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Protein digestion and absorption: the influence of food processing

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Abstract

The rates of dietary protein digestion and absorption can be significantly increased or decreased by food processing treatments such as heating, gelling and enzymatic hydrolysis, with subsequent metabolic impacts, e.g. on muscle synthesis and glucose homeostasis.

This review examines *in vivo* evidence that industrial and domestic food processing modify the kinetics of amino acid release and absorption following a protein-rich meal. It focuses on studies that used compositionally-matched test meals processed in different ways.

Food processing at extremely high temperature at alkaline pH and/or in the presence of reducing sugars can modify amino acid sidechains, leading to loss of bioavailability. Some protein-rich food ingredients are deliberately aggregated, gelled or hydrolysed during manufacture. Hydrolysis accelerates protein digestion/absorption and increases splanchnic utilisation. Aggregation and gelation may slow or accelerate proteolysis in the gut, depending on the aggregate/gel microstructure.

Milk, beef and eggs are heat processed prior to consumption to eliminate pathogens and improve palatability. The temperature and time of heating affect protein digestion and absorption rates, and effects are sometimes non-linear. In light of a dietary transition away from animal proteins, more research is needed on how food processing affects digestion and absorption of non-animal proteins.

Food processing modifies the microstructure of protein-rich foods, and thereby alters protein digestion and absorption kinetics in the stomach and small intestine. Exploiting this principle to optimise metabolic outcomes requires more human clinical trials in which amino acid absorption rates are measured and food microstructure is explicitly considered, measured and manipulated.

Keywords: **food protein: digestion: absorption: kinetics: food processing**

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Introduction

Dietary protein provides the body with amino acids as the building blocks for protein synthesis, but dietary amino acids are also utilised catabolically, and perform important signalling and metabolic roles in the gut and throughout the body[1]. The rate of protein hydrolysis in the gut after a meal determines the time course of local peptide and amino acid concentrations along the gastrointestinal lumen[2]. Local concentrations of amino acids and peptides in turn trigger metabolically important processes in the gut, such as enteroendocrine functions[3].

A high-protein meal elevates plasma amino acid concentrations for several hours, and the rate of amino acid absorption determines the maximum concentration (C_{max}), duration and area-under-the-curve (AUC) of the post-prandial amino acid concentration time courses. Plasma amino acid concentrations control the balance between catabolic and anabolic utilisation of amino acids in many tissues[4] and have an important influence on muscle protein synthesis[5,6] and energy homeostasis[7]. The rate of dietary protein digestion and absorption thus has wide-ranging metabolic effects.

Considerable effort has been devoted to studying the bioavailability of "fast" and "slow" proteins, the most notable examples of which are whey proteins ("fast") and caseins ("slow") from milk[8]. Native whey proteins do not coagulate under gastric conditions[9], and therefore empty rapidly from the stomach, whereas gastric-coagulating caseins empty more slowly[10]. Research has highlighted the key role of gastric emptying rates in controlling the kinetics of proteolysis and absorption[11–13].

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Food processing is known to affect the overall bioavailability of protein[14], but what is less well known is that food processing can alter the rate of protein digestion and absorption, i.e. processing can make a given protein source "faster" or "slower". I review evidence that food processing affects the kinetics of amino acid release and absorption following a protein-rich meal, and discuss the physicochemical phenomena responsible. I focus on studies that used test meals processed in different ways, but with near-identical composition. The evidence suggests that food processing can be an effective tool to optimise protein digestion and absorption for desired metabolic outcomes.

The scope of this review is limited to *in vivo* trials on human volunteers or pigs, which are considered a gold standard animal model of human digestion[15]. The experimental details of studies discussed here are summarised in Table 1.

Protein digestion and absorption

As protein digestion and absorption processes in the human body have been reviewed in detail by others[1,16], I provide only a brief overview here. I use the term digestion to refer to the breakdown of protein into peptides and amino acids, and absorption to refer to the uptake of breakdown products from the gastrointestinal lumen by enterocytes.

Protein digestion begins in the mouth, where food is disrupted by chewing and mixed with saliva, then swallowed. In the stomach, the actions of acid and pepsin, combined with peristaltic mixing, further break down food and initiate proteolysis. Gastric pH typically rises after a meal due to the buffering effect of food materials, and subsequently decreases due to gastric secretions. Pepsin is most active at pH ~2, but it shows partial activity up to pH ~6, so the inhibition of pepsin activity by post-prandial buffering of gastric pH may be relatively short-lived[17]. Proteolysis in the stomach is limited, serving mainly to release peptides and aromatic amino acids that alert gut sensing systems to the composition of the meal[18].

The emptying of stomach contents into the duodenum is influenced by the physical properties of gastric digesta, i.e. foods that coagulate or show high viscosity under gastric conditions empty more slowly than liquid foods[11,12]. A higher energy content of a meal also slows gastric emptying[13].

In the duodenum, bile and pancreatic enzymes are added to the gastric chyme, as well as bicarbonate, to bring pH close to neutral. Further secretions of bicarbonate, water and mucus are added in the jejunum and ileum[19]. During transit through the ileum, proteases in the lumen cleave dietary protein into short peptides and amino acids. Physiological surfactants such as bile acids and phospholipids also contribute to proteolysis by denaturing proteins, which renders them more susceptible to proteases[16]. The villi on the apical surface of enterocytes (the "brush border") contain anchored proteases, but also secrete protease-containing vesicles into the periapical space[20].

Some peptides and amino acids are thought to exert bioactivities through interactions with receptors in the gastrointestinal tract[21,22]. *Ex vivo* studies with animal tissue have shown that casein-derived peptides can stimulate water absorption[23] and decrease intestinal mobility[24]. A recent study with human intestinal biopsy tissue showed that a soybean peptide had immunomodulatory activity[25]. At present, there is no direct evidence of diet-derived exogenous peptides exerting bioactivities in humans, but the aforementioned studies and others suggest that some food-derived peptides may be bioactive in the gut. Exogenous bioactive peptides have been identified in human jejunal aspirates[26], and the effect of protein structure on the kinetics of digestion can alter the time course of peptide and amino acids released into the intestinal lumen[27]. However, the effect of protein digestion rates on peptide bioactivities *in vivo* has not been reported yet.

Dietary protein is not the only source of amino acids in the intestinal lumen; endogenous proteins are secreted into the gut in the form of mucus, enzymes, sloughed off cells, etc. Daily ileal endogenous nitrogen loss has been estimated at 2026±441 mg/d[28]. The amino acids of endogenous proteins are partly recycled through digestion, and endogenous proteins may be an important source of bioactive peptides[29].

Amino acids, di- and tri-peptides are taken up from the intestinal ileum into enterocytes through various apical amino acid transporters, and released into the portal vein blood via different basolateral transporters[30]. The splanchnic tissues use amino acids for protein synthesis and energy, and retention rates differ by amino acid[31]. Overall splanchnic retention of exogenous nitrogen is 29–60 %[32–35], and may be higher for the elderly than for adults[36]. The unused amino acids and peptides are released into circulation.

The true ileal digestibility is the proportion of ingested protein that disappears before the terminal ileum (corrected for endogenous losses), and this proportion is assumed to have been absorbed[1]. True ileal amino acid digestibility values of >80% are common for amino acids in food proteins[37], but a small proportion of protein or peptides may pass into the large intestine, particularly if digesta viscosity is enhanced by dietary fibre[38]. The gut microbiota can hydrolyse protein and metabolise amino acids, and colonocytes may be capable of limited amino acid absorption[39].

