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Impact of human milk pasteurization on digestive kinetics and intestinal absorption using a combination of *in vitro* models

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CONTEXT

Maternal milk is the optimal food for neonates (WHO).
Hospitalized preterm newborns may be fed **pasteurized donor milk** if their own mother's milk is unavailable. Holder pasteurization (62.5°C, 30 min) is conducted for sanitary reasons, but this alters immunological and bioactive components (Akinbi et al., 2010; Van Gysel et al., 2012). As a result, by denaturing the bile salt-stimulated lipase, fat absorption and growth may be reduced in preterm infants (Andersson et al., 2007; Thomas et al., 1999)

Infant

Objective

Impact of human milk pasteurization on premature infants' digestion and intestinal lipid absorption

METHODS

Infant static *in vitro* digestion model

No oral phase **Adapted for preterm infant of 2 kg**

Gastric phase (37°C)

Ratio Meal : Simulated Gastric Fluid of **63:37**

Enzymes : Pepsin (126 U/mL) and Lipase (10 U/mL) from **Rabbit Gastric Extract**

60 min of digestion at pH 5.3

Intestinal phase (37°C)

Ratio Meal : Simulated Gastric Fluid of **39:61**

Enzymes: porcine pancreatin (lipase **: 59 U/mL)**

Bovine bile (1.6 mmol/L)

60 min of digestion at pH 6.6

Pool of human milk (n=2 donors)

Raw Human Milk (RHM) | Pasteurized Human Milk (PHM)

Sample characterization

Microscopic level : laser light scattering
Molecular level :
- Thin Layer Chromatography (TLC)
- Electrophoresis (SDS-Page)

Ménard et al., 2018

In vitro intestinal absorption model

Caco-2/TC7 cell line, derived from a human colon adenocarcinoma, is a well-known lipid absorption model.

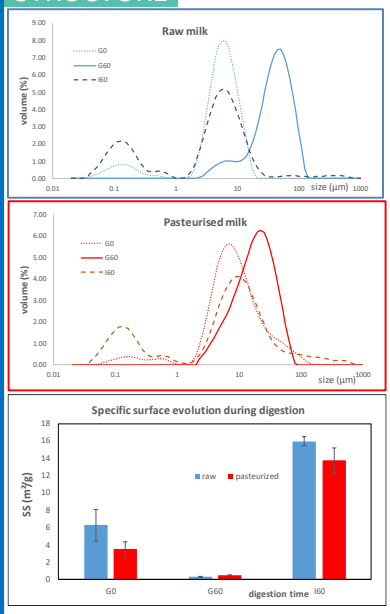
Cells were incubated in Transwell® plates during 16 hours with diluted PHM vs RHM-digestion media.

Cell monolayer integrity was assessed by transepithelial electrical resistance and occludine immunostaining.

Intracellular lipid droplets could be visualized using **Oil Red O staining**.

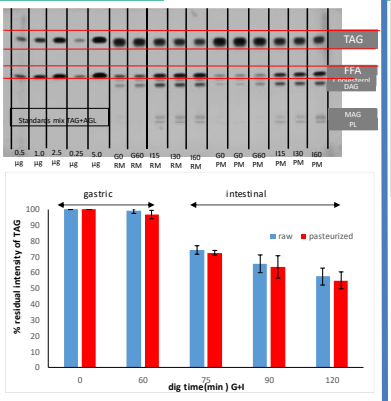
RESULTS

STRUCTURE Particle size distribution



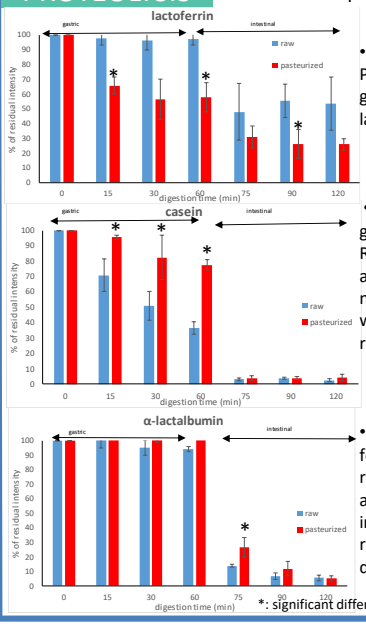
- Before digestion: larger fat globules for pasteurized (D[4;3]=14.5µm) as compared to raw (D[4;3]=6.4µm) is probably due to protein aggregation on globules during pasteurization.
- At 60min of gastric digestion: particle aggregation in both conditions but less in PHM (D[4;3]=23µm) than in RHM (D[4;3]=48µm).
- At 60min of intestinal digestion, same size of mixed compounds (D[4;3]=13.4µm) in both conditions.

LIPOLYSIS RHM vs PHM lipolysis



- No difference between RHM and PHM towards the disappearance of triacylglycerols (TAG) during gastro-intestinal digestion.
- Lipolysis occurs mainly in the intestinal phase.

PROTEOLYSIS RHM vs PHM proteolysis



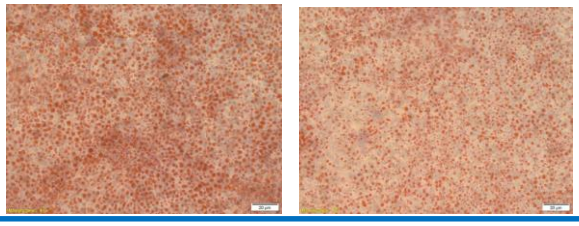
- **Lactoferrin** proteolysis is higher in PHM than in RHM at the end of gastro-intestinal digestion (residual lactoferrin of 26±4 % vs. 53±18%)
- **β-casein** is less susceptible to gastric proteolysis in PHM than in RHM (78±3 % vs. 37±4% remaining at 60 min of gastric digestion) but no difference in the intestinal phase with less than 5% of β-casein remaining.
- Same behaviour of **α-lactalbumin** for both types of milk with a resistance in the gastric phase and an extensive hydrolysis in the intestinal phase (less than 6% remaining at 60min of intestinal digestion).

*: significant difference between RHM and PHM p<0.05 (t.test)

LIPID ABSORPTION

- Incubation of digested human milk on Caco2/TC7 cells did not alter cell monolayer integrity.
- Less lipid droplets seemed to be present within cells incubated with PHM compared to RHM.

RHM **PHM**



CONCLUSION

Holder pasteurization increases milk fat globule size probably due to **protein aggregation around fat globules**. This may be responsible for the observed **different kinetics of proteolysis**, which could impact the release of some bioactive peptides. **Pasteurization did not impact triacylglycerol lipolysis** but might unfavourably modulate enterocytic lipid uptake, thus requiring further investigation.

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