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The structure of infant formulas impacts their lipolysis, proteolysis and disintegration during *in vitro* gastric digestion

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ABSTRACT

Milk lipids supply most of the calories necessary for newborn growth in maternal milk or infant formulas. The chemical composition of infant formulas has been optimized but not the structure of the emulsion. There is still a major difference between the native emulsions of milk fat globules and processed submicronic emulsions in infant formulas. This difference may modify the kinetics of digestion of emulsions in newborns and influence lipid metabolism. To check this, semi-dynamic gastric *in vitro* digestions were conducted on three matrices: a standardized milk emulsion containing native milk fat globules referred to as minimally-processed emulsion and two processed model infant formulas (homogenized or homogenized/pasteurized). Gastric conditions mimicked those reported in newborns. The minimally-processed emulsion was lipolyzed and proteolyzed slower than processed formulas. The difference in initial structure persisted during digestion. The surface of the droplets was the key parameter to control gastric lipolysis kinetics, the pattern of released fatty acids and proteolysis by faster hydrolysis of adsorbed proteins.

1. Introduction

Lipid digestion is essential for adult nutrition since this macronutrient conveys calories, vitamins, hormonal precursors and membrane structural constituents. It is all the more important for infant nutrition in which milk lipids supply 50–60% of the calories necessary for newborn growth ideally under the form of maternal milk or under the form of infant formulas. Paradoxically whereas fat intake per kilogram bodyweight is three to fivefold higher in newborns compared to adults, the main actors of lipid digestion in the adults are immature in newborns (Lindquist & Hernell, 2010). The luminal concentrations of pancreatic triacylglycerol lipase and bile salts necessary for efficient intestinal hydrolysis of triacylglycerides (TAG), for the micellarization of the products of hydrolysis and efficient fat absorption are low compared to adults (Lindquist & Hernell, 2010). On the contrary, human gastric lipase (HGL) which initiates lipolysis in the gastric compartment is mature very early in the newborn (Bourlieu et al., 2014; Sarles, Moreau, & Verger, 1992).

In adult digestion, HGL is responsible for the hydrolysis of 10–30% of TAG and promotes the action of pancreatic lipase through the release of free fatty acids (FFA) which favor the anchoring of the pancreatic lipase-colipase complex at the lipid-water interface (Bakala N'Goma, Amara, Dridi, Janin, & Carrière, 2012; Lengsfeld et al., 2004). HGL could play an even crucial part in neonatal nutrition due to its early maturation and ability to attack directly native milk fat globules (Bernbäck, Blackberg, & Hernell, 1989). The physiological part played by this extremophilic enzyme relies on specific physico-chemical properties such as: a low optimal pH range (4–5) for activity, a stability to pepsin and pH denaturation above 1.5, a high tensioactivity allowing its adsorption at interfaces with low surface tension and in the presence of bile salts (Lengsfeld et al., 2004), and a stereospecificity for the *sn*-3 position of TAG (Bakala N'Goma et al., 2012; Carrière et al., 1997). Very

Abbreviations: CLSM, confocal laser scanning microscopy; DAG, diacylglycerol; FA, fatty acid; FFA, free fatty acid; HD, hydrolysis degree; HGL, human gastric lipase; MAG, monoacylglycerol; RGL, rabbit gastric lipase; *sn*, stereospecific numbering; TAG, triacylglycerol; TLC, thin layer chromatography; SDS, sodium dodecyl sulfate.

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similar properties are shared by the other preduodenal mammalian lipases such as rabbit gastric lipase (RGL). However none of these lipases has been commercially available up to recently, which has limited their use in physiologically pertinent *in vitro* digestion systems (Capolino et al., 2011; Chatterton, Rasmussen, Heegaard, Sorensen, & Petersen, 2004; Garcia, Antona, Robert, Lopez, & Armand, 2014). This limitation has favored the use of acid- and protease-resistant fungic lipases for replacing gastric lipase, but their equivalence to gastric lipase has never been demonstrated so far.

The composition of infant formula is based on human milk composition but the structure of these two matrices differs at different levels of scale. At the molecular level, the nature and structure of TAG on one side (Jensen, Hagerty, & McMahon, 1978), and of casein micelles and whey proteins on the other side, are not similar (Chatterton et al., 2004). At supramolecular level, the emulsions structure, *i.e.* the size of the dispersed milk fat droplets and the nature of their interface, still differs remarkably (Michalski, Briard-Bion, Michel, Tasson, & Poulain, 2005). This structure results for human milk from biological secretion and for infant formulas from a succession of technological treatments. Infant formulas are based on submicronic droplets of 0.5 μm (distribution 0.1–1 μm) whereas human milk contains bigger droplets of 4 μm (distribution 0.1–10 μm) called native milk fat globules. These native milk fat globules are stabilized by a trilayered membrane inherited from their secretory pathway in the mammary gland. The membrane has a typical composition of mammalian cell membrane (phospholipids, sphingolipids, cholesterol, proteins and enzymes) (Lopez, Madec, & Jimenez-Flores, 2011). On the contrary, fat droplets in formulas are stabilized by a neoformed membrane based on caseins, whey protein aggregates and possibly remnants of milk fat globule membrane. Recent studies suggest that these differences in supramolecular structure of emulsions between human milk and infant formulas are key elements accounting for the major long-term health benefits of breastfeeding, such as protection from metabolic syndrome and obesity (Oosting et al., 2014). In parallel to these systemic effects, the luminal phase of milk lipids digestion is very likely to be affected by the difference of structure between native and processed dairy emulsions. It is well established that the rate of lipolysis is controlled by the main physicochemical characteristics of the oil/water interface: the surface area of the lipid droplets (Benzonana & Desnuelle, 1965) and their interfacial composition (Verger & de Haas, 1976). Macierzanka, Sancho, Mills, Rigby, and Mackie (2009) also demonstrated that the adsorption of caseins and β-lactoglobulin at a hydrophobic interface favors their susceptibility to pepsin proteolysis.

