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

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► To cite this version:

Olivia Ménard, Amélie Deglaire, Didier Dupont, J. Picaud, Armelle Penhoat, et al.. Impact of human milk pasteurization on digestive kinetics and intestinal lipid absorption using a combination of in vitro models. 6.International Conference on Food Digestion, Apr 2019, Grenade, Spain. , 2019. hal-02100438

HAL Id: hal-02100438

<https://hal.science/hal-02100438>

Submitted on 2 Jun 2020

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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**

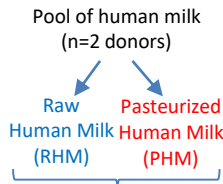
60 min of digestion at pH 5.3

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



Sample characterization

Microscopic level : laser light scattering

Molecular level :

- Thin Layer Chromatography (TLC)
- Electrophoresis (SDS-Page)

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.

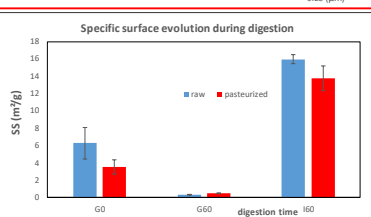
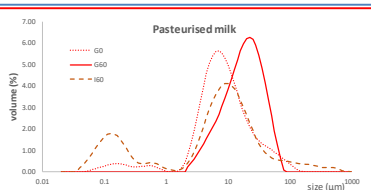
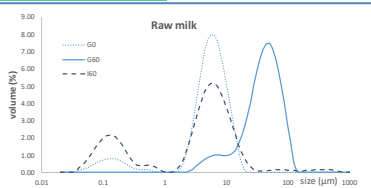
Cell monolayer integrity was assessed by transepithelial electrical resistance and occludine immunostaining.

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

Ménard et al., 2018

RESULTS

STRUCTURE Particle size distribution

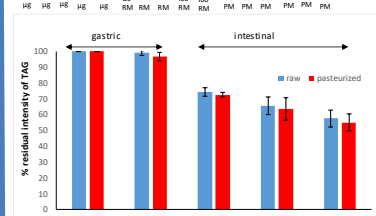
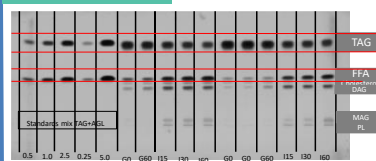


• Before digestion: larger fat globules for pasteurized (D[4;3]=14.5µm) as compared to raw (D[4;3]=6.4µm) is probably due to protein aggregation on globules during pasteurization.

• 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.

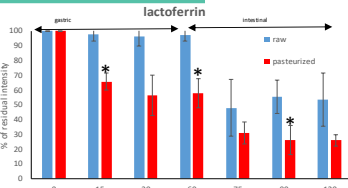
LIPOLYSIS RHM vs PHM lipolysis



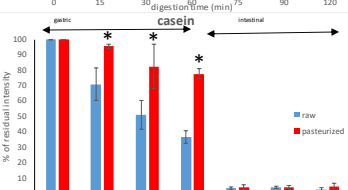
• No difference between RHM and PHM towards the disappearance of triacylglycerols (TAG) during gastro-intestinal digestion.

• Lipolysis occurs mainly in the intestinal phase.

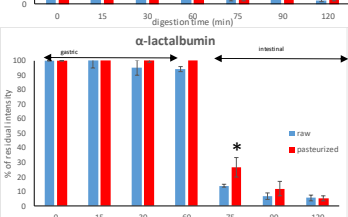
PROTEOLYSIS RHM vs PHM proteolysis



• **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.

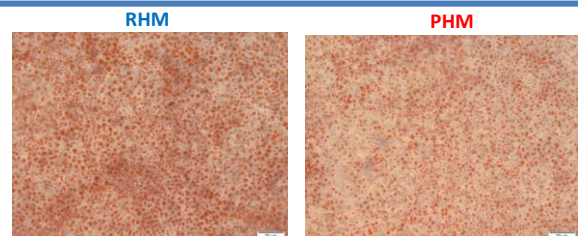


• Same behaviour of **α-lactalbumin** for both types of milk with a resistance in the gastric phase and an extensive hydrolysis in the intestinal phase (less than 6% remaining at 60min of intestinal digestion).

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.



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.