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Postprandial blood amino acid concentrations in older adults after consumption of dairy products: The role of the dairy matrix

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ABSTRACT

This study investigated postprandial aminoacidemia after consumption of different dairy products using a single-blinded cross-over design wherein 10 participants (66.7 ± 4.3 y) received low-fat UHT-treated milk (LF–UHT), low-fat pasteurised milk (LF–PAS), full-fat UHT-treated milk (FF–UHT), full-fat pasteurised milk (FF–PAS), low-fat yoghurt, full-fat cheese, whey protein concentrate (WPC), and micellar casein isolate (MCI). Blood samples were collected postabsorptive and (up to 5 h) postprandial and maximal amino acid concentration (C_{max}), timepoint corresponding to C_{max} (T_{max}) and incremental area under the curve (iAUC) were determined. The highest increase in blood essential amino acid (EAA) levels occurred after WPC and yoghurt consumption, whereas MCI and cheese consumption resulted in extended EAA response curves. Fat delayed the postprandial EAA blood response (FF–UHT versus LF–UHT and FF–PAS versus LF–UHT), whereas no effect of heating milk was found ($P > 0.05$). The findings highlight that the product matrix could be as important as protein composition in postprandial aminoacidemia.

1. Introduction

It has been frequently shown that ingesting whey protein leads to a faster and greater increase in blood essential amino acids and especially leucine concentration than the other class of milk proteins, i.e., casein (Boirie et al., 1997; Dangin et al., 2001; Koopman et al., 2009; Pennings et al., 2011). This is because whey protein contains more leucine (Boirie et al., 1997; Dangin et al., 2001; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009) and typically has faster digestion and absorption kinetics than casein, resulting in a greater increase in postprandial blood amino acid availability, thereby stimulating muscle protein synthesis (Bohe, Low, Wolfe, & Rennie, 2003; Boirie et al., 1997; Dangin et al., 2001; Koopman et al., 2009; Pennings et al., 2011; Walrand et al., 2016). The faster digestion and absorption kinetics of whey protein can be attributed to the fact that whey protein does not coagulate and stays soluble under gastric conditions, whereas casein solutions do tend to coagulate (Lacroix et al., 2006; Wang, Ye, Lin, Han, & Singh, 2018).

In most cases, proteins are not consumed as isolated ingredients, but as a part of food products. These products undergo processing, such as heat treatment, homogenisation, and fermentation, which affects protein structure and interactions and can alter gastrointestinal digestion kinetics and subsequent release of amino acids (Gryson et al., 2014; Nyakayiru et al., 2019; van Lieshout, Lambers, Bragt, & Hettinga, 2019). In vitro it has been shown that heat treatment of milk, which leads to whey protein denaturation, leads to weaker gastric curds and more extensive protein hydrolysis under gastric conditions (Mulet-Cabero, Mackie, Wilde, Fenelon, & Brodkorb, 2019; Ye, Cui, Dalgleish, & Singh, 2017; Ye et al., 2019) and faster gastric emptying (Mulet-Cabero et al., 2019). In vivo, Lacroix et al. (2008) also showed that heat treatment of milk at ultra-high temperature (UHT) conditions improved uptake of amino acids from milk in the blood compared with pasteurisation of milk. During cheese manufacturing, a strong casein curd is formed by the enzymatic coagulation of casein, resulting in very slow gastric digestion in vitro (Fang, Rioux, Labrie, & Turgeon, 2016a; b; Guinot, Rioux, Labrie, Britten, & Turgeon, 2019; Lorieau et al., 2018) as opposed to the much weaker and easily disrupted acid-induced casein aggregates formed during

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yoghurt manufacture leading to an even quicker in vitro protein ingestion than raw milk (Breslaw & Kleyn, 1973).

Besides the effect of processing, the effects of the product matrix on the rate of protein digestion and health outcomes (Geiker et al., 2019; Thorning et al., 2017) have been overlooked since most in vivo human studies on postprandial aminoacidemia of milk proteins have been done with isolated proteins (Koopman et al., 2009; Pennings et al., 2012, 2011; Traylor et al., 2019; van Vliet et al., 2019) rather than products (Burd, Gorissen, van Vliet, Snijders, & van Loon, 2015; Churchward-Venne, Snijders, Linkens, Hamer, van Kranenburg, & van Loon, 2015; Luiking, Abrahamse, Ludwig, Boirie, & Verlaan, 2016). Therefore, the aim of this study was to assess the change in postprandial amino acids concentrations in blood over time, after consumption of different dairy products in an older population. An older population was chosen because of their particular risk of protein energy malnutrition and sarcopenia (Leij-Halfwerk et al., 2019). The different dairy products were selected to compare systematically the effect of relevant composition and processing parameters. In addition to comparing the caseins (in the form of micellar casein isolate; MCI) and whey proteins (in the form of whey protein concentrate; WPC), we compared this in the form of a liquid matrix (milk), a suspension of gel particles (low-fat yoghurt) and a gel (full-fat cheese). Within the liquid matrix, we investigated the effect of fat (low-fat versus full-fat) and heat treatment (pasteurisation versus UHT treatment).

