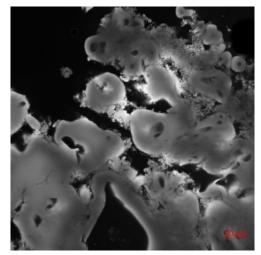


CM forms a large coagulum in the stomach whereas CS mainly remains in the liquid form

Microstructure of gastric chymes

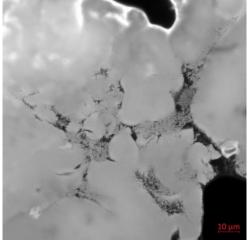


CM



CM Gels (left) are compact and dense =

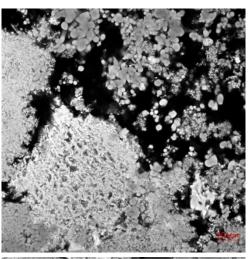
Strong coagulum

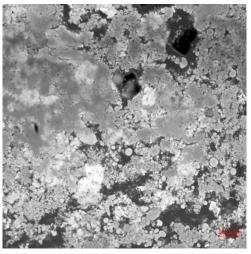


CS Gels (right) are an agglomerate of spherical particles that can easily dissociate. The gel have a very « loose » structure =

Protein precipitate

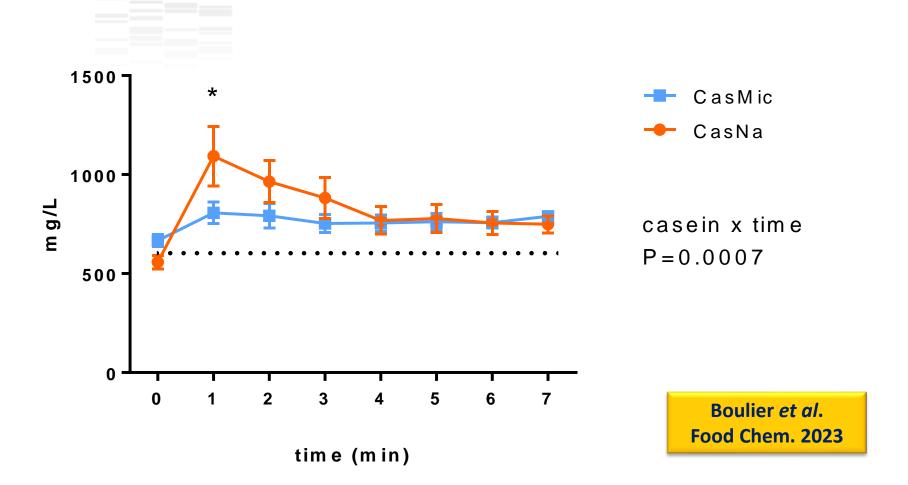
CS





Plasma amino acids





AA peak after 1h for CM whereas the concentration remains stable for CS



Conclusion



- * CM form a strong coagulum in the stomach leading to a slow and constant release of plasma amino acids up to 7 h
- * CS form a loose precipitate in the proximal part of the stomach but most of the caseins remain solubilized in the liquid fraction
- * CS are rapidly metabolized in the small intestine leading to the appearance of a peak of plasma amino acids one hour after protein ingestion

CM = slow caseins, CS = fast caseins

Perspectives

Do some *in vivo* kinetics experiments
Use ¹⁵N labelled-caseins to differentiate endo/exogenous proteins



From the protein molecular structure to the the food microstructure: The case of egg white gels



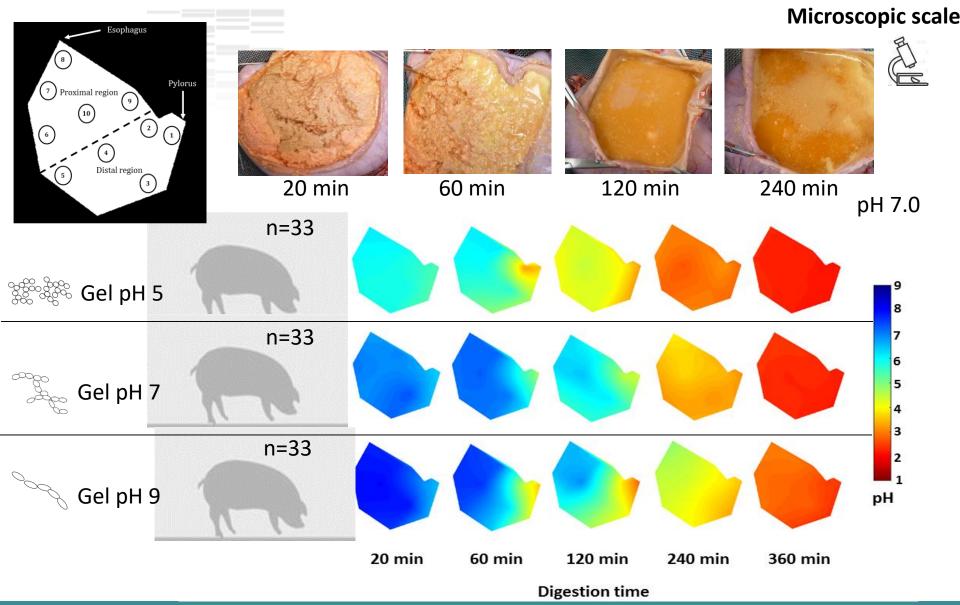
Nau F, & Dupont D. INRAE, Rennes, France



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

80°C/6h pH 7, IS 1M pH 5, IS 1M pH 9, IS 1M branched spherical linear Aggregates Rate of in vitro digestion +++ ++ **Gels** 90°C/2.5 h Nyemb et al. SEM Food Hydro. 2016 Nyemb et al. Food Res Int 2016 **CRYO-TEM** Rate of *in vitro* digestion

