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Emulsion encapsulation in calcium-alginate beads delays lipolysis during dynamic *in vitro* digestion

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ABSTRACT

We assessed the behaviour of encapsulated oil-in-water emulsions in alginate beads under dynamic digestion conditions to test their suitability for ileal brake activation. A dynamic gastrointestinal digestion system (DIDGI) was used with three consecutive compartments simulating the stomach, duodenum and distal small intestine. Digestive media were collected periodically to follow lipolysis, as well as the solubilisation of absorbable lipid species in mixed micelles. Free emulsions (*i.e.*, non-encapsulated) in absence or presence of empty alginate beads were used as controls.

In the free emulsions lipolysis occurred rapidly with micellar solubilisation not rate limiting, which indicates proximal digestion and absorption. Encapsulation of emulsions in calcium-alginate beads delayed lipolysis typically for 3 h by diffusion limitation, after which most probably mechanical disintegration took place. Our findings do not only increase the understanding of the dynamics of lipid digestion, but also directly link to the design of encapsulates (size and mesh size) for long-term weight management strategies.

1. Introduction

Controlling lipid digestion is of growing interest for many reasons. On the one hand, enhancing the gastrointestinal (GI) delivery and bioavailability of health-promoting lipids (*e.g.*, long chain polyunsaturated fatty acids) is desired; on the other hand, delaying lipid digestion can be of interest, to allow undigested lipids to reach distal parts of the GI tract where they activate an intestinal brake mechanism. The ileal brake is a negative feedback mechanism that originates from the ileum, and that targets the proximal GI tract including stomach, gallbladder and pancreas, and also the central nervous system (Alleleyn, van Avesaat, Troost, & Masclee, 2016; Cummings & Overduin, 2007; Maljaars, Peters, Mela, & Masclee, 2008; Van Citters & Lin, 2006). These feedback processes impair food digestion, appetite sensations and food intake, and are able to increase feelings of satiety and satiation (Maljaars et al., 2008; van Avesaat, Troost, Ripken, Hendriks, & Masclee, 2015). To the best of our knowledge, the activation of the ileal brake has only been achieved after direct infusion of nutrients in targeted parts of the human intestine *via* a catheter. In this paper, we focus on a food-based approach that controls lipolysis of encapsulated lipid, which could then activate the ileal brake, and thus be part of a non-invasive treatment for long-term weight management.

Lipolysis is a reaction controlled by the oil–water interface accessibility of lipase, which in turn is controlled by physicochemical characteristics of the surface of oil droplets, such as interfacial area, composition and structure (Armand, 2007). Interfacial area is determined by the emulsion droplet size, and composition is related to the nature of the components present at the interface, which determines the stability in the GI tract (Golding et al., 2011; Lundin, Golding, & Wooster, 2008). It has been postulated that the extent of lipolysis depends on the structure that these components form at the interface, *via* its resistance against adsorption of bile salts and lipase (Corstens, Berton-Carabin, de Vries, et al., 2017). Different designs of emulsion interfaces have been proposed to control and delay lipolysis: synthetic surfactants that provide steric hindrance (Chu et al., 2009; Wulff-Pérez, de Vicente, Martín-Rodríguez, & Gálvez-Ruiz, 2012), thick interfacial films through layer-by-layer adsorption of biopolymers (Corstens, Berton-Carabin, Kester, et al., 2017; Hu, Li, Decker, Xiao, & McClements, 2011; Klinkesorn & McClements, 2010; Mun, Decker, Park, Weiss, & McClements, 2006; Zeeb, Lopez-Pena, Weiss, & McClements, 2015), and particle-based layers (Sarkar et al., 2016; Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013). General trends indicate that these approaches seem insufficient to induce the ileal brake as most of the designed interfaces appeared unstable under gastric conditions and consequently lipid droplets

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emptied in the duodenum rather unprotected and then accessible to lipase.

