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On the role of bile salts in the digestion of emulsified lipids

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ABSTRACT

The objective of this study was to understand quantitatively the role that bile salts play in the digestion of emulsified lipids. The behaviours of digestion by pancreatin (1.6 mg/mL) of sodium-caseinate-stabilized emulsions (0.5 wt% protein) and bile-extract-stabilized emulsions (0.2–5 mg/mL) as influenced by the addition of aqueous bile extract were studied under simulated intestinal conditions (37 °C; pH 7.5; 39 mM K_2HPO_4, 150 mM NaCl; with continuous agitation at ~ 150 rev/min for 3 h). The droplet characteristics (size and ζ-potential) of the sodium caseinate- and bile extract-stabilized droplets were evaluated by light scattering techniques. The kinetics of the total fatty acids released by hydrolysis of the emulsified lipids were monitored by the pH-stat method with or without the presence of continuous phase bile extract. The results suggested that the presence of unadsorbed bile extract markedly enhanced the rate and the extent of lipid digestion. This could be attributed to considerable removal of lipolysis products (free fatty acids, mono- and/or di-acylglycerols) in mixed micelles, which are known to inhibit lipid digestion, by the unadsorbed bile salts. This study provides new insights for the lipid digestion of food formulations.

1. Introduction

The rising levels of obesity and overweight populations are some of the most serious global public health challenges and are creating a huge healthcare cost burden. For this reason, there is a need for the development of effective microstructural strategies to delay the digestion of energy-dense lipids, suppressing appetite and thereby reducing subsequent calorie intake. Consequently, understanding the fundamental aspects of the digestion of emulsified lipids under conditions that simulate the human gastrointestinal tract is of paramount importance to gain insights into the physicochemical and biochemical processes in the physiological milieu that further bioengineer the initial food structure (Golding et al., 2011; Mackie & Macierzanka, 2010; Sarkar et al., 2015; Singh & Sarkar, 2011; Singh, Ye, & Horne, 2009). In the last few years, a significant level of understanding on the gastrointestinal structuring of emulsions after consumption, which typically includes different extents and types of droplet flocculation and coalescence, adsorption/desorption of emulsifiers and binding of metabolites, has been gained (Golding et al., 2011; Hur, Decker, & McClements, 2009; Maldonado-Valderrama et al., 2008; Sarkar, Goh, & Singh, 2009; Sarkar, Goh, & Singh, 2010; Sarkar, Goh, Singh, & Singh, 2009; Sarkar, Horne, & Singh, 2010a; Sarkar, Horne, & Singh, 2010b; Torcello-Gomez, Maldonado-Valderrama, Martin-Rodriguez, & McClements, 2011).

In healthy humans, 70–90% of lipid digestion takes place in the small intestine; it is essentially an interfacial process that involves a complex interplay between lipase/colipase and bile salts. Bile salts are a very peculiar type of biosurfactant that, unlike classical surfactants, do not have a hydrophobic head and a hydrophilic tail group. The facial amphilicity of bile salts originates from the flat steroidal structure, with the polar hydroxyl groups on the concave side and methyl groups on the convex side (Euston, Baird, Campbell, & Kuhns, 2013; Galantini et al., 2015; Maldonado-Valderrama, Muros-Cobos, Holgado-Terriza, & Cabrerizo-Vílchez, 2014). Because of their high surface activity, bile salts play a crucial role in lipid digestion by pushing initial adsorbed materials from the interface and permitting lipase/colipase complexes to act on the bile-coated oil droplets. Recent research has focused mainly on bile-salt-mediated displacement studies, in which an understanding of the orogenic mechanism of this displacement (Maldonado-Valderrama et al., 2008) and the important role of the initial charge (Sarkar, Horne, et al., 2010a) and the type of protein layer (Bellesi, Pizones Ruiz-Henestrosa, & Pilosof, 2014) in determining

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the kinetics of the sequential adsorption or displacement of the adsorbed layer by intestinal bile salts have been revealed. On the other end, the aggregation and self-assembly behaviour of bile salt solutions and their role in absorption/transport have been well established (Holm, Müllertz, & Mu, 2013; Madenci & Egelhaaf, 2010). The aggregation of bile salts in solution is due to hydrophobic interactions and hydrogen bonds between the polar hydroxyl and carboxylate groups (Madenci & Egelhaaf, 2010). Bile salts are believed to facilitate the solubilisation of lipid digestion products into lamellar phase or mixed micelles. This solubilisation results in the removal of digestion products, such as free fatty acids, mono and diacylglcerols from lipid droplets and accelerates further digestion and absorption of lipidic excipients (Small, Cabral, Cistola, Parks, & Hamilton, 1984). However, there is scant information available on the quantitative role of aqueous (unadsorbed) bile salts in the lipid digestion and subsequent fatty acid release as compared to that of the adsorbed phase.

Therefore, the objective of this study was to compare the kinetics and the degree of fatty acid release from sodium-caseinate-stabilized emulsions and porcine-bile-extract-stabilized emulsions and to unravel the role of "free" bile extract in the aqueous phase, using a simple pH-stat-based autotitration technique and theoretical consideration of apparent lipolysis rates. We have introduced the use of porcine bile salts-stabilized emulsions as a relatively new template for understanding digestion of emulsified lipids, to gain some insights into possible role of the presence of bile salts in adsorbed or continuous phase during digestion. Previous studies have generated useful insights into the displacement of protein or phosphatidyl choline-stabilized interface by pure bile salts, such as sodium cholate, sodium deoxycholate, sodium taurcholate, and sodium glycodeoxycholate (Euston et al., 2013; Wickham, Garrood, Leney, Wilson, & Fillery-Travis, 1998). To our knowledge, this is the first study where we used porcine bile extract to initially stabilize oil droplets and understand their *in vitro* lipolysis in absence or presence of continuous phase bile salts.

