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# Milk proteins: Digestion and absorption in the gastrointestinal tract

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## Introduction

In the industrialized world, dairy products constitute an important part of the diet, especially in northern Europe and North America. In these regions, milk products contribute around 30% of the total dietary protein supply and represent about 65% of the intake of animal protein.

The protein content of cow's milk ranges from 32 to 35 g/L. There are two major types of milk protein: the caseins (80%), which are represented by four distinct proteins ( $\alpha_1$ -,  $\alpha_2$ -,  $\beta$ -, and  $\kappa$ -caseins), and the whey proteins (20%), which are represented by proteins such as  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, and lactoferrin. These two families of proteins are opposite in terms of structure. Caseins exhibit a loose and highly flexible structure and are associated into a supramolecular structure called the micelle, whereas whey proteins have a globular, well-defined three-dimensional structure. These structural differences between the two families markedly affect the behavior of these proteins in the gastrointestinal tract and particularly their susceptibility to hydrolysis by the digestive enzymes.

The nutritive value of proteins, including milk proteins, is generally associated with their capacity to provide two components: nitrogen (related to protein quantity) and essential amino acids (related to protein quality). The overall nutritional efficiency of protein is most commonly measured via nitrogen retention, which assesses protein retention. In terms of protein quality, the nutritive value is related to the amino acid composition and the bioavailability of these amino acids. The content and the bioavailability of indispensable amino acids, that is, those that cannot be synthesized in the body and consequently must be supplied through the diet, are of particular concern.

TABLE 20.1 Fecal versus ileal digestibility (%) of milk proteins in humans

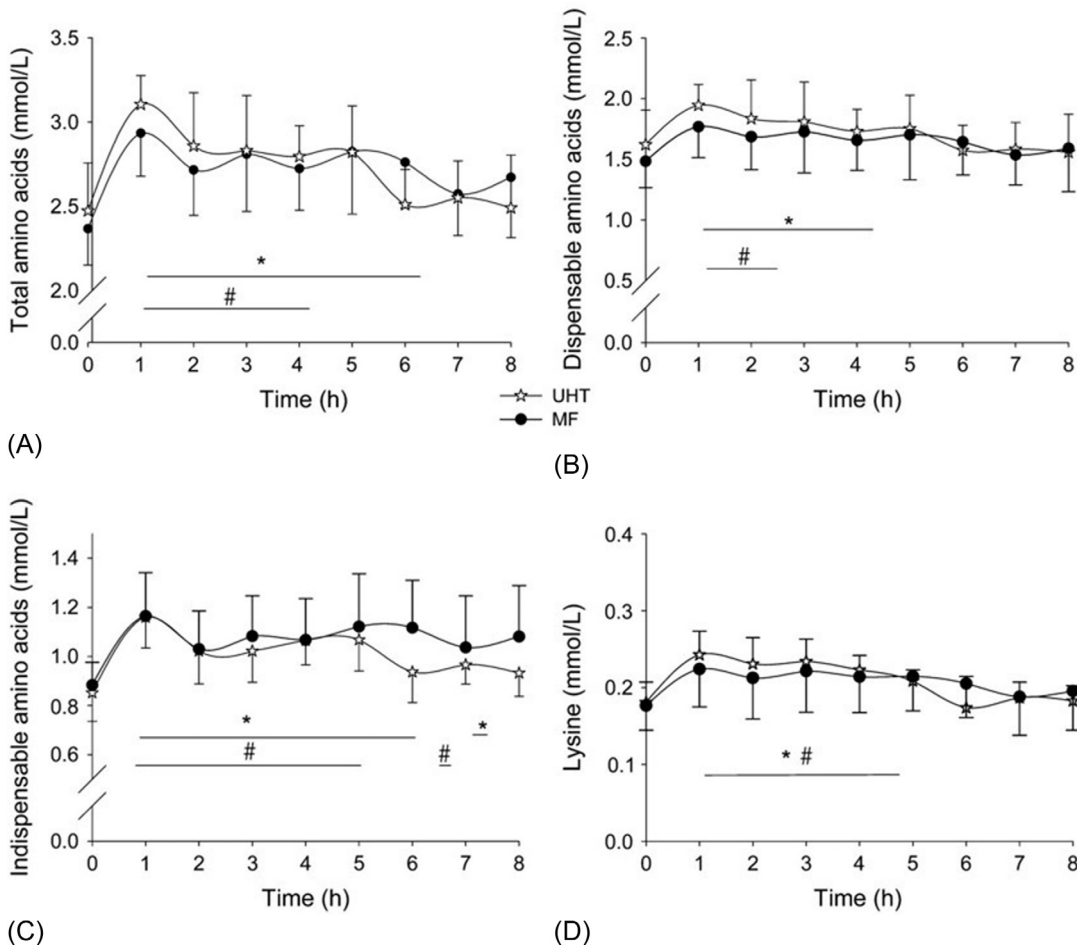
Protein	Fecal True	Ileal		References
		Apparent	True	
Milk protein	96.6	91	95	<a href="#">Bos et al. (2003)</a> , <a href="#">Gaudichon et al. (2002)</a> , <a href="#">Mahe et al. (1994)</a>
Fermented milk	–	90	–	<a href="#">Mahe et al. (1994)</a>
Casein	–	–	94.1	<a href="#">Deglaire et al. (2009)</a>