A high-protein meal produces post-prandial increases in plasma free amino acid concentrations that can last for several hours. Peripheral blood sampling remains the simplest and least invasive way to track dietary protein absorption, but results must be interpreted with caution because plasma amino acid concentrations reflect a multitude of dynamic processes (Figure 1)[1]. Protein synthesis leads to removal of amino acids from the blood stream, and tissue breakdown adds amino acids into the blood stream[40]. Dispensable amino acids in blood partly reflect biosynthesis rates, and various tissues remove circulating amino acids to oxidise them for energy[30].

The relative rates of protein synthesis, protein breakdown and amino acid oxidation contribute to free amino acid homeostasis[31,40], which is under tight hormonal and central nervous system control. Insulin stimulates amino acid uptake into muscle for tissue synthesis[41], but amino acids stimulate insulin release in the pancreas[31], so amino acid homeostasis is intrinsically linked to glucose homeostasis.

After a high-protein meal, the rate of amino acid influx into the intestinal lumen, splanchnic tissue and circulating free amino acid pool determines the magnitude and direction of change

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Table 1. Overview of studies discussed in the main text.

Material	Treatments	Test meal	Subjects and design	Main findings	Study
Process-induced amino acid side chain modification					
Low-heat milk protein powder	Milk protein powder with whey:casein 40:60, 42% protein. treatments: 3% blocked lysine 20% blocked lysine 50% blocked lysine	40 g powder dissolved in 600 mL water (16·8 g protein)	15 men, age 26±1 y, double-blinded randomised crossover	Increasing glycation lowered plasma lysine	Nyakayiru *et al.*[50]
Skim milk	Freeze-dried skim milk (no detectable blocked lysine) Roller-dried skim milk (51% blocked lysine	400g protein-free diets with 400g skim milk powder	Three sub-adult pigs, 52·2 ±2·7 kg	Cumulative lysine in portal blood over 12 h after the meal was reduced by 60% in the roller-dried skim milk	Rérat *et al.*[53]
Aggregation and gelation					
Reconstituted low-heat skim milk	Unheated liquid milk Unheated milk gelled with rennet Unheated milk gelled with acid Heated milk (90°C 10 min) Heated milk gelled with rennet Heated milk gelled with acid Heat milk gelled with acid then stirred	1 kg of treated milk (50 g protein)	Six female minipigs, age 18 months, randomised crossover	Plasma amino acid bioavailability: milk > stirred gel > acid gel > rennet gel	Dupont *et al.*[67] Barbé *et al.*[64] Barbé *et al.*[65]
Dairy products	Whey protein concentrate Micellar casein isolate Low fat pasteurised milk Full fat unhomogenised pasteurised milk Low fat UHT milk Full fat homogenised UHT milk Low fat yoghurt Full fat cheese	Sufficient quantity of product to supply 25 g protein	Five men, five women, age 66·7±4·3 y, single-blinded randomised crossover	Full fat slowed plasma amino acid kinetics compared to low fat. Yoghurt gave faster plasma amino acid kinetics than cheese, and higher C_{max} than low fat UHT milk.	Horstman *et al.*[72]
Plant-based foods	Lab-made pea protein emulsion, commercial products: Seitan Tofu Soy milk	Sufficient quantity of product to supply 30 g protein	Four minipigs, age 8 months, randomised crossover	Apparent, standardised and true ileal digestibility reported. Sulphur amino acids in tofu were less bioavailable than in soy milk	Reynaud *et al.*[123]
Milk protein concentrates, calcium caseinate	Milk protein concentrate (MPC) Mineral-modified MPC (mMPC) Calcium caseinate	Powders in 350 mL water to supply 25 g protein	MPC: 10 men, age 23·7 ±3·3 y mMPC: 10 men age 21·3 ±2·1 y calcium caseinate: 10 men, age 22·7±3·2 y double-blinded randomised parallel	Plasma amino acid bioavailability: mMPC > MPC = calcium caseinate. No difference in myofibrillar fractional synthesis rate among treatments.	Chan *et al.*[85]
Casein ingredients	Calcium caseinate (53% soluble) Micellar casein (5% soluble) Transglutaminase cross-linked sodium caseinate (99% soluble)	Powders in 600 mL water to supply 40 g protein	15 men, age 26±4 y, double-blinded randomised crossover	Plasma amino acid bioavailability: cross-linked sodium caseinate > micellar casein > calcium caseinate.	Trommelen *et al.*[76]
Hydrolysis					
Casein, whey protein and hydrolysates	Whey protein and casein protein (processing unspecified), hydrolysed whey protein and casein protein	Powders in 600 mL isoenergetic solutions to supply 60 g protein	Six men, mean age 30 y (range 27–32 y), randomised crossover	Faster gastric emptying and slightly higher insulinaemia with casein hydrolysate compared with intact casein	Calbet and Holst[90]

Table 1. (*Continued*)

Material	Treatments	Test meal	Subjects and design	Main findings	Study
Micellar casein, casein hydrolysate	[^{15}N]-labelled casein: Intact micellar casein Micellar casein hydrolysed with pancreatin	Semisynthetic meals supplying 320 mmol nitrogen (approximately 28 g protein)	Intact casein: six women, four men, age 32·3 ±8·7 y Hydrolysed casein: six women, five men, age 28·0±8·1 y	Hydrolysed casein gave earlier and higher plasma aminoacidaemia and insulinaemia, and greater splanchnic N utilisation	Deglaire *et al.*[34]
Micellar casein, casein hydrolysate	L-[1-^{13}C]phenylalanine-labelled: micellar casein, micellar casein hydrolysed with commercial enzymes	Powder in 350 mL water to supply 35 g protein	10 men, age 64±1 y, randomised crossover	Hydrolysed casein gave higher insulinaemia and higher plasma amino acid bioavailability, lower splanchnic extraction with hydrolysate.	Koopman *et al.*[88]
Micellar casein, casein hydrolysate, whey protein	L-[1-^{13}C]phenylalanine-labelled: whey protein micellar casein, enzyme-hydrolysed micellar casein	Powder in 250 mL water to supply 20 g protein	Whey: 16 men, age 73±1 y casein: 16 men, age 74±1 y Hydrolysed casein: 16 men, age 74±1 y randomised parallel	Whey and hydrolysed casein gave earlier and higher insulinaemia, plasma amino acid bioavailability: whey > hydrolysed casein > intact casein	Pennings *et al.*[89]
Unspecified milk protein, amino acid mixture	L-[1-^{13}C]phenylalanine-labelled milk protein (processing unspecified), or purified amino acids mixture	Powder in 300 mL water to supply 30 g protein	Intact protein: six men and six women, age 22±2 y amino acids: six men and six women, age 23±3 y double blind randomised parallel	Higher aminoacidaemia and higher net whole body protein balance synthesis with amino acid mixture.	Weijzen *et al.*[91]
Whey protein concentrate (WPC)	WPC or microparticulated WPC (mWPC)	Powder in 350 mL water to supply 20 g protein	WPC: 8 men, age 52·6 ±3·9 y mWPC: 8 men, age 51·0 ±3·5 y, randomised parallel	No detectable difference in plasma amino acid kinetics or muscle anabolic response.	Mitchell *et al.*[93]
Whey protein isolate (WPI), whey protein hydrolysate (WPH)	Commercial whey protein isolate or hydrolysate (degree of hydrolysis 30%)	Powder in 500 mL water to supply 45 g protein	16 men, age 22·5±0·48 y, randomised crossover	Hydrolysate gave greater insulinaemia and higher C_{max} for phenylalanine, but other differences non-significant.	Power *et al.*[92]
WPI, WPH, β-lactoglobulin-enriched WPI	Commercial WPI, WPH or β-lactoglobulin-enriched whey protein isolate (BLG)	500 mL beverage supplying 25g protein	Four men, four women, age 27·0±0·76 y	Plasma leucine and branched-chain amino acids bioavailability: BLG > WPI > WPH, some significant differences	Farnfield *et al.*[94]
Native whey, WPC, hydrolysed WPC, microparticulated WPC, milk	Native whey from ultrafiltration, WPC-80 from cheesemaking, hydrolysed and microparticulated MPC produced from WPC-80, milk (1% fat)	636 mL beverage supplying 3·1–3·3% protein	13 men, age 26·6±7·4 y	Plasma essential and branched-chain amino acids higher with native whey versus other treatments at 30–60 min. No differences in glucose, urea or muscle function.	Hamarsland *et al.*[95]
Heat processing of milk					
Milk	[^{15}N]-labelled milk: microfiltered at 40°C microfiltered and pasteurised (72°C, 20 s) microfiltered at UHT treated (140°C, 5s)	500 mL beverage supplying 23·3 g protein	Microfiltered: five women, three men, age 27·1±7·8 y Pasteurised: four women, four men, age 23·5±6·9 y UHT: five women, four men, age 25·7±6·5 y randomised parallel	Plasma amino acid kinetics not significantly different. UHT milk gave higher N deamination.	Lacroix *et al.*[98]

