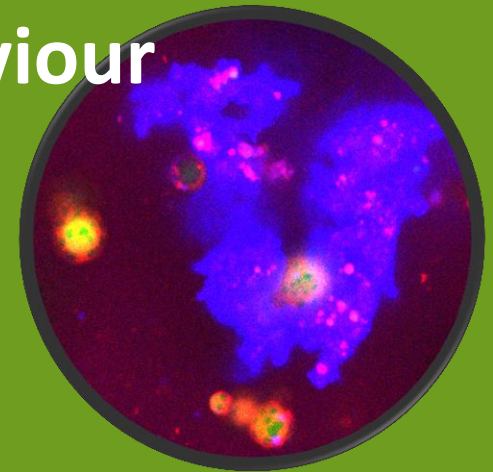


Advances in neonatal nutrition: a step forward infant formula biomimetic of human milk structure and digestive behaviour



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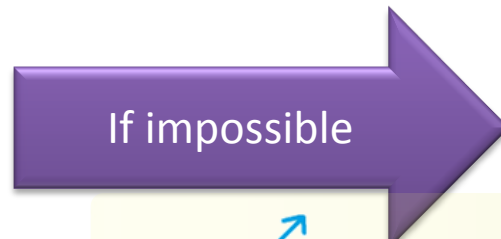


EUROFED LIPID CONGRESS 2017



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

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



Breastfeeding paradox

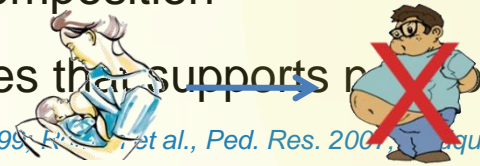
(Rozé et al., 2012)



Infant formula – Optimized chemical composition

Lipids of HM/IF supply 45-55 % calories that supports normal development – 98 % TAG

(Armand et al., Ped. Res ,1996; Jensen, Lipids,1999; Fomon et al., Ped. Res. 2007; Quigley and Hernell, Curr. Opinion Clin. Nutr. Met.Care, 2010, Guesnet et al., OCL, 2013;)



(Victora et al., The Lancet, 2016)

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

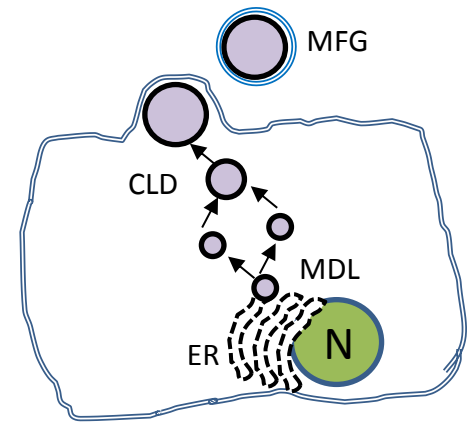
6. Blending during Spray-drying

7. Blending after Spray-drying

8. Big bag packaging

9. Quality control

10. Individual packaging

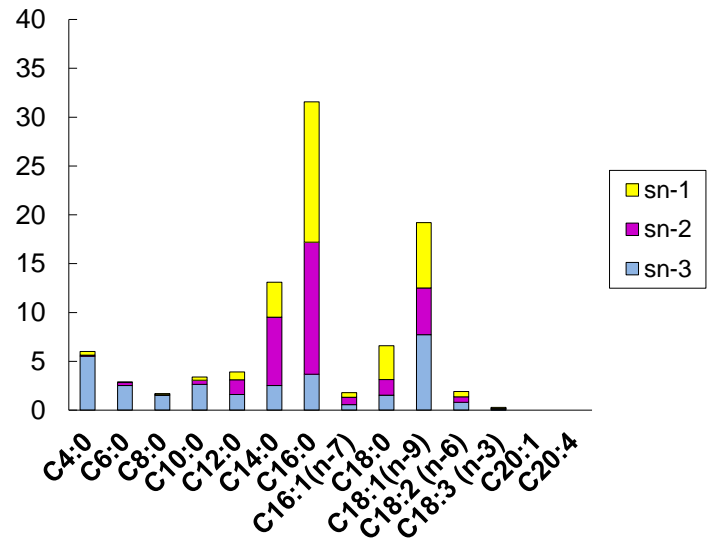
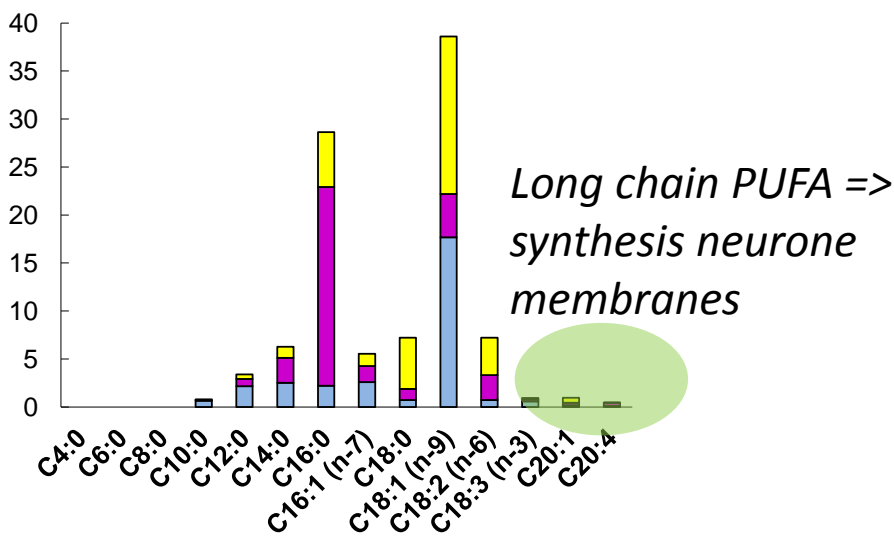
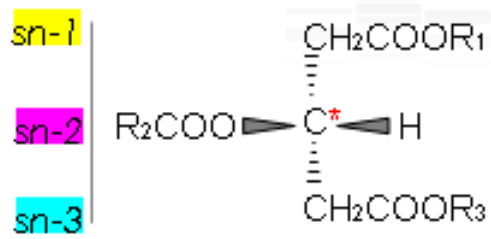


