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Original Research Article

The impact of heat treatment of bovine milk on gastric emptying and nutrient appearance in peripheral circulation in healthy females: a randomized controlled trial comparing pasteurized and ultra-high temperature milk

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A B S T R A C T

Background: Heat treatments of dairy, including pasteurization and ultra-high temperature (UHT) processing, alter milk macromolecular structures, and ultimately affect digestion. *In vitro*, animal, and human studies show faster nutrient release or circulating appearance after consuming UHT milk (UHT-M) compared with pasteurized milk (PAST-M), with a faster gastric emptying (GE) rate proposed as a possible mechanism.

Objectives: To investigate the impact of milk heat treatment on GE as a mechanism of faster nutrient appearance in blood. We hypothesized that GE and circulating nutrient delivery following consumption would be faster for UHT-M than PAST-M.

Methods: In this double-blind randomized controlled cross-over trial, healthy female ($n = 20$; 27.3 ± 1.4 y, mean ± SD) habitual dairy consumers, consumed 500 mL of either homogenized bovine UHT-M or PAST-M (1340 compared with 1320 kJ). Gastric content volume (GCV) emptying half-time (T_{50}) was assessed over 3 h by magnetic resonance imaging subjective digestive symptoms, plasma amino acid, lipid and B vitamin concentrations, and gastric myoelectrical activity were measured over 5 h.

Results: Although GCV T_{50} did not differ (102 ± 7 min compared with 89 ± 8 min, mean ± SEM, UHT-M and PAST-M, respectively; $P = 0.051$), GCV time to emptying 25% of the volume was 31% longer following UHT-M compared with PAST-M (42 ± 2 compared with 32 ± 4 min, $P = 0.004$). Although GCV remained larger for a longer duration following UHT-M (treatment × time interaction, $P = 0.002$), plasma essential amino acid AUC was greater following UHT-M than PAST-M (55,324 ± 3809 compared with 36,598 ± 5673 μmol·min·L^{-1}, $P = 0.006$). Heat treatment did not impact gastric myoelectrical activity, plasma appetite hormone markers or subjective appetite scores.

Abbreviations: BCAA, branched chain amino acid; BSGM, body surface gastric mapping; CAMRI, Centre for Advanced MRI; C_{max}, maximum concentration; CRU, clinical research unit; EAA, essential amino acid; GE, gastric emptying; GCV, gastric content volume; GLP-1, glucagon-like peptide 1; GTV, gastric total volume; MS, mass spectrometer; MBIE, Ministry of Business, Innovation, and Employment; NEAA, nonessential amino acid; PAST-M, pasteurized milk; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PYY, peptide YY; T_{50}, emptying half-time; T_{25}, time to emptying 25% of the volume; T_{75}, time to emptying 75% of the volume; TAA, total amino acid; TG, triacylglyceride; TMAO, trimethylamine N-oxide; UHPLC, ultra high performance liquid chromatography; UHT, ultra-high temperature; VAS, visual analog scale.

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Conclusions: Contrary to expectations, GE was slower with UHT-M, yet, as anticipated, aminoacidemia was greater. The larger GCV following UHT-M suggests that gastric volume may poorly predict circulating nutrient appearance from complex food matrices. Dairy heat treatment may be an effective tool to modify nutrient release by impacting digestion kinetics.
Clinical Trial Registry: www.anzctr.org.au (ACTRN12620000172909).

Keywords: milk, dairy, ultra-high temperature milk, pasteurized milk, food structure, digestion, gastric emptying, MRI, amino acids, body surface gastric mapping

Introduction

Milk is a nutritionally rich food source supporting growth, development, and chronic disease prevention [1]. Proteins, fats, and other nutrients interact within naturally occurring macromolecular structures, influencing the physiochemical properties of ruminant milks. These structures (e.g., casein micelles and milk fat globule membrane) influence milk digestion, altering nutrient breakdown, absorption, and utilization [2]. Milk structures also arise or change during processing, further influencing nutritional impacts [3].

Heat treatment is widely used for most consumed milk, ensuring microbiological safety and extending shelf life [4]. Standard milk heat treatments are pasteurization (72–80°C for 15–30 s) and ultra-high temperature (UHT; 135–150°C for 1–10 s) processing [4]; both can alter macromolecular structures [5–7].

Milk proteins are denatured upon heating, enhancing susceptibility to gastric protein hydrolysis. Individual protein modifications and the resulting impacts on gastric digestion and aminoacidemia have been summarized previously [8]. *In vitro* models of gastric and small intestinal digestion demonstrate that the combination of denatured whey proteins and interference in casein micelle aggregation with increasing degrees of heat treatment causes a more crumbled and fragmented curd, further promoting hydrolysis [5,7,9]. Although predicted physiological effects of milk heat treatment have been summarized, suggesting changes to digestion speed and potentially decreased bioavailability, these implications are largely based on nonhuman experimental models [8].

