10 The Colloidal State and its Relationship to Lipid Digestion

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10.1 INTRODUCTION

The consumption of fats and oils (within reasonable limits) forms an essential part of the human diet, acting as a source of both non-essential and essential fatty acids and as a carrier for oil-soluble micronutrients. Dietary lipids are also contributory to the hedonic properties of foods, serving not only to impart a plethora of textural sensations, such as creaminess, oiliness and melting, but also as a delivery vehicle for lipophilic flavour compounds (Bellamy *et al*., 2009). There is also increasing evidence that perception of fat is not merely textural, with fatty acid components of fats and oils shown to elicit a specific taste response, thereby providing an additional sensory mechanism by which individuals can perceive fat in foods (Stewart *et al*., 2010).

Maintaining a proportionate intake of fat is nutritionally necessary for health and wellbeing; however, overconsumption of dietary lipids can lead to excessive daily energy intake, which is seen as a contributing factor to the obesity epidemic. Additionally, high intake of saturated fats can be related to other health issues, such as increased propensity to coronary heart disease.

Part of the issue is that humans are highly proficient at digesting and assimilating dietary lipids into the body: in excess of 95% efficiency for healthy adults (Lentle *et al*., 2011). This high efficiency is particularly noteworthy, given that apolar triglycerides, the primary lipid components in food oils and fats, are essentially immiscible with the aqueous conditions encountered within the gastrointestinal (GI) environment.

A simplified view of the fat digestion process is the biochemical hydrolysis of dietary triglycerides into free fatty acids and monoglycerides. Whilst these derivatives are more polar than the ingested trig-

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lycerides, they are still of relatively poor solubility in water, and must therefore be assembled into micellar moieties sufficiently small in size for diffusion and transport across the epithelium and uptake into the blood stream (Bauer *et al*., 2005).

Digestive triglyceride hydrolysis is achieved through the adsorption of endogenous lipase enzymes at the oil–water interface (Carey *et al*., 1983; Mu and Hoy, 2004; Bauer *et al*., 2005; Armand, 2007). In principle, the efficacy of lipolysis should therefore be related to the surface area of the oil–water interface, as this will dictate the relative availability of binding sites for the lipase enzyme. Accordingly, a scenario in which ingested lipids remain in a phase-separated state during digestion can be considered inefficient as surface area is minimised. Dispersion of the ingested oil phase as emulsified droplets will of course increase surface area, therefore it is not surprising that the development and maintenance of a colloidal lipid state during GI transit is necessary for fat digestion to be an effective biological process.

An intriguing extension of this digestive mechanism is that there is a high degree of diversity in the material state and structure of lipids present in foods. Variation in the relative surface area of fat distributed in food matrices can span several orders of magnitude; ranging from low surface area non-emulsified fat and oils, such as would be present in fried foods, through to high surface area fine oil-in-water emulsion systems, such as homogenised milks (see Fig. 10.1).

Furthermore, there is considerable variation in the structural arrangement and physical stability of lipids in food systems. For foods containing emulsified fats and oils, lipid microstructure can be influenced by factors such as the interfacial composition of droplets, interactions between droplet and continuous phase, as well the material properties of both the continuous and dispersed phases.

Given this structural diversity, there arises a question of whether the lipid digestion process is influenced by the material state of fats and oils prior to consumption, leading to possible variations in rate and extent of hydrolysis in both stomach and small intestine, and the subsequent absorption of fatty acids across the epithelium (McClements *et al*., 2009a; Singh *et al*., 2009; Golding and Wooster, 2010). The high efficiency of human lipid digestion does imply that the body is consistently able to effectively process fats and oils, irrespective of their structural state on consumption; and, given the human biological imperative to derive nutrition from a broad range of foodstuffs, this is not necessarily surprising. However, there is an additional consideration as to whether the structural properties of dietary lipids can be engineered in order to achieve a specific digestive pathway or outcome. In this way, there is the potential for using the structural design of fats and oils to control satiety and/or lipid uptake as a means of regulating

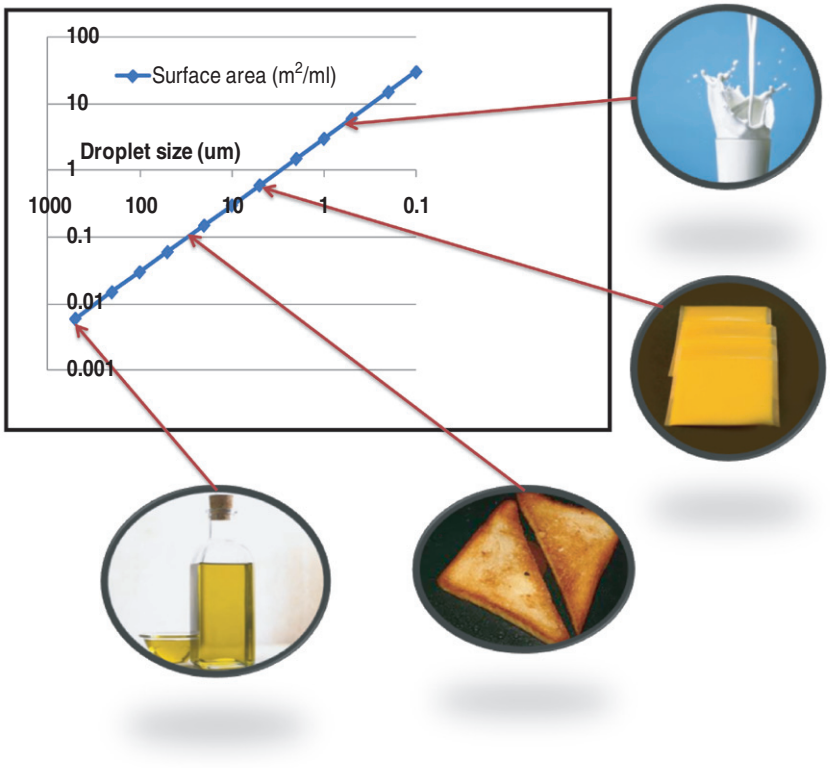

Fig. 10.1 Surface area/particle size plot showing the wide variability in fat surface area for typical fat-containing foods.

energy intake, or alternatively for manipulating the bioavailability of lipophilic micronutrients (Norton *et al.*, 2007; Lundin *et al.*, 2008; Lundin and Golding, 2009).

This chapter will review the lipid digestion process, examining how transit through the GI tract contributes to the development of the colloidal state of fats and oils during digestion, and how lipid microstructure design may, and in turn can, influence the physiological behaviours associate with fat digestion.

10.2 DEVELOPMENT AND DELIVERY OF EMULSION STRUCTURES THROUGH ORAL PROCESSING

The sensory perception of lipids during consumption of foods containing fats and oils is predominantly textural (Bellamy *et al.*, 2009). Lipid structuring during oral processing elicits a broad range of textural

attributes that are specifically associated with the detection of fat, including creaminess, mouthcoating, melting, oiliness and greasiness. As such, the development of such structures provides a significant contribution to our enjoyment of these foods. Oral processing of fats and oils is also an important first step in the lipid digestion processing, ensuring that all ingested fat- containing foods are delivered into the stomach in an emulsified state, thereby providing an interface for adsorption of lipases.

10.2.1 Structures of emulsified foods in the mouth

The oral environment provides a number of factors that contribute to the development of emulsion structures in the mouth: mechanical forces; secretion of mucous for lubrication and bolus assembly; production of amylase and lingual lipase digestive enzymes and a temperature of ~37 °C which contributes to the thermal normalisation of foods prior to swallowing (van Aken *et al*., 2007). The relative contribution of each of these factors is dependent on the initial microstructural state and stability of the lipid component prior to consumption (LeReverend *et al*., 2010). For finely dispersed, well-stabilised oil-in-water emulsions, such as those present in homogenised milks, yogurts, cream liqueurs and mayonnaises, the shear forces in the mouth can be considered insufficient to have a direct impact on particle size distribution as a consequence of breakup. However, the mucosal environment, and to a degree the biochemical environment, can promote interaction between droplets, affecting stability. At the pH conditions in the mouth, negatively charged salivary mucous (a mixture of glycoproteins and mucopolysaccharides) is able to form electrostatic complexes with molecules possessing a net positive charge (Silletti *et al*., 2006, 2007). By extension, emulsions stabilised with cationic emulsifiers become irreversibly flocculated. This has been demonstrated for model emulsion systems in which droplets were stabilised with lysozyme, which possess an isoelectric point (pI) of approximately 10.5 (Vingerhoeds *et al*., 2009). Particle size measurements and microscopy showed extensive droplet aggregation when the emulsion was introduced to saliva (see Fig. 10.2). However, it should be noted that electrostatic association of emulsions with saliva is rarely encountered for food systems at neutral pH, due to the fact that there are few biopolymeric food-grade emulsifiers that are positively charged, and cationic surfactants are invariably toxic.

Flocculation is also observed when emulsions stabilised by non-ionic or (weakly) anionic emulsifiers (which are more commonly used for the stabilisation of food emulsions) are exposed to saliva (Vingerhoeds *et al*., 2005, 2009; Silletti *et al*., 2007). For model emulsion

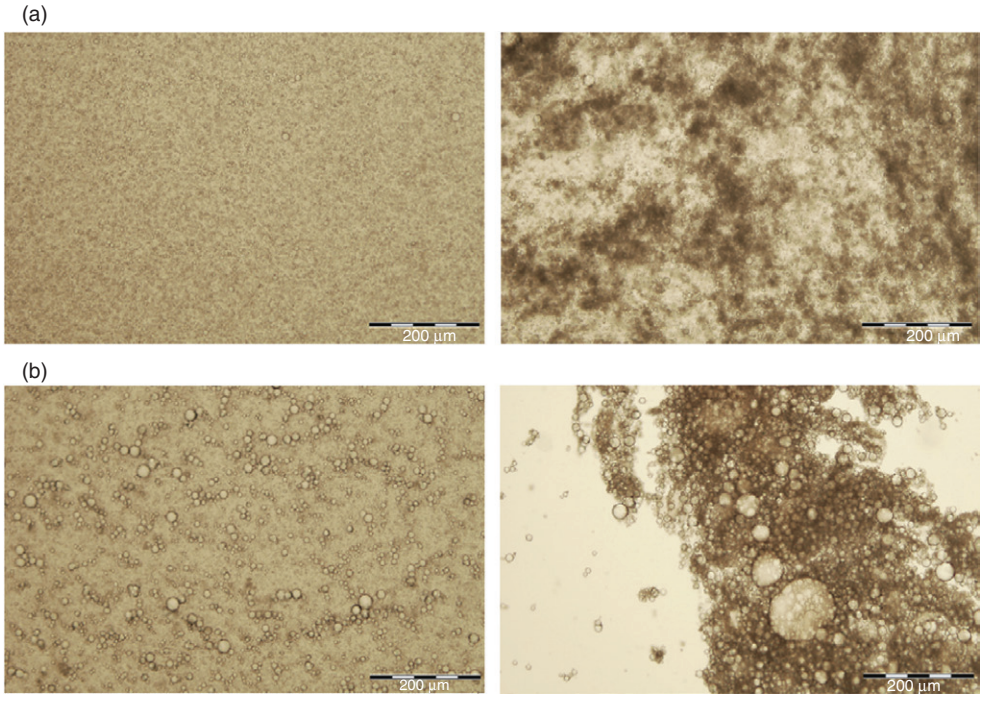

Fig. 10.2 Microscopic images of 10% oil-in-water emulsions stabilised with (a) whey protein isolate or (b) lysozyme before (left image) and after (right image) oral processing. Scale bar represents 200µm. Reproduced with permission from Vingerhoeds *et al.*, (2009). Relating the effect of saliva-induced emulsion flocculation on rheological properties and retention on the tongue surface with sensory perception. *Food Hydrocolloids*, 23(3), 773–785. Elsevier.