Table 1. (*Continued*)

Material	Treatments	Test meal	Subjects and design	Main findings	Study
Reconstituted low heat skim milk	Unheated liquid milk Heated milk (90°C 10 min)	1 kg of treated milk (50 g protein)	Six female minipigs, age 18 months, randomised crossover	Heat treatment slightly increased plasma amino acid bioavailability, but differences not significant	Barbé *et al.*[65]
Dairy products	Whey protein concentrate Micellar casein isolate Low fat pasteurised milk Full fat unhomogenised pasteurised milk Low fat UHT milk Full fat homogenised UHT milk Low fat yoghurt Full fat cheese	Sufficient quantity of product to supply 25 g protein	Five men, five women, age 66·7±4·3 y, single-blinded randomised crossover	Low fat UHT milk gave slightly higher aminoacidaemia than low fat pasteurised, but overall effect not significant.	Horstman *et al.*[72]
Cooking and mincing of beef					
Beef	[^{15}N]-labelled beef: sous vide cooked for 30 min at 60°C, 75°C or 90°C then minced	Meals containing wheat starch, cellulose, water, fat, meat (30 g protein)	Six female pigs, age 12–16 months, randomised crossover	Cooking at 75°C gave more rapid aminoacidaemia, cooking at 95°C delayed aminoacidaemia. Differences significant over first 150 min.	Bax *et al.*[101]
Beef	[^{15}N]-labelled beef cooked in a steam oven at 55°C for 5 min or 90°C for 30 min, then minced.	120 g meat (27 g protein)	beef cooked at 55°C: two women, six men, age 31·0±9·9 y beef cooked at 90°C: three women, five men, age 24·9±4·1 y single-blinded parallel	True ileal digestibility of meat cooked at 55°C for 5 min was slightly lower (90·1 ±2·1% versus 94·1±0·7%, P=0.08). Otherwise no significant differences	Oberli *et al.*[103]
Beef	[^{15}N]-labelled beef cooked in a steam oven at 55°C for 5 min or 90°C for 30 min, then minced.	Sufficient meat to supply 30 g protein	10 men aged 70–82 y, randomised crossover	Plasma amino acid levels were higher at 30–150 min with the meat cooked at 90°C, significant time × treatment effect. Higher whole body protein synthesis with meat cooked at 90°C (56% of leucine versus 40%, P<0.01).	Buffière *et al.*[104]
Beef	[^{15}N]-labelled beef steak or minced beef patty, grilled until inner temperature reached 65 °C	135 g beef, approximately 26 g protein	10 men age 74±2 y, single-blinded randomised crossover	Minced beef gave higher aminoacidaemia and higher overall amino acid bioavailability, as well as more positive whole-body protein balance. Skeletal muscle synthesis rates were not different between treatments.	Pennings *et al.*[105]
Beef	Beef steak cooked sous vide at 80°C for 6 h or pan-fried for 5 min	Steak sandwich, containing 270±20 g steak	14 men, age 18–25 y, randomised crossover	No significant differences in aminoacidaemia, insulinaemia.	Prodhan *et al.*[106]
Cooking of egg protein					
Egg white	[^{15}N]-labelled egg white either raw or microwave-cooked	200g egg white and one egg yolk (25 g protein)	Ileostomates: four women and one man, age 28–76 y	Cooked egg white had higher true ileal digestibility (90·9±0·8% versus 51·3 ±9·8%, P<0.05), higher protein assimilation, slower gastric emptying.	Evenepoel *et al.*[109]
Egg white	Pasteurised egg white adjusted to different pH and ionic strength and heated at 90°C for 15 min. Treatments: pH 5, ionic strength 1 M pH 7, ionic strength 1 M pH 9, ionic strength 0.05 M	1 kg egg white gel (87 g protein)	105 male pigs, 25 kg, 6–7 pigs euthanised per time point	Type of egg white gel affected gastric chyme properties and gastric emptying kinetics.	Nau *et al.*[110]

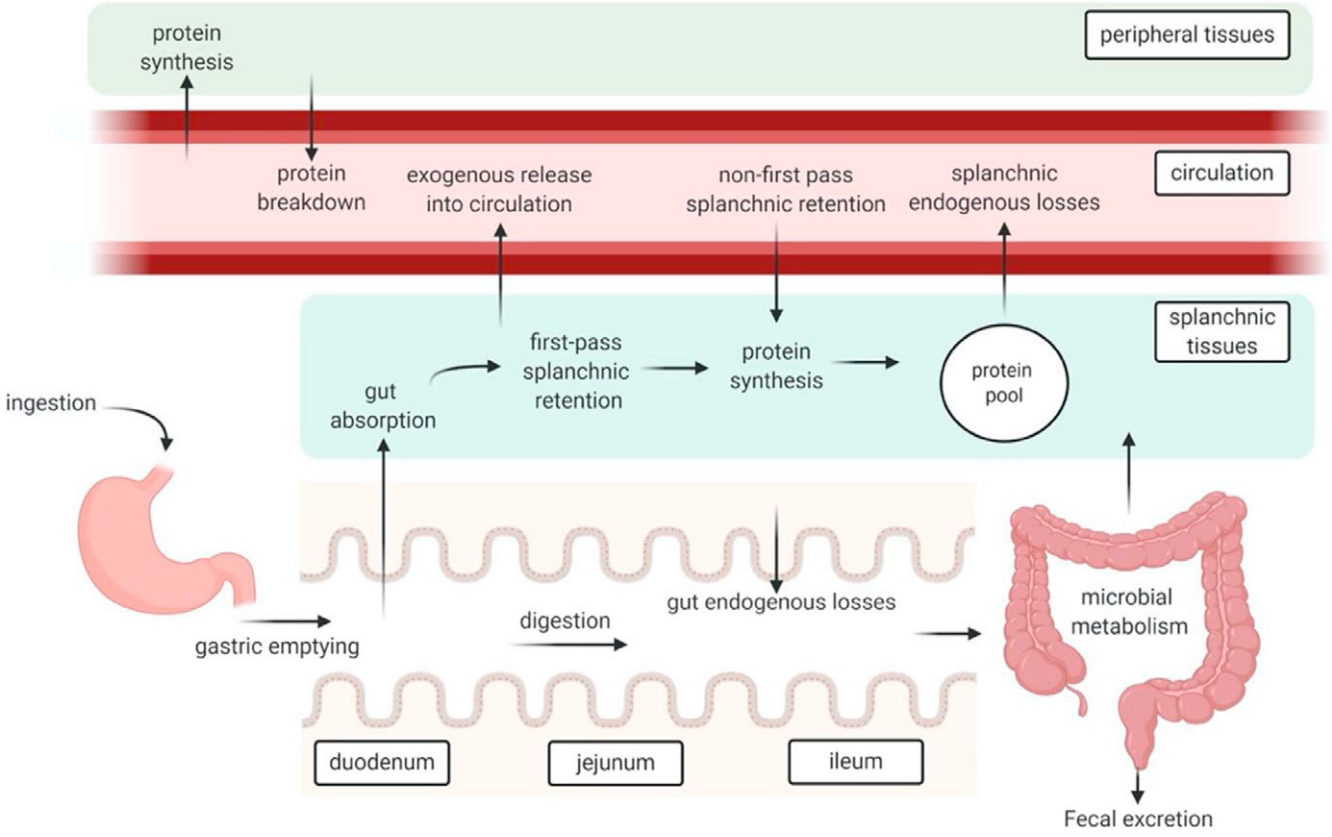

Fig. 1. Schematic representation of protein digestion and absorption processes. After Trommelen *et al.*[1], reproduced with permission.