Lipid droplets are formed in the endoplasmic reticulum (ER) by budding into the cytosolic compartment as very small droplets called microlipid droplets (MLD). Droplets increase in size by fusing, giving birth to larger droplets called cytoplasmic lipid droplets (CLD). At the apical cell surface droplets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

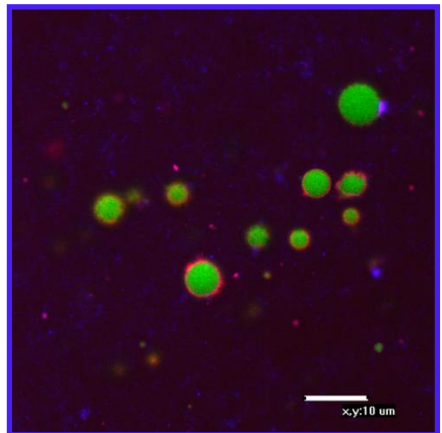
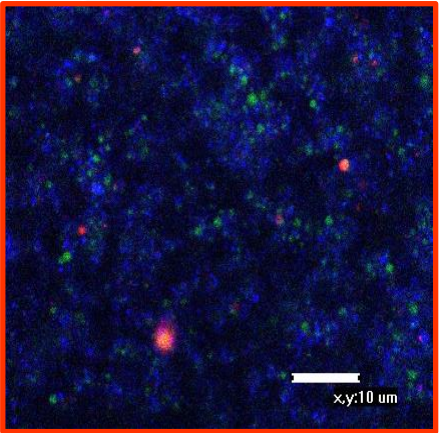
NON RANDOM Distribution of fatty acids (R₁, R₂, R₃) on the glycerol backbone



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

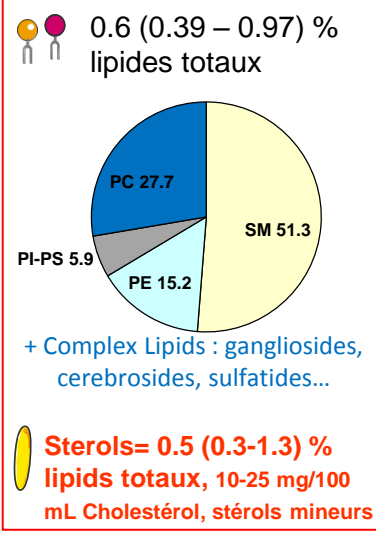
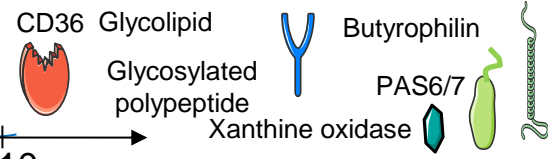
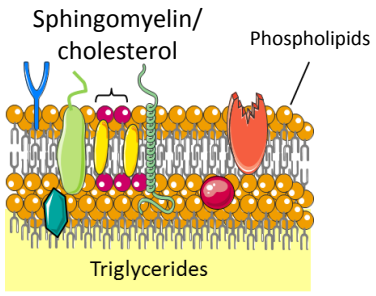
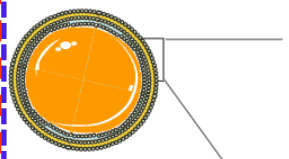
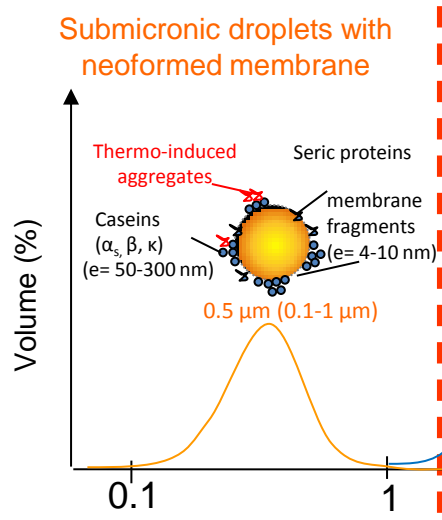


Still a striking difference between HM and IF lipid structure !



3-4.5 g lipids/100 mL

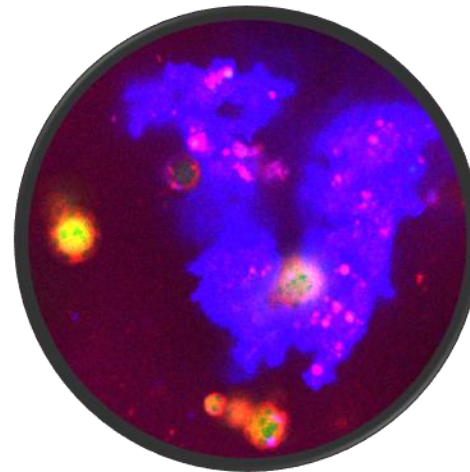
Milk Fat Globule





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

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



Thèse S. Cassia de Oliveira 2013-2016



DIGESTION OF HM IN PRETERM INFANTS

NCT02112331 (ClinicalTrials.gov)

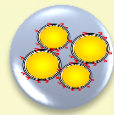
- Randomized controlled trial
- Hospitalized tube-fed preterm infants (GA < 32 wks)
- Alimentation ≥ 120 mL/kg/jour every 3 h
- 6-day experimental period; 2 independent groups

GROUP A

HM from their **own mother**



Raw HM



Past HM

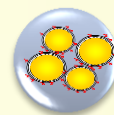
→ collected < 24h
before feeding

→ 1 pool aliquoted in
6 bottles

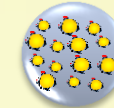
n=12, GA 30.0 ± 1.1 wk, age at first day 27 ± 12 d,
Body weight at first day 1.83 ± 0.41 kg

