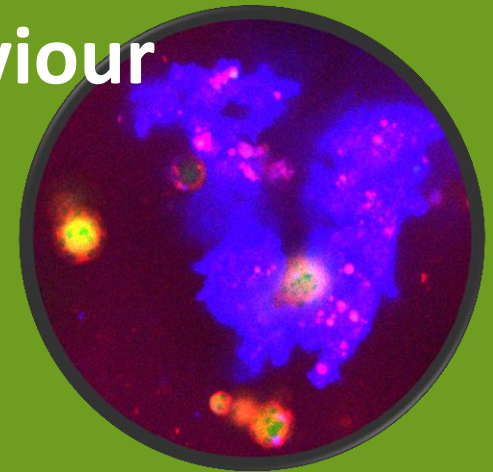


# Advances in neonatal nutrition: a step forward infant formula biomimetic of human milk structure and digestive behaviour



C. Bourlieu<sup>1,2</sup> , A. Deglaire<sup>1</sup>, S. Cassia de Oliveira<sup>1</sup> , O. Ménard<sup>1</sup>, Y. Le Gouar<sup>1</sup>,  
F. Carrière<sup>3</sup>, D. Dupont<sup>1</sup>

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<sup>2</sup>UMR 1208 IATE, Montpellier, France;

<sup>3</sup> CNRS-UMR 7282 EIPL, Marseille, France

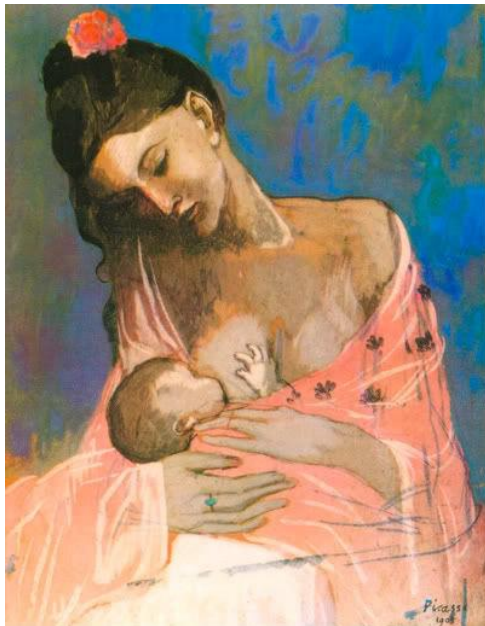


UNIRIO 27/11/2017

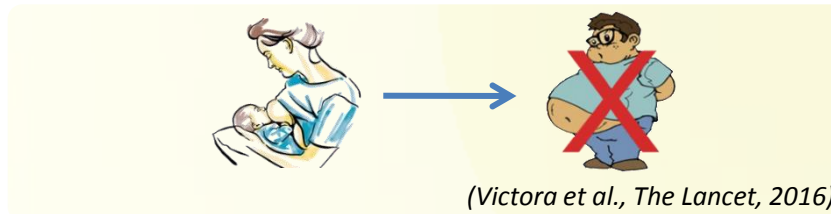
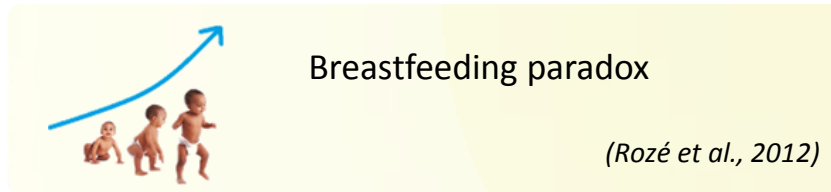


**Recommendations**  
 Exclusive breastfeeding until 6 months  
 Supplemental breastfeeding up to 2 years and beyond

Large array of health benefits: ↘ incidence gravity digestive/respiratory infections, microbiota, ↘ NEC in premies, ↘ diarrhea , ↘ allergy risk ... cognitive, ↘ IBD, T2D risk, obesity...



"LA MATERNITE" Pablo Picasso, 1905



Over the last decade, Lipid structure identified as a parameter involved in early programming (Oosting et al., Ped Res., 2012; Oosting et al., BJN, 2014; Baars et al., BJN, 2016)



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 Exclusive breastfeeding until 6 months  
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Large array of health benefits: ↘ incidence gravity digestive/respiratory infections, microbiota, ↘ NEC in premies, ↘ diarrhea , ↘ allergy risk ... cognitive, ↘ IBD, T2D risk, obesity...



Infant formula – Optimized chemical composition

Lipids of HM/IF supply 45-55 % calories that supports newborn development – 98 % TAG (Armand et al., Ped. Res ,1996; Jensen, Lipids,1999; Roman et al., Ped. Res. 2007; Lindquist and Hernell, Curr. Opinion Clin. Nutr. Met.Care, 2010, Guesnet et al., OCL, 2013;)

Over the last decade, Lipid structure identified as a parameter involved in early programming (Oosting et al., Ped Res., 2012; Oosting et al., BJN, 2014; Baars et al., BJN, 2016)



# Still a striking difference between HM and IF lipid structure !



Ingredients

Skim milk

Liquid preparation

concentrate

powder

packaged powder

1. Blending

2. Pasteurization

3. Vacuum concentration

4. Homogenization

5. Spray-drying

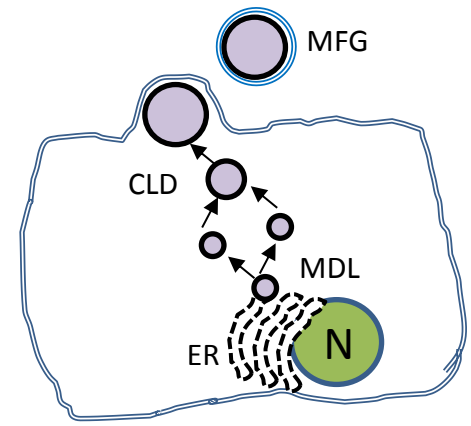
6. Blending during Spray-drying

7. Blending after Spray-drying

8. Big bag packaging

9. Quality control

10. Individual packaging

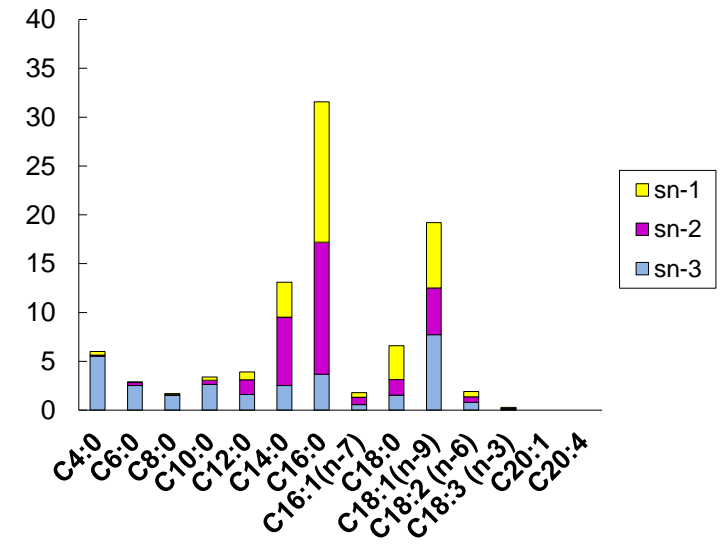
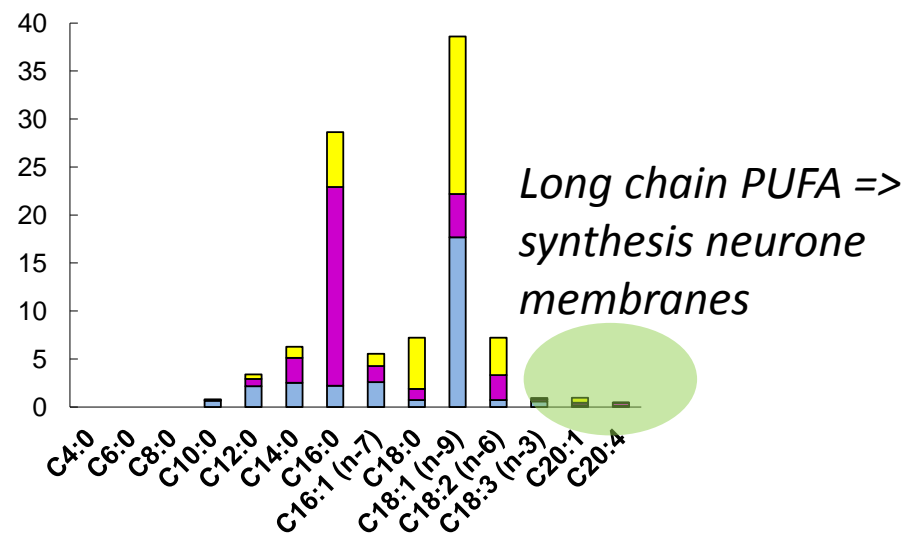
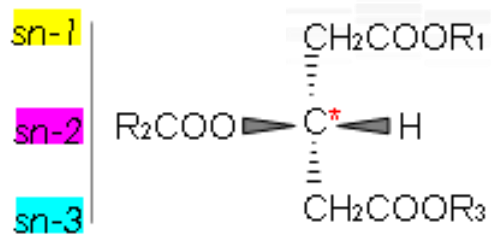


Lipid droplets are formed in the endoplasmic reticulum (ER) by budding into the cytosolic compartment as very small droplets called microlipid droplets (MLD). Droplets increase in size by fusing, giving birth to larger droplets called cytoplasmic lipid droplets (CLD). At the apical cell surface droplets are secreted by envelopment in apical plasma membrane, releasing the milk lipid globule (MLG)

# CONTEXT

70's : specific triacylglycerol (TAG) structure in HM results in improved fatty acids absorption (Tomarelli et al., 1968).

