

Impact of pasteurization and homogenization on the digestion of human milk: an *in vivo* study in the preterm infant

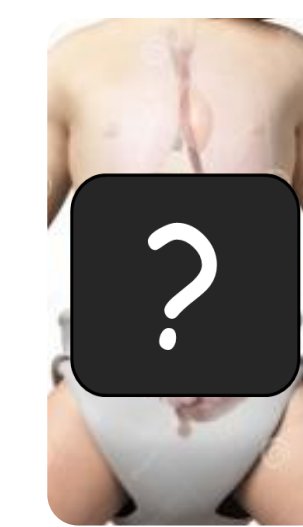
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Context

Ensuring adequate growth of preterm newborns remains a challenge. When breastfeeding not possible → pasteurized human milk from milk banks is preferentially administered. Holder pasteurization (62.5°; 30 min) is applied for sanitary reasons but may reduce fat absorption through the inactivation of milk endogenous lipases. Conversely, homogenization of Holder-pasteurized human milk (PHM) may improve fat absorption and weight gain.

(Thomaz et al., 1999; Rayol et al., 1993; Martinez et al., 1987)

Objective



Impact on digestive kinetics?

→ To investigate the impact of the homogenization and pasteurization of human milk on its gastric digestion in preterm infants

Materials & Methods

ClinicalTrials.gov
NCT02112331

Experimental design



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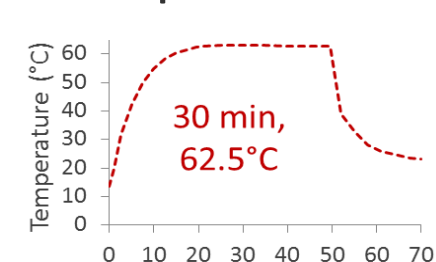
- Randomized controlled trial
- Hospitalized tube-fed preterm infants (GA < 32 wks)
- 6-day experimental period; 2 independent groups

GROUP A
HM from their **own** mother

Raw HM **Pasteurized HM (PHM)**

→ collected < 24h → 1 pool aliquoted in 6 bottles before feeding
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

Holder pasteurization



GROUP B
HM from **anonymous** donor

Pasteurized HM (PHM) **Pasteurized + Homogenized 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

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

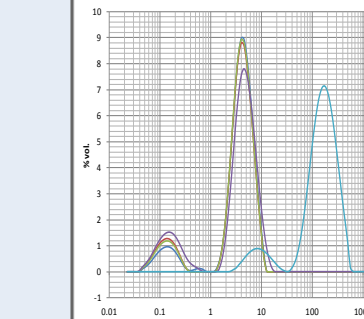
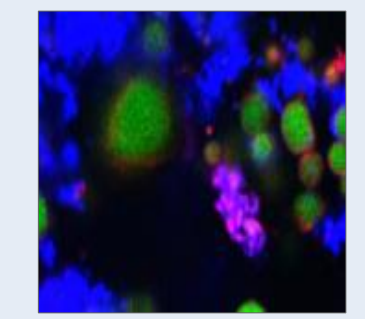
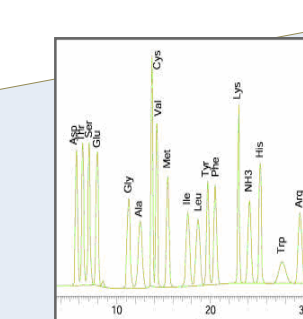
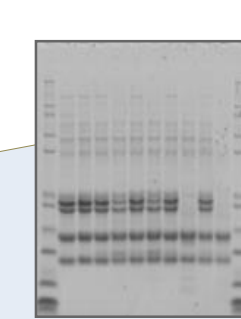
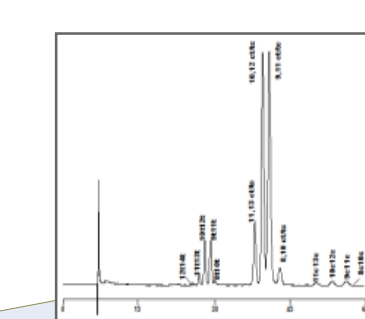


