The Dynamics of Milk Emulsion Structure during *In Vitro* Neonatal Gastric Digestion

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Chalida Lueamsaisuk

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Summary

Efficient fat digestion is an essential part of neonatal development. In this respect, it is noteworthy that the process by which infants digest fat differs from that in adults; key differences include the immaturity of the pancreatic function and elevated gastric pH. The digestion of emulsified lipids may accordingly be rendered less efficient in ambient conditions in the infant gastric lumen. For example, it may be postulated that covariation in optimal conditions of proteolytic and lipolytic digestion may differently affect the digestion and disruption of the droplet membrane, the interfacial accessibility of lipase and the subsequent fatty acid production.

Differences between formulated emulsion structures may therefore influence the rate of digestion; previous human studies have indicated that infants digest formula feeds more slowly than they do breast milk (Splinter and Schreiner, 1999). To further explore this observation, the lipid digestion of native biological milk (human breast milk), commercial infant formulae (liquid and powder), and model emulsions (Intralipid® containing lactoferrin) were investigated in an *in vitro* gastric system. The aim was to gain a better understanding on the changes in emulsion structure and fat digestibility with various interfacial layers and pH environments under simulated gastric conditions.

The introduction and a rationale for the focus of this thesis are shown in **Chapter 1**. **Chapter 2** gives a critical overview and review of the literature pertaining to this thesis, and presents possible explanations of how the properties of milk fat globules and their membranes are related to the digestion outcome in the digestive system of

infants. The review also examines the effects of physicochemical factors on emulsion stability. Then, **Chapter 3** presents the general materials and methods used in the experimental work.

The first experimental design is described in **Chapter 4.** This chapter compares the characteristics and physicochemical properties of different types of milks. Infant formulae are prepared from cow's milk and designed to mimic human milk as much as possible. However, even with the advances of technology, there are still differences observed between the breast milk and commercial infant formulae. Therefore the microstructure, droplet size and droplet charge of these different types of milk (human milk, raw cow's milk, commercial liquid formulae and commercial powder formulae) were examined before studying the emulsion digestibility under simulated infant physiological conditions.

Chapter 5 gives a description on how digestion affects emulsion structure of a typical formula emulsion at different pH levels (2–5.5) in an *in vitro* system that replicates the shear rates that would normally be encountered in the infant stomach. The system is designed to simulate infant gastric conditions using different combinations of porcine pepsin and fungal lipase (*Rhizopus oryzae*). Thus, digestion in the presence and absence of proteolytic and lipolytic enzymes was evaluated by observing changes in microstructure, particle size and surface charge.

In liquid infant formulae, droplet size increased progressively by coalescence during *in vitro* digestion at pHs between 3.5 and 4.5 when both lipase and protease were present, but not when either enzyme was omitted or when pH levels were outside this range. Coalescence was augmented by shear, notably at rates above the normal

physiological range. The fidelity of *in vitro* systems did not appear to be compromised by the use of fungal lipases but compromised by the use of inappropriately high stirring rates. The stability and structural properties of formula emulsions appeared to be influenced by disruption of the proteinaceous oil/water interface during digestion, being most susceptible to the concerted activity of pepsin and gastric lipase over a limited range of pH. Given that the onset of secretion of pepsin, lipase and hydrochloric acid does not occur synchronously in the developing infant stomach, inappropriately formulated milks may lower digestive efficiency.

Chapter 6 progresses the findings from chapter 5, employing a model phospholipid–stabilised emulsion which was digested alone, and in combination with the milk protein lactoferrin. It was postulated that the lactoferrin would form an electrostatic layer-on-layer complex with the phospholipid allowing comparison to be made between digestion of the phospholipid–stabilised emulsion and the emulsion stabilised by lactoferrin–phospholipid complex.

Lipolysis of untreated Intralipid®, as evidenced by the increase in droplet size i.e. d_{43} and by confocal microscopy, took place at pH levels between 3.5 and 5.5. Coalescence was evident with lipase alone and with mixtures of pepsin and lipase at pH 3.5, but did not occur in the presence of pepsin alone. Conversely, no coalescence was evident on digestion of Intralipid® treated with lactoferrin, with lipase alone at pH levels below 5.5. However, coalescence of droplets in treated Intralipid® did take place at pH levels above 2 when both pepsin and lipase were present. Changes in surface potential indicated that interfacial proteolysis was required for lipase-mediated coalescence to occur. Findings indicated that the

interaction of lactoferrin with the oil/water interface of soybean oil droplets may have inhibited the action of lipase pending digestion by pepsin.

The findings of Chapter 5 and 6 demonstrate the co-dependent role of proteolytic and lipolytic enzymes on the stability of emulsions during digestion, and the contribution of pH on enzymatic function. This knowledge should be a key factor for the design of emulsion structures in infant formula emulsions.

Chapter 7 describes how digestion affects the structure of human breast milk. Fat droplets showed no significant propensity towards flocculation and aggregation during incubation both with and without either enzyme at all pH. Additionally, the breast milk emulsion was seen to be resistant to coalescence across all pH's and enzymatic conditions studied. The difference in structural behaviour is attributed to variance in lipid composition of the MFG relative to the emulsion systems studied in chapters 5 and 6. Accordingly, it is suggested that the by-products of lipolysis of the breast milk emulsion may serve to stabilise droplets rather than cause instability. Thus the MFGM of maternal milk is not considered inhibitory to the action of either of these two enzymes (porcine pepsin and fungal lipase) under *in vitro* simulation of infant gastric conditions.

Chapter 8 describes the overall conclusions and addresses the major findings and recommendations for future work.

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α-lac	Alpha-lactalbumin
β–lg	Beta-lactoglobulin
BS	Bile salt
BSDL	Bile salt-dependent lipase
BSSL	Bile salt-stimulated lipase
C	Carbon
CSLM	Confocal scanning laser microscopy
DAGs	Diacylglycerols
EDTA	Disodium ethylene diamine tetra-acetate
FA	Fatty acid
FFA	Free fatty acid
HCl	Hydrochloric acid
LC–PUFA	Long chain polyunsaturated fatty acids
MAGs	Monoacylglycerols
MCFA	Medium chain fatty acid
MCT	Medium chain triglyceride
MFG	Milk fat globule
MFGM	Milk fat globule membrane
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
p*I*	Isoelectric point

PUFA	Polyunsaturated fatty acid
RO	Reverse osmosis
SGF	Simulated gastric fluid
TAGs	Triacylglycerols
Tween 20	Polyoxyethylene sorbitan monolaurate
UHT	Ultra high temperature