

Impact of pasteurization of human milk on the kinetics of peptide release during gastric digestion in the preterm infant



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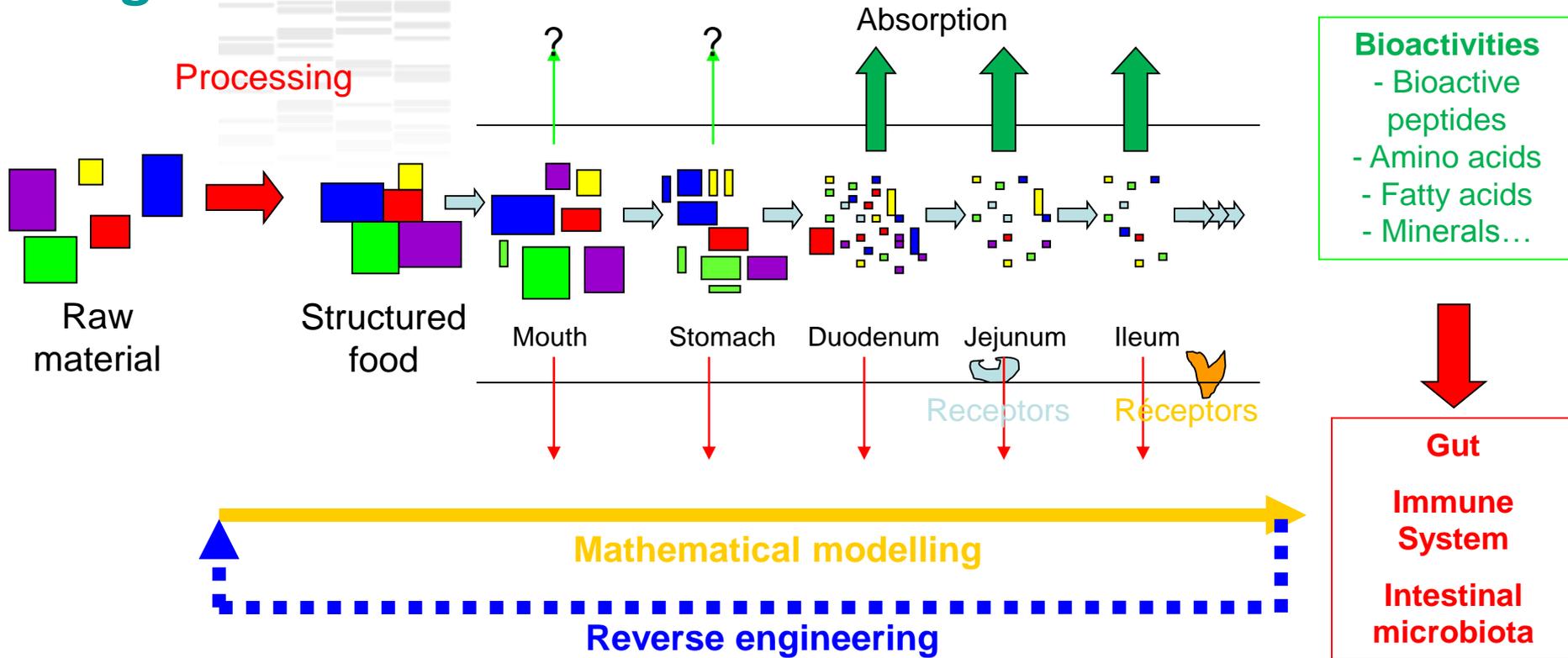
Olivia MENARD

Jordane OSSEMOND

Masters students

Our goals

Healthy Adult/ Neonate/ Elderly



- ☞ 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

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)*

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

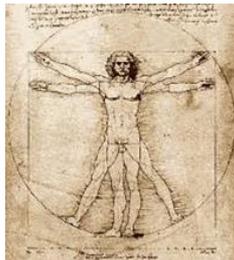


*In vitro dynamic models
(infant, adult, elderly)*

Le Feunteun et al. 2014
Food Bioprocess Tech

*In silico
models*

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



*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

WHO recommendations: Exclusive breastfeeding until 6 months



- ☞ Human milk exhibits important differences in composition and structure with infant formula
- ☞ These differences will modify the behaviour of these foods in the gastrointestinal tract of the neonate:
 - Gastric emptying half-time of human milk is 36 min but 74 min for IF
 - Kinetics of lipid and protein hydrolysis are different

Human/ bovine milk / infant formula

Protein Composition

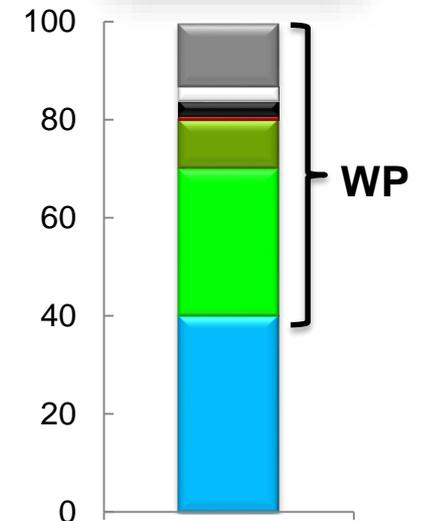
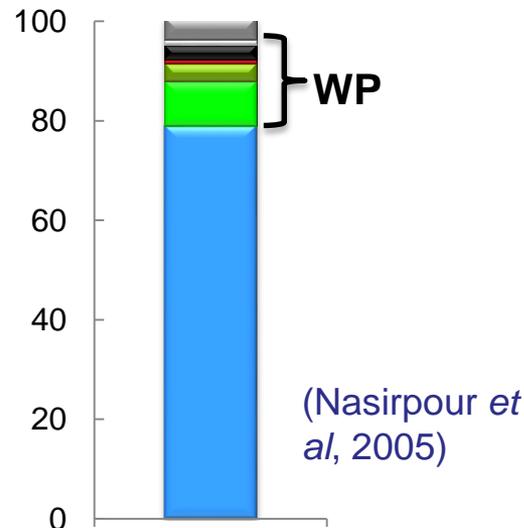
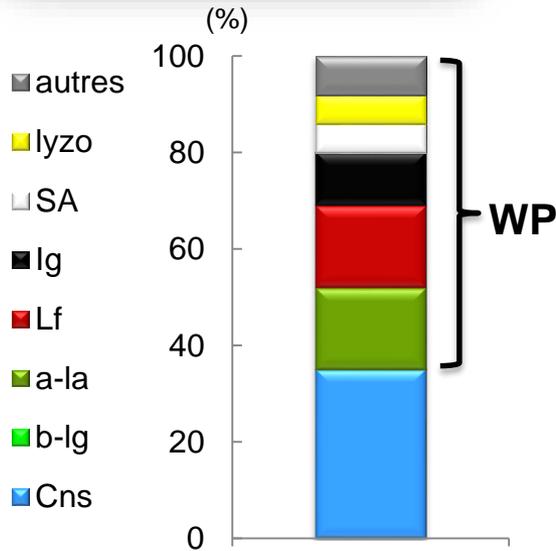
Human milk



Cow's milk



Infant Formula



Human/ bovine milk / Infant Formula

Protein structures

Human milk



Bovine milk

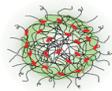


Infant Formula

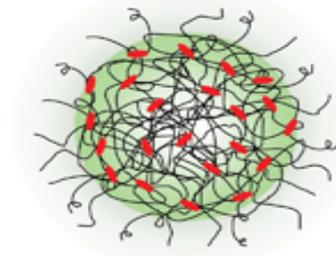


(Turck *et al*, 2010) $\varnothing = 64 \text{ nm}$
(β , κ -casein)

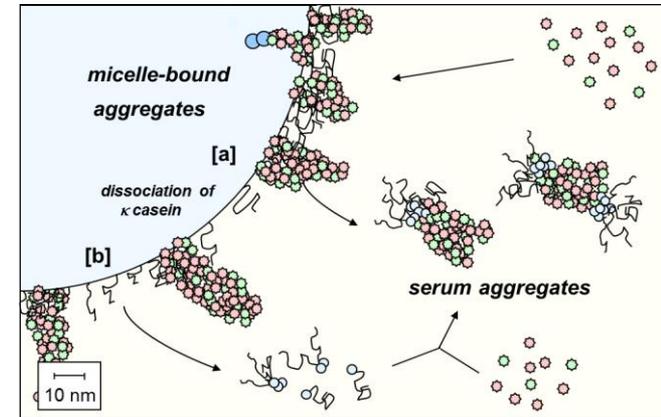
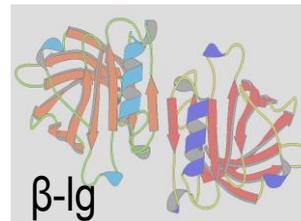
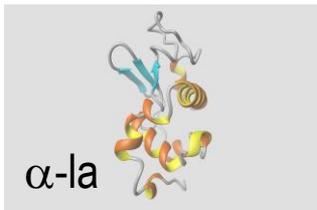
Casein micelle