### Digestion of milk proteins

In the evaluation of the nutritive value of dietary proteins, nitrogen and individual amino acid digestibility, ileal and fecal digestibility, and apparent and true digestibility should be considered ([Fuller and Tome, 2005](#)).

The true digestibility of milk protein, as measured in the ileum, averages 95%, which corresponds to one of the highest digestibilities for dietary proteins ([Table 20.1](#)). The ileal digestibility of caseins has been estimated to be around 93% in pigs and 94% in humans; that of whey proteins appears to be even higher (97%–98%) ([Gilani and Sepehr, 2003](#); [Rutherford and Moughan, 2003](#); [Lacroix et al., 2006](#)) but has never been precisely assessed in humans. Measurement of the true digestibility values of dietary nitrogen and amino acids in healthy human volunteers after the ingestion of milk indicated that ileal digestibility values for the individual amino acids ranged from 92% for serine to 99% for tyrosine, with an average amino acid digestibility of 95.3% ([Gaudichon et al., 2002](#)), that is, the same value as for nitrogen digestibility. In vitro measurement of protein nitrogen and amino acid digestion showed that the digestibility was not different between a goat milk–based infant formula, a cow milk–based infant formula, and human milk ( $78.3\% \pm 3.7\%$ ,  $73.4\% \pm 2.7\%$ , and  $77.9\% \pm 4.1\%$ , respectively) ([Maathuis et al., 2017](#)).

In the context of digestion, although caseins exhibit a structure that makes them highly sensitive to hydrolysis by digestive enzymes, they are considered to be “slow proteins” ([Boirie et al., 1997](#)) because they cause a slow postprandial release of amino acids in the plasma. This contrasts with whey proteins, which rapidly give rise to an intense peak of amino acids in the plasma. This property of caseins has been attributed to their ability to form a coagulum in the stomach through the joint action of acidic secretions and digestive enzymes ([Wang et al., 2018](#)). This coagulum of caseins is retained in the stomach for longer than the whey proteins, which remain soluble and are rapidly delivered from the stomach into the small intestine. These differences in gastric emptying lead to differences in the rate at which dietary amino acids enter the bloodstream ([Mahe et al., 1996](#); [Lacroix et al., 2006](#)). The longer retention of caseins in the stomach leads to a lower level but a longer persistence of amino acids in the plasma than is observed for amino acids from whey proteins ([Fig. 20.1](#)). The type of protein can also specifically influence postmeal aminoacidemia. The chemical composition of whey protein is characterized by high leucine and isoleucine contents, and its ingestion is followed by a peripheral plasma elevation of these amino acids, which are known to be poorly oxidized in the liver. Similarly, the higher plasma proline concentration observed after the ingestion of casein is due to the higher proline content of this fraction ([Lacroix et al., 2006](#)).



**FIG. 20.1** Mean ( $\pm$  standard deviation) changes from baseline in serum total amino acid (AA), indispensable AA (IAA), branched-chain AA (BCAA), and dispensable AA (DAA) concentrations in subjects after the ingestion of total milk protein (TMP;  $n=8$ ), micellar casein (MC;  $n=8$ ), and milk-soluble protein isolate (MSPI;  $n=7$ ). A significant effect of time ( $P=0.0001$ ) and a significant meal-by-time interaction ( $P=0.01$ ) were observed for all variables, as tested on the crude values using a mixed-model analysis of variance (ANOVA) with time as a repeated measure. \*, \*\*, \*\*\* Significantly different from baseline: \* $P=0.05$ , \*\* $P=0.01$ , \*\*\* $P=0.005$  (Lacroix et al., 2006).