systems stabilised by whey protein isolate or β-lactoglobulin, reversible droplet aggregation and network formation was observed (see Fig. 10.2). For these systems, flocculation was attributed to depletion by mucopolysaccharides. It is questionable as to whether depletion flocculation actually takes place in the mouth during the consumption of emulsified foods or beverages, due to a combination of in-mouth shear and short residence time. However, on swallowing, immersion of emulsions in gastric mucosa coupled with ingested oral mucosa could arguably cause emulsions to flocculate in the stomach.

The thermal conditions in the mouth can have an influence on the physical state of fats such as palm, milkfat and coconut during oral processing. Whilst solid at ambient temperatures, these fats can undergo melting on exposure to the oral temperature of ~37 °C. In fact, most food fats and oils are entirely molten at body temperatures. For stable, homogeneously dispersed oil-in-water emulsions, the transition from solid to liquid may have little impact on droplet stability and structure during oral processing. However, for some structured oil-in-water emulsions, and oil continuous emulsions, the change in

material state of the lipid component can have a more profound effect on emulsion properties in the mouth. This is especially true of emulsion-based foods such as ice cream, whipped cream or cheese, in which the solid fat droplets are in a partially coalesced or agglomerated state. Prior to consumption, such structures provide an important role in the stabilisation and material properties of the food system (for example, bubble stabilisation and mechanical reinforcement of foam structures in the case of whipped and ice cream). However, on consumption, these structures undergo melting at in-mouth temperatures, resulting in a transition from partially coalesced agglomerates to fully coalesced droplets. This can have a pronounced effect on the sensory perception of fat in the mouth (Dresselhuis *et al*., 2008), but a second consequence of this effect is that large fat droplets with low surface area are delivered to the stomach, rather than fine emulsion droplets of high surface area.

10.2.2 Colloidal structure development of non-emulsified oils in the mouth

Consumption of free oil (in the absence of other macronutrients) is not particularly palatable, and consequently does not tend to contribute directly to dietary intake of fat. However, it is intriguing to note that emulsification of pure oils still takes place as a consequence of oral processing. This was shown by Adams and co-workers (Adams *et al*., 2007), who used endoscopy to show that rinsing of free oils in the mouth was sufficient for homogenisation of the fat phase into a course emulsion structure with droplets of 10–40 μm. In the absence of any surfactant materials present with the oil component, it can be argued that the glycoproteins present in the mucous are able to provide surface stabilisation of these crude droplets, particularly given that saliva has been shown to both lower surface tension and provide surface elasticity (Glantz, 1997).

Whilst this is a somewhat artificial scenario, it is important to recognise that many consumed fats and oils are in an initially unemulsified state, or are present as oil-continuous emulsions, and it is interesting to consider in what state these are delivered into the stomach. For solid foods such as fried foods, cakes, cheese, chocolate, etc., oral processing can involve melting (if the fat is crystalline) and liberation of oil from the food structure during mastication (Repoux *et al*., 2012). It is known that the release of free oil during eating can provide additional lubrication, reducing chewing time and contributing to bolus assembly. However, there is remarkably little literature investigating the actual structural changes that occur to unemulsified fats and oils, not only during incorporation into the bolus, but also on release from the bolus

during digestion in the stomach. In spite of this, it seems reasonable to hypothesise that the combination of in-mouth mechanical forces and the material changes generated during chewing are able to provide both homogenisation and physical separation of droplets as they are incorporated into the bolus. Droplet stabilisation may be further supported if surface-active materials are available during chewing, which would ensure delivery of lipids into the stomach in at least a crudely emulsified state.

A final consideration of how oral processing may affect the stability and structure of consumed lipids prior to digestion is the role of oral enzymes: lingual amylase and lingual lipase. Lingual amylase is an endogenous enzyme responsible for starch hydrolysis. For oil-in-water food emulsions that are formulated with (gelatinised) starch in the continuous phase (for example, custards), starch hydrolysis will lead to changes to continuous-phase rheology (not only in the mouth, but also in the early stages of gastric digestion), which in turn may affect droplet mobility and interactions. A more unique aspect of amylase effects on emulsion stability occurs when oil droplets are stabilised with octenyl succinic anhydride (OSA) modified starch. OSA starch is amphiphilic and can provide excellent emulsion stability. However, as with other starches, it is susceptible to amylase hydrolysis, and work by Dresselhuis and co-authors (2008) has shown that exposure of OSA-stabilised emulsions to saliva results in an increase in droplet size arising from coalescence. This is a consequence of reduced interfacial stabilisation due to hydrolysis of the starch interface, combined with the shear forces present in the mouth. This mechanism of orally increasing droplet size has already been shown to influence emulsion sensory properties, and might in turn be expected to affect digestibility.

Lingual lipase is a bile-salt-independent lipase that has an optimum pH range of 4.5–5.4. This pH range, coupled with the short residency time of foods in the mouth would indicate that lingual lipase has little or no contribution to emulsion structure or digestion during oral processing. However, it has been postulated that it provides a contribution to fat perception on the basis of liberating small quantities of fatty acids from foods during the eating process (Kawai and Fushiki, 2003; Stewart *et al*., 2010) that may contribute to the taste perception of fatty foods. It is argued that lingual lipase contributes more actively to lipid hydrolysis once consumed food and lipase enters the stomach. For healthy adults the efficacy of lingual lipase in hydrolysing lipids is relatively limited in comparison to pancreatic lipase. However, it plays an important role in lipid digestion for neonates, due to immaturity of the liver and lower availability of both pancreatic lipase and bile salts.

10.3 LIPID STRUCTURE, DIGESTION AND MOTILITY IN THE STOMACH

The conditions in the stomach can induce significant changes to emulsion structure and stability. In the fasted state, luminal pH is typically at 1.9–2.0, but there can be considerable dynamic shifts in gastric pH after food consumption, firstly due to the initial buffering capacity of any ingested meal and secondly as a consequence of ongoing secretion of hydrochloric acid as part of the digestion process (Miles and Shohl, 1927; Standish *et al*., 1927). The biochemical environment also contributes to the evolution of the colloidal state in the stomach, with gastric pepsin able to hydrolyse both aqueous and interfacial protein, and lingual and gastric lipases generating interfacially active fatty acids. Additionally, lingual amylase continues to act on ingested starch, in combination with gastric amylase. Whilst the shear forces present in the stomach are no longer considered sufficient for homogenising lipids into fine emulsions, gastric motility can influence emulsion mixing and the susceptibility of droplets towards coalescence. Finally, the temperature in the stomach can also cause further changes in microstructural state, with continuation of fat melting commenced in the mouth, as well as other thermal transitions, such as melting of gelatine.

10.3.1 Lipid partitioning in the stomach and effect on gastric emptying

These conditions can have a significant impact on the dynamics of emulsion structure and stability during gastric residency, which can alter the distribution of fat within the stomach contents. The partitioning of fat within the stomach is able to influence particular aspects of fat digestion, most notably contributing to the rate of gastric emptying.

Lipids released from the stomach into the intestine can be detected at the intestinal wall. In response to this effect, enteroendocrine cells release the hormone cholecystokinin (CCK), with the additional activation of extrinsic vagal afferent nerve terminals (Foltz *et al*., 2009). The physiological response to this mechanism is to slow the rate of gastric emptying, to ensure the efficient digestion of lipids during intestinal transit. The extent of CCK release is therefore proportionate to the amount of fat being released into the intestine, further regulating the rate of gastric emptying. Regulation of stomach emptying towards slower rates is accompanied by a physiological suppression of hunger as part of controlling further food intake.

The relationship between fat distribution in the stomach and gastric emptying has been demonstrated using model beverage formulations

in a number of separate *in vivo* studies. Research undertaken by Foltz and co-workers (2009) investigated the effects of lipid partitioning on emptying behaviour and associated biomarkers; comparing 325 ml of an orally consumed emulsion with an orally consumed fat-free liquid meal followed by intragastric infusion of non-emulsified oil via a nasogastric tube. For both compositions the lipid fraction was a relatively small component of the overall nutritional profile, comprising only 4 g rapeseed oil in comparison to the inclusion of 15 g protein and 15 g carbohydrate. In spite of this, the manner of lipid delivery was observed to have a noticeable effect on post-prandial behaviour. Intragastric delivery ensured that the lipid fraction would form a separated region that would be layered above the aqueous phase of the meal. Compared to the emulsion meal, this treatment resulted in faster initial gastric emptying of the meal, a lower CCK response for the first 45 minutes after ingestion, but higher for the period 50–120 minutes, and a delayed absorption of lipids. The manner of lipid delivery showed no significant differences in terms of relative hunger, satiety or fullness, which may be due to the greater contributions of protein and carbohydrate on overall satiation.

The specific emptying behaviour of the layered emulsion is attributed to a two-stage mechanism with rapid emptying of the oil-depleted aqueous portion of the meal. With little or no delivery and detection of fat in the intestine, there is minimal secretion of CCK, and accordingly emptying progresses rapidly. Once the fat layer leaves the stomach and enters the intestine there is a corresponding increase in CCK which slows down the rate of emptying. In the case of the emulsified beverage, commencement of gastric emptying results in immediate delivery of lipid into the intestine. The corresponding stimulation of CCK then regulates the rate at which meal contents is released from the stomach and this feedback mechanism proceeds according to the continuing detection of fat on emptying. In comparison to the layered emulsion, this results in a more uniform emptying rate, with markedly slower emptying in the early stages of digestion.