$\varnothing = 182 \text{ nm}$
(α s1, β -casein)



Whey Proteins



Human/ bovine milk / infant formula

Fat Composition

Human Milk



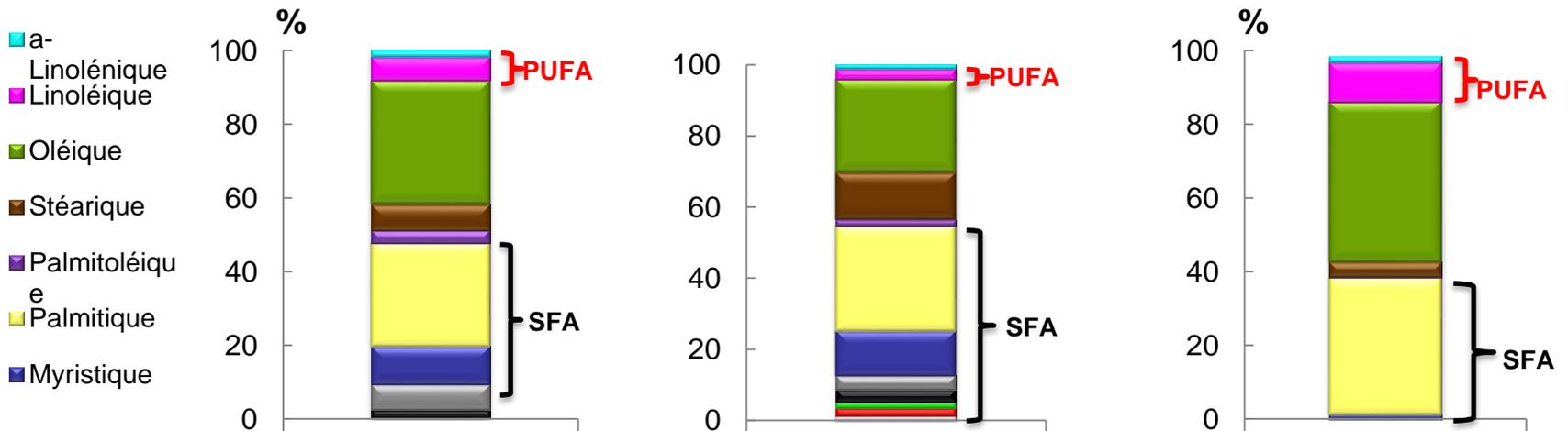
Cow's Milk



Infant Formula



(Malacarne *et al*, 2002)



Human/ bovine milk / Infant Formula

Lipid globule structure

Human milk



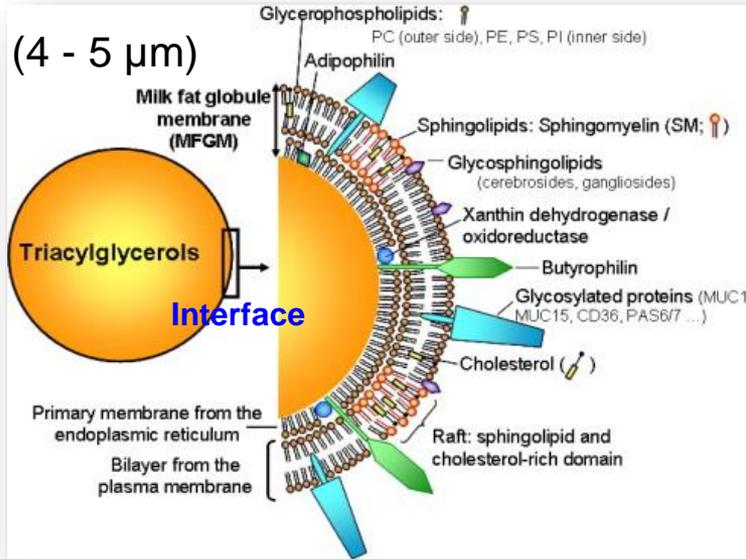
Bovine milk



Infant Formula



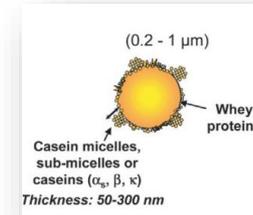
Native milk fat globule



(Lopez, 2010)

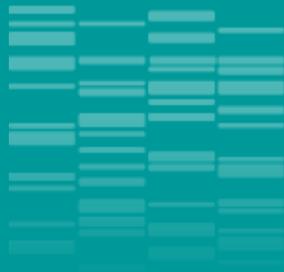
Lipid droplets

Triacylglycerols



(0,2 - 1 μm)

(Lopez and Briard-Bion, 2007)



Understanding how structures of human milk
are disintegrated during digestion to create
new biomimetic infant formula



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

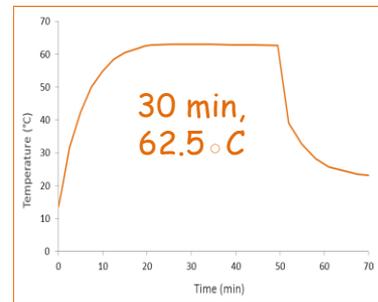
Pasteurized-homogenized human milk

24h before feeding (4°C)

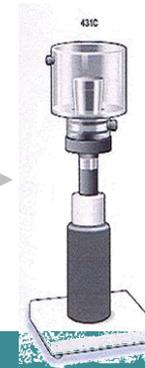
Stored at -20°C
(bank milk)

Stored at -20°C (bank milk)

Bank milk



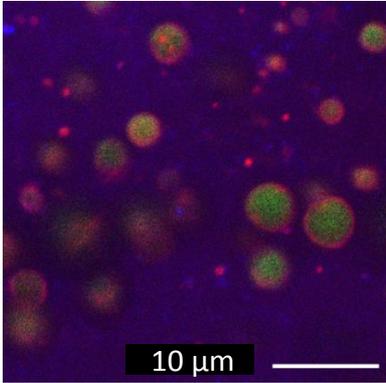
Homogenization by sonication



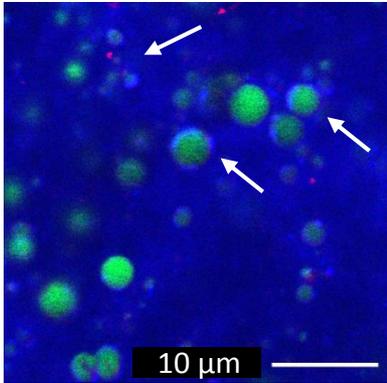
Pasteurization affected the initial structure and the emulsion disintegration of HM

Initial structure

Raw HM



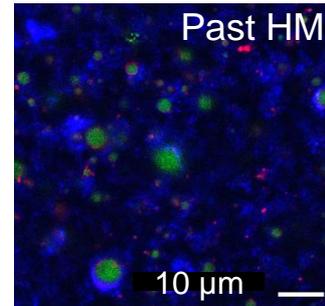
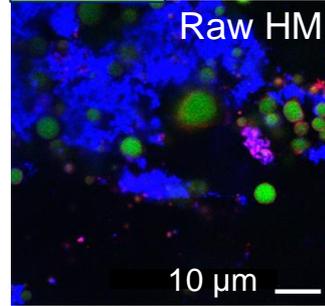
Past HM



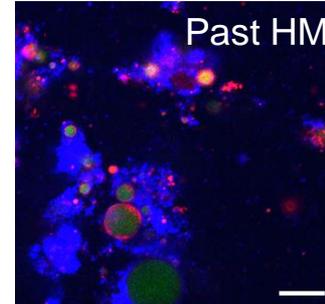
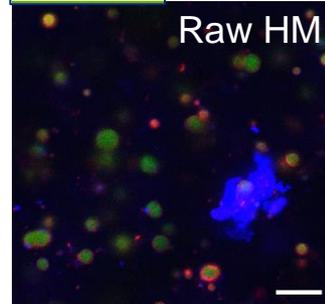
→ Protein heat-induced aggregation

Gastric disintegration

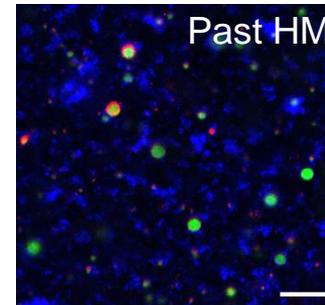
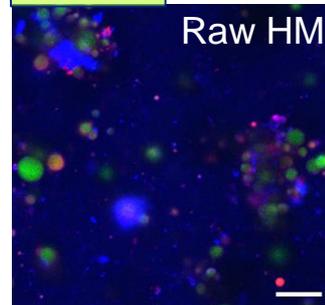
35 min