Only 5 human studies have addressed how milk heat treatment affects nutrient delivery [10–15]. Of 3 studies comparing UHT milk (UHT-M) and pasteurized milk (PAST-M), 2 showed more rapid nutrient appearance in blood following UHT-M [11,12], including greater circulating dietary N and anabolic use [11] and circulating lipid appearance [12]; the third study found no differences in aminoacidemia [15].

Gastrointestinal behavior, particularly gastric coagulation, is an important determinant of postprandial small intestinal protein bioavailability [16] and aminoacidemia [17]. Liquid phase proteins (e.g., whey) empty from the stomach more rapidly, whereas solid phase proteins in the curd empty more slowly, subsequently delaying small intestinal protein availability [16]. Dairy food structure alteration (e.g., cheese, butter, fermentation, and hydrolysis) impacts gastric emptying (GE) rates [18–21], influencing postprandial responses such as aminoacidemia [15,20,22] or lipemia [18,19,23]. Hence, although faster GE is a proposed mechanism for faster nutrient appearance with UHT-M [11], supported by both greater protein retention in humans and the known influences of dairy structure on nutrient delivery *in vitro*, this has not yet been assessed in humans.

The aim of this study was to compare the GE kinetics of homogenized UHT-M and PAST-M, and to assess corresponding appearance in peripheral circulation of nutrients responsive to acute ingestion, including amino acids, lipids, and B vitamins. We hypothesized that because of the different heat processing impacts on macromolecular milk structures, UHT-M consumption will result in more rapid GE, digestion, and circulating nutrient delivery compared with PAST-M.

Methods

Experimental design

This Temperature treatment of Milk impacts on MRI digestion rates and nutrient delivery (TuMMI) Trial was a double-blinded, cross-over randomized controlled trial to compare the impact of consumption of a single 500 mL serving of either homogenized PAST-M or UHT-M in female habitual dairy consumers. The prescribed washout between interventions was a minimum of 3 d and a maximum of 28 d to minimize any cross-over effects from study procedures.

The sequence of treatment arms was randomly generated by an independent statistician and subjects were allocated by an independent researcher through a password-protected database before the first milk intervention. The milk intervention was distributed by an independent researcher. Investigators and participants were blinded to the identity of treatments for the duration of the data analysis of the primary outcome. No sensory masking of milk was used.

The primary outcome of the study was the GE half-time (T_{50}) of the gastric content volume (GCV). Secondary MRI outcomes were changes in gastric total volume (GTV) and GCV over time including $AUC_{0–180}$, the GTV T_{50}, the GCV and GTV parameters for time to emptying 25% of the volume (T_{25}) and time to emptying 75% of the volume (T_{75}), GE rate at T_{50}, and Vmax. Other secondary outcomes were the assessment of amino acid appearance in blood, lipid and B vitamin responses, and subjective metabolic and digestive responses (patient-reported outcomes) to UHT-M and PAST-M ingestion. The secondary outcome of body surface gastric mapping (BSGM) assessed digestive responses and aimed to establish the feasibility in the context of meal variation and measurement alongside MRI. Further secondary outcomes not reported here are other metabolome responses to UHT-M and PAST-M ingestion, and other physiological assessments of digestive responses.

Ethics approval and trial registration

Ethics approval was granted by the Central Health and Disability Ethics Committee (New Zealand, 19/CEN/205). The clinical trial was prospectively registered at www.anzctr.org.au on February 17, 2020 (Trial ID: ACTRN12620000172909). The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and written informed consent was obtained from all subjects.

Setting

The study began recruitment February 25, 2020, and the intervention was conducted from March 4, 2020, to July 6, 2021, at which point all follow-up was complete. The study was temporarily halted from March 25, 2020 to June 8, 2020; August 12, 2020 to

October 7, 2020; and February 14, 2021 to March 12, 2021, because of restrictions put in place by the New Zealand Government in response to the COVID-19 global pandemic. All participant assessments were carried out at the University of Auckland's Centre for Advanced MRI (CAMRI) and the Maurice and Phyllis Paykel Clinical Research Unit (CRU), Liggins Institute.

Intervention

Both milks were homogenized, commercially available, and sourced from the same supplier (Fonterra Co-operative Group Limited). The UHT milk (preheated 95°C, 90 s, processed 140°C, 4 s, 160 bar; heating parameters matching others [6,7,24]) was received in 3 batches. Of 20 participants, 1 participant received the UHT from Batch 1, 14 participants received Batch 2, and 5 participants received Batch 3. PAST-M (75°C, 15 s, 160 bar; heating parameters matching others [7, 24]) was obtained ad hoc, with a best before date between 5 and 10 d from the assessment date. The nutritional composition of each milk (Table 1) was analyzed by standard methods (Supplemental Methods).

