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TI: Impact of protein gel porosity on the digestion of lipid emulsions

Authors: Anwesha Sarkar, Jean-Marc Juan, Eric Kolodziejczyk, Simone Acquistapace, Laurence Donato-Capel, Tim J Wooster[#]

- corresponding author: timothyjames.wooster@rdls.nestle.com

Abstract:

The present study sought to understand how the structure of protein gels impacts lipolysis of gelled emulsions. The selected system consisted of an o/w emulsion embedded within gelatine gels. The gelatine gelled emulsions consisted of a discontinuous network of aggregated emulsion droplets, dispersed within a continuous network of gelatine. The rheology of the gelled emulsions was dominated by the rheological behaviour of the gelatine, at no point was yielding or brittle fracture observed suggesting a gelatine continuous structure rather than a bi-continuous gel. A direct relationship between the speed of fat digestion and gel average mesh size was found, indicating that the digestion of fat within gelatine gelled emulsions is controlled by the gels ability to slow lipase diffusion to the interface of fat droplets. Digestion of fat was facilitated by deposition of the gelatine network, which mainly occurred via surface erosion catalysed by proteases. Overall this work has demonstrated that protein gels can considerably slow the kinetics of lipolysis of gelled emulsions, key for the future development of structures to control fat digestion and or the delivery of nutrients to different parts of the gastrointestinal tract.

Introduction:

The increasing incidence of nutrition-related illnesses and their adverse impact on quality of life has led to strong efforts to understand how food is sensed, processed and digested within the gastrointestinal (GI) tract (*1*). The overconsumption of highly appealing energy dense foods is seen by some as a key contributor to this issue, and the consumption of diets rich in lipids is often cited as a leading cause of obesity (*2*). However, lipids are a good source of energy, essential fatty acids and play an important role in the absorption of micronutrients (vitamins, minerals), anti-nutrients (cholesterol) and class II pharmaceuticals (*3-8*). What is unknown is how much the balance of the role of fat in our diet is evolutionary or environmental. Hence, there is keen interest in understanding how foods in general and lipids in particular are transformed during eating, digestion and absorption (*4, 9, 10*).

In healthy adult humans fat digestion occurs both in the stomach (by acid stable gastric lipase) and the small intestine (primarily by co-lipase dependant pancreatic lipase but also by other minor lipases) (*11, 12*). The resulting fatty acids not only assist vitamin/nutrient absorption via forming mixed micelles, but also participate in short term control of food intake via negative feedback pathways that modulate gastrointestinal motility, primarily through the duodenal break mechanism,(*13, 14*) but also potentially through the ileal break mechanism (*15, 16*). The presence of free fatty acids in the duodenum stimulates cholecystokinin (CCK) secretion which slows gastric emptying by suppressing the muscular contractions of the antrum and enhancing the contractions of the pylorus (*14, 17*). The magnitude of this effect is a function of both the site and duration of small intestinal exposure to fatty acids, and is therefore largely affected by the way emulsions are emptied from the stomach and the speed of their digestion (*18-20*). The implications that different rates/extents of fat digestion have on human health are increasingly being recognised and understood (*21, 22*), hence there has been consistent interest in understanding how to control fat digestion (*23-25*).

The digestion of lipids is an interfacial process which is predicated by the adsorption of lipases to the interface of emulsified fat droplets (*3, 26, 27*). In healthy adult humans, fat digestion occurs both in the stomach by acid stable gastric lipase, and in the small intestine primarily by co-lipase dependant pancreatic lipase but also by other minor lipases. Combined the digestive capacity of the various lipases is generally a slight excess relative to the amount of fat ingested (*28, 29*). Because of the slight excess of enzyme relative to its substrate, the main mechanisms that have been used to

regulate fat digestion are to control lipases' ability to bind to the interface of emulsified fat droplets, either by; stopping enzyme activity (*30, 31*), controlling the composition of the interface (*32-35*), controlling the area of the interface (*22, 36*) or encapsulation (*37*). A number of groups have demonstrated in vitro and/or in vivo that such approaches can have considerable effects on; the rate (*22, 36*) and/or the extent (*31, 38*) of fat digestion. Whilst most of these studies have focused on controlling the adsorption of lipase to the interface of fat droplets, there have been relatively few studies that focus on controlling the transport of lipase to interface, using hydrogels for example.