- 2 test meal/day, for each test meal, **two gastric effluent collections**: before the meal and **35, 60 or 90 min** after the ingestion start

Multi-scale characterization of HM and digested samples

Biochemical composition: kinetics of lipolysis and proteolysis

Structural disintegration



Human Milk analyzer

Gas and thin layer chromatography

Titrimetry
SDS-Page + densitometry;
Cation exchange chromatography

Confocal microscopy

Laser light scattering

Instantaneous lipolysis level:

→ % of free fatty acids vs. total acyl chains present at a given time

Lipolytic activity in gastric aspirates:

→ Titration of butyric acid released from tributyrin at pH 4.5 and 37 °C (Gargouri et al., 1986)

% of intact protein remaining at a given time:

→ Lactoferrin, α-lactalbumin, serum albumin, β-casein (data not shown here)

Particle size distribution

Fluorescent probes:

→ Proteins (FastGreen®), apolar lipids (LipidTox®) and polar lipids (Rhodamine-PE®)

→ Results were expressed as means ± SD

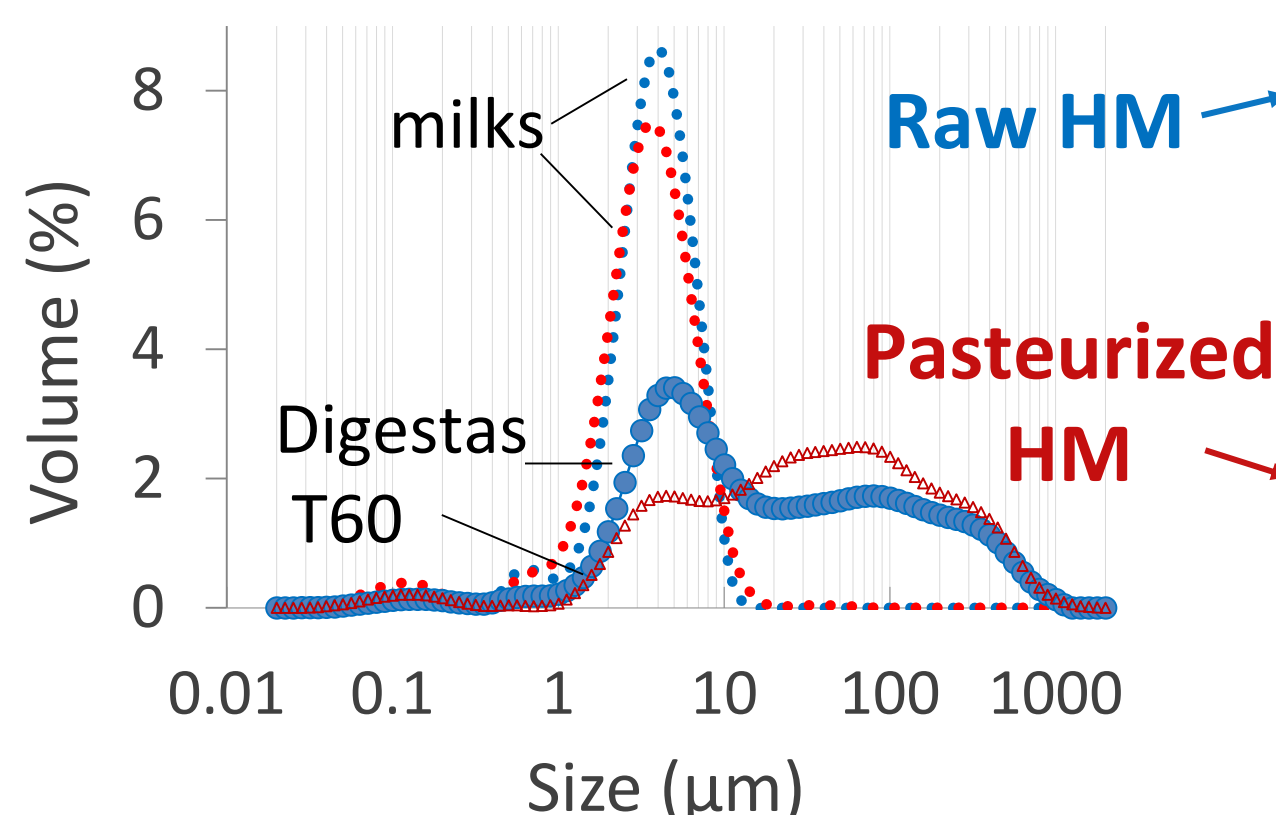
P < 0.001 (***); P < 0.01 (**); P < 0.05 (*); P > 0.05 (NS).

