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Short title: *Dairy food structure and nutrient bioavailability*

Abstract

Beyond nutrient composition matrix plays an important role on food health potential, notably acting on the kinetics of nutrient release, and finally on their bioavailability. This is particularly true for dairy products that present both solid (cheeses), semi-solid (yogurts) and liquid (milks) matrices. The main objective of this narrative review has been to synthesize available data in relation with the impact of physical structure of main dairy matrices on nutrient bio-accessibility, bioavailability and metabolic effects, *in vitro*, in animals and in humans. Focus has been made on dairy nutrients the most studied, *i.e.*, proteins, lipids and calcium. Data collected show different kinetics of bioavailability of amino acids, fatty acids and calcium according to the physicochemical parameters of these matrices, including compactness, hardness, elasticity, protein/lipid ratio, P/Ca ratio, effect of ferments, size of fat globules, and possibly other qualitative parameters yet to be discovered. This could be of great interest for the development of innovative dairy products for older populations, sometimes in protein denutrition or with poor dentition, involving the development of dairy matrices with optimized metabolic effects by playing on gastric retention time and thus on the kinetics of release of the amino acids within bloodstream.

Keywords: Dairy products, food structure, protein, lipid, calcium, bio-accessibility, bioavailability

Introduction

Today there are sufficient scientific evidences to include the matrix effect for defining food health potential, not only nutrient composition (Fardet, 2014a; Fardet, 2015a; Fardet, 2015b; Fardet et al., 2013). Indeed, at similar composition (nutrients and calories) two foods with distinct matrix structure - resulting from either different technological processes or different degree of chewing - may give different health potential, as shown for example with apple products (Haber et al., 1977), durum wheat pasta versus bread (Granfeldt et al., 1991), whole carrot versus carrot nutrients (Moorhead et al., 2006), almonds of different particle sizes (Grundy et al., 2016), casein versus corresponding aminoacid mixture (Dangin et al., 2001) and grilled beef meat differently chewed (Rémond et al., 2007). Concerning the carbohydrate fraction, according to starch structure (Fardet, 2015b), food particle size (Holt and Miller, 1994) and/or food density (Burton and Lightowler, 2006) it is well recognized that blood glycaemic responses may be rather different leading to different insulinemic responses with important consequences for diabetic subjects (Fardet, 2014b). Concerning the protein fraction, it has also been shown that depending on chewing (Rémond et al., 2007) or protein structure (Boirie et al., 1997) the subsequent protein metabolism and protein gain are different, also with important implications, notably in elderly populations (Dangin et al., 2003). Finally, the same is true for the lipid fraction, and depending on lipid droplet size, lipids are not metabolized similarly with again implication in clinically ill patients, as for lipase pancreatic insufficiency (Armand et al., 1999). On the other hand, concerning the fiber fraction, depending on their physico-chemical properties such as water-holding capacity, porosity, swelling behaviour and/or degree of crystallinity, they are not fermented according to the same profiles leading to production of different short-chain acid profiles within colon (Fardet, 2016). Therefore, for all these important nutrient it is not exaggerated to define slow and rapid fractions, each one impacting differently metabolism. In all these examples this is not the composition which matters but the physicochemical characteristics of the food matrix. If such effects have been widely studied for plant-based foods this is less true for animal-based foods, notably dairy products. Yet, main dairy products exhibit three different types of food structure or texture as defined by rheological science, i.e., liquid (milks and some fermented milks), semi-solid (yogurts and some fresh cheeses) and solid (most of cheeses). And in each category are encountered numerous different food structures, especially in cheeses.

Otherwise it is not only to know the food composition to estimate which nutrient fraction will be actually used by the organism. For example, in starchy foods, a fraction of starch is not digested within small intestine and arrived to the colon where it is fermented as resistant starch fraction leading to the production of butyric acid (Birt et al., 2013). In brief, foods are first broken down during chewing and they release within mouth, stomach and small intestine a fraction of their

nutrient which is called the bioaccessible fraction (Fardet, 2015b). Secondly, this fraction is not always fully absorbed and reaches the colon. Thirdly, the absorbed fraction is not always fully metabolized and used by organism, a fraction being released in urine *via* the kidney, *e.g.*, as for polyphenols (Scalbert et al., 2002). The final fraction really used by our organism and exerting a defined physiological function is what is generally called the bioavailable fraction.

The bioaccessible fraction is generally measured *via* dynamic or static *in vitro* digestive systems more or less mimicking that of humans (McClements and Li, 2010). In some cases a semipermeable (cellulose) membrane or dialysis bag is added allowing to measure a dialysable fraction or *in vitro* bioavailable fraction (Bouayed et al., 2012). Sometimes this is coupled with model cells such as Caco2 cells allowing to estimate the fraction uptake by the cell (Haraldsson et al., 2005). *In vivo*, comparing what is ingested with what is excreted or collected in feces allows estimating an apparent digestibility (Kristensen et al., 2008), generally performed in animals (Li et al., 2016). But to reach the real bioavailable fraction in humans, more expensive studies are needed, measuring nutrient content in plasma (Fenech et al., 1999), golden standard studies involving radiolabelled foods with isotopes to distinguish between exogenous and indigenous nutrient (Bruno et al., 2006). And these latter studies are very rare. However, in these studies the initial food is generally consumed alone whereas other foods may interact and modify the bioavailable fraction.

The impact of food structure on health is therefore an important research topic, but still in progress. Notably there is no review about the impact of dairy food structure on their health potential. Therefore the main objective of this narrative review has been to synthesize available data in relation with the impact of physical structure of main dairy matrices (*i.e.*, milks, yogurts, fermented milks and cheeses) on nutrient bio-accessibility, bioavailability and metabolic and health effects, *in vitro*, in animals and in humans. A particular focus has been made on dairy nutrients the most studied, *i.e.*, proteins, lipids and calcium.

Dairy food structure and macronutrient bioavailability

Food matrix disintegration and the protein fraction

In vitro studies

Milks, yogurts and fermented milks

Seven studies investigating the digestive fate of milks (Almaas et al., 2006; Dupont et al., 2010; Rinaldi et al., 2014) (Kopf-Bolanz et al., 2012; Tunick et al., 2016; Ye et al., 2016), fermented milk (Matar et al., 1996) and yogurts (Dupont et al., 2010; Rinaldi et al., 2014) have been identified. Concerning milks, different types of milks were compared : goat *versus* cow milks (Almaas et al., 2006), pasteurized and homogenized milk (Kopf-Bolanz et al., 2012), heated *versus* raw milk

(Almaas et al., 2006; Ye et al., 2016), and pasteurized *versus* sterilized milks (Almaas et al., 2006; Dupont et al., 2010; Rinaldi et al., 2014).

Before comparing different milks it is worth beginning with the study of the mere *in vitro* gastrointestinal digestion of a whole pasteurized and homogenized milk (Kopf-Bolanz et al., 2012). After oral and gastric digestion, all proteins except β-lactoglobulin were degraded, with peptides being further degraded at the end of intestinal digestion. Most identified peptides came from digestive enzymes and only a few from milk. Concerning the β-lactoglobulin authors suggested that fatty acids and triglycerides would link to it preventing proteases access, and therefore requesting bile for further digestion (Gass et al., 2007). Indeed, when *in vitro* digestion was completed 54% of total proteins were degraded into free amino acids, dipeptides and tripeptides, and only 43% without bile. Average peptide sizes was of 5-6 aminoacids. Finally while undigested milk contains 45% essential aminoacids, the free aminoacids coming from the 10% protein fraction degraded after complete digestion contains 94% essential aminoacids.

Compared to skimmed cow milk, skimmed caprine goat milk proteins are digested faster, caprine β-lactoglobulin being less resistant to digestion (23% remaining after gastro-duodenal digestion) than cow milk (83% remaining) (Almaas et al., 2006). Difference in tertiary structure and surface hydrophobicity could be involved in such differences (El-Zahar et al., 2005). For goat milk, milks with high and low content (typical of some Norwegian goats) in α_{S1}-casein were also studied, but no significant difference was observed for protein digestion. Upon heating (pasteurization at 72°C for 15 seconds or sterilization at 100°C for one minute) protein digestion was lower with both caprine and ovine milks due to protein denaturation and aggregation through heating. However, while a higher degradation of the pasteurized milk than the high heated milk proteins was observed for cow milk there was no difference for goat milk.

Heating milk has important impact on the hydrolysis kinetics of its protein fraction. In a recent study Ye and Sui have investigated the *in vitro* gastric digestion of unheated and heated (90°C for 20 minutes) skimmed milk (Ye et al., 2016). Both milks formed a gastric clot after 10 minutes digestion but with very different structures: while unheated milk « showed a closely knitted network with numerous small pores interspersed throughout the matrix » heated milk exhibited « a network structure with larger voids ». Contrary to unheated milk the heated milk clot includes both casein and whey proteins within a loose structure. As a result, casein digestion is much slower, and β-lactoglobulin and α-lactalbumine remained globally intact with unheated milk. In heated milk whey proteins were readily hydrolyzed and only peptides were released within small intestine. Upon longer time of gastric digestion clot structures tightened and became less permeable consequently influencing casein digestion by pepsin. Clot formation is therefore initially governed by the action of pepsin towards casein κ leading to the destruction of the protective effect that

casein *k* have on casein micelles. Heating clearly influences clot structure and this may find application through the creation of clot with different structures to control protein bioavailability and also of non protein compounds to influence glycemic, lipidic and aminoacid metabolisms.

Rinaldi *et al*. went further by studying *in vitro* gastrointestinal protein digestibility of three commercial distinct dairy matrices, *i.e.*, sterilized and pasteurized milks (liquid matrices), and stirred-yogurt (semi-solid matrix) (Rinaldi et al., 2014). As suggested in the previous study, the differences in both caseins and whey protein digestions were mainly driven by the gastric digestion phase. After buccal digestion, soluble proteins were lower with yogurt than with milks but the difference decreased at the end of gastric digestion and disappeared after duodenal digestion. However, between each phase of digestion, the highest increase in soluble proteins was observed with yogurt. Casein digestion began in stomach and was slower for pasteurized milk than with sterilized milk and yogurt. At the end no casein was present after duodenal digestion and traces of whey proteins were observed for pasteurized milk and yogurt. Besides, the release of free aminoacids during the duodenal phase varied according to their nature (acid, basic, neutral or hydrophobic) and seemed to be driven by enzyme specificity, but hydrophobic aminoacids were released at higher amounts for all matrices. On the other hand, a greater proportion of free aminoacids and short peptides was released from sterilized milk than from other dairy matrices; which leads authors to suggest that more than food structure, processing, especially heating, had impact on protein digestion. Hence the importance of a good mastery of heating process.

The degree of complexity was still increased in the following study in which a yogurt, three raw (whole, homogenized, skimmed), two pasteurized (82°C for 30 seconds, whole, homogenized) and three sterilized (120°C for 10 minutes, whole, homogenized, semi-skimmed) milks were manufactured from the same original milk batch, and subjected to *in vitro* digestion using an infant gut model (Dupont et al., 2010). The primary objective of this study was to determine whether processing could modify the resistance of caseins to digestion and to identify resistant regions capable of eliciting an allergic response in infant, not to study the protein digestive fate of each dairy product. Globally caseins resisted digestion, especially casein κ and α_{s1} due to hydrophobic fragments and/or post-translational modifications such as phosphorylation and glycosylation. Thus, at gastric level it seems that there was a relationship between hydrophobicity at pH 3.0 (gastric pH) and resistance to digestion, each type of casein having its own hydrophobic fraction: 149-199 for casein α_{s1}, N terminal moiety for casein α_{s2}, 55-92 for casein β and 120-169 for casein κ; these area becoming more hydrophilic at duodenal level and therefore more accessible to digestive enzyme. Otherwise, milk processing leads to different peptide profiles and milk heating increased the number of peptides in digested samples (as shown in previous study). For all three matrices, at gastric level intact casein all disappeared after 20 minutes of digestion, and β-lactoglobulin was

more digested with yogurt. Authors identified 19 peptides from casein α_{s1} with raw milk, 16 for pasteurized milk and 51 for yogurt. Finally it was concluded that milk processing increases casein resistance to digestion in the condition of this study.