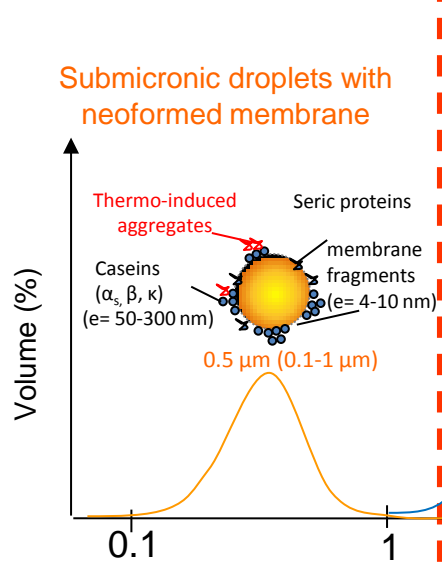
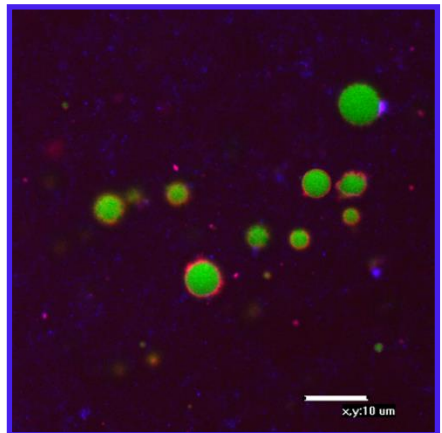
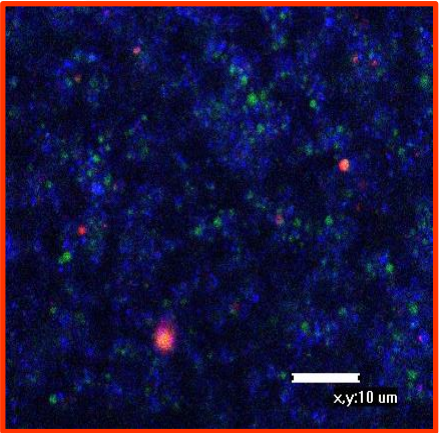
**NON RANDOM** Distribution of fatty acids (R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>) on the glycerol backbone



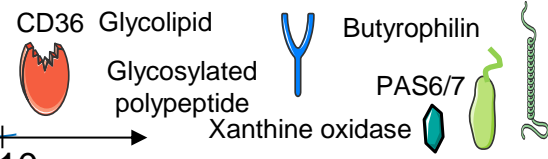
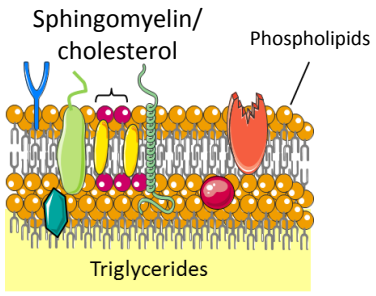
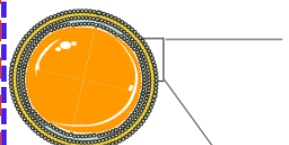
Comparison of the composition and regiodistribution of FA in human and bovine milks (% mol main FA > 0.5 % total)



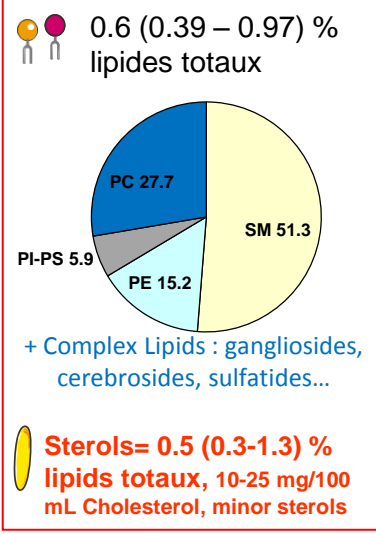
# Still a striking difference between HM and IF lipid structure !



**Milk Fat Globule**

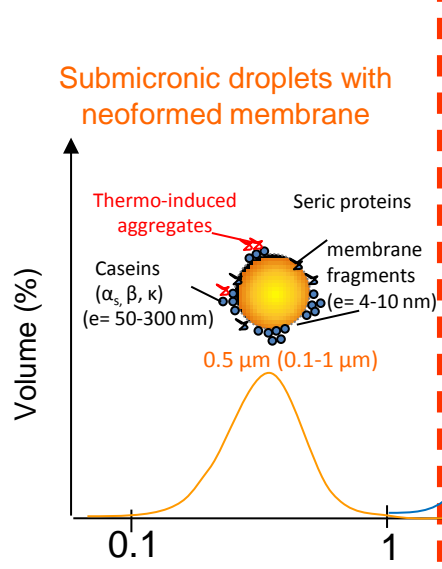
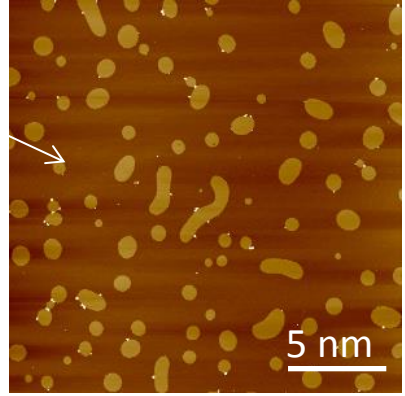
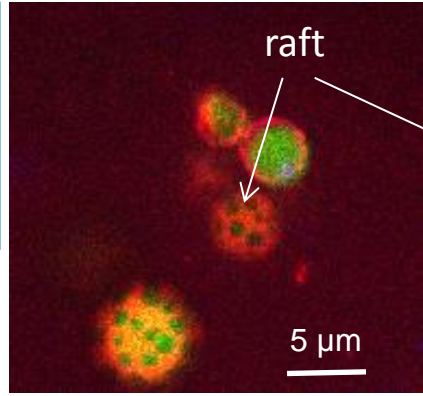
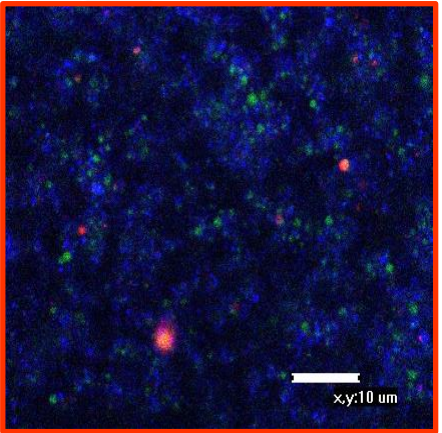


**Milk Fat Globule Membrane**

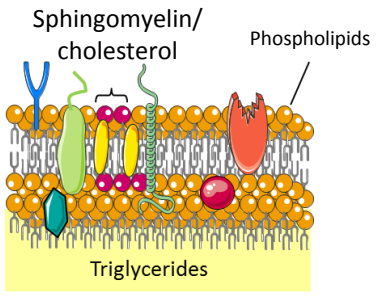
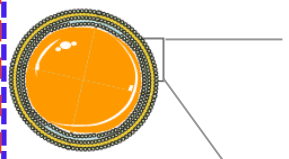




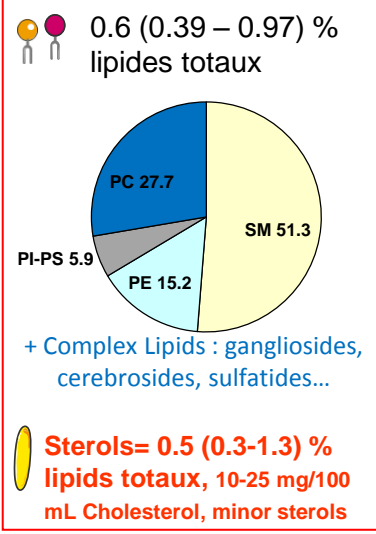
# Still a striking difference between HM and IF lipid structure !



## Milk Fat Globule



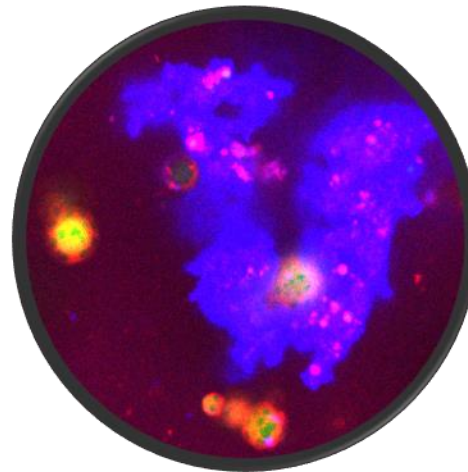
## Milk Fat Globule Membrane





Designing IF that mimic HM digestive behaviour supposes prior characterization of this behaviour

→ **PRIORITY COLLECT DATA ABOUT HM DIGESTIVE BEHAVIOUR**



Human Milk in gastric phase seen in confocal microscopy



*Thèse S. Cassia de Oliveira 2013-2016*





## DIGESTION OF HM IN PRETERM INFANTS

NCT02112331 (ClinicalTrials.gov)

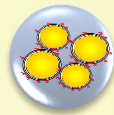
- Randomized controlled trial
- Hospitalized tube-fed preterm infants (GA < 32 wks)
- Feeding  $\geq 120$  mL/kg/jour every 3 h
- 6-day experimental period; 2 independent groups

### GROUP A

HM from their **own mother**



Raw HM



Past HM

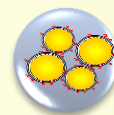
→ collected < 24h  
before feeding

→ 1 pool aliquoted in  
6 bottles

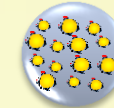
n=12, GA  $30.0 \pm 1.1$  wk, age at first day  $27 \pm 12$  d,  
Body weight at first day  $1.83 \pm 0.41$  kg

### GROUP B

HM from **anonymous donor**



Past HM

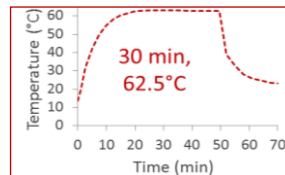


P+Homog HM

→ The same pool from one donor  
was used for the two types of milk  
n=8, GA  $29.5 \pm 1.5$  wk, age at first day  $32 \pm 21$  d,  
Body weight at first day  $1.73 \pm 0.48$  kg

### HM bank

Holder pasteurization



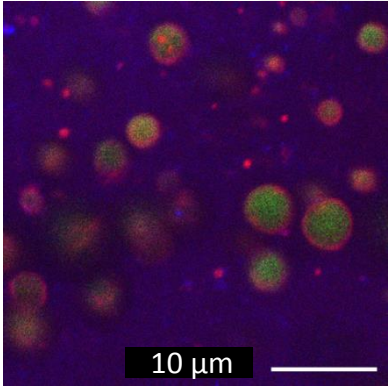
Indirect homogenization by ultrasonication



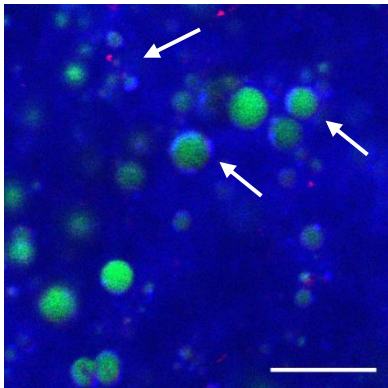
→ 595 W, 3 periods of 5  
min interrupted by 30s  
of pause

# GROUP A GASTRIC BEHAVIOUR OF HM IS DOMINATED BY AGREGATION AROUND MILK FAT GLOBULE AND INFLUENCED BY PASTEURIZATION

Raw HM



Pasteurized HM

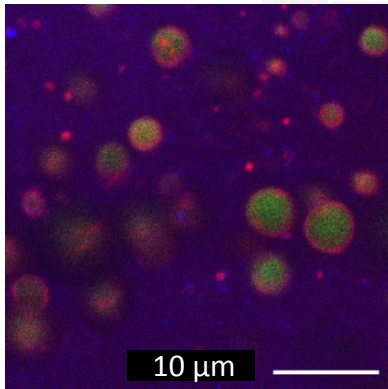


■ Apolar lipids ■ Amphiphiles ■ Proteins

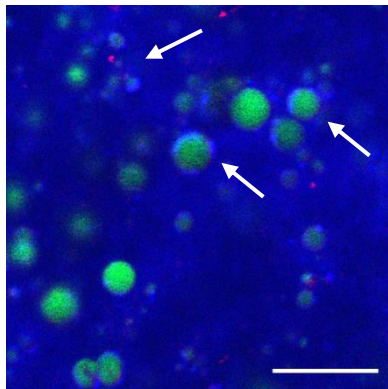
(De Oliveira et al., AJCN, 2017)