2. Methods and materials

2.1. Participants

In a randomised, single-blinded cross-over trial, 10 healthy, recreationally active, older men (n = 5) and women (n = 5) [age, 66.7 ± 4.3 y; BMI, 25.6 ± 2.6 kg m^{-2} (values are the mean ± SD)] were recruited by inviting all persons over 60 years (n = 1000) from a database (Wageningen University, Department of Human Nutrition). Interested participants received written information about the study (n = 152) and were invited to an information meeting (n = 55). None of the participants had (a self-reported) food allergy or sensitivity to dairy ingredients or acetaminophen, used glucose lowering drugs or medications known to affect protein digestion, amino acid absorption, or protein metabolism, used protein supplements, or had a history of inflammatory bowel disease, hepatitis, pancreatitis, ulcers, gastrointestinal or rectal bleeding, major gastrointestinal tract surgery (such as gastrectomy, gastroenterostomy or bowel resection), known or suspected gastrointestinal (GI) disorders, colon or GI tract cancer. Before written informed consent was obtained, all participants were informed about the purpose of the study, the experimental procedures, and possible risks involved with participation.

Non-smoking volunteers over the age of 60 with a BMI between 21 and 30 kg m^{-2} and having regular Dutch eating habits (3 main meals per day) who had signed for informed consent (n = 46) and who were deemed healthy as assessed by the NIZO lifestyle and health questionnaire underwent a medical screening to assess their medical history, suitability of their veins for cannulation and blood sampling and to measure height, weight, fasting blood glucose and Hb (both from finger puncture). Nineteen out of 46 participants screened were not eligible. Of the 27 eligible participants, 5 men and 5 women were enrolled by lot.

This study was approved by the Medical Ethics Committee of Wageningen University (NL53912.081.15). The study was registered at clinicaltrial.gov as NCT02546141. The procedures followed were in accordance with the latest version of the Declaration of Helsinki. The trial was conducted at the facilities of the WUR, Wageningen, the Netherlands, in September and October 2015 by NIZO (Ede, the Netherlands). Participants' characteristics are presented in Table 1.

2.2. Experimental trial

The day before the test days, no strenuous physical activity or alcohol consumption was allowed. On the evening before the test days, a similar meal was consumed each week. Participants were free to choose this meal prior to the first test day. After an overnight fast (no eating or drinking after 21:00 h the day before), participants arrived at the laboratory at 7:30 h by the same means of transportation each week, and with least activity and no rush. A cannula was inserted into an antecubital vein and kept patent with a saline drip. After taking a baseline blood sample the study product was taken (ingestion within a 10-min period, finished at t = 0 min). Subsequent venous blood samples were collected at t = 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240 and 300 min during the entire (0–5 h) postprandial phase. Serum amino acid concentrations, plasma glucose concentrations and plasma insulin concentrations were measured.

2.3. Study products

Participants randomly received 8 products: 6 dairy products [low-fat UHT milk (LF–UHT), low-fat pasteurised milk (LF–PAS), homogenised full-fat UHT milk (FF–UHT), unhomogenised full-fat pasteurised milk (FF–PAS), low-fat yoghurt, and full-fat cheese] and 2 spray-dried milk protein ingredients (WPC80 and MCI). All products, with the exception of FF–PAS, were commercial products produced by FrieslandCampina (Amersfoort, The Netherlands). FF–PAS was produced on pilot scale at FrieslandCampina (Wageningen, The Netherlands). Products were supplied on 8 separate test days, with a 1 wk washout period between treatments. For each test product, an appropriate amount of the product to ensure 25 g of protein intake was consumed. For the milk protein ingredients (WPC and MCI), this was achieved by dissolving an appropriate amount of powder in water to attain a solution of 700 mL containing 25 g of protein. To standardise the volume for all products, water was added to a total of 700 mL of volume ingested. For the milk samples, the water was mixed with the milk. For yoghurt and cheese, the water was provided separately to the product. Table 2 shows the nutrient composition of the products and servings used in the study.

2.4. Blood analyses

2.4.1. Glucose

Blood glucose concentrations (in mmol L^{-1}) were determined immediately in fresh drawn blood (at t = −5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240 and 300 min) in a portable glucometer (FreeStyle Freedom Lite with FreeStyle Lite test trips; Abbott

Table 1
Participants' characteristics at baseline.[a]

Parameter	Value	Range (min – max)
Age (y)	66.7 ± 4.3	60–75
Gender (% female)	50	
Body weight (kg)	74.1 ± 9.1	60.1–89.1
Height (cm)	170 ± 9.4	155–187
BMI (kg m^{-2})	25.6 ± 2.6	21–29
Hb (mmol L^{-1})	9.1 ± 0.5	8.5–9.6
Fasting glucose (mmol L^{-1})	5.4 ± 0.6	4.7–6.8

[a] Abbreviations are: BMI, body mass index; Hb, haemoglobin. Values (except gender) are means ± SD (n = 10).

Table 2
Composition of ingredients and products and servings used in this study.[a]

Parameter	WPC	MCI	LF–UHT	LF–PAS	FF–UHT	FF–PAS	Yoghurt	Cheese
Quantity used per serving	31 g	29 g	676 mL	694 mL	694 mL	694 mL	532 mL	97 g
Ingredient/product composition								
Energy (MJ 100 g^{-1} or 100 mL^{-1})	1.64	2.01	0.15	0.15	0.28	0.28	0.17	1.69
Protein (g 100 g^{-1} or 100 mL^{-1})	80	87	3.7	3.6	3.6	3.6	4.7	25.7
Carbohydrate (g 100 g^{-1} or 100 mL^{-1})	6.6	10	5	4.7	4.7	4.7	4	0
Fat (g 100 g^{-1} or 100 mL^{-1})	5	1	0.1	0.1	3.6	3.6	0	33.5
Intake per serving								
Energy (MJ)	0.51	0.58	1.03	1.01	1.93	1.91	0.89	1.64
Protein (g)	24.8	25.2	25.0	25.0	25.0	25.0	25.0	24.9
Carbohydrate (g)	2.0	2.9	33.8	32.6	32.6	32.6	21.3	0
Fat (g)	1.6	0.3	0.7	0.7	25.0	25.0	0.0	32.5

[a] Abbreviations are: WPC, whey protein concentrate; MCI, micellar casein isolate; LF–UHT, low-fat UHT-treated milk; LF–PAS, low-fat pasteurised milk; FF–UHT, full-fat UHT-treated milk; FF–PAS, full-fat pasteurised milk, unhomogenised.