Subsequently, the study of Tunick *et al*. (Tunick et al., 2016) investigated the role of milk processing treatments on proteins and lipids digestion. Raw skim milk, raw whole milk, homogenized raw whole milk, pasteurized whole milk, homogenized HTST-pasteurized whole milk, homogenized UHT-sterilized whole milk, HTST-pasteurized skim milk and UHT-sterilized skim milk (Tunick et al., 2016) were prepared. Before digestion, the protein profiles of most milks were similar except for homogenized UHT-sterilized whole milk and UHT-sterilized skim milk which showed a smearing of the bands indicative of new disulfilde bond formation (Wada and Loennerdal, 2014). During gastric digestion, homogenized UHT-sterilized whole milk and UHT-sterilized skim milk showed faded bands for β-lactoglobulin and darker bands for the peptides below 5 kDa. This is in agreement with previous study (Rinaldi et al., 2014) where denatured β-lactoglobulin is sensitive to pepsin hydrolysis favoring the production of smaller peptides. Also, whole milks and skim milks exhibited different protein digestion profiles. Independently of the processing treatment applied, fader protein bands were observed for skim milk samples during gastric and duodenal digestion suggesting a faster protein bioaccessibility. For skim milk, HTST-pasteurized or UHT-sterilized have no effect on protein digestion according to the SDS-PAGE protein profile. However for whole milks, the processing treatments have an impact. Homogenized HTST-pasteurized whole milk was more resistant during intestinal digestion compared to the others milks. This may be explained by the residual large fat droplets stabilized by proteins found at the end of the intestinal digestion for homogenized HTST-pasteurized whole milk. This could not be confirmed for homogenized UHT-sterilized whole milk as the confocal microscopy images were not presented by the authors.

It is obvious that thermal processing have important impact on the kinetics of nutrients release (peptide production). But altering the composition by modifying the casein to whey protein ratio (Rioux and Turgeon, 2012) or by the addition of stabilizer (Rinaldi et al., 2015) may also affect protein digestion. Indeed, three yogurt formulations with casein to whey protein ratios of 4.5:1 (ratio of regular milk), 2.8:1 (ratio usually used in the yogurt industry) and 1.5:1 (ratio containing more whey proteins) were formulated (Rioux and Turgeon, 2012). Yogurt with a 1.5:1 ratio was more hardly digested. The heat treatments used during yogurt-milk processing favors the formation of denatured whey proteins which have the capacity to form large aggregates or complexes with κ-casein. These were proposed to have a larger size in yogurts with a 1.5:1 ratio, affecting pepsin proteolytic activity on caseins. The addition of stabilizers (starch, pectin or β-glucan) also modifies milk protein digestion during the gastric phase (Rinaldi et al., 2015). Faster

proteolysis, lower release of large peptides, and a higher proportion of free amino acids was found for yogurts formulated with pectin or β-glucan than those with starch or without stabilizer. Stabilizers and proteins are not always compatible when found in mixture and sometimes they segregate (found in separate phases). It was hypothesized that β-glucan and dairy proteins segregate during *in vitro* gastric digestion explaining the faster proteolysis found due to concentration of pepsin and proteins in a separated phase. This could be considered as "micro-reactors" favoring protein digestion. In milk beverage enriched or not with alginate (negatively charged polysaccharides) or konjac glucomannan (neutral polysaccharides), milk proteins digestion was reduced (Borreani et al., 2016). Under the condition studied, alginate is interacting with dairy proteins preventing pepsin hydrolysis explaining a decrease in protein digestion compared with the other beverages.

In the last study Matar *et al*. evaluated the effect of lactic acid bacteria on protein hydrolysis during milk fermentation (by *Lactobacillus helveticus*) and on the subsequent release of aminoacids and peptides after simulated peptic and pancreatic digestion (Matar et al., 1996). Fresh, unpasteurized skim milk, pasteurized skim milk, pasteurized skim milk containing cells of *Lactobacillus helveticus*, pasteurized skim milk fermented for 12 h with *Lactobacillus helveticus* with pH controlled at 6, and pasteurized skim milk fermented for 12 h with *Lactobacillus helveticus* without pH control were tested upon *in vitro* digestion. Overall, there were already free amino acids in fermented milk, and milk fermentation impacted the release of some amino acids during *in vitro* digestion, *e.g.*, the availability of proline. In milk, proline release is very slow due to the resistance of imine bond (*i.e.*, compound containing a carbon-nitrogen double bond) to pepsin and pancreatin activities. Specific proteolytic activity of *Lactobacillus helveticus* leads to faster release of proline and short peptides containing proline. Fermentation also favours modification of protein structure and the release of peptides having different hydrophobicity. The bacterial activity also generated some bioactive peptides such as peptide 114-119 from casein β and peptide 98-106 from casein κ. Authors concluded that the fermentation of milk by proteolytic bacteria may therefore provide a new mechanism leading to the release of novel peptides from milk proteins after digestion.

Cheeses

Cheese matrices are complex in both structure and composition. Recently Fang *et al*. have carried out two *in vitro* digestion studies on different cheeses varying in texture and fat content (Fang et al., 2016a; Fang et al., 2016b).

The first study have been led with five commercial cheeses, covering a range of textural properties: a Camembert (soft texture, but described as "firm" in this study), a smear cheese, a young Cheddar (uncooked pressed cheese), an aged Cheddar and a string Mozzarella (Fang et al.,

2016a). After gastric digestion disintegration and proteolysis profiles were different according to cheeses considered. Disintegration was higher when initial hardness, cohesiveness and chewiness were lower. Thus the Camembert needed more strength for its matrix to be broken up. The buccal disintegration was already rapid then gastric pepsinolysis decreased cohesive strengths that maintain the matrix, increasing furthermore disintegration. For mozzarella, initial cohesiveness and hardness were higher leading to a slower and continuous gastric disintegration. Otherwise, an important result was that fat content and proteolysis impacted textural characteristics that in return impacted disintegration; and cheese disintegration content was positively correlated with fat content. Thus elastic mozzarella was three times less disintegrated than matured soft cheese (Camembert and aged Cheddar) with higher fat contents. Then, peptides/protein releases were in agreement with disintegration kinetics, *i.e.*, two-fold less with mozzarella. Overall at the end of orogastric digestion Camembert and mozzarella were disintegrated by around 75 and 18%, respectively while peptide/protein releases were of around 40 and 17%, respectively. Duodenal digestion exhibited a very important role on disintegration and proteolysis so that there were no more significant difference for disintegration and peptide/protein release after both 5 and 180 minutes digestion for Camembert and mozzarella. The results of this first study well showed that it is possible to control cheese manufacturing parameters, *i.e.*, structure and texture, to modulate further protein digestion and impact post-prandial proteic metabolic response.

In the second study, the effect of fat content and texture was further investigated through *in vitro* digestion of Cheddar (33% fat), light Cheddar (21% fat), Mozzarella (20% fat) and light Mozzarella (14% fat) cheese particles (Fang et al., 2016b). For the four cheeses the disintegration profiles were clearly modulated by texture. Thus at the end of gastric digestion, considering all cheeses together disintegration was negatively correlated with hardness, cohesiveness, resilience, fat content, proteolysis degree, chewiness and adhesiveness. At the end of duodenal digestion, disintegration was negatively correlated with hardness, elasticity, cohesiveness, resilience, proteolysis degree and chewiness. Again firmer and more cohesive cheeses were disintegrated more slowly, and nitrogen compounds followed disintegration profiles. Disintegration continue at duodenal level but the time required for complete disintegration differed according to cheeses. Without enzymes, disintegration occurred but it was weaker with an average of around 75% with enzymes and of around 30% without enzymes. Concerning the protein release there was no significant difference after oral digestion; while after gastric digestion it was the highest with regular Cheddar (≈ 52%) and the lowest with light Mozzarella (≈ 40%). Differences were leveled after duodenal digestion but regular Cheddar always exhibited a significantly higher level of disintegration than three other cheeses. Finally, duodenal cheese disintegration was negatively predicted (total variance explained: 93%) by solid non-fat, chewiness, hardness and resilience.

Authors reached the same conclusion than in their first study, *i.e.*, « the modification of fat content in cheese, affecting cheese texture and microstructure, could regulate the protein digestion, which may further affect acute protein metabolic response and some physiological functions » (Fang et al., 2016b).

Similar results were obtained by the same research team in 2012 (Lamothe et al., 2012). Mild Cheddar (31% lipid), aged Cheddar (34% lipid), light Cheddar (23% lipid) and Mozzarella (25% lipid) cheeses were also submitted to *in vitro* gastro-intestinal digestion and investigated for the association between texture and disintegration rate and extent. After two hours of gastric digestion aged Cheddar was clearly the more rapidly disintegrated compared to other three cheeses (≈ 65 versus ≈ 20-30%); but differences were again leveled after two hours duodenal digestion even if kinetics differed. In all cheeses tested, the most important changes in matrix disintegration were observed within the first 30 min of the duodenal phase. Finally the fat content also affected degradation in the duodenal phase.

Qureshi *et al*. studied *in vitro* gastrointestinal protein digestibility of two other types of cheese (Norwegian): the Gamalost (granular cheese, ripened for 10 and 30 days) and the Norvegia (hard cheese, ripened for 90 days) (Qureshi et al., 2013). The Gamalost is prepared by boiling milk of lean cow without adding rennet, contrary to what is customary in Europe. The Norvegia cheese is a trademark of cheese with secret recipe and close to Gouda. It melts easily with heat. The two cheeses therefore have different matrices. Overall Gamalost cheese gave more peptides after digestion than for Norvegia cheese, most of them derived from β-casein, some of casein α_{s1} and only a few from casein α_{s2} and κ. After digestion, both cheeses led to increase amounts of released aromatic and positively charged amino acids, and leucine; but considering all amino acids, Gamalost cheese released higher quantities than Norvegia cheese. Some peptides remain intact after digestion and more peptides were generated after gastric than duodenal digestion. Such results again emphasized an important matrix effect for cheeses with different initial texture.

In the end, cheeses were more resistant to protein and lipid digestion than liquid or semi-solid matrices were. No direct relationship could be established between disintegration kinetics and cheese rheological properties (Lamothe et al., 2017).

Animal studies

The matrix effect was first tested in minipigs consuming yogurt or milk (Gaudichon et al., 1994).This study was of great interest because it was a *sensu stricto* bioavailability study using a radiolabeled yogurt and milk with nitrogen ^{15}N allowing differentiation between endogenous and exogenous nitrogen. First, there was no effect of both dairy matrices on nitrogen endogenous secretions. Compared to milk, yogurt delayed intestinal release of liquid phase and of chyme

nitrogen fraction. One hour after consumption, 84 and 64% of exogenous nitrogen remained in the stomach for yogurt and milk, respectively; but after four hours more yogurt nitrogen remained in the stomach (19%) compared to milk (32%). Overall both matrices were highly digestible as 93% of exogenous nitrogen has disappeared twelve hours after meal. The most striking result was the high correlation between absorbed exogenous nitrogen and kinetics of released nitrogen at intestinal level (r = 0.999 for milk and r = 0.974 for yogurt). Once in intestine milk proteins were rapidly absorbed. Gastric emptying therefore appears to be a major factor for controlling the kinetics of nitrogen absorption, the higher viscosity of yogurt allowing a more regular release of nitrogen into intestine leading to a more gradual absorption and distribution of dietary nitrogenous compounds within organism.

The next study, also carried out in minipigs, is also particularly relevant for evaluating the impact of dairy food matrices on protein digestion because from the same ULH ("ultra low heat") skimmed milk powder were processed four different matrices: 1) heated liquid skimmed milk (90°C for 10 minutes); 2) unheated liquid skimmed milk; and 3-4) corresponding dairy gels (coagulation with rennet at 20°C for 24 hours) (Barbé et al., 2013). First dairy gels slowed down the flow of the meal from stomach with low level of proteins at duodenal level. This consequently slowed down amino acid absorption and bioavailability. In the stomach casein and β-lactoglobulin from unheated dairy matrices were sensitive and resistant, respectively, to hydrolysis; whereas with heated dairy matrices they all exhibited similar digestion β-lactoglobulin becoming susceptible to hydrolysis. Compared to other matrices, casein from heated gel seemed slightly more retained in the stomach. With heated liquid matrix β-lactoglobulin showed a fast duodenal appearance while being very low for gels. Otherwise liquid versus solid matrices had not the same effect on CCK (anorexigenic) and ghrelin (orexigenic): for example the increase in CCK (cholecystochinine) concentration was faster with unheated liquid dairy matrix than with unheated gel; but unheated gel induced a more prolonged increase in CCK than unheated liquid matrix. Finally post-prandial decrease in ghrelin concentration was more pronounced with unheated gel than with unheated liquid. The main conclusion of this study was that both microstructure and macrostructure were affected by heating and gelification processes, respectively, and that both impacted differently proteolysis steps, microstructure acting more on hydrolysis and macrostructure more on amino acid absorption and bioavailability.

