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Dairy structures and physiological responses: a matter of gastric digestion

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Abstract

Digestion and health properties of food do not solely rely on the sum of nutrients but are also influenced by food structure. Dairy products present an array of structures due to differences in the origin of milk components and the changes induced by processing. Some dairy structures have been observed to induce specific effects on digestion rates and physiological responses. However, the underlying mechanisms are not fully understood. Gastric digestion plays a key role in controlling digestion kinetics. The main objective of this review is to expose the relevance of gastric phase as the link between dairy structures and physiological responses. The focus is on human and animal studies, and physiological relevant *in vitro* digestion models. Data collected showed that the structure of dairy products have a profound impact on rate of nutrient bioavailability, absorption and physiological responses, suggesting gastric digestion as the main driver. Control of gastric digestion can be a tool for delivering specific rates of nutrient digestion. Therefore, the design of food structure targeting specific gastric behaviour could be of great interest for particular population needs e.g. rapid nutrient digestion will benefit elderly, and slow nutrient digestion could help to enhance satiety.

Key words:

dairy foods, food structure, food matrix, gastric digestion, bioaccessibility, bioavailability,

Abbreviations:

AA, amino acid; GI, gastrointestinal; GE, gastric emptying; UHT, Ultra High Temperature; β-Lg, β-Lactoglobulin; MFGM, milk fat globule membrane; TAG, triacylglyceride; CVD, cardiovascular diseases.

1.Introduction

Dairy products have been established as excellent foods due to their high nutritional value. However, several controversies have been arisen from their consumption in relation for instance to their high contribution of saturated fats, which are linked to cardiovascular diseases (CVD), through the increase of blood lipids in particular low-density lipoprotein (Griffin, 2017). Nevertheles, there is a paradox in the fact that increasing evidence has shown the consistent neutral or even benefitial associations between dairy food consumption and CVD as shown in meta-analysis of prospective cohort studies (Givens, 2017), and several other diseases (Thorning, et al., 2016). A multinational cohort study in 21 countries from five continents was recently published showing that the consumption of dairy products (milk, yoghurt and cheese) was associated with lower risk of mortality and CVD (Dehghan, et al., 2018).

This discrepancy is probably due to the generalised approach of evaluating the health benefits of a food according to its individual components rather than the structure (or matrix). The food matrix is defined as the arrangement of food constituents and their interactions at multiple spatial length scales (Parada & Aguilera, 2007), see Figure 1. The structure of food either occurs naturally (i.e. milk) or is created or destroyed by processing (e.g. yoghurt and cheese) and preparation. Furthermore, specific conditions of processing (e.g. the pressure of milk homogenisation and ripening time in cheese) will modulate the physico-chemical properties and the structure of the final product at the different length scales. This will probably affect the digestion and metabolism of the foods in different ways. Therefore, when investigating the health effects of dairy products, the whole matrix and its specific structure has to be considered (Thorning, et al., 2017).

The physico-chemical characteristics of food components and the whole matrix impact how individual components interact and behave within the gastrointestinal (GI) tract. Research has mainly focussed on the small intestine digestion in order to control nutrient digestion using different mechanisms such as the ileal break (Maljaars, et al., 2008) by manipulating interfacial composition of lipid droplets for instance, that alters the access to enzymes and bile and might slow the release of lipid.

58 However, possibly having a more significant impact, the stomach can play a key role in controlling the
59 rate of nutrient absorption and subsequent physiological responses. A schematic diagram illustrating
60 this approach is displayed in Figure 2. There are several complex processes occurring in the gastric
61 compartment: enzymatic (pepsin and gastric lipase), physical (e.g. peristalsis) and chemical (e.g. pH
62 decrease and ionic composition).

63 The behaviour of food structures will depend on their physico-chemical properties in the gastric
64 environment. This might cause restructuring, i.e. change of the initial structure, which will alter the
65 food's functional characteristics. Several types of colloidal behaviour might be observed depending on
66 the stability of the structure. Golding, et al. (2011) showed that colloidal structures can be tailored to
67 exert different behaviour under the acidic conditions of the stomach. The properties of food structure
68 adopted in the stomach will profoundly impact gastric disintegration and the rate of gastric emptying
69 (GE), i.e. gastric contents gradually delivered into the duodenum. Marciani, et al. (2007) showed that
70 gastric acid-unstable emulsions led to the formation of an intragastric oil layer to be formed on top of
71 the chyme in the stomach. This accelerated GE of the aqueous phase followed by a slow emptying of
72 the intragastric oil layer in contrast to the slow GE of gastric acid stable emulsions. Then, due to
73 differences in the rate of delivery of nutrients to the duodenum, they might be absorbed and metabolised
74 at different rates, altering hormonal secretion and physiological responses. For instance, the slower GE
75 of the acid stable emulsion in the study by Marciani, et al. (2007) study led to a greater secretion of
76 CCK and greater satiety. A slow GE has been also shown to help diabetics by reducing peaks in lipemia
77 (Rayner, et al., 2001). Therefore, control of GE by intragastric behaviour of digesta can be essential for
78 ensuring optimal digestion addressed to specific physiological responses. For instance, foods with slow
79 nutrient digestion might induce fullness for longer, which could be useful for obese/overweight
80 population. In contrast, fast protein digestion and uptake would be beneficial for elderly individuals and
81 athletes by enhancing muscle synthesis. This approach should be further studied and exploited to design
82 healthier food structures in the future.

83 To achieve this, a deep understanding of the mechanisms of food breakdown in the stomach is critical.
84 This research should progress further in the light of the development of sophisticated in vivo techniques

such as magnetic resonance imaging (MRI). Despite the gold standard for investigating nutrient digestion being the human body, there are several reasons that constrain its use; it is highly variable, costly, time-consuming and might generate ethical issues. *In vitro* models are widely used, and they are mainly classified into static and dynamic. Sophisticated dynamic models can simulate the dynamics of stomach physiology, but they are also expensive and restrictive. One example is the human gastric simulator (HGS) developed at the University of California, Davis (Kong & Singh, 2010). In contrast, static models are cheap, simple and useful in predicting the overall nutrient hydrolysis and end-points values of the digestion *in vivo* (Egger, et al., 2017), however they do not consider the structural changes and nutrient breakdown kinetics that occur in response to dynamic changes in gastric digestion conditions.

In an effort to fill the gap between *in vitro* digestion models, a semi-dynamic method was recently developed (Mulet-Cabero, et al., 2019; Mulet-Cabero, et al., 2017), which can mimic the main gastric dynamics of gradual acidification, fluids and enzyme secretion and emptying at low-cost. The development of a standardised protocol for this semi-dynamic model is ongoing and the method will be published in 2019 by INFOGEST members.

This literature review therefore aims to illustrate how the physiological responses and patterns of digestion observed following consumption of dairy products with different structures might be linked to gastric digestion and the need for its further study. The review will focus on assessing how dairy structures at several length scales affect nutrient bioaccessibility and bioavailability kinetics leading to different metabolic effects, in light of gastric digestion. In that view, *in vivo* studies and *in vitro* studies using dynamic and semi-dynamic models are mainly considered. Dairy structures from bovine origin are only considered, excluding studies where milk from other animal sources was used. The key conclusions of the main research articles to date have been summarized in Table 1, and these findings will be discussed in more detail in the following sections.

2.Effect of dairy proteins on digestion and physiological responses

Milk proteins are classified as high-quality proteins considering human amino acid (AA) requirements and digestibility. They have high true digestibility and postprandial protein utilization of 95-96% and 74%, respectively (Bos, et al., 1999). Milk proteins can be generally considered as a better source of protein compared to plant proteins since the later proteins are less digestible and deficient in one or more essential amino acids (EAAs) and their leucine (Leu) content is 6-8%, compared to 10-13% in dairy proteins (Gorissen & Witard, 2018). The EAAs, in particular branched chain AAs (BCAAs), are important since they exert a key role in muscle protein synthesis (Wolfe, 2002). Milk protein ingestion has been suggested to have benefits on cardiometabolic health (Fekete, et al., 2016). Moreover, milk proteins have important biological functions, casein micelle carries calcium and phosphate for efficient absorption. Several peptides from milk proteins have been reported to exert certain functions such as antihypertensive, opioid-like activity and antithrombotic properties (Fekete, et al., 2015; Jauhiainen & Korpela, 2007).