Spatial-temporal evolution of pH during an in vivo digestion

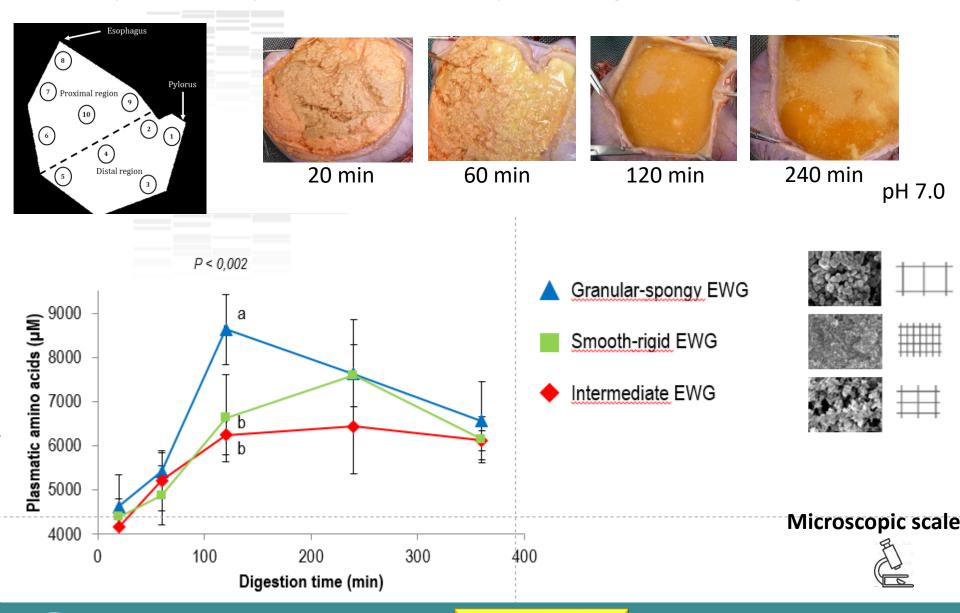








Spatial-temporal evolution of pH during an in vivo digestion









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



Le Feunteun S, Menard O, Dupont D. INRAE, Rennes, France



composition but different structure Macroscopic scale macrostructure unheated milk Ultra Low Heat rennet gel pH 6.6 ("raw" milk) powder 24h-20°C, rehydration in rennet 0.003 % v/w water 14.5% heat treatment 90°C-10 min pH 6.6 rennet gel microstructure 24h-20°C. heated milk rennet 0.3 % v/w 24h-20°C, acid gel pH 4 GDL 3 % w/w 24h-20°C, GDL 3 % w/w + stirred acid gel mixer 2 min pH 4 Fat-free matrices: 40 g/L caseins, 10 g/L whey proteins,