Results

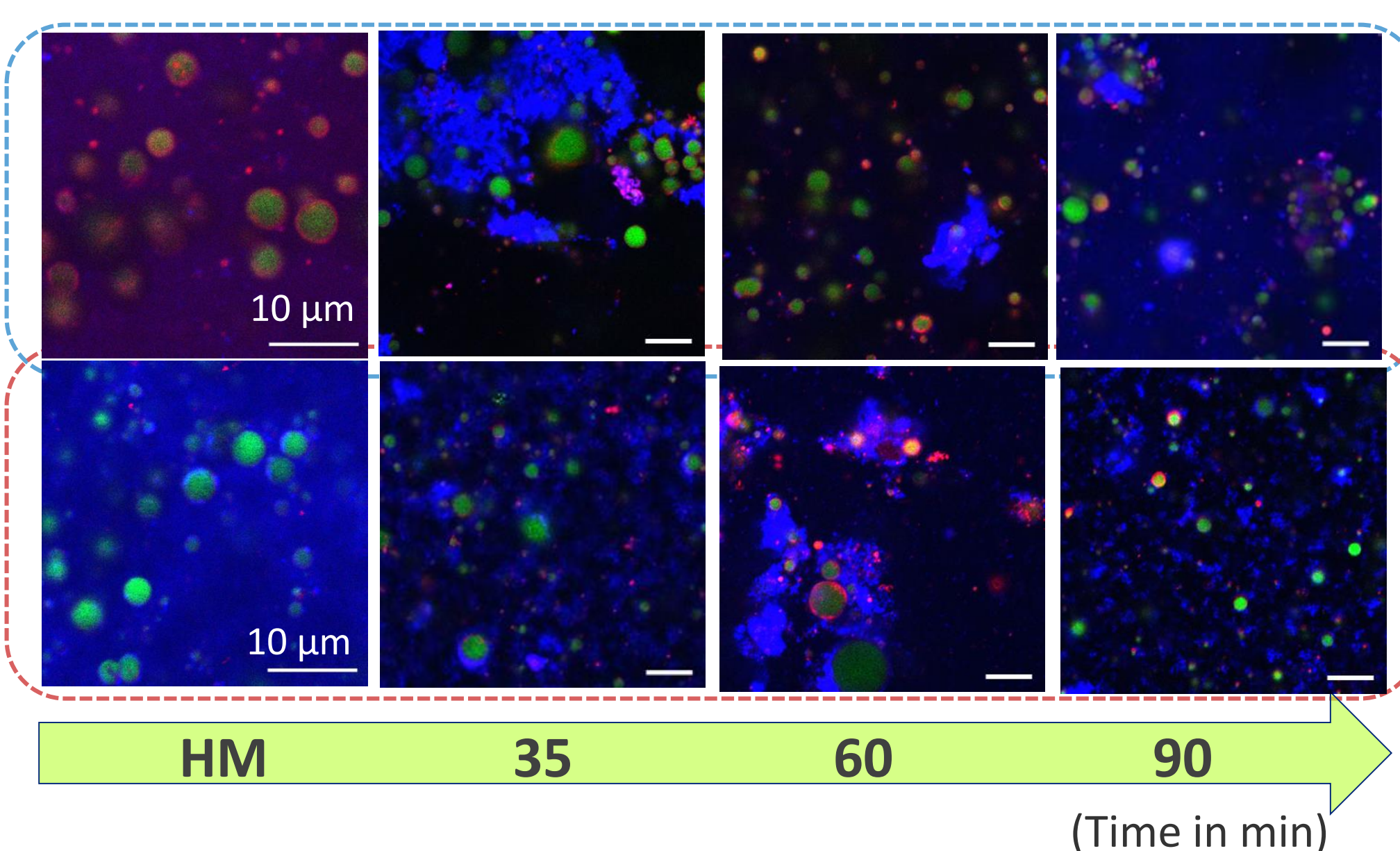
GROUP A

Pasteurization affected the emulsion disintegration of HM

Particle size distribution



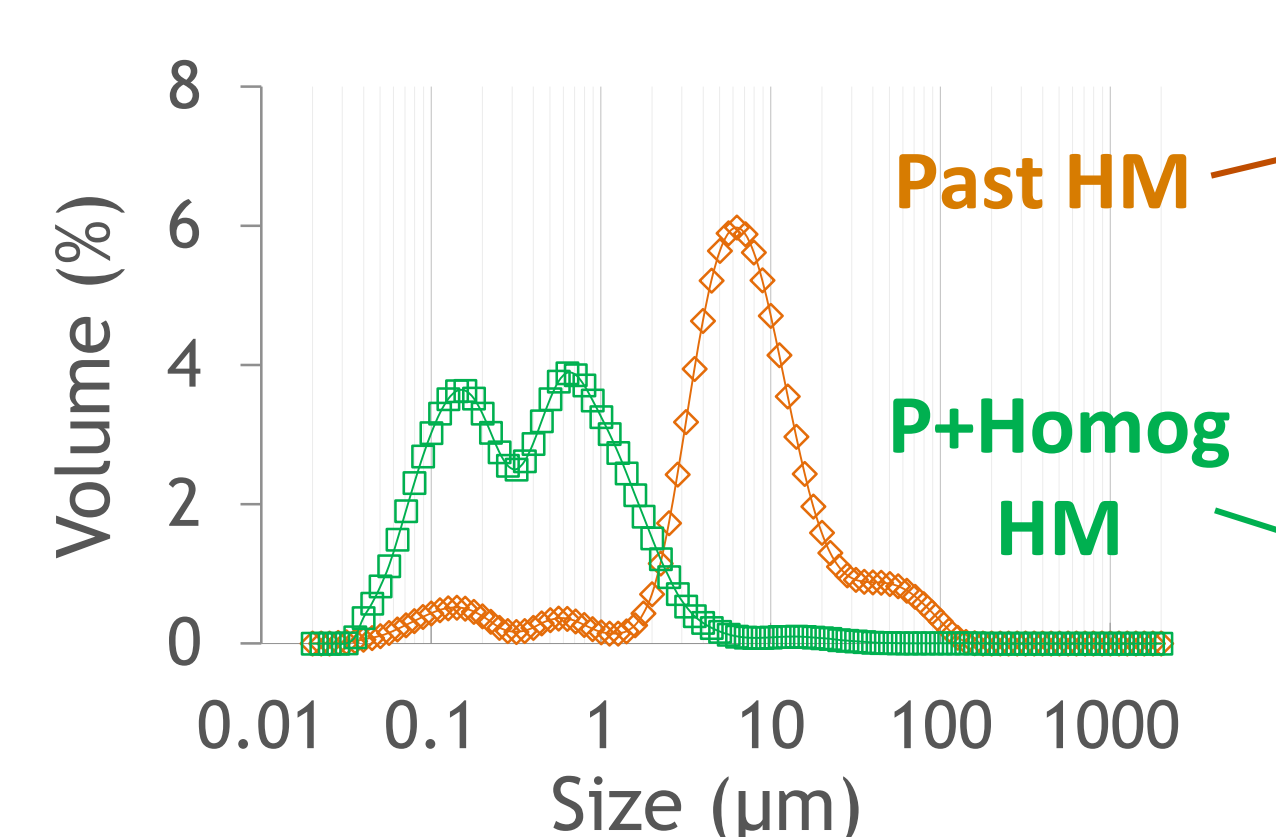
■ Apolar lipids ■ Amphiphiles ■ Proteins



GROUP B

Homogenization affected the initial structure and the emulsion disintegration of HM

Particle size distribution in HM



→ Six-fold increase in the specific surface area after homogenization: from $4.1 \pm 1.2 \text{ m}^2/\text{g}$ to $25.5 \pm 3.8 \text{ m}^2/\text{g}$ of fat