2.1.Bioaccessibility of dairy proteins

The nature of dairy proteins in terms of their molecular structure and physico-chemical properties has a strong impact on gastric and intestinal digestion which subsequently affects the bioaccessibility of nutrients. The main milk proteins have different physico-chemical properties, which are governed by their structure. Caseins have a relatively open and flexible conformation forming ordered structures known as casein micelles and are insoluble at their isolectric point, pH 4.7. In contrast, whey proteins have globular, compact structure and are soluble under acidic conditions.

At the molecular level, caseins have been reported to be more easily digested than whey proteins during the simulated gastric phase (Egger, et al., 2017; Macierzanka, et al., 2009). In addition, the same authors Macierzanka, et al. (2009) showed that the degree of proteolysis in β-Lactoglobulin (β-Lg) was increased when emulsified with oil, which was due to the partial unfolding of the β-Lg secondary structure improving accessibility to pepsin. These studies were performed in static conditions in which the pH of the gastric phase remain constant at pH 3.

This contrasts with *in vivo* results showing that caseins are slowly digested and whey proteins are rapidly digested. In general, it is assumed that caseins coagulate in the gastric conditions whereas whey proteins remain relatively soluble. The coagulation of caseins could be mainly driven by the action of pepsin that cleaves the Phe^{105}-Met^{106} band in k-casein, together with the gradual pH decrease occuring in the stomach (Figure 3).

Despite the fact that there are several indirect indications suggesting this behaviour occurs, no direct visual evidence of the restructuring of caseins occurring in the human stomach has been reported so far. Some studies reported the markedly different GE and digestion kinetics between the main milk proteins (Mahé, et al., 1991; Mahé, et al., 1996). However, there are contradictory studies in which no differences in GE for the different milk proteins were found (Calbet & Holst, 2004; Lang, et al., 1998). In the latter studies, sodium or calcium caseinate was used whereas milk or micellar casein were digested in those studies where the GE was different. Therefore, the state of the caseins and its processing history seems to strongly influence its digestion behaviour in the stomach. Caseins in the micellar state are coagulated by pepsin (Tam & Whitaker, 1972) and low pH (Dalgleish & Corredig, 2012). This contrasts with caseinate, i.e. a mixture of caseins with the calcium phosphate removed, which does not have the same micellar-type structure and is not coagulated by pepsin. In this review, studies using caseins in micellar form will be mainly discussed because it is the most relevant structural form found in natural dairy products.

In that context, Miranda and Pelissier (1981) showed in rats that the rate of GE in skimmed milk was slower compared with that of a mixture of denatured caseins and the proteolysis in the stomach was much lower in the skimmed milk reporting that α_{s1}-casein and β-casein were almost undergraded and κ-casein was converted into para- κ-casein.

The distinct rate and composition of the products delivered to the small intestine after the ingestion of different milk proteins suggest a different time of residence and behaviour in the stomach. Mahé, et al. (1996) investigated the digestion kinetics of intrinsically labelled ^{15}N β-Lg and casein drinks, which were fed to healthy young volunteers. The effluents from the jejunum were collected by a nasal tube and their protein contents and flow rate were assessed. The jejunal flow rate peaked in the first 20 min

after β-Lg digestion, which was present mostly in the intact form. In contrast, caseins were more slowly recovered in the jejunum in a more degraded form. These results were supported by a more recent human study (Boutrou, et al., 2013) where the authors found that, after casein ingestion, the delivery of dietary protein in the jejunum was progressive for 6 hours and in the form of medium size-peptides (750-1,050 Da). In contrast, the ingestion of whey protein induced the release of larger-size peptides (1,050-1,800 Da) and was completed after 3 hours. The authors suggested that the coagulation of caseins could lead to a slower rate of emptying compared to a more rapid emptying of the whey proteins in solution. Therefore, caseins coagula could be more exposed to pepsin hydrolysis leading to the emptying of more degraded products.

2.2. Absorption and protein metabolic utilization of dairy proteins

The distinct pattern of protein digestion in the GI tract has been reflected in different rates of AA absorption, which might modulate the postprandial metabolism of whole body protein synthesis, breakdown and oxidation in the liver. Boirie, et al. (1997) performed a study on young healthy subjects using intrinsically ^{13}C-Leu labelled whey protein and micellar casein drinks, which were matched for Leu content but were not isonitrogenous. The postprandial whole-body Leu balance, considered as an index of protein deposition, was assessed by tracing samples from blood and breath. The plasma AA appearance, i.e. aminoacidemia, was fast, high and transitory after whey protein drink ingestion, which led to an increase in whole body protein synthesis (68%) but no support in whole body protein breakdown. In contrast, the ingestion of a casein drink resulted in a lower, slower and prolonged release of AAs. This was associated with a markedly higher inhibition of whole protein breakdown (34% for 7 hours), but just slight stimulation of whole protein synthesis (31%) compared to whey protein drink. However, the Leu balance was positive for the casein drink over 7 hours, promoting protein deposition whereas no effect was provided from whey protein drink. The authors classified, in relation to that postprandial behaviour, whey proteins and casein as ‘fast’ and ‘slow’ digested proteins respectively, which has been widely considered in the literature. Similarly, Lacroix, Bos, et al. (2006) showed in healthy volunteers that the aminoacidemia for the first hour in whey proteins ingestion was rapid and

high followed by a decrease reaching values below the baseline of total plasma AAs after 3 hours, which resulted in hypoaminoacidemia.

Whey proteins have a higher Leu content than caseins, therefore, one might think that this compositional difference, not their protein digestion kinetics, might induce different dietary nitrogen postprandial metabolism. This was addressed in a study by Dangin, et al. (2001). Casein and whey protein drinks were matched in AA composition and nitrogen contents but designed to have different digestion rates. The fast-digested drinks were whey protein and casein hydrolysate, and the slow-digested drinks were caseins and whey proteins consumed at repeated times during digestion. In accordance with the previous studies illustrated, they showed that fast-digested drinks induced rapid, pronounced and transient increase of aminoacidemia, which led to high and immediate stimulation of protein synthesis. In contrast, slow-digested drinks induced moderated and prolonged aminoacidemia resulting in the inhibition of protein breakdown. Similarly, Bos, et al. (2003) demonstrated that the availability of AAs by digestion kinetics was the main driver for protein metabolism by using milk protein compared to soy protein.

The inclusion of other nutrients in the protein matrix might affect protein utilization. Gaudichon, et al. (1999), investigated whether the addition of sucrose or milk fat affected the net postprandial protein utilization of milk protein. Sucrose, but not fat, significantly reduced the postprandial transfer of [^{15}N]-milk nitrogen to urea, which could be mainly due to a delayed GE of the meal because of the higher energy density. Nevertheless, the total amount of dietary nitrogen recovered over an eight-hour period after meal ingestion was not different. The absence of any effect in the presence of fat was unexpected because the energy density was similar to that of the sucrose meal and the authors suggested that lipid may have separated and formed a layer on top of the meal in the stomach and emptied after the aqueous phase of the meal. However, having similar behaviour to the control milk protein sample, fat could be entrapped in the coagula possibly formed in the stomach.

Mariotti, et al. (2015) investigated the effect of caseins and whey proteins in triaglycerides (TAG) response in a mixed high-fat meal using a crossover design in healthy overweight men. The authors showed that caseins, compared to whey proteins, markedly reduced postprandial TAG and formation

of plasma chylomicrons, which was suggested to be caused by low solubility and phase separation of casein in gastric conditions. This contrasts with other studies showing whey proteins to be more efficient in lowering effects on blood lipids (Mortensen, et al., 2009; Pal, et al., 2010). Therefore, there is a gap in understanding the influence of other nutrients included in the food matrix on metabolic effects, which should be studied considering the digestive kinetics and gastric behaviour.