Marciani and co-workers (Marciani *et al*., 2006, 2007, 2009), also provided evidence of how the partitioning of the fat phase in the stomach affected emptying. The 500 ml beverage emulsion systems used in these studies were designed to be either gastric stable, i.e. retaining a homogeneous distribution of fat in the stomach after consumption, or gastric unstable, in which the emulsion would rapidly phase separate after consumption, leading to a layering of oil on top of the aqueous component. Unlike the previous study, there was no inclusion of protein or carbohydrates in the compositions, and therefore physiological markers such as satiety would be purely in response to changes in fat digestion and metabolism. The distribution of fat in the

stomach was visualised using magnetic resonance imaging (MRI), which was able to clearly distinguish between the gastric-stable and gastric-separating compositions (see Fig. 10.3).

Due to the higher fat content (50 g) in the emulsion formulations and the absence of other macronutrients, differences in emptying rates, CCK response and appetite scores were considerably more pronounced in comparison to the Foltz study (Foltz *et al.*, 2009), although the same trends were observed. Gastric emptying was again seen to be initially more rapid for consumption of the gastric-unstable emulsion, due to release of the oil-depleted aqueous phase into the intestine, with the emptying rate significantly decreasing on release of the oil-separated portion into the intestine. In comparison, the gastric-stable emulsion showed a slower and more uniform rate of emptying, due to consistent release of fat from the stomach during the emptying period (see Fig. 10.3). The differences in emptying behaviour were seen to correlate to CCK response, with the gastric-unstable emulsion showing significantly lower measured plasma CCK in comparison to the gastric-stable emulsion, for up to 6 hours after initial consumption of the emulsion. Data from satiety assessment also indicated that the acid-stable emulsion was more effective at suppressing hunger.

Similar behaviour was observed in a third *in vivo* study by Keogh and co-workers (2011), in which consumption of 330 ml of a phase-separating emulsion beverage showed faster initial gastric emptying and lower CCK response relative to a gastric-stable emulsion (with both emulsions containing 30 g and no additional protein or carbohydrate). An interesting feature of this particular example is that the phase-separating emulsion was specifically designed to undergo extensive partial coalescence in the stomach (i.e. formation of agglomerated structures comprising crystalline or partially crystalline droplets). This was achieved by formulating the lipid component of the emulsion droplets to have a solid fat content of 25% at 37 °C, in combination with poor interfacial stabilisation under gastric conditions. *In vitro* studies clearly showed for this formulation the formation and separation of large agglomerates of solid fat in simulated gastric fluid, which would be anticipated to cause layering of the emulsion under *in vivo* gastric conditions.

Whilst consistent gastric emptying behaviour can be observed *in vivo* for model beverage emulsion systems, as evidenced by these examples, meal intake is predominantly based on consumption of solid foods. The emptying of solids and solid liquid foods has been a subject of research interest (Collins *et al.*, 1996), including how the introduction of solid foods affects the distribution and emptying of lipids from the stomach (Edelbroek *et al.*, 1992; Horowitz *et al.*, 1993). Relative to simple emulsion formulations, there is a considerably greater level

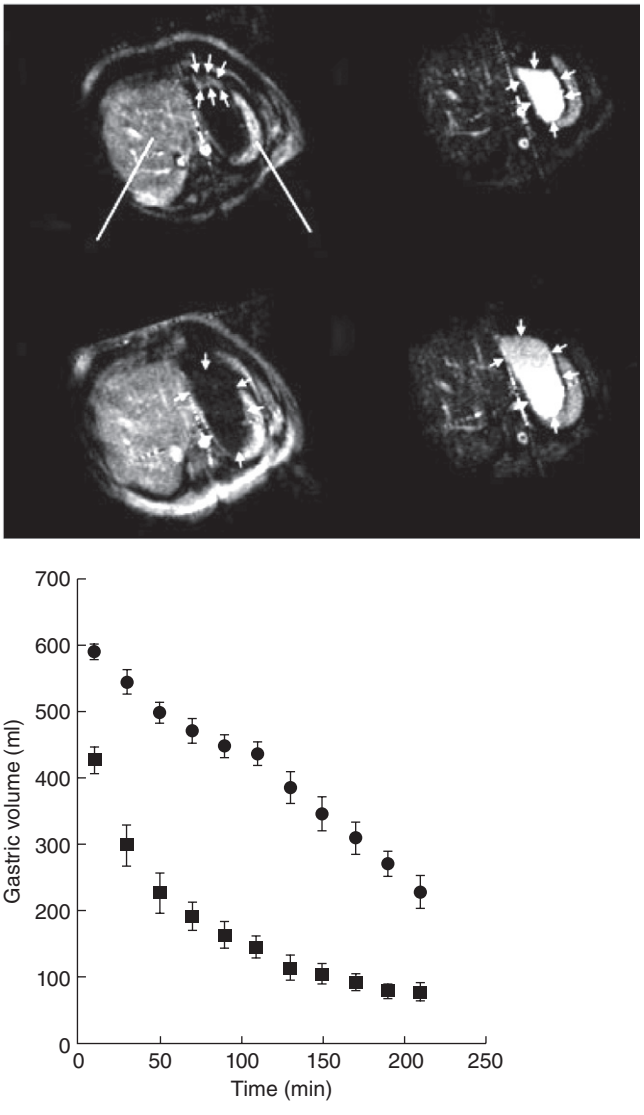

Fig. 10.3 MRI Scan showing cross-section of stomach contents for gastric-unstable (top scans) and gastric-stable emulsions. Gastric-unstable emulsion shows separation and partitioning 40 minutes after ingestion, while the gastric stable emulsion retains a homogeneous disutribution. Lower plot shows relative rate of emptying for the two emulsions (■, gastric unstable; •, gastric stable). Gastric-unstable shows faster rate of emptying, whle gastric-stable shows uniform emptying. Reproduced with permission from Marciani *et al.*, (2009) Effect of intragastric acid stability of fat emulsions on gastric emptying, plasma lipid profile and postprandial satiety. *British Journal of Nutrition*, 101(6), 919–928. Cambridge University Press.

of complexity in the dynamics of emptying due to not only the material state of the food systems, their bolus/chyme properties and the relative nutrient density, which may all impact on gastric motility.

A case in point is demonstrated by Kunz *et al*. (2005) who carried out an *in vivo* study investigating gastric fat distribution for a mayonnaise-type emulsion (37 g fat), which was consumed either before or after a fat-free pasta meal. Curiously, whilst consumption of the emulsion before the pasta meal resulted in a significantly faster emptying of fat from the stomach, there was no significant difference in total emptying between the two experimental conditions. This would seem to indicate that for solid food matrices, the role of fat release into the intestine and stimulation of CCK does not have a significant effect on regulating emptying. In addition to emptying behaviour, the study also investigated the spatial arrangement of the fat. The use of MRI was able to show that layering of the fat on the stomach contents was observed irrespective of meal ordering, with a greater extent of layering occurring when the emulsion was consumed first. Factors such as gastric pH, aqueous phase viscosity and relative position of the lipid/meal phases in the antrum and distal regions of the stomach were considered potential reasons for the specific distribution of fat in the stomach.

10.3.2 Dynamics of emulsion structuring during gastric digestion

It has been shown that, at least for liquid meal systems, the spatial arrangement of fat in the stomach can be correlated to the emptying rate of lipids into the intestine. The examples provided in the previous section are based predominantly on structural extremes, comparing a gastric-stable emulsion to either a delivered oil layer, or a poorly stabilised, rapidly separating emulsion. To achieve these structural states actually requires careful consideration as to how an emulsion or lipid composition will behave on entry to the stomach, allowing emulsions to be formulated appropriately. The conditions in the stomach can impact on continuous-phase properties, interfacial composition and interactions, as well changes to the material state of droplets. Accordingly, exposure of food emulsions to the gastric environment has the potential to lead to a broad range of structural outcomes, from gastric-stable, to flocculated, to partially coalesced, coalesced and broken, depending on initial formulation design (Golding *et al*., 2011). Furthermore, structural changes may not occur immediately on introduction to the stomach, but may occur dynamically over the residence period, and may involve the formation of transitional structural states.

In recent years, there has been considerable interest in how formulation design impacts on the structural state and digestibility of

predominantly oil-in-water emulsions under gastric and intestinal conditions. The development and use of *in vitro* techniques has been widely used to demonstrate the diversity of emulsion behaviours in simulated gastric and intestinal conditions. Whilst this has greatly increased knowledge of how lipid systems respond to specific physiological stimuli, the limitations of *in vitro* models in relation to actual in-body behaviours must be recognised (Li *et al*., 2012b). This is particularly true when considering the challenges of replicating in-body mechanical forces, biochemical environment and aspects such as hormonal regulation of digestive function across the entire GI tract. The advantage of *in vitro* is, of course, that it allows high throughput investigations of emulsion behaviours, as well as being selective in probing specific aspects of GI digestion.

10.3.2.1 *Structure and stability of protein-stabilised emulsions in the stomach*

Proteins are widely used as emulsifiers in the food industry, due to their natural consumer perception and extensive functionality. Depending on the product, proteins can to impart either stabilisation or controlled destabilisation, contributing to material/textural properties. On ingestion, the conditions in the stomach can impact significantly on the stability and structure of protein-stabilised emulsions, due to the acidic pH environment, interaction with gastric mucins, interfacial proteolysis and hydrolysis, and the mixing environment in the stomach.

It is notable that a common structural feature of many protein-stabilised emulsions under (simulated) gastric conditions is flocculation. This has been most extensively observed *in vitro* for milk-protein-stabilised emulsions, including both whey and casein fractions, but has also been observed for emulsions comprising non-dairy proteins, such as soy (Nik *et al.*, 2010; Golding *et al*., 2011; Li *et al*., 2012a).

The stability of protein-stabilised emulsions against flocculation can be particularly sensitive to pH. As indicated, the pH of the stomach is the fasting state is typically 1.9–2.0. However, this can rise significantly on ingestion of food due to buffering effects of the food system, and this is particularly true of protein-based compositions. In the case of protein-stabilised emulsions, the initial elevation of gastric pH is followed by gradual lowering back towards fasting levels, which can effect a transition through the isoelectric point for some protein systems, such as the caseins (van Aken *et al*., 2011) (see Fig. 10.4).