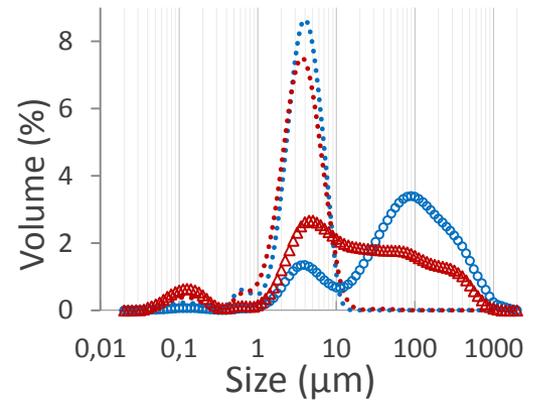
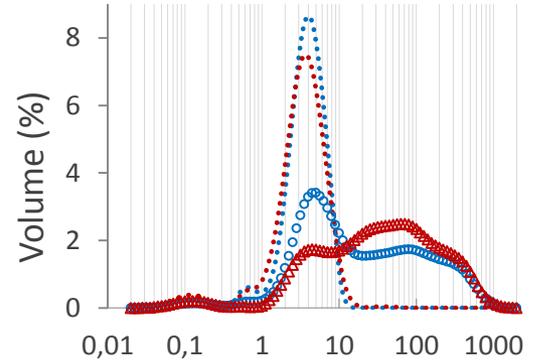
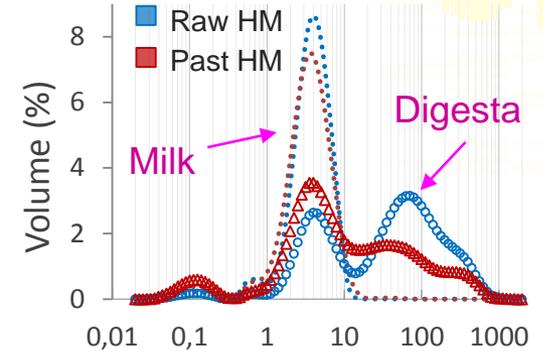
60 min



90 min



(n = 6 infants)



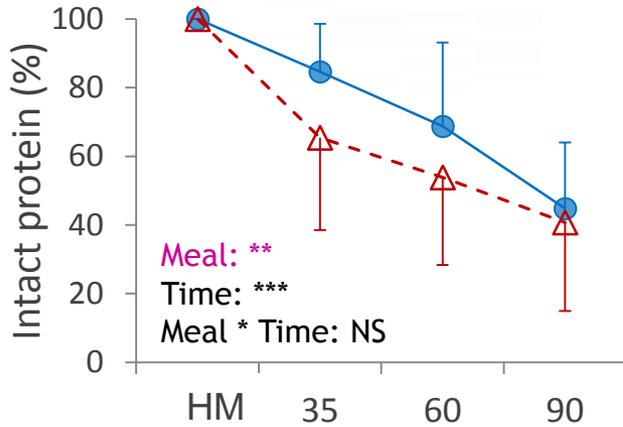
■ Apolar lipids ■ Amphiphiles ■ Proteins

(De Oliveira et al., AJCN, under revision)

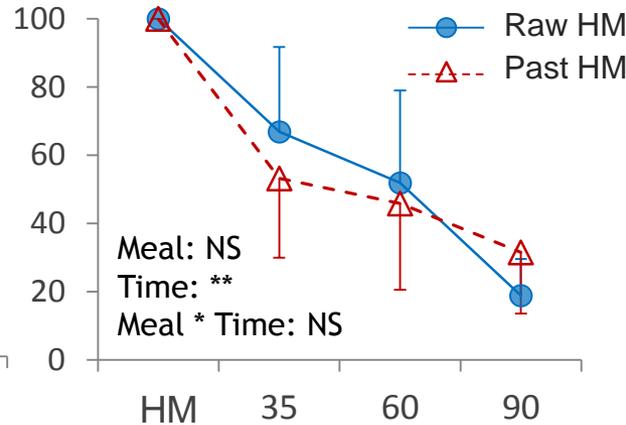
Pasteurization selectively impacted gastric proteolysis



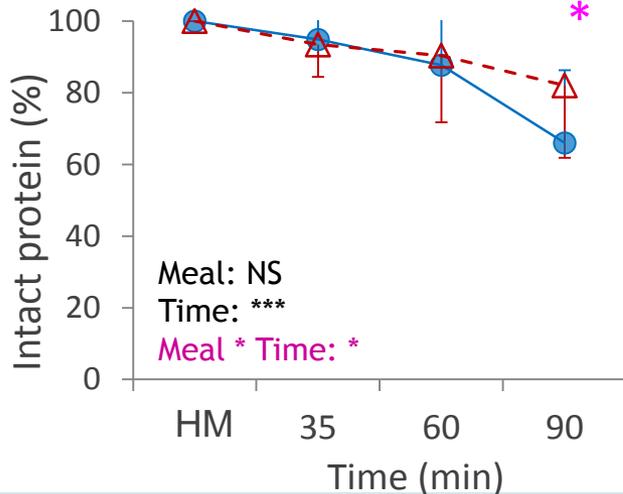
Lactoferrin



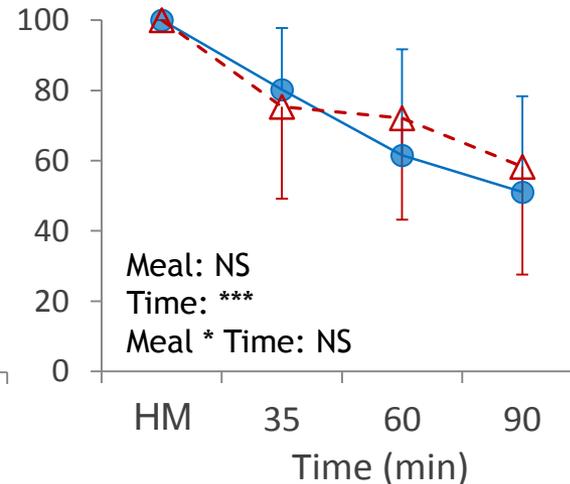
Serum albumin



α -lactalbumin



β -casein



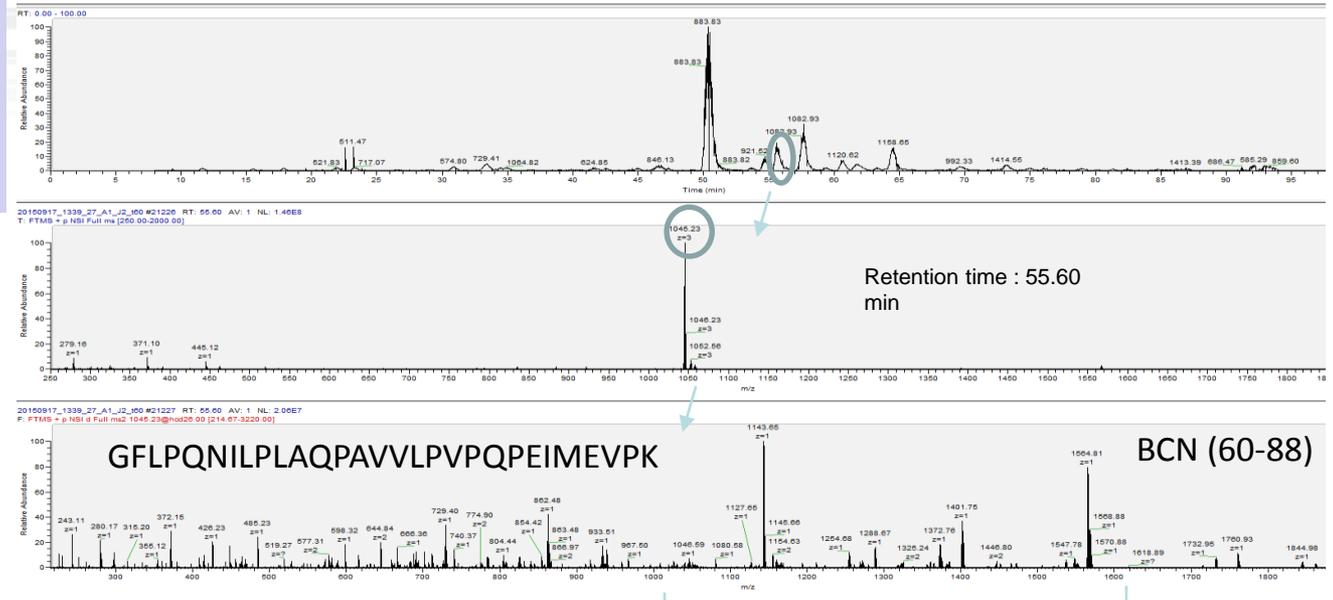
- Faster decrease of lactoferrin in Past HM
- Slower decrease of α -lactalbumin in Past HM (only at 90 min)
- α -lactalbumin was the most resistant to gastric digestion

Impact on protective qualities of the native lactoferrin?