2.3. Dairy proteins and skeletal muscle mass

Muscle mass maintenance is regulated by the balance between muscle protein breakdown (catabolism) and synthesis (anabolism) rates, which has been dependent on physical activity and food intake. The postprandial muscle protein synthetic response to feeding is regulated on factors including dietary protein amount, source and digestion, AA absorption and uptake by muscle and intramyocellular signalling (Gorissen, et al., 2015). Muscle protein synthesis (MPS) is of particular interest to athletes, active people and the elderly. Ageing can result in a diminished muscle protein synthetic response after protein intake, which is often accompanied with the progressive decline of skeletal muscle mass, known as sarcopenia. Some studies have shown that faster digestion of whey proteins resulted in an enhancement of MPS responses in elderly men (Burd, et al., 2012; Dangin, et al., 2003; Pennings, et al., 2011; West, et al., 2011), in elderly men after resistance exercise (Burd, et al., 2012) and also in young men at rest and after resistance exercise (Tang, et al., 2009). In general, it has been shown that the specific pattern in plasma aminoacidemia after the consumption of whey proteins, i.e. rapid and pronounced AA peak, was the main driver. Moreover, a strong correlation was reported between plasma Leu levels and muscle protein accretion (Pennings, et al., 2011). The stimulation of MPS is driven primarily by essential AAs (Volpi, et al., 2003), from which Leu has been reported as the main signal (Drummond & Rasmussen, 2008). Therefore, a direct comparison between the effects of the absorption rates of these two proteins related to MPS is conflicted by their differing AA contents. In healthy men after resistance exercise, whey proteins ingested as a single bolus was compared to the same amount of protein but taken in repeated small drinks, which aimed to simulate slower digested protein (West, et al., 2011). The authors showed that whey proteins consumed as a bolus caused a rapid and greater increase in aminoacidemia, which was reflected in a greater stimulation in MPS response at an early

stage (1-3 hours) compared to the whey protein consumed repeated times (3-5 hours). They concluded that the pattern of aminoacidemia, not the net AA exposure, was the main driver for the effect in skeletal muscle. These results were supported by other studies using casein and casein hydrolysate (Pennings, et al., 2011).

The ingestion of other macronutrients together with dairy proteins is another factor to consider in relation to MPS (Churchward-Venne, et al., 2015; Gorissen, et al., 2014). Co-ingestion of carbohydrates with casein resulted in no differences in muscle synthetic response in both young and elderly men (Gorissen, et al., 2014). However, this resulted in a delay of protein digestion and absorption, attributed to the decrease of GE rate. In contrast, other studies showed a greater AA uptake into peripheral tissues in resting subjects when milk proteins were combined with lipid and sucrose (Mariotti, et al., 2000). Elliot, et al. (2006) showed that milk ingested as a whole food stimulated net MPS following resistance exercise, suggesting that its consumption would be suitable during recovery. Interestingly, the uptake of AAs, based on Thr and Phe, was greater for whole milk compared to fat-free milk. However, the reason of this outcome was not clear for the authors suggesting that the extra energy could help the nitrogen balance process. Therefore, further work should be performed in investigating the role of lipid in AA uptake.

2.4.Dairy proteins and satiety

The ingestion of protein stimulates the release of gut hormones involved in appetite and food intake regulation, such as CCK, GLP-1, PYY and insulin (Anderson & Moore, 2004). Whey proteins might be considered to have a greater effect on satiety compared to caseins since a higher content of BCAAs can greatly stimulate insulin secretion (Nilsson, et al., 2004). However there is no consistent evidence supporting one milk protein to be more satiating than the other (Bendtsen, et al., 2013). Hall, et al. (2003) investigated in healthy lean volunteers the appetite responses of whey proteins and casein drinks containing both lipid and carbohydrate with their total energy matched. The authors found that a whey-based drink was satiating for 180 min after ingestion according to the appetite subjective score and was more efficient at decreasing energy intake in an *ad libitum* lunch, served 90 min after ingestion, than a casein drink. The secretion of the most important hormones in the role of satiety, i.e. CCK and GLP-1,

increased by 60% and 65% respectively after whey proteins ingestion compared to caseins. Similarly, Veldhorst, et al. (2009) reported a decrease in appetite after, in particular, 20 min of consumption of 15 g of whey proteins (as a part of a standard breakfast) compared to casein or soy protein, which was in accordance to the higher concentration of GLP-1 and insulin observed following whey protein consumption. Accordingly, Luhovyy, et al. (2007) showed that whey proteins greatly suppressed food intake more quickly, i.e. at 90 min compared to the latter time of 150 min observed in casein after meal consumption. In contrast, casein consumption was reported to induce a greater satiety promoting effect than whey proteins in a study by Acheson, et al. (2011), in which subjective appetite sensations were measured for 330 min. This was supported by Alfenas, et al. (2010) with normal weight subjects, in which casein consumption led to a daily lower energy intake with 7-day supplementation compared to whey protein. Calbet and Holst (2004) did not find significant differences in the hormonal secretion of GLP-1 and PYY when the casein and whey proteins were compared with their hydrolysates. The variability of results can be explained by the protein source, quantity and time of measurements used in the studies. Overall, it seems that fast digested protein could have a greater satiety promoting power in the short term in contrast to the long-term effect shown by the slow digested protein of casein. Then the different digestion and subsequent AA absorption rate of casein and whey proteins might affect the secretion of GI hormones and subsequent satiety and food intake.

The ingestion of milk as a whole was more effective in increasing satiation and reducing subsequent food intake than consuming the isocaloric drinks containing casein or whey proteins alone (Lorenzen, et al., 2012), which could be as the result of a greater increase of CCK and GLP-1 (Diepvens, et al., 2008). Moreover, milk consumption has been reported to promote satiety and decrease food intake when compared to other drinks such as fruit juice (Dove, et al., 2009).

2.5. Conclusions

In this section, we showed evidence of how the single milk proteins affect the physiological functions differently after their digestion. Casein and whey proteins have been categorised as ‘slow’ and ‘fast’ protein respectively, according to their plasma AA appearance rate. Several studies have shown that the different aminoacidemia patterns, rather than protein composition, have a profound effect on protein

296 metabolism, in particular protein synthetic response of skeletal muscle. Despite the fact that it is widely
297 suggested that gastric digestion could be the main driver for these outcomes, there is still no direct
298 evidence. As far as it is known, there is no *in vivo* study showing the changes in gastric behaviour during
299 the digestion of the main dairy proteins.

300 **3. Micro-scale dairy structures: digestion, absorption and physiological responses**

301 Some of the most common processes used in the dairy industry are homogenisation and heat treatment.
302 These processing conditions will vary the physico-chemical properties and macronutrient organisation
303 at the microscopic length scale, providing different microstructure. This could, in consequence, affect
304 the nutritional properties.

305 **3.1.Dairy microstructure changes induced by heat processing**

306 **3.1.1 Changes of heat processing**

307 Structural and functional modifications of milk proteins following heating have been extensively
308 reported (de la Fuente, et al., 2002; Singh, 2004). However, the impact of heat treatments on the
309 digestion of milk proteins has been much less studied and it is still subject of debate. The main chemical
310 modifications to milk proteins during heating are denaturation of whey proteins, in particular β-Lg,
311 casein-whey protein interactions and glycosylation by Maillard reaction. Despite the significant
312 biochemical alterations induced by heat, some studies have shown no impairment of digestibility in the
313 intestine (Efigênia, et al., 1997; Rutherfurd & Moughan, 2005), and nitrogen availability (Lacroix,
314 Léonil, et al., 2006) after pasteurisation and ultra-high temperature (UHT) treatment in rats.

315 **3.1.2. *In vivo* digestion**

316 From our knowledge, there is only one study in humans assessing the nutritional impact of milk heat
317 treatment. Lacroix, et al. (2008) compared the treatments of pasteurisation (72°C for 20 s) and UHT
318 (140°C for 5 s) with non-heated milk. The kinetics of postprandial utilization of dietary nitrogen for the
319 transfer into serum protein and AA, body urea and urinary urea over 8 hours were significantly faster
320 for UHT milk compared to pasteurised milk and non-heated milk, which had a similar protein metabolic

pattern. This shows that the digestive kinetics were more rapid after UHT milk ingestion. There were no firm conclusions regarding the mechanisms behind this observation.