Lipolytic and proteolytic processes are therefore highly dependent on the structure of food emulsion, which can be considerably altered by the environment and physicochemical conditions encountered in the gastro-intestinal tract (Gallier, Ye, & Singh, 2014; Singh, Ye, & Horne, 2009). Similarly, the initial structure of infant formula resulting from technological treatments may seriously impact gastric lipolysis and proteolysis. A very recent study (Garcia et al., 2014) has compared the lipolysis extent of milks containing native or homogenized milk fat using a static *in vitro* digestion model mimicking adult gastric digestion and concluded that homogenization did not affect the gastric lipolysis extent (9% hydrolysis of TAG). However, the study did not compare directly batches of milk having the same composition nor established the kinetics of gastric lipolysis.

The purpose of the present study was to determine to what extent homogenization and heat treatment had an impact on the gastric hydrolysis and disintegration of infant formulas. The results of this study could be useful to food scientists and infant food manufacturers as it highlights the influence of the structure of the emulsion on the luminal part of neonatal gastric digestion.

In this study, three model infant formulas, based on bovine milk and representative of native, homogenized or homogenized and heat-treated emulsions were formulated. The model formulas were then subjected to *in vitro* digestion using a semi-dynamic model mimicking the infant gastric phase.

2. Materials and methods

2.1. Materials

Unless otherwise stated, chemicals were from commercial origin (Sigma–Aldrich, Saint-Quentin Fallavier, France).

2.2. Preparation of model infant formula emulsions

Raw cow's milk (20 L) was purchased from a local dairy plant (Entremont, Montauban de Bretagne) and transformed into three model infant formulas with similar chemical composition but three distinct structures. At reception milk was submitted to a minimal heat treatment to inactivate endogenous lipase (54 °C, 20 min) without denaturing milk proteins (Deeth, 2006). Milk was then skimmed (elecrem, type 125, Châtillon, France; rate: 315 L/h 45 °C) and standardized at 3.3% fat content and at 2.1% protein content by redispersion of the cream in the skim milk and dilution of the skim milk with milk ultrafiltrate (5 kDa). The casein/whey protein ratio was readjusted from its initial value of 80:20 w/w to 40:60 w/w by addition of milk whey protein concentrate (Prolacta®95).

The matrix obtained after this standardization was called M1 and can be considered as 'minimally processed' as it was composed of native milk fat globules dispersed in a standardized colloidal phase whose protein composition mirrored that of infant formulas. The preservation of the milk fat globules after skimming was checked by apparent zeta-potential assessment in the experiments (Zetasizer Nano series Nano-ZS, Malvern Instruments, Malvern, UK): the apparent zeta-potential of the raw milk dispersed in a buffer mimicking milk ionic strength (pH 7, 5 mM $CaCl_2$, 50 mM NaCl, 20 mM imidazole; Dalgleish, 1984) was −10.5 (0.5); the apparent zeta-potential of the cream redispersed in the same buffer was −10.1 (0.7) indicating that the skimming had been performed gently and preserved the milk fat globule membrane. Other experimental evidence was collected indicating that the milk fat globules in M1 were preserved: – granulometry analysis did not present a range of particles of larger size which would have been formed by aggregation during skimming, – confocal microscopy observations revealed individual fat globules surrounded by intact membrane without aggregation or coalescence, – lipolysis experiments were also conducted on heat-treated (54 °C, 20 min) milk without skimming nor standardization and gave very similar rate of free fatty acids release indicating that the minimal treatments applied to process M1 (skimming and standardization) had minimal impact on native milk fat globules. The second matrix M2 was obtained by homogenization of M1 using a two-stage high-pressure homogenizer at 220-20 bars (Rannie slow model LAB 16/15, APV France, Evreux, France). Eventually a pasteurization treatment (72 °C, 15 s) was applied to M2 to obtain the matrix M3.

Two batches of raw milk were purchased and processed to obtain the three matrices in duplicate. Their main macronutrients composition with regards to fat [%], protein [%] and lactose [%] was respectively: 4.33 ± 0.00, 3.50 ± 0.01, 4.89 ± 0.02 for the first batch of initial milk; 4.25 ± 0.00, 3.46 ± 0.01, 4.91 ± 0.02 for the second batch of initial milk; 3.27 ± 0.01, 2.08 ± 0.00, 4.72 ± 0.01 for the first batch of model emulsion and 3.26 ± 0.00, 2.20 ± 0.01, 4.65 ± 0.02 for the second batch of model emulsion.