Hydrogels have often been used in drug delivery and controlled release because the release of polymeric active ingredients can be controlled by designing; hydrogel porosity (through the combination of polymer volume fraction and cross-link density) and/or hydrogel-active affinity (*39-44*). In food natural (and modified) biopolymers such as polysaccharides (e.g. alginate, agar, agarose) and proteins (e.g. gelatine) are used to design the properties of hydrogel network (*45-48*). In particular the porosity (mesh size) of biopolymer hydrogels can be tailored by factors impacting structuring elements (volume fraction, distribution and sizes of pores …) of the gel such as chemical (e.g. hydrogen, covalent bonds, enzymatic crosslinking)/physical bindings (e.g. hydrophobic bonds) (*46-50*). For example, Klak *et al* recent demonstrated that the release of different molecular weight dextrans could be retarded by transglutaminase cross-linked gelatine gels (5 wt%) (*51*). Klak *et al* also demonstrated that the release of high Mw dextran was accelerated due to enzymatic degradation of the gel, highlighting the polymer release from hydrogels can occur via a combination of diffusion control and surface erosion (*51*). Emulsions have also been incorporated into biopolymer gels to impact the textural and flavour release properties of complex food systems (*52-57*). Somewhat surprisingly there has only been limited work on the control of lipid emulsion digestion (*23, 24*). Li *et al* encapsulated MCT emulsions stabilised by β-lactoglobulin into Ca-Alginate beads demonstrated that the kinetics of fatty acid release was affected (modestly) by alginate bead size, and considerably by alginate volume fraction and calcium concentration (*23*). Another recent study by Guo et al. suggested that the structure as well as gel strength of whey protein emulsion gel had an effect on delaying intestinal digestion by 60 min (*25*).

Whilst both Li *et al* (*23*) and Guo et al (*25*) related the retardation of lipolysis to the structure of the gel, mechanistic understanding of how gel architecture (particularly porosity and network architecture), impacts the transport of enzymes/digesta through a hydrogel and how this relates to the rate of lipolysis is missing. Therefore the present study sought to understand how the structure of protein gels impacts lipolysis of gelled emulsions. The selected system consists in a o/w emulsion embedded within gelatine gel. The structure of the gel was modulated with different protein concentrations. *In vitro* digestion of the systems was performed and the impact of different gel structures on lipase diffusion was assessed. In addition model experiments were conducted in order to understand the mechanism by which protein gels slow/retard lipase access to the fat droplets (diffusion control vs. surface erosion).

2.0 Experimental:

2.1 Materials

For the preparation of emulsion, sunflower oil (Coop, Switzerland) and whey protein isolate (WPI) containing 95.8% protein (Bipro, Davisco Foods International, US) were used without any further purification. Commercial gelatine Type B was obtained from Gelita (240 Bloom, 30 mesh, 98% protein). Milli-Q water (water purified by treatment with a Milli-Q apparatus; Millipore Corp., Bedford, MA, USA) was used as the solvent for all the experiments. Pancreatin (Porcine, USP $\times$ 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).

2.2 Methods

2.2.1 Emulsion preparation

The aqueous phase containing 5 wt% protein, was prepared by dissolving WPI in Milli-Q water and the pH was adjusted to pH 8. Protein dispersion was stirred at ambient conditions for 1 hour. Sunflower oil was then added to the aqueous protein solution to form final emulsions containing 40 wt% oil. Pre-emulsion was prepared by homogenizing the mixture of the aqueous phase with the oil phase using a Silverson L5M-A rotor-stator mixer (Silverson machines, UK) at 7000 rpm for 2 minutes. Then the pre-emulsion was further homogenised by two passes through a two-stage valve homogenizer (NIRO Soavi Panda, GEA, France) operating at 1000 bars for the first stage and 160

bars for the second stage. Sodium azide (0.05 wt%) was added to avoid microbial growth. Emulsions were stored for not more than 1 week at 4 °C.

2.2.2 Gelled emulsion preparation for in vitro digestion

Gelatine was solubilized of different concentrations (2.5-15 wt%) in 0.1 M NaCl solution at 60 °C. The pH of the gelatine solution was adjusted to pH 7.0 using 1 M NaOH. Sodium azide (0.05 wt%) was added to avoid microbial growth. Stock gelatine gels were prepared by allowing gelation in 100 mL glass containers at 4°C for 24 hours.