**De Oliveira et al.,
Am J Clin Nutr 2017**

Peptidomic Analyses

Nano-RSLC – MS – MS
 Q Exactive™ Quadrupole-Orbitrap
 Electrospray Ionisation
Precision : 0.001Da (1 ppm)
Sensitivity : 0.3 pmol/mL



Peptide identification
 6 to 51 AA – 0.7 to 5.7 kDa

Relative quantification
 Abundancy

X!Tandem

Possible Post-Translational Modifications :

- serine or threonine phosphorylation
- methionine oxidation
- lysine or arginine lactosylation
- cyclisation of Glu into pyroglutamic acid
- acetylation in N-ter position



Article
pubs.acs.org/jpr

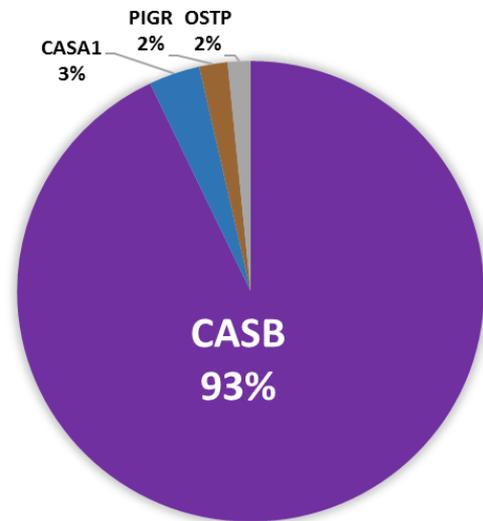
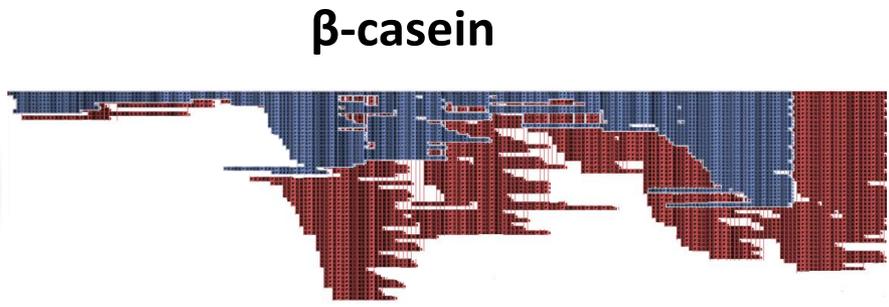
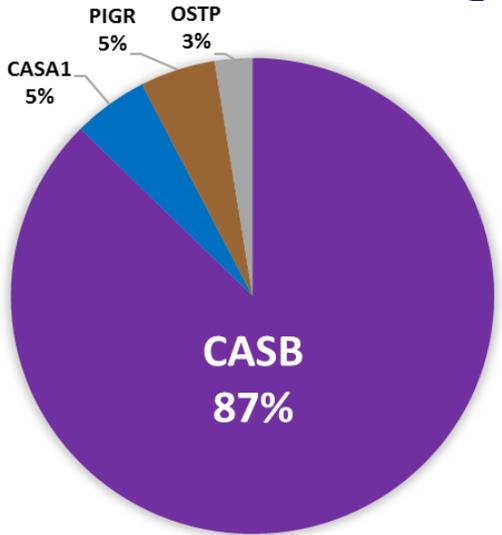
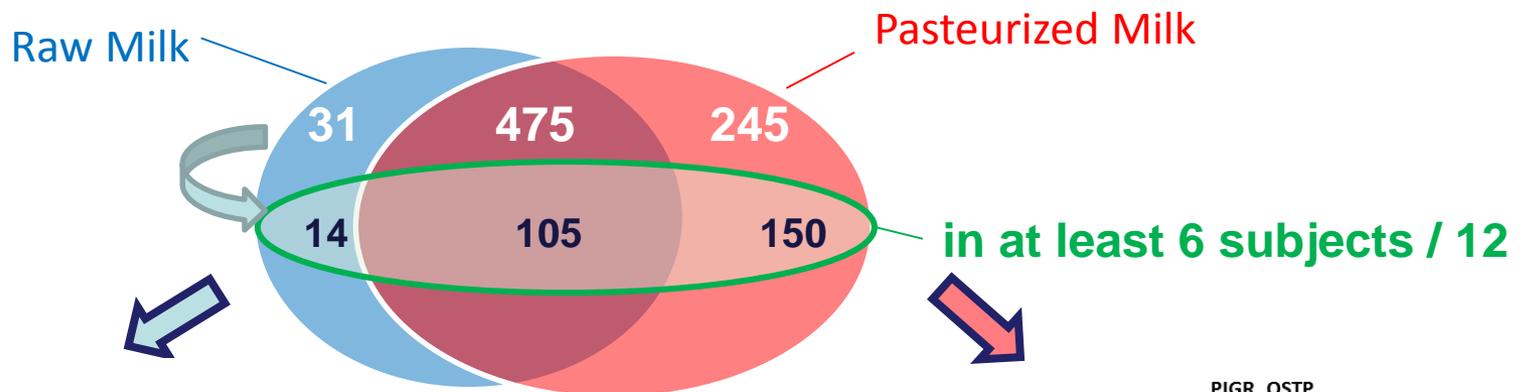


Proteome Mapping of Human Skim Milk Proteins in Term and Preterm Milk

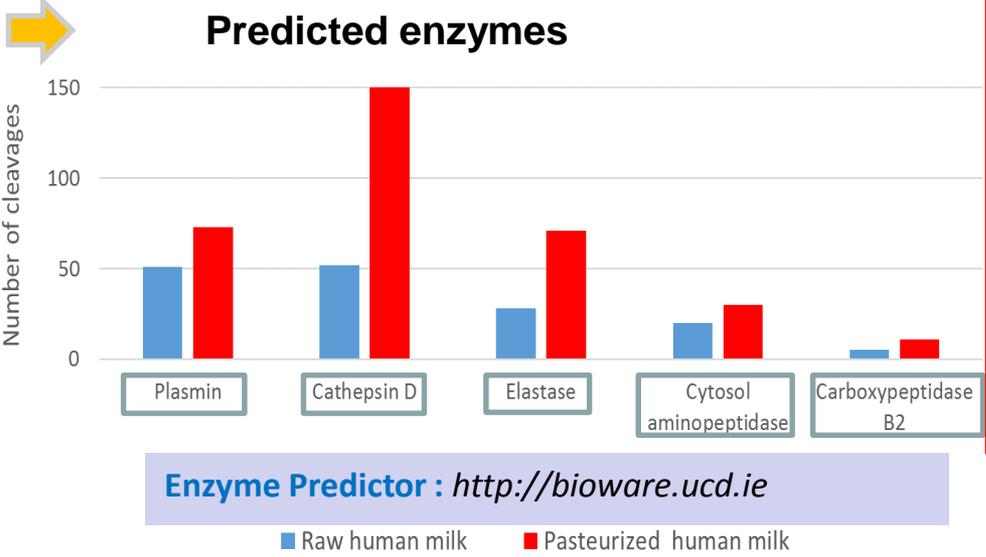
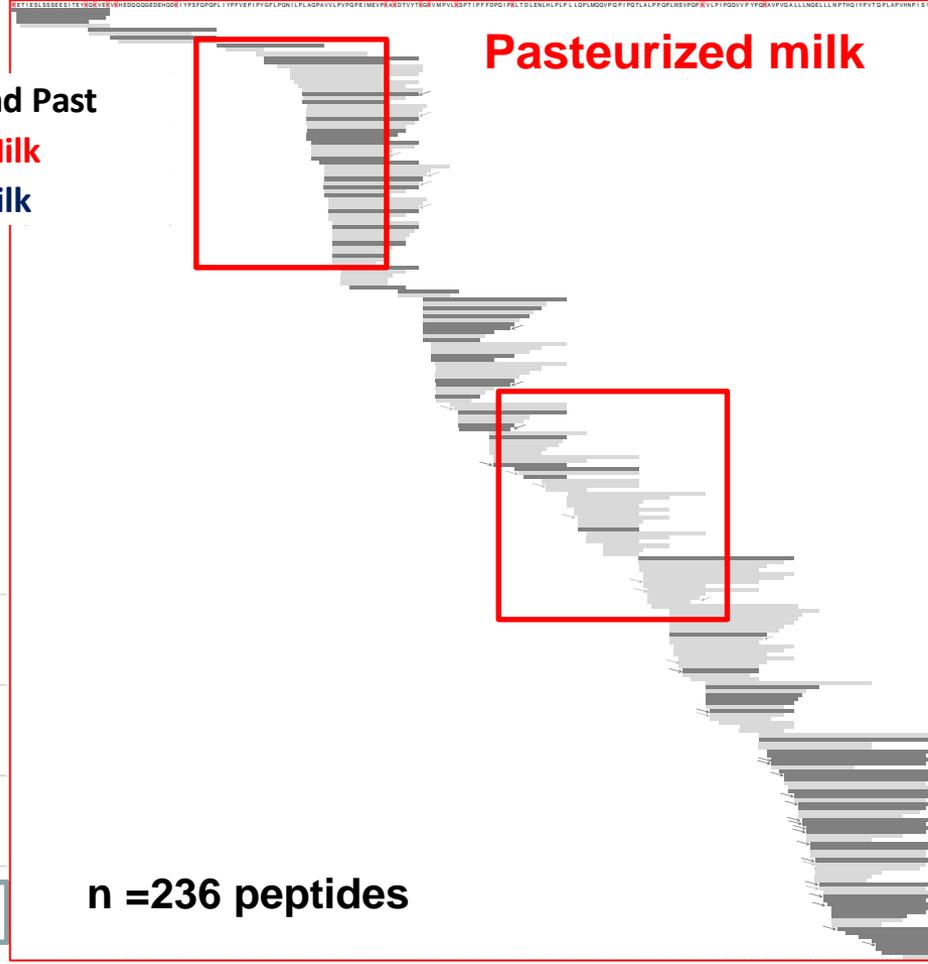
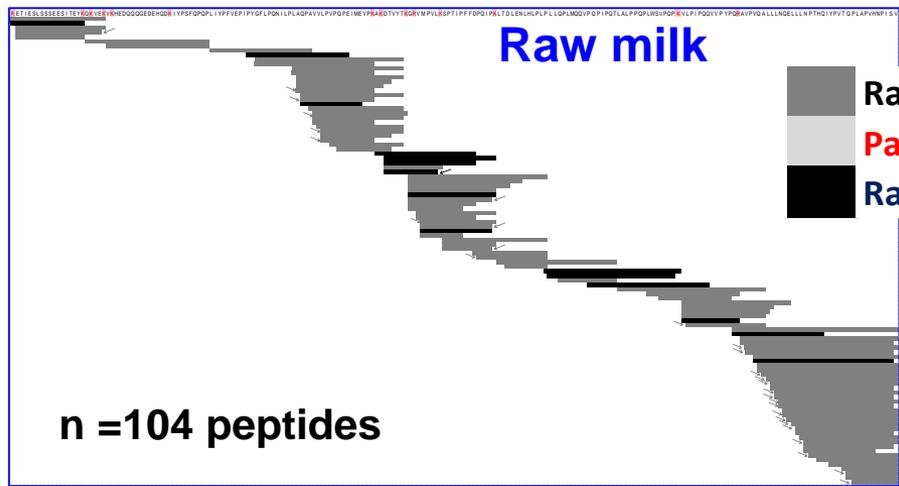
Claire E. Molinari,^{*,†} Ylenia S. Casadio,[†] Ben T. Hartmann,[‡] Andreja Livk,[§] Scott Bringans,[§] Peter G. Arthur,[†] and Peter E. Hartmann[†]