## Milk protein hydrolysis in the intestinal lumen

### Caseins

Caseins are extensively degraded during the gastric phase of digestion, an observation that is consistent with the fact that pepsin has a preference for mobile, loosely structured polypeptides. All *in vitro* studies on purified proteins have clearly demonstrated that caseins are hydrolyzed within the first minutes of pepsin hydrolysis, in adults and in infants (Fig. 20.2).

More recently, an animal trial conducted on minipigs fed skim milk and yogurt also showed a rapid and extensive hydrolysis of caseins during the first minutes of digestion, with intact caseins being detected for only 20 min after the meal intake (Barbé et al., 2013).

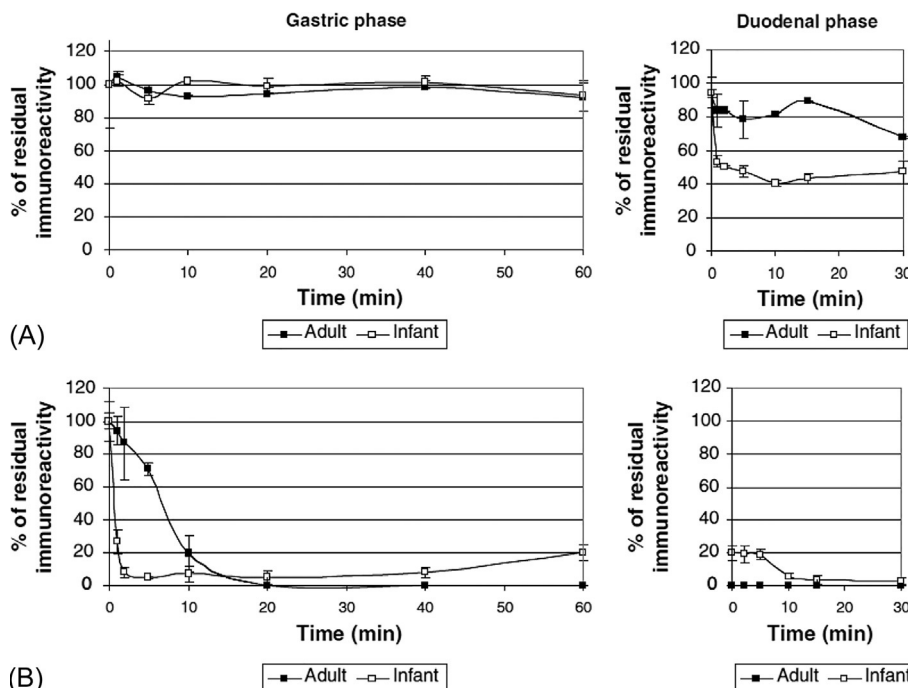


FIG. 20.2 Evolution of residual immunoreactivity of (A)  $\beta$ -lactoglobulin and (B)  $\beta$ -casein during in vitro gastric (left) and duodenal (right) digestion using an infant model ( $\square$ ) and an adult model ( $\blacksquare$ ), as determined by inhibition enzyme-linked immunosorbent assay (data are the results of three independent determinations made in duplicate).

Similarly, in piglets fed milk-based infant formulas, caseins were shown to be more rapidly hydrolyzed than whey proteins, with only 23% of intact caseins being present in the stomach 30 min after ingestion (Bouzerzour et al., 2012).

An in vivo study on human volunteers fed caseins showed extensive release of medium-sized peptides (750–1050 kDa) in the jejunum during the first 6 h after the meal intake. Most of the identified peptides originated from the two major caseins, that is,  $\beta$ -casein (61%) and  $\alpha_{s1}$ -casein (25%), and most contained two or more proline residues; the largest contained seven proline residues out of the 26 residues in its sequence (Boutrou et al., 2013). This is in agreement with the generally reported resistance of proline-containing peptides to gastric and pancreatic digestive enzymes (Vanhoof et al., 1995; Agudelo et al., 2004) and to epithelial proteases (Bauchart et al., 2007). Protein degradation in human jejunum after oral ingestion of casein was also compared with the digests of the same substrate using a standardized in vitro protocol; no intact casein was detected, neither in the jejunum nor in the in vitro samples taken during the intestinal phase (Sanchon et al., 2018).