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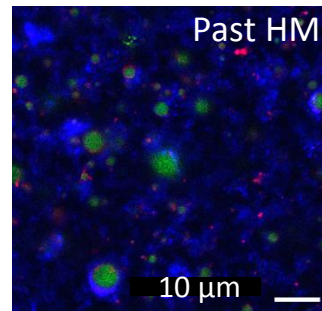
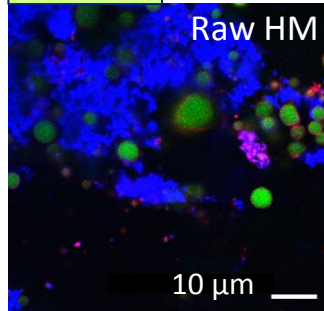
Raw HM



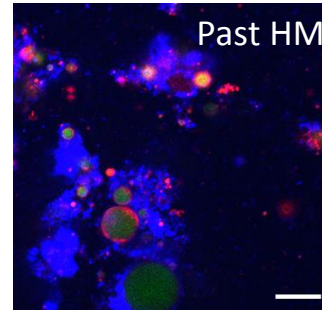
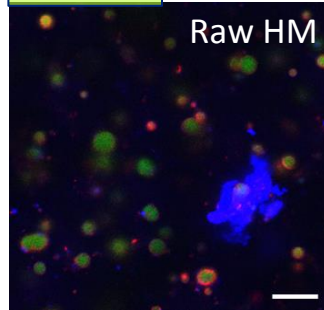
Pasteurized HM



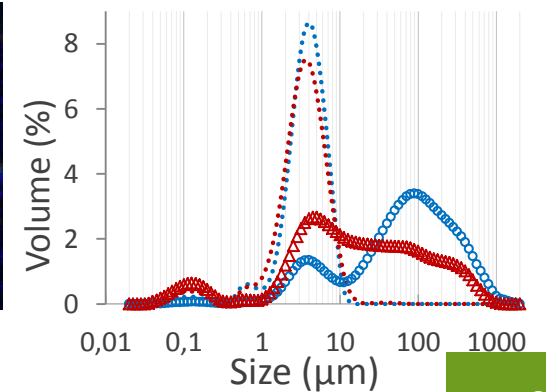
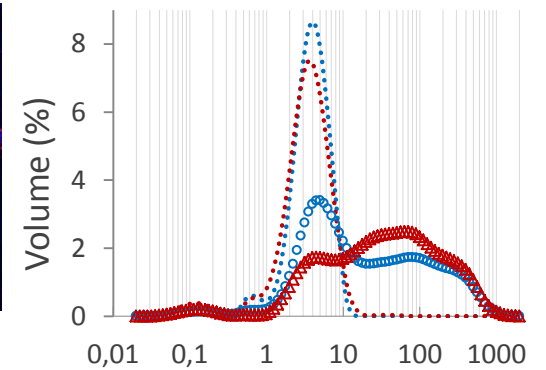
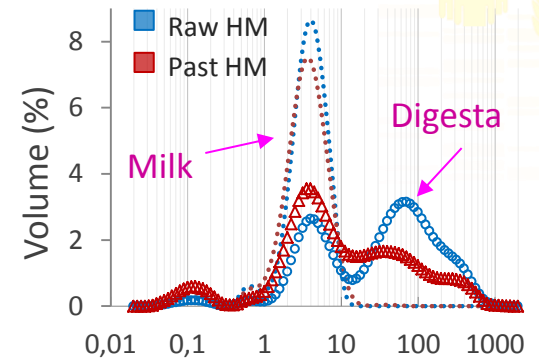
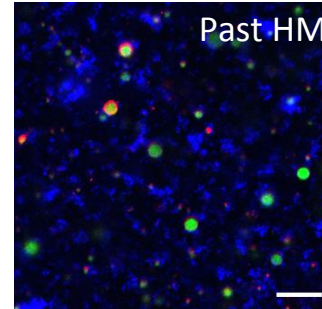
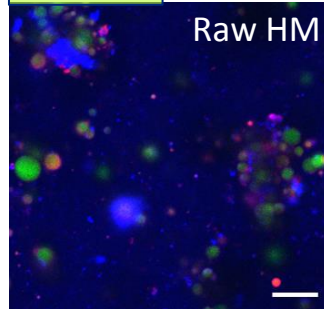
35 min



60 min



90 min

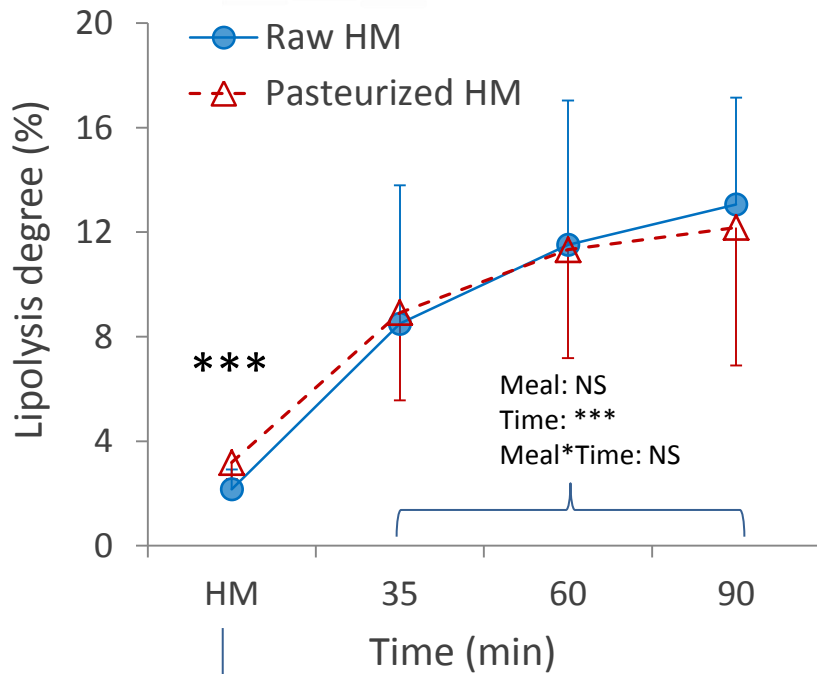


■ Apolar lipids 
 ■ Amphiphiles 
 ■ Proteins

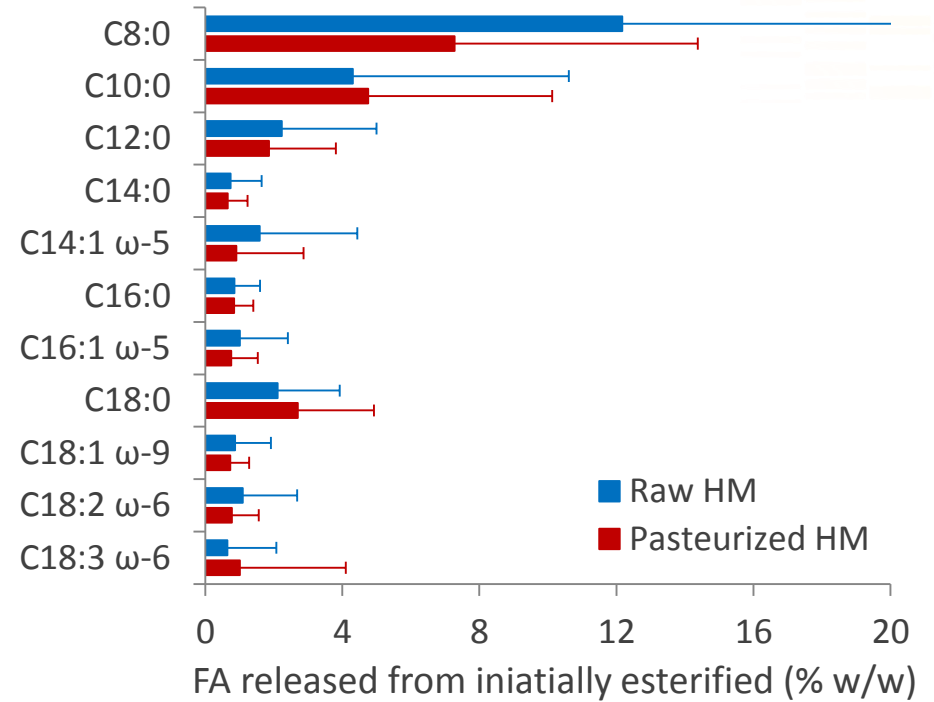
(De Oliveira et al., AJCN, 2017)