Diabetes Care Inc., Hoofddorp, The Netherlands; CV 4.3%). Five different glucometers were used and all measures for one participant during the whole study were performed with the same glucose meter.

2.4.2. Insulin

Blood samples for measurements of plasma insulin (at t = −5, 15, 30, 45, 60, 75, 90, 120 and 180 min) were collected in pre-chilled lithium-heparin-containing tubes. Tubes were kept on ice until centrifuging and plasma was centrifuged within 15 min, for 10 min at 1192×*g* at 4 °C. All samples were immediately stored at −70 °C. Insulin was measured by a solid-phase enzyme-labelled chemiluminescent immunometric assay. Intra- and inter-assay variation at a concentration of 8.61 mU L^{-1} were SD 0.189 and SD 0.389, respectively; at a concentration of 22.61 mU L^{-1} they were SD 0.803 and SD 0.803, respectively; and at a concentration of 36.6 mU L^{-1} they were SD 0.933 and SD 1.633, respectively.

2.4.3. Amino acids

Blood samples were collected in regular serum tubes, which were left to stand at room temperature for at least half an hour before centrifuging for 10 min at 1192×*g* at 4 °C. Aliquots of serum were frozen in liquid nitrogen and stored at −70 °C. Free amino acids in serum were quantified using the EZ:faast amino acid kit (Phenomenex, Torrance, CA, USA). The EZ:faast amino acid procedure consists of a solid phase extraction followed by a derivatisation and a liquid/liquid extraction step. Derivatised samples were analysed by liquid chromatography-mass spectrometry (LC-MS) on a EZ:faast AAA-MS column (Phenomex) using a water–methanol gradient with both solvents containing ammonium formate as outlined by the manufacturer. Reproducibility (based on one sample analysed 6 times with complete procedure as described above) was between 0.8 and 4.6% for all reported amino acids. Limit of detection was between 1 and 10 pmol mL^{-1} for all amino acids reported. In addition to single amino acids, total serum amino acid (TAA) and essential amino acid (EAA) concentrations were summarised and included in the analyses (TAAs: arginine, glutamine, serine, asparagine, glycine, threonine, alanine, methionine, proline, lysine, aspartic acid, histidine, valine, glutamic acid, tryptophan, leucine, phenylalanine, isoleucine, cysteine and tyrosine; EAAs: phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine).

2.5. Statistical analyses

All data are expressed as mean ± SEM. Paired sample t-tests were used for comparing maximal EAA concentration values (C_{max}), the time point corresponding to C_{max} (T_{max}) and the incremental area under the curve (iAUC; i.e., area under the curve minus baseline value at t = −5 min using the trapezoid method). Repeated Measures ANOVA was used for the kinetics of the essential amino acid time curves. Statistical significance was set at $P < 0.05$. All calculations were performed using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). The 9 pre-defined contrasts were MCI versus WPC, MCI versus cheese, WPC versus yoghurt, yoghurt versus cheese, yoghurt versus LF–UHT, LF–UHT versus LF–PAS, LF–UHT versus FF–UHT, FF–UHT versus FF–PAS and FF–PAS versus LF–PAS.

3. Results

3.1. Glucose and insulin concentrations

Serum glucose and insulin concentrations after ingestion of the different products are shown in Fig. 1. C_{max} for glucose significantly differed between LF–PAS (highest peak) on the one hand, and WPC ($P < 0.001$), FF–PAS ($P = 0.036$) and yoghurt ($P = 0.001$) on the other hand. iAUC for glucose was not overall different between products ($P = 0.99$). Serum insulin concentrations increased after protein intake. Peak plasma insulin concentrations were highest after intake of the 4 milk samples and yoghurt (average 55 ± 3 mU L^{-1}) and lowest after MCI and cheese intake (average 22 ± 2 mU L^{-1}). This corresponds well with the lactose content in products (Table 2). A stronger insulin response for WPC was found than for MCI ($P < 0.001$), which was expected considering the more branched-chain amino acids in whey and faster digestion kinetics compared with casein (Nilsson, Stenberg, Frid, Holst, & Bjorck, 2004; Pal & Ellis, 2010).

3.2. Serum amino acid concentrations

Per product, postprandial TAA, EAA and leucine serum concentrations are shown in Fig. 2. Peak postprandial blood TAA concentrations increased most after intake of whey protein (from 1.67 ± 0.09 to 2.07 ± 0.12 mmol L^{-1}) and yoghurt (from 1.62 ± 0.06 to 2.20 ± 0.11 mmol L^{-1}), with T_{max} at 52.5 ± 6.4 and 58.5 ± 4.2 min, respectively. Consumption of MCI and both full fat milks (PAS and UHT) resulted in the lowest C_{max} (1.81 ± 0.08, 1.83 ± 0.08 and 1.89 ± 0.10 mmol L^{-1}), with T_{max} at 81.0 ± 12.7, 61.5 ± 9.1 and 63.0 ± 11.4 min, respectively.