The digestions of cow milk proteins and goat milk proteins were compared in vitro by varying pH, enzyme concentrations, and incubation times to simulate infant and young child gastric conditions (Hodgkinson et al., 2018). Caseins reacted to pH changes differently from whey proteins, with less digestion of casein at pH 3.0 than at pH 5.0. Caseins from goat milk tended to be more efficiently digested than caseins from cow milk, and the peptide profiles from goat milk were distinct from those from cow milk.

## Whey proteins

In contrast to caseins, whey proteins, because of their globular structure, are known to be extremely resistant to proteolysis. This is particularly the case for  $\beta$ -lactoglobulin, which is not affected during gastric digestion, being virtually unaltered after 60 min of simulated digestion (Fig. 20.2A) (Schmidt et al., 1995; Mandalari et al., 2009; Dupont et al., 2010a). It has also been shown that the molecular interaction of  $\beta$ -lactoglobulin with phosphatidylcholine from the gastric mucosa protects the protein from duodenal digestion by trypsin and chymotrypsin (Mandalari et al., 2009). However,  $\beta$ -lactoglobulin has been found to be more sensitive to pepsinolysis when located at the interface of a lipid droplet than when in solution, because of drastic conformational changes (Macierzanka et al., 2009). A comparison of the standardized in vitro digestion model (Infogest) with in vivo digestion data from human jejuna showed that intact  $\beta$ -lactoglobulin was visible both in the samples taken at 1 h in human digestion and in the in vitro gastrointestinal simulation (Sanchon et al., 2018). When the digestions of cow and goat milk proteins were compared in vitro to simulate infant and young child gastric conditions, digestion of higher molecular weight whey proteins increased with decreasing pH and higher enzyme concentrations of the young child gastric digestion conditions compared with the infant conditions, and  $\beta$ -lactoglobulin was poorly digested under all gastric digestion conditions (Hodgkinson et al., 2018). Conflicting results have been published for the second major bovine whey protein, that is,  $\alpha$ -lactalbumin. Whereas some have found that  $\alpha$ -lactalbumin is even more resistant to simulated digestion than  $\beta$ -lactoglobulin (Inglingstad et al., 2010), others have found that  $\alpha$ -lactalbumin is susceptible to hydrolysis in solution (Nik et al., 2010). In contrast to  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin appears to be more resistant to digestion when located at the oil-water interface than when in solution. The same protective effect of phospholipids from the gastric mucosa on the susceptibility of  $\alpha$ -lactalbumin to hydrolysis by pancreatic enzymes has been described (Moreno et al., 2005). More recently, an in vivo study on human milk-fed preterm neonates showed that  $\alpha$ -lactalbumin was the human milk protein that was most resistant to gastric digestion in aspirates collected through a nasogastric probe (de Oliveira et al., 2017).

In contrast to  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin, lactoferrin has been shown to be extensively degraded during simulated gastric digestion (Furlund et al., 2013). Multiple sequence analyses of the identified peptides indicated a motif consisting of proline and neighboring hydrophobic residues that could restrict proteolytic processing. Further structure analysis showed that almost all proteolytic cleavage sites were located on the surface and mainly on the nonglycosylated half of lactoferrin.

## Peptides released during digestion

The hydrolysis of milk proteins in the gastrointestinal tract will result in the production of a myriad of peptides (Jahan-Mihan et al., 2011), some of which have been shown to exert biological activities such as antihypertensive (Martínez-Maqueda et al., 2012), antiatherogenic (Ricci-Cabello et al., 2012), antimicrobiological, and immunomodulatory (Agyei and Danquah, 2012). Mass spectrometry is the best tool for tracking peptides released during digestion, and the concept of nutritional peptidomics has recently been proposed (Panchaud et al., 2012).