The faster nitrogen utilisation in highly heat treated milk might be due to faster gastric digestion. Miranda and Pelissier (1987), using a rat model, compared skimmed milk samples heated using UHT and autoclaved (120°C for 20 min) conditions with non-heated skimmed milk. The heat treatment, in particular autoclaved, accelerated the GE rate of total nitrogen and casein hydrolysis. This contrasts with the work by Barbé, et al. (2013), in mini-pigs, in which a higher mean retention time in the stomach of heated skimmed milk (90°C, 10 min) was observed when compared to a non-heated system. However, in the latter study, they used chromium-EDTA, which is a non-hydrolysable and non-absorbable marker of the liquid phase and thus might not be representative of the entire gastric contents of the heterogenous structures formed in the stomach. In general, there is limited research on the GE rate of heated milk samples to draw any firm conclusions.

However, a faster GE induced by heating might be more in accordance with some evidence showing reduced milk protein coagulation in the stomach. The study by Kaufmann (1984), using mini-pigs, appears to be the only *in vivo* study reporting visually that heat treatment modifies the structure of the coagulum formed in the gastric compartment. This study also demonstrated the restructuring of milk in the stomach, whereby the coagulum formed with UHT sterilized milk in the stomach was less firm and had crumbly structure compared to pasteurised milk and more so with raw milk. Moreover, Meisel and Hagemeister (1984) reported a significant effect of UHT treatment on acidification and protein emptying during gastric digestion. The pH at 360 min of gastric digestion was 4.32 and 1.80 for raw and UHT+homogenised milk, respectively. The protein content in chyme after 360 min digestion was approximately 75% in raw milk compared to 25% in UHT+homogenised milk. This could potentially be related to the differences in pepsin activity found. The analysis of the chyme at 360 min showed that pepsin activity was approximately 1,000 U/g protein in raw milk in contrast to the approximate value of 33,000 U/g protein measured in UHT+homogenised milk.

3.1.3. *In vitro* digestion

347 Using the *in vitro* dynamic model HGS, Ye, et al. (2016b) showed the formation of different structures
348 of the coagula formed in the simulated stomach between unheated and heated skimmed milk (90°C for
349 20 min). Unheated milk formed a dense, solid structure with small pores in contrast to the loose and
350 particulate structure of the heated milk. Gastric behaviour also had an impact on the composition of
351 nutrient emptying, such that whey proteins were preferentially emptied in unheated milk since they
352 were not involved in the formation of the coagulum (Ye, et al., 2016b). This contrasts to the small
353 amounts of caseins and almost no intact whey proteins that were firstly emptied in heated milk. The
354 rate of release of fat globules from the coagulum was also influenced by the matrix structure. In whole
355 milk, fat globules were entrapped in the protein matrix but fat globules of unheated milk were
356 distributed more evenly within the matrix compared to those in heated milk (Ye, et al., 2016a). This
357 seemed to affect the release of lipid from the coagula, which was faster in heated milk. Therefore, this
358 study demonstrated the significant influence of heat treatment on the gastric digestion behaviour and
359 nutrient digestion kinetics of milk. However, the heating conditions of this study (90°C for 20 min)
360 were not comparable to those used conventionally in the milk industry, which might have different
361 effects on changes in the protein molecular structure.

362 Mulet-Cabero, et al. (2019) using a semi-dynamic gastric model, investigated the gastric behaviour of
363 whole milk after controlled and standard industrial heat treatments, pasteurisation (72°C for 15 s) and
364 UHT (140°C for 3 s). The authors showed the formation of coagula during gastric digestion, in which
365 the consistency was profoundly affected by heat treatment. They reported, by rheology, that the higher
366 the temperature of the treatment, the softer the coagulum obtained. Moreover, this different gastric
367 restructuring had an impact on the emptied products. The gastric emptying of caseins from raw and
368 pasteurised milk was delayed due to a more compact coagulation in the stomach whereas caseins were
369 mostly emptied in hydrolysed form in UHT-treated milk. The gastric behaviour also had an impact on
370 the emptying kinetics of the total lipid and protein content.

371 **3.1.4. Protein hydrolysis and products of digestion**

372 As pointed out in the previous studies, pepsin seems to play a key role in the disintegration of protein
373 matrices in the gastric digesta. Aggregation induced by heat treatment might limit or modify the

accessibility to some cleavage sites by digestive enzymes, which might affect the peptides released during digestion. There is very little research in this aspect on *in vivo* systems. Barbé, et al. (2014), using mini-pigs, compared the release of peptides into the duodenum from raw or heated (90°C, 10 min) skimmed milk. The number of peptides identified was slightly lower in heated milk compared to the non-heated sample. However, β- and α_{s1}-caseins, which are very similar in their sequence length and abundance in milk, produced a different number of peptides. The number of peptides deriving from α_{s1}-casein was less abundant than β-casein, showing more resistance to proteolysis probably due to differences in secondary structure and phosphorylation. This contrasts to Sánchez-Rivera, et al. (2015), using the same samples but digested in the dynamic gastric model available in INRA (France), that found a higher resistance of β-casein regions to digestion compared to other caseins. The latter results were in accordance with the study performed by Dupont, et al. (2010) using an infant *in vitro* static digestion. Moreover, Sánchez-Rivera, et al. (2015) found rapid hydrolysis of caseins after just 4 min in non-heated milk whereas intact caseins were visible for up to 50 min in heated milk, which was assessed by SDS-PAGE. The authors suggested that heat induced aggregation between caseins and whey proteins might be the cause of this behaviour. However, this is opposite to the results reported above with other dynamic gastric models. It seems that the gastric colloidal behaviour was completely different, unfortunately there was no reference to that in the study by Sánchez-Rivera, et al. (2015). This highlights the need for full characterisation of the structure and behaviour of the matrices and also the physical and chemical environments of the dynamic models that could induce differences in the coagulation and digestion behaviour.

As illustrated, there are still conflicting results regarding casein digestion, as this appears to be sensitive to the matrix structure, which in turn is very sensitive to the environmental conditions and processing. In fact, Li and Zhao (2019) showed the profound effect of heating on the behaviour of casein coagulation, both by acid and rennet. However, the effect of heat on whey protein digestion is much simpler and more generally accepted. Native β-Lg is resistant to pepsin digestion while heating β-Lg promotes its hydrolysis. Temperatures above 75°C denature whey proteins, in particular β-Lg which unfolds and exposes hydrophobic groups. This susceptibility to proteolysis has been observed in several

digestion systems; mini-pigs (Barbé, et al., 2013), dynamic gastric model (Ye, et al., 2016b) and static digestion model (Islam, et al., 2017).

3.2. Dairy microstructure changes induced by homogenisation processing

3.2.1. Changes of homogenisation

Homogenization is another common process used in the dairy industry, which disrupts the milk fat globule membrane (MFGM) and reduces the fat globule size. This leads to the formation of a new interface on the fat globules, which mainly consists of adsorbed milk protiens and native MFGM frgments (Lopez, 2005). Studies of the effect of droplet size on dairy nutrient digestion have been mainly performed using *in vitro* static models in particular with regard to lipolysis in infant formula and human milk (Bourlieu, et al., 2015). It is generally believed that the reduction in droplet size following homogenisation enhances lipid digestion because of the overall larger interface area available for lipase action. However, other factors such as protein matrix and droplet interfacial composition play an important role and it is complex to distinguish the main driver of the digestion outcomes (Garcia, et al., 2014).

