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

C. Bourlieu\textsuperscript{1,2} et A. Deglaire\textsuperscript{1}, S. Cassia de Oliveira\textsuperscript{1}, O. Ménard\textsuperscript{1}, Y. Le Gouar\textsuperscript{1}, F. Carrière\textsuperscript{3}, D. Dupont\textsuperscript{1}

*claire.bourlieu-lacanal@inra.fr
amélie.deglaire@agrocampus-ouest.fr

\textsuperscript{1} INRA/Agrocampus Ouest - UMR 1253 STLO, Rennes, France;
\textsuperscript{2} UMR 1208 IATE, Montpellier, France;
\textsuperscript{3} CNRS-UMR 7282 EIPL, Marseille, France
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...

If impossible

Breastfeeding paradox

Infant formula – Optimized chemical composition

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

Over the last decade, Lipid structure identified as a parameter involved in early programming

Lipid structure supply 45-55% calories that supports newborn development – 98% TAG

(Rozé et al., 2012)


(Victora et al., The Lancet, 2016)

(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!

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

Long chain PUFA => synthesis neurone membranes

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!

Submicronic droplets with neoformed membrane

Thermo-induced aggregates
Caseins (α, β, κ) (e= 50-300 nm)

Membrane fragments (e=4-10 nm)

0.5 µm (0.1-1 µm)

4 µm (0.1-10 µm)

Milk Fat Globule

Sphingomyelin/cholesterol
Phospholipids

Seric proteins

Glycolipid

Glycosylated polypeptide

Butyrophilin

CD36

Xanthine oxidase

PAS6/7

3-4.5 g lipids/100 mL

0.6 (0.39 – 0.97) % lipides totaux

Sterols= 0.5 (0.3-1.3) % lipides totaux, 10-25 mg/100 mL Cholestérol, stérols mineurs

+ Complex Lipids : gangliosides, cerebrosides, sulfatides...

INRA
SCIENCE & IMPACT

(Michalski et al. JDS, 2005; Michalski et al., EJLST, 2009)

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

→ **PRIORITY COLLECT DATA ABOUT HM DIGESTIVE BEHAVIOUR**
DIGESTION OF HM IN PRETERM INFANTS

- 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

NCT02112331 (ClinicalTrials.gov)

Poster HENU-015
GROUP A GASTRIC BEHAVIOUR OF HM IS DOMINATED BY AGREGATION AROUND MILK FAT GLOBULE AND INFLUENCED BY PASTEURIZATION (De Oliveira et al., AJCN, 2017)

(Diagram showing changes in milk fat globule aggregation in raw and pasteurized HM over 90 minutes)

- **35 min**
  - Raw HM
  - Pasteurized HM

- **60 min**
  - Raw HM
  - Pasteurized HM

- **90 min**
  - Raw HM
  - Pasteurized HM

Graphs showing volume distribution over time for milk and digesta.
**GROUP A PASTEURIZATION DID NOT LOWER LIPOLYSIS NOR BIOACCESIBILITY BUT MODULATED PROTEOLYSIS**

**Instantaneous Lipolysis**

- **Raw HM**
- **Pasteurized HM**

**Bioaccessibility of FA**

- Raw HM
- Pasteurized HM

**FA released from initially esterified (% w/w)**

**Pre-lipolysis:** n = 4 infants
- **Raw HM** = 2.2 ± 0.8%
- **Past HM** = 3.2 ± 0.6%

**Expression, freezing, delivery to the HMB (storage at -20 °C)**
- **Thawing and pool of independent collections**
- **Holder Pasteurization, quick cooling and storage at -20 °C**
- **Thawing and distribution**

*(De Oliveira et al., AJCN, 2017)*
GROUP B HOMOGENIZATION AFFECTED THE INITIAL STRUCTURE AND THE EMULSION DISINTEGRATION OF HM

Past HM: 4.1 ± 1.2 m²/g of fat

P+Homog HM: 25.5 ± 3.8 m²/g of fat

(De Oliveira et al., Clinical Nutrition ESPEN, 2017)
HOMOGENIZATION IMPACTED GASTRIC LIPOLYSIS AND EMPTYING

**Instantaneous lipolysis level**

- **Pasteurized HM**
- **Homogenized HM**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Lipolysis degree (%)</th>
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<tbody>
<tr>
<td>HM</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
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</tbody>
</table>

**Meal:** **; **Time:** ***

**Meal:** * Time: NS

- Hormonal feedback triggered by higher lipolysis level
- Free fatty acids in the duodenum $\rightarrow$ Cholecystokinin secretion $\rightarrow$ Pylorus contractions and gastric emptying
  
  *(Grider, 1994; Yamagishi & Debas, 1978)*

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

**Gastric emptying**

- **PHM emptying**
- **P+HoHM emptying**

<table>
<thead>
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<th>Time (min)</th>
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<tbody>
<tr>
<td>0-30 min: HM ingestion</td>
</tr>
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<td>30</td>
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<td>90</td>
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**Meal:** ***

- **Time:** ***
- **Meal:** * Time: NS

**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)*
Innis et al. (2010) n-6/n-3 perinatal → intestinal development, sensibility to inflammation later in life. H: eicosanoid metabolite (PGE2) < ARA

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

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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

PN16 NUTURIS® PN42 Western diet PN126 (Oosting et al. Translational Inv., 2012)

CONTROL

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
- HM probiotics/ synbiotics
- Lipid structure/ reintroduction of dairy lipids / LCPUFA / programming
- 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
  

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

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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)


2) MORE BIOMIMETIC OF HM : LARGE DROPLETS EMULSIONS

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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)

Michalski et al., JDS, 2005)
TAKE Home messages

We are at a scientific and technological important cross-road ... far beyond the nutritionnal 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!!