The loss of charge stabilisation close to the isoelectric point can induce flocculation, as evidenced for sodium caseinate emulsions (for which the protein possesses a net pI of 4.6) (Li *et al*., 2012b). As the pH returns towards fasting- state levels, most protein-stabilised emulsions will become increasingly positively charged. On the assumption

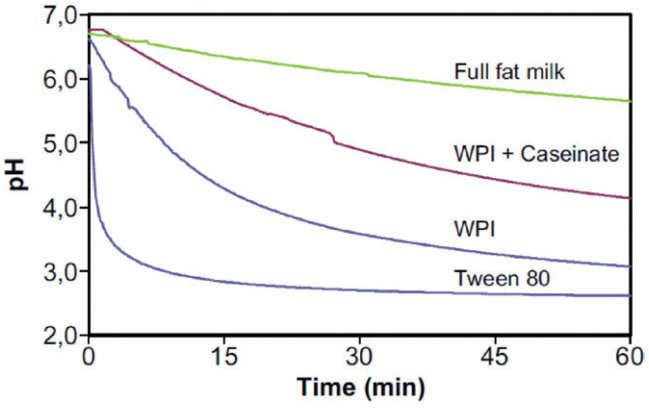

Fig. 10.4 Dynamic changes to pH for emulsions (3.5–5% fat) over one hour incubation in simulated gastric fluid. Reproduced with permission from van Aken *et al.*, (2011) Differences in in vitro gastric behaviour between homogenized milk and emulsions stabilised by Tween 80, whey protein, or whey protein and caseinate. *Food Hydrocolloids* 25(4), 781–788. Elsevier.

that pH is the only mechanism by which gastric flocculation occurs, it might be expected that some dissociation through charge repulsion might occur. However, other contributing factors are able to contribute to gastric flocculation of ingested emulsions, such as the interaction of emulsion droplets with gastric mucin.

In vitro studies, primarily using porcine gastric mucin, show similar emulsion behaviour to that observed for the salivary mucous system. In separate studies Ritzoulis *et al*. (2012), Vingerhoeds *et al*. (2005) and Sarkar *et al*. (2009) showed that mixing of emulsions with porcine gastric mucin induced significant droplet flocculation. For the study by Ritzoulis *et al*. (2012) emulsion droplets were stabilised by sodium caseinate. Here it was demonstrated that flocculation was charge dependent. At pH above the isolectric point of the protein flocculation was governed by depletion effects generated by the presence of high-molecular-weight mucosal glycoprotein; behaviour consistent with the observations of Vingerhoeds *et al*. (2005). However, at pH < pI, flocculation was attributed to an electrostatic bridging mechanism between the now positively charged interfacial protein layer, and the still negatively charged mucin.

Similar behaviour was observed by Sarkar *et al*. for an emulsion system in which the interfacial layer was stabilised by β-lactoglobulin, for which flocculation behaviour at pH 1.9 was determined to be electrostatic bridging (Sarkar *et al*., 2010a). Whilst the *in vitro* studies clearly demonstrate the effect of porcine gastric mucins on droplet stability, there is less known about the consequences of exposure of food emulsion systems to gastric mucin *in vivo*, particularly in relation

to the extent by which ingested emulsions are actually able to mix with the mucosal layer, and the specific effects of dilution and pH.

Whilst it can be considered that mucosal depletion may act as a generic flocculation mechanism, irrespective of interfacial composition, for protein-stabilised droplets proteolysis represents a third mechanism which may contribute to gastric emulsion stability and flocculation. The primary gastric protease, pepsin, is expressed in the stomach in the pro-form pepsinogen. The acidic gastric environment results in unfolding and autocatalytic cleavage of pepsinogen to pepsin. Pepsin is most active at in-body temperatures and over an acidic pH range of 1.5–2.5. Whilst not site specific, it tends to preferentially cleave proteins at sites comprising hydrophobic residues, particularly those containing aromatic residues.

Accordingly, for some globular proteins, such as β-lactoglobulin, the burial of hydrophobic protein domains within the tertiary structure of the native protein, provide a measure of protection against protein hydrolysis by pepsin, imparting pronounced resistance of the protein during the gastric stage of proteolysis. However, it has been shown that adsorption and partial unfolding of β-lactoglobulin at the oil–water interface results in greater exposure of hydrophobic domains, and therefore these regions are made more accessible to pepsin. Relative to non-adsorbed protein in solution, surface-adsorbed β-lactoglobulin is not only more readily hydrolysed, but results in the formation of different digestive peptides (Macierzanka *et al*., 2009; Maldonado-Valderrama *et al*., 2012). For β-lactoglobulin, Maldonado-Valderrama and co-authors (2012) also demonstrated that the composition of the oil phase had an impact on interfacial hydrolysis. In comparison with olive oil, β-lactoglobulin adsorbed at a tetradecane interface was seen to be less susceptible to proteolysis. This was attributed to increased orientation and interaction of hydrophobic domains towards the less polar tetradecane oil phase, thereby reducing the availability of cleavage sites for the pepsin. A further study by Nik and co-workers (Nik *et al.*, 2010) compared the gastric proteolysis of β-lactoglobulin with α-lactalbumin. Findings for β-lactoglobulin were broadly consistent with other studies (Sarkar *et al*., 2010a; Maldonado-Valderrama *et al*., 2012), showing increased propensity of interfacial protein towards hydrolysis in comparision with non-adsorbed protein. However, in contrast to the behaviour of the β-lactoglobulin system, they showed that the adsorbed α-lactalbumin was actually more resistant to proteolysis than non-adsorbed α-lactalbumin.

In comparision to the globular whey proteins, the structurally disordered casein proteins provide greater accessibility of hydrophobic domains for pepsin proteolysis (in spite of their propensity for self-association) and are rapidly and extensively hydrolysed in solution.

Casein proteins adsorbed at the oil–water interface are likewise readily hydrolysed, as the hydrophobic domains are again relatively exposed to the enzyme (Macierzanka *et al*., 2009; Li *et al*., 2012a).

Proteolytic cleavage of a proteinaceous adsorbed layer can have a pronounced effect on the interfacial composition and structure, with consequences for emulsion stability. For protein-stabilised emulsions, stability against flocculation is imparted through a combination of electrostatic and steric repulsion provided by the adsorbed layer. Protection against coalescence is also provided by the protein layer, which is able to inhibit film thinning due to extension of hydrophilic domains into the aqueous phase, and by imparting a mechanically robust interface which provides resistance to film rupture. Pepsin proteolysis of the adsorbed protein layer can result in detachment of hydrophilic regions of the molecule. Whilst hydrophobic domains remain adsorbed at the interface, hydrophilic peptide fragments will be released from the interface and be solubilised in the aqueous phase. As a consequence, the net hydrophobicity of the interface increases as the surface-stabilising charged and polar domains are detached, which in turn increases the propensity of the emulsion droplets towards flocculation via attractive hydrophobic interactions (McClements *et al*., 2009b; van Aken, 2010).

The effect of pepsin hydrolysis on protein adsorbed layer and emulsion properties has been demonstrated in a number of studies. At the planar oil–water interface, Maldonado-Valderrama and co-authors (2010) used a combination of atomic force microscopy (AFM) and dilational interfacial rheology (at the air–water interface) to study how proteolysis under gastric conditions affected the structural and mechanical properties of the adsorbed layer. The showed that whilst partial pepsin hydrolysis of the interfacial protein layer took place, resulting in a reduction in surface tension, atomic force microscopy showed that to a degree the interconnected interfacial network remained intact. Accordingly, dilational elastic modulus was only partly lowered as a consequence of enzyme digestion of the interface. The retention of the interfacial network was speculated to be due to strong hydrophobic interactions, not only between protein and the interface, but between neighbouring protein molecules.

In the case of an emulsion system stabilised with β-lactoglobulin, Sarkar *et al*. (2010a) showed that *in vitro* exposure of the emulsion systems to gastric fluid at pH 1.2 and containing pepsin resulted in a time-dependent reduction in zeta potential from +50 mV (15 minutes exposure to SGF) to +17.6 mV (two hours exposure to SGF). The low pH environment is significantly below that of the protein pI, hence the large initial positive value for zeta potential. The reduction in zeta potential is a consequence of the detachment of charged domains of the

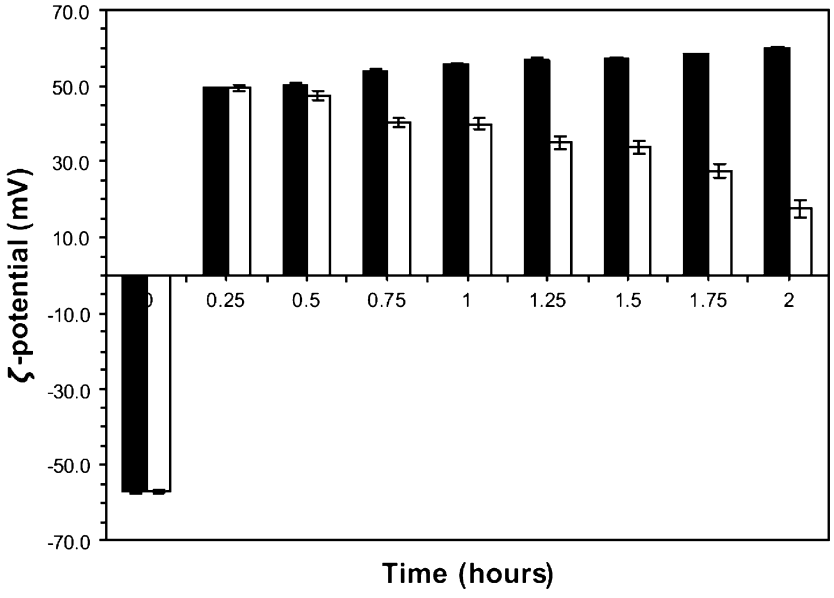

Fig. 10.5 Dynamic change in ζ-potential for β-lactoglobulin-stabilised emulsions (10% oil-in-water) across two hours exposure to simulated gastric fluid (SGF) at pH 1.2 (except at $t = 0$ where pH = 7.0). Shaded bars indicate changes where pepsin has been excluded from SGF; hollow bars indicate changes where pepsin has been included. Reproduced with permission from Sarkar *et al.*, (2009). Behaviour of an oil-in-water emulsion stabilised by beta-lactoglobulin in an in vitro gastric model. *Food Hydrocolloids* 23(6), 1563–1569. Elsevier.

protein layer from the interface (see Fig. 10.5). Additional SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) measurements were used to show that after two hours digestion only ~20% of the interfacial protein membrane remained intact.