To date, milk peptides have been identified by submitting food to simulated *in vitro* digestion (Dupont et al., 2010b; Picariello et al., 2010; Kopf-Bolan et al., 2012). In the *in vitro* situation, identifying and quantifying the peptides in digested samples is rather easy because most of the proteins in the samples originate from the food itself. However, it is still questionable whether mimicking digestion with *in vitro* models perfectly reflects the physiological reality. Only a few *in vivo* studies have been conducted; detection of dietary peptides is made more difficult by the presence of endogenous proteins secreted in the different compartments of the gut. In a pioneer work, milk caseinomacropeptide, that is,  $\kappa$ -casein (f106–169), was detected in the jejunum of humans fed  $^{15}\text{N}$ -labeled casein, whey protein, and yogurt (Ledoux et al., 1999). Similarly, caseinophosphopeptides were identified in the effluent collected from milk-fed humans (Meisel et al., 2003). In 2013, the peptidome of jejunal effluents collected from milk protein-fed humans was established (Boutrou et al., 2013). Totals of 356 and 146 peptides were detected and sequenced in the jejunum following casein and whey protein ingestion, respectively. Protein degradation and peptide release in human jejunum after oral ingestion of casein were compared with the digests of the same substrate using a standardized *in vitro* protocol (Sanchon et al., 2018). *In vivo* and *in vitro* digests showed comparable peptide profiles and a high number of common sequences. Most of the sequences found in the jejunum, some of them not previously described, were also identified in the simulated digests. Common regions that were resistant to digestion were identified, revealing that the *in vitro* protocol constitutes a good approximation to the physiological gastrointestinal digestion of milk proteins. However, the subjects were fed pure protein fractions, and the possible effect of the food structure was not investigated. Indeed, the structure of the chyme, as affected by food structure, could limit or modify the accessibility of digestive enzymes to some cleavage sites. When the digestions of cow and goat milk proteins were compared *in vitro* to simulate the infant and young child gastric conditions, the caseins from goat milk tended to be more efficiently digested than the caseins from cow milk, and the peptide profiles from goat milk were distinct from those from cow milk (Hodgkinson et al., 2018). Generated peptides, identified using liquid chromatography coupled to a mass spectrometer, showed both similarities and differences in the cow milk and goat milk postdigestion profiles (Hodgkinson et al., 2019). The majority of peptides were from casein proteins, 50% representing  $\beta$ -casein, with many peptides unique to each species. Low and no peptides from  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, respectively, suggest that these proteins were highly resistant to infant gastric digestion, as reported by others. Minor milk proteins, comprising 5% peptides, were represented by different proteins from the cow and the goat. Peptides with known bioactivities were also observed, both in common and unique to each species.

More recently, the gastric and intestinal peptidomes of raw or pasteurized human milk, digested using a preterm neonate dynamic digestion model, were characterized exhaustively (Deglaire et al., 2019); 1531 peptides were clearly identified and arose from 27 different proteins.  $\beta$ -Casein was shown to be the protein that elicited the highest number of peptides. Surprisingly, the most major human whey proteins, that is,  $\alpha$ -lactalbumin and lactoferrin, led to the release of a small number of peptides, confirming their resistance to gastric digestion. Similar work was conducted *in vivo*, that is, by analyzing the content of gastric aspirates collected from infants (Dallas et al., 2014). The results showed that around 200 peptides were already present in human milk before ingestion, indicating that proteolysis events caused by



indigenous proteases such as plasmin were occurring in milk during expression and storage. Then 649 peptides were identified during digestion in the stomach, and as for the *in vitro* experiment,  $\beta$ -casein was shown to be the major source of peptides. Finally, bioactive peptides known to carry various biological activities, such as immunomodulating, opioid, antihypertensive, and antibacterial activities, were shown to be released during digestion and were present in the stomachs of the infants.

In all the previous examples, peptides released from foods of different compositions were identified but not peptides released from foods of identical compositions but different structures. In recent work, the impact of the structure of the dairy matrix on the number and nature of milk peptides released in the duodenum was investigated using cannulated minipigs fed a dairy liquid, an acid dairy gel, or a rennet dairy gel, all of identical composition. The formation of peptides *in vivo* was followed by tandem mass spectrometry over a postprandial period of 5 h after the ingestion of the dairy matrices by the minipigs. The effect of the meal structure was investigated at two levels: the microstructure, as modified by thermal treatment, and the macrostructure, as modified by milk gelation. More than 16,000 peptides were sequenced and unambiguously identified. The results obtained showed that the structure of the dairy products had only little influence on the location of the cleavage sites on the protein sequences (Barbé et al., 2014a). However, the structure markedly impacted the number of peptides identified, especially for the rennet dairy gel; about three times fewer peptides were detected than for the other matrices. This effect was attributed to greater extents of dilution by digestive secretions associated with longer gastric retentions for the rennet gel. Potential bioactive peptides were also produced over time, and their identification has increased our knowledge of the peptides present in the lumen *in vivo*. Our results indicate that the structure of dairy matrices markedly affects the kinetics of milk protein digestion *in vivo*, more than the mechanism of proteolysis itself.