In Figs. 3 and 4, the comparisons of postprandial blood EAA concentrations between the specific products are depicted. As expected, WPC intake resulted in higher peak postprandial EAA concentrations compared with MCI (C_{max}, $P < 0.001$; T_{max}, $P = 0.014$; iAUC, $P = 0.034$). Yoghurt consumption resulted in earlier and higher peak postprandial blood EAA concentrations compared

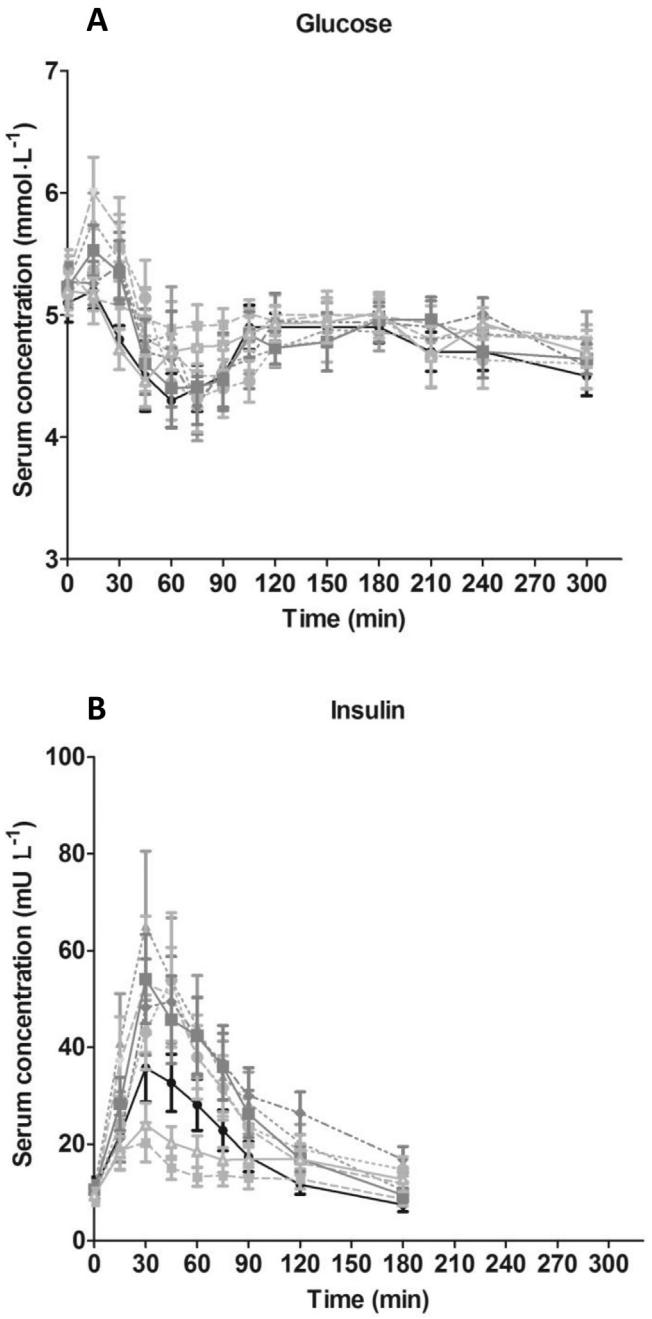

Fig. 1. Mean ± SEM serum glucose (mmol L^{-1}) (A) and insulin (mU L^{-1}) (B) concentrations during the fasted state and after ingestion of 25 g of protein in healthy older adults (n = 10): ●, whey protein concentrate; ■, micellar casein isolate; ▲, low-fat UHT-treated milk (LF−UHT); ▼, low-fat pasteurised milk (LF−PAS); ◆, full-fat UHT-treated milk (FF−UHT); ●, full-fat pasteurised milk, unhomogenised (FF−PAS); ■, yoghurt;, cheese.

with cheese (C_{max}, $P = 0.003$; T_{max}, $P = 0.010$; iAUC, $P = 0.457$), but in lower peak EAA concentrations compared with WPC (C_{max}, $P = 0.005$; T_{max}, $P = 0.005$; iAUC, $P = 0.496$). Postprandial blood EAA concentrations did not differ after cheese versus MCI intake (C_{max}, $P = 0.067$; T_{max}, $P = 0.873$; iAUC, $P = 0.592$). Fat in the milk products resulted in lower peaks and more prolonged postprandial blood EAA responses with no differences in iAUCs ($P > 0.05$) (C_{max}, $P = 0.001$; T_{max}, $P = 0.015$ for LF versus FF−UHT, and C_{max}, $P = 0.053$; T_{max}, $P = 0.191$ for FF versus LF−PAS). No effect of heating of milk was found (FF−UHT versus FF−PAS and LF−UHT versus LF−PAS) on postprandial aminoacidemia. Blood leucine concentrations rapidly increased following protein ingestion in all groups ($P < 0.001$), reaching highest values after intake of whey protein (0.415 ± 0.028 mmol L^{-1}) and yoghurt (0.292 ± 0.013 mmol L^{-1}).

4. Discussion

In the present study, we observed clear differences in blood EAA concentrations after ingestion of 8 dairy products and ingredients. Upon the ingestion of dairy products containing 25 g protein, a higher increase in EAA concentrations in blood was observed after consumption of yoghurt, compared with milk and cheese (summarised pictorially in Fig. 5).