Proteolytic removal of the electrostatic and steric stabilising layer is contributory to the flocculation of protein-stabilised emulsions (Hur *et al.*, 2009; Golding *et al.*, 2011), promoting droplet association through increased hydrophobic interactions. However, a further consequence of the hydrolytic breakdown of the interfacial layer is a reduction in the mechanical strength of the interface (Maldonado-Valderrama *et al.*, 2009). For flocculated, weakly stabilised emulsion droplets, even mild shear conditions are sufficient to induce coalescence. For liquid-oil-stabilised emulsions this has been shown using microscopy in a number of separate studies (Sarkar *et al.*, 2008; Hur *et al.*, 2009), which has shown coalescence susceptibility as a consequence of prolonged exposure to the simulated gastric environment.

Whilst proteolysis alone has been shown to be consequential in the destabilisation of protein-stabilised emulsions, it is important to recognise that pepsin is not the only gastric enzyme capable of acting at

the droplet interface. Gastric lipase is an acid-stable, bile-salt-independent lipase with an optimum pH range of 3–6. During digestion, gastric lipolysis can account for up to ~30% of the total lipid hydrolyisis. In contrast to pancreatic lipase, gastric lipase is Sn1,3 specific, hydrolysing triglycerides to one free fatty acid and one diglyceride. From the perspective of emulsion digestion, the generation of significant concentrations of surface-active free fatty acids additionally contributes to the dynamic interfacial and structural properties of emulsions during exposure to the gastric environment (Pafumi *et al*., 2002). However, most *in vitro* studies ignore the role of gastric lipase in emulsion properties in the stomach. This is primarily due to limited availability of human gastric lipase for such studies. To try and account for the lipolytic contribution to emulsion properties, a number of studies have incorporated acid-stable fungal lipase into the *in vitro* model design (Golding *et al*., 2011; van Aken *et al*., 2011). Such lipases have similar pH activity and specificity to gastric lipase. In their gastric *in vitro* study van Aken *et al*. (2011) compared dynamic structural changes to emulsions stabilised with whey protein isolate, mixed whey/caseinate, Tween 80 and homogenised full-fat milk. Flocculation was observed for full-fat milk, even though the buffering capacity of the system retained pH appreciably above pI and this was attributed to the presence of both pepsin and lipase, with the additional production of lipolytic fatty acids leading to flocculation due to competitive displacement of the protein-adsorbed layer, and a subsequent weakening of electrostatic repulsion.

The ability to control the structural and digestive properties of protein- stabilised emulsions during the gastric stage of digestion has potential implications for subsequent processing of lipids further down the GI tract. *In vitro* models suggest a reasonably generic model of flocculation and limited coalescence during gastric residency for simple protein-stabilised emulsions. Manipulation of the interfacial properties of the adsorbed protein layer provide a potential means for altering the stability and structure of emulsions during both gastric and intestinal stages of digestion. The use of Maillard complexes to provide a more resistant interface to digestion has been considered in a number of studies (Oliver *et al*., 2009; Lesmes and McClements, 2012). Lesmes and McClements (2012) investigated the conjugation of β-lactoglobulin with dextrans of varying molecular weight, and the behaviour of these Maillard complexes under *in vitro* gastric and intestinal conditions. Emulsions stabilised with the conjugate protein were shown to be more resistant to flocculation as a consequence of a reduction in pH. Acid stabilisation was seen to be further enhanced with increasing molecular weight of dextran. This was attributed to greater steric stabilisation of droplets, particularly given that the zeta-potential of the emulsions was

reduced close to zero for the high-molecular-weight dextran-β-lactoglobulin conjugates, indicating little electrostatic contribution to droplet repulsion. High-molecular-weight dextran conjugates also appeared to show increased resistance to proteolysis, with only minimal increases to emulsion particle size distribution after two hours incubation in simulated gastric fluid containing pepsin. The reduction in apparent proteolysis may be a consequence of the dextran moiety limiting access of the pepsin to the protein-stabilised interface.

On the basis of the observations made by Lesmes and McClements, it stands to reason that other mechanisms used to manipulate the interfacial structure and mechanical properties of the adsorbed protein layer could impact changes to emulsion stability during gastric digestion. Previous studies have been used to probe the effects of heat (Roth *et al*., 2000), enzymatic modification (e.g. transglutaminase) (Romoscanu and Mezzenga, 2005; Macierzanka *et al*., 2011; Ercili-Cura *et al*., 2012) and physical crosslinking (e.g. ionic interactions) (Zeeb *et al*., 2011) on the properties of protein-stabilised interfaces, under both planar and emulsified conditions. However, thus far the effects of surface structuring under gastric conditions are not yet widely reported (Nik *et al.*, 2010).

10.3.2.2 *Structure and stability of non-protein stabilised emulsions in the stomach*

Whilst proteins are widely used for emulsification in many food applications, the stabilisation and structuring of droplets is not constrained to the use of proteinaceous ingredients. Non-protein components generally fall into one of two classes: small- molecular-weight surfactants and biopolymers. Permitted small-molecular-weight surfactants widely used for the stabilisation of oil-in-water type emulsions can be ionic (e.g. sodium stearoyl lactylate, lecithin), zwitterionic (lecithins with cationic functional groups) or non-ionic (the Tweens, high hydrophilic–lipophilic balance (HLB) sucrose esters). Low HLB non-ionic emulsifiers such as monoglycerides, the Spans and polyglycerol polyracinoleate, are widely used for the stabilisation of water-in-oil type emulsions. Non-protein biopolymers with amphiphilic properties capable of stabilising oil-in-water emulsions include octenyl succinic anhydride modified starch, propylene glycol alginate, methylcellulose and acetylated pectins (Huang *et al*., 2001; Dickinson, 2009).

A generic feature of both classes of emulsifiers is that under gastric conditions pepsin will have no effect on the structure of the interfacial layer or the stability of the emulsion, due to the absence of protein. However, other effects in the stomach may impact on changes to emulsion stability.

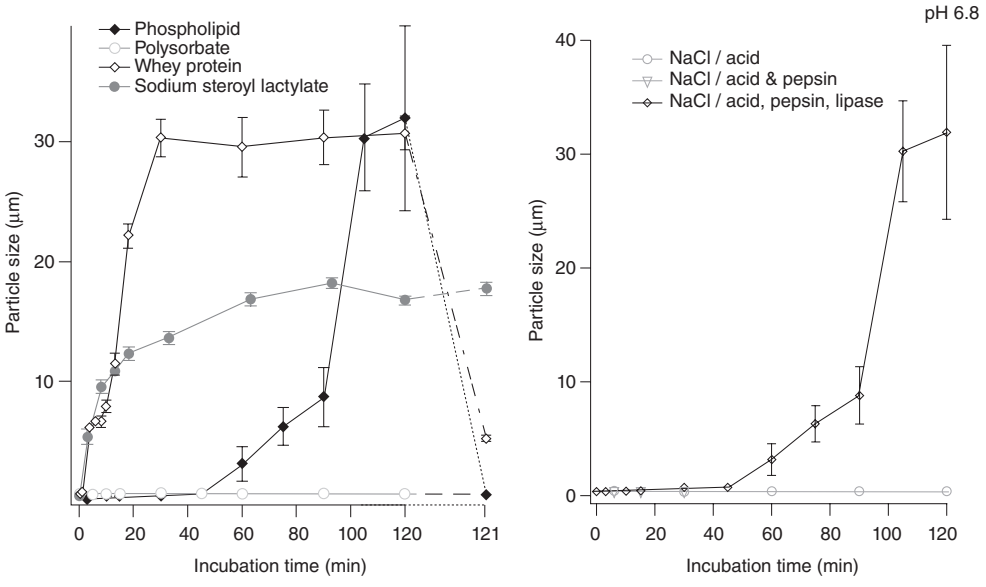

Fig. 10.6 Changes in average particle size for 20% oil-in-water emulsions stabilised by various emulsifiers during incubation in simulated gastric fluid. Left-hand image compares phospholipid, polysorbate, whey proteins and sodium stearoyl lactylate emulsifiers; right-hand image shows the effects of enzyme addition on particle size for phospholipid-stabilised emulsion. Reproduced with permission from Golding *et al.*, (2011) Impact of gastric structuring on the lipolysis of emulsified lipids. *Soft Matter* 7(7), 3513–3523. Royal Society of Chemistry.

Surfactant-stabilised emulsions show variable behaviour depending on the type of surfactant used (see Fig. 10.6). Fine oil-in-water emulsions ($d[4,3] < 1\,\mu m$) stabilised with either non-ionic high HLB polyglycerol esters or Tween 80 were found to be stable to pH-induced flocculation under *in vitro* gastric conditions (Yin *et al*., 2008; Golding *et al*., 2011; van Aken *et al*., 2011), as were emulsions stabilised by phospholipids (Golding *et al*., 2011). In particular, changes in particle-size distribution for the Tween-stabilised emulsions did not vary significantly over the two-hour gastric incubation period. These findings support the *in vivo* MRI studies made by Marciani *et al*. (2007, 2009) that Tween-stabilised emulsions were acid stable under physiological gastric conditions and remained homogeneously distributed in the stomach during digestion. In comparison, the lecithin-stabilised emulsions, whilst initially stable under *in vitro* gastric conditions, displayed a progressive increase in droplet size after 45 minutes incubation time.

An interesting feature of both studies was the inclusion of an acid-stable fungal lipase in the *in vitro* model, which was intended to be representative of human gastric lipase. Findings from the Golding *et al*. (2011) study confirmed that when lipase was excluded from

the model, no change in particle size distribution was observed for the phospholipid-stabilised emulsion. However, when the lipase was included, a significant increase in droplet size was indeed seen (see Fig. 10.6). As indicated earlier, the conversion of triglycerides to diglycerides and free fatty acids may affect the surface composition over time, with fatty acid displacement resulting in flocculation due to a loss of charge and steric stability. In contrast, inclusion of lipase had considerably less effect on the stability or particle size distribution of the Tween emulsions, indicating that the Tween interface is more resistant to the adsorption of lipase to the interface, either due to the surface tension differential between the surfactant and the enzyme or possibly due to binding of aqueous Tween to the lipase.