# GROUP A PASTEURIZATION DID NOT LOWER LIPOLYSIS NOR BIOACCESSIBILITY

## Instantaneous Lipolysis



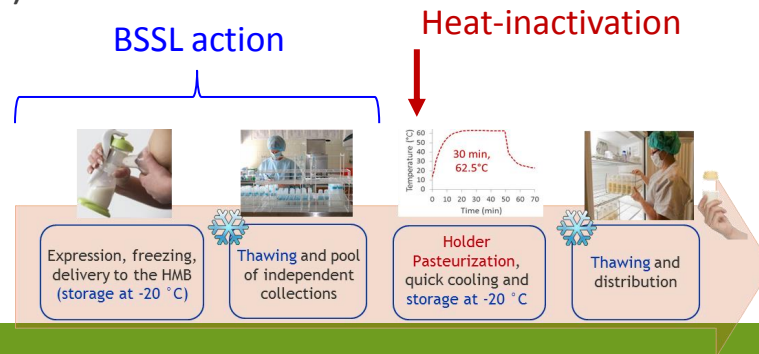
## Bioaccessibility of FA



Pre-lipolysis: n = 4 infants

Raw HM =  $2.2 \pm 0.8\%$

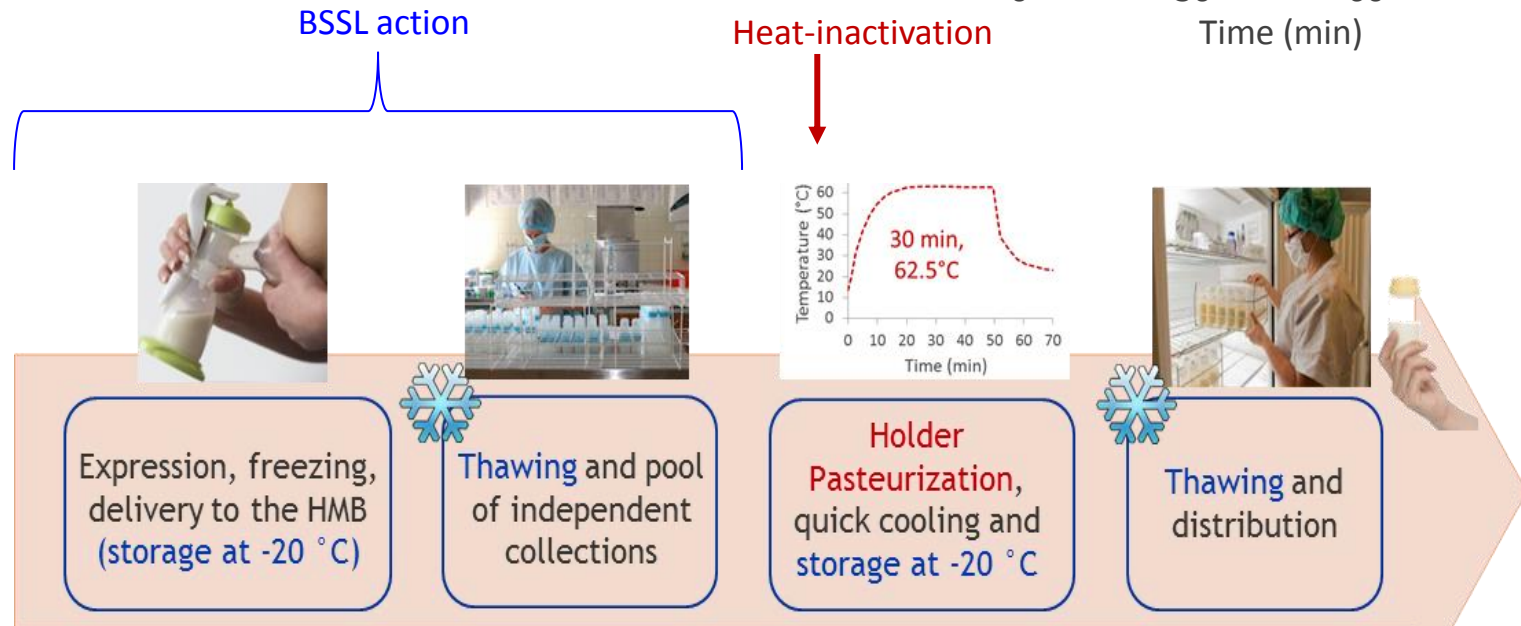
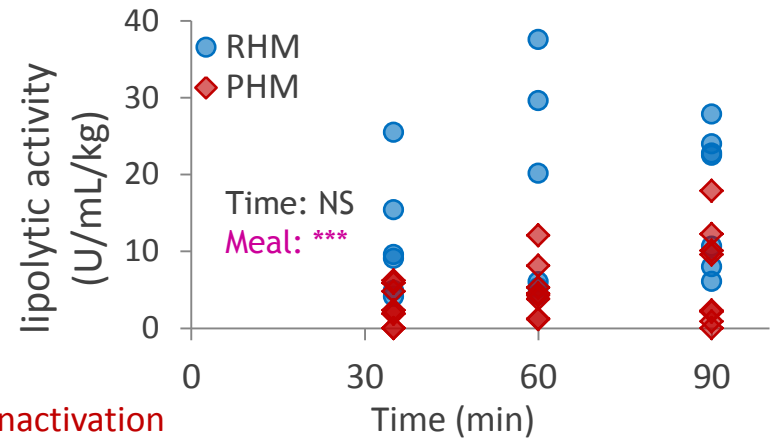
Past HM =  $3.2 \pm 0.6\%$



(De Oliveira et al., AJCN, 2017)

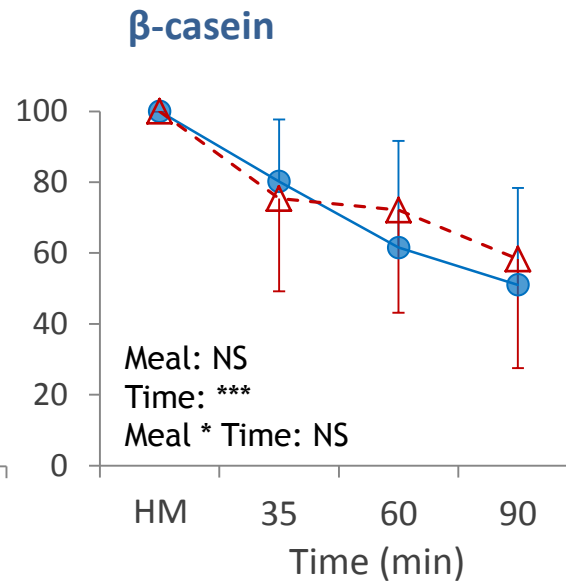
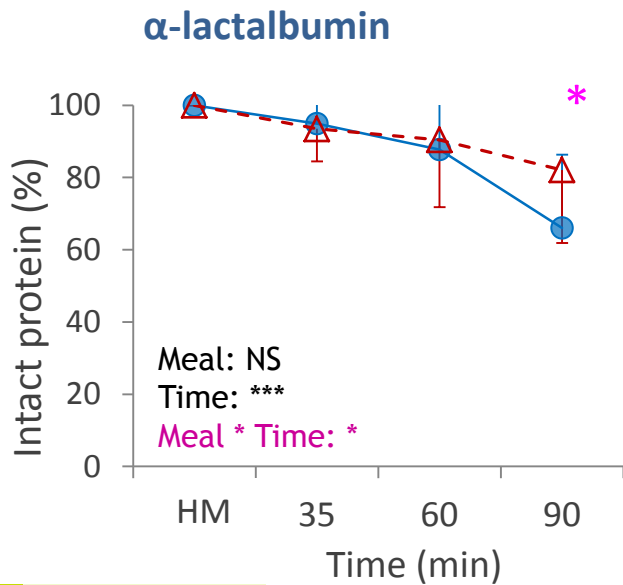
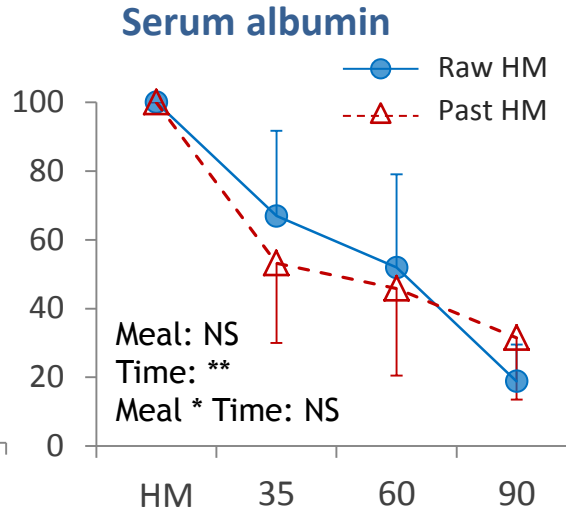
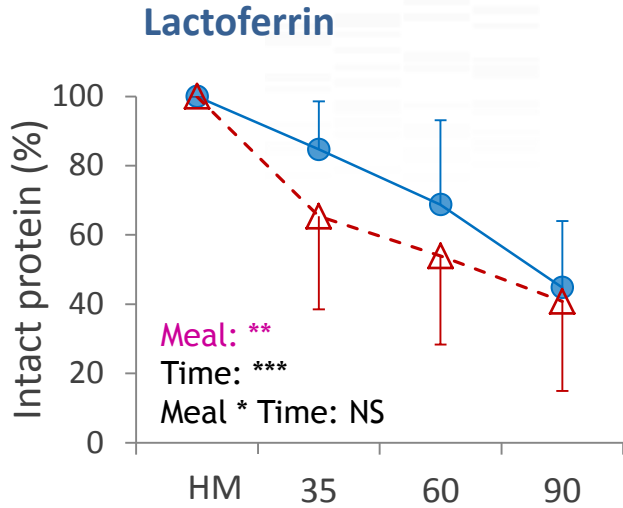
# GROUP A PASTEURIZATION DID NOT LOWER LIPOLYSIS NOR BIOACCESSIBILITY

→ And yet Lipolytic activity was higher after ingestion of raw HM



(Casper et al. PLOS ONE. 2016)

# GROUP A PASTEURIZATION SELECTIVELY IMPACTED GASTRIC PROTEOLYSIS



→  $\alpha$ -lactalbumin was the most resistant to gastric digestion

→ Faster decrease of lactoferrin in Past HM

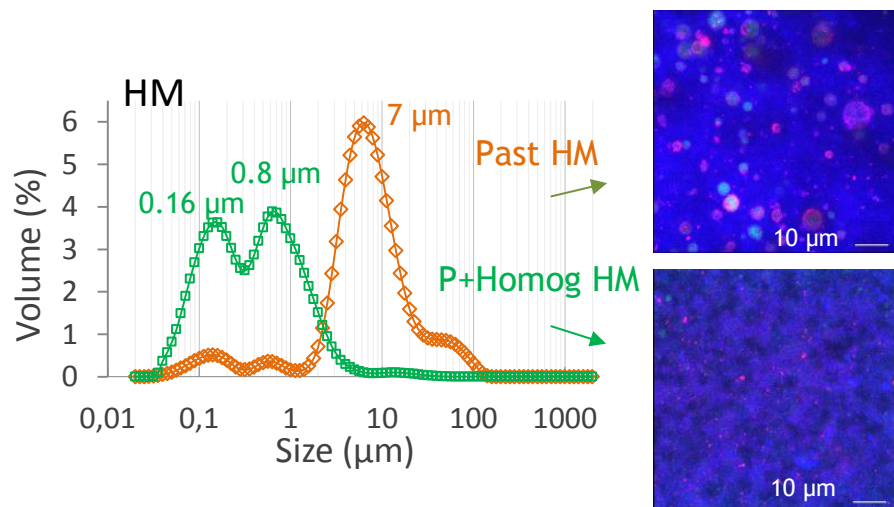
→ Slower decrease of  $\alpha$ -lactalbumin in Past HM (only at 90 min)

**Impact on protective qualities of the native lactoferrin?**

(De Oliveira et al., AJCN, 2017)

