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Tailoring the digestion of structured emulsions using mixed monoglyceride–caseinate interfaces

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ABSTRACT

The destabilisation of emulsions within the stomach alters their droplet size and surface area, which in turn influences the rate and extent of fat digestion. In this study, we sought to gain further understanding of the mechanisms of the colloidal destabilisation of emulsions during digestion by examining how the composition of the interface impacts on these destabilisation processes. Understanding of emulsion destabilisation within the stomach was then linked to the extent of fat digestion through *in vitro* lipolysis measurements and *in vivo* triglyceride absorption studies. Two factors were examined; 1) co-variance of protein and monoglyceride composition at the droplet surface and 2) fat phase composition. Of the two emulsifiers present, caseinate provided the colloidal stability to the emulsion via a combination of electrostatic and steric repulsion. The acidic pH of gastric fluid resulted in a loss of electrostatic charge and a collapse of the casein steric layer, ultimately causing the emulsion to flocculate. The presence of monoglyceride influenced the emulsions susceptibility to flocculation in gastric juice and the resistance of the interface to film rupture which impacted the degree of droplet coalescence. It appeared that there was an optimum ratio between monoglyceride and protein at the interface for emulsion destabilisation. An excessive decrease in protein at the interface as monoglyceride concentration increased limited initial droplet flocculation, because there were fewer junction points for protein bridging between droplets. These changes to emulsion droplet structure had an impact on the *in vitro* rate and the extent of lipolysis. However triglyceride absorption *in vivo* was only significantly impacted when the coalesced droplet structure (e.g. emulsion containing solid fat) was maintained until the intestine. The principle cause of the altered lipolysis profile was the destabilisation of the emulsion within the stomach. These results highlight that the complexity of real food systems (i.e. multiple/mixed ingredients) can have an important impact on the digestion of emulsions, and have implications for the creation of functional foods aimed at obesity and/or diabetes.

1. Introduction

Fat is the most energy dense, but least effective of the three macronutrients at suppressing feelings of hunger and food intake (Mattes, 2007). The weak satiating effect of fat could lead to its overconsumption which in turn results in energy intake outstripping energy expenditure, a leading cause of obesity (Blundell & Macdiarmid, 1997; Lissner & Heitmann, 1995). Reducing fat content in food products is one of the common approaches being exploited by food manufacturers in an attempt to reduce total energy intake. However, such a strategy has its own issues. The poor satiety and fullness associated with the consumption of low fat products means that benefits from calorie reduction are simply negated by overeating in total. One possible cause of overconsumption is that fat plays an important role in the sensorial perception of food, in particular fat texture is associated with pleasantness of food in the mouth (Rolls, 2011). Given the considerable interest in the role that fat plays in our diet, understanding how food structure impacts on lipid digestion is critical and may lead to more effective ways of maintain a healthy diet without reducing sensorial quality of eating.

The digestion of fat is an interfacial process due to the apolar nature of lipids which are the substrate of water-soluble lipases (Armand et al., 1997; Golding & Wooster, 2010; Porter, Trevaskis, & Charman, 2007; Reis, Holmberg, Watzke, Leser, & Miller, 2009). Fat digestion is initiated in the stomach by acid-stable gastric lipase which in adults generally hydrolyses 10–15% of triglycerides (Armand, 2007; Carriere, Barrowman, Verger, & Laugier, 1993;

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Carriere et al., 2005). The fatty acids released during fat digestion are thought to aid further emulsification of ingested fat in the stomach and change the interfacial composition of the emulsion droplets (Carey, Small, & Bliss, 1983). The major part of the lipolysis of emulsified fat takes place in the duodenum by the dual action of gastric lipase and co-lipase dependant pancreatic lipase in the presence of surface active bile salts (Armand et al., 1997; Carriere et al., 1993; Fave, Coste, & Armand, 2004). Generally, in healthy adult humans, the amount of enzyme secreted by the pancreas has a high capacity for fat digestion and changes in response to medium term changes in diet (i.e. over several weeks) (Armand, 2007; Carriere et al., 2005). Therefore the rate of lipolysis is controlled, not by the amount of enzyme, but by its ability to access the interface of its emulsified substrate (Marangoni, 1994). This in turn is controlled by the physicochemical characteristics of the oil/water interface; such as interfacial structure/composition and droplet surface area (Armand, 2007; Armand et al., 1997; Carriere et al., 2005; Fave et al., 2004).

There has been a considerable interest in the role that food, emulsion and interfacial structure has on fat digestion. Different approaches have been studied, for example, inhibition of lipase activity (Carriere et al., 2001; Daher et al., 1997); controlling the ability of lipase to bind to the interface by altering the interfacial composition of the emulsions (Chu et al., 2009; Hur, Decker, & McClements, 2009; Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011; Wickham, Garrood, Leney, Wilson, & Fillery-Travis, 1998) and controlling the oil droplet interfacial area by inducing droplet coalescence (Golding et al., 2011; Seimon et al., 2009). These studies have shown that alteration of interfaces or emulsion structure can have considerable effects on the lipase activity and consequently the rate and/or the extent of fat digestion. However, the impact of different emulsifiers and interfacial structures on fat digestion *in vivo* is largely negated by orogenic displacement of the emulsions' interface by bile salts (Maldonado-Valderrama et al., 2008, 2011), or the action of enzymes on the emulsifier (i.e. pancreatic lipase-related protein 2 in the case of galactolipids (Amara et al., 2010). Furthermore, inhibiting fat digestion through active site inhibitors leads to un-acceptable side effects. It would seem then that one of the more effective, practical means to impact fat digestion is to alter the dispersion state of emulsified lipids.