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





95 g/L lactose and minerals



Comparison of 6 dairy products of identical



SOLID

LIQUID

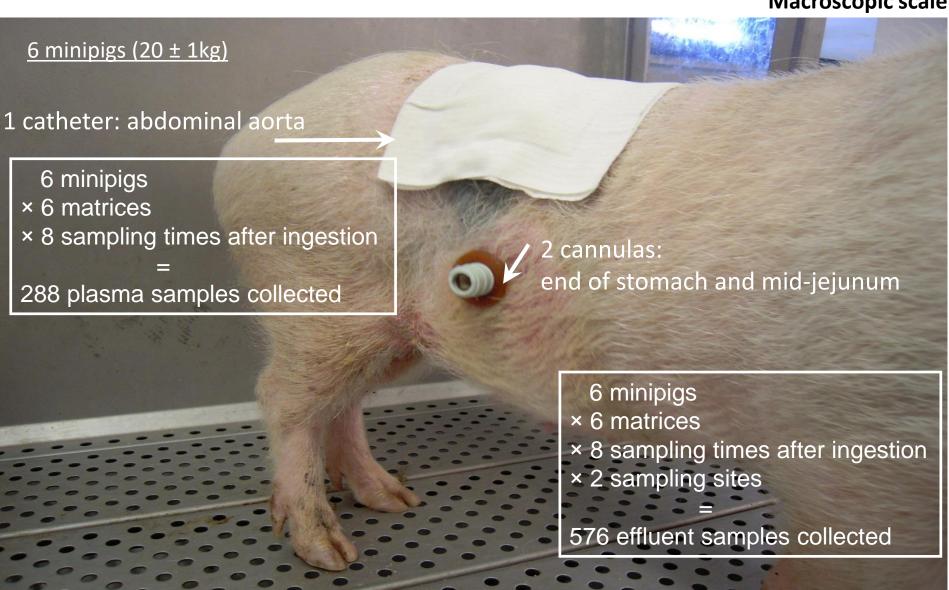
The multi-canulated mini-pigs







Macroscopic scale



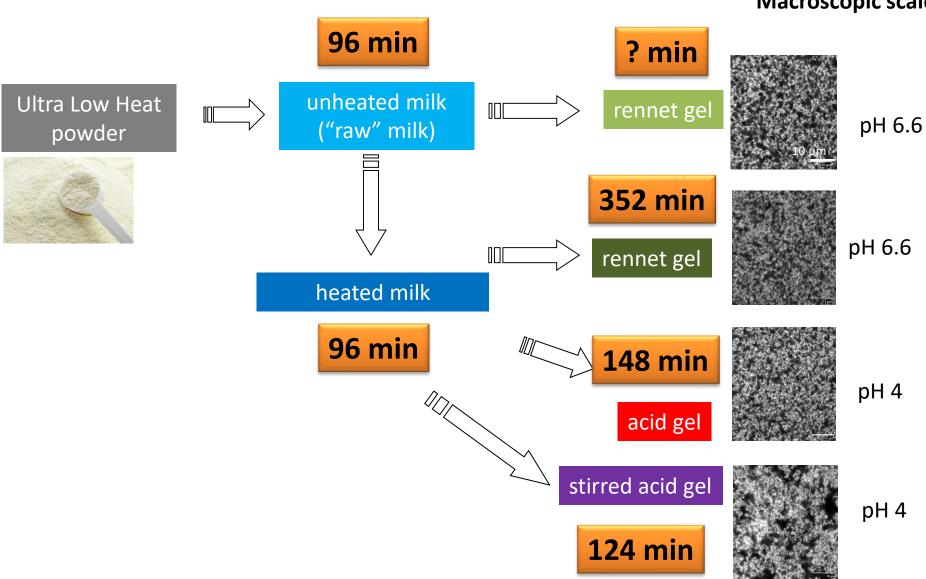
Gastric emptying half time







Macroscopic scale



The liquid-gel transition

Barbé *et al*.
Food Chem 2013

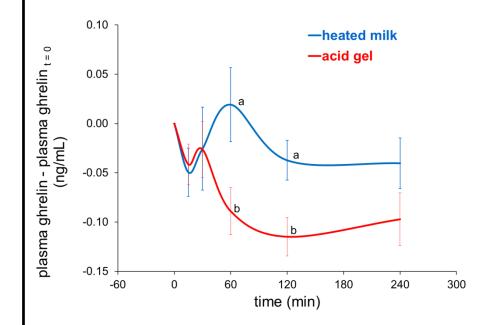




Macroscopic scale

Potential effect on satiety

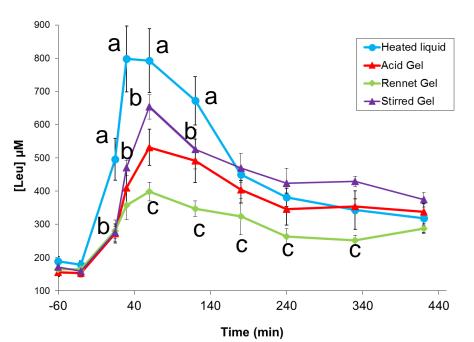
ghrelin (gastrointestinal hormone → appetite stimulation)



milk gelation:

postprandial ghrelin concentration = satiety?

Effect on absorption



milk gelation:

→ delayed proteins transit → delayed AA absorption maximal AA concentration in the plasma







Bioactive peptides released during digestion differ from one matrix to another



SOLID



LIQUID

Macroscopic scale

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

						_ ` `		٦,	- -		ĺ
Protein	Sequence	Activity	Reference	4	20	50	105	165	225	315	١,
αs1	1-23	EMUL	Shimizu et al. (1984)								ľ
αs1	23-34	HYP	Maruyama & Suzuki (1982)								ı
αs1	30-45	MB	Meisel et al. (1991)								ı
αs1	40-52	MB	Adamson & Reynolds (1996)								ı
αs1	43-58	MB	Meisel et al. (1991)								ı
α s 1	91-100	STRE	Miclo et al. (2001)								ı
αs1	99-109	MIC	McCann et al. (2006)								ı
αs1	167-180	MIC	Hayes et al. (2006)								ı
α s 1	180-193	MIC	Hayes et al. (2006)								ı
α s2	1-24	MB	Miquel et al. (2005)								ı
α s2	124-146	MB	Miquel et al. (2005)								ı
α s2	183-206	TRAN	Kizawa et al. (1996)								ı
α s2	183-207	MIC	Recio & Visser (1999)								ı
α s2	189-197	HYP	Maeno et al. (1996)								ı
α s2	190-197	HYP	Maeno et al. (1996)								ı
β	1-24	MB	Bouhallab et al. (1999)								ı
β	33-52	MB	Miquel et al. (2005)								ı
β	60-80	OPI	Jinsmaa & Yoshikawa (1999)								ı
β	98-105	OXI	Rival et al. (2001)								ı
β	114-119	OPI	Jinsmaa & Yoshikawa (1999)								ı
β	132-140	HYP	Robert et al. (2004)								ı
β	192-209	IMM	Coste et al. (1992)								ı
β	193-202	IMM	Kayser & Meisel (1996)								ı
β	193-209	IMM	Coste et al. (1992)								ı
κ	18-24	HYP	Lopez-Exposito et al. (2007)								ı
κ	106-116	THR	Jolles et al. (1986)								ı
β –lg	32-40	HYP	Pihlanto-Leppala et al. (2000)								1
β –lg	92-100	MIC	Pellegrini et al. (2001)								ı
β –lg	142-148	HYP	Mullally et al. (1997)								ı
											ı
											1

Protein	Sequence	Activity	Reference Adamson & Reynolds	4	20	50	105	165	225	315
αs1	40-52	MB	(1996)							
αs1	43-58	MB	Meisel et al. (1991)							
α s1	99-109	MIC	McCann et al. (2006)							
αs1	167-180	MIC	Hayes et al. (2006)							
αs1	180-193	MIC	Hayes et al. (2006)							
α s2	1-24	MB	Miquel et al. (2005)							
α s2	189-197	HYP	Maeno et al. (1996)							
β	33-52	MB	Miquel et al. (2005)							
β	166-175	HYP	Hayes et al. (2007)							
β	193-202	IMM	Kayser & Meisel (1996)							
βlg	92-100	MIC	(8))							
β –lg	142-148	HYP	(9))							

Rennet Gel

- 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

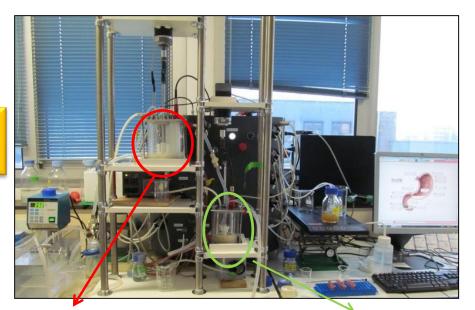
Acid Gel

Barbé et al. 2014 Food Res Int

Differential behaviour of acid/rennet gels in gastric conditions

- Acid/Rennet gel: identical composition, similar rheological properties and pore size
- - How can we explain this difference? Dynamic *in vitro* digestion of the 2 gels

Ménard *et al*. Food Chem 2014



Emptying:

Elashoff's model

DIDGI®

StoRM® software

Stomach

- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCI



Small intestine



- 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





Formation of a strong coagulum with rennet gel \rightarrow 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