among protein synthesis, protein breakdown and amino acid oxidation processes[40]. Protein digestion and absorption also influence other processes via the signalling roles of certain free amino acids. For example, K-cells in the intestinal epithelium release glucose-dependent insulinotropic polypeptide (GIP) in response to increased concentrations of free amino acids and dipeptides in the intestinal lumen[42]. The hypothalamus senses the post-prandial increase in blood leucine concentration and suppresses glycogenolysis and gluconeogenesis[7].

Here I discuss evidence that food processing modulates protein digestion and absorption rates via effects on food microstructure. Trials with food ingredients and formulated foods are discussed separately from wholefood trials because natural food microstructures and heterogenous composition in wholefoods introduce extra complexity into digestion processes. The wider metabolic effects of differential protein digestion rates are not well understood, but present *in vivo* results suggest that rational control of protein digestion and absorption by careful selection of food processing conditions could be a useful tool to optimise metabolic response to protein-rich meals.

Food ingredients and formulated foods

Process-induced amino acid side chain modifications

Food processing can involve extremes of temperature and pH. Under these conditions some amino acid sidechains are susceptible to undergoing chemical reactions[14,43] that cause permanent loss of bioavailability[44]. Amino acid side chains can undergo acid-/alkali- and water-catalysed reactions such as oxidation and deamination, or can react with other amino acids, leading to non-native cross-links[43]. Side chains can react with other food components, for example Maillard reactions with reducing sugars, leading to glycation and further reactions[45].

The indispensable amino acids histidine, lysine, methionine, threonine and tryptophan are particularly reactive, and reactions catalysed by heat, oxidising conditions or alkaline pH will compromise their bioavailability[46,47] and may give rise to toxic derivatives[46,48]. Special precautions are required when analysing lysine in processed food or feed, because Maillard-reacted lysine (which is not bioavailable) reverts to lysine during the acid hydrolysis step in amino acid analysis[49].

Nyakayiru *et al.*[50] measured the effect of glycation on lysine peripheral bioavailability with milk protein powders that had minimal glycation (3%), or glycation of 20% or 50% after dry heating at 50°C. In 15 healthy volunteers, glycation of 20% and 50% reduced post-prandial plasma lysine iAUC (incremental AUC) by 35% and 92%, respectively (Figure 2). Although the level of lysine glycation was artificially manipulated in this study, site-specific glycation rates of ≥20% have been observed in commercial milk products whose manufacture involves severe heating[51], and this is thought to be responsible for reduced lysine bioavailability[52].

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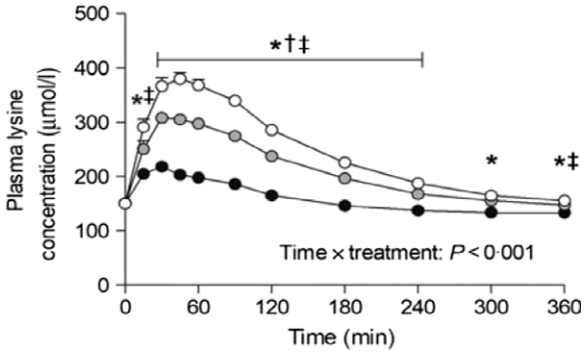

Fig. 2. Post-prandial plasma lysine concentrations for volunteers consuming milk protein powders with lysine glycation of 3% (open circles), 20% (grey circles) or 50% (black circles). After Nyakayiru *et al.*[50], reproduced with permission. *Significantly lower concentrations following ingestion of 50% glycation than 3% glycation (P<0·001). †Significantly lower concentrations following ingestion of 20% glycation than 3% glycation (P≤0·029). ‡Significantly lower concentrations following ingestion of 50% glycation than 20% glycation (P<0·001).

The effect of Maillard reactions on lysine bioavailability was also seen by Rérat *et al.*[53] in pigs after feeding heat-treated skim milk powder (51% lysine blockage). The cumulative appearance of lysine in portal blood was reduced by 60% relative to unheated skim milk powder. Valine absorption was also reduced (−34%) by heat treatment, but cystine absorption increased (+37%). Other *in vitro* and *in vivo* studies of processing-induced amino acid modifications to dairy proteins were reviewed by van Lieshout *et al.*[54].

Relatively little is known about process-induced amino acid modifications in foods other than dairy products, partly due to the diversity of reaction products and the complexity of mass spectrometric analysis. In a survey of commercial soybean meal ingredients, Troise *et al.*[55] reported that 26 out of 80 samples had ≥20% blocked lysine. Lassé *et al.*[56] reported that boiling egg white protein created a wide range of oxidative and other amino acid modifications. In blanched navy beans, Deb-Choudhury *et al.*[57] found lysinoalanine cross-links and heat-induced amino acid modifications.

Early Maillard reaction products appear to have low digestibility, but they are absorbed to some extent and excreted in urine[53,58]. Low digestibility may derive from the inability of trypsin to recognise lysine-adjacent cleavage sites (according to Keil rules[59]) when lysine is glycated, or digestive enzymes may be sterically hindered from accessing cleavage sites near glycated and/or cross-linked lysine[54]. Alkali exposure induces cross-linking of certain amino acids with the ϵ-NH_2 group of lysine via elimination–addition reactions[60], as well as amino acid racemisation[60]. Alkali-induced cross-links may similarly obstruct digestive enzymes, and lysinoalanine does not appear to be absorbed in rats[58] or humans[61], but can be absorbed by ruminants[62]. D-amino acids are utilised by humans to different extents, but D-lysine is not utilised[63].

Collectively, these data suggest that certain food processes involving extreme heating in the presence of reducing sugars and/or alkaline pH can modify amino acid side chains in ways that impair bioavailability. In contrast, more moderate heat treatments can improve protein digestibility through a combination of inactivating enzyme inhibitors[46] and denaturing proteins so that they are more susceptible to enzymatic cleavage[64].

Aggregation and gelation

Many food proteins will gel under certain conditions, i.e. they will aggregate into supramolecular protein–protein networks that percolate across an entire sample and give it a self-supporting semi-solid form. Familiar foods such as cheese, yoghurt, gelatin jelly and tofu are examples in which proteins are responsible for the gelled texture.

A series of studies by a group of French scientists[65–68] illustrated the magnitude of gelation effects on plasma amino acid kinetics using a pig model of human digestion. In these studies, heated milk (90°C 10 min) was gelled with rennet (cheese-like texture), gelled with acid (set yoghurt texture), or gelled with acid and then stirred (stirred yoghurt texture).The kinetics of amino acid appearance in the blood were strongly altered by gelation (Figure 3), and the different gel types produced significant differences. The effects of gelation and gel type were attributed to alteration of gastric emptying rate.