GROUP B

HM from **anonymous donor**



Past HM

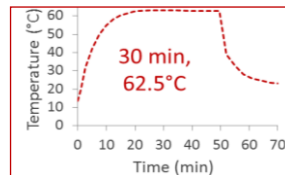


P+Homog HM

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

HM bank

Holder pasteurization



Indirect homogenization by ultrasonication

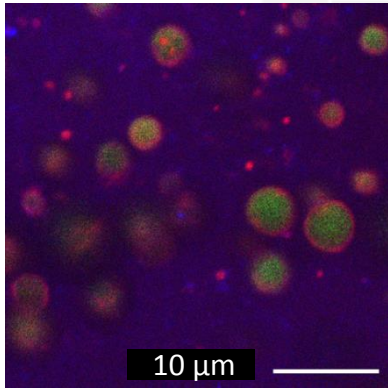


→ 595 W, 3 periods of 5
min interrupted by 30s
of pause

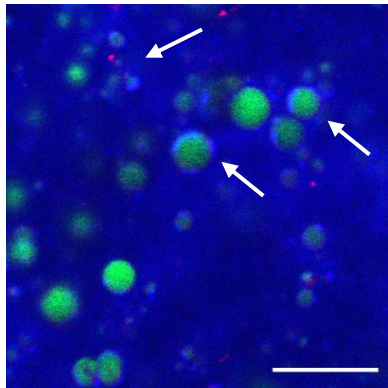
Poster
HENU-015

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

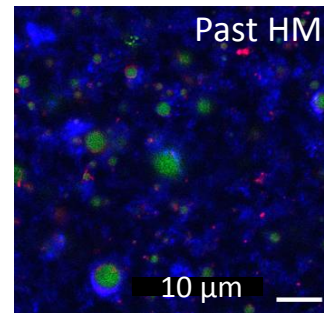
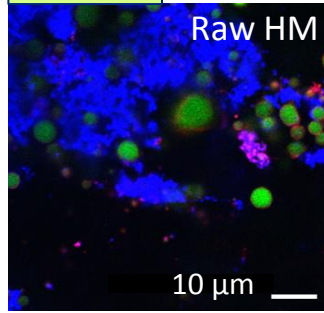
Raw HM



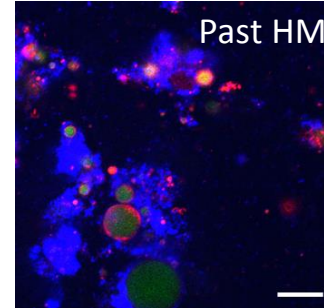
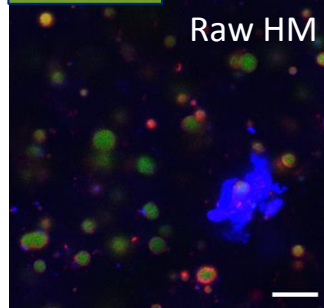
Pasteurized HM



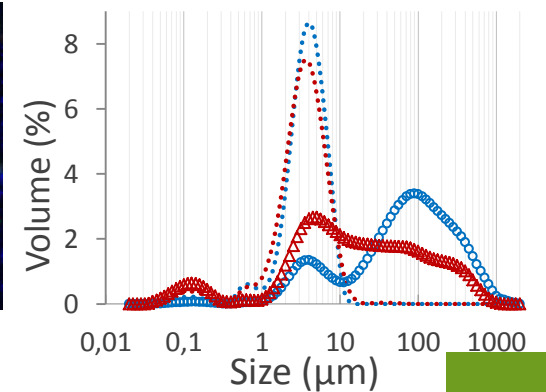
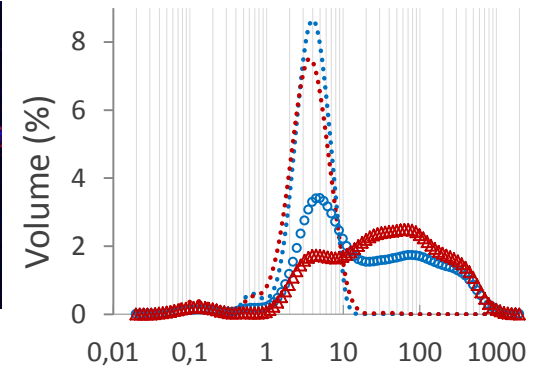
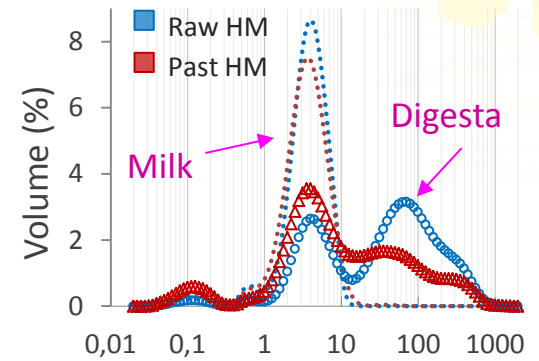
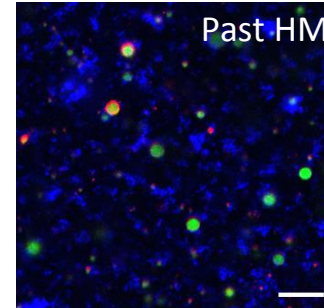
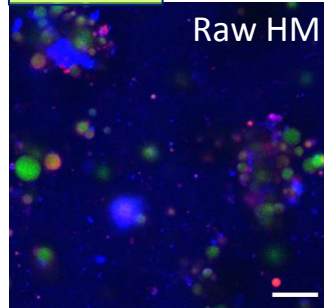
35 min