For preparation of gelatine gelled emulsions, the emulsion prepared as above and the gelatine dispersion were placed in water bath at 60°C. Emulsion was gently added to the gelatine solutions (10-25 wt%) to reach final concentration of 10 wt% oil, 0.7 wt% BLG and 2.5-15 wt% gelatine in 100 mM NaCl at pH 8. The emulsion/gelatine dispersion gelatine were mixed at 60 °C in a water bath in 100 mL glass container for 30 min and cooled down by storage in a fridge set at 4 °C for 24 hours to allow the gels to set. These gels were further used for in-vitro digestion and microscopy measurements.

2.2.3 Confocal microscopy

For the confocal imaging, the gels were sliced manually into 1 x 1 x 0.2-.03 mm slices, then stained overnight in a 10-6 M solution of Rhodamine 6G (Sigma #83697) containing Nile red (Sigma, #N3013: 100 µl of a 0.025 % ethanol solution per 10 ml of the rhodamine solution). The confocal imaging was carried out with a Zeiss LSM 710 confocal microscope, at excitation wavelengths of 514 nm for the Rhodamine 6G (emission bandwidth: 520-560) and 561 nm for the Nile red (emission bandwidth: 565-700 nm).

Lipase diffusion experiment: For the diffusion experiments, the gels were cooled down in petridishes and punched with the capillary end of glass Pasteur pipettes. This capillary end, with the sample inside was then glue at the bottom of a 3 mm deep Plexiglas observation chamber. Finally, this experimental setup was covered with the solution containing the lipase (50 µl labelled lipase in 1 ml of PBS). The continuous image acquisition was carried out at an excitation wavelength of 488 nm for 30 minutes (120 images per run). The experiments were carried out in triplicate.

2.2.4 Characterization of visco-elastic properties of gelled emulsion by small deformation rheology

Oscillatory measurements were carried out using a Physica MCR 501 rheometer (Anton Paar, Graz, Austria). The measuring geometry was a concentric cylinder (CC-27; the inner diameter of the cup is 24.5 mm and the diameter of bob is 23 mm) with a peltier temperature control system. 20 milliliters of gelatine emulsion dispersion described in 2.2.2 and as control, corresponding gelatine dispersion with similar concentration were placed in the measuring cell at 60 °C, and the surface of the sample was covered with mineral oil to avoid evaporation. The sample was then submitted to temperature ramp mimicking the temperature ramp of sample prepared for in vitro digestion protocol (described in 2.2.7) allowing gelation in situ of equipment. Temperature was decreased from 60 °C to 4 °C at -5 °C/ min. Then, the temperature was maintained for 2 hours at 4 °C. Finally, the temperature was raised to 25 °C at +5 °C/min. The temperature ramp was done within linear regime with strain of 0.5% at 1 Hz frequency. A frequency sweep test was then performed from 0.1 to 100 Hz at 0.5 % strain at 25°C. Finally a strain sweep test was performed from 0.1 to 20 % strain at 0.1 Hz at 25°C. Critical gelation temperature was defined as G', G'' crossover. Final gel strength was defined as final G' modulus obtained at 25 °C after temperature ramp was completed. Triplicate gelatine measurements were performed for each system. Gel point was defined as the cross over between G' and G'' (*58, 59*). Final gel strength was taken as value of G' measured at the end of temperature profile at 25°C.

2.2.5 In vitro digestion

The digestion kinetics of gelled emulsion systems was examined using a simulated intestinal digestion model at 25°C with conditions similar to Wooster *et al*, (*24*). Briefly, a single piece of gelled emulsion (20x 20x5 mm ~ 2 grams) was mixed with 8.5 mL of simulated intestinal fluid (SIF) to achieve a final composition of 10 mM $CaCl_2$, 12 mM mixed bile salts, 0.75 mM phospholipid, 150 mM NaCl and 4 mM tris(hydroxymethyl)aminomethane buffer. The temperature and pH were stabilised for at least 2 min at 37 ± 0.5°C and 6.8 ± 0.02 respectively, the titration was then initialised with the addition of pancreatin solution (200 µL, containing 150 mM NaCl, 4 mM Tris HCl and 125 mg/mL pancreatin). The digestion was carried out over 3 h whilst maintaining the pH at 6.8

by addition of 0.05 M NaOH using a pH-STAT (TIM 854, Radiometer). During the intestinal phase of digestion, the kinetics of digestion were followed using a pH-STAT technique and expressed as titratable acid (rather than fatty acid) and was calculated by the equation:

$$TA = VNaOH \times 0.05 \times 1000 \quad \text{Equation 1.}$$

TA: Total titratable acid released, µMol; VNaOH: volume of NaOH used to titrate the released acid in 3 h, mL.