## **Impact of processing on milk protein digestion and absorption**

Milk proteins are introduced into the human diet as processed milk products. It is therefore critical to determine the impact of the major processing technologies on milk protein digestion.

### **Heat treatment of milk**

One of the most common processes applied to milk is heat treatment to ensure product safety. Because of the structural differences already mentioned, heat treatment affects caseins and whey proteins quite differently. Heat treatment modifies the three-dimensional structure of the whey proteins markedly, resulting in an “opening” of the globular structure and making the whey proteins more sensitive to the action of digestive enzymes, as demonstrated for  $\beta$ -lactoglobulin (Barbé et al., 2013) and  $\alpha$ -lactalbumin (Inglingstad et al., 2010). In contrast, caseins, with their loose and highly flexible structure, are not strongly modified by heat treatment. Heat treatment at high temperature results in an increased resistance of the caseins to simulated digestion (Almaas et al., 2006; Dupont et al., 2010b; Barbé et al., 2013), which has



been attributed to the formation of thermally induced aggregates between caseins and between caseins and whey proteins.

## Homogenization of milk

Homogenization of milk results in the disruption of the milk fat globule membrane. Lipids are present as smaller droplets that are stabilized by milk proteins covering the oil-water interface.  $\beta$ -Lactoglobulin and  $\beta$ -casein have been shown to be more susceptible to pepsinolysis when they are adsorbed to an oil-water interface than when they are in solution (Macierzanka et al., 2009; Sarkar et al., 2009). This has been attributed to the unfolding of the proteins at the droplet surface, which improves their accessibility to pepsin. It has been found that the rate of gastric digestion of  $\beta$ -casein is twice as fast when it is adsorbed to the oil-water interface than when it is in solution. In the small intestine, proteins are displaced from the interface by bile salts (Sarkar et al., 2010), making triglycerides more accessible to the pancreatic lipase.

Recently, Ye et al. (2017) investigated the combined effect of both homogenization and heat treatment on the formation and the breakdown of clots during gastric digestion of whole milk using a dynamic digestion simulator. They showed that these processing conditions led to the formation of a coagulum with fragmented and crumbled structures compared with the coagulum formed from raw whole milk. The combination of homogenization and heat treatment resulted in a greater incorporation of protein and fat globules in the coagulum, leading to the formation of more pores. These pores allowed a better diffusion of the digestive enzymes during a simulated digestion, leading to a greater rate of proteolysis. This can result in an increased bioavailability of amino acids. Change in the structure of the coagulum during gastric digestion is also probably of major importance for the regulation of gastric emptying and the transit of food in the gastrointestinal tract.

## Physicochemical modifications of proteins

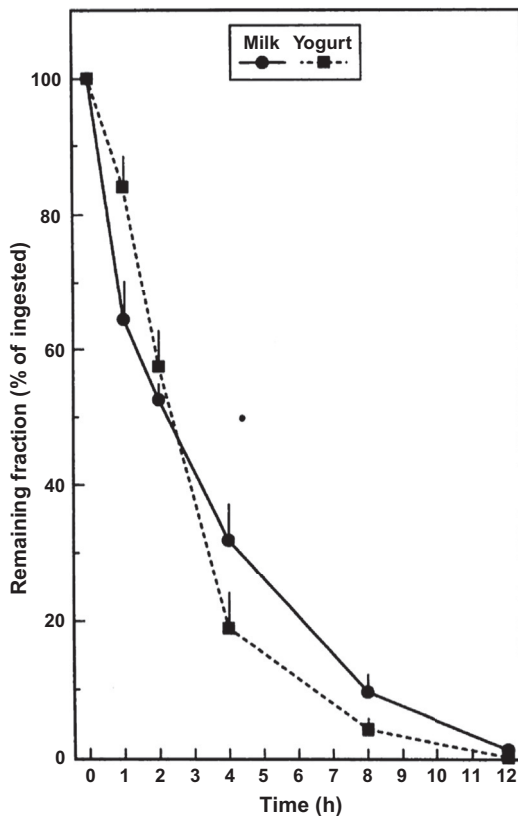
Milk protein modification with cross-linking enzymes such as transglutaminase (TG) has been used extensively to change the functionality of proteins and thereby to improve the textural quality and the stability of protein-based food products. In dairy products, TG-induced cross-linking can increase the firmness and water-holding capacity of acid-induced gels in products with low solids and fat contents or can improve the stability of emulsions and foams. The effect of the TG-induced cross-linking of sodium caseinate on postprandial metabolic and appetite responses was recently investigated in 13 healthy individuals (Juvonen et al., 2012). The results indicated that enzymatically cross-linked sodium caseinate and native sodium caseinate had comparable metabolic responses in a liquid matrix, suggesting similar digestion and absorption rates and first pass metabolism despite the structural modification of the cross-linked sodium caseinate.