# GROUP B HOMOGENIZATION AFFECTED THE INITIAL STRUCTURE AND THE EMULSION DISINTEGRATION OF HM

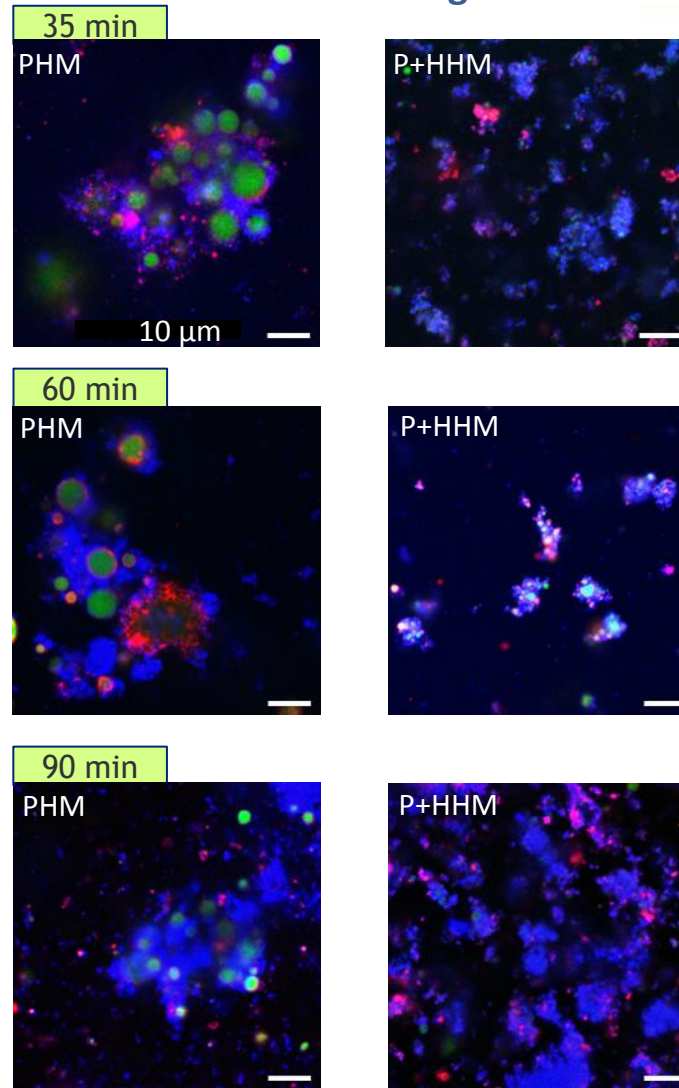
Initial structure



Past HM:  $4.1 \pm 1.2 \text{ m}^2/\text{g}$  of fat

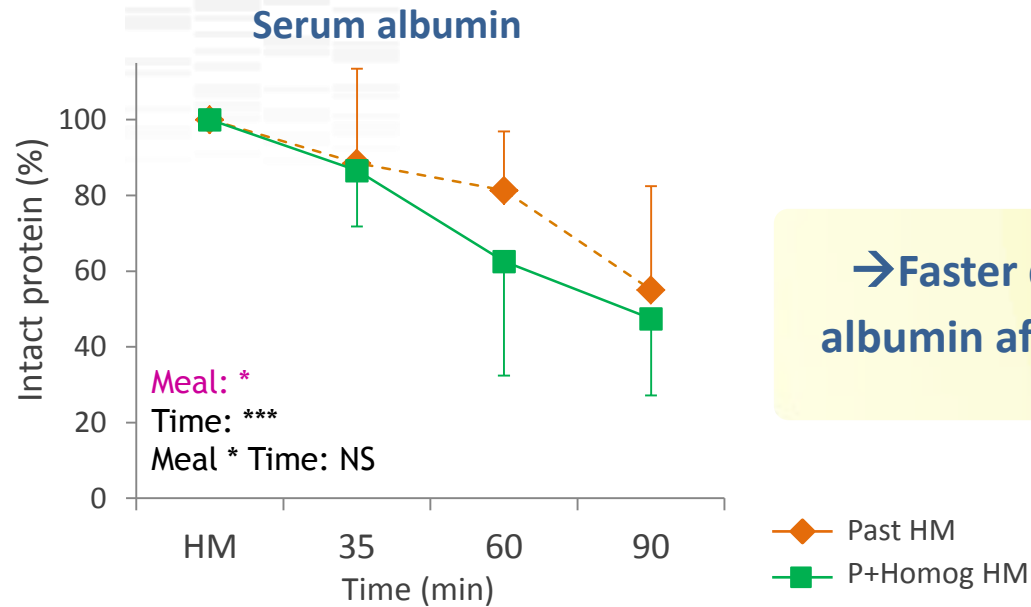
P+Homog HM:  $25.5 \pm 3.8 \text{ m}^2/\text{g}$  of fat

Gastric disintegration



(De Oliveira et al., Clinical Nutrition ESPEN, 2017)

## GROUP B HOMOGENIZATION SELECTIVELY IMPACTED GASTRIC PROTEOLYSIS

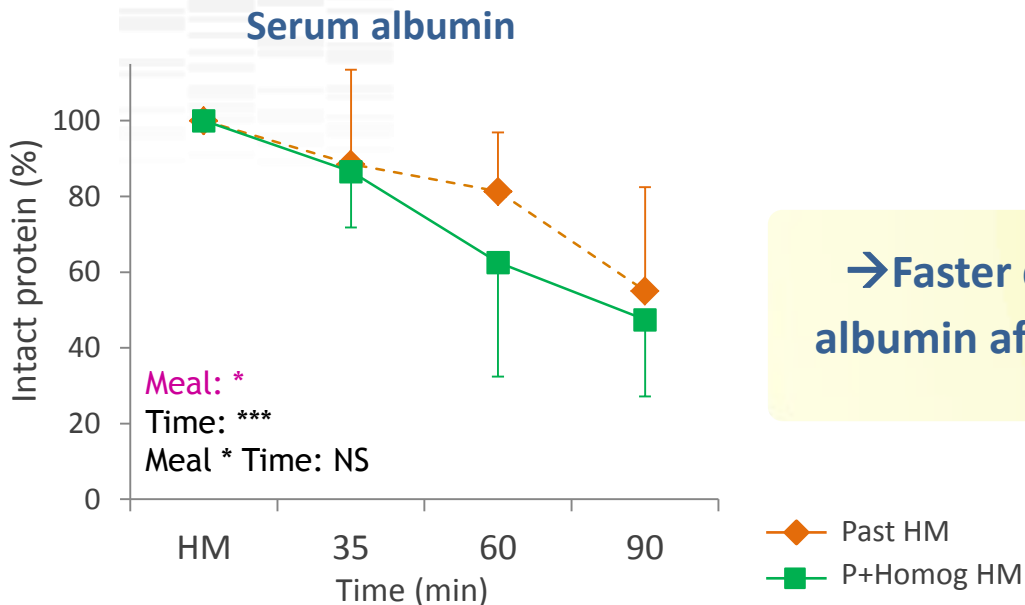


→ Faster decrease of serum albumin after homogenization

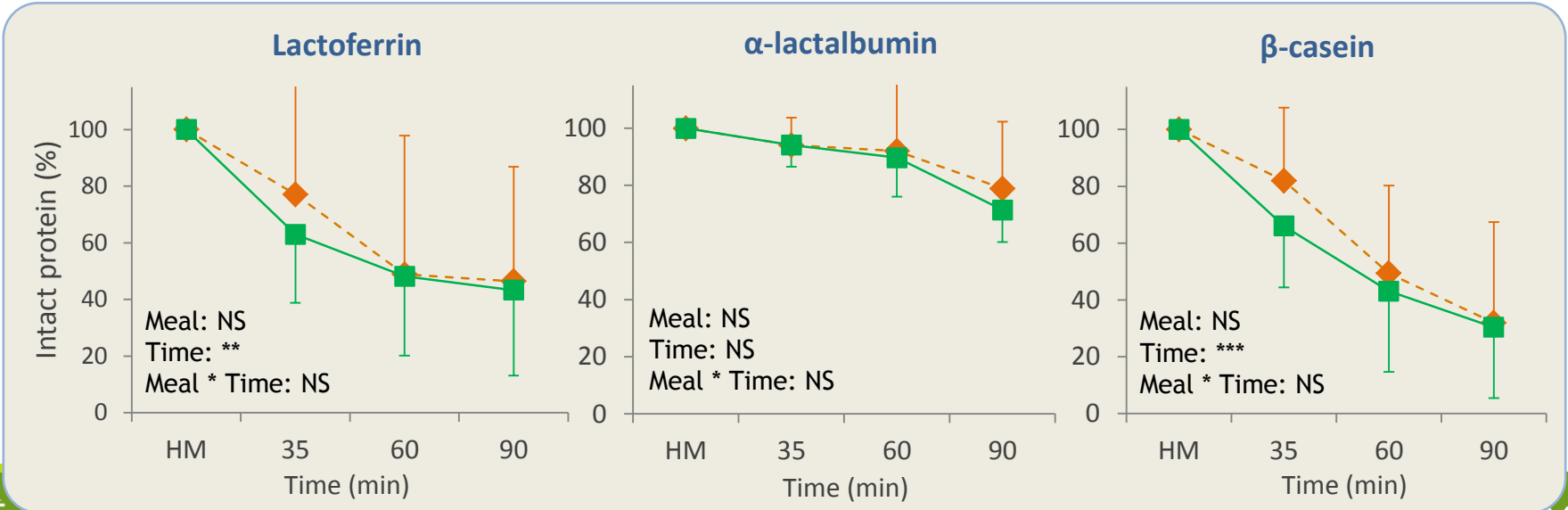
(De Oliveira et al., Clinical Nutrition ESPEN, 2017)



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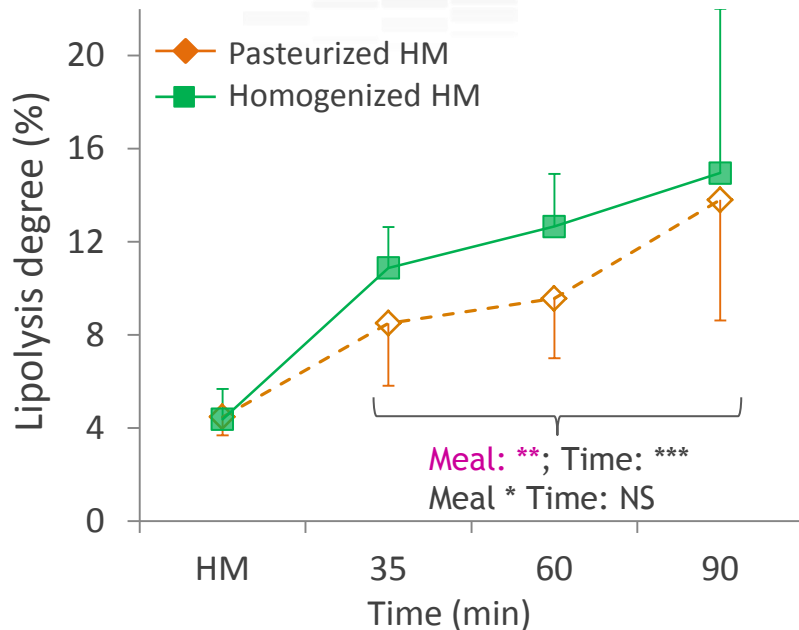


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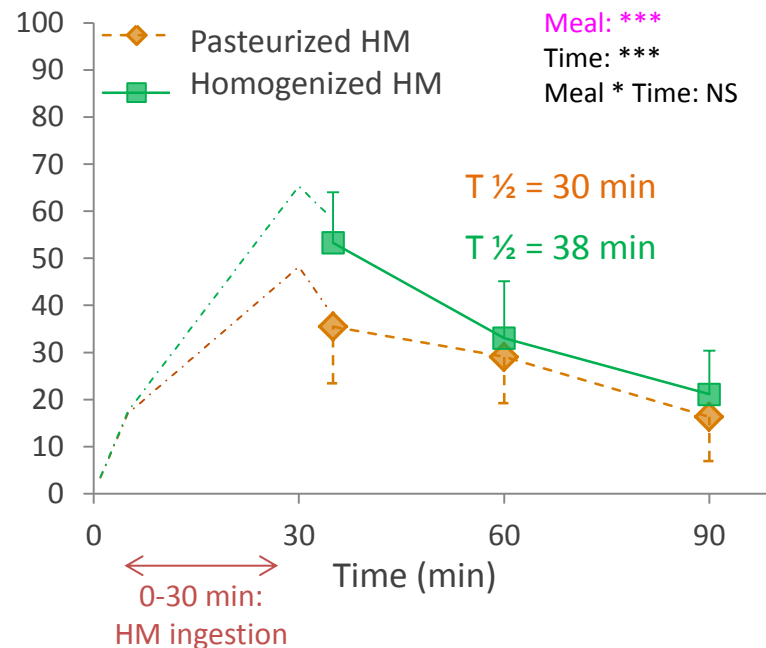


# GROUP B HOMOGENIZATION IMPACTED GASTRIC LIPOLYSIS AND EMPTYING

## Instantaneous lipolysis level



## Gastric emptying



## Hormonal feedback triggered by higher lipolysis level

↗ Free fatty acids in the duodenum → ↗ cholecystinin secretion → ↘ pylorus contractions and gastric emptying  
(Grider, 1994; Yamagishi & Debas, 1978)