The study by Golding *et al*. (2011) also investigated the behaviour of emulsions stabilised by an anionic food-grade emulsifier, sodium stearoyl lactylate (SSL). Under neutral conditions, the emulsifier imparts a negative charge to droplets, providing excellent emulsion stability. However, under simulated gastric conditions a significant loss of emulsion stability was observed, with confocal microscopy showing extensive coalescence. A combination of initial flocculation as pH is lowered (Zinoviadou *et al*., 2012) and poor mechanical stability of the interface caused the flocculated droplets to undergo rapid coalescence into significantly larger droplets. An intriguing extension of this mechanism is the formulation of the lipid composition of emulsions droplets to contain 25% solid fat at 37 °C. Confocal microscopy shows that the system, rather than forming fully coalesced droplets, undergoes partial coalescence at in-body temperatures, forming large, irreversibly associated agglomerates of droplets (see Fig. 10.7).

To date, there are few studies relating how the direct interfacial stabilisation of emulsions by non-protein biopolymers affects emulsion properties under either *in vivo* or *in vitro* GI conditions. However, some studies have begun to explore the use of biopolymers as adjuncts in controlling emulsion digestion properties. This includes the complexation of biopolymers with the interfacial layer, such as studies on the electrovalent crosslinking of lecithin- or caseinate-stabilised emulsions with chitosan and/or pectin (Li *et al*., 2010; Hu *et al*., 2011) (although this has primarily been evaluated under *in vitro* intestinal conditions).

An alternative structuring route has been the entrapment of emulsion systems within a biopolymer-based gel matrix (so-called gel hydrospheres) to provide a physical barrier to digestion (Matalanis and McClements, 2012), such as the encapsulation of protein-stabilised emulsion droplets within a coacervated chitosan–calcium-alginate hydrogel (Li and McClements, 2011). In this particular study, the gel beads were found to remain stable and intact under acidic conditions,

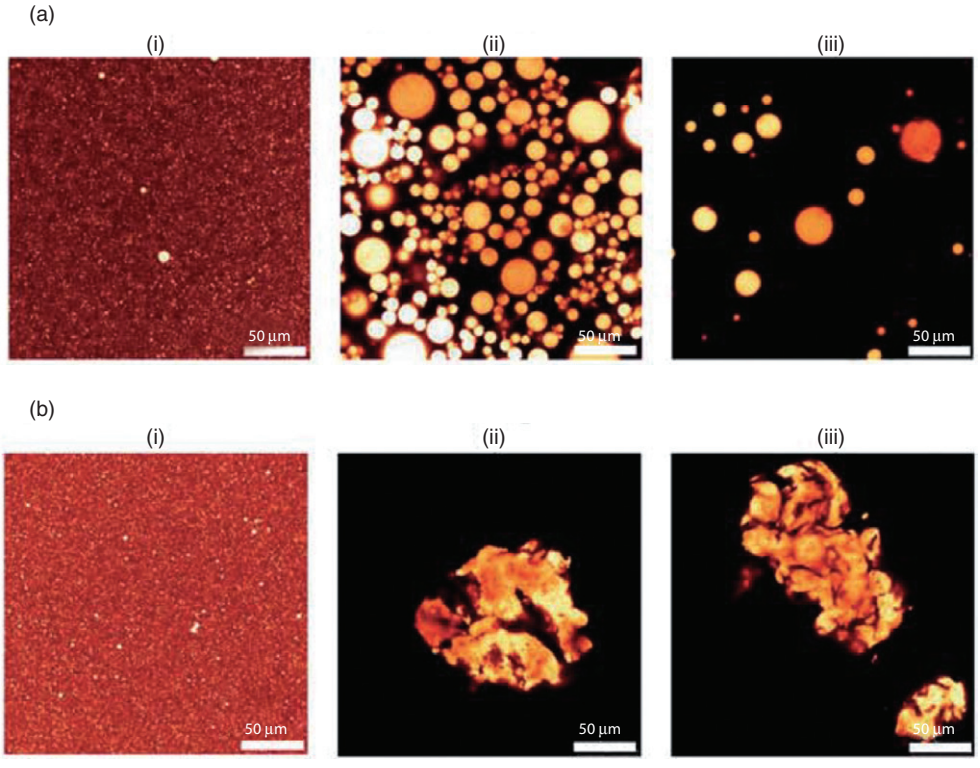

Fig. 10.7 Confocal microscopy showing changes to emulsion structure for 10% oil-in-water emulsions stabilised with sodium stearoyl lactylate and comprising droplets of: (a) liquid oil; (b) solid/liquid oil (25% solid fat content). Micrographs show structure: (i), prior to simulated gastrointestinal treatment; (ii), 30 minutes after exposure to simulated gastric fluid; (iii), 30 minutes after exposure to simulated intestinal fluid. Bar = 50 µm. (a and b) Reproduced with permission from Golding *et al.*, (2011) Impact of gastric structuring on the lipolysis of emulsified lipids. *Soft Matter* 7(7), 3513–3523. Royal Society of Chemistry.

whilst undergoing aggregation and sedimentation with pH closer to neutral. The encapsulation of the lipid phase within the stable gel matrix was seen to have an appreciable effect on inhibiting digestibility of the emulsion.

10.4 LIPID STRUCTURE, DIGESTION AND MOTILITY IN THE INTESTINE

10.4.1 The intestinal environment in facilitating lipolysis

Gastric lipolysis typically accounts for 10–30% of total lipid hydrolysis in healthy adults, with the remainder taking place in the small intestine. In addition to completion of triglyceride hydrolysis to fatty acids, the

conditions in the small intestine facilitate the formation of the so-called mixed micellar state, in which fatty acids undergo self-assembly with bile salts and other biological surface-active material into nanostructured moieties of sufficiently small size to diffuse and transport across the intestinal lumen, mucin layer and epithelium (Armand, 2007).

Lipolysis in the small intestine is mediated through co-lipase-dependent human pancreatic lipase, which hydrolyses triglycerides at the sn-1 and sn-3 positions to generate two free fatty acids and an sn-2 monoglyceride. Pancreatic lipase is active under the neutral to mildly alkaline conditions found in the small intestine, with optimal activity at ~6.5. Whilst able to catalyse lipolysis through direct adsorption to an interface, its adsorption is more readily inhibited by the presence of any surface-active components already occupying the interface. The efficiency of intestinal lipolysis is greatly improved by the secretion of co-lipase and bile salts (Blackberg *et al*., 1979). Co-lipase is a non-enzymatic protein co-factor. It is a more amphiphilic molecule than pancreatic lipase, and is able to form complexes with the C-terminal region of the enzyme. The net effect of this interaction is the generation of an extended hydrophobic domain which provides a more favourable environment for adsorption at the oil–water interface (Lowe, 1997). Whilst this arrangement facilitates adsorption at the interface, it may still be confounded by highly surface-active molecules adsorbed to emulsion droplets on entry to the small intestine. For example, stabilisation of an emulsion system with the small molecule surfactant Tween 80 was shown to render the interface impervious to both pancreatic lipase, and complexed lipase–co-lipase (Gargouri *et al*., 1983).

The secretion of endogenous biosurfactants, bile salts and phospholipids by the liver plays an important role in the intestinal lipolysis of fats and oils (Maldonado-Valderrama *et al*., 2011). Whilst bile salts are amphiphilic, their structure is unusual in that they do not exhibit the classical headgroup–tailgroup arrangement of typical small-molecule surfactants. The basic structure of bile salts can be described as a rigid steroid backbone comprising hydrophobic and hydrophilic faces which is attached to a flexible aliphatic region. The primary bile salts secreted as part of digestion are cholate, chenodeoxycholate and deoxycholate, with the structure of deoxycholic acid shown in Fig. 10.8, including a schematic as to how the molecule orients at the oil–water interface.

In spite of the notable structural differences to classical surfactants, bile salts are highly surface active. That said, the equilibrium surface pressure of bile salts is still generally lower than that of synthetic surfactants such as SDS and Tween 20, being shown to be only reach surface pressures of up to 30 mN/m compared to ~50 mN/m for the

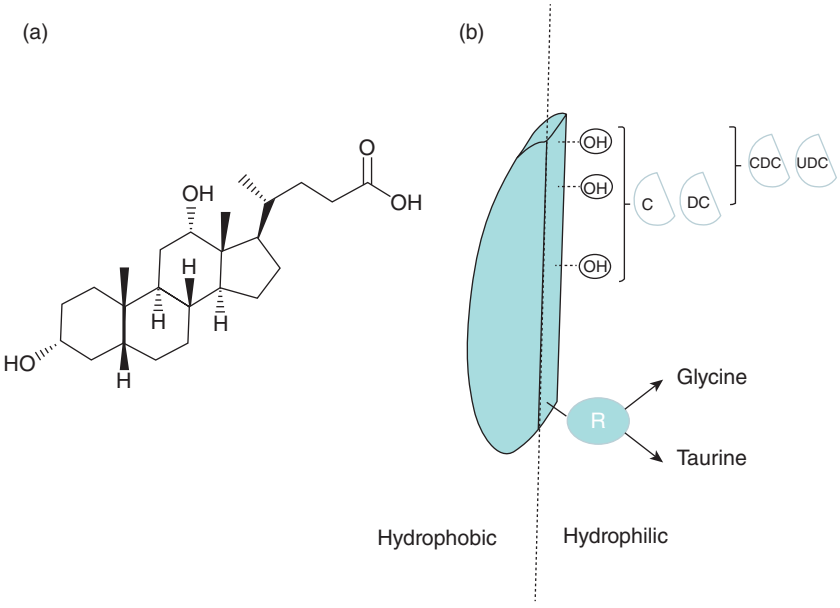

Fig. 10.8 Molecular structure (a) and interfacial orientation (b) of deoxycholic acid. Reproduced with permission from Maldonado-Valderrama *et al.*, (2011). The role of bile salts in digestion. *Advances in Colloid and Interface Science* 165(1), 36–46. Elsevier.

more surface-active synthetic surfactants (Maldonado-Valderrama *et al.*, 2011). The relatively lower surface pressure exhibited by bile salts is attributed to the parallel orientation of the molecule at the surface, rather than the perpendicular arrangement of headgroup–tailgroup-type surfactants, which leads to less efficient packing at the interface than for polar lipid emulsifiers. The adsorption of bile salts to an oil–water interface depends therefore on the surface properties of the molecule in residence. In comparison to most proteins, bile salts are more surface active, and accordingly will displace protein through a mechanism of orogenic displacement (Maldonado-Valderrama *et al.*, 2008, 2009).

It is worth noting that under biological conditions the interfacial layer of an emulsion will have already been hydrolysed, rendering the interface more susceptible to bile-salt displacement (Sandra *et al.*, 2008). This mechanism of displacement can have a significant impact on the mechanical properties of the interface, as shown in Fig. 10.9, which illustrates the rapid and extensive reduction in dilational surface elasticity generated by the displacement of β-lactoglobulin by bile salts.