Both milks were chilled at 7°C for ≥12 h prior to consumption and were served chilled in plasticware. A serving size of 500 mL was chosen to align with previous investigations of GE dynamics [19,25, 26] and nutrient appearance in blood following milk ingestion [11,12].

Inclusion and exclusion criteria

Participants were self-reported healthy females, 18–40 y of age, with a BMI (kg/m^2) between 18 and 30 and self-described habitual dairy consumers as assessed by average weekly liquid milk consumption of 3 × 250 mL [27], consumed as a drink or as fluid milk in other forms. Habitual dairy consumption was based on dairy consumption patterns aligning with typical dairy consumption rates in New Zealand (~280 mL/d [27]) and globally (~125 mL/d [28]). The inclusion was limited to females to reduce variability between sexes in hormonal GE influences [29] or circulating amino acid concentrations [30]. BMI >25 was selected to account for demographic changes in average BMI within New Zealand [31] and to ensure that demographics with higher mean BMI (e.g., Māori and Pacific ethnicity [31], age, higher socioeconomic deprivation [31]) would not be excluded from the research.

Participants were ineligible if they had known bovine milk allergy or lactose intolerance or were classified as lactose intolerant using a screening tool to assess perceived abdominal cramps, rumbling, flatulence, diarrhea, and vomiting (score >70/500 mm) [32]. Known significant gastrointestinal, cardiovascular, or metabolic disorders, irritable bowel syndrome and/or gastroesophageal dysfunction based on the ROME IV criteria, or current medication expected to interfere with normal digestive or metabolic processes were also exclusion criteria. Participants were also ineligible if they were pregnant or lactating, had conditions or metal implants precluding MRI scans, or had a self-reported alcohol intake >28 units per week.

Study procedures

Following informed consent, participants responded to demographic and dairy consumption and tolerance questions. On the day prior to each clinical visit, participants were instructed to abstain from vigorous physical exercise and avoid dairy and fiber-rich food. Participants were provided with a standardized low-fat, low dietary fiber dinner of ad libitum 150 g lean protein (chicken, white fish, or egg depending on dietary requirements), 150 g basmati rice, and 150 g vegetables (pumpkin, green bean, and carrot) seasoned with salt,

TABLE 1
Proximate and amino acid composition of 500 mL of the UHT-M and PAST-M

Component	PAST-M[1]	UHT-M[1]
Total energy (kJ)	1320	1340
Fat (g)	17	17
Protein (g)	16.5	17.5
Lactose[2] (g)	24	24
Sodium (mg)	200	200
Calcium (mg)	585	610
Amino acid (g)		
Total	16.74 ± 0.56	17.87 ± 0.69
Essential amino acids		
Leucine	1.54 ± 0.05	1.66 ± 0.05
Lysine	1.33 ± 0.03	1.38 ± 0.08
Valine	1.03 ± 0.03	1.10 ± 0.06
Isoleucine	0.83 ± 0.02	0.88 ± 0.06
Phenylalanine	0.78 ± 0.01	0.84 ± 0.03
Threonine	0.71 ± 0.02	0.77 ± 0.03
Histidine	0.44 ± 0.01	0.46 ± 0.03
Methionine	0.34 ± 0.08	0.41 ± 0.01
Tryptophan	0.22 ± 0.02	0.25 ± 0.00
Nonessential amino acids		
Glutamic acid[3]	3.50 ± 0.10	3.77 ± 0.09
Proline	1.56 ± 0.04	1.68 ± 0.05
Aspartic acid[3]	1.24 ± 0.03	1.33 ± 0.05
Serine	0.89 ± 0.02	0.95 ± 0.02
Tyrosine	0.79 ± 0.03	0.80 ± 0.07
Alanine	0.55 ± 0.03	0.58 ± 0.02
Arginine	0.57 ± 0.01	0.57 ± 0.04
Glycine	0.30 ± 0.01	0.33 ± 0.01
Cysteine	0.11 ± 0.03	0.13 ± 0.01
Fatty acid[4]		
Pooled fatty acid (g)		
Saturated fat	12.5	11.5
Monounsaturated fat	3.5	4.0
Polyunsaturated fat	0.5	1.0
Trans fat content	n.d.	0.5
Individual fatty acid (mg)		
Butyric, C4:0	470	500
Caproic, C6:0	305	340
Caprylic, C8:0	170	190
Capric, C10:0	405	430
Lauric, C12:0	1000	650
Myristic, C14:0	2100	1900
Myristoleic, C14:1	260	215
Pentadecanoic, C15:0	195	200
Palmitic, C16:0	5600	4900
Palmitoleic, C16:1	290	220
Margaric, C17:0	85	100
Stearic, C18:0	1250	1700
Oleic, C18:1n-9	2600	2850
Octadecenoic, C18:1n-7	55	55
Linoleic, C18:2n-6	145	150
Conjugated linoleic, C18:2 9c, 11t	95	175
Alpha linolenic, C18:3n-3	55	100
B vitamin[5] (μg)		
Thiamine	n.d.	115
Riboflavin	1425	1280
Total vitamin B_3 (niacin + nicotinamide)	n.d.	n.d.
Total vitamin B_6	70	55
Folic acid	0.600	n.d.
Vitamin B_{12}	1.95	0.35
Biotin	19.05	18.25

Abbreviations: n.d., not detected; PAST-M: pasteurized milk; UHT-M: ultra-high temperature milk.