3.0 Results & Discussion

3.1 *Microstructure of gelled emulsions observed by CLSM*

The mixing of the whey protein isolate (WPI) stabilised emulsion with different gelatine concentrations lead to the formation of gels whose structure changed with increasing gelatine concentration (Figure 1 A-F). The WPI-stabilised emulsion itself presents as individual droplets with a log-normal particle distribution and an average diameter, $D_{4,3}$ of 0.32 µm (Figure 1A). Mixing WPI emulsion (10 wt%) with 2.5 wt% gelatine and gelling at 4^{o}C results in a homogeneous structure where emulsion droplets are evenly distributed throughout the gel (Figure 1B). Increasing the gelatine concentration to 5 wt% (Figure 1C) results in a change in gelled emulsion structure, the emulsion droplets appear as aggregates and light green voids (protein) appear between the aggregates of droplets. Increasing the gelatine concentration higher to 7.5, 10 and 15 wt% causes an increase in the size/number of both the emulsion aggregates and the void spaces between the aggregates (Figures 1D-F). The voids between the emulsion droplets consist of regions rich in gelatine as indicated by the dark green hue in the confocal images (Figure 1D inset) and by visual observations that these gels are very firm. These observations indicate that as gelatine concentration is increased the resulting structure is a discontinuous network of aggregated emulsion droplets contained within a continuous network of gelatine. The aggregation of the emulsion droplets in the gel could either be due to the presence of salt (100 mM) or the addition of gelatine. Given the strong increase in aggregation with increasing gelatine concentration, and the fact that similar emulsions (β-lg) have been shown to be (relatively) stable to the presence of 120

mM NaCl (*60*), we suggest that the main driving force for aggregation is depletion interactions caused by the self-association of gelatine during the gelation process.

3.2 Viscoelastic properties of gelled emulsion

The gelation behaviour of gelatine is known to be mainly driven by temperature and gelatine concentration such that lower temperatures and higher concentrations resulted in faster and stronger gel formation (*54, 61*). Figure 2 shows the time-dependent storage modulus G' and loss modulus G" of 15.0 wt% gelatine gelled emulsion and the corresponding 15 wt% gelatine gel as a function of temperature. A steep increase in G' and G'' was observed as the temperature was decreased to 4 °C, after which they remained fairly constant during ageing at 4 °C for two hours. Both G' and G'' decreased upon heating to 25 °C, due to weakening of the H-bonded triple helix. Similar changes in G' and G'' as a function of temperature were observed for all gelatine concentrations used to make simple gels or gelled emulsion systems.

In order to understand the impact that incorporating an emulsion has on the properties of gelatine gels, dynamic rheological experiments were conducted to assess gel point and gel viscoelasticity as a function of gelatine concentration. Figure 3 shows the gel point (A) and storage/loss moduli (B) (at 25^{o}C) for simple gels and gelled emulsion systems as a function of gelatine concentration. As gelatine concentration increased from 2.5 to 10 wt%, the gel points of both systems increased linearly from 7°C to 20°C then increased slightly to 23°C at 15 wt% gelatine. No significant differences in gel point were found between the gelled emulsion systems and simple gelatine gels. The magnitude of the storage modulus *G*' at 25^{o}C increased from 0.95 Pa to 2325 Pa for the gelled emulsion system as the gelatine concentration increased from 2.5 wt% to 15.0 wt%. Simple gels containing 2.5 wt% gelatine showed a higher storage modulus (6.9 ± x vs. 0.95 ± 0.26 Pa) and a similar loss modulus (0.57 ± vs. 0.69 ± Pa) compared to gelled emulsion systems. At and above 5 wt% gelatine it was the gelled emulsion systems that exhibited higher loss and storage moduli (Figure 3B).