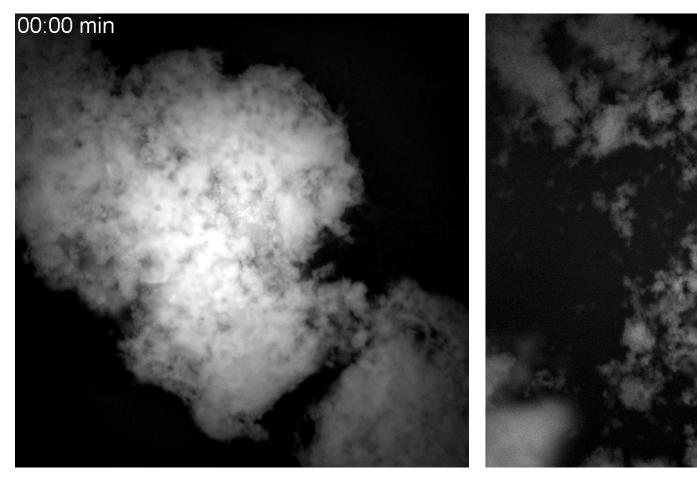


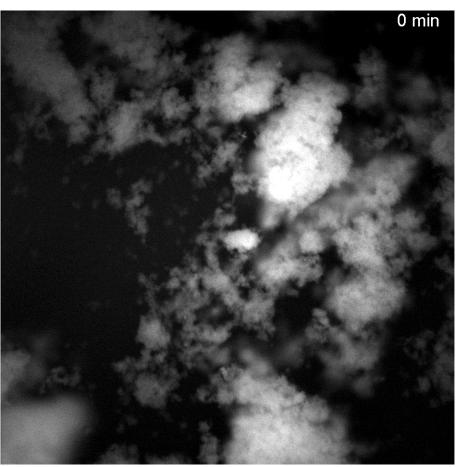
DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light

Allow the imaging of protein intrinsic fluorescence with a UV microscope



Kinetics of gel particles disintegration





Rennet Gel

Acid Gel

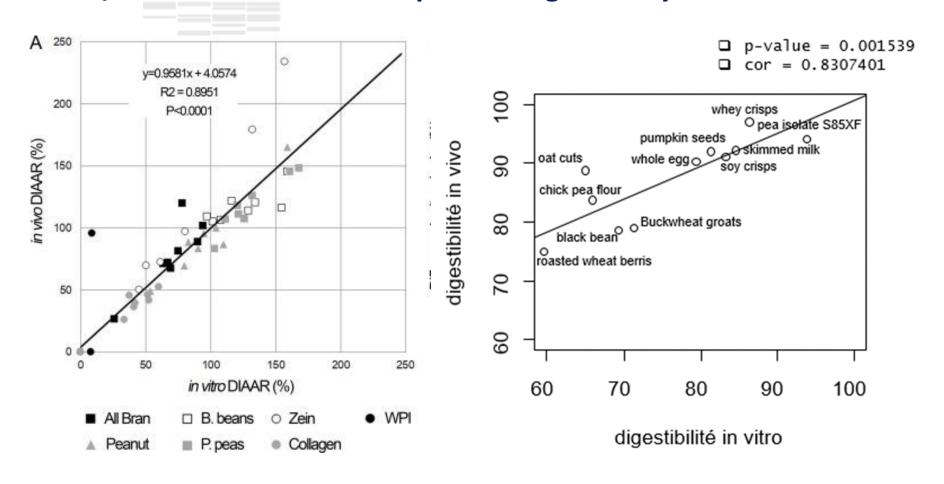
Can we estimate plant protein digestibility with in vitro digestion models?



Le Feunteun S, Menard O, Dupont D. INRAE, Rennes, France



In vitro/ in vivo correlation for protein digestibility measurement



Sousa et al. 2023

Nau et al. unpublished

Overall, good correlation are observed but some differences between studies persist







Protein digestibility with a dynamic in vitro digestion model

Study of 4 plant-based foods: 2 solids / 2 liquids

Tofu



Seitan



Soymilk



Pea Emulsion









Reynaud et al. 2021

Food Chem. 341

In vitro digestibility (%)

Dynamic in vitro digestion DiDGi®



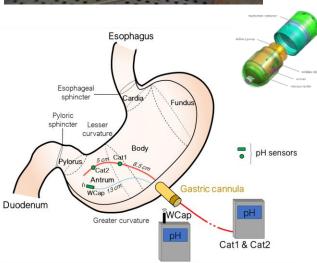


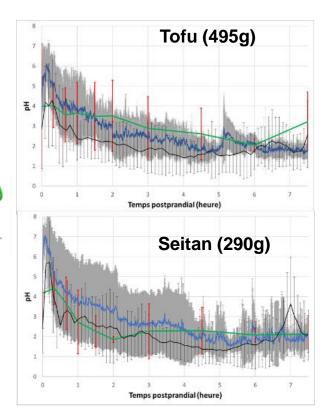


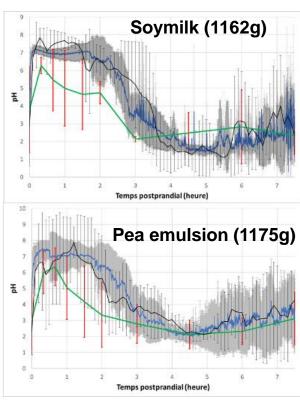
In vivo data are needed to program the digestion simulator