3.2.2. *In vitro* digestion

To our knowledge, the effect of homogenisation of a milk matrix on the digestive kinetics has not been studied in adult humans to date. However, *in vitro* systems have been used to get some understanding of this relationship. Mulet-Cabero, et al. (2019), using a semi-dynamic model, showed a strong impact of homogenisation in the gastric behaviour. Homogenised milks ($d_{4,3}$ of 0.4 µm approximately) showed creaming in the gastric phase. This was regardless the heat treatment applied but it was more pronounced when homogenisation was combined with UHT treatment compared to pasteurisation or without heat tretment. The authors suggested that this colloidal behaviour was driven by both droplet destabilisation and aggregate density. However, the phase separation observed in Mulet-Cabero, et al. (2019) was not reported in Ye, et al. (2017) using the HGS. It is important to note that this dynamic model does not allow the visualisation of the digesta in the course of gastric digestion. Nevertheless, they reported, in line with Mulet-Cabero, et al. (2019), that the structure of the coagula of the

427 homogenised milk was more fragmented than raw milk but this effect was much less profound than
428 when heat was applied. In terms of protein hydrolysis, homogenisation did not affect the pattern of
429 digestion (Mulet-Cabero, et al., 2019; Ye, et al., 2017). However, the gastric behaviour had an impact
430 on the nutrient content emptied. The homogenised samples seemed to release more lipid at the end of
431 the gastric digestion (Mulet-Cabero, et al., 2019). Moreover, they reported a lower lipid/protein ratio in
432 the serum of the homogenised sample suggesting an easier incorporation of the droplets in the coagulum
433 and closely linked to the protein matrix. This coagulum could be present with a lower density in the
434 gastric phase. Indeed, Michalski, et al. (2002) reported that homogenised fat globules interacted with
435 the casein network, participating in the coagulum structure (rennet and acid gelation) whereas native
436 milk fat globules were entrapped in serum pores of milk coagulum without much interaction.

437 The protein network in which lipid droplets are embedded seems to be a critical factor in the hydrolysis
438 of lipid. This protein matrix can be induced by restructuring in the stomach as the studies cited above
439 or formed initially (emulsion gel). Guo, et al. (2014) investigated the effect of the droplet size (1, 6 and
440 12 µm) in whey protein emulsion gels in the oral and gastric compartments using the HGS. The initial
441 gel structure strongly influenced its disintegration during the gastric phase, resulting in significant break
442 down of particles and coalescence during digestion for the 6 µm- and 12 µm-droplet gels, which caused
443 the creaming observed after 300 min of gastric digestion. In contrast, the oil droplets of the 1 µm-droplet
444 gel bolus, with an initial evenly distributed structure, largely remained within the protein matrix during
445 gastric digestion. Moreover, the hydrolysis rate of whey proteins was higher in 12 µm-droplet gel. This
446 could be attributed to the loose protein structure induced to the higher droplet size, which could promote
447 the access to pepsin to the cleavage sites.

448 **3.2.3. Intragastric colloidal stability and physiological responses**

449 The effect of the droplet size in relation to colloidal stability in gastric environment has been less studied
450 which can be attributed to the limitations of *in vitro* systems, in particular static models. Some clinical
451 studies have shown a significant impact of emulsion droplet size and intragastric stability not only on
452 nutrient delivery and absorption (Golding, et al., 2011; Marciani, et al., 2007) but also on satiety
453 (Marciani, et al., 2008), and subsequent regulation of energy intake (Hussein, et al., 2015).

Unfortunately, there a few studies in which a dairy based ingredients is the main focus. This highlights the gap of knowledge in understanding the physiological response to consumption of dairy structures.

Peters, et al. (2014) studied the effect of droplet size (3 versus 0.1 µm) in a milk shake. They did not find any significant effect in CCK released, satiety and food intake for 180 min after drink ingestion. This is in contrast to the significant enhancement in satiety for the smaller size droplets observed by Maljaars, et al. (2012). In the latter study, the same milk shake but without lipid was orally administrated and the lipid droplets were infused directly to the small intestine. In the Peters, et al. (2014) study the sample including protein could potentially coagulate in the stomach entrapping the lipid droplets, which could delay or change the effect on satiety that was expected. Unfortunately, the gastric behaviour was not investigated in the study. Again, this illustrates the crucial importance of studying the gastric phase and the importance of the effect of the matrix within which the components of interest are arranged.

Droplet size is also thought to influence food intake behaviour. However, the study of satiety is quite complex because it also involves cognitive factors as well as physiological factors. Lett, et al. (2016) aimed to modulate satiation and satiety by controlling oil droplet size ($d_{4,3}$ of 2 versus 50 µm) of an emulsion (1% wt sodium caseinate and 15% wt sunflower oil). The smaller droplet size within an emulsion preload resulted in a significant reduction in food intake (62.4 kcal) at a subsequent *ad libitum* meal. The authors did not find any conclusive primary mechanism responsible for this effect on satiety, but it is likely from earlier discussions, that this difference in droplet size would affect the lipid profile emptied from the stomach, and the subsequent feedback and control of gastric emptying could influence satiety.

3.3. Conclusions

The literature reviewed in this section illustrated that processing of milk can be seen as a tool to modulate digestion kinetics which does not imply an imparment of protein quality. In terms of heat processing, only drastic temperatures seem to have significant effects on protein bioavailability as reported by the increase of nitrogen availability and utilisation in the body after high heat treatment in humans. This is supported by the restructuring in the gastric phase observed in relevant *in vitro* models.

480 The important role of pepsin, and the mechanisms by which it controls this process needs to be further
481 investigated. Also, the effect of heat on caseins digestion is still controversial and needs further
482 investigation taking into account the digestion model and heating conditions. Moreover, there are no
483 reports about the effect of heat treatment on satiety in dairy products.

484 Homogenisation has been observed to cause a profound effect on milk gastric behaviour *in vitro* but it
485 needs confirmation in humans. It is generally accepted that lipolysis is enhanced by reduction of droplet
486 size. However, droplet interface interactions with protein matrix are less considered and play a crucial
487 role in the lipolysis. Therefore, the effect of droplet size should be investigated together in a food matrix
488 to assess the gastric colloidal behaviour that will impact not only on lipid absorption rate but
489 physiological responses such as satiety.

490 **4. Micro-scale dairy structures: digestion, absorption and physiological responses**

491 Milk processing will not only vary the macronutrient organisation at the microscopic level but at larger
492 scale (i.e. macrostructure) providing products with different rheological properties with liquid, semi-
493 solid and solid consistencies (i.e. yoghurt, cream, butter and cheese). These process-induced physical
494 structures might influence nutrient release and absorption.

495 **4.1. Effect of macrostructure on digestion**

496 Scanff, et al. (1990) showed, using calves, that caseins from yoghurt were emptied constantly from the
497 stomach in both intact and degraded forms in contrast to the longer retention of caseins in milk. In
498 humans, Mahé, et al. (1994) compared the digesta of proximal jejunum and terminal ileum between
499 milk and yoghurt. They found, in milk, an early emptying of whey proteins, which remained in solution
500 whereas caseins coagulated. In contrast, the greater viscosity of yoghurt provided a more delayed and
501 regular release of nitrogen into the duodenum compared to milk. This was suggested to be due to the
502 different GE pattern, the gastric half emptying time (measured using ^{14}C-PEG-4000) was shorter in
503 milk (35 min) than in yoghurt (60 min). However, this did not affect the overall extensive digestion of
504 milk proteins from both matrices; approximately 91% of nitrogen was absorbed between the stomach
505 and the terminal ileum in 240 min. These outcomes were supported in humans (Gaudichon, et al., 1995)

and mini-pigs (Gaudichon, et al., 1994), in which the dependence of liquid to solid ratio of the gastric digesta in GE was highlighted. The viscosity of yoghurt also influenced the postprandial lipid profile in humans by delaying the peak in plasma TAG whereas milk provided a lower but more long-lasting rise of TAG in plasma (Sanggaard, et al., 2004). Interestingly, Mahé, et al. (1994) reported, in humans, that the absorption of calcium in the duodenum was significantly higher after ingestion of yoghurt (67%) compared to that of milk (44%), probably due to a higher casein availability in yoghurt. This shows that the effect of intestinal delivery rates does not only affect macronutrients but micronutrients as well.