Frequency sweep test spectra of selected gelled emulsion containing different concentrations of gelatine as well as simple gelatine gels is shown in Figure 4. Similar behaviour was observed for both systems at all concentrations above 5.0 wt% (data not shown for 7.5% and 15%). In presence of 5-15 wt% gelatine, a strong gel like behaviour is observed characterized by predominance of G'

over G'', the absence of crossover points between the G' and G" and also by weak dependence on frequency (*62*). The dependence was slightly higher at 5% than 10% and above. In contrast, gelled emulsion containing 2.5 wt% gelatine presented a mechanical behaviour of rather a weak gel with G' close to G'' at low frequency with little differences between the moduli, and cross over at high frequency (*59*). Additional strain sweep test (not shown) formed with 2.5% gelatine broke at very low strain (0.2%) whereas gelled emulsion systems with higher gelatine concentration did not break when strain increased up to 100%. Similarly, the gelatine gel also showed strain breakage only for 2.5 wt% gelatine gels but at higher strain level (16.2%).

Overall results suggests that the rheology of gelled emulsion systems is mostly governed by gelatine gelation at concentrations equal or above 5 wt% gelatine leading to strong gels with higher moduli than the corresponding simple gelatine gels. At 2.5 wt% gelatine, the gelled emulsion system was weaker than simple gelatine gel suggesting a different structure. This mechanical behaviour confirms the above description of the CLSM microstructure suggesting a discontinuous network of aggregated emulsion droplets dispersed within a continuous network of gelatine which govern the overall mechanical properties of the gelled emulsion. At gelatine concentration higher than 2.5 wt%, aggregation of emulsion droplets was visible which could be related to the higher storage modulus of the gelled emulsion compared to simple gelatine gels. Previous studies have shown that incorporation of particles or oil droplets could lead to an increase in gelatine gel rigidity depending upon the gelatine concentration (*53, 63*). Oil droplets seem to contribute to the stiffening of the gel as they are mechanically connected to the gel matrix and so contribute to the gel strength (*52*). At 2.5 wt%, the lower storage modulus compared to gelatine system might be related to the discontinuous dispersion of emulsion within the continuous gelatine network (*55, 64*)

3.3 Estimation of gel porosity using rheological data.

A key hypothesis of this work is that the digestion of gelled emulsions is a related to gelatine network structure through the decrease in network porosity within increasing gelatine concentration. In order to understand this relationship, the average mesh size (ie. pore size) of simple gelatine gels and gelled emulsions was calculated using equation 2 (*51*):

$$\xi^3 = \kappa_B T/G' \qquad \text{Equation 2.}$$

where, ξ is the mesh size and G' represents the storage modulus (Pa), K_B is the Boltzmann constant and T is the temperature.

Figure 5 shows the variation of mesh size as function of gelatine concentration for gelled emulsion systems and simple gelatine gels. The mesh size of gelled emulsion systems decreases from 164 nm to 20 nm with increase in gelatine concentration from 2.5 to 7.5 wt% and to 12 nm with 15 wt% gelatine (Figure 5). The corresponding simple gelatine gels showed slightly higher mesh sizes at 5 and 7.5 wt% gelatine (38, 30 nm; vs. 24, 20 nm) and similar mesh sizes at 10 and 15% concentration (18.5 nm; 12 nm). At 2.5 wt% gelatine, the simple gelatine gels had significantly smaller average mesh size (85 nm) compared to gelled emulsion (164 nm). These results can be understood from the structural changes observed by CLSM by dynamic rheology. The presence of emulsion within the gelatine network at 2.5 wt% modified its structure suggesting a less dense weaker network structure than that of the simple gelatine gel. Thus, the gelled emulsion has an increased mesh size as compared to the gelatine system. As gelatine concentration increased, this effect was less visible since the emulsion droplets start to aggregate locally and the gelatine network becomes the main continuous network in the gelled emulsion systems. Consequently, the average mesh size decreased in the gelled emulsion as compared to the corresponding pure gelatine gels. Overall these results suggest that at 2.5 wt % gelatine, the emulsion droplets are evenly distributed in the gelatine network decreasing its connectivity and overall gel strength compared to gelatine systems. At 5 and 7.5 wt% gelatine, the increase in gelatine concentration leads to decrease in average mesh size and the local aggregation of the emulsions droplets further contributes to the increase in gel strength compared to gelatine systems. As gelatine concentration increases further (10 and 15 wt%), less differences compared to gelatine systems were visible suggesting that the local aggregation of emulsion droplet does not contribute significantly to the overall mechanical behaviour of gelled emulsion. Above 10 wt% gelatine, the structure is mainly governed by the gelatine continuous network and thus mesh sizes decrease to minimum level as gelatine concentration increased in both the gelatine gel and gelled emulsion systems.