60 min



90 min

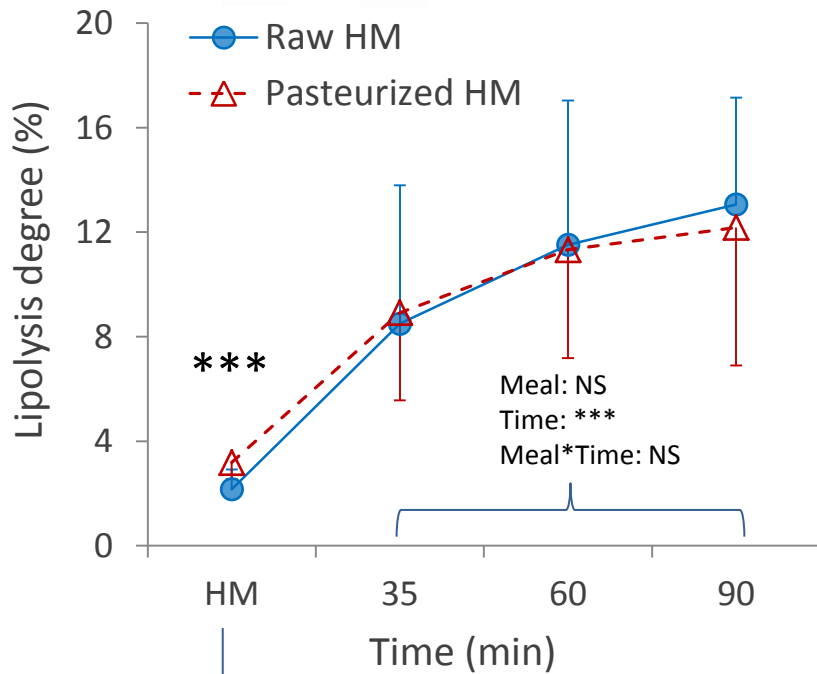


■ Apolar lipids ■ Amphiphiles ■ Proteins

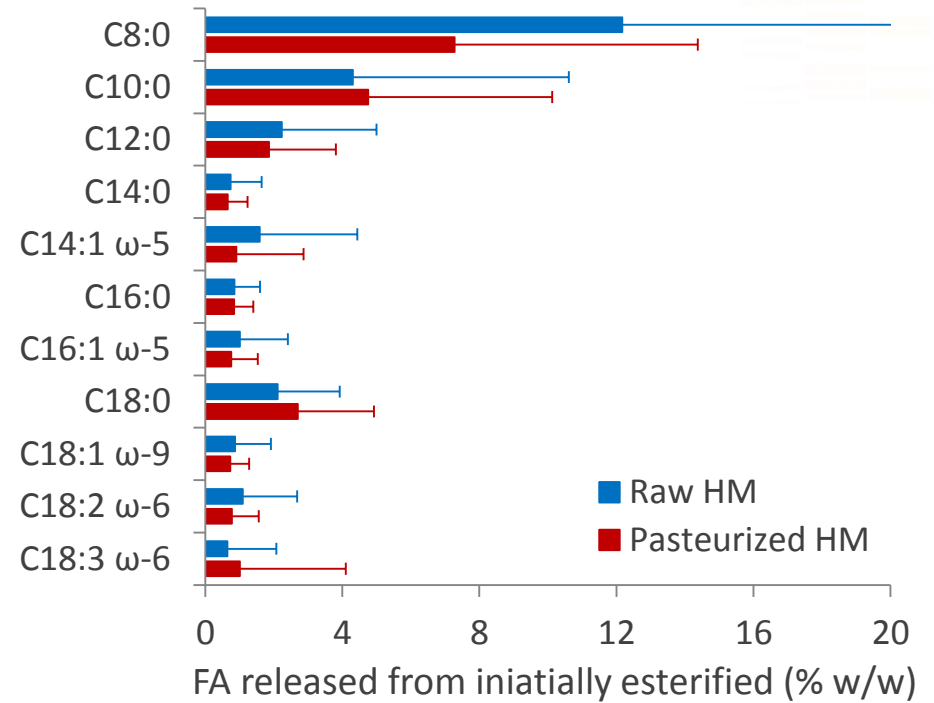
(De Oliveira et al., AJCN, 2017)