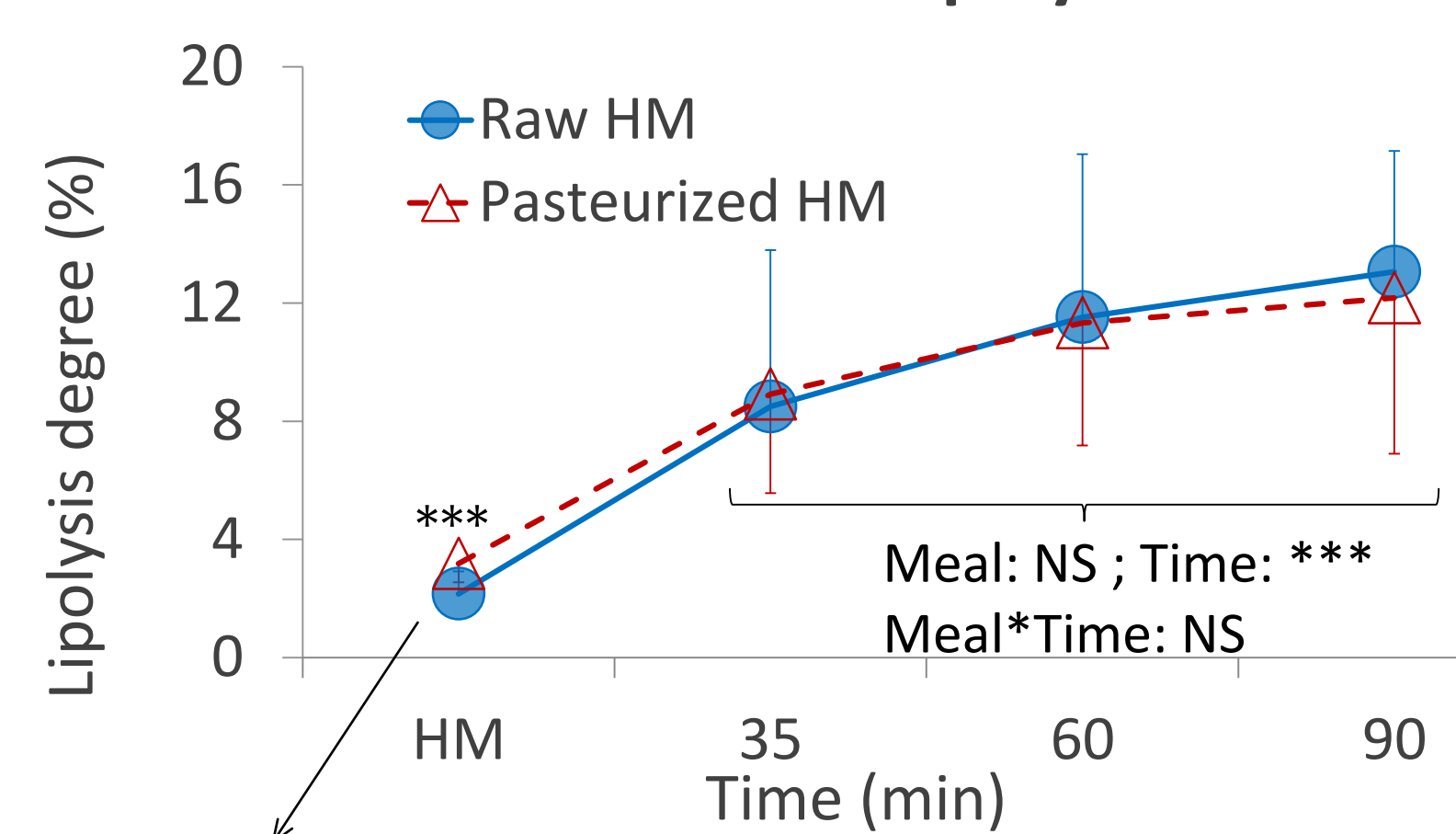
→ Past HM and P+Homog HM (n = 4 pools): same macronutrients composition, same pre-lipolysis degree ($4.4 \pm 1.0\%$) but **different structure**