The following year Barbé *et al*. published another study in multi-canulated minipigs to investigate the protein digestion of two distinct dairy gel matrices with the same composition, similar rheological and structural properties but differing for their type of coagulation (acidification *versus* renneting) (Barbé et al., 2014b). While the acid gel led to a duodenal peak of casein and β-lactoglobulin after only 20 minutes of digestion and to a peak of plasmatic amino acids after 60

minutes of digestion the rennet gel led to lower levels of duodenal proteins and plasmatic amino acids. In addition the rennet gel appeared more statietogenic (plasma CCK). Authors proposed the following explanation for the observed effects, *i.e.*, a combined action of three mechanisms: 1) a strong resistance of the rennet gel to hydrolysis in the stomach, 2) combined with a longer gastric retention time, and 3) a substantial dilution by gastric juices; besides, while acid gel flocculated in stomach without apparent syneresis the rennet gel exhibited an extensive syneresis indicating a strong contraction of gel particles leading to a more pronounced retention and a delayed gastric emptying. In addition particle contraction has limited enzyme accessibility to cleavage sites of proteins (Kong and Singh, 2008). Therefore, the time of gastric residence might induce satiety with potential application in developing diets for loosing weight in case of obesity (Kong and Singh, 2008). On the contrary, the impacted bioavailability of amino acids may have a potential effect on splanchnic and peripheric protein metabolism with potential application in elderly populations for which the preference for acidified milk would induce a more efficient muscular synthesis.

In their third study Barbé *et al*. went further through identification (by LC-MS/MS) of released peptides in the gastrointestinal tract of minipigs from six distinct dairy matrices with similar composition but with different physical structure (Barbé et al., 2014a). Duodenal effluents were collected upon five hours after consumption of the six following dairy matrices (which were prepared from Ultra Low Heat (ULH) skim milk powder): 1) heated milk (90°C for 10 minutes), 2) raw milk, 3-4) corresponding rennet gels, 5) acid gel from heated milk, and 6) stirred acid gel from heated milk. Authors reported that 16 000 peptides were clearly sequenced and identified and the dairy structure had only a little influence on the localization of cleavage sites as regards with protein sequences. However structure strongly impacted the number of identified peptides more specifically for rennet gel that led to three-fold less identified peptides. As for the previous study effect was attributed to the the more important degree of dilution in the stomach by digestive secretions plus a longer gastric retention. These results led to an important conclusion: more than proteolysis mechanisms the dairy matrix structure strongly impact dairy protein digestion kinetics.

From data collected in minipigs towards the digestion of different dairy matrices, Le Feunteun *et al*. developed a mathematical model for dairy protein digestion (Le Feunteun et al., 2014). For collecting data they used six 18-month-old female adult mini-pigs and six distinct dairy matrices (same as those described in the paragraph above) with identical composition based on a skimmed milk powder. Model was therefore developed to correspond to *in vivo* data. To summarize the collected data clearly showed that dairy matrix structures impact protein digestion kinetics, and the great differences between amino acid absorption kinetics can be entirely understood by considering the behaviour of dairy matrices in the stomach. Thus the raw rennet gel clearly exhibited the more sustained and low appearance of plasma amino acids while raw milk led to the

highest peak of plasma amino acids only after 20-30 minutes digestion. Other matrices had intermediary behavior but the tendency for amino acid absorption appears to be in decreasing order: milks > acid gels > rennet gels. These results well emphasize that it may exist slow and rapid dairy protein only according to matrix structure, not composition, as was demonstrated earlier with whey *versus* casein (Boirie et al., 1997).

Although composition of infant formula has been significantly improved during the last decade, major differences with the composition and structure of breast milk still remain and might affect nutrient digestion and gut biology. The incorporation of dairy fat in infant formulas could modify their physiological impacts by making their composition closer to that of human milk. The effect of milk fat and milk fat globule membrane (MFGM) fragments in infant formulas on gut digestion, mucosal immunity and microbiota composition was evaluated (Le Huërou-Luron et al., 2016). Three formulas containing either (1) vegetable lipids stabilized only by proteins (V-P), (2) vegetable lipids stabilized by a mixture of proteins and MFGM fragments (V-M) and (3) a mixture of milk and vegetable lipids stabilized by a mixture of proteins and MFGM fragments (M-M) were automatically distributed to 42 newborn piglets until slaughter at postnatal day 7 or 28, and compared to a fourth group of sow's suckling piglets used as a breast-fed reference. At both postnatal day, casein and β-lactoglobulin digestion was reduced in M-M proximal jejunum and ileum contents compared to V-P and V-M ones leading to more numerous β-casein peptides in M-M contents. In conclusion, the incorporation of both milk fat and MFGM fragments in infant formula modifies protein digestion.

Human studies

All collected human studies were led in healthy adults; but only one study *stricto sensu* studied the effect of dairy matrices - as eaten - on protein metabolism in humans (Gaudichon et al., 1995). Other studies used casein or whey fractions (Boutrou et al., 2013; Churchward-Venne et al., 2015; Gaudichon et al., 1999), or compared cottage cheese with eggs (Nuttall and Gannon, 1990).

The bioavailability study by Gaudichon et al. pointed out to the role of dairy matrices on protein digestibility in sixteen healthy adults (average age of 30 years) by using radiolabelled milk and yogurt (from the same milk) with ^{15}N (Gaudichon et al., 1995). Both products significantly stimulated endogenous nitrogen secretion 20-60 minutes after consumption, and upon four hours endogenous nitrogen flow rate did not differ between both matrices. An expected high level of proteolysis was also observed for both matrices. However the yogurt led to delayed gastric emptying as regards with exogenous nitrogen flow rate as compared to milk. Otherwise when evaluating the net gastro-jejunal exogenous nitrogen absorption there was not significant difference between milk (57%) and yogurt (51%). Finally, concerning non-protein nitrogen, the measured flow

rate for yogurt tended to be higher to that of milk between 40-100 minutes after consumption. As shown with mini-pigs it seems that the stomach step plays an important role in controlling the delivering of protein in the small intestine and for the further absorption of amino acids with potential application for protein anabolism especially in elderly populations.

The study by Boutrou *et al*. does not relate to complex dairy matrices as usually consumed but casein and whey proteins (Boutrou et al., 2013). However, since these two milk proteins have different structures, the results obtained give interesting information on the peptide profiles and the presence of bioactive peptides after digestion at the jejunal level according to these two protein structures. The study has been led during 6 hours post-prandially in thirteen healthy adults (18-40 years old) consuming 30 g of either radiolabelled (^{15}N) casein or lactoserum. First, the recovery of dietary nitrogen indicated that 50% and 60% of ingested nitrogen had already been absorbed after 6 hours at the jejunal level in WP and casein groups, respectively. Then the jejunal peak of exogenous nitrogen after whey consumption was largely higher than that of casein at 0.5-1 hour after consumption; but casein showed a more regular and lower nitrogen profile in agreement with previous results about rapid *versus* slow protein (Boirie et al., 1997). And the peak of jejunal peptides was around 0.5-2.5 hours for casein and one hour for whey. Casein was a more important peptide precursor at jejunal level with 356 identified and sequenced peptides against 146 for whey. Casein peptides also presented varied biological activities and were of average size, *i.e.*, 750-1050 KDa during 6 hours while whey presented during 3 hours peptides with larger size, *i.e.*, 1050-1800 KDa. Moreover, the quantities of casomorphins β (caseins β 57, 58, 59 and 60-66) and casein β 108-113 released were sufficient to obtain a biological action of these peptides (*i.e.*, respectively opioid and antihypertensive).

The second study by Gaudichon *et al*. did not compare different dairy matrices but it deserves to dwell on it because it indirectly evaluates the "matrix effect" of milk by comparing the protein digestion of radiolabeled casein alone and in the presence of sucrose or milk lipids (Gaudichon et al., 1999). Thus, 25 healthy male and female subjects (mean age of 29 years) consumed three different diets, each containing 30 g of defatted milk proteins labeled with ^{15}N: 1) milk protein alone; 2) milk proteins + sucrose (100 g); and 3) dairy protein + 43 g of dairy lipids (from 36 g of butter and 46 g of cream). Sucrose clearly slowed down protein absorption on longer time than lipids but in the end ileal digestion did not differ between the three diets (around 95%). Sucrose also significantly reduced post-prandial transfer of nitrogen from milk to urea but not lipids again. Secondly the net post-prandial milk protein utilization (8 hours after meals) was significantly higher with sucrose (85%) compared to protein alone or with lipids (80%). According to authors « This study shows that energy nutrients do not affect the nitrogen absorption but modify the metabolic utilization of dietary protein in the phase of nitrogen gain » (Gaudichon et al., 1999).

In the following study, Churchward-Venne *et al*. have hypothesized that the digestion and kinetics of protein absorption and the subsequent response of muscle protein synthesis after ingestion of micellar casein is modulated by the dairy matrix (Churchward-Venne et al., 2015). Their assumption or hypothesis was based on the well-known fact that the slow digestion and slow kinetics of amino acid uptake from isolated micellar casein are believed to be responsible for the relatively lower postprandial response of muscle protein synthesis compared to those obtained with more rapidly digested proteins such as isolated whey. In this study subjects were older than in previous one (average age of 71 years) and consumed 600 mL casein with ^{13}C leucine in either bovine milk serum or water. The reconstructed dairy matrix (casein + bovine milk serum) did not significantly modulate the post-prandial rate of myofibrillar protein synthesis compared to isolated casein; and there was no significant difference for ^{13}C phenylalanine enrichment of muscular proteins. Therefore if casein of a given dairy matrix delays digestion and absorption of protein it does not modulate post-prandial muscular protein synthesis as compared to isolated micellar casein. More generally authors concluded that « These findings demonstrate that the anabolic response to micellar casein protein is not compromised or enhanced by the milk matrix » (Churchward-Venne et al., 2015).

Although the following study is quite ancient and does not compare two different dairy matrices, but that of cottage cheese and egg white, its results are interesting to analyze because both products have quite closed protein (12.1 *versus* 10.6%, respectively), carbohydrates (2.2 *versus* 0.9%) and lipids (3.9 *versus* 0.2%) compositions, but with different matrices (Nuttall and Gannon, 1990). The study was led in seven healthy adults (average of 30 years) consuming either 480 mL water or 50 g cottage cheese or egg white. After 8 hours the area under curve for plasma nitrogen from amino acids and urea was lower by 50% for egg white compared to cottage cheese, notably between 0-4 days. This was associated with a 50% lower protein conversion in urea. Overall 70% of cottage cheese might explain the urea formation and only 47% for egg white. Finally cottage cheese exhibited a better protein digestibility.

Conclusions on protein fraction

First of all, it should be noted that there are, in the end, very few studies comparing the digestive evolution, more particularly that of proteins, of dairy products as consumed by man and presenting different matrices. From the synthesis of *in vitro*, animals and human studies, it is difficult to draw firm conclusions about the influence of the dairy matrix on digestibility, bio-accessibility, bioavailability and nutritional effects of the protein fraction. However, some conclusions, although still partial, can be proposed in relation to the studies described above (see also Table 1):

Milks versus acid gels versus rennet gels (mini-pigs)

In pigs, based on dairy matrices of the same composition but differing in their internal structures, the large differences in the kinetics of absorption of amino acids can be fully understood by considering behavior of dairy matrices in the stomach. Compared to gelling by rennet, acid gelling results in a higher peak of duodenal proteins as well as for amino acids, suggesting resistance of rennet gel to gastric digestion; but the differences are leveled at the jejunal level. More generally, gelation slows gastric emptying and subsequent absorption of amino acids, and decreases their bioavailability in peripheral blood. Moreover, the structure of dairy matrices strongly affects the kinetics of digestion of proteins rather than the mechanisms of proteolysis *per se*. Another main conclusion is that both microstructure and macrostructure are affected by heating and gelification processes, respectively, and that both impact differently proteolysis steps, microstructure acting more on hydrolysis and macrostructure more on amino acid absorption and bioavailability.

Cheeses (in vitro)

Depending on their texture, cheeses such as Camembert (soft cheese), blue-veined cheese, young and aged Cheddar cheese, and Mozzarella (elastic structure) have different disintegration and digestion patterns during gastric digestion. Thus, the disintegration at the end of gastric digestion is higher when the initial hardness, cohesion and chewability are lower. Thus, Mozzarella, with higher initial cohesion and hardness values, is disintegrated slowly and continuously during gastric digestion. At the end of gastric digestion, elastic-type cheeses (Mozzarella) are about three times less disintegrated in comparison with ripened soft cheeses with a high fat content (Camembert, aged Cheddar); and the release of peptides/proteins, in agreement with the disintegration kinetics, is globally two times lower. Textural properties and structural characteristics such as protein matrix density and fat distribution also affect degradation during the duodenal phase.

Concerning Norwegian cheeses Gamalost (grained cheese) *versus* Norvegia (firm cheese) more peptides are detected in digested Gamalost compared to Norvegia cheese. Moreover, most of the Gamalost peptides are derived from β-caseins, some from casein αs1, and very little from casein αs2 and casein κ. Gamalost and Norvegia cheeses therefore have different protein digestibility profiles, underlining an important "matrix" effect.