Within the gut there are several biophysical factors that can alter the dispersion state of emulsified lipids, including pH, dissolved biopolymers and gastrointestinal mixing. The stomachs' acidic environment can cause emulsions, especially protein stabilised emulsions, to flocculate and mucins in mouth and stomach can also induce flocculation of emulsions via electrostatic and/or depletion interactions (Sarkar, Goh, & Singh, 2009). Where there is a weak interfacial membrane, such flocculation can lead to coalescence of the emulsion, thus significantly reducing the interfacial area available for lipolysis (Marciani, 2007). By carefully selecting surfactants and/or tailoring emulsion fat composition, we have previously demonstrated that emulsion droplet surface area could be controlled through different emulsion instabilities induced within the gut. Large changes in droplet surface area due to the emulsions undergoing extensive partial coalescence under gastric conditions, resulted in a dramatic reduction in the rate of lipolysis (*in vitro*) and *in vivo* triglyceride absorption (Golding et al., 2011; Keogh et al., 2011). The reduction in the rate of fat digestion due to emulsion coalesce also slowed down the fat absorption and lowered systemic concentrations CCK, GLP-1 and PYY hormones (Keogh et al., 2011).

In our previous studies, we used various emulsion systems consisting of a single surfactant and different lipid compositions to create different emulsion instabilities *in vivo* (Golding et al., 2011; Keogh et al., 2011). However, food is complex and emulsions or foams are rarely stabilised by one type of molecule. A mixture of proteins, surfactants and lipids, either naturally present in the food or added as ingredients, all compete for the surface (Bos & van Vliet, 2001; Gunning, Mackie, Gunning, Wilde, & Morris, 2005). Both small molecule surfactants and proteins are surface active and can co-exist at emulsion interfaces, but there are differences in the mechanisms by which surfactants and proteins stabilise emulsions (Dickinson, 2006). Competition between these molecules can result in complex interfacial structures which impact the subsequent colloidal behaviour (Clark, Wilde, & Wilson, 1991; Courthaudon, Dickinson, & Dalgleish, 1991; Davies, Dickinson, & Bee, 2000; Euston, Singh, Munro, & Dalgleish, 1995). Addition of surfactants to protein stabilised emulsions can result in displacement of protein from the interface (Courthaudon, Dickinson, & Christie, 1991; Dickinson, 2006; Euston et al., 1995; Mackie & Wilde, 2005; Mackie, Gunning, Wilde, & Morris, 2000). This is due to the competition between emulsifiers and proteins which leads to reduction in surface thin film stability and can result in destabilisation and coalescence of the emulsion (Dickinson, 2006; Kragel, Wustneck, Husband, Wilde, Makievski, Grigoriev, et al., 1999). However, in some food applications, controlled "partial" coalescence is preferred in order to develop an internal structure of agglomerated fat that imparts favourable texture and physical appearance of the product (Goff, 1997). This can be achieved through a partially crystalline emulsion (e.g. implying refrigerated temperatures for milk fat) and/or by addition of small molecule surfactants (e.g., monoglycerides, diglycerides, and polysorbates) to protein-stabilised emulsions to promote partial coalescence by displacing proteins from the surface.

Following on from our previous studies on the digestion of gastric structured emulsions, this study sought to establish if one can structure emulsions in the more complex systems commonly found in food i.e. mixed interfaces. A particular focus was whether the competitive adsorption of proteins and surfactants at emulsion interfaces can be exploited to control emulsion 'partial' coalescence under gastric conditions. It was thought that such destabilisation will enable one to control the surface area available for fat digestion further down the gastrointestinal tract. Different ratios of protein to surfactants were used to determine how the emulsion instability under (simulated) digestive conditions might be maximised and thereby dictate lipolytic efficiency.

2. Experimental

2.1. Materials

Sodium caseinate (90.1% protein, Fonterra, New Zealand) and monoglyceride (DIMODAN, R-T PEL/B Danisco) were used as the surfactants for the emulsion. Crisco Canola Oil (20LTR, Goodman Fielder Limited), or in combination with hydrogenated vegetable oil (Sett® S 69, 25 kg, Cognis Australia) was used as the oil phase. Phospholipid stabilised emulsion, the Ivelip® 20%, was purchased from Baxter Healthcare Australia. Small amounts of other ingredients such as flavour (Coffee or Banana International Flavours & Fragrances Inc., Australia), sweeteners (Splenda® tablet, Johnson & Johnson Pacific and Splenda® Sucralose granular, DDF-1, TATE & LYLE) and colourants (Queen Fine Foods Pty Ltd, Queensland, Australia) were added to the emulsions to enhance their palatability for the clinical study.

Pepsin (Porcine, 800–2500 U/mg, Sigma–Aldrich P7000, Actual activity: 882 U/mg), pancreatin (Porcine, USP × 8, Sigma–Aldrich P7545) and bile extract porcine (total bile salt content = 49 wt%; with 10–15% glycodeoxycholic acid, 3–9% taurodeoxycholic acid, 0.5–7% deoxycholic acid; phospholipids 5 wt%) were used as obtained from Sigma–Aldrich (B8631). Fungal lipase (80 U/mg, Fungal