GROUP A PASTEURIZATION DID NOT LOWER LIPOLYSIS NOR BIOACCESSIBILITY BUT MODULATED PROTEOLYSIS

Instantaneous Lipolysis



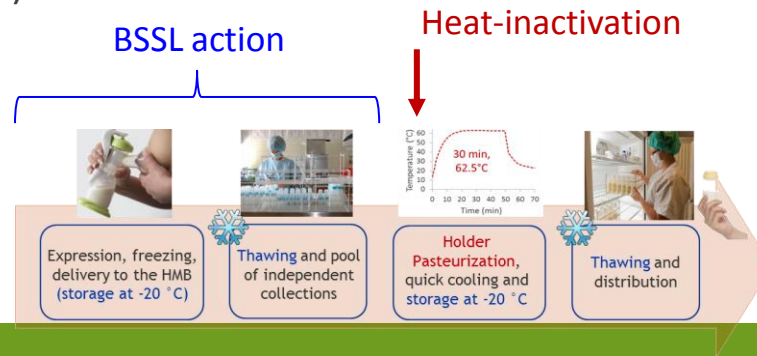
Bioaccessibility of FA



Pre-lipolysis: n = 4 infants

Raw HM = $2.2 \pm 0.8\%$

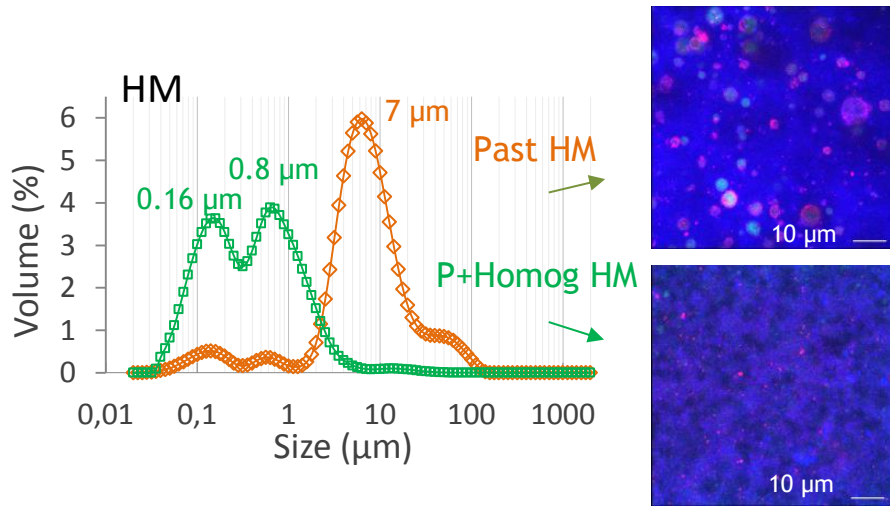
Past HM = $3.2 \pm 0.6\%$



(De Oliveira et al., AJCN, 2017)

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

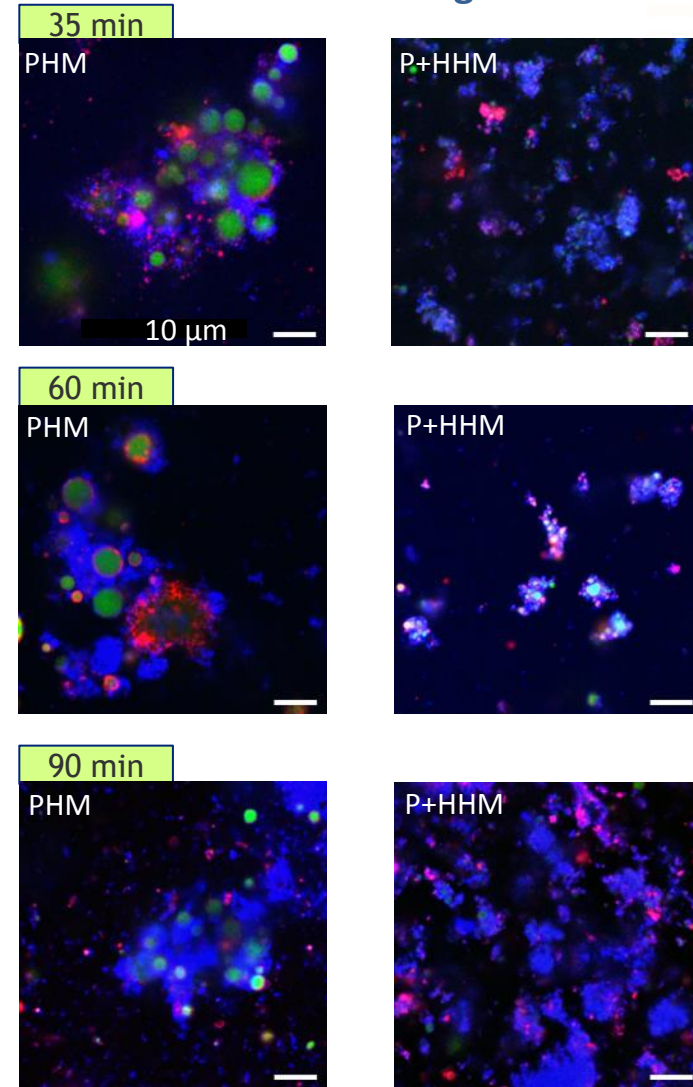
Initial structure



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

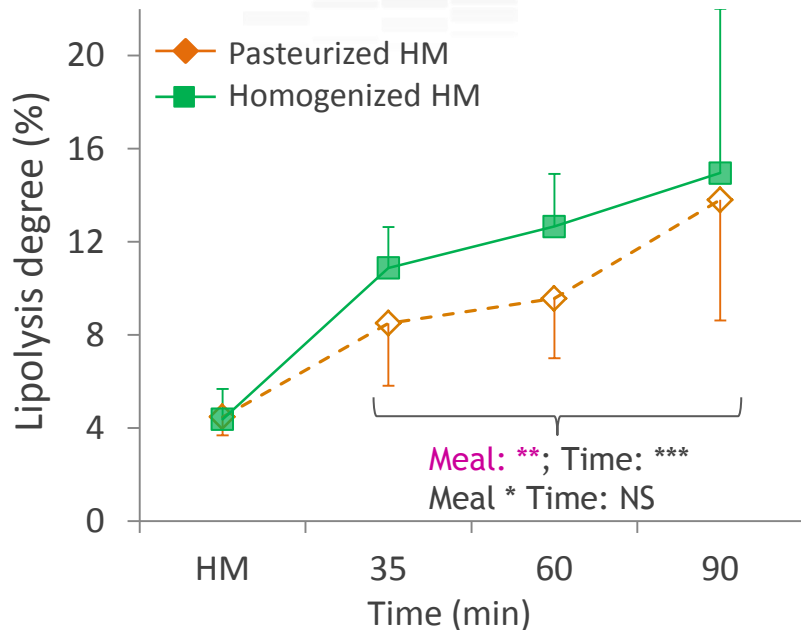


(De Oliveira et al., Clinical Nutrition ESPEN, 2017)