As lipid digestion is an interfacial process, the surface area of lipid droplets is expected to have an impact on the binding of lipase and the formation of fatty acids, with smaller droplets resulting in an increased level of fatty acid release and vice versa (Armand et al., 1992). Hence, we also compared the droplet characteristics of protein-stabilized and bile-extract-stabilized emulsions using light scattering techniques.

2. Materials and methods

2.1. Materials

Sodium caseinate (Nacas) was obtained from Fonterra Co-operative Group Ltd, Auckland, New Zealand. Porcine bile extract B8631 and porcine pancreatin (P1750, 4 × USP) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Porcine BE used in this study had a total bile salt content of 49 wt%, of which the majority of the bile acid species were glycodeoxycholic acid (10–15 wt%) followed by taurodeoxycholic acid (3–9 wt%) and deoxycholic acid (0.5–7 wt%) (Zangenberg, Müllertz, Kristensen, & Hovgaard, 2001). The key phospholipid was phosphatidyl choline (6 wt%) and the content of Ca^{2+} was less than 0.06% (w%). Based on the phospholipid/bile acid ratio, it can be suggested that the phospholipid was present as mixed micelles in conjunction with bile salt (Wickham et al., 1998). Commercial soy oil (refined, bleached and deodorized) was obtained from Davis Trading Company, Palmerston North, New Zealand. All other chemicals were of analytical grade and were obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma–Aldrich Chemical Company unless otherwise specified. Prior to experiments, solutions were freshly prepared using Milli-Q water (water purified by treatment with a Milli-Q apparatus; Millipore Corp., Bedford, MA, USA) as the solvent.

2.2. Preparation of emulsions

Aqueous solutions of Nacas (0.5 wt%) were prepared by dispersing Nacas in Milli-Q water and stirring gently for at least 2 h at 20 °C to ensure complete dissolution. Similarly, aqueous solutions of bile extract (BE) (0.1, 0.5 and 1.0 wt%) were prepared by dispersing quantities of BE in Milli-Q water and stirring for 3 h at 45 °C until all the BE had dissolved. The pH was adjusted to 7.0 using 1 M NaOH or 1 M HCl. Initially, pre-emulsions were prepared by blending 20.0 wt% soy oil with 80.0 wt% aqueous Nacas solution or BE solution using a conventional high speed mixer (Silverson L4RT, OFI Testing Equipment, Inc., Houston, TX, USA) at 6500 rev/min for 3 min. These coarse emulsions were then passed twice through a mini two-stage valve homogenizer (12.5H, Rannie, Copenhagen, Denmark) operating at 250 bar and 50 bar in the first and second stages respectively. The Nacas and BE emulsions were prepared at least in duplicate.

2.3. Droplet size determination

The mean droplet size distribution was monitored by static laser light scattering using a particle analyser (Mastersizer 2000, Malvern Instruments Ltd, Malvern, Worcestershire, UK). The relative refractive index (N) of the emulsion was taken as 1.095, i.e. the ratio of the refractive index of soy oil (1.456) to that of the aqueous phase (1.33). The absorbance value of the emulsion droplets was taken as 0.001. The sizes of emulsion droplets were reported as the surface-weighted mean diameter $d_{3,2}$ (μm) and were calculated using the equation $d_{3,2} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$, where n_i is the number of particles and d_i is the diameter of emulsion droplets.

2.4. ζ-Potential measurements

The zeta-potential (ζ-potential) of emulsions was determined by a laser Doppler velocimetry and phase analysis light scattering (M3-PALS) technique using a Malvern Zetasizer Nano ZS (ZEN 3600) instrument (Malvern Instruments Ltd). One millilitre of sample diluted to approximately 0.005 wt% droplet concentration was placed in a folded capillary cell (Model DTS 1070, Malvern Instruments Ltd). An individual ζ-potential measurement was calculated from the mean and the standard deviation of at least five readings from an individual sample.

2.5. Preparation of simulated intestinal fluid (SIF) and mixing of emulsions with SIF

The SIF contained 39 mM K_2HPO_4 and 150 mM NaCl and the pH was maintained at 7.5 (Convention, 1995). For *in vitro* intestinal digestion with SIF, freshly prepared emulsions were diluted with SIF buffer (without added pancreatin) and water at a ratio of 1:4, which resulted in a final oil concentration of 4 wt%. Post dilution, the final concentrations of BE in the BE-stabilized emulsions (0.1, 0.5 and 1.0 wt%) were 0.2, 1 and 2 mg/mL respectively. The mixture of BE emulsion or Nacas emulsion with SIF was digested by the addition of pancreatin (1.6 mg/mL) in powdered form in the presence of 0.2, 1, 2 or 5 mg/mL of BE during digestion. In some experiments involving the digestion of Nacas emulsions, the aqueous phase bile salts were removed by centrifugation at 48,000 *g* for 30 min before addition of the pancreatin. During the digestion of the emulsions, small aliquots were withdrawn periodically for analysis.