3.3 Impact of gelatine gels on fat emulsion digestion

A central concept of this work is that surrounding a fat emulsion in a protein gel will slow down the digestion of the fat because the protein network slows lipase diffusion to the emulsion droplet. In order to assess the impact of gelatine gels on the digestion of emulsions, gelled emulsion of differing gelatine concentrations were assessed by simulating digestion only the intestine phase of

digestion (at 25^{o}C) using a pH-STAT technique (Figure 6).[1] As control the emulsion alone was also measured. The emulsion underwent rapid digestion with an initial acid release rate of 100 uMol min^{-1}, and released 580 uMol acid after 15 minutes before going through a point of inflection after which the rate of acid release slowed to 0.6 uMol min^{-1}. Surrounding the emulsion in a gelatine gel progressively decreased the rate of acid release (Table 1). The WPI emulsion in 2.5 wt% gelatine had an initial acid release rate of 35 uMol min^{-2}, this systematically slows being 11 uMol min^{-2} at 7.5 wt% gelatine and 3.5 uMol min^{-2} at 15 wt% gelatine. Each sample released a similar maximum amount of acid of 700 uMol. Each exhibited an inflection point in the acid release curve from fast to slow acid release at increasingly longer times as gelatine concentration increased. A solution of 10 wt% gelatine, had an acid release rate of 0.32 uMol min^{-2} and released 17.5 uMol acid after 60 minutes showing that proteolysis of the gelatine itself contributed minimally to the observed differences in acid release and that the kinetics of acid release and essentially be regarded as kinetics of fat digestion.

It is apparent from Figure 6 and Table 1 that there is a systematic relationship between gelatine concentration in emulsion gelled and the kinetics of lipolysis. As described earlier the gelatine concentration impacts on overall structure of the gelling particular its porosity. It is well know that the porosity of a gel will affect the diffusion of large molecules through the gel. Hence, an understanding of the porosity of these gelled emulsions as a function of gelatine concentration was obtained from rheology data using equation 2 (*51*). Figure 6 shows the relationship between the kinetics of lipolysis and mesh size of gelled emulsion. It is apparent that there is a direct almost linear relationship between gel mesh size and the kinetics of lipolysis. This suggests that smaller mesh size of the gelatine gel (at higher gelatine concentration) restricts the transport of lipase to the emulsion droplet. The question is whether digestion is slowed because lipase diffusion through the gel is slowed or because lipase is not able to diffuse into the gel and proteolysis of the gel is first required in-order for lipolysis to occur.

In order to understand the mechanisms by which protein gels slow the digestion of gelled emulsions two experiments were conducted; i) digestions of the gelled emulsion in the presence of

[1] A highly artificial digestion model was used to test the concept that protein gels can slow fat digestion. A temperature of 25oC was used to prevent gelatine melting; this is not representative of human physiology (37^{o}C). In addition, oral or gastric steps of digestion were omitted – both phases are likely to impact digestion through trituration and proteolysis.

pure lipase (Figure 7) ii) measurement of lipase transport through pure gelatine control gels (Figure 8). Figure 7 compares the digestion of the 10 wt% O/W emulsion trapped within at 10 wt% gelatine gel by pancreatin and pure pancreatic lipase in the presence of 12 mM mixed bile and 10 mM $CaCl_2$ at pH 6.8 25^{o}C. Digestion of the gelled emulsion by pancreatic results in a moderate rate of acid release, having a constant rate of 8.9 ± 0.3 uMolmin^{-1} over the first 60 minutes, gradually slowing over the next 60 minutes to reach 90% digestion after 120 minutes. In contrast, digestion of the same composition by pure pancreatic lipase (with co-lipase) resulted in a tenfold decrease in digestion kinetics (Figure 7ii). After an initial lag phase of ~30 minutes acid release reaches a constant rate of 0.76 ± 0. 095 uMolmin^{-1} between 60 and 180 minutes (2-15% digestion) and a constant rate of 0.45 ± 0.036 uMolmin^{-1} between 420 and 840 minutes (30-60 % digestion). The differences between digestion by pancreatin and pure pancreatic lipase corresponds to a 11 fold reduction during the initial stages of digestion and 20 fold reduction during the majority of digestion. What is interesting is that the rate of acid release from the gelled emulsion digested with pancreatin is 10 times lower than the rate of digestion of the parent emulsion or the melted gelled emulsion (100 uMol min^{-1}). These results highlight that the gelatine gel is able to slow the diffusion of both lipase and proteases.