## Difference of colloidal behavior between the native MFG and submicronic droplets

Half gastric emptying times reported in preterms

30 38

↗ Infant formula

PP Time (min)

# → STRUCTURE OF HM LIPIDS AND EARLY PROGRAMMING CONCEPT



Gaillard et al. (1989)

**ARA (20:4 n-6)**  
**precursor of prostacyclin in preadipocytes**

Ailhaud et al. (2006)

**LA/LNA ratios** perinatal period → **important programming effect on body composition**

Innis et al. (2010)

**n-6/n-3** perinatal → **intestinal development, sensibility to inflammation** later in life.

H: eicosanoid metabolite (PGE2) < ARA

Oosting et al. (2010) **n-3 LCPUFA-rich neonatal diet** → **adult body composition** (↘ 30 % fat accumulation WSD challenge) & metabolic homeostasis

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Oosting et al. (2010) **n-3 LCPUFA-rich neonatal diet** → **adult**  
**body composition** (∇ 30 % fat accumulation WSD challenge) &  
 metabolic homeostasis

- **(Oosting et al., 2012)** Administration of concept IF (NUTURIS®) during neonatal period in mice over 3 weeks



➔ NUTURIS® => in adulthood to lower fat accumulation, lower fasting plasma leptin, resistin, glucose and lipid (TG and total cholesterol)

## → FIELDS OF RESEARCH AND INNOVATION IN IF

### Fields of research and innovation

Prebiotics/ HM oligosaccharides / other carbohydrate than lactose

**Lipid structure/ reintroduction of dairy lipids /LCPUFA /programming**

HM probiotics/ synbiotics

Protection of protein conformation/ lactoferrin/alternative sources of protein/ peptidome

### Emerging trends

Lactosomes, exosomes, miRNA, HM variability

Sources : Clinical trials, espacenet patent, WOS

*(Bourlieu, Deglaire et al. OCL, 2017)*

# → 1) REINTRODUCTION OF DAIRY LIPIDS : ADDITION OF BOVINE MILK LIPID FRACTIONS

- Reintroduction of cow's milk TAG favours DHA accumulation in brain

*(Delplanque et al. Prostaglandins Leukot. Essent. Fatty Acids, 2013)*

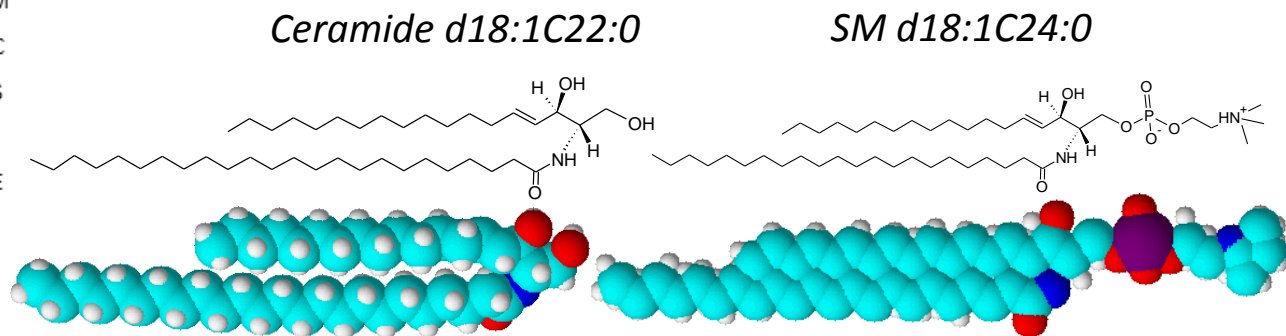
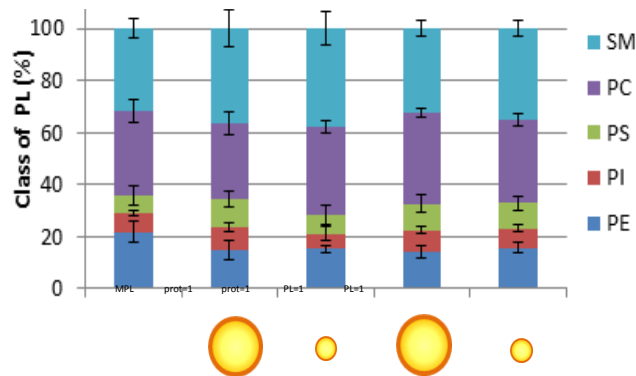
- Reintroduction of MFGM (buttermilk, butterserums and derivatives)

- Taken individually proven bioactivities of polar lipids

*(Hirabayashi & Furuya, Progress Lipid Res., 2008; Kullenberg et al., Lipids Health Dis. 2012; Lonnerdal, AJCN, 2014)*

- Associated as MFGM : anti-bacterial/viral, anti-inflammatory, immune protection

*(Zanabria et al. Food Func., 2013; Zanabria et al. JDS, 2014; Bourlieu and Michalski, COCN 2015)*



*(Bourlieu et al. EJLST, 2015)*

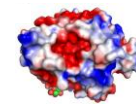
*(Bourlieu, Cheillan, et al. Food chem 2018)*



- lipase



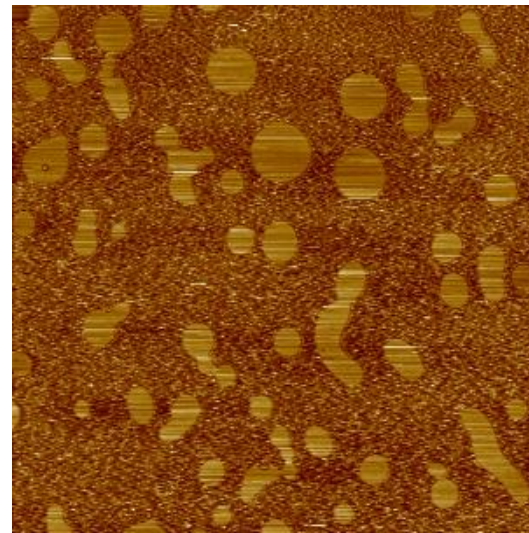
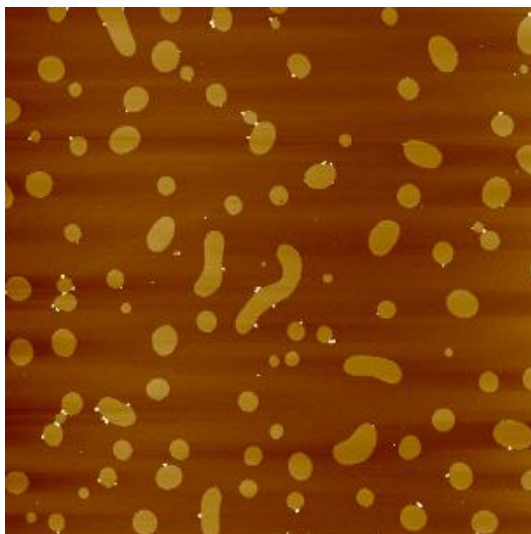
+ lipase



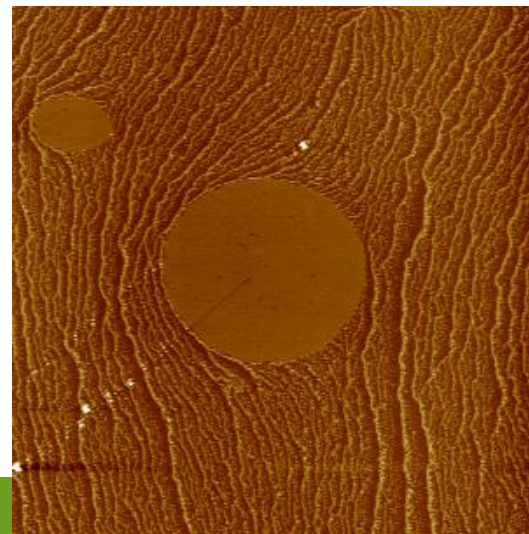
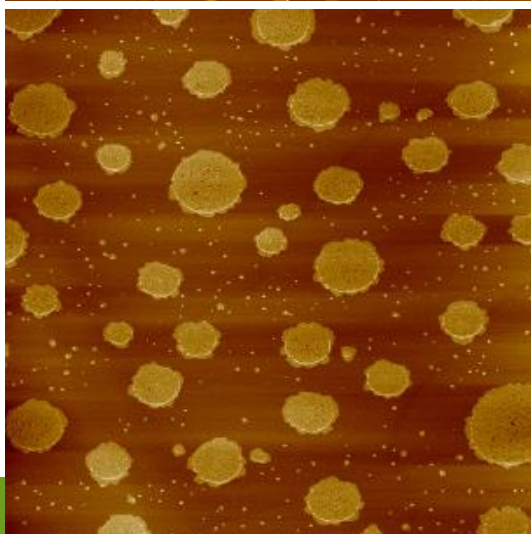
AFM images  
(20 × 20 μm)



Human MFGM



Bovine MFGM



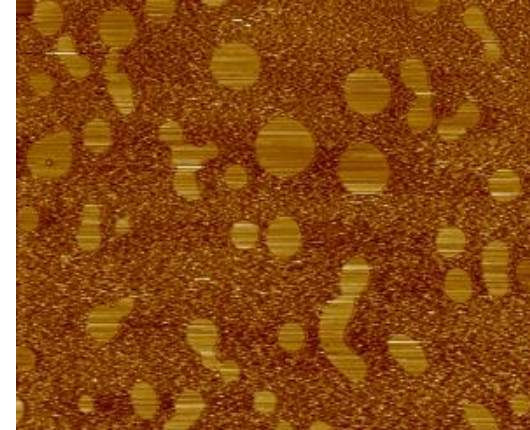
AFM images  
(20 × 20 μm)

+ lipase

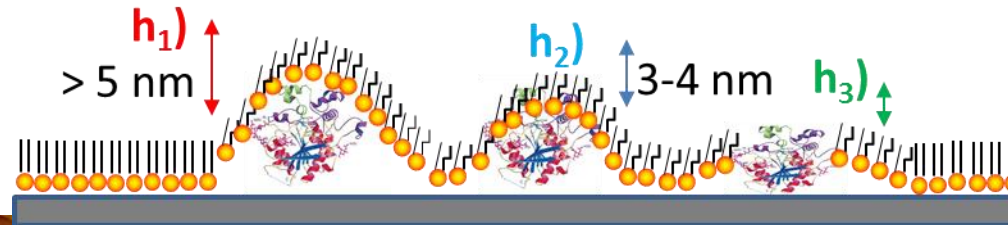
- lipase



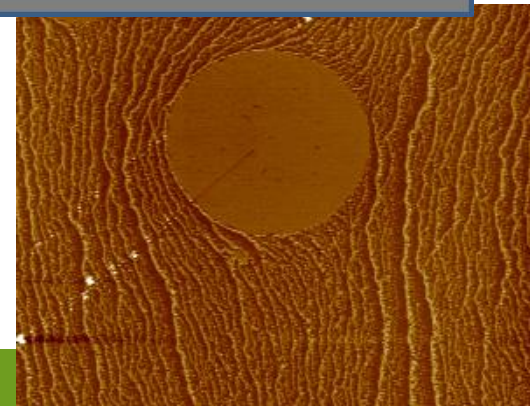
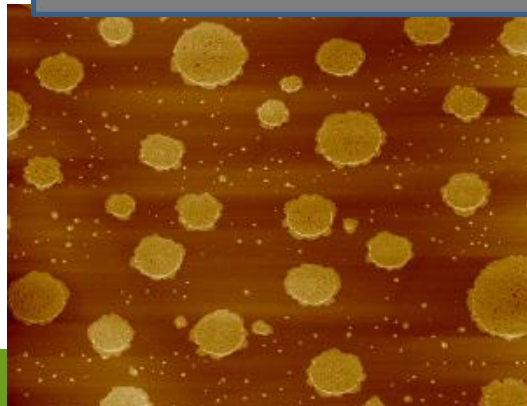
Human MFGM