Particulate or coagulated foods may retain their microstructure in the stomach, and gastric conditions may catalyse coagulation of non-particulate foods, e.g. by destabilising colloidal casein micelles[10]. Particles or coagula that are too large to pass through the gastric pylorus are gradually eroded by the actions of pepsin and peristaltic mechanical shear. Mechanical breakup of protein coagula depends on properties such as elasticity and brittleness, and the rate of pepsin diffusion into protein coagula depends on microstructural factors such as pore size and degree of cross-linking[69,70]. Thus, the rate of gastric emptying is determined by the competing dynamics of microstructure formation versus mechanical/enzymatic erosion in gastric digesta. In the aforementioned studies with milk gels, gelling in different ways was thought to retard the erosion of gastric coagula to differing extents[66,71].

In a study with adults aged 66·7±4·3 y, Horstman *et al.*[72] reported a small but significant difference in peak serum amino acid concentration after consuming stirred yoghurt or low-fat ultra-high temperature treated (UHT) milk. The maximum concentration of serum essential amino acids was 13% higher with yoghurt (P<0.005), but area under the curve and other parameters were not significantly different.

According to Figure 3, acid gelation of pre-heated milk as part of yoghurt making slows the digestion of milk proteins, and this effect is partially reversed by stirring, which breaks up large aggregates. The findings of Horstman *et al.*[72] indicate that the protein in stirred yoghurt is still digested and absorbed faster than the protein in UHT milk, despite the semi-solid form of stirred yoghurt. This is probably the result of the more severe heat treatment during yoghurt manufacture (e.g. 90°C for 10 min) compared with UHT treatment (e.g. 140°C for 5 s), which results in greater whey protein denaturation[72–74].

Some protein-rich food ingredients are deliberately aggregated as part of their manufacture, either as a way to concentrate and fractionate proteins with different properties (e.g. casein and whey) or to modify their functionality for food manufacture[75]. Protein-rich food ingredients usually take the form of uniform

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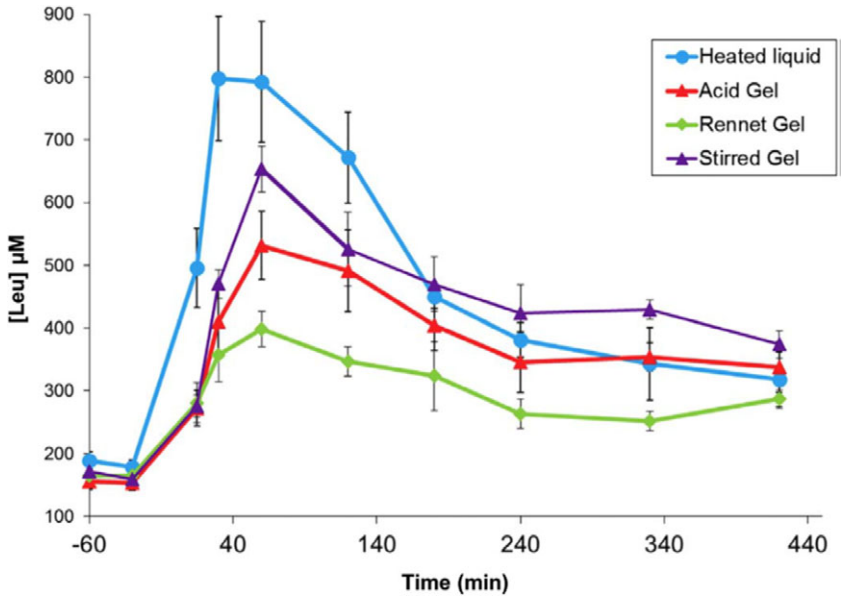

Fig. 3. Post-prandial plasma leucine kinetics in pigs after consuming milk in liquid or gelled forms. After Dupont *et al.*[68], reproduced with permission.

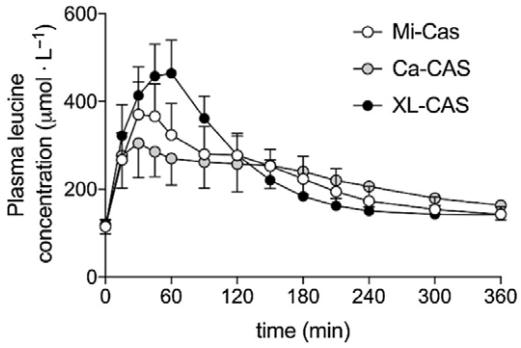

Fig. 4. Plasma leucine kinetics in volunteers after consuming different milk protein solutions. Mi-Cas, micellar casein; Ca-CAS, calcium caseinate; XL-CAS, cross-linked sodium caseinate. After Trommelen *et al.*[76], reproduced with permission.

fine powders, but with diverse microstructures that have measurable effects on protein digestion and absorption.

Trommelen *et al.*[76] measured amino acid uptake from three casein-derived food ingredients: calcium caseinate, a form of casein that has been acid precipitated and resolubilised with calcium hydroxide; micellar casein, which is produced by microfiltration of milk and retains its native micelle structure; and sodium caseinate cross-linked with the enzyme transglutaminase. The kinetics of amino acid appearance in blood plasma were similar for calcium caseinate and micellar casein, but markedly different for cross-linked sodium caseinate (Figure 4). The C_{max} and iAUC for branched-chain and essential amino acids were significantly higher for cross-linked sodium caseinate.

The materials used in this study differed widely in apparent solubility after 2 h of stirring: 99%, 53% and 5% soluble for cross-linked sodium caseinate, calcium caseinate and micellar casein, respectively[76]. In comparison with other studies[72,77], all casein ingredients in this study gave plasma leucine time courses (Figure 4) more characteristic of whey protein than of casein. This can be understood by considering the behaviour of the three test materials under gastric conditions.

Sodium caseinate powder dissolves rapidly[75] and coagulates slowly under gastric conditions to form a loose coagulum[10,72]. Cross-linking with transglutaminase slows down coagulation[78]. The rapid absorption of transglutaminase cross-linked sodium caseinate in Figure 4 probably reflects rapid dissolution followed by emptying from the stomach before coagulation can occur.

Calcium caseinate powder dissolves slowly and incompletely: even after 2 h of stirring at 25°C most material remains as suspended particles 10–100 µm in diameter[75,79], i.e. two to three orders of magnitude larger than casein micelles[80]. Particles in this size range sediment slowly under centrifugal force, and may appear soluble. Suspended calcium caseinate particles do not coagulate under gastric conditions[72], probably because of low collision frequency (high viscous drag[81]) and/or low collision efficiency[82,83] due to relatively large size, so they will also empty from the stomach rapidly. The overall bioavailability of the calcium caseinate in the study of Trommelen *et al.*[76] was lower than that of cross-linked sodium caseinate, which may be because a fraction of calcium caseinate particles remained partially undissolved in intestinal fluid and was therefore inaccessible to digestive enzymes.

Micellar casein powder also dissolves slowly, but ultimately dissolves completely, e.g. within ~30 min at 25°C[75]. It gave a peak in plasma leucine at 30 min, and overall bioavailability was intermediate between those of calcium caseinate and cross-linked sodium caseinate[76]. The timing of the leucine peak suggests rapid emptying of most micellar casein from the stomach, i.e. failure to coagulate significantly under gastric conditions. The slightly higher leucine bioavailability at 150–300 min may reflect either partial coagulation in the stomach with more advanced pH decrease, or slower dissolution of this specific micellar caseinate powder compared with that tested by Ji *et al.*[75].