For polar lipid surfactants with greater relative surface activity than bile salts, a mechanism of orogenic competitive displacement does not apply. Instead, it has been shown that for phospholipid monolayers, a

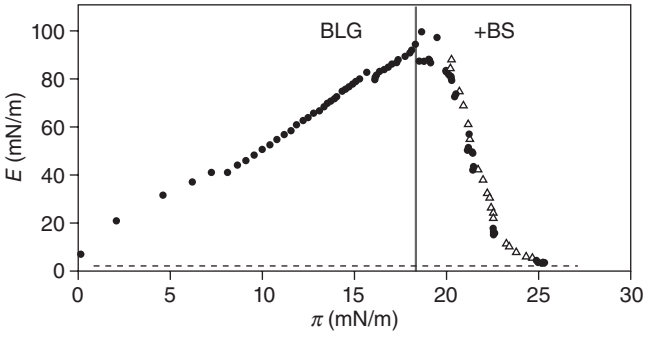

Fig. 10.9 Change in dilational surface elasticity and surface pressure of β-lg interface on addition of bile salts (BS). Reproduced with permission from Maldonado-Valderrama *et al.*, (2011). The role of bile salts in digestion. *Advances in Colloid and Interface Science* 165(1), 36–46. Elsevier.

synergistic interaction between the bile salts and the polar lipid interface allows for bile salts to be co-adsorbed at the surface, as evidenced by a lowering of interfacial tension beyond that of the surfactant system alone.

The adsorption of bile salts at the interface is an important step in the digestive process, as it provides binding sites for the colipase–lipase complex at the interface, allowing lipolysis to proceed. Therefore, whilst in the absence of bile salts, the lipolysis of Tween emulsions is inhibited, inclusion of bile salts and subsequent adsorption enables lipolysis to progress (Gargouri *et al*., 1983). On this basis, any interfacial mechanism that restricts direct access of bile salts to the droplet surface is likely to have an inhibitory effect on lipolysis. This has been shown to be the case for droplets stabilised with non-ionic galactolipids. In comparison to ionic phospholipids, galactolipids displayed both reduced propensity for interaction with bile salts and a more densely packed interface, which imparted a steric barrier inhibiting bile salt accessibility to the oil surface. Galactolipid-stabilised droplets were accordingly less susceptible to lipolysis (Chu *et al*., 2009, 2010).

The formation of multilayered structures may also provide an effective barrier against bile-salt adsorption. For example, the formation of charged multilayers in which an interfacial layer of anionic lecithin was complexed with cationic chitosan was shown to have a reduced rate of lipolysis under *in vitro* intestinal conditions, compared to a control emulsion solely stabilised by lecithin (Mun *et al*., 2006). Intriguingly, whilst the effects of surface inhibition clearly resulted in a modified lipolysis rate under *in vitro* conditions, when carried out under *in vivo* conditions (in a mouse study), modification of emulsion surface properties did not appear to have any influence on key digestive biomarkers

relating to fat uptake when compared to an unmodified control (Park *et al*., 2007).

10.4.2 Development of intestinal emulsion structures and relationship to digestion

Lipase accessibility to an interface is not the only mechanism by which emulsion lipolysis in the small intestine can be influenced. Since lipolysis is an interfacially mediated process, then hydrolysis efficiency should be limited by the relative available surface area. Reducing the droplet size of emulsions entering the small intestine will, in principle, lead to more efficient lipolysis due to increased availability of lipase binding sites as a consequence of increasing surface area. *In vitro* studies in which emulsions undergo controlled destabilisation during gastric treatment do appear to show that the rate and extent of pancreatic lipolysis is suppressed as emulsion surface area is reduced (Golding *et al*., 2011), with emulsions displaying coalescence exhibiting lower levels of released fatty acids in comparison to stable emulsions retaining high surface area. Additional *in vitro* studies, in which the droplet size of the emulsions is controlled prior to exposure to simulated intestinal fluid, also show that lipolysis is correlated to surface area (Seimon *et al*., 2009a; Li *et al*., 2011).

Under *in vivo* conditions the situation is more ambiguous. In cases where emulsions have been delivered directly into the small intestine through the use of intubation, a relationship between droplet size and lipid digestion efficiency does appear to exist. In one particular study (Seimon *et al*., 2009b) the effect of duodenal intubation of emulsions varying in size from 0.26–170 μm was investigated. Findings showed that emulsion droplets with the highest surface area generated statistically significant greater levels of CCK and PYY secretion, as well as higher levels of plasma triglycerides. Finer emulsions also had a pronounced effect on intestinal motility, effectively slowing down intestinal transit to ensure full digestion and uptake of fat. A separate study, also looking at the effects of direct intubation showed that increasing surface area influenced not only digestive biomarkers, but also relative food intake.

However, in determining a correlation between surface area and intestinal digestibility of fat these studies choose to ignore the contributions of the oral and gastric environments on the emulsion structure dynamics across the digestion process. A notable *in vivo* study by Armand and co-authors (1999) took a more integrated approach, employing intubation of two emulsions of differing size (10 and 0.7 μm) into the stomach and monitoring changes to size distribution, gastric and pancreatic lipase activities and fat digestion. Whilst lipolysis under

gastric conditions was seen to be greater for the fine emulsion, this was accompanied by an increase in droplet size during residence in the stomach. A difference in lipolysis was also observed in the duodenum; again, greater for the fine emulsions. However, the overall plasma triglyceride counts were not significantly different between the two emulsions, indicating that overall fat assimilation was not affected by initial droplet size. One other aspect of note from this study was the fact that the peak point for plasma triglyceride was significantly delayed in the case of the fine emulsion, indicating that differences in fat distribution during gastric digestion may have affected the rate of stomach emptying of the two emulsions.

This study was later extended to investigate whether initial emulsion droplet size had any impact on the uptake of the oil-soluble vitamins A and E (Borel *et al*., 2001), using similar mean drop sizes of 0.7 and 10.1 μm. Gastric emptying rate, chylomicron response, and gastric and intestinal aspirates were measured. The conclusions of the study showed that the initial size of fat droplets had no impact on the overall absorption of vitamins A or E. Curiously, the gastric emptying rates for the two emulsions were found to be similar.

This apparent normalisation of lipid digestion is understandable, given the biological imperative to achieve efficient fat uptake, irrespective of the initial structural state or surface area of the ingested fat or oil. In this respect, bile salt/phospholipid adsorption appears to be an important factor in regulating the colloidal state during the intestinal stage of digestion. A number of *in vitro* studies (Sarkar *et al*., 2010b; Golding *et al*., 2011; Nik *et al*., 2011; Li *et al*., 2012b) have shown that for fine emulsions (droplet size typically < 1 μm), significant coalescence takes place during incubation in simulated intestinal fluid containing bile salts (irrespective of surface composition). Whilst bile salts are effective at adsorbing at the oil–water interface and lowering surface tension, there is little molecular interaction at the interface between adjacent molecules, and consequently bile salts do not impart particularly effective mechanical stability at the interface, as shown in Fig. 10.9. Therefore, droplets in close proximity are susceptible to coalescence. It should be noted that the extensive coalescence observed in all the above examples appears to be mediated by extensive flocculation of all the emulsions taking place under gastric conditions. In the flocculated state, there are high numbers of droplets in close contact. Bile salt displacement at the interface in these close-packed systems can therefore be expected to promote extensive coalescence and increase in particle size, as shown in Fig. 10.10. It is interesting to note that in the absence of flocculation, for example in the case of the polysorbate-stabilised emulsion in Fig. 10.10, less coalescence is observed after exposure to the simulated intestinal fluid (Golding *et al*., 2011). Bile

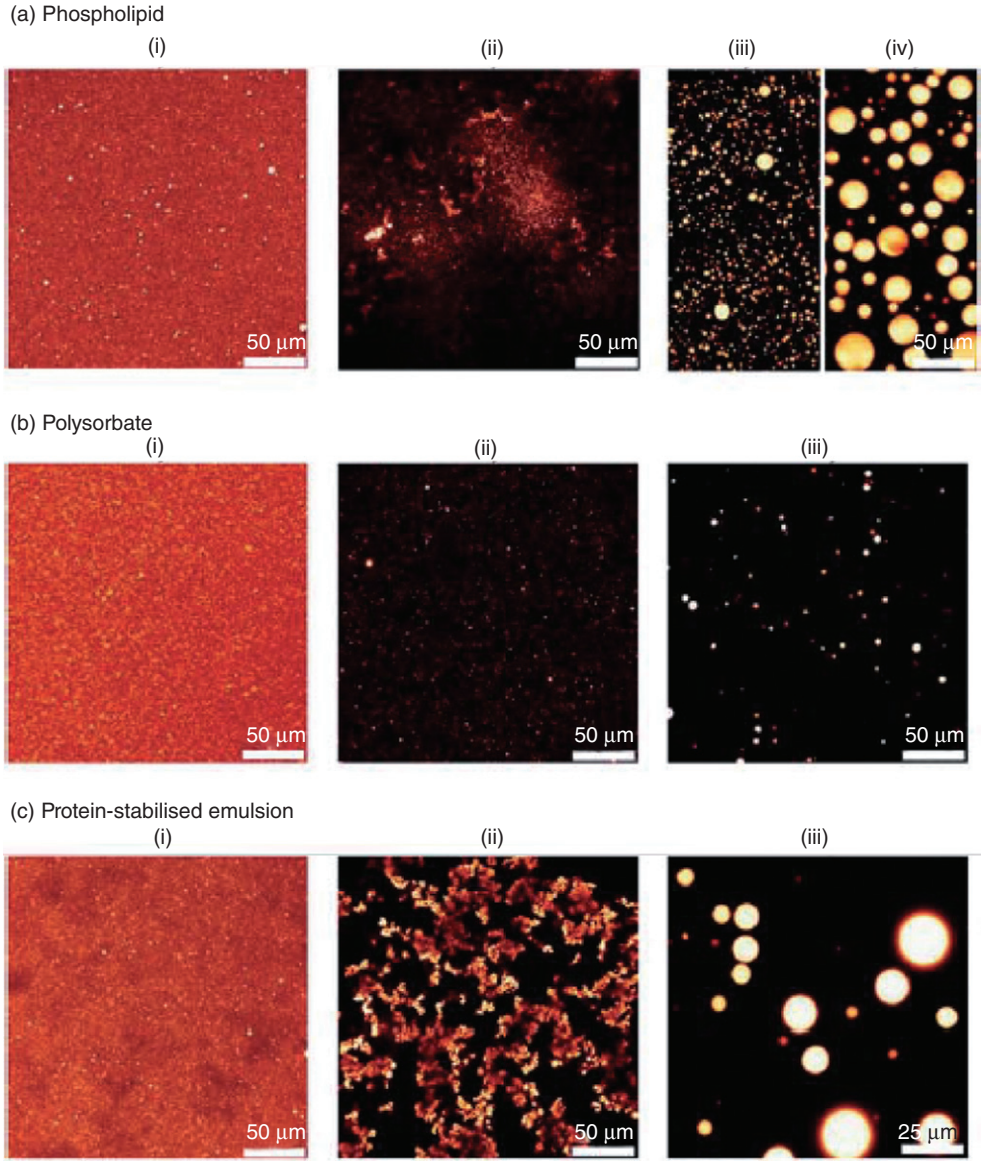

Fig. 10.10 Confocal micrographs showing changes in emulsion structure for 10% oil-in-water emulsions with different interfacial composition. Images taken: (i) prior to incubation in simulated gastrointestinal fluid; (ii) 30 minutes after exposure to simulated gastric fluid; (aiii) five minutes after exposure to simulated intestinal fluid (for phospholipid emulsion); (aiv), (biii) and (ciii) after 30 minutes exposure to simulated intestinal fluid. Bar = 50µm. Reproduced with permission from Golding *et al.*, (2011). Impact of gastric structuring on the lipolysis of emulsified lipids. *Soft Matter* 7(7), 3513–3523. Royal Society of Chemistry.