Protein content and volume were kept constant between the 8 test servings, with the consequence that, e.g., energy, lactose and fat content differed. Other factors that varied between the test products were pH, structure, viscosity, homogenisation and heat treatment. By comparing full-fat and low-fat variants for both pasteurised and UHT milk, the effect of the presence of fat in the product could be assessed. We expected that fat in a composition would slow down digestion, since it is known that fat globules can become part of the coagula and cream in the stomach (Mulet-Cabero et al., 2019). We indeed found lower peaks and more prolonged blood EAA responses after ingestion of full-fat versus skim milk (Figs. 3 and 4), which is in line with earlier findings by Bergeim, Evvard, Rehfuss, and Hawk (1919) who observed delayed gastric emptying of full-fat milk compared with low-fat milk. In contrast, Gorissen et al. (2017) did not find an effect on postprandial protein handling when co-ingesting milk fat with micellar casein. This might be due to the fact that the product was only blended, which just leads to coarse emulsions, for which the fat globules most probably did not become part of the casein coagula in the stomach.

By comparing UHT and pasteurised milks, the effect of heat treatment was evaluated. We did not find a significant effect of heating (FF−UHT versus FF−PAS and LF−UHT versus LF−PAS) on C_{max} and T_{max} (Fig. 4). A typical UHT-treatment (5 s at 140 °C) leaves ~30% of the whey protein native (McMahon, Yousif, & Kaláb, 1993) whereas a typical pasteurisation (15 s at 72 °C) leaves > 80% of the whey protein native (Resmini, Pellegrino, Hogenboom, & Andreini, 1989). A notable part of the denatured whey protein becomes associated with the casein micelles and these whey protein-coated casein micelles are notably more stable to enzyme-induced coagulation. In cheese-making, heat-induced association of whey proteins with casein micelles results in poorly coagulable milk (Gazi & Huppertz, 2015), and this is also the case for coagulation of milk during in vitro gastric digestion (Mulet-Cabero et al., 2019; Ye et al., 2017, 2019).

Although in the current study, the blood EAA curves for the UHT-treated milk seem consistently higher than for the pasteurised milk, both for skim milk and full-fat milk, there are no significant differences in C_{max}, T_{max} or iAUC between the UHT-treated and pasteurised milk (Figs. 3 and 4). This could be due to the fact we used a relatively mildly treated UHT milk (processed with direct steam injection; DSI) compared with more traditional UHT milk treated by indirect heating (Tran, Datta, Lewis, & Deeth, 2008) and certainly with sterilised milk. Hence there might not have been enough contrast in the whey protein denaturation. A similar effect has been shown for rennet coagulation of directly versus indirectly UHT-treated milk, with the former giving higher denaturation and much weaker coagulation (Perkin, Henschel, & Burton, 2009).

When comparing yoghurt versus cheese, we found a more rapid and higher increase in postprandial EAA blood concentrations after yoghurt versus cheese intake (Figs. 3 and 4). Also, yoghurt versus skim (LF−)UHT milk was compared, showing higher peak