Evolution of gastric pH







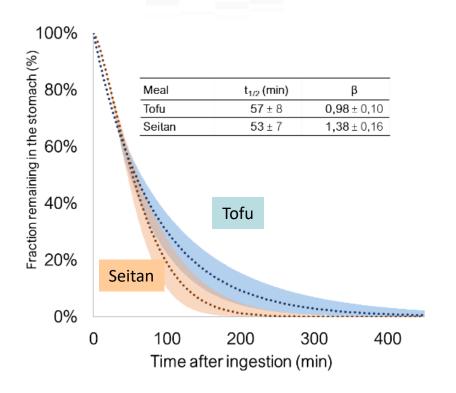


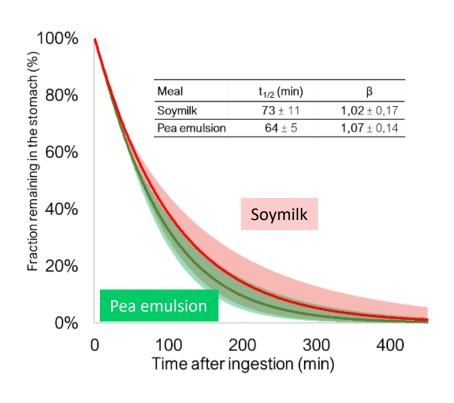






In vivo data are needed to program the digestion simulator Gastric emptying





Reynaud et al. 2020 Food Res Int, 128



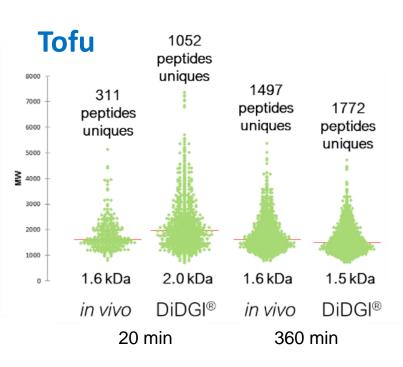


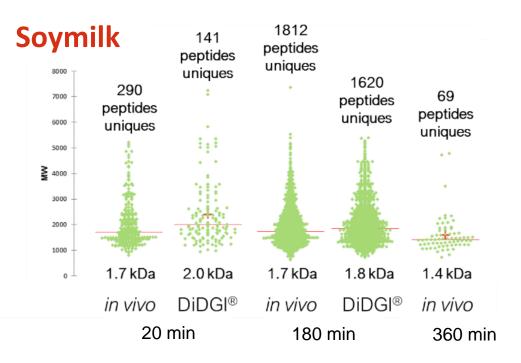


Comparison between pig and in vitro data

Model	Digestibility	Tofu	Soymilk
in vivo	True	97.1 ± 4.8%	99.4 ± 2.2%
	Apparent	56.5 ± 7.8% ^b	71.3 ± 2.5% ^a
in vitro	Apparent simulated	63.7 ± 3.5% ^b	72.7 ± 1.4% ^a

Comparison of the gastric peptidome









Improving DHA delivery by encapsulation and design of functional foods



Wang J, Pedrono F, & Dupont D. INRAE, Rennes, France



General strategy

DHA oil encapsulation

DHA bioaccessibility

DHA bioavailability, accretion and metabolism

17-HDoHE

15-HETE

8-HETE

12-HETE

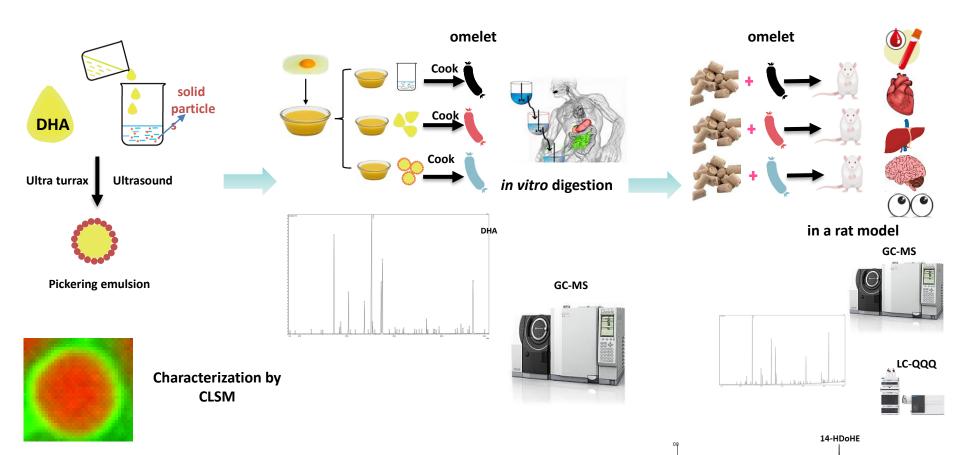
5-HETE

13-HODE

9-HODE

TXB2 PGE2

PGF2a



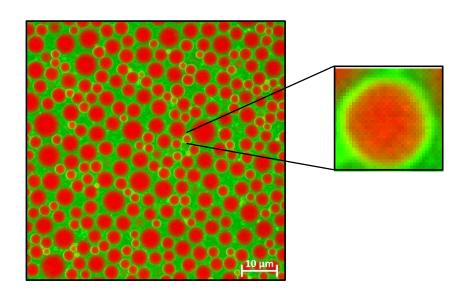
Wang et al.
Food Res Int 2022

Wang et al.
Frontiers in Nutr
2022

Wang et al.
Nutrients 2023

DHA oil in emulsion and omelet

In emulsion

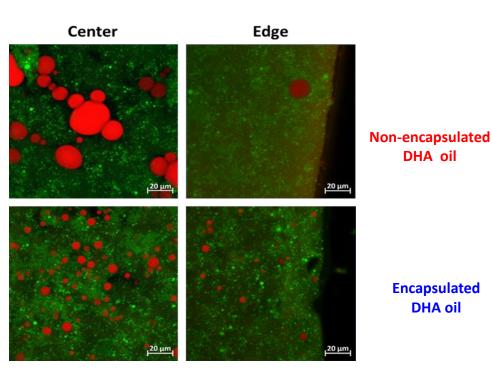


Encapsulated DHA oil with heatdenatured WPI

DHA oil stained with Nile Red and proteins stained with Fast Green.