If we can attempt a first general conclusion on the influence of cheese matrices on proteolysis, it would appear that more cohesive, compact, elastic and structured cheeses disintegrate less rapidly during digestion, especially at the gastric level, resulting in slower proteolysis. This result would be in line with what has been observed with breads, *i.e.*, those with more dense crumb give more time-spreading glycemic responses and have lower glycemic indexes (Burton and Lightowler, 2006; Saulnier and Micard, 2012).

Milks versus yogurts versus fermented milks (in vitro, mini-pigs and humans)

The digestion of casein begins during the gastric phase and appears slower for pasteurized milk than for yogurt. At the end of duodenal digestion, no intact casein is present in the different dairy matrices, while small bands of whey proteins are still visible for pasteurized milk and yoghurt compared to sterilized milk. The release of free amino acids during the duodenal phase varies according to their nature (acid, basic, neutral or hydrophobic) and appears to be governed by the specificity of the enzymes. For both types of matrix, the hydrophobic amino acids are released in significantly greater amounts than other types of amino acids. But overall, total protein digestibility seems to be identical between milk and yoghurt even though yogurt digestion results in different peptide profiles. Whether for milk or yogurt, digestion of β-casein gives a very significant number of peptides covering almost the entire sequence of this protein. It is believed that lactic fermentation is responsible for the formation of new peptides during gastrointestinal digestion. Compared to milk, yoghurt could also delay intestinal nitrogen delivery, but not final absorption. Concerning fermented milks, there is little data: fermentation of milk affects the release of certain amino acids during *in vitro* digestion. Proteolysis during fermentation can lead to the formation of new peptides during gastrointestinal digestion.

In mini-pigs, milk and yogurt proteins are also highly digestible (93%); and the kinetics of exogenous nitrogen delivery in the intestine is highly correlated with that of exogenous nitrogen uptake for milk and yoghurt. Gastric emptying also appears to be a major factor for controlling the kinetics of nitrogen absorption, the higher viscosity of yogurt allowing a more regular release of nitrogen into intestine leading to a more gradual absorption and distribution of dietary nitrogenous compounds within organism. Finally, in man fermentation only modifies the rate of gastric emptying of nitrogen (delayed with yoghurt) and does not affect the level of hydrolysis of milk and yoghurt made from the same milk, stimulation of endogenous nitrogen and digestibility rate. Whether in the mini-pig or man stomach seems to play a very important role in controlling the emptying of more or less hydrolyzed proteins in the small intestine.

The lipid fraction

In vitro studies

Milks, yogurts and derived products

Very few studies have investigated *in vitro* lipolysis of dairy products with different matrix. The first study was led with fresh raw bovine milk submitted to *in vitro* gastrointestinal digestion (Gallier et al., 2012). Briefly fat globules were stable in acidic conditions within stomach due to phospholipids. Then at intestinal level, lipolysis products (released from the hydrolysis of

triglyceride core) destabilized fat globules and provoked their coalescence or aggregation. Thereafter lipolysis products accumulated to the surface of fat globules forming a lamellar phase. Their solubilisation by bile salts led to the formation of disk-shapes micelles. Finally the free fatty acid release after one hour of gastric digestion and two hours of intestinal digestion followed a logarithmic-type curve (without lag phase), and reached 114 μmol free fatty acids/mL of subsample.

Initially the study by Kopf-Bolanz *et al*. was dedicated to validate an *in vitro* digestion system for investigating macronutrient decomposition in human; and homogenized and pasteurized milk was chosen as an example of a complex food matrix (Kopf-Bolanz et al., 2012). Although there is no comparison between different dairy matrices, the results on digestion of the lipid fraction deserve to be reported: first, the digestion process increased free fatty acid content from 1,04 to 62,8 mmol/L, *i.e.*, 41% of total milk fat content (however, assuming that all triglycerides present in whole milk were hydrolyzed into two free fatty acids and one monoacylglycerol, this value corresponded to 100% of the triglycerides); second, as regards with fatty acid distribution, more short-chain fatty acids (*i.e.*, around 11%) were released after digestion compared to relative total fatty acid content before digestion (*i.e.*, around 6%).

Subsequently, in the study of Tunick *et al*. (Tunick et al., 2016), described above for protein digestion, authors investigated the role of milk processing treatments on proteins and lipids digestion. Homogenization increases free fatty acids release attributed to the increase surface area improving lipase efficiency (Armand et al., 1992). This was confirmed by the confocal microscopy images during *in vitro* digestion showing smaller lipid droplets for homogenized HTST-pasteurized whole milks. The fatty acids profiles were also determined but their release was not affected by the processing treatments used in this study.

Finally, the *in vitro* study by Ye *et al*. provides interesting evidence on the role of the matrix effect of milk by comparing the digestion of the fat globules of a raw native milk and a recombinant milk from skim milk mixed with 4 % of anhydrous milk fat (Ye et al., 2010). The fat globules in raw milk are coated by natural milk fat globule membrane that is composed predominantly of phospholipids and milk fat globule membrane proteins whereas the fat globules in recombined milk are coated by casein micelles and whey protein. Overall there was a lower lipolysis in the raw milk than in the recombined milk. Authors suggested that « this is probably because of the inhibitory effect of components in the milk fat globule membrane on the activity of lipase » (Ye et al., 2010). Thus, the conversion of fat globules with their membrane (as in raw milk) into fat globules with superficial layers of casein and serum proteins (as in recombinant milk) increases lipolysis rate of dairy fat, involving an inhibitory effect of the membranes of the fat globules on the hydrolytic action of the pancreatic lipase. In this study authors also reported that the rate and degree of

lipolysis appears not to be influenced by the initial size of the fat globules. Finally after 5 hours of hydrolysis the extent of fatty acid release was equivalent for both milks.

Cheeses

The casein-calcium interactions are mainly responsible for the structuring of the protein network in the cheese, and therefore for its textural properties (Lucey and Fox, 1993; Lucey et al., 2003). Thus, in the most recent study Cheddar cheeses with different levels of calcium were made to establish how this compound impacts the *in vitro* digestion of cheese lipid fraction: 1) cheese control (25.0 mg calcium/g protein); 2) high-calcium cheese (29.6 mg calcium/g protein); and 3) very high calcium cheese (46.4 mg calcium/g protein) (Ayala-Bribiesca et al., 2016). As expected, the hardness of the cheese increases with the calcium content, resulting in a slower disintegration during digestion. However, despite more rapid disintegration, control cheese has the slowest lipolysis progression. This can be attributed to the fact that calcium increases lipolysis rates, an effect that can be explained by the depletion of fatty acids at the lipid-water interface. The final lipolysis rates after 300 min of digestion were 73.6%, 77.9%, and 72.5% for the control, high-calcium, and very high-calcium cheeses, respectively, with a statistical difference between the last two ($P = 0.0148$). Finally, this study suggests that lipolysis depends on the calcium content and the matrix modulating the access of enzymes to their substrates. To further understand the role of calcium on dairy fat lipolysis, Cheddar cheese were prepared with anhydrous milk fat with low (olein: unsaturated fatty acids and short chains fatty acids) or high (stearin: triacylglycerol and long chain fatty acids) melting point fatty acids (Ayala-Bribiesca et al., 2017). A control was also prepared with melted butter. For each Cheddar cheese prepared, two levels of calcium were also studied: regular (7,136 µg/g of cheese) or high (9,466 µg/g of cheese). The addition of calcium had no impact on cheese hardness at the opposite of the type of anhydrous milk fat. The cheese prepared with stearin anhydrous milk fat was 50% harder when the texture was measured at 22°C. A large proportion of the control and olein anhydrous milk fat was liquid at the measurement temperature as shown in the calorimetry thermogram. When measured at 37°C, most of the fat is liquid for all cheeses and no significant difference in cheese hardness was observed. The stearin cheese was still higher and retained its shape after compression mainly attributed to the residual solid fat content. This had a significant impact on cheese disintegration where stearin cheese were less disintegrated during gastric digestion compared to the other cheese matrices. In addition, no effect of calcium was found for the disintegration. The lipolysis was significantly modulated by the type of anhydrous milk fat and the calcium concentration. For the regular calcium cheeses, the stearin cheese had a lower lipolysis at the end of the duodenal digestion (below 50%) compared to the other cheeses. Short- and medium-chain fatty acids are more soluble and easily cleaved by lipase

(Mu and Porsgaard, 2005) favoring an increased fatty acid release for the control and olein anhydrous milk fat cheeses, both rich in short- and medium fatty acids (Ayala-Bribiesca et al., 2017). For high calcium cheeses, the kinetics of lipolysis was significantly different during digestion. Within the first 15 min, lipolysis was significantly increased for olein cheese compared to stearin and was maintained until 240 min. At the end of the duodenal digestion, lipolysis increased for the stearin cheese to reach a similar value than the other cheeses.

Lamothe *et al*. added that although composition factors (total lipid and calcium concentrations) are known to influence the lipolysis rate, the characteristics of the cheese matrix are also an important factor affecting the release of fatty acids, in particular *via* the restriction of enzyme and fatty acids diffusion (Lamothe et al., 2012). In their study, lipids are released from cheese matrices differently during the gastric phase, with aged Cheddar releasing fats faster than mild Cheddar and Mozzaralla, than light Cheddar, but the release rates are the same after 4 hours of gastroduodenal digestion for all cheeses (Lamothe et al., 2012). Authors explained that the stronger, denser and more cohesive matrix structure of light Cheddar could explain the lower rate of lipid digestion relative to other cheeses during gastric digestion. In addition free oil was strongly correlated with cheese disintegration during the different stages of cheese digestion. However, concerning free fatty acid release following lipid hydrolysis during the duodenal digestion the higher moisture and lower fat content of light Cheddar cheese make it easier for the enzymes to diffuse within the cheese matrix and react with fat droplets. On the contrary, according to authors the much lower degree of cohesion of aged Cheddar could promote matrix disruption into fine particles for which the greater firmness and lower porosity could contribute to the resistance of lipids to lipase attack. Then the greater extent of matrix disruption for aged Cheddar probably explains why its rate and extent of free fatty acids production were slightly greater after 60 min of duodenal digestion compared to mild Cheddar.

Therefore, for cheeses, greater fatty acid release could not be related to faster matrix disintegration, suggesting that the lipid droplet size dispersion was more important than matrix breakdown was for the modulation of lipid digestion kinetics (Lamothe et al., 2017).

Infant formulas

The effect of homogenization on gastric lipolysis was studied by investigating the semi-dynamic gastric *in vitro* digestion of three matrices (three model infant formulas): a standardized milk emulsion containing native milk fat globules referred to as minimally-processed emulsion, and two processed model infant formulas (homogenized or homogenized/pasteurized) (Bourlieu et al., 2015). Gastric conditions mimicked those reported in newborns. The minimally-processed emulsion was lipolyzed and proteolyzed slower than processed formulas. The difference in initial structure

persisted during digestion. The surface of the droplets was the key parameter to control gastric lipolysis kinetics; it ranged from 1.81 m²/g of lipid for minimally-processed formula to 31.90 m²/g of lipid for the most processed one. The pattern of released fatty acids and proteolysis by faster hydrolysis of adsorbed proteins was also significantly affected by homogenization.

Animal study

The objective of the study by Fruekilde *et al*. was to examine in the rat for the effects of the physicochemical properties of dairy products on lipid absorption at the lymphatic level (Fruekilde and Hoy, 2004). Although with very different fat contents (21 to 80%), the five dairy products tested (cream cheese, cream, sour cream, butter and melted butter) differ little in terms of their fatty acid composition. Administration of cream and sour cream leads to a faster absorption of the lipid fraction at the lymphatic level than with cream cheese, butter and melted butter, and a higher cumulative absorption after 8 hours. The lymphatic absorption of the fat after administration of the cream cheese is similar to the absorption after consumption of butter and melted butter for up to 4 hours; then it increases to a level between that obtained with the consumption in the rat of cream or sour cream, and of butter or melted butter. Overall, these results demonstrate different profiles of lymphatic absorption of dairy fat by their physicochemical properties. Because the fatty acid composition of dairy products differs only slightly, this indicates that the viscosity, the type of emulsion, the particle size and possibly also the protein content, influence the digestion and absorption of the dairy fat, and probably affects the lipemic response.

Human studies

Human studies comparing the effect on lipemia of different ready-to-eat usual dairy matrices are not so numerous. In the ten selected studies milk, fermented milk, butter, cheese, cream and yogurt have been tested against lipemia mainly in healthy adults, but also in subjects with metabolic syndrome, type 2 diabetes, and mildly hypercholesterolemic.