This dramatic reduction in lipolysis can be understood from the fact that the tight mesh of the gelatine gel restricts lipase transport to the emulsion droplets embedded within the gel. The average pore size of this system was estimated to be 18.5 nm (10 wt% gelatine), and the radius of gyration of the pancreatic lipase/co-lipase complex is 25-26 A (*65*). Given that the average pore size is approximately 3-4 times larger than the pancreatic lipase/co-lipase complex, lipase should be able to diffuse through the gel, which was confirmed by digestion experiments with pure lipase. In addition, confocal microscopy measurements show that the diffusion of lipase through 5 wt% gelatine gels is much faster than the diffusion through 10 wt% gelatine gels (Figure 8). Whilst it is apparent that Lipase can diffuse through the gelatine gel, the lipolysis rate is considerably retarded compared to the free emulsion (~130 times slower), indicating that the gel provides a (restricted) diffusion barrier to the digestion of the emulsion. The fact that digestion occurs at 10-20 times faster in the presence of protease indicates that the main decomposition mechanism is surface erosion of the gel by proteases. A limitation of this work is that the gel systems studied are thermo-reversible and melt at in-body temperatures. However, these results do demonstrate that protein gels can act to slow lipolysis. Some might consider that the gel pieces at 20 x 20 x 5 mm are

too large to empty from the stomach and anyway would undergo erosion during chewing and trituation (*66*). However, the effective diffusion distance is thinnest dimension of the gel, which at 5 mm is within the range of particles that empty from the stomach (*66*). Overall this work highlights a new approach to control the digestion of lipid emulsions by controlling lipase access to the interface of emulsions via a diffusion barrier adjacent to the interface. The new insights in this work provide new design principles for the controlled digestion of food to impact on satiety and weight management or the controlled delivery of active ingredients at different times and/or places within the digestive tract.

4 Summary & Conclusions

The present study sought to understand how the structure of protein gels impacts lipolysis of gelled emulsions. The selected system consists in o/w emulsion embedded within two different protein gel matrices gelatine gels and bovine serum albumin gels. The structure of the gel was modulated with different protein types and concentrations. *In vitro* digestion of the systems was performed and the impact of different gel structures on lipase diffusion was assessed. The gelatine gelled emulsions consisted of a discontinuous network of aggregated emulsion droplets, dispersed within a continuous network of gelatine. Rheological measurements found that the base gelatine gel exhibited thermo-reversible elastic type behaviour characteristic of gelatine. Incorporation the emulsion into low protein concentration gels weakened the gels due to interruption of network connectivity. Incorporation of the emulsion into gels with ≥ 5wt% gelatine, lead to a slight strengthening of the gel. The rheology of the gelled emulsions was dominated by the rheological behaviour of the gelatine, at no point was yielding or brittle fracture observed suggesting a gelatine continuous structure rather than a bi-continuous gel. The speed of fat digestion of gelatine gels correlated with protein concentration within the gel. The average gel mesh size (ξ) was estimated from the storage modulus (equation 2), and a direct relationship between lipolysis kinetics and average gel mesh size was found. This direct relationship between Klip and ξ indicates that the digestion of fat within gelatine gelled emulsions is controlled by the gels ability to slow lipase diffusion to the interface of fat droplets. Studies using pure enzymes identified that the tortuous gelatine network significantly slowed lipase diffusion into the gel, but did not stop it. The main mechanism of gel breakdown was surface erosion catalysed by proteases. Overall this work has demonstrated that protein gels can significantly slow the kinetics of lipolysis of gelled emulsions.

This information can be of great use in the design of foods and nutraceuticals to control fat digestion.

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Table 1:

	Gelatine concentration in 10 wt% gelled emulsions					
	0 wt%	2.5 wt%	5 wt%	7.5 wt%	10 wt%	15 wt%
Klip (uMol min^{-1})	100	35	18.5	11	6	3.5
T infect (min)	13	24.5	55	90	149	260

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