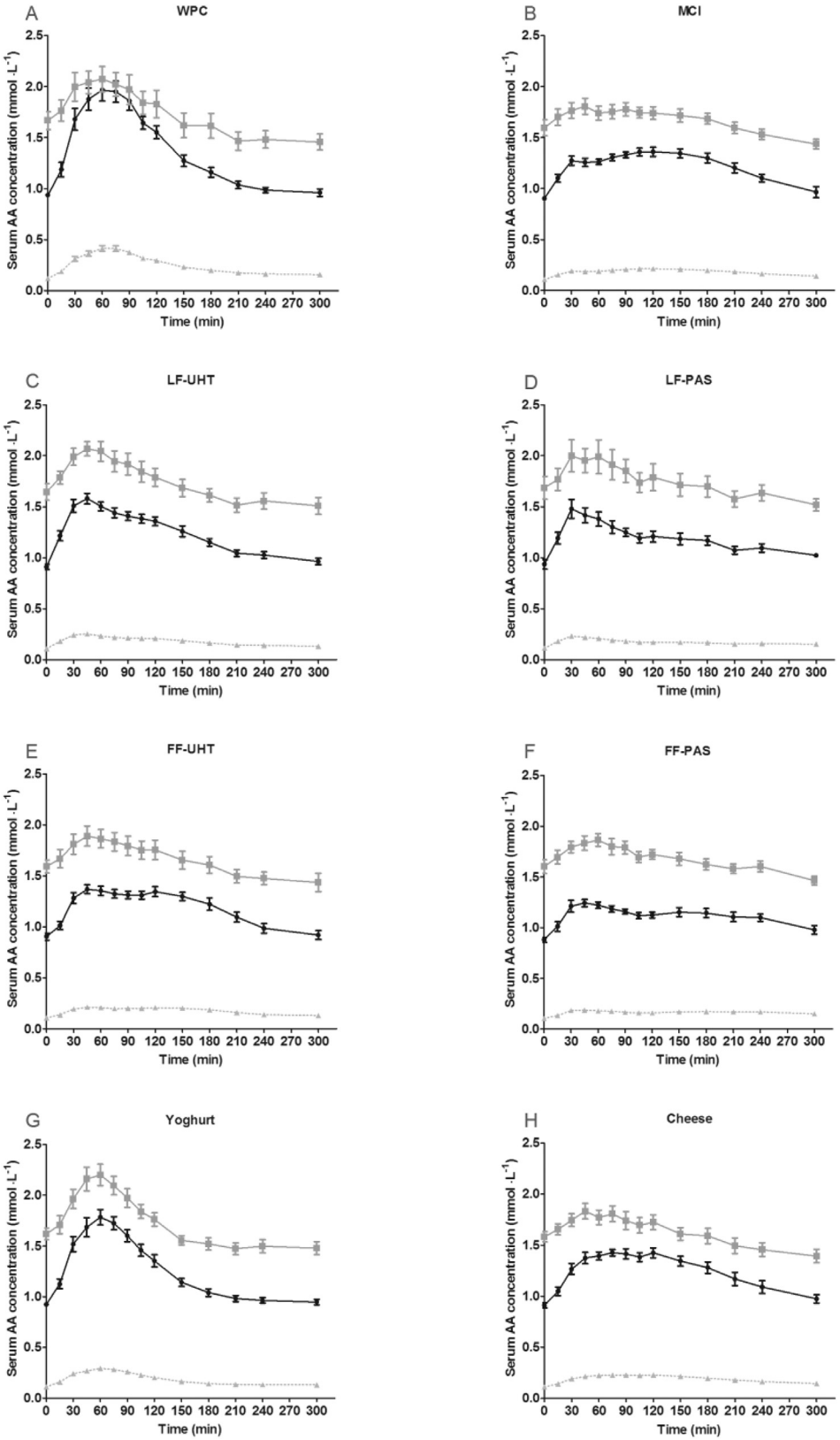

Fig. 2. Mean ± SEM serum total amino acid (▒), essential amino acid (●) and leucine (▲) concentrations (mmol L^{-1}) in healthy older adults (n = 10) after the ingestion of 25 g of protein from (A) whey protein concentrate (WPC), (B) micellar casein isolate (MCI), (C) low-fat UHT-treated milk (LF–UHT), (D) low-fat pasteurised milk (LF–PAS), (E) full-fat UHT-treated milk (FF–UHT), (F) full-fat pasteurised milk (FF–PAS), (G) low-fat yoghurt, and (H) full-fat cheese.

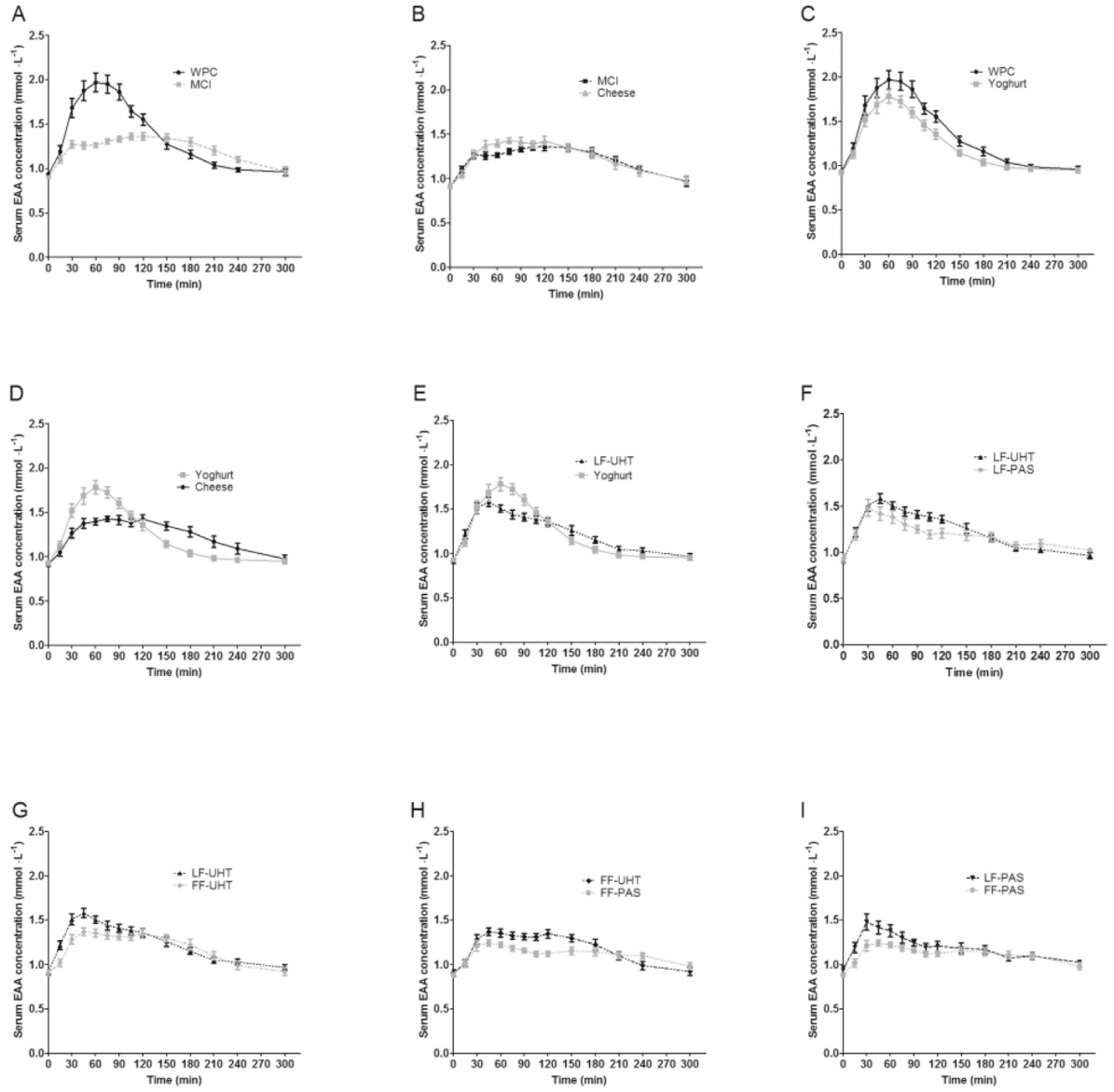

Fig. 3. Mean ± SEM serum essential amino acid concentrations (mmol L^{-1}) in healthy older adults (n=10) after ingestion of 25 g of protein in A: whey protein concentrate (WPC) vs micellar casein isolate (MCI), B: MCI vs cheese, C: WPC vs yoghurt, D: yoghurt vs cheese, E: yoghurt vs low-fat UHT-treated milk (LF-UHT), F: LF-UHT vs low-fat pasteurized milk (LF-PAS), G: LF-UHT vs full-fat UHT-treated milk (FF-UHT), H: FF-UHT vs full-fat pasteurized milk (FF-PAS), and I: FF-PAS vs LF-PAS. Interaction effects (time x product): A: P<0.001. B: =0.139. C: P=0.026. D: P<0.001. E: P<0.001. F: P=0.001. G: P<0.001. H: P<0.001. I: P=0.001.