In the first and recent randomized and crossover study the impact of the cheese matrix on postprandial lipemia in healthy humans has been evaluated (Drouin-Chartier et al., 2017). Subjects consummed 33 g fat from a firm cheese (young cheddar), a soft cream cheese (cream cheese), or butter (control) incorporated into standardized meals that were matched for macronutrient content. After 4 h there was no difference in serum increases in triglyceride concentrations and in the triglyceride $iAUC_{0\text{-}8\ h}$ (area under curve) for the three dairy matrices. The most notable result was the significantly higher triglyceride response induced by the cream cheese (change from baseline: +44%) than those induced by butter (change from baseline: +24%) and cheddar cheese (change from baseline: +16%). According to authors the fat globule organization within cheese matrices

may have modulated the postprandial lipid response. In effect homogenization of the cream cheese increases the number and stability and decreases the size of lipid droplets. Thus, the diameter of a lipid droplet from cream cheese is ≈0.5 μm, approximately one-sixth the size of a cheddar cheese lipid droplet (≈3.0 μm), increasing contact surface for lipase.

The second study has been led during 8 weeks in 153 Norvegians aged above 18 years with (n = 44) and without (n = 102) metabolic syndrome (Nilsen et al., 2015). The subjects were divided into three groups: 1) Gamalost cheese (50 g/day) which is a traditional Norwegian skimmed milk cheese naturally free of salt and fat, containing only 160 mg calcium/100 g, and which has a high protein content and a high amount of bioactive peptides; 2) Norwegia, a gouda-type cheese (n = 50) (80 g/day) that contains 27% fat and 800 mg/100 g calcium; 3) control (n = 50). After intervention, no increase in LDL and total cholesterol was observed in the 'cheese' groups compared to the control. However, a stratified analysis showed that subjects in the Norvegia group with the metabolic syndrome showed significant reductions in total cholesterol at the end of the intervention compared to the control group (-0.70 mmol/L, p = 0.013) and also of triglycerides. In the Gamalost group, subjects who had a high total cholesterol level at baseline showed a significant reduction in total cholesterol compared to control (-0.40 mmol/L, p = 0.035).

In the next randomized 3 x 2 week crossover study semi-hard cheese was compared to skimmed milk in 15 healthy young men (Soerensen et al., 2014). Focusing on the specific potential effects of cheese calcium *versus* calcium in liquid milk, Soerensen *et al*. compared the effects of consumption of three isocaloric diets with similar lipid content and composition and varying in calcium intake. The calcium intakes in each of the three intervention periods were 400 mg/day without dairy (control), or 1700 mg/day including 800 mg/day via dairy products, either as 670 mL/day of skimmed milk, or 120 g/day of semi-hard cheese. Compared to the control diet, each of the two dairy-based diets limited total cholesterol and LDL cholesterol blood increases, but had no effect on triglycerides and HDL-cholesterol (no difference between the two dairy products), and resulted in a higher fecal lipid loss, which seems to be explained by their calcium content. Therefore, although the effect on blood lipids do not differ substantially between milk and cheese consumption with similar calcium contents, results of this study support that calcium-rich dairy foods may be less atherogenic than their calcium-poor counterparts.

The following intervention study was performed in 159 moderately hypercholesterolemic French patients (without treatment) consuming twice daily 30 g of Camembert or 250 g of whole yogurt for 5 weeks (Schlienger et al., 2014). No significant difference for all lipid parameters was observed both between groups and after the intervention, *i.e.*, total cholesterol, HDL- and LDL-cholesterol and triglycerides.

In 2011, a randomized cross-sectional study compared in 49 healthy adult males and females the effects of daily consumption of hard cheese (Samsø with 27% fat) for 6 weeks compared to butter (bringing the same amount of lipids, accounting for 13% of energy intake) (Hjerpsted et al., 2011). The cheese-based diet resulted in plasma LDL cholesterol concentrations 6.9% lower than the butter-based diet. Given the composition of the diets, the authors ruled out that this may be due to differences in calcium and protein concentrations between cheese and butter, and suggest mechanisms related to fermentation and a "matrix effect" due to the dispersion of the fat globules in the solid casein matrix of the cheese.

In another randomized cross-sectional study, healthy volunteers were subjected to a diet in which 20% of their total energy intake was provided by dairy fat, either in the form of hard cheese, milk or butter (Tholstrup et al., 2004). On the fourth day of each 3-week intervention period, an acute postprandial test was performed with the test product. These tests revealed no difference in the amount or profile of chylomicron fatty acids from cheese, milk or butter. However, a long-term effect was observed: daily cheese consumption resulted in lower total cholesterol and LDL-cholesterol than that of butter. These results are in agreement with the previous observation of a lower total cholesterol level after consumption of cheese ('Jarlsberg', Swiss type) than butter (Biong et al., 2004), both studies using diets where dairy fat constituted 20% of the energy intake. At equal fat content the cheese can therefore be less hypercholesterolemic than butter (no differences for triglycerides and HDL-cholesterol).

The last study with cheese has been led in diabetic subjects (Clemente et al., 2003). Test meals containing 30 g of fat in the form of Mozzarella cheese or milk resulted in a peak plasma of triglycerides earlier than the same amount of butterfat, although the cumulative absorption of triglycerides on 6 hours (area under the curve) was similar in all groups. In addition, the rate of gastric emptying is significantly faster with Mozzarella than with butter or milk. The authors conclude that « while the physical structure of fat-rich foods has no major effect on postprandial plasma triglyceride concentrations, it is able to influence the timing of triglyceride peak; gastric emptying time does not play a major role in modulating the postprandial response of triglycerides and glucose ».

In the last two studies fermented milk (Sanggaard et al., 2004) and yogurt (Rossouw et al., 1981) were compared to milk towards lipemia. The study by Sanggaard *et al.* had the main objective of comparing the effects of whole milk and fermented whole milk (with a mixture of *Lactobacillus acidophilus* and *Lactococcus cremoris*) on postprandial lipid and carbohydrate metabolism, rate of gastric emptying and appetite (Sanggaard et al., 2004). Lactose was added to the fermented milk to equalize the lactose contents of both products. Fermented milk results in a significantly slower rate of gastric emptying (p <0.001), probably because of higher viscosity. In

addition, fermented milk results in a greater increase and a more rapid decrease in the triglyceride content in all lipoprotein fractions (LDL fractions, $p < 0.005$; other fractions HDL, VLDL and chylomicrons, $p < 0.001$). The more prolonged and lower triglyceridemic response to milk consumption may be due to coagulation of milk in the stomach. These effects were attributed to the higher viscosity of the fermented milk and could therefore be applied to fresh cheeses. Finally, Rossouw et al. conducted a short, 3-week randomized controlled study in 32 healthy adolescents who consumed 2 liters of skimmed milk, yoghurt or whole milk cream per day (Rossouw et al., 1981) in addition to their usual diet. Compared with pre-intervention values, plasma LDL-cholesterol concentrations were reduced in the skimmed milk group but increased in the yoghurt and cream groups.

Finally in a randomized controlled trial, eight hospitalized tube-fed preterm infants were their own control to compare the gastric digestion of pasteurized human milk and of homogenized pasteurized human milk. Pasteurized human milk was obtained from donors and, for half of it, was homogenized by ultrasonication (de Oliveira et al., 2017). Over a six-day sequence, gastric aspirates were collected twice a day, before and 35, 60 or 90 min after the start of pasteurized human milk or homogenized pasteurized human milk ingestion. The impact of homogenization on pasteurized human milk digestive kinetics and disintegration was tested using a general linear mixed model. Results were expressed as means ± SD. Homogenization led to a six-fold increase in the specific surface ($P < 0.01$) of lipid droplets. The types of aggregates formed during digestion were different between pasteurized human milk and homogenized pasteurized human milk, but the lipid fraction kept its initial structure all over the gastric digestion (native globules in PHM vs. blend of droplets in PHHM). Homogenization increased the gastric lipolysis level ($p < 0.01$), particularly at 35 and 60 min (22 and 24% higher for homogenized pasteurized human milk, respectively). Homogenization of pasteurized human milk increased the gastric lipolysis level. This could be a potential strategy to improve fat absorption, and thus growth and development in infants fed with pasteurized human milk; however, its gastrointestinal tolerance needs to be further investigated.

Conclusions on the lipid fraction

Overall, these works show that differently structured dairy products, including cheeses, can result in different profiles of postprandial lipemia. First, fatty acid release in the intestinal phase was much faster when matrices were produced from homogenized milk (Lamothe et al., 2017). Second, the composition of the cheeses, their viscosity and the presence of a matrix that is more or less solid, as for a pressed cooked cheese compared to a fresh cheese, could affect the kinetics of digestion of the lipids of cheeses. Indeed, the higher the viscosity of the food bolus, the longer the gastric emptying time, which results in delaying the peak of postprandial triglyceridemia. This raises the question of

the effects of the viscosity of different cheeses (soft fresh cheeses *versus* hard cheeses in particular) on the metabolism of fat, especially when consumed as such in the French way, and not primarily in a melted form as in North America. On the basis of the *in vitro* and *in vivo* studies presented, additional conclusions can be proposed:

In vitro studies

The comparison of a native milk with a recombined milk shows that the lipolysis rate of milk fat is increased when the fat globules with their membrane (as in raw milk) are converted into fat globules with superficial layers of casein and serum proteins (as in recombined milk), and that the rate and degree of lipolysis appears not to be influenced by the initial size of the fat globules; which could be attributed to changes in the physicochemical properties of fat globules during lipid hydrolysis.

The casein-calcium interactions are mainly responsible for the structuring of the protein network in the cheese and the hardness of the cheeses thus increases with the calcium content, resulting in a slower disintegration during digestion; but although the final rate of lipolysis did not differ over three hours, the slowest lipolysis was observed with the fastest disintegrating cheese. Calcium thus influences both the structure of the cheese and the kinetics of lipolysis. Moreover, aged Cheddar (pressed) releases fat faster than mild Cheddar and Mozzaralla (spun pasta of "elastic" type) but the final rate of release does not differ after four hours of digestion.

In vivo studies

In the rat, lymphatic absorption of lipids is faster for cream and sour cream compared to a cream cheese and butter. Viscosity, type of emulsion, particle size and protein content could play a role in the degree of lipolysis with identical composition of fatty acids. In humans, postprandial or multi-week intervention studies were performed either in healthy subjects or at risk for chronic diseases. Their results give indirect indications on "dairy matrices" effects according to the differences of lipemiae observed:

1) Hard cheeses cause LDL-cholesterol levels - and probably total cholesterol - to be significantly lower than butter; but this does not mean that the butter is hypercholesterolemic;
2) A semi-hard cheese does not give a different cholesterolemia than a skim milk;
3) Two different cheeses can have a different impact on total cholesterol;
4) A Camembert and a whole yogurt give similar cholesterolemia;
5) Compared to native milk, fermented milk results in a slower gastric emptying than liquid milk, as well as a higher increase and a faster decrease in triglyceride levels in all plasma

lipoprotein fractions. These effects were attributed to the higher viscosity of the fermented milk and could therefore be applied to fresh cheeses;

6) While the physical structure of the different dairy products does not appear to have a major effect on plasma levels of postprandial triglycerides, it is still able to influence the time of onset of the triglyceride peak, and gastric emptying time does not seem to play a major role in modulating the triglyceridemic postprandial response.

Dairy food structure and calcium bioavailability

In vitro studies

In vitro are measured only the bioaccessibility or the solubilisation of the calcium: the percentages do not reflect in any way the actual bioavailability of calcium *in vivo*. However, they may give an indication of its potential absorbability by the intestine, notably after dialysis.

In the study by Unal *et al*. several dairy products purchased from local Turkish supermarkets were tested for the bioavailability of calcium *in vitro* (after dialysis): whole or skimmed milk, whole or skimmed milk yogurt, fermented milks, and various cheeses (Turkish pickled white Cheese, tulum cheese, cream cheese, kashari cheese and whey cheese) (Unal et al., 2005). The products differ in their acidity (percentage of lactic acid), ranging from 0.11% for whole milk to 1.78% for Turkish pickled white cheese. The skimming of milk does not significantly alter the dialysability of calcium, which is about 26-28%. For yogurts as well as for milks, no significant difference is measured (28-33% bioavailability), leading the authors to conclude that the milk fat content has no effect on bioavailability of calcium. On the other hand, significant differences are observed for cheeses: from 14% for cream cheese to 32% for whey cheese. The study also shows that there is no significant correlation between cheese acidity and calcium bioavailability. In accordance with the literature (Allen, 1982), the authors attribute differences in bioavailability to the phosphorus/calcium (P/Ca) ratio of cheeses, cream cheese having the highest ratio and whey cheese the lowest. Finally, of all dairy products, skimmed milk yoghurt has the highest calcium bioavailability (33%, $p < 0.05$). In another study the value obtained for cow's milk dialysability was 20%, a value lower than that calculated in the study by Unal et al. (Unal et al., 2005). Shen *et al*. also measured the dialysability of calcium from whole and skimmed cow's milk using *in vitro* digestion (Shen et al., 1995). The values obtained are of the same order of magnitude as the previous studies, namely 19.6% for whole milk and 23.2% for skimmed milk, the difference not being significant.