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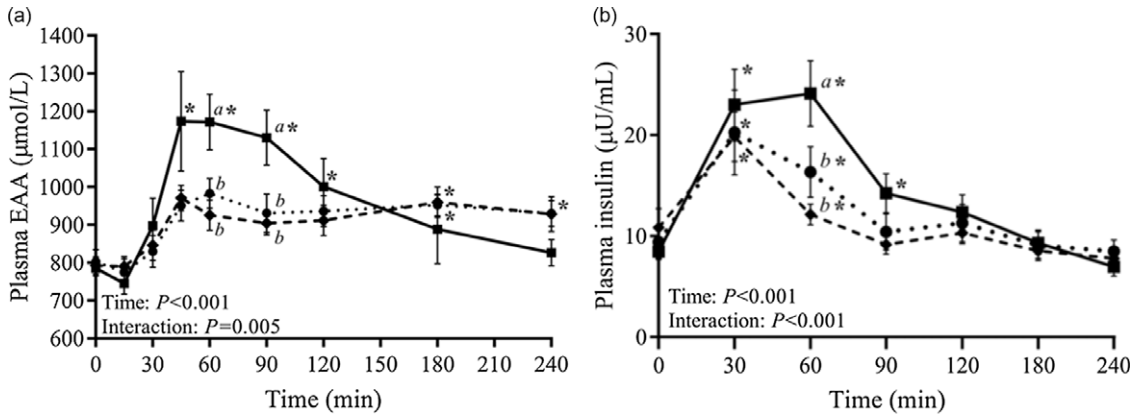

Fig. 5. Plasma concentrations of essential amino acids (EAA) (a) and plasma insulin concentration (b) after consumption of milk protein concentrate (dotted line, circles), mineral-modified milk protein concentrate (solid line, squares) or calcium caseinate (dashed line, diamonds). After Chan *et al.*[85], reproduced with permission.

All of the materials tested by Trommelen *et al.*[76] were substantially different to native casein micelles in liquid milk, which coagulate rapidly and effectively under gastric conditions[10]. Dissolution is a necessary precursor of coagulation due to hydrodynamic considerations. If a test meal consists of a slow-dissolving protein powder suspended (rather than dissolved) in water, then dissolution may not occur during gastric residence, and coagulation is thereby impaired.

Casein coagulation under gastric conditions is partly driven by the presence of calcium, and partial removal of calcium using zeolite treatment during casein manufacture can weaken the ability of caseins to coagulate[84]. In the work of Chan *et al.*[85], calcium-depleted milk protein concentrates (mMPC) gave significantly higher plasma concentrations of essential amino acids at 45–90 min post-prandially, compared with conventional MPC or calcium caseinate (Figure 5A). The MPC versus mMPC difference was attributed to weaker gastric coagulation with mMPC as a result of calcium depletion, and led to significantly higher insulin at 60 min (Figure 5B).

Aggregation and/or gelation as a result of food processing alters the physical form of food proteins, and thereby affects food digestion processes. The susceptibility of protein to enzymatic proteolysis may be increased or decreased, depending on aggregate structure. Gastric coagulation slows protein digestion, and processing that affects gastric coagulation significantly impacts protein digestion rates.

In a comprehensive overview of milk protein digestion studies, Horstman and Huppertz[86] suggested that the main factors controlling the rate of milk protein digestion are the potential of a given milk protein material to coagulate in the stomach and the energy content of a meal. Horstman and Huppertz[86] noted that not all forms of casein will coagulate under gastric conditions. This often-overlooked nuance can have a substantial effect on plasma amino acid kinetics, as seen in the studies by Trommelen *et al.*[76] and Chan *et al.*[85] (Section 3.2). Homogenising milk causes casein micelles to adsorb to milk fat globule interfaces, and this substantially alters the structure and mechanical properties of coagula formed under gastric conditions[87]. It remains to be seen what the consequences of homogenisation are for milk protein digestion and absorption rates.

Hydrolysis

Food proteins can be hydrolysed by acid or enzymes prior to ingestion, and this may affect digestion and absorption kinetics, depending on the degree of hydrolysis and the presence of other food components.

Deglaire et al.[28,34] quantified post-prandial nitrogen flows in adult humans after consuming intact casein or casein hydrolysed with pancreatin. Ileal exogenous nitrogen flow was higher for the hydrolysed casein over the first 3 h[28], and this was reflected in plasma amino acid kinetics[34], particularly for indispensable amino acids (Figure 6A). Endogenous nitrogen and amino acid flows and net post-prandial protein utilisation were not significantly different for the two diets. However the hydrolysed casein diet elicited greater splanchnic retention and lower peripheral uptake, as well as significantly higher insulin production[34] (Figure 6B).

In other studies comparing intact micellar casein with commercial casein hydrolysate, Koopman *et al.*[88] and Pennings *et al.*[89] reported significantly higher post-prandial plasma amino acid and insulin concentrations with hydrolysates (20 g or 35 g protein bolus). Calbet and Holst[90] reported similar differences in plasma amino acid kinetics with a 60 g bolus of casein or hydrolysed casein.

In the most extreme case, intact protein can be compared to a compositionally-equivalent mixture of amino acids. Weijzen *et al.*[91] reported large and significant differences in plasma amino acid kinetics in humans after consuming milk protein (processing unspecified) or a corresponding amino acid mixture (30 g bolus). Plasma amino acid levels, insulin peak concentration and net protein balance were higher with the amino acid mixture, but muscle protein synthesis rate was not significantly different.

There are conflicting results regarding the effect of processing on whey protein digestion. Some studies have shown very similar plasma amino acid kinetics, whether native, hydrolysed[90,92] or microparticulated.[93] Others have reported that intact whey protein produces higher plasma leucine than hydrolysed whey protein[94,95], although this was attributed to higher leucine content in one case[95]. The differences resulting from intact versus hydrolysed whey protein were much smaller than for intact versus hydrolysed caseins.

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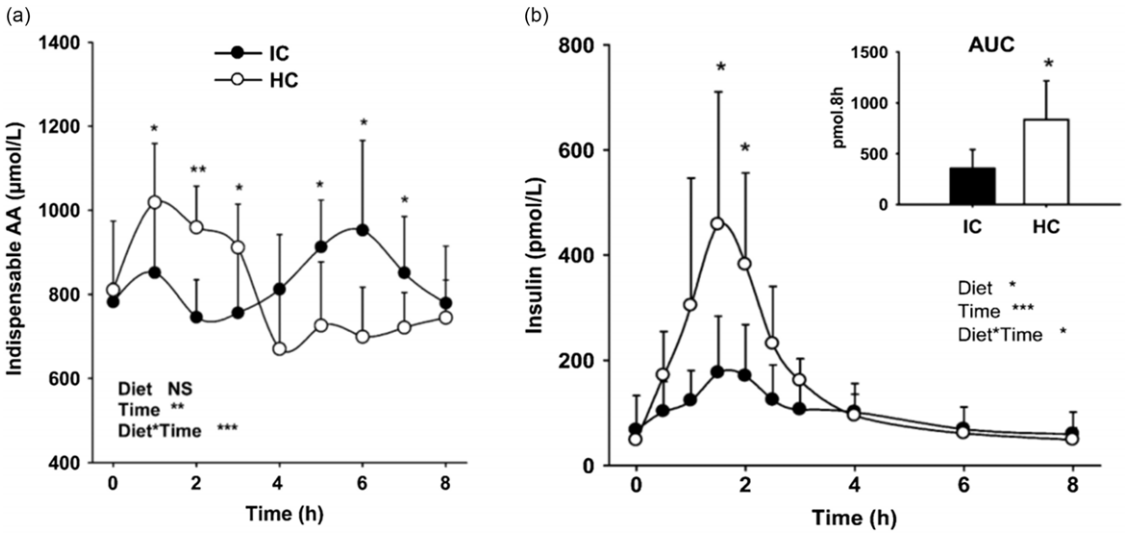

Fig. 6. Plasma amino acid (a) and plasma insulin (b) in adult humans following consumption of a meal containing intact casein (C) or hydrolysed casein (HC). After Deglaire *et al.*[34], reproduced with permission.

