Title:

Structural and physicochemical changes in human milk during *in vitro* dynamic infant gastric digestion

Authors & affiliations:

Linda Nezbedova, Riddet Institute, Massey University, Palmerston North, New Zealand

Aiqian Ye, Riddet Institute, Massey University, Palmerston North, New Zealand
Alejandra Acevedo-Fani, Riddet Institute, Massey University, Palmerston North, New Zealand
Louise Brough, School of Food Technology and Natural Sciences, Palmerston North, New Zealand
Harjinder Singh, Riddet Institute, Massey University, Palmerston North, New Zealand
Debashree Roy, Riddet Institute, Massey University, Palmerston North, New Zealand

Abstract:

Human milk is the primary source of nutrition for infants, with gastric digestion representing the first critical step in nutrient breakdown and absorption. Despite its importance, limited information is available on the gastric digestion of human milk, particularly regarding protein and lipid behaviour. This study investigated the structural and physicochemical changes of human milk using an infant dynamic *in vitro* human gastric simulator (iHGS).

Fresh pooled human milk from exclusively breastfeeding mothers was digested in the iHGS under physiologically relevant conditions. Gastric contents were analysed for colloidal stability (static light scattering), microstructure (confocal and transmission electron microscopy), nutrient delivery (compositional analysis), and protein hydrolysis (SDS-PAGE, OPA assay).

The results showed that human milk underwent intragastric destabilisation, with visible signs of protein aggregation and a top fat layer formation after 160 min of gastric digestion i.e. pH < 3.5, along with progressive increase in particle size during digestion. Microscopic measurements confirmed casein micelle aggregation, coalescence of fat globules, and disruption of milk fat globule membrane with digestion. Gastric emptying results showed that in comparison to proteins, lipids were emptied relatively slowly to the small intestine, which is likely due to the creaming phenomenon observed. SDS-PAGE analysis indicated time-dependent hydrolysis of both caseins and whey proteins (alpha-lactalbumin, lactoferrin), although caseins were hydrolysed relatively faster. Time-dependent increase in free amino groups concentration was observed, indicating progressive hydrolysis and emptying of digested proteins from the iHGS. These results indicate that under infant gastric conditions, as a result of pH dependent pepsin and lipase-mediated hydrolysis, coalescence and creaming of milk fat globules might occur in the stomach, resulting in phased delivery of nutrients.

This study provided new insights into fresh human milk digestion, highlighting the influence of structural changes on gastric emptying, which may play a key role in regulating nutrient delivery to the small intestine during early infancy.