In the Cameroonian study of Ngounou *et al*. wild strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, isolated from Cameroonian zebu milk, were used to ferment skimmed

UHT milk (Ngounou et al., 2003). The determination of the soluble calcium in the fermented milks obtained shows, whatever the bacterial strain, an increase in the solubility of this mineral. The association of the two strains, while maintaining a high level of solubilization of the minerals, appears less effective than the strains in individual cultivation. For example, with a single strain, solubilization of calcium can reach almost 80%, whereas with two associated strains, the maximum does not exceed 70%. The authors give no explanation for these differences and conclude that the efficacy of these wild strains in solubilizing calcium justifies the interest of their biochemical characterization in order to better define their contribution to the quality of the local fermented milks.

In their review of 2014, Klobukowski *et al*. recalled that in an *in vitro* experiment carried out in 2005 all the calcium in the milk was released in solution after digestion (Klobukowski et al., 2014). Then, Klobukowski *et al*. briefly reported the results of a study conducted in 2005 but unpublished with respect to the solubilization of milk calcium in the presence of cereal products. Breakfasts based on milk alone or with cereal products were digested *in vitro*. Almost all calcium from milk alone is released in solution. However, while corn and barley flakes and semolina slightly decrease the amount of calcium measured in solution after digestion, wholemeal bread and rolls retain about 50% of the calcium; which is a surprise for the authors as the rolls were made from white flour. Then the authors reported the results of an earlier *in vitro* digestion study by Skibniewska *et al*. wherein the calcium solubility of a commercial yoghurt was measured in the presence or absence of cereal products (*i.e.*, corn flakes, wheat flakes, muesli, oat flakes, wheat and rye bread slice, and slice of wholemeal bread) (Skibniewska et al., 2010). While the calcium fraction released from the yogurt after digestion is about 67%, this fraction is always lower in the presence of any cereal products, *i.e.*, from about 38 (oat flakes) to 65 % (slice of wheat and rye bread). In a second experiment, 9 commercial yogurts with various cereal additives (unspecified) were also digested *in vitro*: the released calcium fraction varies from 28.5 to 77.9% (Skibniewska et al., 2010). These results led authors to wonder, in view of the general deficiency of calcium in the diet in humans, whether or not dieticians should recommend mixing milk and cereal products.

Animal studies

Calcium is by far the most studied mineral in animals. However, a significant number of studies evaluated the bioavailability of calcium from fortified dairy products. These studies will therefore not be discussed here unless a non-fortified control group was included in the experimental design. Otherwise calcium bioavailability was also evaluated *via* bone criteria such as bone mineralization, bone resistance and/or bone content.

Dairy products

In the five following studies several distinct dairy products were compared in rats for their calcium bioavailability.

In the first study, the objective was to compare the biological availability (measured by apparent absorption and bone mineralization) of calcium in rats fed diets based on milk and various dairy products (semi-hard/hard cheese L'Envol made from skimmed milk with 4% fat, skimmed milk yoghurt and skimmed milk powder) containing various proportions of dairy proteins and calcium chemical forms (Delisle et al., 1995). The apparent absorption of calcium from cheese was higher (≈35%) than from skimmed milk, yoghurt and skimmed milk powder (≈13-18%) in young rats (6 weeks) whereas in adult rats (16 weeks) the apparent absorption of calcium is lower from cheese than from other dairy products. Nevertheless, the deposition of calcium at the level of the tibia and the femur and bone resistance were the same for all the diets.

The study by Buchowski *et al*. has compared the bioavailability of double-labeled calcium from different dairy matrices, *i.e.*, skimmed milk, yoghurt and fresh cheese curds (Buchowski et al., 1989). The radiolabeling was carried out intrinsically on goat milk with ^{45}Ca (injected into the jugular vein of the goat) and extrinsically (calcium solution added before the final incubation of 24 hours at 4°C for the 3 products) with ^{47}Ca. First, the apparent absorption of ^{47}Ca is 69, 72 and 59% for milk, yogurt and cheese, respectively; and the apparent retention of ^{47}Ca is 61, 64 and 54%, respectively. In the tibia, there were no significant differences in retention (4.0-4.4%) and absorption (3.3-3.5%) of ^{47}Ca. Expressed as a percentage of the dose administered, absorption of ^{47}Ca was also strongly correlated with bone content in ^{47}Ca and ^{45}Ca ($R = 0.923$ for the correlation between the ^{47}Ca and ^{45}Ca content of the tibia). For each product tested, the extrinsic ^{47}Ca was absorbed similarly to the intrinsic ^{45}Ca. Finally, the percentage of radioactive dose retained in bone appears to be a valid indicator of the relative bioavailability of dietary calcium.

In the study by Poneros *et al*. dried skimmed milk powder, freeze-dried Mozzarella cheese powder and calcium carbonate with or without 0.05% ascorbic acid were administered in rats (Poneros and Erdman, 1988). Ascorbic acid has no influence on the bioavailability of calcium. The cheese had a higher calcium bioavailability (105%) - but not significant - compared to skimmed milk (95%). The calcium content of the tibia (calcium tibia/calcium ingested) was also not significantly different. It is surprising to find such high values, in particular greater than 100%. However, relative bioavailability was measured from the calcium tibia content according to the formula: tibia calcium (mg)/total consumed calcium (g); with a value of 100% attributed to calcium carbonate without ascorbic acid.

In the study by Kaup *et al*. several groups of rats have received different diets in which the calcium carbonate provides either the whole of the calcium or half which is then added half by

calcium from 1) dried skimmed milk with or without lactic acid, 2) traditional yogurt (cultured with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*), 3) yoghurt termed acidophilus (cultured with *S. thermophilus* and *L. acidophilus*) (Kaup et al., 1987). First, the ash bone content increased with milk but not with yogurt; second, rats fed with milk with added lactic acid had thicker femurs than with yogurts; thirdly, rats given yogurts and acidified milk had femurs with higher bending moments (induced reaction when an external force or moment is applied to an element causing its flexion) than rats given unacidified milk; finally bone strain decreased and the modulus of elasticity increased as ingested calcium increased. Authors concluded that « the improved bone characteristics in rats given milk, and particularly in those given acidified or cultured milk, in diets at constant lactose content indicate that lactose is not the only contributor to the high bioavailability of calcium in milk products and that lactic acid may also be a factor » (Kaup et al., 1987).

In the last study Dupuis *et al*. have studied the use of calcium from calcium carbonate (lactose-free control group) and from three dairy matrices (skimmed milk, yogurt and processed cheese) in growing (8 weeks) and ageing (14 months) rats (Dupuis et al., 1985). First, the calcium of milk products was better utilized than in the control. During ageing and lactation, the milk and yoghurt diets had the lowest bone exchanges while being higher in the control diet in the absence of Ca utilization factors. Authors suggested that lactose may be responsible for differences in bone exchanges of calcium within the milk products used.

Milks

In the four following studies calcium absorption and retention were measured in mice and rats. In the first study carried out in mice, although not clearly explained, the milk used appears to be an unprocessed whole cow's milk (Singh et al., 2007). The percentages of absorption and retention of calcium are respectively 50 and 44%, values lower than those observed in the rat in the next studies. Thus in the study by Toba *et al*. in 7-week-old rats the apparent uptake of calcium was approximately 80% and only 45% in 12-week-old rats (Toba et al., 1998). The study by Brink *et al*. was carried out in rats fed, inter alia, with a sterilized cow's milk in a diet supplemented with a mixture with or without minerals and proteins in a first experiment, and with or without a mixture of magnesium, sodium and potassium in a second experiment (Brink et al., 1992). In the first experiment the absorption of calcium is 77% for normal milk and only 59% with the addition of a mixture of minerals and proteins. In the second experiment, the absorption of calcium is similar and is 74%, the addition of magnesium, sodium and potassium having only a small impact on this absorption reduced to 70%. The last study compared the bioavailability of calcium in rats from dried skim milk, white bread and wholemeal bread (Ranhotra et al., 1981). The bioavailability of

calcium compared to that of the control group receiving calcium sulfate (bioavailability considered 100%) is higher with milk (113.3%) than with bread (99.4 and 104.4%).

Yogurts

The objective of the El-Gawad *et al*. study was to evaluate and compare the effect of cow's milk (3.2% fat) and various yogurts with (*Bifidobacterium lactis Bb-12* or *Bifidobacterium longum Bb-46*) or without probiotics on the bioavailability of calcium and bone mineralization in 48 male albino rats fed 45 days (El-Gawad et al., 2014). The serum calcium levels did not differ significantly between the different dairy matrices. However, the highest apparent calcium absorption was observed in rats fed the yogurt-based diet cultured with *Bifidobacterium lactis Bb-12* (83%) followed by the yogurt cultured with *Bifidobacterium longum Bb-46* (82%), plain yogurt (78%) and cow's milk (76%). The authors suggest that peptides released by the degradation of caseins by digestive enzymes can promote the absorption of calcium by intestinal cells by preventing the precipitation of insoluble salts (Meisel et al., 2003). Otherwise, bone mineral density of the femur is significantly higher with yogurt cultured with *Bifidobacterium lactis Bb-12* compared to other dairy matrices. Moreover, the rupture force of the femur does not differ according to the matrices. According to the authors, the suggested mechanisms of the positive effect of probiotics on mineral bioavailability and bone properties may include: 1) increased mineral solubility due to increased bacterial production of short-chain fatty acids, and 2) enlargement of the absorptive surface due to the promotion of enterocyte proliferation mediated by bacterial fermentation products (Perez-Conesa et al., 2007). It is also possible that short-chain fatty acids can directly stimulate calcium absorption in the rat colon and that at least calcium could pass through the cell membrane more readily in the form of a less charged complex (acetate-calcium) *via* a passive route (Lutz and Scharrer, 1991).

Cheeses

Different types of cheese have been tested in rats for their calcium bioavailability. First, based on studies in animals, Klobukowski *et al*. (Klobukowski et al., 2014) recall in their review that the highest percentages of calcium apparent absorption and retention are observed for fresh cheese produced either by a conventional method (acid coagulation) (90.3% and 84.2%, respectively) or with the addition of probiotic *Lactobacillus plantarum* (90.5% and 86.3, respectively) (Kłobukowski et al., 1997b); this compared with the refined cheese (59.6-76.3% and 64.4-81.3%, respectively) produced by immersion in traditional (2% NaCl) or modified (NaCl and KCl solution at 1:1) brines or unrefined Ricotta-type cheese (57.4-60.5% and 52.0-52.2%, respectively) produced by the heat-acid protein coagulation method (Table 2) (Kłobukowski et al., 1997a). The following

study, carried out in the rat, tested the absorption of calcium from a white cheese supplemented or not with the probiotic *Lactobacillus plantarum* (Klobukowski et al., 2009). No significant differences in calcium retention percentages (≈84-86%) and calcium absorption (≈90%) were observed for both cheeses.

The objective of the study by Mora-Gutierrez et al. was to evaluate the apparent uptake and deposition of calcium (bone mineralization) in male rats fed diets containing Monterey Jack cheese (semi-hard cheese) based on bovine or caprine milk and enriched or not with milk calcium (Mora-Gutierrez et al., 2007). Five groups of rats were therefore given a control diet without casein or one of the following four experimental diets: (1) diet with bovine milk cheese; (2) diet with calcium-enriched bovine milk cheese; (3) diet with goat milk cheese; and (4) diet with calcium-enriched goat milk cheese. Significant differences are reported for calcium absorbability (referred to in this study as "calcium digestibility") in the following order: (4) 68% > (3) 61% > (2) 57% > (1) 53% > (control) 41%. Significant increases are also observed for bone mineral content, bone mineral density and femoral bone rupture strength in the following order: (4) > (3) > (2) > (1) > (control). The authors concluded that goat milk-based cheese characterized by high content of peptides from casein α_{s2} and β, enriched or not enriched with calcium, may be more protective against bone fragility than cheese from bovine milk, notably by increasing the absorption of calcium.

Similar to the previous study, Kato *et al*. investigated the calcium bioavailability of a cheese, enriched or not with milk calcium, by examining bone strength and bone mineral density in growing male rats for 31 days (Kato et al., 2002). Unfortunately, the authors did not give percentages of absorbability. They reported the following results: compared to the control group "without cheese", the "cheese" groups had a significantly higher bone mineral density of the 4th lumbar vertebra; however, only the "calcium-enriched cheese" group had significantly greater strength and breaking strength of the femur than the control group; no significant difference in serum calcium concentration was observed between the three groups.