postprandial blood EAA levels after yoghurt intake with no difference in T_{max} or iAUC (Fig. 4). Low-fat yoghurt can be distinguished from skim milk products based on several criteria. During processing, yoghurt milk is pre-heated extensively (typically 90–95 °C for 5–10 min), as a result of which virtually all whey proteins are denatured (Gazi & Huppertz, 2015).

Post-fermentation, the yoghurt gel is subsequently sheared to produce a stirred yoghurt product that is essentially a (concentrated) suspension of microgel particles, with particles in the size range of 50–250 μm, i.e., approximately an order of magnitude smaller than the maximum size of particles deemed suitable for gastric emptying. Under gastric conditions, these particles show little tendency for aggregation (Buchheim, 1984; Pfeil, 1984), which makes them suitable to rapid gastric emptying. In addition, the small particles have a proportionally large surface area, facilitating access for gastric and intestinal proteases for digestion.

We indeed show a rapid and high increase in amino acid concentrations after yoghurt intake (Figs. 2 and 3). In contrast, Gaudichon et al. (1994) showed the opposite effect, as both the intestinal delivery of the liquid phase and the nitrogenous fraction of the chyme were more delayed in pigs fed yoghurt than milk. Further research also indicated delayed gastric emptying of the yoghurt compared with milk in vivo in humans (Gaudichon et al., 1995). However, it is not reported whether set yoghurt (as opposed to stirred yoghurt) was used and whether the milk in those studies had been frozen, which can impact the digestive properties. In vitro studies indicate that yoghurt structure has a notable effect on protein digestion in yoghurt (Rinaldi, Rioux, Britten, & Turgeon, 2015) and that if raw milk is fermented, it digests faster (Doan & Dizikes, 1942; Pfeil, 1984). Our results show that the combined heat-treatment and fermentation in the yoghurt-making process

WPC

	WPC	yoghurt	P
iAUC	121.9 ± 32.6	92.2 ± 19.7	0.005 *
C_{max}	2066.0 ± 103.2	1822.2 ± 69.0	0.005 *
T_{max}	64.5 ± 20.1	60.0 ± 10.0	0.496

	WPC	MCI	P
iAUC	121.9 ± 32.6	94.3 ± 22.8	0.034 *
C_{max}	2066.0 ± 103.2	1464.0 ± 30.0	<0.001 *
T_{max}	64.5 ± 20.1	117.0 ± 53.3	0.014 *

MCI

	MCI	Cheese	P
iAUC	94.3 ± 22.8	96.9 ± 17.2	0.592
C_{max}	1464.0 ± 30.0	1520.9 ± 29.9	0.067 $
T_{max}	117.0 ± 53.3	112.5 ± 57.6	0.873

yoghurt

	yoghurt	LF-UHT	P
iAUC	92.2 ± 19.7	93.6 ± 15.0	0.794
C_{max}	1.82 ± 0.07	1.61 ± 0.05	0.005 *
T_{max}	60.0 ± 10.0	51.0 ± 26.6	0.382

	yoghurt	cheese	P
iAUC	92.2 ± 19.7	96.9 ± 17.2	.0457
C_{max}	1822.2 ± 69.0	1520.9 ± 29.9	0.003 *
T_{max}	60.0 ± 10.0	112.5 ± 57.6	0.010 *

cheese

FF-UHT

	FF-UHT	FF-PAS	P
iAUC	81.6 ± 19.3	71.3 ± 20.4	0.331
C_{max}	1.43 ± 0.05	1.34 ± 0.03	0.158
T_{max}	94.5 ± 44.2	48.0 ± 46.8	0.349

	FF-UHT	LF-UHT	P
iAUC	81.6 ± 19.3	93.6 ± 15.0	0.152
C_{max}	1.42 ± 0.05	1.61 ± 0.05	0.001 *
T_{max}	94.5 ± 44.2	51.0 ± 26.6	0.015 *

	FF-PAS	LF-PAS	P
iAUC	71.3 ± 20.4	75.4 ± 17.9	0.673
C_{max}	1.34 ± 0.03	1.52 ± 0.09	0.053 $
T_{max}	73.5 ± 66.1	48.0 ± 46.8	0.191

FF-PAS

LF-UHT

	LF-UHT	LF-PAS	P
iAUC	93.6 ± 15.0	75.4 ± 17.9	0.066 $
C_{max}	1.61 ± 0.05	1.52 ± 0.09	0.250
T_{max}	51.0 ± 26.6	48.0 ± 46.8	0.856

LF-PAS

Fig. 4. Mean ± SEM iAUC (mmol L^{-1} 5 h), C_{max} (mmol L^{-1}) and T_{max} (min) for serum essential amino acid concentrations in healthy older people (n = 10) after ingestion of 25 g of protein (*$P < 0.05$; $ $P < 0.10$). C_{max}, maximal concentration values; FF–PAS, full-fat pasteurised milk; FF–UHT, full-fat UHT-treated milk; iAUC, incremental area under the curve; LF–PAS, low-fat pasteurised milk; LF–UHT, low-fat UHT-treated milk; MCI, micellar casein isolate; T_{max}, time point corresponding to C_{max}; WPC, whey protein concentrate.