→ The lipid fraction kept its initial structure all over the gastric digestion (**native globules** vs. **blend of droplets**)

→ Hormonal feedback triggered by higher lipolysis level
→ Difference of colloidal behavior

But did not impact gastric lipolysis

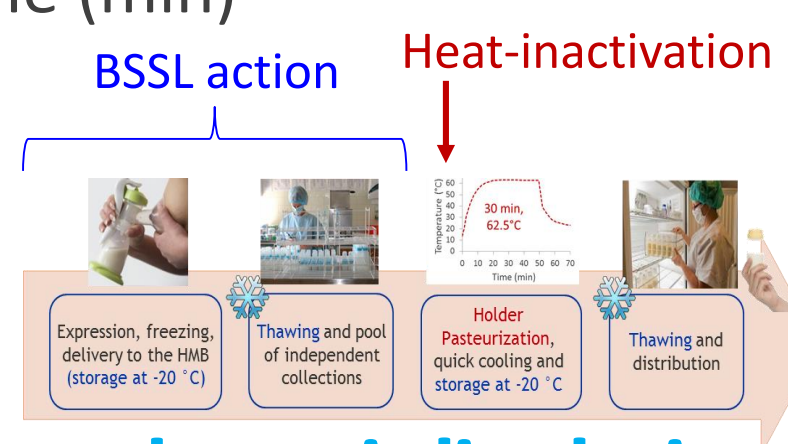
Instantaneous lipolysis level



Pre-lipolysis: n = 4 infants

Raw HM = $2.2 \pm 0.8\%$

Pasteurized HM = $3.2 \pm 0.6\%$



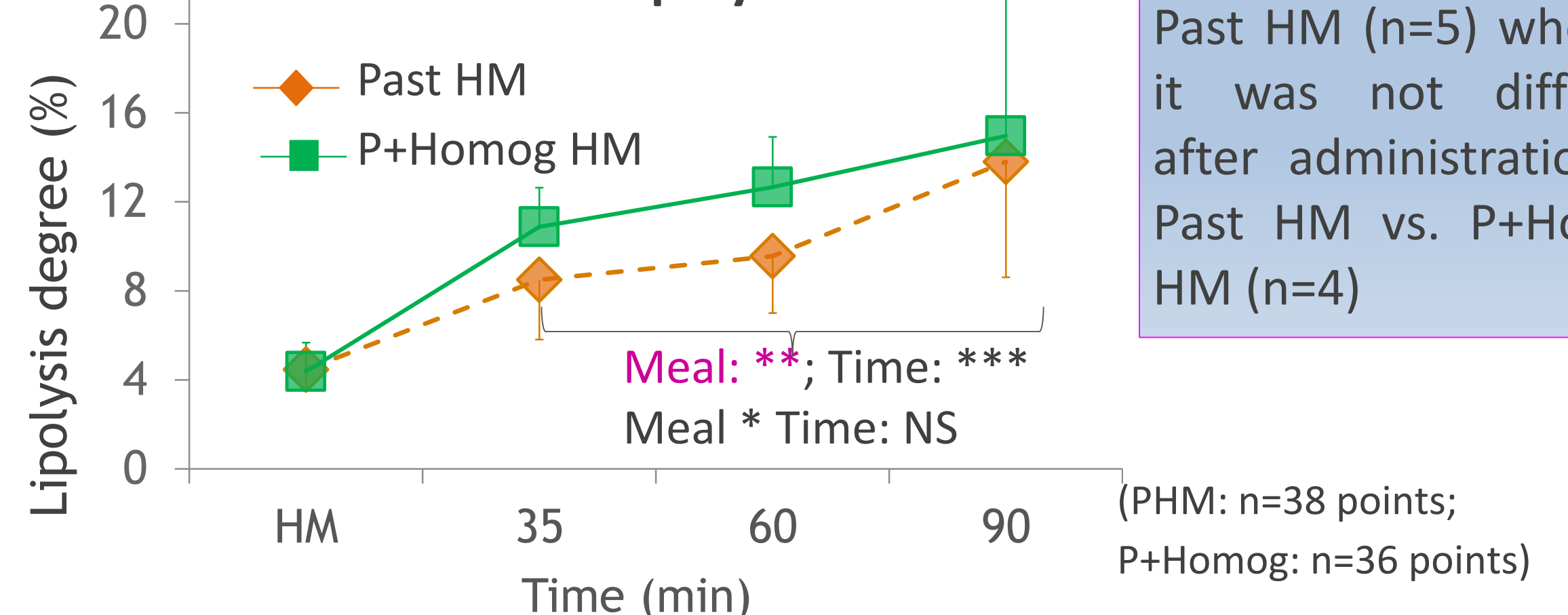
Lipolytic activity

→ Pre-lipolysis occurred prior to pasteurization in all milks and groups ranging between 2.2-4.0%. The global gastric lipolytic activity detected in fasted state averaged $17.5 \pm 2.9 \text{ U/mL/kg}$ at pH 4.5.

→ Postprandial lipolytic activity increased with time and was higher after administration of Raw HM compared to Past HM (n=5) whereas it was not different after administration of Past HM vs. P+Homog HM (n=4)