Then, Delisle *et al*. have studied the bioavailability of calcium from three Cheddar (hard cheeses) differing in composition (31% fat ±0.35% whey proteins, and 7% fat) in ovariectomized rats (Delisle et al., 1997). The results interestingly showed that whey proteins do not affect the apparent absorption of calcium compared to the reference Cheddar at 31% fat (from 20% at 4 weeks to about 7% at 16 weeks), while a high level of lipids improves the apparent absorption of calcium and delays, although slightly, bone resorption (*i.e.*, reduction of bone loss between 8 and 16 weeks) with age compared to 7% fatty Cheddar. As in the previous study by Toba *et al*. (Toba et al., 1998), an "age" effect in favor of a decrease in apparent calcium absorption is highlighted.

In the last study, based on the lack of information on the effect of cheese making, specifically the ripening time, on the bioavailability of calcium Buchowski and Miller compared the

bioavailability of calcium in rats from $CaCl_2$ (28 mM), $CaCO_3$, milk (fresh and adjusted to pH 5.35 - Cheddar pH) and Cheddar cheeses (≈35% fat) ripened for various periods - using double-labeled cheeses with ^{45}Ca and ^{47}Ca (Buchowski and Miller, 1990). The cheeses were made from pasteurized cow's milk. The bioavailability of calcium was determined by two methods: 1) the measured absorption based on the whole body radioactivity count; and 2) availability for bone metabolism assessed by bone radioactivity measurements. Calcium absorption varies from 72.8 (Cheddar at 0 day ripening) to 81.2% (Cheddar as a slurry in a lactose solution). The ripening time of the cheese had no significant effect on calcium absorption. The calcium absorption from milks and $CaCl_2$ and $CaCO_3$ was similar around 75.7-79.3% ($p > 0.05$). There was no significant difference in the extent of calcium absorption in the tibia for all tested products (between 2.7 and 2.9%).

Human studies

It is important to note that bioavailability studies in humans are generally rather old, probably due to (1) the development of more recent, cheaper and faster *in vitro* digestion studies, (2) the high cost of studies using stable isotopes, (3) non-authorization of radioisotopes, and (3) investment in time for this type of study.

Dairy products

Two human studies have simultaneously compared milk, yogurt and cheese for their calcium fractional absorption. In the first one, four different dairy matrices were tested in healthy young Caucasian women, and no significant difference was observed between pasteurized and homogenized cow's milk (32.8%), Cheddar cheese (37.4%), yoghurt (24.2%) and processed cheese (33%) (Nickel et al., 1996). In the second study in healthy postmenopausal women, calcium absorption was not significantly different between whole milk (26.7%), chocolate milk (23.2%), yoghurt (made from whole milk with 2% fat) (25.4%), a milk substitute (prepared from milk and non-dairy products) (22.4%), a cheese (no information about its variety) (22.9%) and calcium carbonate (22.0%) (Recker et al., 1988).

Milks

There are more studies comparing calcium fractional absorption in milk as compared with other foods or beverages (especially mineral waters), and also calcium carbonate.

Milk vs mineral waters

Brandolini *et al*. recalled that it is well known that the intestinal availability of calcium from mineral waters rich in calcium is equivalent to that of milk (Brandolini et al., 2005). However, the

effect of associated anions on urinary calcium losses has not been studied. Their study was carried out in cross-over in 37 healthy women over a period of 4 x 3 weeks and consuming the same amount of calcium from a SO_4-rich milk or mineral water (for anions SO_4^{2-}). Urinary excretion of calcium is higher (+ 0.5 mmol/day) with water than with milk ($p < 0.001$), suggesting that calcium balance is better with milk intake than with SO_4-rich water. The acidogenic action of SO_4 would be responsible for this effect.

In the study by Couzy *et al*. the bioavailability of milk calcium (UHT sterilized, 2.8% fat) is compared to that of mineral water in healthy young women (Couzy et al., 1995). The fractional absorption of calcium from milk (25%) did not differ significantly from that of mineral water (23.8%).

The study by Heaney and Dowell also compared the fractional absorption of milk calcium (no details given on technological treatments) and Italian mineral water in healthy women, and found no significant difference between both products (43.3 versus 47.5%, respectively) (Heaney and Dowell, 1994). However, differences in percent bioavailability between this study and the previous one may be surprising.

Again, in the study by Halpen *et al*. the bioavailability of calcium (^{45}Ca) in milk (no data on technological treatments) was compared with calcium-rich mineral water in healthy adult males (Halpern et al., 1991). The authors did not give percentages of absorption but report that the bioavailability of calcium from mineral water is generally as good as or better than that of milk.

Again with a view to knowing whether calcium derived from mineral waters is better absorbed or not than from whole milk, Sheikh *et al*. have evaluated calcium absorption of different calcium salts dissolved in water with that of milk in healthy subjects (Sheikh et al., 1987). The absorption of whole milk calcium (31%) was not significantly different from calcium salts.

Milk vs other foods

Based on the assumption that the recommended intake of calcium may be difficult to achieve with only dairy products, Gonnelli *et al*. wanted to evaluate the calcium absorbability (marked with ^{44}Ca) of a new calcium-fortified orange beverage in cross-over and compare it with milk in healthy adults (Gonnelli et al., 2007). The relative changes in calcium absorption from the two test drinks were similar (23.0% for milk and 20.9% for the orange beverage).

Hitz *et al*. have compared the bioavailability of calcium from calcium carbonate with or without cholecalciferol, and from milk (no data on applied technological treatments) in young women (mean age 30 years) and a group of elderly people of both sexes (mean age of 66 years) (Hitz et al., 2005). Each diet contains 1200 mg of calcium. Taking into account all participants, urinary excretion rates (mmol/day) of calcium were 4.41 for the placebo group (tablets), 5.17 for

milk, 5.83 for calcium carbonate and 6.06 for calcium carbonate supplemented with cholecalciferol. Compared to placebo, urinary excretion rates were all significantly different. The increase in calcium excretion with the "milk" diet is significant only for the elderly group. Using calcium excretion as an indirect measure of intestinal calcium absorption, the bioavailability of calcium from milk and calcium carbonate with or without cholecalciferol has been therefore demonstrated in healthy young and elderly individuals. Furthermore, the bioavailability of calcium in men and women over 60 years of age and healthy appears to be the same as in younger healthy women.

Zhao *et al*. studied the calcium bioavailability of soy milk fortified with calcium carbonate and that of cow's milk (without further details on the nature of the milk) in healthy young women (average age 23 years) (Zhao et al., 2005). The fractional absorption of calcium did not differ between the two products (21.1% for soya milk and 21.7% for cow's milk). Similarly, Weaver *et al*. have studied the bioavailability of calcium from tofu and cow's milk in healthy pre-menopausal women of Caucasian and Asian origin with ^{44}Ca (Weaver et al., 2002). In Caucasian women (whole milk), absorption of calcium is 54.8%, and 39.8% in Asian women (skimmed milk), a percentage not significantly different from that of tofu. Although studies by Zhao *et al*. and Weaver *et al*. have both used ^{44}Ca, their method of calculation differs a little to measure the absorption of calcium; but this does not explain the values of single to double reported (from 21.7% to 54.8%).

In the study by Griessen *et al*. the bioavailability of calcium was tested in normal male students but also lactase deficient (Griessen et al., 1989). Three products were evaluated: 1) a reconstituted powdered milk containing lactose, 2) a reconstituted powdered milk where the lactose was replaced by glucose, and 3) water. The fractional absorption of calcium was 21.4% in normal subjects for reconstituted powdered milk containing lactose and nearly 30% in lactase-deficient subjects ($p < 0.05$).

Finally, in healthy adult women, Heaney *et al*. reported an absorbability of milk calcium (no data on technological treatments) of 31.7% *versus* 4.6% for spinach and 41% for kale (Heaney et al., 1990); and of 27.6% for milk and 5.1% for spinach in a previous study (Heaney et al., 1988).

Yogurts

Smith *et al*. studied the absorption of calcium from whole milk and yogurt in normal and lactose-deficient subjects (Smith et al., 1985). Normal and deficient subjects equally absorbed calcium from milk (17.0 *versus* 19.8%, respectively) and yogurt (15.1 *versus* 20.6%, respectively).

Cheeses

The first study did not measure directly calcium bioavailability, but rather the relative effectiveness of calcium supplementation from cheese or pills with or without vitamin D supplementation for

bone mass accrual during the rapid growth period in 195 healthy girls aged 10-12 years with dietary calcium intakes < 900 mg/day (Cheng et al., 2005). Four groups involved 1) 1000 mg calcium carbonate + 200 IU (5 µg) vitamin D daily; 2) 1000 mg calcium carbonate daily + vitamin D placebo; 3) 1000 mg calcium daily from supplemented dairy products (mainly low-fat cheese); and 4) calcium placebo + vitamin D placebo identical to the effective pills. First, calcium supplementation with cheese resulted in a higher percentage change in cortical thickness of the tibia than did other three groups, and in higher whole-body bone mineral density than did placebo treatment. However, when controlling for growth velocity, these differences disappeared. Such results suggested that increasing calcium intake through consuming cheese would be more beneficial for cortical bone mass accrual than the consumption of tablets containing a similar amount of calcium.

An indirect method of bioavailability measuring the parathyroid response to an oral load of calcium has also been used (Karkkainen et al., 1997). Thus, the comparison of nine adult women between Emmenthal cheese, milk, spinach, sesame seed and a mixture of medicated calcium salts (lactate, gluconate, carbonate) was in favor of the calcium of the cheese for a significant decrease in serum levels of intact parathyroid hormone, better than milk and equivalent to calcium salt, and much higher than calcium in spinach and sesame seeds (rich in oxalic or phytic acids) (Karkkainen et al., 1997). On the other hand, urinary calcium excretion was 141% ($p = 0.001$) with cheese, 107% ($p = 0.004$) with milk and 75% ($p = 0.02$) with calcium salt, above that of the control session. The authors concluded that their results indicate that fermented cheese may be a better dietary source of calcium than milk when the metabolic effects of a food are considered.

Van Dokkum *et al*. studied the bioavailability of calcium (^{44}Ca) derived in particular from a fresh cheese (Petit Gervais with fruit), another fresh cheese (no further details on the product) and the same fresh cheese enriched with iron in healthy women (van Dokkum et al., 1996). The fractional absorption of calcium was 42.2, 37.7 and 38.8% ($p > 0.05$), respectively. The urinary excretion of calcium over 24 hours also did not differ.

Finally, in the following study, the influence of the Emmental matrix (cooked pressed paste) on the bioavailability of calcium relative to that of calcium carbonate was evaluated; and on the basis of serum calcium, no significant difference was observed between both sources of calcium (Fardellone et al., 1993).

Conclusions on the calcium fraction

Specific conclusions on cheeses

In 2008, the calcium bioavailability from cheeses has been reviewed and some first conclusions were already proposed (Guéguen, 2008) : 1) The actual absorption coefficient of calcium in milk

varies in adult between 25 and 35%; 2) Even if it is sometimes difficult to extrapolate to human the results obtained in rats or *in vitro*, at least it can be seen that calcium is as bioavailable, or even more, in cheeses than in milk; 3) Recent data in humans are few. They show that the calcium absorption of a cooked pressed cheese is significantly higher than that of calcium from various plants (Falcou and coll., 1988); and that the comparison between various dairy products and calcium carbonate confirmed the good intestinal absorbability of calcium in cheese, equivalent to that of carbonate (Recker et al., 1988).

Guéguen continued: « The absorbability of calcium in cheese thus seems good and equivalent to that of calcium in milk, despite the differences in constituents that could theoretically be unfavorable. Thus, the fact that most cheeses are poor in lactose could theoretically be unfavorable to calcium absorption, although the relative importance of this beneficial effect of lactose in a meal has been questioned (Gueguen and Pointillart, 2000). On the other hand, if the phosphopeptides are likely to increase the *in vivo* absorption of calcium (which is also debatable), their presence in a greater proportion in the cheese resulting from the degradation of the proteins during acidification and ripening should be a favorable factor. Finally, the risk of intestinal formation of insoluble calcium soaps from the long saturated fatty acids provided by non-lightened cheeses could be an unfavorable factor. In fact, we have shown that the possible formation of soaps occurs in the distal small intestine and thus insolubilizes calcium not previously absorbed and which, in any case, would no longer be so (Guéguen, 1992). It seems well established that saturated lipids do not interfere with the intestinal absorption of calcium, while excess calcium is likely to decrease absorption of free saturated fatty acids, which is beneficial » (pages 7-8) (Guéguen, 2008).