At sufficiently high concentration, whey proteins will denature and form a gel when heated. *In vitro* experiments indicate that whey protein gels are rapidly hydrolysed under gastric conditions, because denaturation makes whey proteins more susceptible to pepsinolysis.[96] In conclusion, hydrolysis almost always increases the rate of protein digestion, except where protein digestion rates are already high, as in the case of whey protein.

Whole foods

Heat processing of milk

Heating is a common way to kill pathogens that may be present in raw milk, and heat treatments also extend the shelf life by killing non-pathogenic microorganisms that cause spoilage. In the dairy industry, the most widespread heat treatments for liquid milk are high temperature, short time (HTST) pasteurisation, e.g. 72°C for 15–20 s, and UHT heat treatment, e.g. 140°C for 5 s. The major effect of heat processing milk at >80°C is to denature whey proteins, which adsorb to the surface of casein micelles and weaken gastric coagulation of casein[97]. Extreme heating can also cause amino acid side chain modifications, as discussed in Section 3.1.

Lacroix *et al.*[98] reported post-prandial nitrogen flows in humans after consuming milk treated by heat pasteurisation, UHT treatment or microfiltration (a low-temperature pasteurisation method). Post-prandial plasma amino acids were elevated above baseline levels for all treatments, but the type of milk processing did not produce significant differences. However, a higher rate of deamination and a lower rate of retention was seen with UHT milk, relative to microfiltered milk. The authors suggested that the different nitrogen utilisation pattern for UHT milk could be a result of more rapid gastric emptying and/or heat-related lysine damage[98]. Lysine damage in UHT milk is minor[52], and *in vitro* simulated digestion studies support the hypothesis of more rapid gastric emptying for UHT milk as a result of weaker gastric coagulation[10].

In another study focused on dairy product processing, Horstman *et al.*[72] reported slightly higher post-prandial plasma amino acid levels following consumption of low-fat UHT milk, compared with low-fat pasteurised milk (P=0.066 for the iAUC comparison). In this case, the UHT treatment was applied by direct steam injection, and Horstman *et al.*[72] suggested that the observed differences may be even larger with conventional indirect UHT treatment, which exposes milk to high temperatures for a longer time.

Barbé *et al.*[65] also examined the effect of a more severe heat treatment (90°C for 10 min) on post-prandial plasma amino acid kinetics in pigs after consumption of liquid milk. Consuming heated milk resulted in slightly higher aminoacidaemia than consuming unheated milk, but the differences were not significant.

Other *in vivo* and *in vitro* studies of milk protein digestion have been reviewed by Horstman and Huppertz[86] and Li *et al.*[99]. *In vitro* studies report differences in proteolysis rates between UHT-treated and pasteurised milks[74], but *in vivo* studies have found only small differences in amino acid bioavailability.

Cooking and mincing of beef

Cooking transforms raw meat into an edible form, as well as eliminating pathogens and enhancing flavour. Meat undergoes extensive microstructural and biochemical changes during cooking, including partial or complete denaturation of myofibrillar, sarcoplasmic and connective tissue proteins, shrinkage and expulsion of water, amino acid side chain modifications[100] and Maillard reactions between proteins and sugars. These cooking-related changes affect post-prandial nitrogen flows, including plasma amino acid kinetics.

One illustration of cooking effects on post-prandial amino acid kinetics is the study by Bax *et al.*[101] using pigs that were

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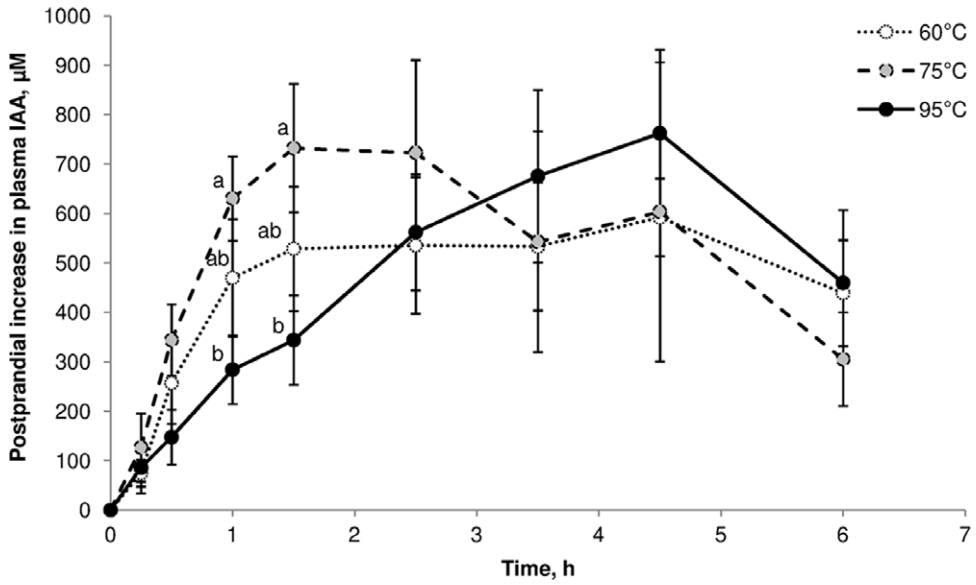

Fig. 7. Post-prandial kinetics of indispensable amino acids in pigs fed beef cooked for 30 min at 60°C, 75°C or 95°C. After Bax *et al.*[101], reproduced with permission.

fed beef samples cooked for 30 min in a water bath at 60°C, 75°C or 95 °C. Plasma amino acid levels showed significant differences over the first 1.5 h (Figure 7). Kinetic parameters were not significantly different, but it was clear that heating at 75°C led to a faster increase in plasma indispensable amino acids, and heating at 95°C resulted in slower aminoacidaemia.

Based on the results of a parallel simulated *in vitro* digestion study with pork[102], Bax *et al.*[101] proposed that low-temperature cooking led to protein denaturation, which enhanced proteolysis by exposing more cleavage sites. Cooking at a higher temperature led to protein aggregation, which made cleavage sites less accessible to proteases.

In a comparison of beef cooked at 55°C for 5 min with that cooked at 90°C for 30 min, Oberli *et al.*[103] reported higher ileal nitrogen flow and slightly lower digestibility in healthy young adults for the higher cooking temperature, but plasma amino acid kinetics were not significantly different. The authors hypothesised that the chosen cooking treatments gave rise to meat microstructures that resisted digestion to a similar extent, either through retained native microstructure (cooking at 55°C) or extensive protein aggregation (cooking at 90°C), as suggested by Bax *et al.*[102].

In a subsequent study using the same cooking treatments, Buffière *et al.* reported significantly different plasma amino acid kinetics for elderly volunteers[104]. Plasma leucine and plasma indispensable amino acids had significantly higher peaks for the beef cooked at 90°C than for beef cooked at 55°C, but the time at which plasma amino acid concentrations peaked was unaffected by cooking method. Whole body protein synthesis was higher with the well-cooked meat[104]. The contrast between the findings of Oberli *et al.*[103] and those of Buffière *et al.*[104] highlights the effect of age on digestion, i.e. amino acids from rare and well-cooked meat were equally bioavailable for adults, whereas amino acids in well-cooked meat were more bioavailable for elderly volunteers.