Before ingestion : proteolysis occurs in the milk

Number of peptides identified in the milk prior to ingestion



β-casein peptides in milk



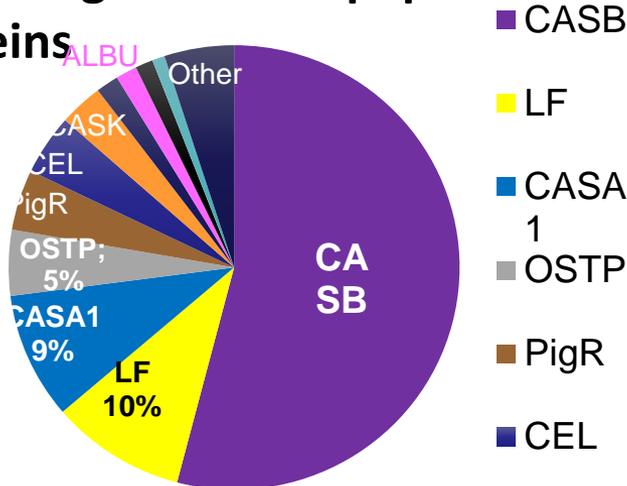
→ **Plasmin** and **Cathepsin D** (indigenous milk proteases) are involved in milk pre-digestion

→ β-Casein : preferentially hydrolysed by plasmin (Dallas 2015)

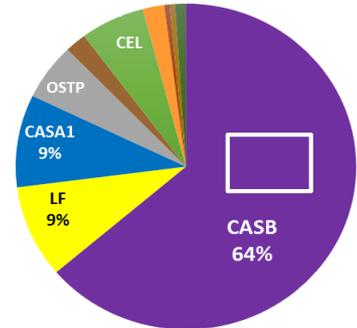
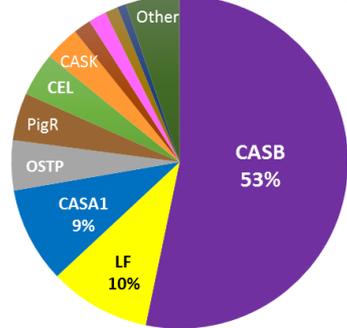
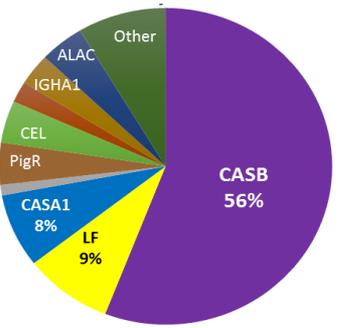
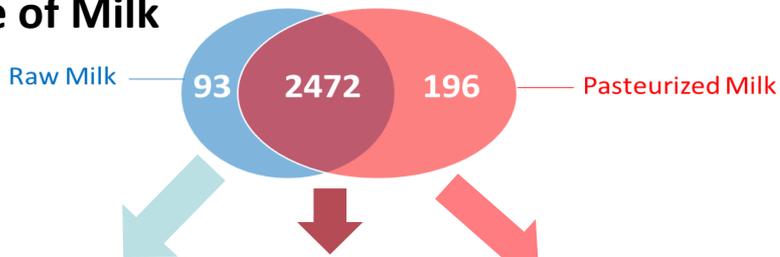
Identification of the peptides released in the stomach

→ In all milks and digesta: 2761 peptides

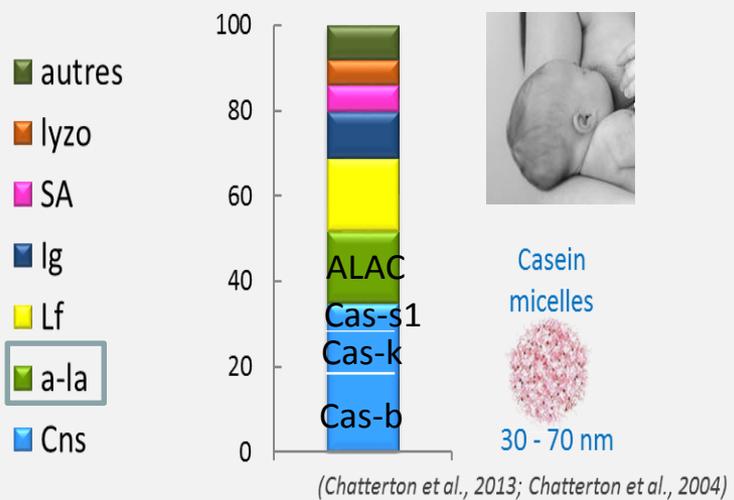
→ From 18 proteins



→ By type of Milk



Human milk composition



- Peptides mainly from β -casein
- α -lactalbumin (ALAC) : 1% of the peptides
 - Digestion resistance
 - 4 disulfide bonds on 123 AA
- More specific peptides in Pasteurised Milk, in greater proportion from β -casein



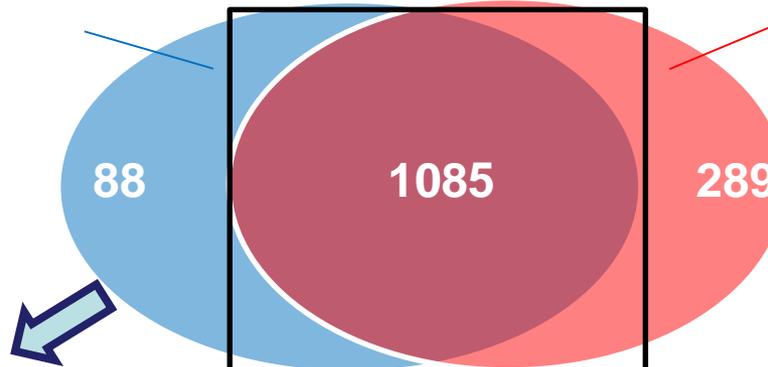
Identification of the peptides released in the stomach