The hydrolysis of milk proteins has been widely used to reduce their allergenicity properties in infant nutrition. However, hydrolysis could also be considered to be a “predigestion” of proteins, facilitating their digestion and absorption in the gastrointestinal tract. This was confirmed in a study on 10 elderly subjects who received either intact or hydrolyzed caseins (Koopman et al., 2009). The plasma amino acid concentrations increased extensively (25%–50%) after the ingestion of the hydrolyzed casein, compared with the intact casein ( $P < 0.01$ ).

## Coagulation (liquid/gel/solid transition) of milk

Milk coagulation is used extensively in the dairy industry, especially for yogurt and cheese manufacture, even though the mechanisms of milk clotting for these two types of product are quite different. Studies on the digestion of dairy matrices (yogurt and cheese) are scarce, compared with studies on purified fractions of casein or whey protein. [Gaudichon et al. \(1994\)](#) showed, using minipigs, that the half gastric emptying time of the liquid phase was not different between milk and yogurt. However, the intestinal deliveries of both the liquid phase and the nitrogenous fraction of the chyme were more delayed in pigs fed yogurt than in pigs fed milk ([Fig. 20.3](#)). The kinetics of exogenous nitrogen delivery into the intestine were correlated with the kinetics of exogenous nitrogen absorption. These results suggest that milk proteins are rapidly absorbed after they reach the intestine and that gastric emptying is a major factor controlling the kinetics of milk nitrogen absorption.

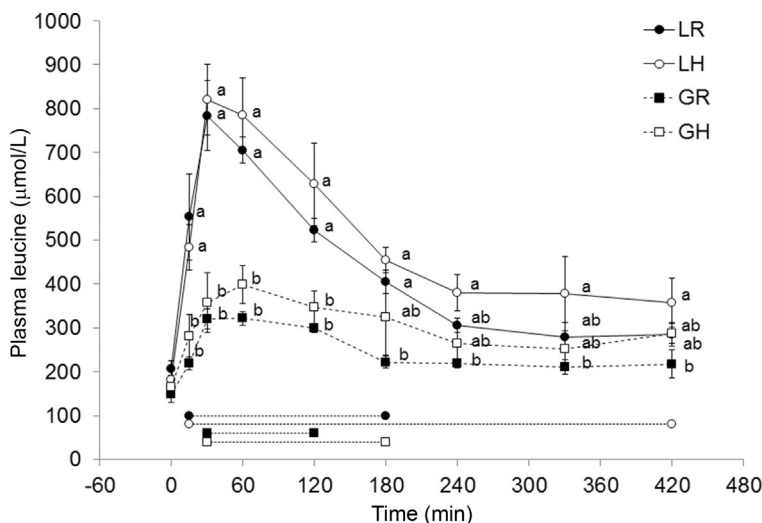
[Rychen et al. \(2002\)](#) examined the postprandial portal absorption of  $^{15}\text{N}$  in the growing pig after the ingestion of milk, yogurt, and heat-treated yogurt. Although the total portal absorption was similar between the three products, yogurt nitrogen was absorbed more



**FIG. 20.3** Remaining fraction of exogenous nitrogen in the stomach of minipigs after the ingestion of 500 mL of milk or 500 g of yogurt. The ingested milk and yogurt contained 17 and 18 g of nitrogen, respectively. Values are means  $\pm$  standard error of the mean (SEM) for three or four pigs. No significant differences were found by ANOVA,  $P < 0.05$ .

slowly than milk nitrogen, with significant differences being observed after 30, 60, and 180 min. Heat-treated yogurt showed similar behavior to milk; it was hypothesized that heat treatment of the gel was responsible for destroying the natural body and viscosity of the yogurt. These effects were therefore attributed to different emptying rates between milk, yogurt, and heat-treated yogurt.