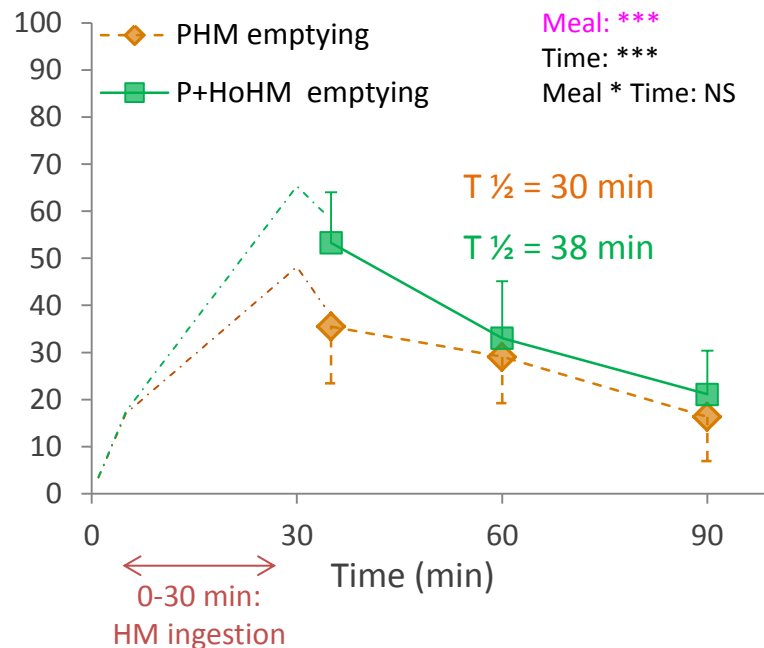


GROUP B HOMOGENIZATION IMPACTED GASTRIC LIPOLYSIS AND EMPTYING

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)

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

→ 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

- **(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

Lantosomes, 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)

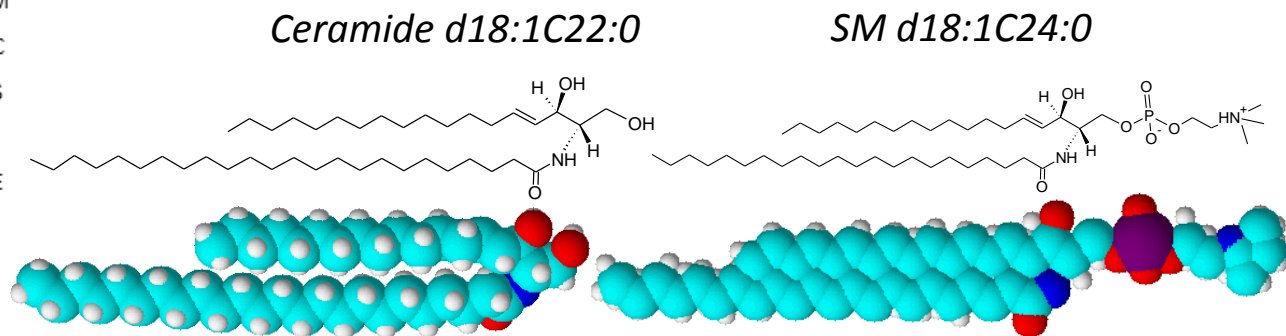
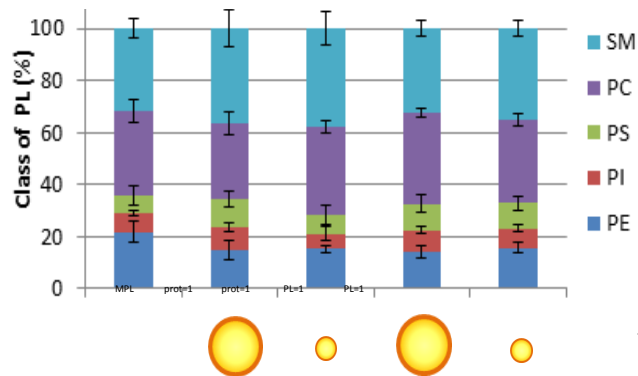
- Reintroduction of MFGM (buttermilk, butterserums and derivatives)

- 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)

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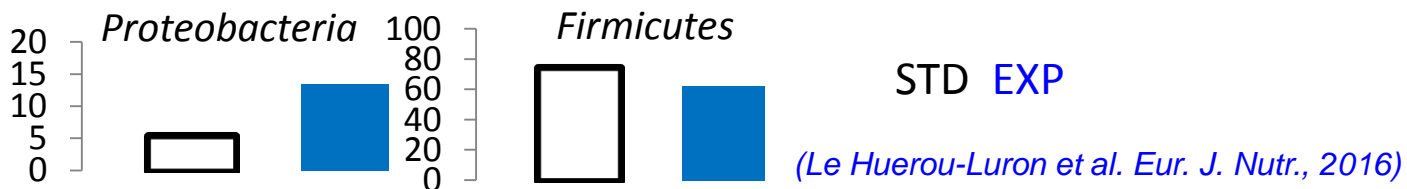
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- Reintroduction of TAG and MFGM

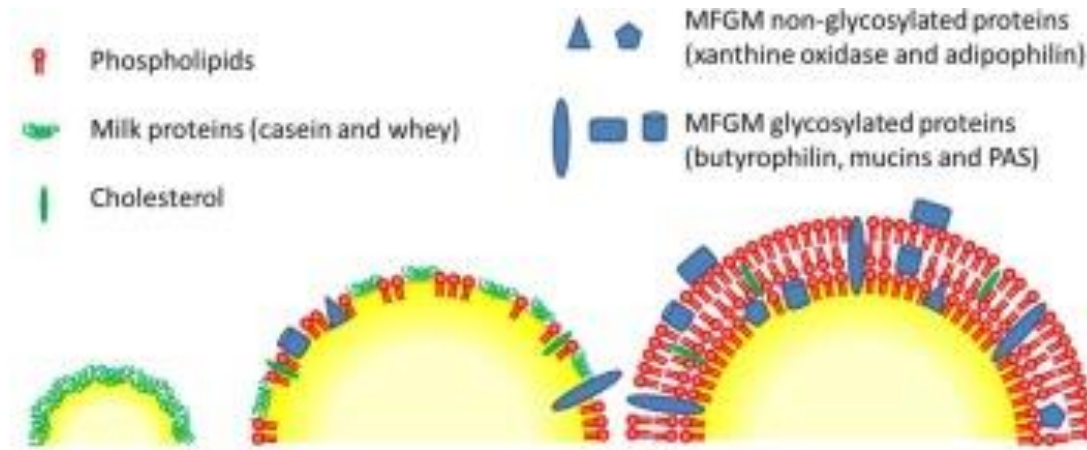
- In piglets => favour s intestinal and immune development and positively influenced microbiota composition