salts carry an appreciable negative charge at intestinal pH, which may be sufficient to prevent droplet approach and thus coalescence, as long as droplets are not in contact. In this way, even large droplets entering the small intestine environment can be maintained in the colloidal state (Hur *et al*., 2009). This is illustrated in Fig. 10.10a, which shows that for a sodium stearoyl lactylate emulsion that has already undergone significant coalescence in the gastric stage of digestion, there is little further change to droplet size distribution on introduction to simulated intestinal fluid (Golding *et al*., 2011). *In vivo* findings by Armand and co-authors (1999) also showed that large droplets of 10 μm delivered intragastrically did not appreciably change in particle size on entry into the small intestine.

10.4.3 The role of fat composition on lipid digestion

Fats and oils can display wide-ranging variations in material properties and solid fat content depending on temperature. Highly mono- and polyunsaturated oils are generally fully liquid from chilled to ambient temperature conditions. Fats containing higher levels of saturated fatty acids, such as butter, coconut and palm fat, tend to have higher melting points, and can therefore be solids from chilled to ambient. However, at 37 °C almost all natural food fats and oils are fully molten, and accordingly most studies do not give consideration to the material state of the dispersed phase.

Nevertheless, it is entirely possible to formulate emulsion systems to have a specific solid fat content at in-body temperature, such that droplets are at least partially crystalline. Under digestion conditions, the solidification of droplets results in some interesting behaviours. This is exemplified in a study investigating *in vitro* intestinal digestion an emulsion system stabilised with SDS in which solid droplets containing tripalmitin were compared to a liquid droplet system (Bonnaire *et al*., 2008). It was observed that whilst the rate and extent of lipolysis was lower for the emulsion systems comprising solid particles, nevertheless this emulsion still underwent an appreciable amount of lipid digestion (<35% after two hours incubation time).

When combined with an additional structuring mechanism, the presence of solid fat in an emulsion can have a more pronounced effect on lipid digestibility (Keogh *et al*., 2011). This was demonstrated for emulsion systems formulated to contain 25% solid fat at 37 °C, and comprising a mixed interface of milk protein and emulsifier. Under gastric conditions a combination of protein-induced flocculation and poor interfacial stabilisation (due to the presence of monoglyceride) resulted in extensive droplet partial coalescence being observed, to the extent that agglomerates could be visually observed. This resulted in a signifi-

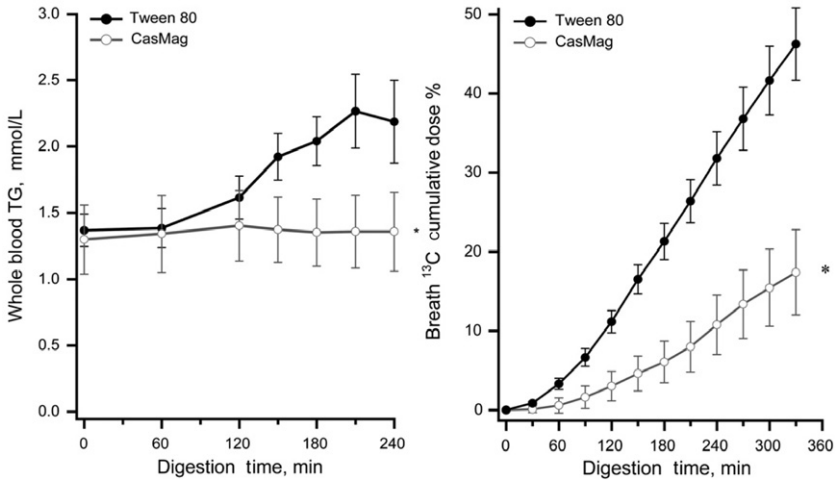

Fig. 10.11 *In-vivo* data comparing post-prandial lipid digestion markers for non-structuring emulsion stabilised with Tween 80 relative to a partially coalescing emulsion stabilised with sodium caseinate and monoglyceride. Study participants consumed 350 ml beverage emulsion with 30 g fat loading. Left-hand image shows whole blood triglyceride during the four-hour period after meal consumption. Right- hand image shows ^{13}C breath data for participants during the six-hour period post meal consumption. Reproduced with permission from Keogh *et al.*, (2011). Slowly and rapidly digested fat emulsions are equally satiating but their triglycerides are differentially absorbed and metabolized in humans. *Journal of Nutrition* 141(5), 809–815. American Society of Nutrition.

cant reduction in lipid surface area, not only during gastric treatment, but also during exposure to the intestinal environment (see Fig. 10.10).

Under *in vitro* conditions, the formation of these partially coalesced structures resulted in a significant reduction in rate and extent of lipolysis compared to stable emulsions comprising liquid droplets. Intriguingly, when applied to an *in vivo* study, consumption of a milkshake designed to partially coalesce in the stomach showed statistically significant suppression of plasma triglyceride, lower levels of CCK and PYY, and markedly different gastric emptying behaviour when compared to a stable control emulsion comprising liquid oil droplets (see Fig. 10.11); indicating that the behaviours observed under simulated conditions had been retained in the human study (Keogh *et al.*, 2011). It is speculated that the biomechanical forces present in the gut have no significant impact on the structural properties of agglomerates once they are formed, and as a consequence, surface area remains low over the course of digestion.

10.4.4 Droplet dissolution and mixed micelle formation

Prolonged exposure in the intestinal environment should be expected to result in gradual dissolution of emulsions due to generation and

movement of fatty acids and monoglycerides into mixed micelles prior to transport across the epithelium. This has been observed under *in vitro* conditions for four emulsion systems stabilised by separate emulsifiers (lecithin, polysorbate, whey protein and caseinate). After initial exposure to intestinal conditions, all emulsions displayed similar large droplet size distributions. However, after two hours of incubation, mean droplet size had been reduced to ~1 μm. A similar time-dependent decrease in particle size distribution was observed for whey-protein- and soy-protein-stabilised emulsions during simulated duodenal digestion, attributed to the liberation and self-assembly of fatty acids and monoglycerides generated during lipolysis (Nik *et al.*, 2011). The biological dissolution of emulsion droplets in mixed micelles, which enables fatty acid transport across the epithelium, is appreciably complex and remains a subject of research interest as part of determining a definitive mechanism (Bauer *et al.*, 2005).

During lipolyisis, the surface of emulsion droplets becomes enriched with surface- active components: bile salts, phospholipids, fatty acids, monoglycerides. This is understood to allow the surface-mediated assembly of mixed micelles, into which fatty acids are incorporated. Surface-assembled micelles subsequently desorb from the surface of droplets. Dissociation of these entities from the interface provides new binding sites for adsorption of bile salts and lipase–co-lipase complex, thereby continuing the lipolysis process (Porter *et al.*, 2007). An alternative theory has speculated that surfactant mesophases generated by lipolysis are able to penetrate the core of droplets, and that accordingly dissolution may occur across the entire droplet and not simply as a surface effect (Lentle *et al.*, 2011).

Neutron scattering has shown that the primary molecular structure of the mixed micellar moiety is in fact cylindrical (Hjelm *et al.*, 1995). However, it is also held that further dynamic transitions to the structural state of micellar assemblies takes place during the digestion cycle, depending on relative concentrations of lipid digestion products and endogenous surfactants (bile salts, phospholipids and cholesterol), pH environment and local ionic strength (Madenci and Egelhaaf, 2010). Irrespective of mechanism of formation or structure, micelles formed as a consequence of triglyceride hydrolysis to fatty acids are rendered discrete enough to diffuse across the mucus layer coating the intestinal epithelium, allowing transport of fatty acids into the enterocyte. The efficiency of this process ensures that up to 95% of dietary lipids are absorbed during intestinal transit, and accordingly the concentration of lipid components entering the colon is usually very low (Lentle *et al.*, 2011).

10.5 CONCLUSION

The digestion of fats and oils is a complex biological process. The immiscibility of lipids with the aqueous digestive environment requires that fat digestion is an interfacial process, and that accordingly the colloidal state is of significant importance in ensuring digestive efficiency. In this respect it has been shown that, in the absence of any gastrointestinal maladies, humans are remarkably efficient at processing and absorbing dietary lipids, irrespective of the state in which they are initially consumed. However, in comparison with other macronutrient components, it is the structural diversity of fats and oils that perhaps provides the greatest opportunity for manipulating digestive behaviour.

There is rapidly growing research interest and capability aimed at developing understanding as to how the dynamics of surface structures, continuous phase properties and macrostructural properties of emulsion systems behave during gastrointestinal transit. Through this, it is increasingly becoming evident that emulsion compositions with specific, and consistent, digestion behaviours can be designed. In this way, the lipid component of food systems can be more effectively targeted towards improved health and nutrition, for example by reduction in uptake, or for improved delivery of bioactive components.

It is recognised that to achieve full benefit from this approach requires the development of harmonised and verified *in vitro* models, fully representative of physiological conditions, and correlation of *in vitro* findings and properties into statistically meaningful outcomes under *in vivo* conditions (Dupont *et al*., 2011). Perhaps the greatest challenge still remains the adaptation and retention of novel digestive-fat structuring pathways in food products consumed as part of our daily food intake.

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