An alternative approach is to control the diffusion of lipase toward its substrate, by trapping lipid droplets in a hydrogel matrix. The latter can be either digestible, such as proteins (Dekkers, Kolodziejczyk, Acquistapace, Engmann, & Wooster, 2016; Sarkar et al., 2015), or indigestible, such as alginate (Corstens, Berton-Carabin, Elichiry-Ortiz, et al., 2017; Li, Hu, Du, Xiao, & McClements, 2011; Zhang et al., 2016). Proteolysis of protein-based hydrogels leads to surface erosion (Sarkar et al., 2015), whereas indigestible hydrogels remain intact and hence can limit lipase diffusion, and in that way control lipolysis. This process can be fine-tuned by variation in gel bead size and pore size, as recently shown for a static *in vitro* model using oil-in-water (O/W) emulsions ($d_{32} \sim 25\,\mu m$) encapsulated in calcium-alginate hydrogel beads (bead diameter 0.5–1.7 mm; pore size 5–10 nm) (Corstens, Berton-Carabin, Elichiry-Ortiz, et al., 2017). These encouraging results were obtained under static *in vitro* conditions, which do not include the dynamics of lipid digestion; therefore, we further test the behaviour of these beads under dynamic *in vitro* conditions that are closer to *in vivo* physiological conditions.

Several *in vitro* equipment are available to take into account part of the dynamic aspect of human digestion (Dupont et al., 2018; Guerra et al., 2012; Oosterveld, Minekus, Bomhof, Zoet, & van Aken, 2016; Verhoeckx et al., 2015). The most used dynamic *in vitro* model of the upper GI tract is the TNO gastrointestinal model 1 (TIM-1), which contains four compartments: stomach, duodenum, jejunum, and ileum. It is able to control the GI transit, regulate pH, secretion, and absorption from the small intestine (Minekus, Marteau, Havenaar, & Huis in 't Veld, 1995). The French National Institute for Agricultural Research (INRA) has developed an alternative model of the upper GI tract that is simpler, cheaper and easier: the dynamic gastrointestinal dynamic digestion system (DIDGI) (Ménard et al., 2014; Verhoeckx et al., 2015). The DIDGI system has been validated with *in vivo* data for protein digestion using only two compartments: stomach and small intestine that are computer-controlled in regard to transit times, pH kinetics, and secretions (Ménard et al., 2014). For the current study, a third compartment was added to mimic the second part of the small intestine (jejunum + ileum).

Our objective was to assess the dynamic gastrointestinal digestive behaviour of free emulsions and emulsion-alginate beads to test their potentiality to induce the ileal brake. For encapsulation, a calcium-alginate hydrogel matrix was used as described previously (Corstens, Berton-Carabin, Elichiry-Ortiz, et al., 2017). Both free and encapsulated emulsions were mixed with a fat-free yogurt that mimics a meal and then introduced in a dynamic three-compartment model of the upper GI tract (DIDGI). The potential effect of the encapsulation material itself on lipolysis was tested with a control meal made of fat-free yoghurt mixed with free emulsion and empty calcium-alginate beads. Aliquots of digestive media were sampled over time and analysed for both the total extent of lipolysis and the bioaccessible fraction (*i.e.*, solubilized in mixed micelles), which allowed us to assess the potential of our encapsulate to delay lipolysis.