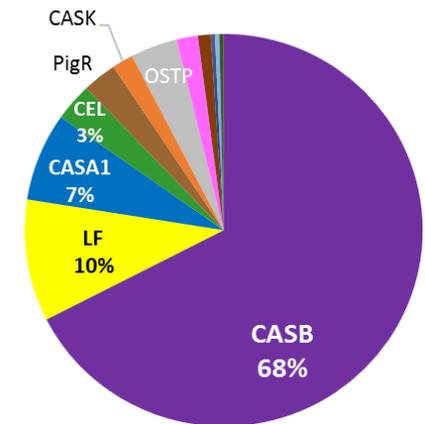
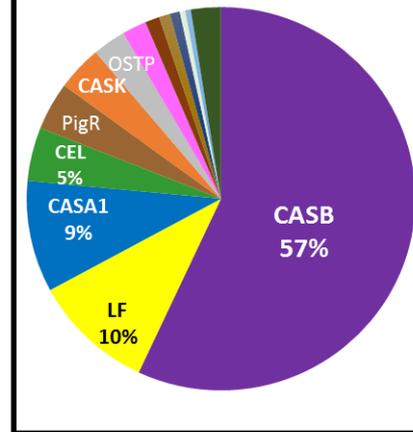
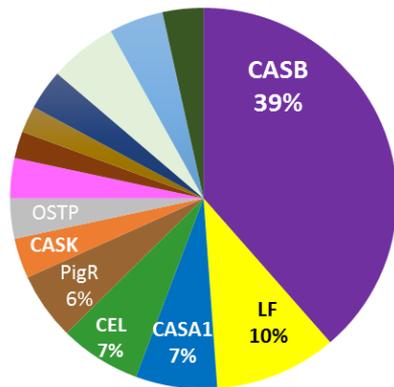
→ Over all milks and digesta : 2761 peptides
 → Common to Milks : 2472 peptides
 → detected on at least 6 subjects
 1462 peptides

Raw Milk

Pasteurized Milk

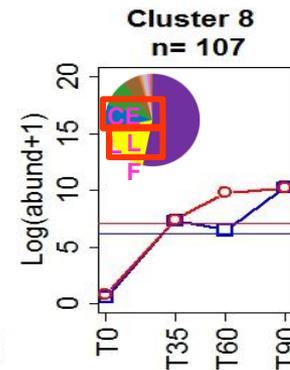
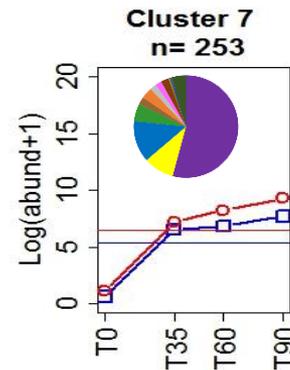
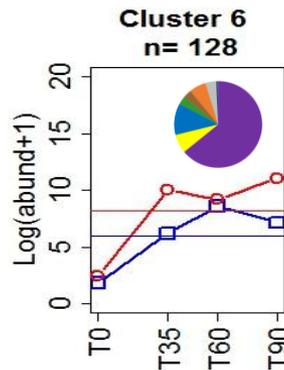
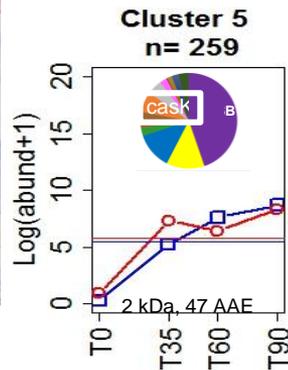
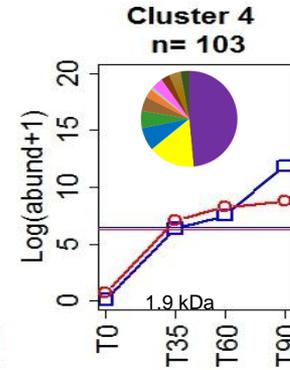
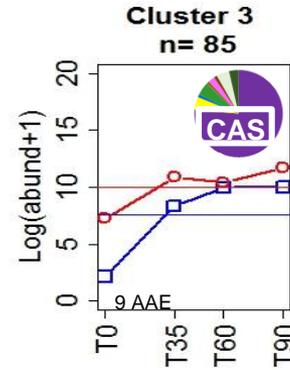
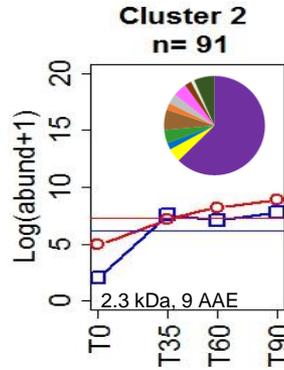
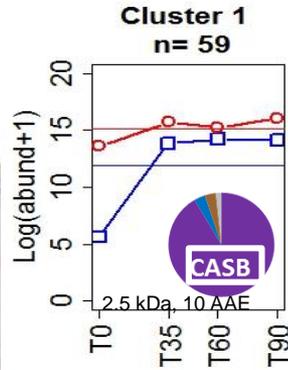
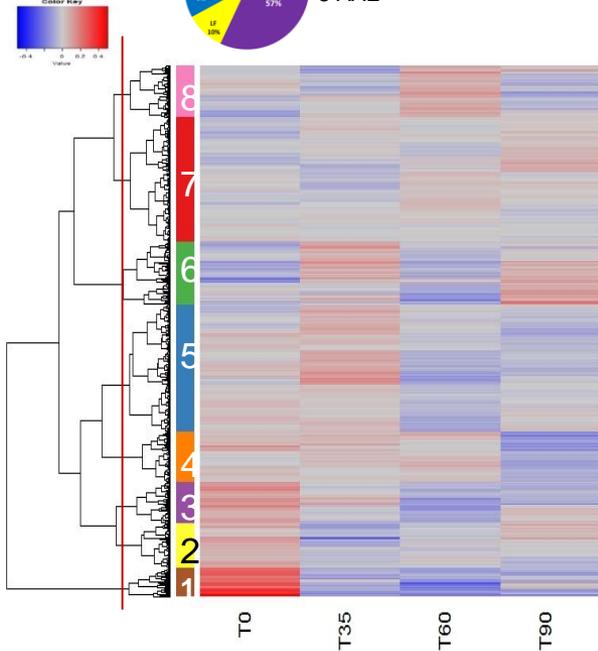
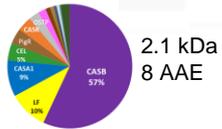


- CASB
- LF
- CASA1
- CEL
- PigR
- CASK
- OSTP
- ALBU
- Apo-J
- IGHA1
- ALAC
- H-FABP
- MFG-E8
- Other



Identification of the peptides released in the stomach

Overall (n = 1085)



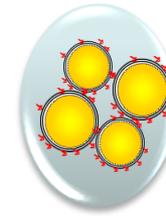
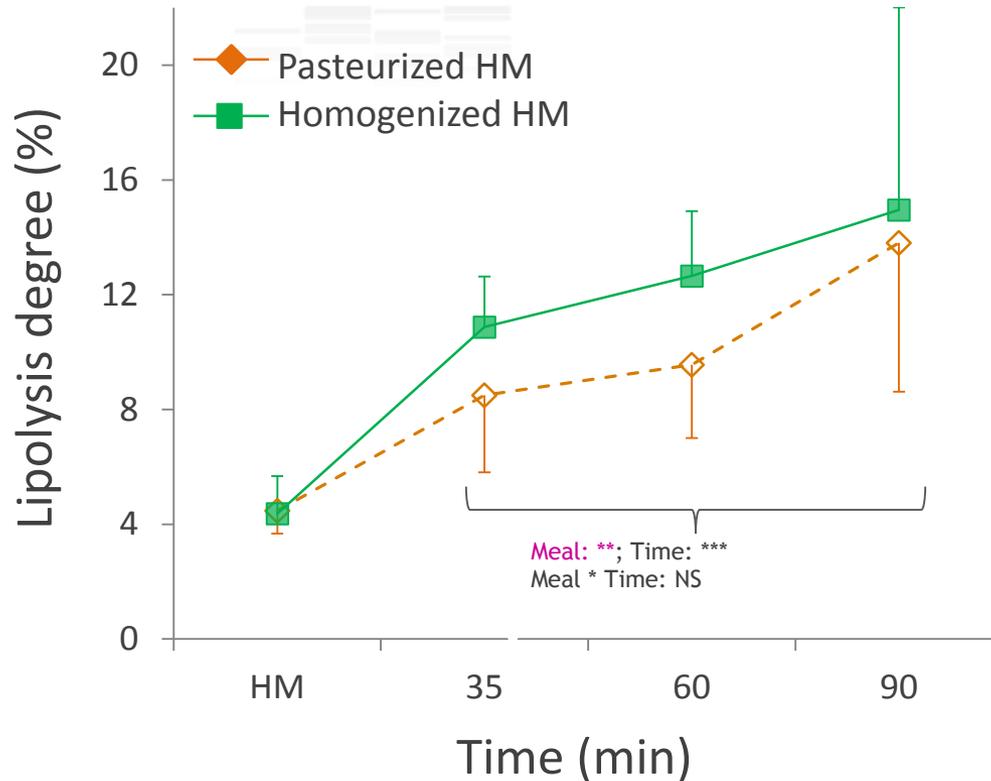
- Raw Milk
- Past. Milk



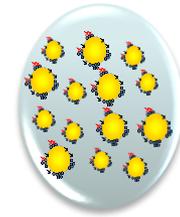
Most discriminant factor :
Protein origin
 Molecular Weight
 Number of Essential AA

→ Pepsin and **Plasmin** involved in the gastric digestion (Demers-Mathieu et al., 2017)
 → **Cathepsin D** from human milk can also hydrolyse proteins at gastric pH (Demers-Mathieu et al., 2017)

Homogenization impacted gastric lipolysis



Past HM



P+Homog HM

- Same milk composition, initial pre-lipolysis degree and inactivation of BSSL

- Different structure**

➔ Increase of specific surface of droplets facilitating HGL adsorption

Homogenization of human milk: a new way of stimulating the growth and development of pre-term neonate?