Using similar macrostructures, Barbé, et al. (2013) compared the effect of unheated skimmed milk and its correspondent gel produced using rennet in mini-pigs. The gel matrix ingestion induced significantly lower and prolonged Leu levels in plasma throughout a 7-hour period after meal ingestion, whereas the liquid structure peaked after 30 min of meal ingestion. It was suggested that the gel structure could slow down proteolysis and AA absorption, which could be reflected physiologically. However, the levels of the GI hormones measured in this study, CCK and ghrelin, did not present any significant difference between the matrices over a 4-hour period after meal ingestion. Despite the different AA uptake rate that was obtained, the authors found no significant differences in the mean retention time in the stomach. The measurement was based on chromium-EDTA which, as mention before, might not be representative of the entire gastric contents. The effect of these matrices (milk liquid versus gel) was further studied in relation to the pattern of peptides released into the duodenum at different times using mini-pigs (Barbé, et al., 2014). The authors reported that the food matrix did not affect the accessibility of enzymes to the cleavage sites as seen, due to the identification of the same peptides over the digestion time but the structure had a great impact on the quantity of identified peptides. The gel structure presented lower amounts of free AAs but higher number of peptides, when compared to the matrix ingested in a liquid state, even though the latter was supposed to coagulate in the stomach. The mechanisms behind these results were not clearly identified, which illustrates the difficulty of interpretation of results in a system with several contributory factors, such as matrix structure, gastric restructuring and emptying and enzyme accessibility. The mechanisms behind the results of these dairy structures were investigated by mathematical modelling, which accounted for the main digestive events

533 including gastric behaviours of coagulation and syneresis in the stomach (Le Feunteun, et al., 2014). It
534 was shown that the gastric retention controlled by the physico-chemical properties of the matrix was
535 the limiting step explaining the differences in the mini-pig data. Importantly, the gastric phase was
536 determined to have the crucial, rate-limiting effect in explaining the kinetics of AA absorption observed
537 *in vivo*.

538 **4.2. Effect of macrostructure on appetite**

539 Only a few studies have investigated the impact of the physical structure of dairy matrices in relation
540 to appetite. Sanggaard, et al. (2004), did not find any difference in appetite sensation, measured by
541 visual analogue scale, and insulin and glucose between yoghurt and whole milk in eight healthy men,
542 despite the significant slower rate of GE observed after yoghurt consumption. However, the level of
543 GIP was twice higher in yoghurt between 30 and 120 min but the measured gut hormones remained
544 elevated for longer time after milk ingestion, in agreement with the postprandial lipid profile. The rapid
545 first emptying of whey proteins and the subsequent slow emptying of the casein coagulum might
546 counteract the effect of viscosity in the yoghurt with the more homogenous nutrient emptying and lead
547 to similar appetite sensations. Dougkas, et al. (2012) studied the effect of three isocaloric dairy products
548 (semi-skimmed milk, yoghurt and cheese) consumed as a snack on appetite and subsequent *ab libitum*
549 lunch energy intake in overweight men. The yoghurt intake reduced the rating of hunger of 8 and 10%
550 compared with cheese and milk, repectively, whereas they did not present any difference in the overall
551 energy intake or the satiety hormones of ghrelin, PYY and insulin. Similarly, Mackie, et al. (2013)
552 compared two isocaloric samples (same lipid, protein and carbohydrate content) with a different
553 physical structure. The liquid sample was a milk protein-stabilised emulsion and the semi-solid sample
554 was a mixture of grated cheese and yoghurt. The authors showed a different behaviour in the stomach
555 using MRI; the semi-solid matrix presented sedimentation whereas liquid matrix presented creaming.
556 The semi-solid sample promoted greater fullness over the three-hour study, which was linked to the
557 volume of the gastric contents remaining by the slower GE rate over the first hour. However, the
558 effective reduction of hunger of the semi-solid meal was not reflected in the plasma CCK level, which
559 was lower over the first hour and then similar for both meals. This shows that the rationale of the satiety

560 biomarkers does not always guarantee high perceived satiety, since there is not a mathematical
561 association (Veldhorst, et al., 2008). The understanding of the underlying mechanisms of the GE
562 patterns would imply consecutive measurements of the chyme, which is difficult in humans. Mulet-
563 Cabero, et al. (2017) further investigated the same liquid and semi-solid samples using a semi-dynamic
564 gastric model that could simulate the behaviour observed in the human stomach. The liquid system
565 showed a delayed nutrient release, in particular lipid, due to the formation of the cream layer during
566 gastric digestion. In contrast, the sedimentation in semi-solid system led to the early emptying of high
567 nutrient content since lipid was prolonged entrrapped in the protein matrix. This caused a faster nutrient
568 hydrolysis in the small intestine, which might enhance nutrient absorption. This shows that the
569 evaluation of the nutrient delivery during gastric digestion could provide useful information to shed
570 light on the *in vivo* observations. Therefore, the approach of combining the strengths of *in vivo* and *in*
571 *vitro* models could provide more relevant data in order to understand the mechanisms linking food
572 structure and physiological responses.

573 Currently, there are no conclusive results of the impact of dairy products on appetite and energy intake,
574 and research of the satiating power of solid versus liquid matrix remains inconsistent (Almiron-Roig,
575 et al., 2003). The potential for dairy products to help individuals control body weight needs further
576 investigation, which should importantly include the study of the behaviour in the stomach. Also, the
577 study of the rate of GE usually provides contradictory outcomes when compared to the rate of nutrient
578 absorption; most of the labelled substrates used only reflect the behaviour of the liquid phase of the
579 digesta so it is important to understand the structural changes of the whole food matrix within the
580 stomach.

581 **4.3. Solid dairy foods with different textures**

582 **4.3.1. Effect of viscosity and gel strength**

583 Protein gel texture induced by processing might affect disintegration kinetics during gastric digestion
584 and subsequent AA bioavailability. Guo, et al. (2015) used the HGS to study the gastric disintegration
585 between two whey protein emulsion gels, i.e. hard gel (69.9 N hardness) and soft gel (19.2 N hardness).

586 Prior to the gastric digestion simulation, the samples went through an oral phase simulating the bolus
587 obtained in an *in vivo* study (Guo, et al., 2013). In the gastric phase, the soft gel disintegrated faster than
588 the hard gel, suggesting that the main mechanism of disintegration for hard gel was abrasion whereas
589 soft gel presented both abrasion and fragmentation during gastric digestion. The disintegration in the
590 stomach was accelerated by the action of pepsin, in particular after 180 min of digestion for the soft gel.
591 This could be attributed to the weakly crosslinked protein structure observed in the soft gel, compared
592 to the intimately linked protein matrix of the hard gel, which could hamper pepsin accessibility. The
593 behaviour of the gastric disintegration had a significant impact on the GE, which was related to the
594 retention of solids in the HGS as a function of time. The GE was faster in soft gel after 180 min of
595 digestion whereas the gastric content retention before 120 min in soft gel was higher, which was
596 attributed to a larger particles size of the original bolus (Guo, et al., 2013). This shows the relevant
597 importance of the oral phase in the process of gastric disintegration. The rate of GE between the matrices
598 was almost the same at the end of gastric digestion (300 min) indicating that slower emptying of the
599 soft gel at the beginning of digestion due to particle size of bolus was compensated by the more rapid
600 disintegration due to easier hydrolysis by pepsin.

601 Cheeses have different consistencies with different structures, which might affect the disintegration rate
602 and release of nutrients. Tran Do and Kong (2018) used the HGS to study the gastric disintegration of
603 Cheddar, Mozzarella and Parmesan cheeses. Mozzarella cheese formed a more dense outer layer during
604 the dynamic gastric digestion that led to a lower protein hydrolysis, compared to the other cheeses.

605 A recent study using pigs investigated the effect of viscosity in yoghurt on GE by gama scintigraphy
606 and protein digestion using the dynamic model DIDGI® (Ménard, et al., 2018). The low and high
607 viscosity yoghurt with the same nutrient composition but different viscosity (2.2 versus 0.3 Pa·s) were
608 compared a control yoghurt with lower protein and fiber content, and intermediate viscosity (1.3 Pa·s).
609 The authors showed that the enrichment of protein and fibre slowed down GE whereas the viscosity
610 seemed not to be a controlling parameter in emptying since low and high viscosity yoghurts presented
611 no significant difference. However, since the control yoghurt differed in both nutrient composition and
612 viscosity, it is not possible to draw any conclusive outcome. Moreover, the pepsin hydrolysis of whey

proteins was higher in the high viscosity yoghurt compared to the low viscosity sample, which was suggested by the authors to be due to the different behaviour observed when entering the small intestine.