More recently, a determination of the kinetics of milk protein digestion and amino acid absorption after the ingestion of liquid or gelled (acid and rennet gels) dairy matrices by six minipigs showed that the gelation of milk slowed down the outflow of the meal from the stomach, slowed down the subsequent absorption of amino acids, and decreased their bioavailability in peripheral blood (Fig. 20.4) (Barbé et al., 2014b). The nature of the matrix seemed to affect the release of the gastrointestinal hormones involved in appetite regulation, with the gel matrices appearing to be potentially more satiating. It was also shown that two gels with the same composition and similar rheological and structural properties, but differing in their mode of coagulation (acidification/renneting), exhibited different behaviors during digestion. Indeed, ingestion of the rennet gel resulted in lower levels of both proteins in the duodenum and lower levels of amino acids in the plasma, compared with the ingestion of the acid gel. This was probably due to the formation of a coagulum with high stiffness after the ingestion of the rennet gel, under the simultaneous action of the stomach acidity and the rennet, leading to a very long retention of the rennet matrix in the stomach (Barbé et al., 2014b).



**FIG. 20.4** Plasma leucine concentration ( $\mu\text{mol/L}$ ) in minipigs over a 7-h period after the ingestion of liquid (L) and gel (G) matrices, from unheated (R) and heated (H) milk products. Values are means  $\pm$  SEM calculated for four minipigs ( $n=4$ ). The data were analyzed using a mixed-model ANOVA. The time effect was significant ( $P < 0.001$ ), and the lines at the bottom of the figure indicate a significant difference ( $P < 0.05$ ) from baseline for each curve. The time-by-matrix interaction was significant ( $P < 0.001$ ), and at a given time, differences between matrices are indicated by different letters, a and b ( $P < 0.05$ ).

The plasma cholecystokinin and ghrelin concentrations suggested a potentially more satiating effect of the rennet gel than the acid gel.

Studies on the digestion of cheese are scarce. A recent study compared the kinetics of the matrix degradation of different cheeses in a gastrointestinal environment ([Lamothe et al., 2012](#)). The relationship between the physical characteristics of the cheeses (rheological properties and microstructure) and their digestion patterns was also studied. Rheological measurements and compositional and microstructural analyses were performed on mild cheddar, aged cheddar, light cheddar, and mozzarella cheeses. Mozzarella cheese showed the highest rate of matrix degradation. Aged cheddar cheese showed rapid degradation during the gastric phase but was more resistant to the duodenal environment. Light cheddar cheese showed the opposite behavior, being highly resistant to the gastric environment; however, it underwent extensive degradation at the end of the duodenal phase. The extent of matrix degradation for mild cheddar cheese was similar to that for mozzarella cheese in the gastric phase but was much lower than that for the other cheeses in the duodenal phase. The results suggest that degradation of the cheeses was driven mainly by their physical characteristics.

The production of Parmigiano-Reggiano cheese is closely related to the nutritional quality of the final product; in particular, the high digestibility of its proteins is claimed to be proportional to the ripening stage of the cheese. The effect of the aging of cheese on the kinetics of protein digestion was recently investigated. Two different kinds of Parmigiano-Reggiano, young (aged 15 months) and old (aged 30 months), were separately digested using an *in vitro* system that simulated digestive processes in the mouth, stomach, and small intestine ([Bordoni et al., 2011](#)). The results indicated that the digestion of cheeses with different aging times, although starting from different initial compositions, concluded in similar ways, in terms of free amino acids and small organic compounds, but evolved with different kinetics of hydrolysis and peptide formation, discriminating the young cheese from the old cheese.

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## Conclusions

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The digestion and the absorption of milk proteins have been extensively studied, and the mechanisms involved are well described. However, many of the studies have been performed either *in vitro* or with purified protein fractions, and more work is needed to better understand the disintegration of real dairy products in the human gastrointestinal tract. Nevertheless, it appears that casein and whey protein exhibit different behaviors in the gastrointestinal tract because of differences in their structure and physicochemical properties and that processing has a significant impact on the kinetics of protein digestion by modifying the residence time of the products in the stomach. In the context of the nutritional properties of food, it appears that the micro- and macrostructures of a meal, resulting from technological processes used in the food industry, markedly affect the different steps of milk protein digestion. Thus, the design of food matrices at the technological level is of particular interest in the control of the delivery of nutrients, especially for specific subpopulations, such as the elderly or overweight people.

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