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

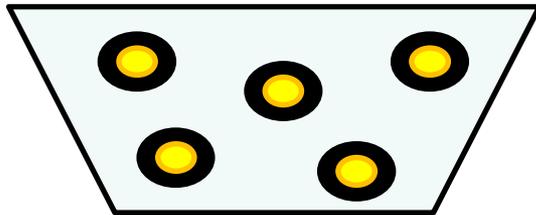


Infant formulas
Can we create lipid structures
biomimetic on the native fat globule?



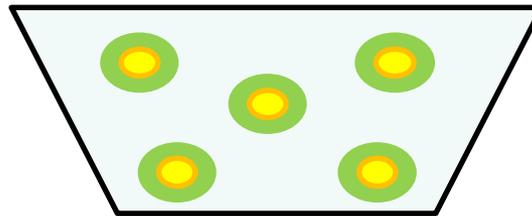
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula
T1



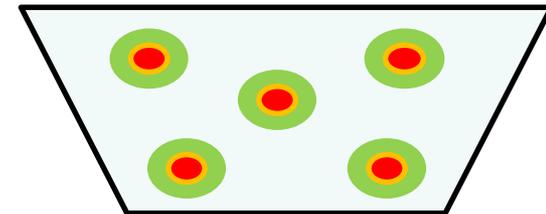
Interface 100 % Proteins
100% vegetable oil

Formula
T2

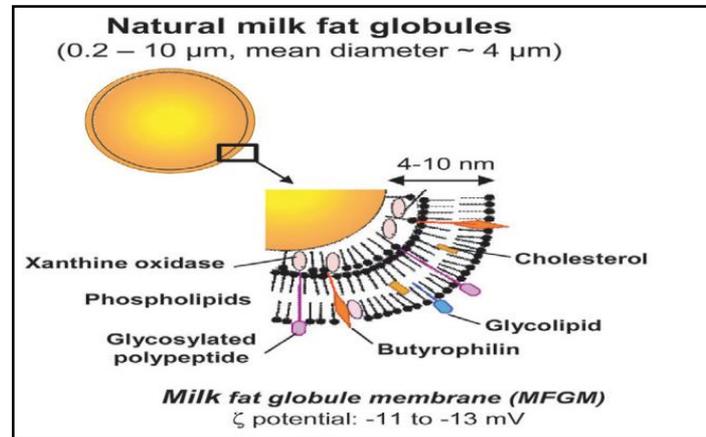


Interface 100 % phospholipids
100% vegetable oil

Formula
T3

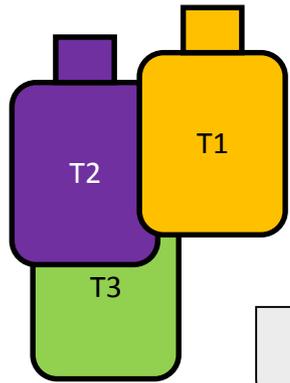


Interface 100 % phospholipids
40% vegetable oil + 60% milk fat



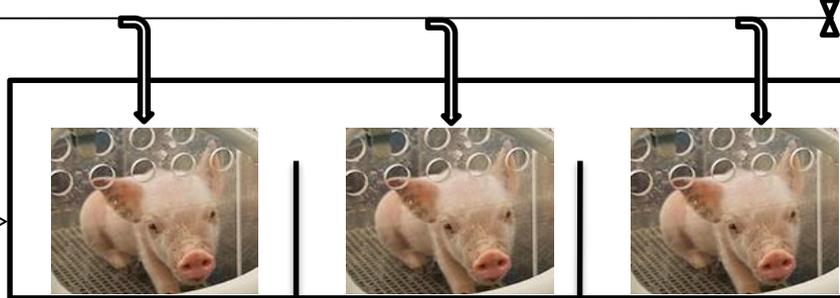
Lopez, (2007)

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

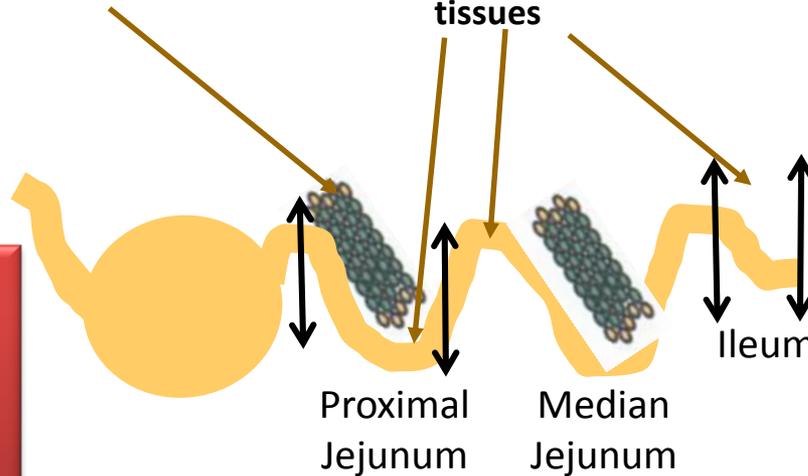
7 days

28 days

(90 min postprandial)

Mesenteric Lymph Nodes (MLN)

Collect of effluents and tissues



Effluents:
-SDS-PAGE
-Elisa

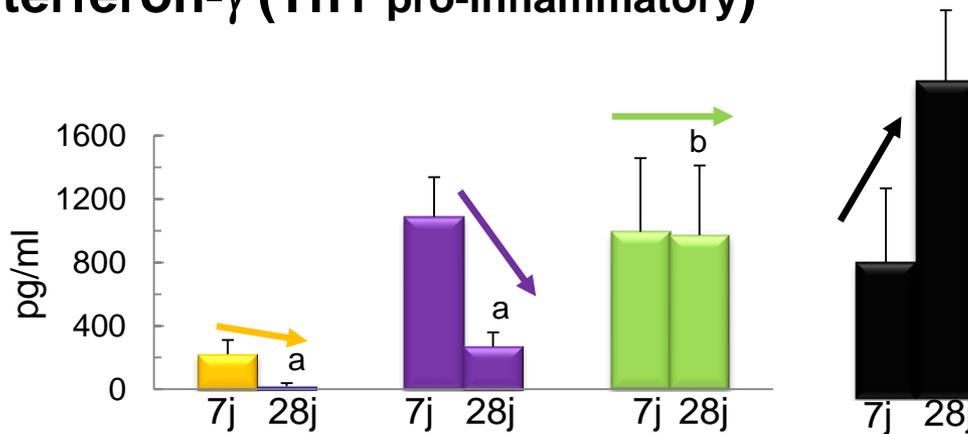
Tissues:

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

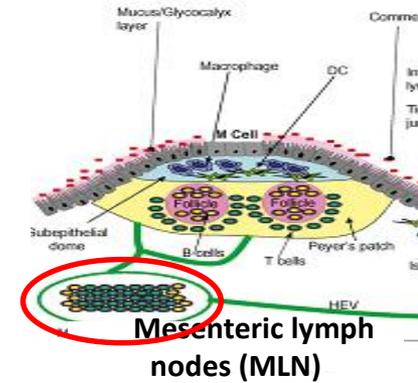
Maturation of the immune system during infancy