4.3.2. Effect on lipid digestion and physiological responses

Examples of dairy products in which oil droplets are dispersed in a semi-solid/solid protein matrix are yoghurt and cheese. The influence of the protein matrix on the release of lipids might impact absorption and lipaemia, which can have potential effects on risk markers of cardiovascular diseases. Drouin-Chartier, et al. (2017) compared the lipid absorption from hard and soft cheeses and butter, matched in total calories and macronutrient content. There were no differences in serum TAG, free fatty acid and apoB-48 in the incremental area under the curve over 8 hours. However, it seemed that the soft cheese induced greater increase in TAG concentration at 2 hours and attenuated the low dense lipoprotein of apoB-48 compared to the firm cheese. These results showed that the physical structure may not necessarily influence the overall magnitude of postprandial lipemia but more importantly the timing and magnitude of the TAG peak value. This could be related to the protein network and lipid droplet arrangement within the cheese matrix. The authors suggested that the homogenised lipid droplets in soft cheese are enclosed in a loose protein gel, which causes easier access for both pepsin and gastric lipase in the stomach. Moreover, the lipid droplets were smaller, giving the food an overall larger surface area, which might facilitate lipolysis. Interestingly, they did not find any differences between hard cheese and butter. This could be attributed to a limited availability of the nutrients; hard cheese could take longer to be disintegrated in the stomach and the formation of layering could be possible in the case of butter delaying the delivery of lipid. Similar results were obtained in a study comparing milk, butter and mozzarella-cheese (Clemente, et al., 2003). There was no significant difference in the average of postprandial plasma TAG but in the peak time (315, 277 and 225 min for butter, mozzarella and milk respectively). This contrasts with the GE rate, using ultrasonographic measurements of the antrum-pylorus section, in which mozzarella cheese presented a faster emptying compared to milk and butter. This study showed that GE might not play a critical role in modulating postprandial lipids in blood, using this specific methodology. However, it should also be noted that the study was performed with type 2 diabetic patients, which could modify the outcome when compared with healthy subjects. It is

640 important to note that lipemia can be affected by other factors such as fatty acid composition (degree of
641 saturation and length of fatty acid chain) and the properties of the lipid droplet interface.

642 There are some studies suggesting that the consumption of fat in cheese form has different effect on
643 blood lipids by reducing low density lipoprotein cholesterol, when compared to the same amount of fat
644 consumed in butter form (Hjerpsted, et al., 2011; Tholstrup, et al., 2004). Indeed, Feeney, et al. (2018)
645 showed that dairy fat in form of cheese lower the total cholesterol levels compared with that of equal
646 amount of fat, casein and calcium content in different matrices, suggesting the synergistic effect of the
647 constituents in the cheese matrix. The role of calcium in the fat absorption has been seen one important
648 factor controlling the metabolic responses observed (Thorning, et al., 2016) but this has to be proven in
649 humans and, in general, more research is needed to understand the role of the food matrix on gastric
650 digestion and lipaemia, and metabolic effects, which should be in the context of the lipid/protein
651 organisation and interaction and their behaviour in the gastric compartment.

652 **4.4. Conclusions**

653 This section illustrated that dairy products with different physical structures can affect the rates of
654 nutrient hydrolysis as well as absorption, which is mainly driven by the physico-chemical effect of the
655 structure on gastric digestion. It seems that solid and semi-solid dairy structures have slower digestion
656 than liquid meals. However, the restructuring of liquid meals in the stomach through, for instance
657 coagulation and phase separation, should also be considered. Moreover, factors of the initial food matrix
658 such as hardness, viscosity and pH are also relevant for the breakdown of the food, and how these
659 properties evolve within the GI tract are crucial in nutrient digestion. For instance, studying the
660 rheological properties of the chyme during gastric digestion could provide valuable information about
661 the effect on gastric digestion time. Protein digestion is usually overlooked when assessing lipid
662 digestion in complex matrices. However, research has shown that disintegration of a protein matrix in
663 the stomach is crucial for lipid accessibility and subsequent digestion. This is important not only for the
664 initial design of structures but also the structures that can be formed within the gastric compartment. It
665 has been evident from the literature that there is a complex relationship between structuring in the
666 stomach, the content and rate of nutrients emptied from the stomach and the rate of nutrient absorption.

667 This could be due to the methodology used for measuring GE and also the characterisation of the food
668 matrix. It is difficult to assess both absorption of nutrients and bioaccessibility in relation to gastric
669 behaviour. For that, using both *in vivo* and relevant *in vitro* gastric digestion systems could be an
670 interesting approach to gain more insight into the mechanisms of nutrient digestion. There is currently
671 no evidence of how the different physical dairy structures can influence nitrogen metabolism, and also,
672 more research on satiety responses is needed. Moreover, some research in cheese (Fang, et al., 2016;
673 Lamothe, et al., 2012) has shown that lipid digestion rates can depend on the hardness, cohesiveness
674 and elasticity of the cheese type, which constitutes an important factor in gastric disintegration.
675 However, these studies applied an *in vitro* static model for digestion and there are no clinical studies
676 showing this influence.

677 **5. General conclusions and perspectives**

678 Dairy structures impact nutrient digestion and physiological responses. This impact has been shown at
679 different length scales, from molecular level of the individual milk proteins to physical macrostructure
680 of, for instance, cheese. There are several physiological responses associated with dairy structures but
681 little is known about the mechanisms behind them. Some research has suggested that the gastric phase
682 is the critical step explaining the outcomes observed. However, the gastric phase has been mostly
683 overlooked and few studies have shown direct evidence. The research shown in the present review
684 highlights the importance of the interactions between the dairy products and the gastrointestinal tract,
685 in which the stomach was shown to play a key role. The conditions of the gastric phase drive the
686 restructuring of the initial matrix, which will govern the flow of the digesta and, consequently, nutrient
687 digestion kinetics. It has been shown that there is a gap in the literature about the structural changes and
688 nutrient emptying kinetics of dairy products of dairy matrices that will allow us to understand the
689 postprandial responses. In general, more research is needed to understand the behaviour of foods inside
690 the stomach, and potentially control it. This will provide the means for regulating nutrients digestion
691 and metabolism kinetics.

692 The control of emptying kinetics from the stomach should be targeted by engineering specific gastric
693 behaviour. In this vein, several approaches can be used to design specific structures, having particular

694 physico-chemical properties, to induce certain behaviour in the gastric environment. For instance,
695 modulation of rheological properties of viscosity or hardness since they are key in gastric emptying.
696 This could be achieved by designing gels with physical properties or induce gelation with different
697 consistencies in the gastric environment or droplet interfacial properties that could induce specific
698 intragastric behaviour such as creaming.

699 Future foods may be structured in such a way to have specific gastric digestion profiles that may allow
700 the development of mainstream foods with particular physiological properties targeted to specific
701 population needs such as the elderly, athletes and those at risk of metabolic disorders such as type 2
702 diabetes.