Cooked beef was minced in the work of Buffière *et al.*[104], Oberli *et al.*[103] and Bax *et al.*[101] to eliminate the effects of inter-individual variation in chewing patterns. Cooking-related differences were attributed to different meat meso- and microstructures. Pennings *et al.*[105] examined the effect of millimetre-scale meat structure, i.e. beef steak versus minced beef, using L-[1-^{13}C]phenylalanine intrinsically labelled beef. Minced beef produced a higher plasma enrichment of L-[1-^{13}C]phenylalanine than steak (Figure 8), suggesting more rapid digestion and absorption. Consequently, whole-body protein balance in the subjects (10 men 74±2 y old) was higher with minced beef.

Whereas previous studies compared minced beef cooked at different temperatures and times, or compared minced beef with steak, Prodhan *et al.*[106] compared beef steaks cooked with different methods: *sous vide* cooking at 80°C for 6 h or pan frying for 5 min. In young men taking part in the crossover trial, postprandial plasma amino acid kinetics and hormone levels were not significantly affected by the cooking method. Results from *in vitro* studies of cooking effects on meat digestion have been reviewed by Bhat *et al.*[107].

As with other food systems, processing of meat influences protein digestion via alterations to microstructure and physical form. Gross alterations such as mincing speed up protein digestion. Mild-to-moderate cooking increases proteolysis rates due to protein denaturation, whereas more extensive cooking can retard proteolysis as a result of extensive protein aggregation

Cooking of egg protein

Egg protein digestion has been studied *in vitro*[108], but few *in vivo* studies have examined how food processing affects egg protein digestion and absorption. Evenepoel *et al.*[109] reported that a raw egg meal had substantially lower ileal digestibility than the same meal that had been microwave-cooked (51·3±9·8% versus 90·9±0·8%). Gastric emptying was significantly faster with raw egg, which was attributed to its liquid consistency.

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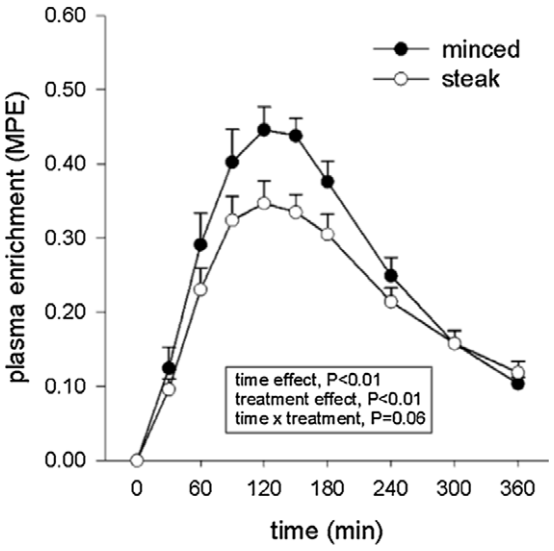

Fig. 8. Plasma enrichment with L-[1-^{13}C]phenylalanine following consumption of beef steak (open circles) or minced beef (closed circles). After Pennings *et al.*[105], reproduced with permission.

Nau *et al.*[110] took a more detailed look at gastric processing of cooked egg white in pigs, using egg white gels with different microstructures, which were created by adjusting pH and ionic strength before heating[111]. Gel structure affected gastric acidification rates and gastric emptying kinetics. The differences were attributed to the effect of egg white gels on the rheological properties of gastric chyme. Parallel *in vitro* studies suggested that egg white gel structure could affect protein hydrolysis rates[111], as well as the type and amount of peptides released by hydrolysis[112].

Concluding Remarks

From the *in vivo* evidence reviewed here, it is clear that several food processing operations affect the kinetics of post-prandial protein digestion and absorption. A wide variety of tissues and metabolic processes are sensitive to amino acid concentrations in peripheral blood, so the impacts of food processing on the body's response to protein intake are potentially wide ranging.

In many cases, the effect of food processing on digestion and absorption is mediated by protein structure effects at millimetre, micrometre and molecular length scales, which affect the physicochemical behaviour of protein-rich materials in the gastrointestinal tract. The solubility and gastric coagulation potential of food proteins can substantially alter the kinetics of protein digestion and absorption. These co-variate factors are often overlooked when comparing proteins from different sources and may be responsible for some of the variability reported in studies comparing protein sources.

Processing effects on protein digestion rates can be non-linear, as protein denaturation increases susceptibility to proteolysis, and subsequent aggregation inhibits proteolysis. Harsh processing causes chemical reactions that render indispensable amino acids permanently non-bioavailable. Hydrolysis increases the rate of protein digestion and absorption for slowly digestible proteins such as casein, but has little effect for whey proteins, which are rapidly digested and absorbed even when intact.

Food processing effects on protein digestion processes cannot be generalised, because the type and degree of alterations to protein structure depend on specific conditions, especially time, temperature and pH. Few studies have examined the effect of age on protein digestion. The contrasting findings in studies of minced beef digestion by adult[103] or elderly[104] volunteers suggest that processing-related effects on protein digestion may be larger in the elderly.

A lot is known about the digestion of milk and meat products processed in different ways, but the effects of food processing on the digestion of proteins from fish, egg and non-animal sources have received little attention. It is likely that similar principles will apply, i.e. food processes that produce aggregation, hydrolysis or amino acid modifications are likely to alter gastric emptying and/or the accessibility of peptide bonds to proteases. For example, heat-induced aggregation of bean proteins decreases digestibility in rats[113]. Recent comparisons of soy milk and tofu have found slower *in vitro* gastric proteolysis with tofu[114], corresponding to sustained elevation of intragastric pH in pigs after consuming tofu but not soy milk[115]. Additionally, food processes that eliminate the anti-nutritional factors found in pulses are reported to improve overall digestibility[116], and may increase the rate of protein digestion and absorption. With increasing public interest in reducing consumption of animal proteins, there is an acute need for better understanding of how food processing affects the digestion and absorption of non-animal proteins from traditional and novel sources.

The connection between protein digestion/absorption kinetics, protein structure and food processing reveals an opportunity to optimise protein digestion rates using carefully designed food processing treatments to improve health outcomes. Research with casein and casein hydrolysates (Table 1) has shown that protein digestion and absorption rates can have large effects on post-prandial insulin secretion profiles. Other effects on splanchnic nitrogen extraction and muscle synthesis are more subtle, and sometimes opposite effects are seen in young versus elderly subjects[103,104,117]. Satiety is likely to be influenced by food processes that alter the kinetics of protein digestion and absorption[66]. Food processing to increase satiation could produce foods for appetite control and weight management.

As we age, we need to consume more dietary protein to maintain muscle mass and function, but increasing our protein intake is often a challenge due to declining appetite and the poor palatability of high-protein foods[118,119]. There is a need for high-protein foods that are more palatable and less satiating. The protein in these foods should be rapidly digested and absorbed to provide sufficiently high peak plasma amino acid levels to overcome the anabolic resistance or "blunting" of muscle responsiveness that develops with age[120]. Such foods will be particularly challenging to produce with plant-derived proteins, which often have lower digestibility and/or lower essential amino acid content[121].

Food processing technologies are constantly evolving. New technologies such as high hydrostatic pressure processing and

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microwave-assisted pasteurisation introduce physical or electromagnetic stimuli that denature proteins in different ways to traditional heating or cooking processes[122]. These technologies offer new ways to modify protein digestion and absorption rates to beneficially influence physiological responses to food intake. Food processing has strong potential to optimise food protein digestion, but realising this potential will require more human clinical studies in which amino acid absorption rates are measured, and food microstructure is explicitly considered, measured and manipulated.

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Conflict of interest

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Authorship

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