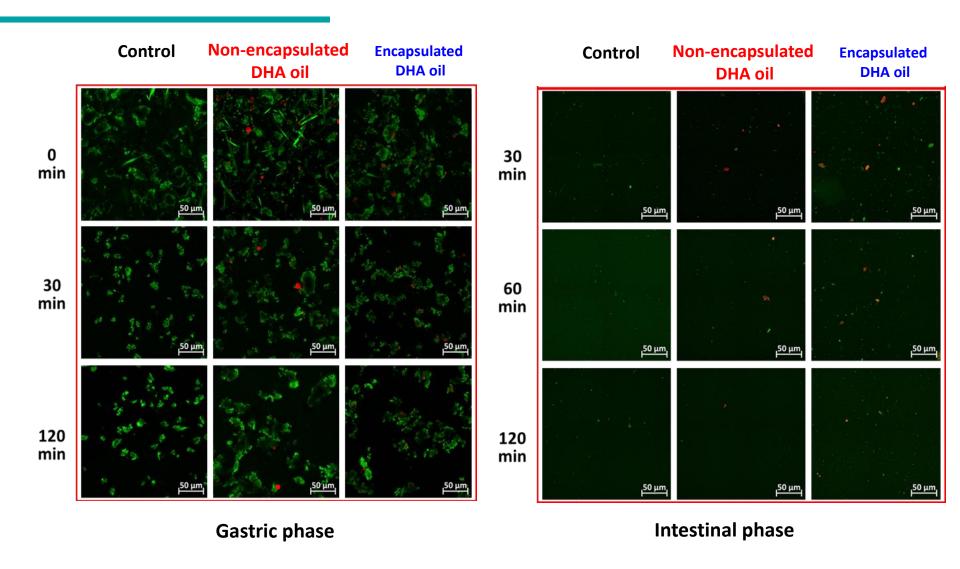
The particle size of heat-denatured WPI on average is 42 nm.

In omelet



Distribution of non-encapsulated and encapsulated DHA oil in omelets.

DHA oil in omelet during digestion

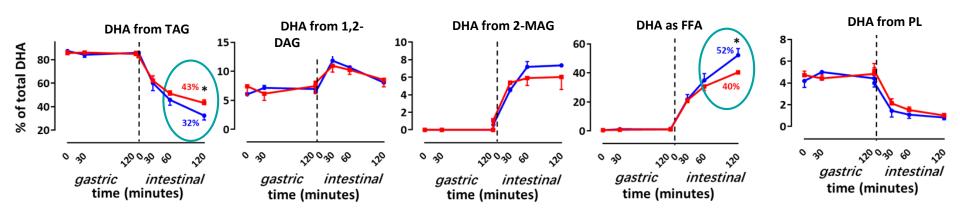


DHA oil and proteins were mainly hydrolyzed in intestinal phase

The release of DHA from different lipid species during digestion



Encapsulated DHA oil



The evolution DHA from different lipid species during digestion.

In gastric phase (pepsin and RGE):

☐ DHA oil was not hydrolyzed in gastric phase.

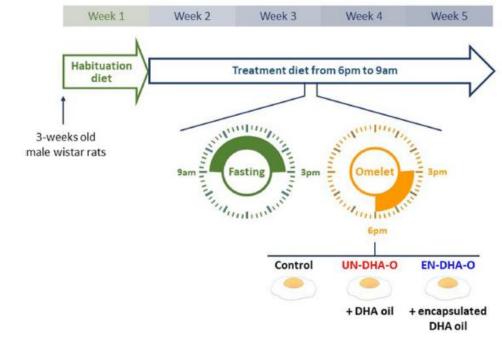
In intestinal phase (bile salt and pancreatin):

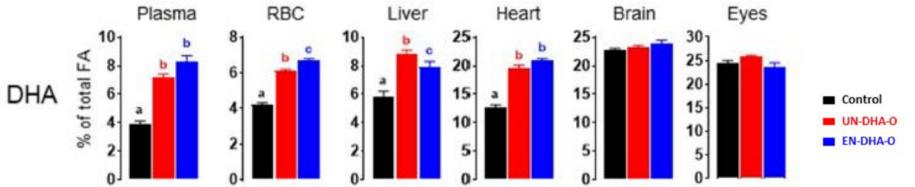
■ Hydrolyzed TAG and released FFA encapsulation > unencapsulation

- □ Larger interaction area between DHA oil and pancreatic lipase made by emulsification (Maljaars, 2012).
- ☐ Around 10-25% and 40-70% of ingested TAG can be hydrolyzed in gastric and intestinal phase, respectively (Bauer et al; Carriere et al., 1993).

What happens in vivo?

Encapsulation increased DHA concentration in plasma and red blood cells





Encapsulation increased oxylipins in heart and brain (precursors of protectins and maresins that limit inflammation and infection)



Food structure affects the delivery of hydrophilic and lipophilic micronutrients



Hiolle M., Dupont D. & Nau F.

INRAE, Rennes, France



Objective of the study:

Demonstrate the effect of the food macrostructure on the bioavailability of Vit B9, Vit B12, Vit D and lutein

Development of food matrices

Identical composition on dry matter:

- 17 % proteins
- 30 % lipids
- 52 % carbohydrates
 Enriched in micronutrients



Biscuit DM = 97 %

Sponge cake DM = 57 %





Pudding DM = 51 %

Custard DM = 31 %

Hiolle *et al*.
Food Chem. 2020

Characterisation of the matrices

Extensive biochemical characterization

Macrostructure : texture analysis

Microstructure : confocal microscopy

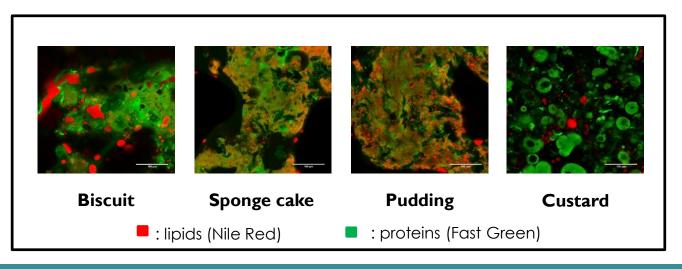
Clinical study

12 healthy volunteers (20-30 y) Randomized, controlled, crossover study

Postprandial analyses over 8h

Quantification in the plasma:

- Vit B9, B12, D and lutein



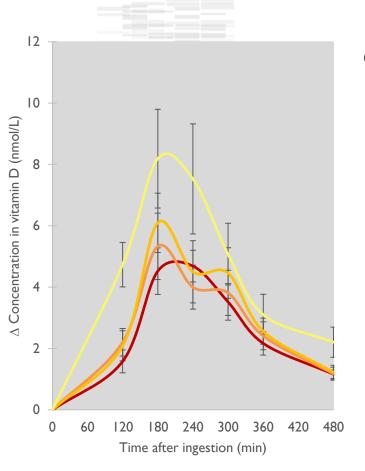






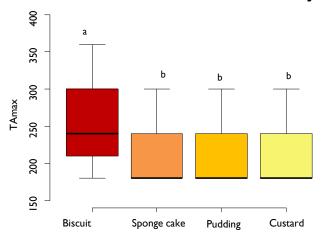


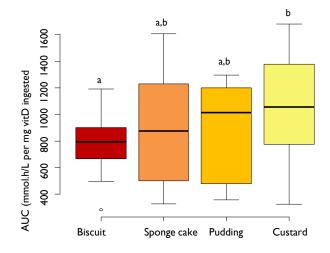
Food structure affects micronutrient kinetics of release and bioavailability



Vitamin D

Clinical study





Buffière *et al*. Food Chem. 2020

Increased AUC for vitamin D when provided via a custard









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.

INRAE, Rennes, France

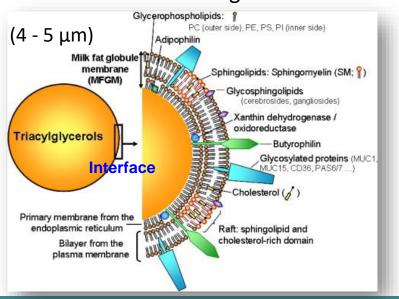


Human/ bovine milk / Infant Formula

Lipid globule structure



Native milk fat globule

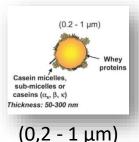


(Lopez, 2010)



Lipid droplets

Triacylglycerols



(Lopez and Briard-Bion, 2007)

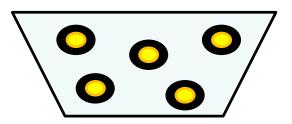






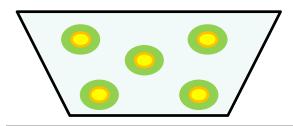
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula T1



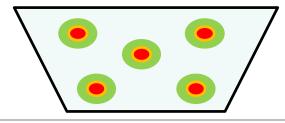
Interface 100 % Proteins 100% vegetable oil

Formula T2

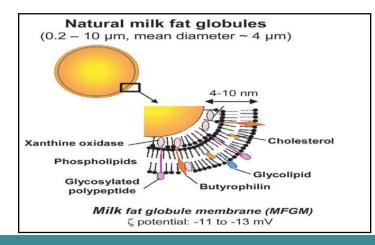


Interface 100 % phospholipids 100% vegetable oil

Formula T3



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



Lopez, (2007)

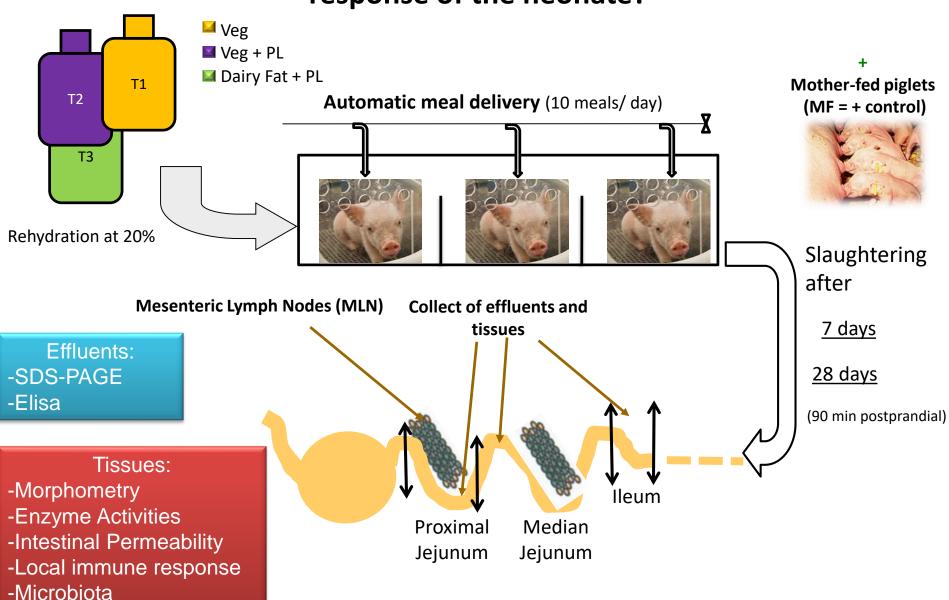






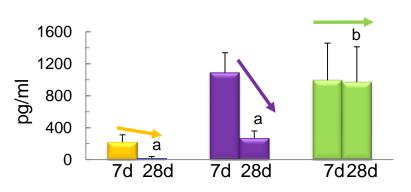


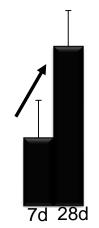
Can the composition of infant formula modulate the physiological response of the neonate?



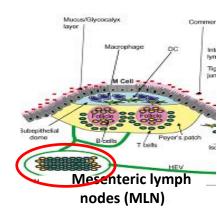
Secretory activity of MLN

Interferon-g (Th1 pro-inflammatory)

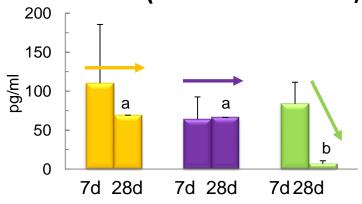








Interleukine-10 (Th2 anti-inflammatory)





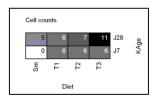
Milk lipids → maturation of the piglet's immune system more similar than with sow's milk