*Lipase in LE  
only and at 3  
height levels*



Bovine MFGM





# → 1) REINTRODUCTION OF DAIRY LIPIDS : ADDITION OF BOVINE MILK LIPID FRACTIONS

- Reintroduction of cow's milk TAG favours DHA accumulation in brain

*(Delplanque et al. Prostaglandins Leukot. Essent. Fatty Acids, 2013)*

- Reintroduction of MFGM ( buttermilk, butterserums and derivatives)

- Taken individually proven bioactivities of polar lipids

*(Hirabayashi & Furuya, Progress Lipid Res., 2008; Kullenberg et al., Lipids Health Dis. 2012; Lonnerdal, AJCN, 2014)*

- Associated as MFGM : anti-bacterial/viral, anti-inflammatory, immune protection

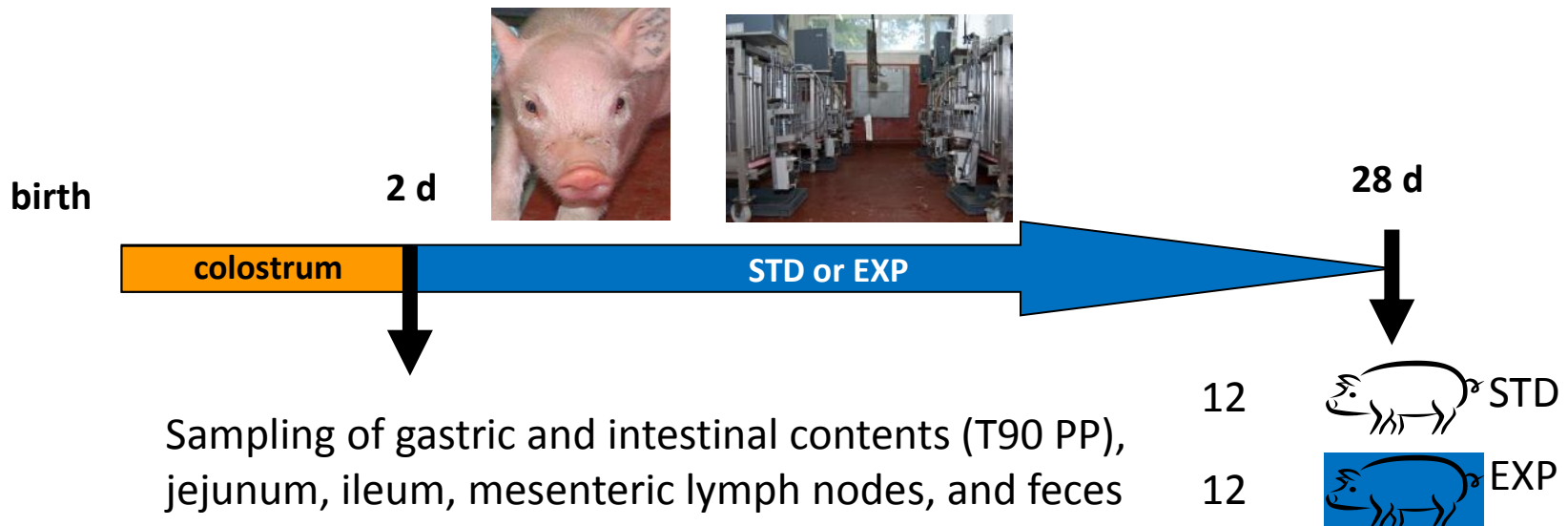
*(Zanabria et al. Food Func., 2013; Zanabria et al. JDS, 2014; Bourlieu and Michalski, COCN 2015)*

# → 1) REINTRODUCTION OF DAIRY LIPIDS : ADDITION OF BOVINE MILK LIPID FRACTIONS

- Reintroduction of TAG and MFGM
  - Pairs of littermate piglets
  - Allocation to STD or EXP formula

+ Sow milk-fed piglets (N=6)

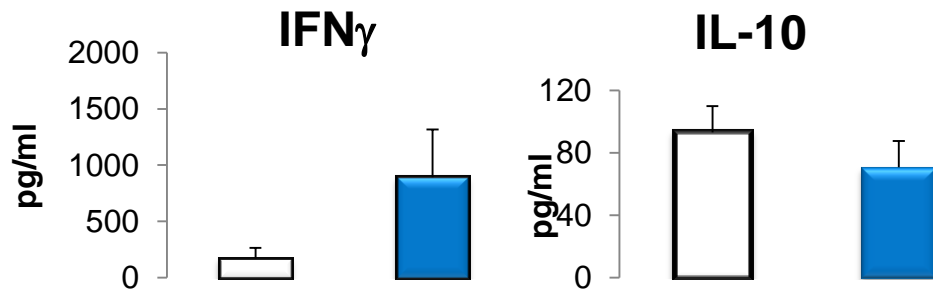
	% DM	STD	EXP
Protein		17.7	17.7
Fat		44.5	44.5
<i>Fat source</i>		<i>Palm oil</i>	<i>Milk fat</i>
		<i>Rapeseed oil</i>	<i>MFGM</i>
		<i>Sunflower oil</i>	<i>Rapeseed oil</i>
			<i>Sunflower oil</i>
Carbohydrate		32.2	32.2



# → 1) REINTRODUCTION OF DAIRY LIPIDS : ADDITION OF BOVINE MILK LIPID FRACTIONS

## ■ Reintroduction of TAG and MFGM

➤ In piglets => favour s intestinal and immune development and positively influenced microbiota composition

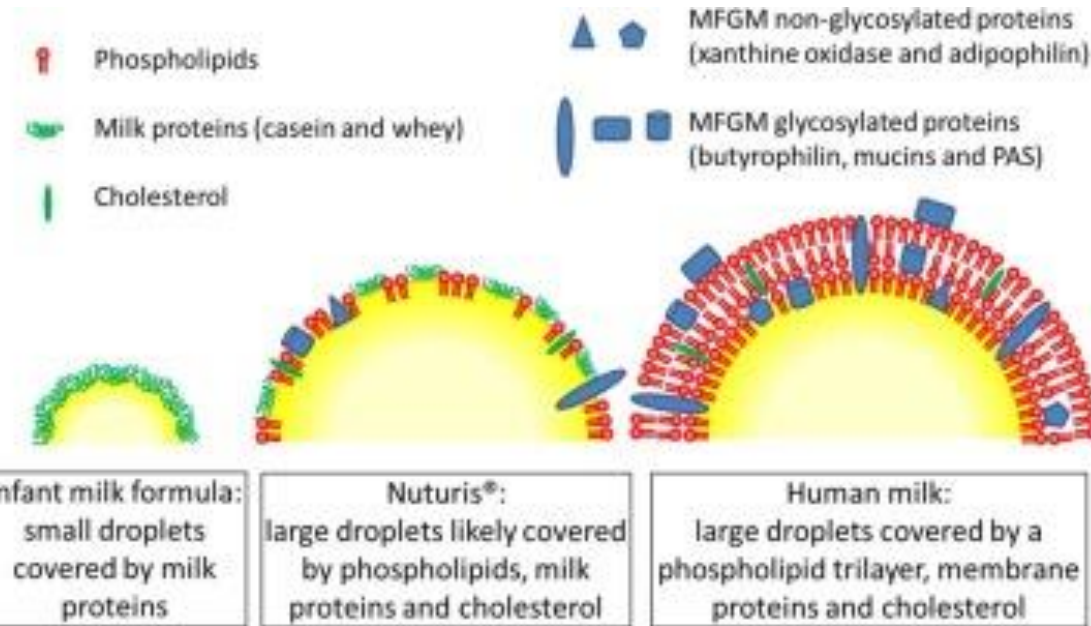


*Mononuclear cells have been isolated from MLN and cultured in vitro to evaluate their cytokine secretory pattern (ELISA)*



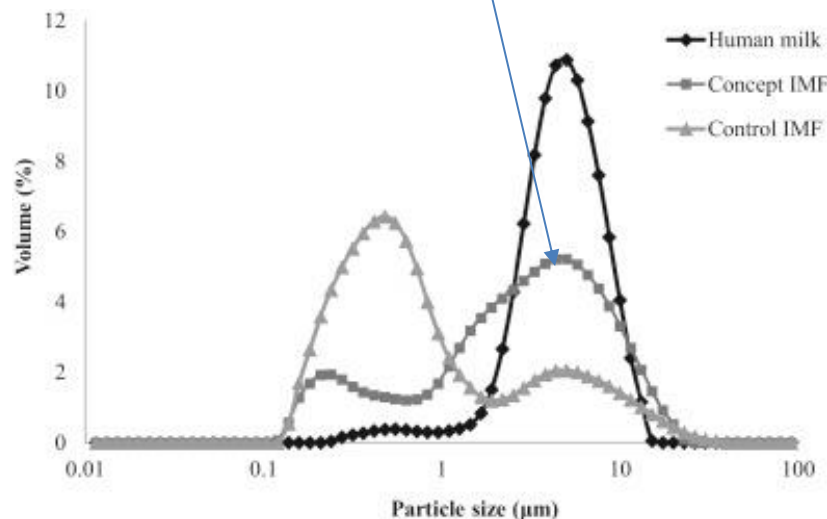
*(Le Huerou-Luron et al. Eur. J. Nutr., 2016)*

## → 2) MORE BIOMIMETIC OF HM : LARGE DROPLETS EMULSIONS



Nuturis®

(WO2013135739A1)