In 2017, based on this review, we propose new updated conclusions for all dairy products and calcium bioavailability:

In vitro studies

In vitro studies generally measure different parameters such as bioaccessibility, solubilization and/or dialysability *via* generally Caco2 cells, somehow measuring a form of *in vitro* absorption or bioavailability. The previously detailed studies provide the following potential conclusions:

1) The milk calcium is apparently almost completely solubilized *in vitro*, and its fermentation could further increase this solubilization;

2) The calcium dialysability of milk varies from 20 to 28% according to studies without effect of the fat content;

3) The calcium dialysability of yogurts varies from 28 to 33% without effect of the fat content or the acidity of the products;

4) The calcium dialysability of cheese varies from 14 to 32%, and may depend on the ratio of phosphorus/calcium (P/Ca);

5) In the presence of cereal products, solubilization of calcium from milk is reduced: while maize and barley flakes and wheat semolina slightly decrease the amount of calcium measured in solution after digestion, wholemeal bread and small rolls retain about 50% of the calcium ;

6) While the calcium fraction released from the yogurt after digestion is about 67%, this fraction is always lower in the presence of any cereal products, *i.e.*, about 38 (oat flakes) to 65% (slice of wheat and rye bread).

Murine models

On the basis of studies in the adult rat the percentages of apparent absorption of calcium are summarized in Table 2. It should be noted, however, that the sometimes significant variability of the apparent absorption percentages for the same product must be attributed to the experimental conditions more than to true natural variability. The available studies therefore make it possible to arrive at comparative comparisons and to propose conclusions or certain qualitative trends:

1) The apparent absorption of calcium from fermented milks is higher than that of a normal yogurt and that of milk; and the bone mineral density of the femur follows the same classification; also probiotics would increase the solubility of calcium, and increased bacterial production of short-chain fatty acids in the presence of probiotics would increase colonic absorption of calcium ;

2) Milk and yoghurt have similar calcium absorption but higher than cheese;

3) Absorption and retention of calcium is higher for fresh cheese compared to ripened or unripened cheese;

4) The absorption of calcium from skimmed milk and Cheddar cheese is decreased with aging, which would correspond to a decrease in calcium requirements of the body;

5) The lipid content of cheese may increase calcium absorption and decrease bone resorption;

6) There is no significant difference in calcium absorption in the tibia for Cheddar cheese (with different ripening times) and fresh milk; and also for a skim milk, a yoghurt and a goat cheese curd;

7) The ripening time of a cheddar cheese has no significant effect on calcium absorption;

8) There is a correlation between calcium absorption and bone calcium content;

9) In addition to lactose (which may play a role in differences in calcium absorption between dairy products), lactic acid may play a role in the high bioavailability of calcium in some dairy products.

10) Some recent studies based on bone tests have confirmed the hypothesis formulated in 1992 (Guéguen, 1992) on the equivalence of milk and cheese for the bone efficiency of calcium (Kato et al., 2002) (Delisle et al., 1995; Mora-Gutierrez et al., 2007).

Human studies

The different studies carried out in healthy subjects, except for lactase deficient subjects, suggest the following conclusions:

1) The absorption of milk calcium is between 17 and 55%, with the majority of studies giving values between 20 and 30%; which joins the conclusions of the previous review (Gueguen, 2005);

2) The fractional absorption of milk calcium is similar to that of calcium from mineral water;

3) Cheddar cheese has a fractional absorption of calcium (37%) slightly higher than milk (33%), processed cheese (33%) and yogurt (24%);

4) The fractional absorption of yogurt calcium seems to be between 15 and 25%;

5) Fresh cheeses (Petit Gervais type) appear to have a higher calcium absorption at around 42%.

In the end, it remains difficult to draw firm conclusions as experimental designs and dairy products differ from one study to another. However, the fractional absorption of calcium measured in the rat is generally greater than that measured in humans. Moreover, according to the matrices, it is probably with the cheeses that the variations of the absorption of the calcium are the strongest, especially in the rat (Table 2). This is not surprising given the multiplicity of matrices and nutritional compositions in the large « family » of cheeses. Finally, Dr. Guéguen recalls that "The absorbability of calcium depends relatively little on its solubility in water, but especially on its solubility in acid medium (Heaney et al., 1990). If the absorption of calcium from milk is often taken as a reference, it must be admitted that it is not significantly higher than that of most calcium mineral salts (carbonate, phosphate, sulfate, chloride, etc.) and is sometimes a little lower than that of organic salts (lactate, gluconate, citrate, citromalate, etc.). However, differences between good sources of calcium remain low (Gueguen and Pointillart, 2000), the actual absorption coefficient (in humans) being most often found, under the most favorable physiological conditions, for milk as for good calcium salts, between 25 and 30% » (page S9) (Gueguen, 2005).

General conclusions and perspectives

Depending on the nature of their matrix, milks, yogurts, fermented milks and cheeses have different effects on the bio-accessibility and bioavailability of their nutrients (Table 1 and Figure 1).

Milks have a high protein digestibility of more than 95%. The structure of proteins (casein *versus* whey) and the physico-chemical characteristics of lipid droplets play a predominant role in the kinetics of bioavailability of amino acids and fatty acids, respectively. In particular, the viscosity, the type of emulsion, the size of the particles and possibly also the protein content, influence the digestion and absorption of the milk fat, and probably affects the lipemic responses. Concerning calcium, absorption in humans is around 20-30%. As with milks, yogurts have a high

digestibility of their protein fraction (≥ 95%). For the lipid fraction, the data are too still few to conclude. Concerning calcium, its absorption is around 15-25% in humans. Fermentation appears to increase calcium solubilization during digestion. Since cheeses display a wide variety of matrices and nutritional composition, it is difficult to propose solid conclusions for the parameters studied in this report. However, some data can be synthesized: 1) the digestibility of cheese proteins is very high, as for milk and yogurts, *i.e.*, greater than 95%; 2) the kinetics of digestibility of the lipids depend in particular on the ratio of proteins/lipids and the texture of the matrix; 3) in human, the absorption of calcium is probably between 20 and 40% depending on the cheese matrices considered.

The "matrix" effect of dairy products on the bioavailability of protein, lipid and calcium fractions remains a topic to be explored. In all cases, data collected up to now seem to show different behavior of kinetics of bioavailability of amino acids, fatty acids and calcium according to the physicochemical parameters of these matrices, including compactness, hardness, elasticity, protein/lipid ratio, P/Ca ratio, effect of ferments, size of fat globules, and possibly other qualitative parameters yet to be discovered. This could be of great interest for the development of innovative dairy products for older populations, sometimes in protein denutrition or with poor dentition, involving the development of dairy matrices with optimized metabolic effects by playing on gastric retention time and thus on the kinetics of release of the amino acids within bloodstream.

Conflict of Interest

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Abbreviations

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Figure legends

Figure 1. A synthetic view of the matrix effect of main dairy products on metabolim

1697 **Table 1.** Influence of main dairy matrices on the digestibility, bioaccessibility and/or bioavailability of proteins, lipids and calcium, and satiety[a]

	Milks	Yogurts and fermented milks	Cheeses
Proteins	*In vitro* : - After oral and gastric digestion, all proteins except β-lactoglobulin were degraded, with peptides being further degraded at the end of intestinal digestion - Concerning the β-lactoglobulin fatty acids and triglycerides would link to it preventing proteases access, and therefore requesting bile for further digestion - Skimmed cow milk > skimmed caprine goat milk - Heating milk has important impact on the hydrolysis kinetics of its protein fraction *In vivo* : - Digestibility ≥ 95% - The rate of digestion influences postprandial protein retention: casein (slow protein) versus whey (fast protein) - In the elderly, a fast protein results in better postprandial protein gain than a slow protein, contrary to what is observed in the young subject	*In vitro* : - Digestion of yoghurt results in different peptide profiles than milk *In vivo* : - Digestibility ≥ 95% - Proteolysis during fermentation may lead to the formation of new peptides during gastrointestinal digestion	*In vitro* : - More cohesive, compact, elastic and structured cheeses would disintegrate less rapidly during digestion, especially at the gastric level, resulting in slower proteolysis *In vivo* : - Digestibility ≥ 95%
Lipids	*In vitro* : - The digested milk contains nearly 41% free fatty acids with respect to the total milkfat (this value corresponded to 100% of the triglycerides) ; - More short chain fatty acids are released after digestion compared to the relative distribution of total fatty acids in milk before digestion ; - The size of the lipid droplets influences the kinetics of lipolysis ; - The initial size of fat globules controls the type of free fatty acids generated but not the degree of	*In vivo* : - Fermented milk results in a significantly slower rate of gastric emptying, probably because of higher viscosity ; - Fermented milk results in a greater increase and a more rapid decrease in the content of triglycerides in all lipoprotein fractions.	*In vitro* : - The properties of the cheese matrix influence the access to the lipids of the cheese by controlling the release of the free fat and also via the restriction of the diffusion of the enzyme and the fatty acids ; - Calcium influences both the structure of the cheese and the kinetics of lipolysis. *In vivo* : - Hard cheeses cause levels of LDL cholesterol, and probably total cholesterol, significantly lower than butter ;

	lipolysis ; - The composition of the milk fat cell membrane affects the rate of lipid hydrolysis. *In vivo* : - Short- and medium-chain fatty acids are released faster than long-chain fatty acids ; - The viscosity, the type of emulsion, the size of the particles and possibly also the protein content, influence the digestion and absorption of dairy fat, and probably affects the lipemic response ; - The small fat globules release probably less atherogenic fatty acids.		- Two different cheeses can have a different impact on total cholesterol.
Calcium	*In vitro* : - Calcium is apparently almost completely solubilized, and fermentation of milk could further increase this solubilization ; - The dialysability of calcium varies from 20 to 28% without apparent effect of the fat content ; - In the presence of cereal products, solubilization of calcium is reduced. *In vivo* : rats - The apparent absorption of calcium varies from 74 to 80% ; - The absorption of calcium is decreased with aging. *In vivo* : humans - The absorption of calcium is between 17 and 55%, with a majority of studies giving values between 20 and 30% ; - The fractional absorption of milk calcium is similar to that of mineral water.	*In vitro* : - The dialysability of calcium varies from 28 to 33% without effect of the fat content or the acidity of the products ; - While the calcium fraction released is about 67%, this fraction is always lower in the presence of cereal products or additives. *In vivo* : rats - The apparent absorption of calcium varies from 72 to 83% ; - The apparent absorption of calcium from fermented milks is higher than that of normal yoghurt and that of milk. *In vivo* : humans - The fractional absorption of calcium seems to be between 15 and 25%.	*In vitro* : - The calcium dialysability of cheese ranges from 14 to 32%, and may depend on the ratio of phosphorus to calcium (P/Ca). *In vivo* : rats - The apparent absorption of calcium varies from 12 to 90% according to cheese matrices ; - Absorption and retention of calcium is superior for fresh cheese compared to ripened or unripened cheese; - Calcium absorption of Cheddar cheese is decreased with aging; - Lipid content may increase calcium absorption and decrease bone resorption; - The refining time of a cheddar cheese has no significant effect on the absorption of calcium; - The absorbability of calcium is not increased by the addition of lactose (considered favorable to absorption and absent or in small quantity in most cheeses) *In vivo* : humans - There is a high probability that the bioavailability of calcium in cheese will not be different from that of calcium in milk; - Cheddar cheese has a fractional absorption of calcium (37%) slightly higher than milk (33%), processed cheese (33%) and yogurt (24%);

	- Fresh cheeses appear to have a higher calcium absorption at about 42%; - The calcium absorption of a cooked pressed cheese is significantly higher than that of calcium from various plants.

[a]Syntheses and conclusions based on data from the literature presented in this report

Tableau 2. Calcium apparent absorption from different dairy products as measured in adult rats*

Dairy products	Apparent absorption (%)
Whole cow milk	76, 74-77, 77
Skimmed milk	15, 69[a], 80
Plain yogurt	15, 72[a], 78
Probiotic yogurt	82-83
Fresh cheese (acid coagulation)	90
Ripened cheese	60-76
Unripened Ricotta cheese	57-61
White cheese	90
Monterey Jack cheese	53
Cheddar (31% fat)	20
Cheddar (7% fat)	12
Cheddar (35% fat, 0 day ripening)	78
Cheddar (35% fat, 70 days ripening)	73
L'Envol cheese (4% fat)	35
Goat fresh cheese curds	59[a]

* Percentages separated by a comma correspond to separate studies. [a]Goat milk

Milks: liquid matrices

- Whey: rapid protein
- Casein: slow protein
- Small fat globule → less atherogenic?
- Short/medium-chain released faster than long-chain fatty acids

Yogurts: semi-solid & viscous matrices

- New peptides during fermentation

Cheeses: solid & semi-solid matrices

- More cohesive/compact/elastic → slower proteolysis
- ↑ Protein/lipid ratio → ↓ proteolysis & lipolysis (denser protein network)
- Different effect on cholesterolemia according to matrices

Proteins: > 95% digestibility for 3 matrices

Lipids:

- Similar digestibility for 3 matrices but different kinetics
- Influence of calcium on lipolysis in cheese
- Role of fat globule size

Calcium:

- 20-30% (milk)
- 15-25% (yogurts)
- 20-40% (cheeses)
- Role of P/Ca ratio
- ≈ mineral water

Metabolic effect:
bioavailable fraction

Absorption → Urine

Bio-accessible fraction by enzymes