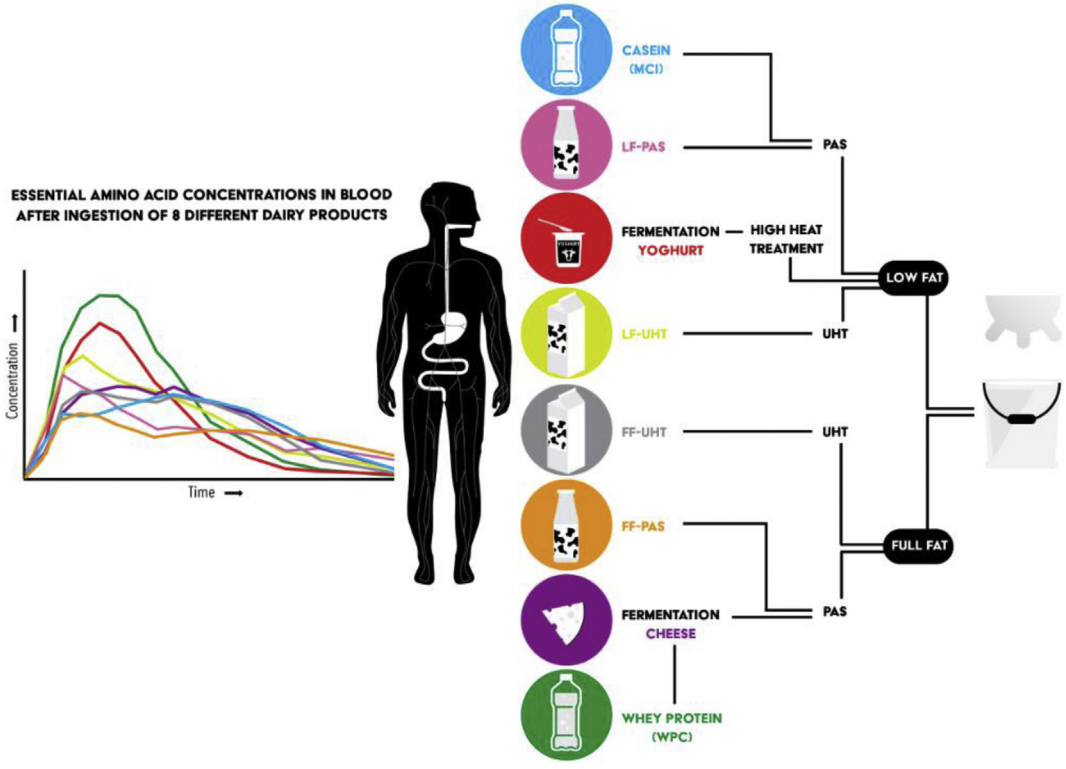

Fig. 5. Pictorial summary of the main results.

leads to higher (1.82 ± 0.07 versus 1.61 ± 0.05 mmol L^{-1}, $P = 0.005$), but not significantly faster, postprandial EAA blood profiles compared with LF–UHT milk (Fig. 4), even though the protein fraction of both products consists of 20% whey protein and 80% casein. In addition, although protein in yoghurt consists mainly of casein, our results interestingly show a similar total AA profile in blood after the ingestion of yoghurt compared with WPC (Figs. 3 and 4).

Our last comparison was cheese versus micellar casein. From a protein:fat ratio, heat-load and state of the fat globules, cheese is most comparable with whole milk, but it is virtually devoid of whey proteins and thus differs in protein composition. Furthermore, a main differentiating factor is that cheese is a highly concentrated coagulated casein matrix, from which most water has been removed (Walstra, Walstra, Wouters, & Geurts, 2005) and needs to be masticated to form a bolus before swallowing and enter the stomach as bolus particles with sizes larger than those suitable for gastric emptying. These gel particles are unlikely to aggregate or synerese, but diffusion of materials, including digestive enzymes into the cheese matrix will be extremely slow, as is also clear from studies on rennet gels by Barbé et al. (2013) and Le Feunteun et al. (2013).

In line with this, the results of the current study do not show any differences between blood EAA concentrations after ingestion of semi-matured full-fat cheese and micellar casein isolate. Interestingly, similar reactions occur in the stomach, where pepsin hydrolyses κ-casein, leading to aggregation of para-casein micelles, followed by syneresis of the formed particles. Gamlath, Leong, Ashokkumar, and Martin (2017) showed that not only denatured whey protein can hinder the aggregation of para-casein micelles, but that also the presence of native whey proteins can hinder the aggregation of para-casein micelles. Hence, in the aggregation of casein micelles from MCI, stronger gels are expected than those from milk due to the higher casein:whey protein ratio in the former.

5. Conclusions

Consumption of the same amount of milk protein from different dairy products with similar volume results in different blood amino acid concentrations over time. A higher increase in (essential) amino acid concentrations was observed after consumption of yoghurt, compared with milk and cheese. In addition, fat in a composition slowed down digestion. Our results clearly show that, besides the protein composition, the product matrix and processing are very important for systemic amino acid concentrations. Understanding the behaviour of different dairy products in the stomach can provide direction for modulating the rate of amino acid appearance in blood.

Author contributions

Astrid M.H. Horstman: Formal Analysis, Investigation, Visualization, Roles/Writing – original draft, Writing – review&editing.

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