Homogenization increased gastric lipolysis

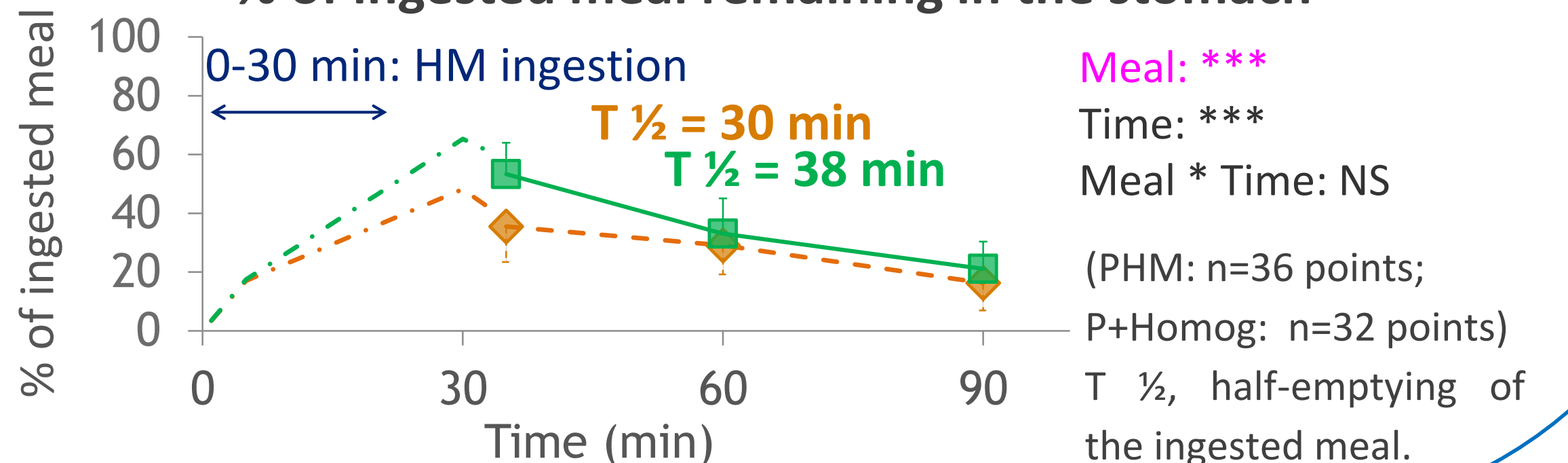
Instantaneous lipolysis level



(PHM: n=38 points; P+Homog: n=36 points)

Homogenization reduced the meal emptying rate

% of ingested meal remaining in the stomach



Meal: ***
Time: ***
Meal * Time: NS
(PHM: n=36 points; P+Homog: n=32 points)
T ½, half-emptying of the ingested meal.

Conclusion

- The initial structure of HM modulated the hydrolysis kinetics (lipolysis and proteolysis) and the emulsion disintegration during the gastric digestion in preterm infants
- Overall, *in vivo* data gathered here are crucial for a better understanding of milk neonatal digestion and help supporting the nutritional management of preterm infants.