Le Huerou et al. Eur J Nutr 2017









Microbiota by DHPLC

Cell counts

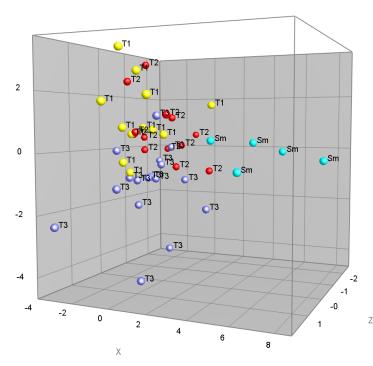
5 6 7 11 J28 95

E C C C

D7 & D28

D28

T2



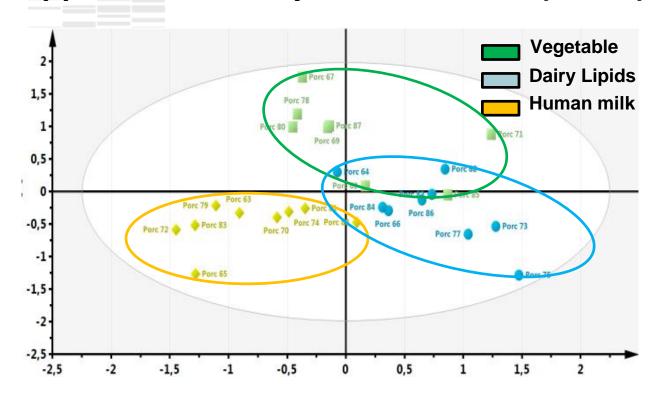
The composition/structure of the infant formula « orientates » the microbiota

More Proteobacteria with milk fat / More Firmicutes with plant oil

_T1 CT1 -5 -10 0 -10 milk

Bourlieu et al. Eur J Lipid Sci Technol 2016

What happens when they become older (140 d)?



If animals are submitted to a nutritional stress (high fat/sugar diet), some differences remain in:

- * the microbiota composition
- * the fecal metabolome with different metabolites (including propionate)
- * the immune system with a reduced susceptibility to inflammation with milk lipids









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 team at INRA Rennes

Head Didier DUPO

Didier DUPONT- Senior Scientist

Scientists

Amélie DEGLAIRE – Lecturer
Juliane FLOURY – Lecturer
Catherine GUERIN - Lecturer
Steven LE FEUNTEUN – Senior Scientist
Joëlle LEONIL – Senior Scientist
Martine MORZEL – Senior Scientist
Françoise NAU – Professor
Frédérique PEDRONO – Lecturer

Xiaoxi YU — Post-doc Guilherme FURTADO — Post-doc



PhD students

Yohan REYNAUD (2016-2019) Amira HALABI (2017-2020) Jun WANG (2018-2021) Lea SALLELES (2018-2021) Elise CHARTON (2019-2022) Lucile CHAUVET (2019-2022) Ousmane SUWAREH (2019-2022)

Technicians

Gwenaële HENRY Yann LE GOUAR Nathalie MONTHEAN

Engineers

Julien JARDIN Olivia MENARD Jordane OSSEMOND

Masters students









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

International Network

Dr. Didier DUPONT, Senior Scientist, INRAE, France







Main objective: understanding the mechanisms of food digestion

- Develop new in vitro, in vivo and in silico digestion models including some for specific populations (infant, elderly)
- Harmonize the methodologies and propose guidelines for performing experiments
- Validate in vitro models towards in vivo data (animal and/or human)
- Identify the beneficial/deleterious components that are released in the gut during food digestion
- Determine the effect of the matrix structure on the bioavailability of food nutrients and bioactive molecules





Industry involvement





































































LACTALIS























GRANAROLO

















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Vice-chair Alan Mackie - UK



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



Isidra Recio



Pasquale Ferranti



Linda Giblin



Myriam Grundy



Daniela Freitas



Choi-Hong Lai



Andre Brodkorb



Lotti Egger



Uri Lesmes



Brigitte Graf



Frederic Carriere



Anabel Mulet-Cabero



Bin Zhang

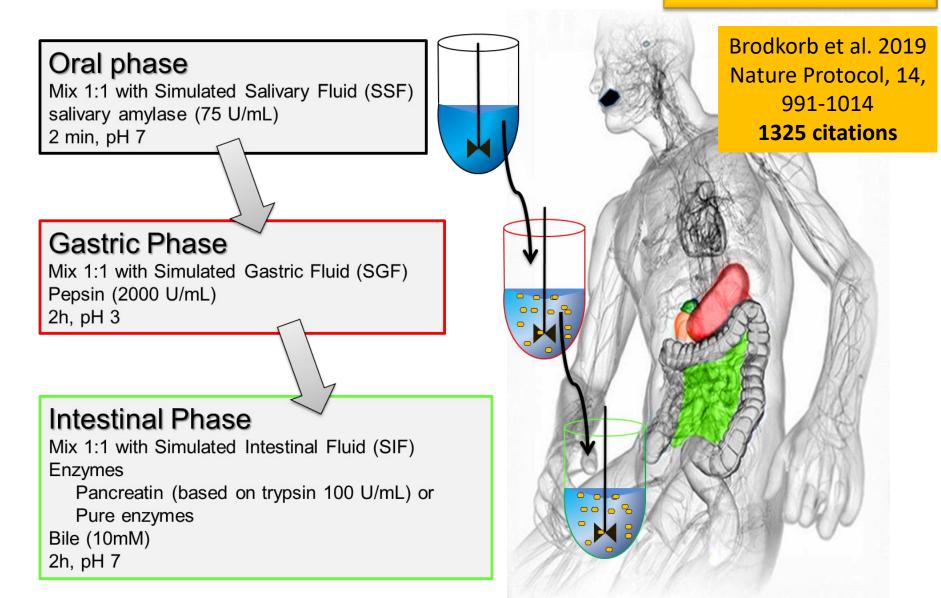


Steven Le Feunteun

Some outputs

In vitro gastrointestinal digestion Consensus INFOGEST protocol

Minekus et al. 2014
Food & Function, 5, 11131124
3125 citations







Porto, Portugal 2024

We are pleased to announce the next 8th International Conference on Food Digestion



in Porto, Portugal, 9-11 April 2024