→ 2) MORE BIOMIMETIC OF HM : LARGE DROPLETS EMULSIONS

Nuturis®

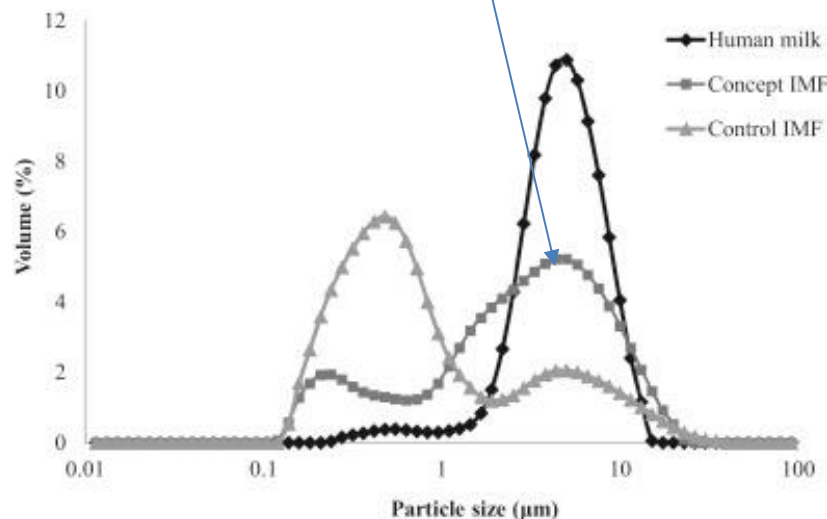
(WO2013135739A1)



Infant milk formula:
small droplets
covered by milk
proteins

Nuturis®:
large droplets likely covered
by phospholipids, milk
proteins and cholesterol

Human milk:
large droplets covered by a
phospholipid trilayer, membrane
proteins and cholesterol



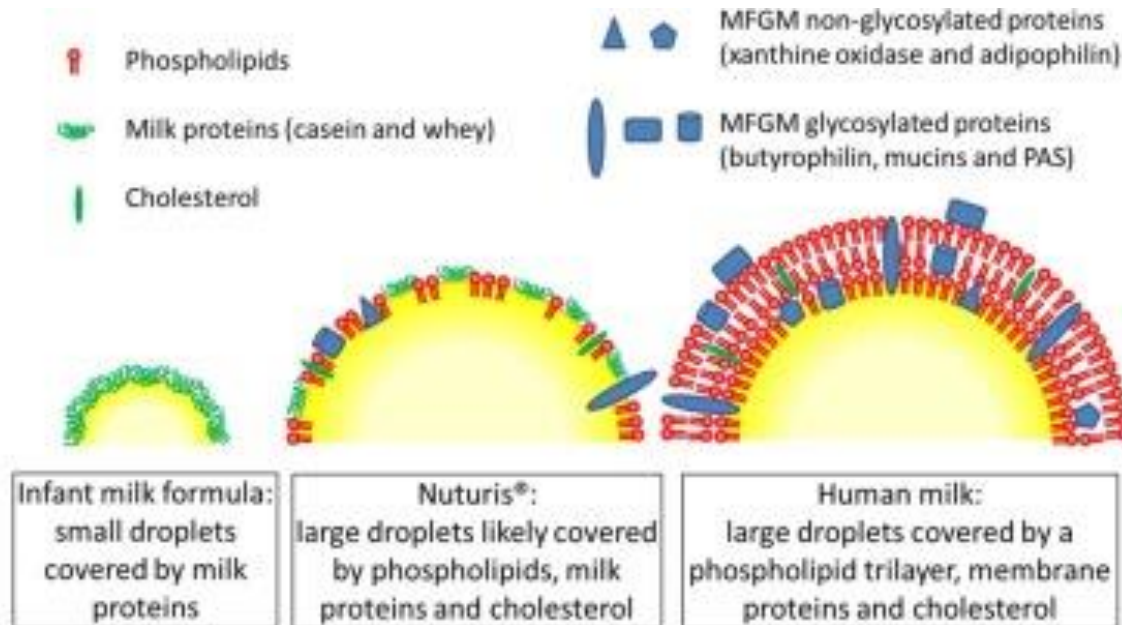
stable 'minimally processed emulsions' — 6 patents in 2016 (US2016219911, NZ631411, US2016278413, NZ627997, US2016295895, US2016302611)

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

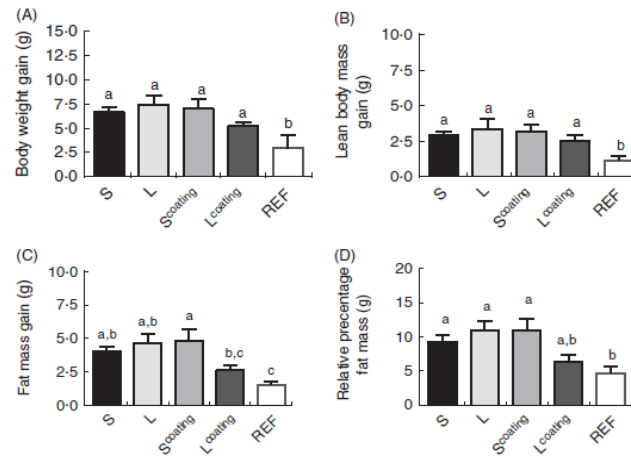
→ 2) MORE BIOMIMETIC OF HM : LARGE DROPLETS EMULSIONS

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(WO2013135739A1)