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Table 1. Influence of dairy matrices at the different length scales on the bioaccessibility, bioavailability of nutrients, and physiological responses.[a]

	Molecular Level (Caseins vs whey proteins)	**Microstructure** (Matrix reorganisation due to processing, i.e. heat treatment and homogenisation)	**Macrostructure** (Different rheological properties: liquid, semi-solid and solid consistencies, i.e. yoghurt, cream, butter and cheese).
Bioaccessibility (Nutrient digestion)	*In vitro*: Caseins were more easily digested than whey proteins during the static gastric digestion. The digestibility of	*In vitro*: - Harsh thermal treatment of milk formed loose and particulate coagula in contrast to the dense, solid structure of	*In vitro*: - Dairy protein gel texture induced by processing affected disintegration kinetics during gastric digestion.

	the latter increased by the partial unfolding of the whey proteins. *In vivo*: - Rapid apperance of mostly intact protein form in jejunum after β-Lg digestion whereas caseins were more slowly recovered in a more degraded form.	unheated milk. - The gastric behaviour also impacted the emptying kinetics of both lipid and protein; The gastric emptying of caseins from raw and pasteurised milk was delayed whereas caseins were mostly emptied in hydrolysed form in UHT-treated milk. - Homogenised milks showed creaming in the gastric phase and seemed to release more lipid at the end of the gastric digestion. - Droplet interactions with protein matrix could play a crucial role in lipolysis. *In vivo*: - Drastic heat treatment softened casein coagula and increased gastric emptying in pigs.	Soft gels disintegrated faster than hard gels. - Solid and semi-solid dairy structures had slower digestion than liquid meals. However, the restructuring of liquid meals in the stomach throughcoagulation and phase separation, should also be considered. - In cheese, lipid digestion rates can depend on the hardness, cohesiveness and elasticity of the cheese type. *In vivo*: Caseins in yoghurt were emptied constantly from the stomach in both intact and degraded forms in contrast to the longer retention in the stomach for caseins in milk.
Bioavailablity (Nutrient Absorption)	*In vivo*: - According to plasma AA appearance, whey proteins and caseins are considered as 'fast' and 'slow' digested proteins respectively.	- Only drastic temperatures seem to have significant effects on protein bioavailability as reported by the increase of nitrogen availability and utilisation in the body in humans following UHT treatment.	-Gel matrix ingestion induced significantly lower and prolonged AAs levels in plasma compared to liquid structures - Soft cheese induced greater increase in TAG concentration compared to firm cheese.
Physiological Responses	- Caseins consumption inhibited whole protein breakdown but only a slight increase in whole protein synthesis compared to whey proteins.	- Smaller emulsion droplets in liquid drinks resulted in a significant reduction in food intake.	- Yoghurt intake reduced the rating of hunger compared with cheese and milk. - Semi-solid meal promoted greater fullness compared to a liquid meal which was related to the behaviour in the stomachof

	- Whey proteins stimulated muscle protein synthesis. - Whey proteins seemed to have a greater satiety promoting power in the short term in contrast to the long-term effect shown by the slow protein digestion of caseins.		sedimentation and creaming, respectively.

[a] Conclusions based on data from the literature presented in this review.

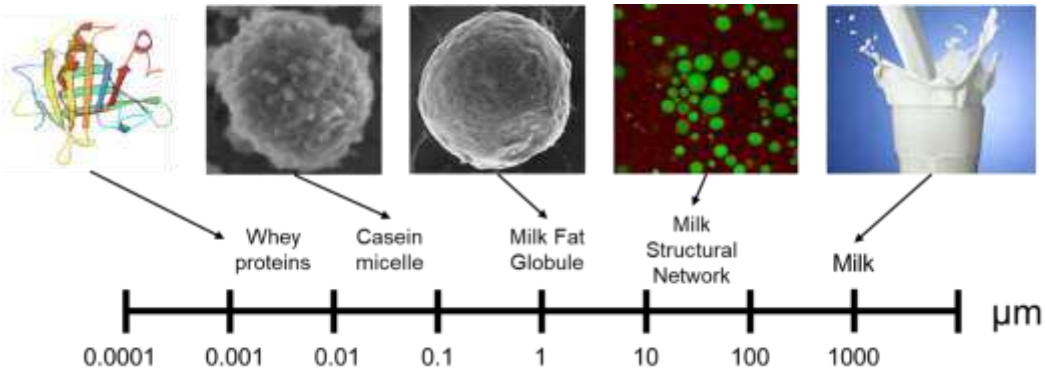
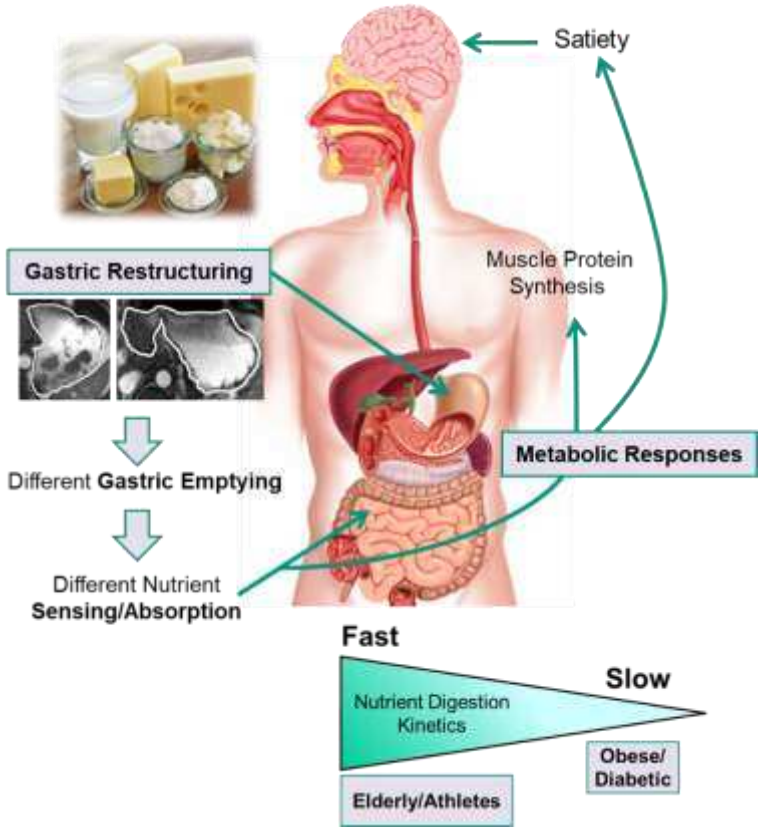


Figure 1. Structural elements and relevant length scales of milk, as an example of the concept of food matrix, i.e. the arrangement and interactions of structural elements at multiscales. Electron micrographs of an individual casein micelle and milk fat globule are from Dalgleish *et al.* (2004) and Luo *et al.* (2014), respectively.



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1052 Figure 2. Schematic representation of the approach showing the role of gastric digestion in
1053 controlling nutrient delivery and absorption by the restructuring of food in the gastric
1054 conditions. This might, subsequently, exert different physiological responses that can be
1055 helpful to specific population groups. Images of the stomach by magnetic resonance imaging
1056 of are from Mackie *et al.* (2013).

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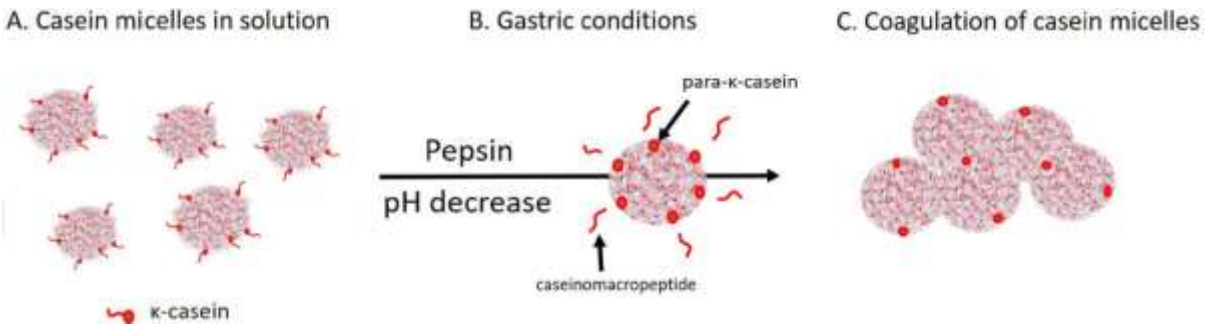

Figure 3. Schematic representation of the possible mechanism for the coagulation of casein micelles in the stomach. (A) Caseins are assembled in micelles, with κ-casein on the surface providing steric and electrostatic stability. (B) During gastric digestion, pepsin is secreted and there is a gradual decrease of pH due to gradual acid secretion and emptying. Pepsin cleaves the Phe105-Met106 bond, which separates para-κ-casein from caseinomacropeptide. (C) These gastric conditions destabilise the casein micelles leading to coagulation.