2. Materials and methods

2.1. Materials

Safflower oil was purchased from De Wit Specialty oils (19200 Safflower Oil High Linoleic Refined, the Netherlands), and consists mostly of linoleic acid, as shown in supplementary Table S.1. Fat-free yoghurt (composition per 100 g, as given by the manufacturer: 0 g lipids, 4.0 g carbohydrates, 4.7 g proteins, 135 mg calcium) was purchased at a local supermarket, and produced by Campina (the Netherlands; pH around 4.4). Whey protein isolate (WPI) was obtained from Davisco Foods International (BiPro, Eden Prairie, Minnesota, USA; purity 97.5%). From Sigma Aldrich (St. Louis, MO, USA, and Saint-Quentin Fallavier, France) we purchased sodium alginate, sodium bicarbonate, sodium chloride, calcium chloride, sodium phosphate dibasic, sodium phosphate monobasic, hydrochloric acid, potassium chloride, heptadecanoic acid (GC standard), porcine gastric mucosa (3200–4500 U mg^{-1} protein), Amano Lipase A from *Aspergillus niger* (120 U mg^{-1} at pH 6.5, 45 °C), pancreatin from porcine pancreas (8x USP specification; including trypsin, amylase, lipase, ribonuclease, protease), lipase from porcine pancreas (Type II, 100–500 U mg^{-1} protein on olive oil), porcine bile extract (crude extract, purity estimated to be 30–60% containing glycine and taurine conjugates of hyodeoxycholic acid and other bile salts according to the supplier). From Carlo Erba Reagents (Val de Reuil, France) we purchased ammonia solution (30%), cyclohexane, and propan-2-ol; and from Biosolve (Valkenswaard, the Netherlands) methanol absolute (HPLC supra-gradient), *n*-hexane (HPLC), chloroform (ethanol-stabilized, HPLC). All materials were used directly without further purification. Millipore ultrapure water (18.2 MΩ) was used throughout the study.

2.2. Methods

2.2.1. Meal preparation

Emulsion preparation. Safflower oil was mixed with WPI solution (1 wt% in 10 mM phosphate buffer pH 7.0) in a ratio of 1:4 (w/w), using a rotor–stator homogenizer (Silent Crusher, Heidolph basic Ultra-Turrax homogenizer, Germany) for 5 min at $13 \cdot 10^3$ rpm, 2 min rest, and 2 min at $15 \cdot 10^3$ rpm to reach an average droplet size of ~25 µm. Emulsions were used within 2 h either to prepare a meal or to encapsulate in calcium-alginate beads.

Bead preparation. The alginate solution (10 mM phosphate buffer pH 7.0) was hydrated overnight at 4 °C, and then mixed with the emulsion (magnetic stirrer). The final composition of the mixture was 10 wt% lipid and 3 wt% alginate in the continuous phase. This mixture was injected in a 0.45 M $CaCl_2$ bath to form the gel beads (average size of 0.64 mm) as described previously (Corstens, Berton-Carabin, Elichiry-Ortiz, et al., 2017). The formed emulsion-alginate beads were stored overnight at 4 °C to allow hardening, and were washed and filtered before further use. Empty beads were produced with 1% alginate solution using the same method.

Meal preparation. The maximum meal volume that can be introduced into the DIDGI in a single dose through a syringe pump was 120 mL. Fat-free yogurt (37.5 g) was enriched with 2.5 g emulsified safflower oil (2.1% lipid in the meals) either encapsulated as emulsion-alginate beads (total 25 g beads, 10 wt% lipid), or as a free emulsion (12.5 g emulsion, 20 wt% lipid) with or without empty calcium-alginate beads (22.5 g beads, 0 wt% lipid). Water was added to obtain a similar total volume for all meals. The composition of the three tested meals is summarized in Table 1. The meals were stirred for homogeneity (1 h, 300 rpm, 4 °C) before introduction into the dynamic *in vitro* digestion system. The caloric content of the meals ranged from 37 to 40 kcal: fat-free yoghurt with free emulsion 37 kcal (free emulsion meal), with free emulsion and empty beads 38 kcal (free emulsion meal with empty beads), and with emulsion-alginate beads 40 kcal (encapsulated

Table 1
Composition of the meals.

(gram)	"Free emulsion"	"Free emulsion with empty beads"	"Encapsulated emulsion"
Fat-free yoghurt	37.5	37.5	37.5
Emulsion-alginate beads	–	–	25
Empty alginate beads	–	22.5	–
Emulsion (20% oil)	12.5	12.5	–
Water	80	57.5	67.5

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