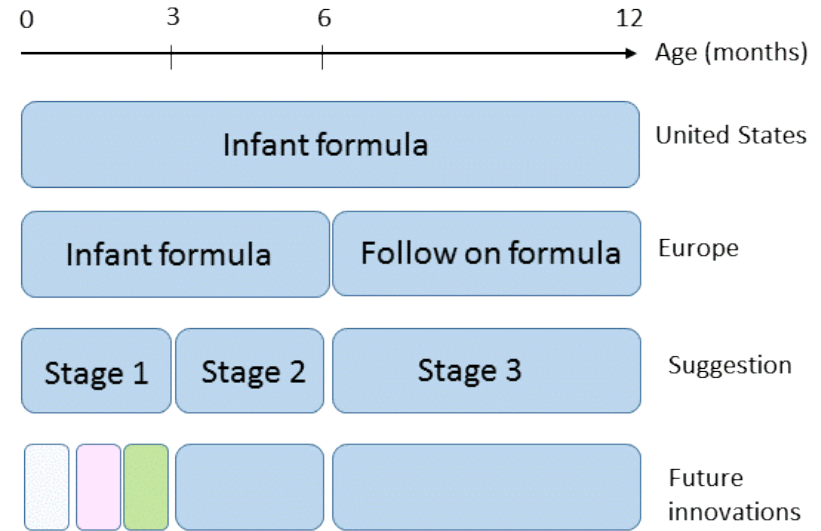
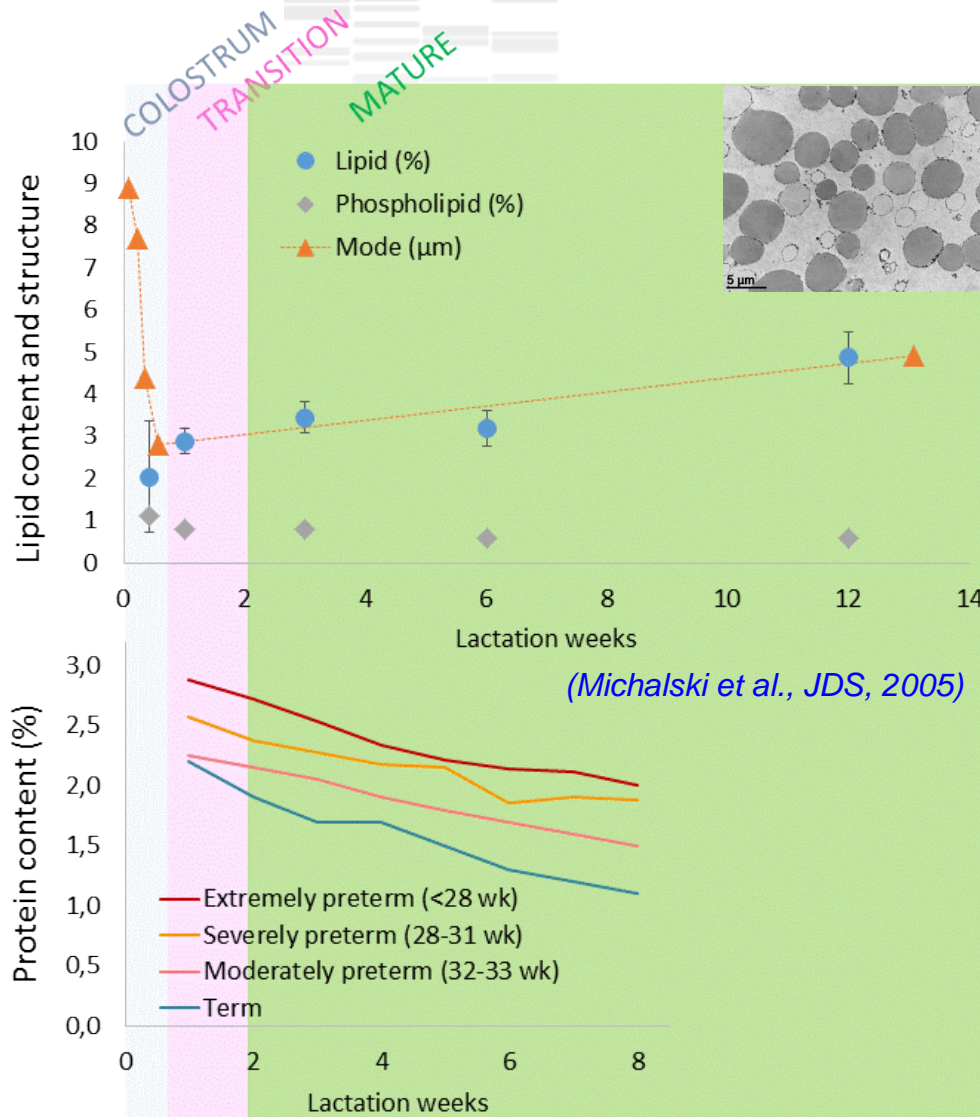


- Protective effect against fat accumulation in adulthood arising from the droplet size and/or from the droplet membrane ?



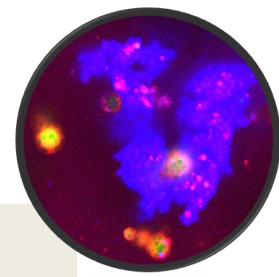
(Baars et al. BJN, 2016)

→ 3) STAGING CONCEPT TO BETTER APPROACH MILK DYNAMICS



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

TAKE Home messages

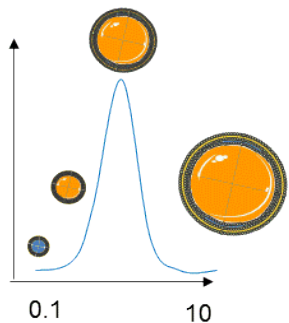


We are at a scientific and technological important cross-road ... far beyond the nutritional part played by each individual compounds

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,
Thanks for your kind attention !!**