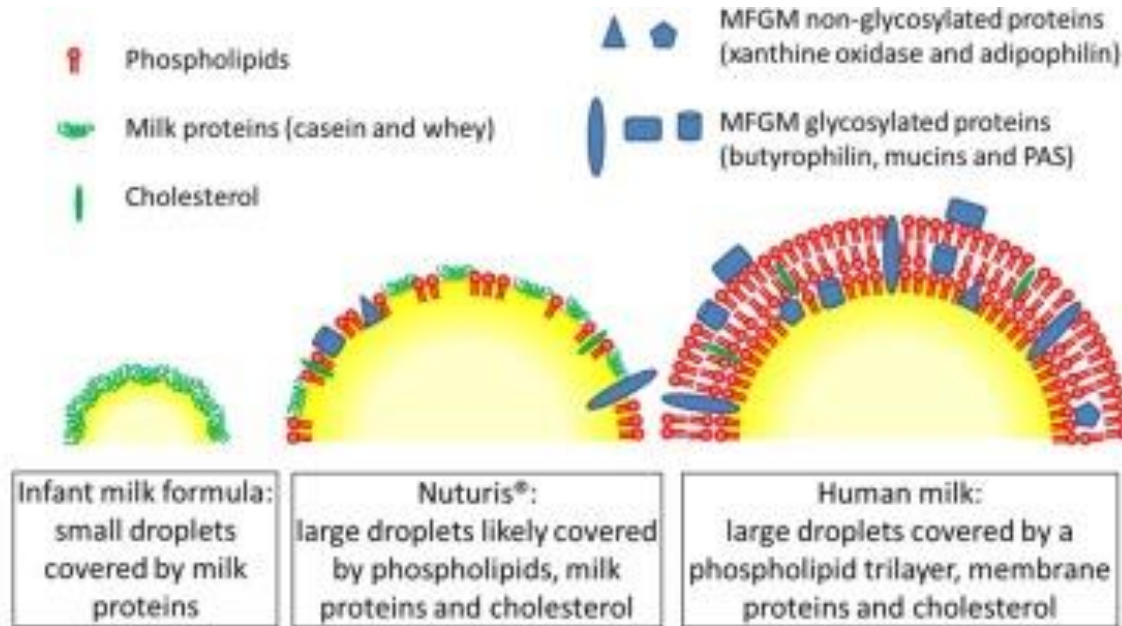
*stable 'minimally processed emulsions' — 6 patents in 2016 (US2016219911, NZ631411, US2016278413, NZ627997, US2016295895, US2016302611)*

(Gallier et al. Coll. Surf. B, 2015)

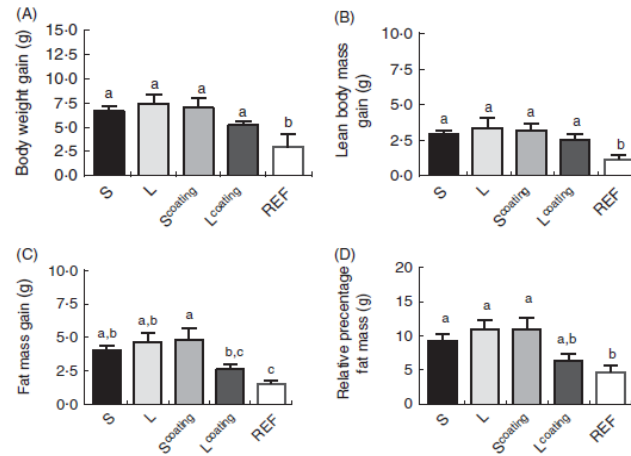
## → 2) MORE BIOMIMETIC OF HM : LARGE DROPLETS EMULSIONS

Nuturis®

(WO2013135739A1)

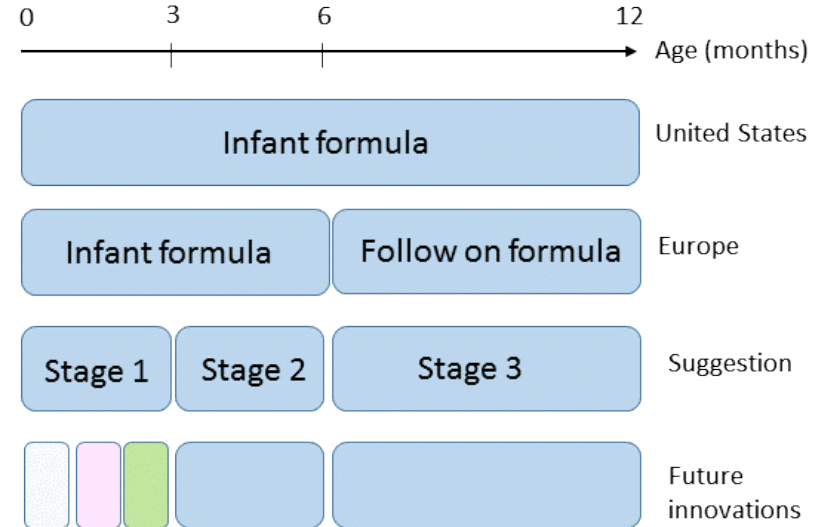
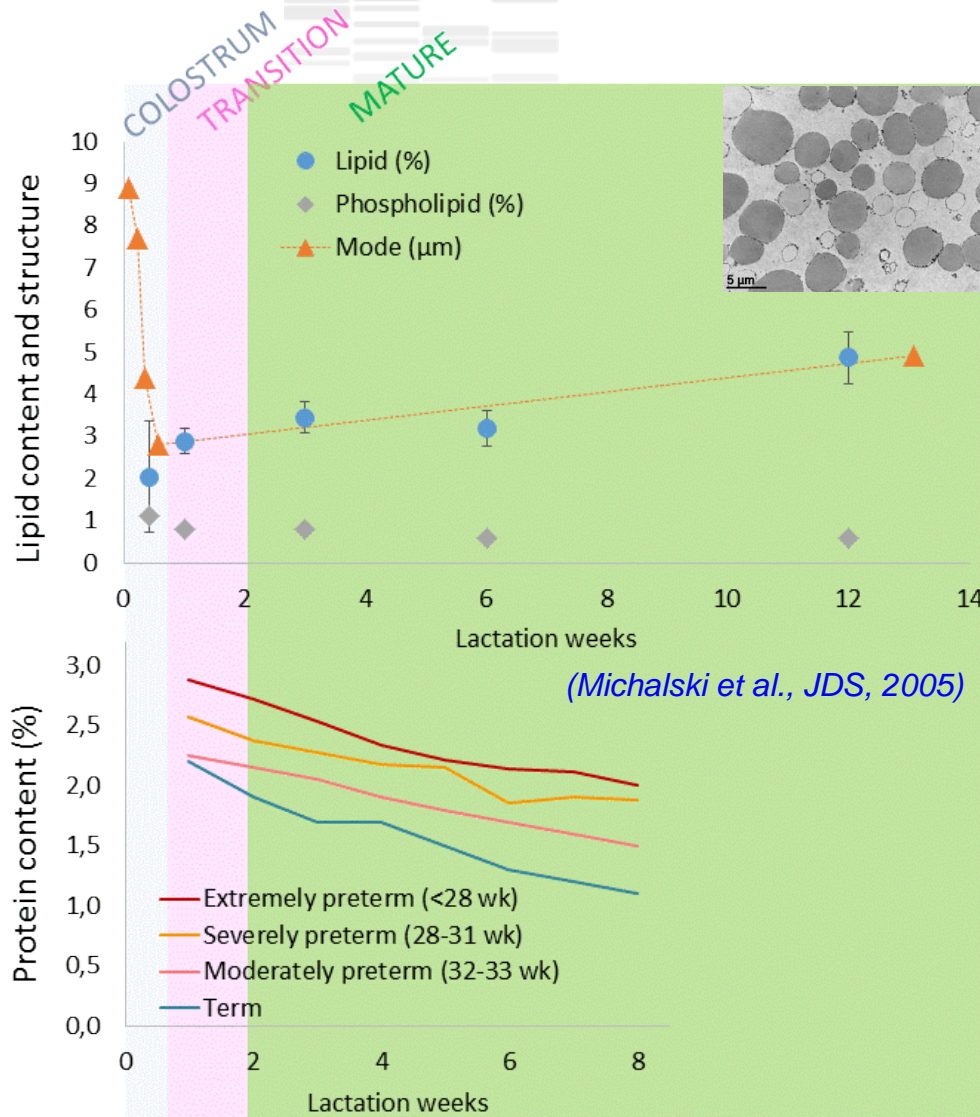


- Protective effect against fat accumulation in adulthood arising from the droplet size and/or from the droplet membrane ?



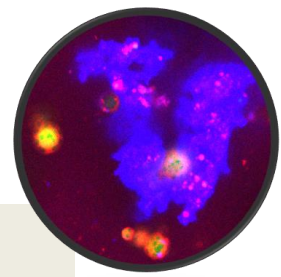
(Baars et al. BJN, 2016)

# → 3) STAGING CONCEPT TO BETTER APPROACH MILK DYNAMICS



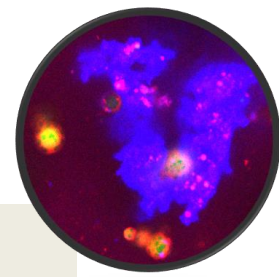
*(Lonnerdal & Hernell, J Ped. Gastr. Nutr., 2016)*

## TAKE Home messages



**We are at a scientific and technological important cross-road ... far beyond the nutritional part played by each individual compounds**

# TAKE Home messages

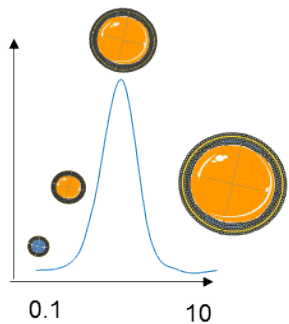


We are at a scientific and technological important cross-road ... far beyond the nutritional part played by each individual compounds

Reproduce digestive behaviour of HM when structuring IF lipids is a priority

## - Size of droplets in emulsion

- Directly determines surface available for lipase adsorption and lipolysis kinetics
- Increases proteolysis via casein adsorption
- Influences gastric aggregates size and emptying



## - Size of droplets and MFGM extracts at interface

- Preprogram body mass and metabolism in model animal. Clinical trial in healthy term infants (NCT01609634)...

## - Regiodistribution of FA on TAG

- Induces specific release of FA and favours PUFA absorption



*Reintroduction of cow's milk fat in IF up to 50%*



# TAKE Home messages

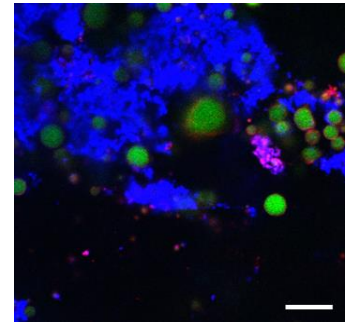
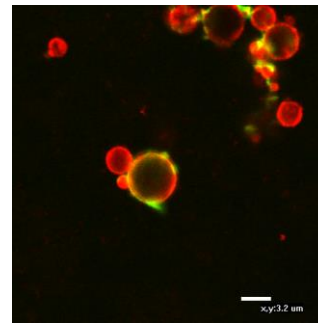
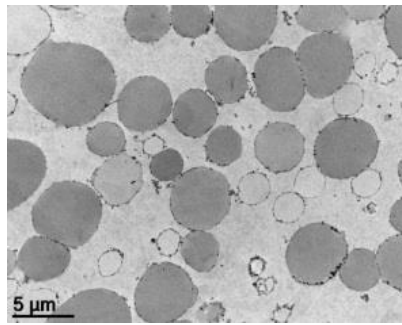
Data on the structure of HM throughout lactation / digestive behaviour of specific fractions of HM lipids still needed to build a sound basis for the optimization of IF

Tendency to combine effects in recent clinical trials about IF :

- NCT01197365 Galacto-oligosaccharides, Beta-palmitate structured TAG and acidified milk

More staging ?

**THANKS To INRA CEPIA, CORECT-CHU Rennes, lactarium de Rennes and associated TEAMS**



Muito obrigada pela atenção  
Muito obrigada a UNIRIO, PPGAN, Gluten Team  
que me permitiram participar deste programa