Secretory activity of Mesenteric Lymph Nodes

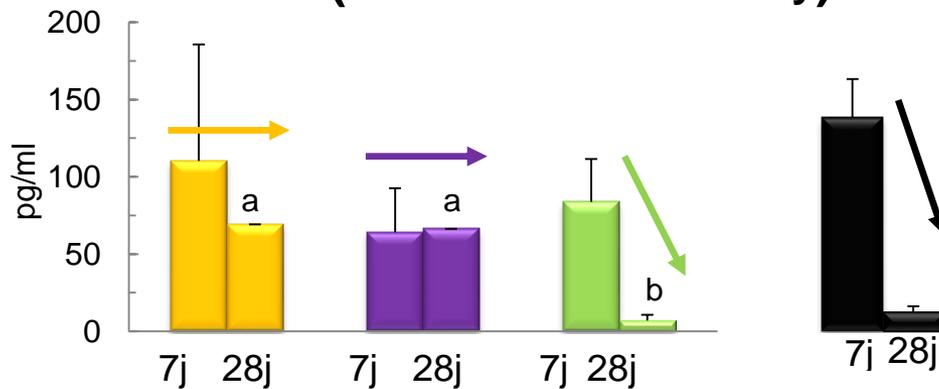
Interferon- γ (Th1 pro-inflammatory)



- Veg
- Veg + PL
- Dairy Fat + PL
- Piglets MF



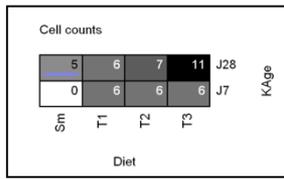
Interleukine-10 (Th2 anti-inflammatory)



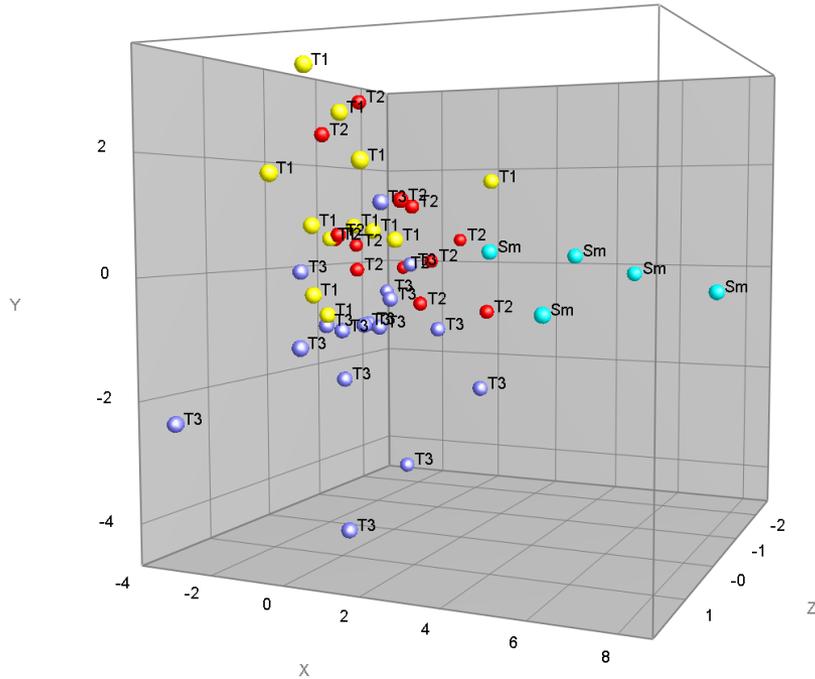
Milk lipids → maturation of the piglet's immune system more similar than with sow's milk

Le Huerou et al.
Eur J Nutr 2017

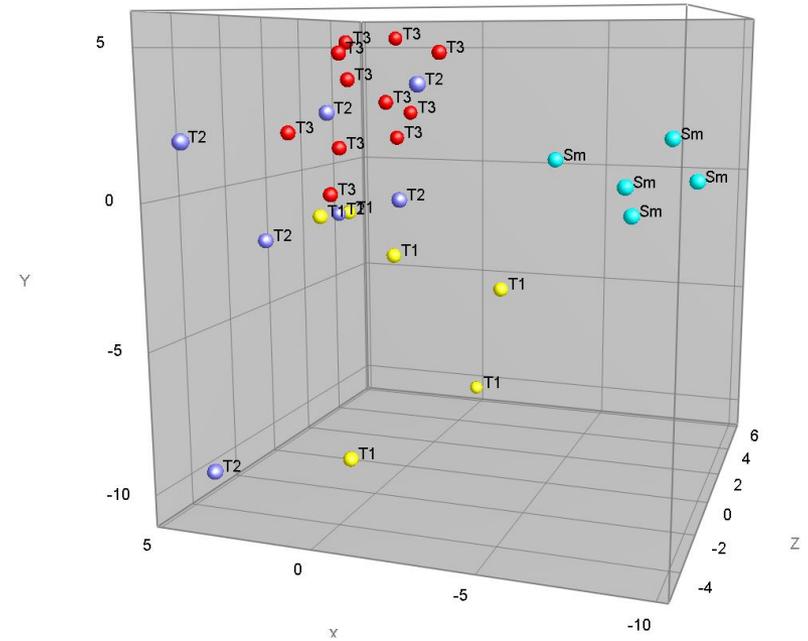
Microbiota by DHPLC



D7 & D28

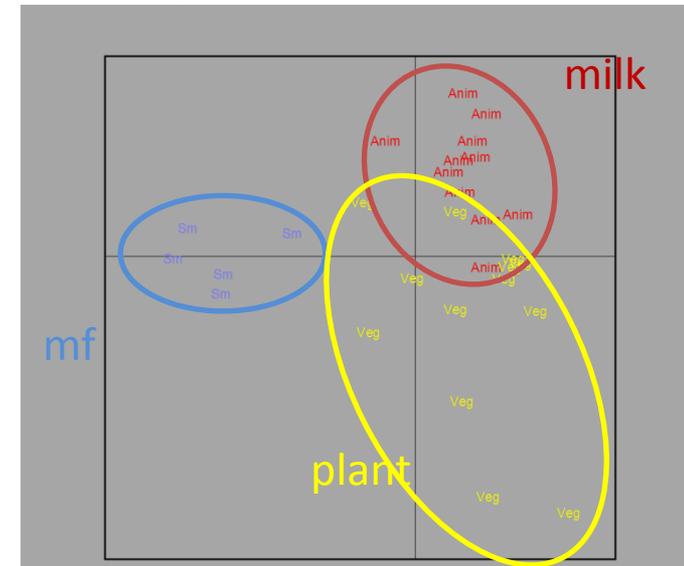


D28



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

More Proteobacteria with milk fat /
More Firmicutes with plant oil



Conclusion

Processing affects the structure of the emulsion and modifies the kinetics of protein and lipid digestion by the neonate

Pasteurisation leads to protein denaturation and aggregation changing the resistance of proteins to the action of digestive enzymes affecting the peptidomes

Homogenization increases the specific surface of the lipid droplets making the adsorption of the gastric lipase easier. Gastric lipolysis is consequently accelerated

Understanding the mechanisms of digestion of the human milk macronutrients should help us to design a new generation of biomimetic infant formula

Infant formula with less heat-denatured proteins and lipid droplets similar to the milk fat globule would certainly behave identically to the human milk in the neonate's GI tract

↳ objective of a new research project at INRA in 2018

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

International Network

Dr. Didier DUPONT, Senior Scientist, INRA, France

●
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380 scientists - 140 institutes – 40 countries

Industry involvement

☞ ~ 40 European companies are involved in INFOGEST





Chair
Didier Dupont - France



Vice-chair
Alan Mackie - UK

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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

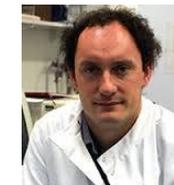


Guy Vergeres

The "Mind-the-Gap" group



Frederic Carriere



Fred Warren



Steven Le Feunteun



INFOGEST
International Conference
Granada, Spain
April 2019

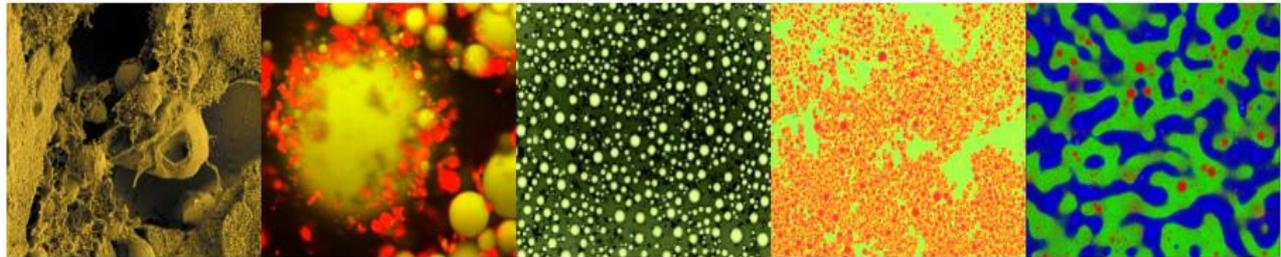
We are pleased to announce the next
6th International Conference on Food Digestion



in Granada, Spain, 2-4 April 2019



The next INFOGEST Workshop will be held on the 12-13 of April 2018 in Leeds UK connected to:



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