[1] Total energy, fat, protein, lactose, sodium, and calcium based on the Nutrient Information Panel. Amino acid quantity reported for 2 pasteurized milk samples from 2 points during trial (best before dates December 11, 2020, and July 16, 2021), and 3 UHT batches (best before dates September 8, 2020,

January 13, 2021, and September 16, 2021) (mean ± SD). Fatty acid and B vitamin analysis reported for 1 sample of PAST-M and UHT-M, respectively.

[2] Carbohydrate content is equal to lactose content.

[3] Results for aspartic acid and glutamic acid may include contributions of asparagine and glutamine, respectively, converted during hydrolysis.

[4] Fatty acids detected below the detection limit not reported. Trans fat detection limit 0.1 g/100 g.

[5] B vitamin detection limits: thiamine 20 μg/100 g; total vitamin B_3 200 μg/100 g; folic acid 0.100 μg/100 g.

pepper, and lemon juice (Muscle Chow NZ Limited). After completing dinner, they were to remain fasted from 10.00 pm.

Study participants arrived at CAMRI fasted. On arrival, digestive symptoms were recorded using a visual analog scale (VAS). Blood samples were drawn by inserting a venous cannula into the forearm. Participants were then provided with the intervention milk prior to their first MRI scan. Following ingestion of milk within 5 min, assessments were carried out at regular intervals over 3 h (MRI at CAMRI) and for ≤5 h (bloods, VAS, physiological assessments at CRU from 3 h), as detailed below. During the washout period between assessments, no dietary or lifestyle restrictions were required.

A 3 h duration was chosen to capture the T_{50}, expected to be similar to mixed macronutrient liquid meals (~84 ± 35 min [33]) and within range of other liquid and solid meals (10 min [25] ≤115 min [34] for solid meals). Previous MRI-based studies have used durations of 120 [33] to 270 min [34] to sufficiently capture GE T_{50}. For nutrient appearance in peripheral blood circulation following a meal, a 5 h duration was chosen to capture peak exogenous amino acid and lipid responses [11,12] while limiting the assessment to a single meal period.

Analysis methodology

MRI protocol

Participants underwent MRI scans following the consumption of 500 mL of milk on a research-dedicated Siemens 3.0T MRI scanner (Magnetom Skyra; Siemens Medical Solutions) located at CAMRI. Participants were positioned supine in the MRI scanner with a 16-channel abdominal receiver coil wrapped around the abdomen. The participants were imaged using a rapid-acquisition HASTE (Half Fourier Acquisition Single Shot Turbo Spin Echo) coronal sequence (slices = 20, echo time = 87 ms, repetition time = 1500 ms, flip angle = 90° for excitation and 150° for refocusing pulse, field of view = 440 mm, thickness = 5.0 mm, gap 0.5), covering the whole abdominal region of interest. The participants were asked to hold their breath during 2 breath-holds for this sequence to minimize respiratory motion and the image acquisition time lasted for 46 s at each timepoint. MRI scans captured images of gastric contents at multiple timepoints ≤3 h (i.e., at 5, 10, 20, 30, 60, 90, 120, and 180 min) following milk ingestion. Participants stayed supine in the scanner between the 5–20 min scans. Participants were supine for 5 min during the other timed scans and were upright when not in the scanner.

MRI image analysis

MRI image analysis was completed at the Liggins Institute in collaboration with the University of Nottingham. In this study, the T2-weighted HASTE sequence provided a good contrast between the milk loads and the surrounding organs and gas. The gastric content (i.e., milk load) exhibited high signal intensity, whereas intragastric air appeared black, facilitating the manual segmentation of regions of interest.

Briefly, the volume of the gastric contents was measured from the images obtained from the MRI scans at each timepoint by manually tracing a region of interest around the stomach wall (GTV, reflecting total stomach volume as in meal plus gas) and stomach contents (GCV, reflecting just the meal in the stomach without any gas) of each slice by using a polygon selection tool in the Medical Image Processing, Analysis, and Visualization software (MIPAV version 10.0, National Institutes of Health) [35].

The GCV were computed by summing across the slices at each timepoint. The surrounding organs and gastric gas (if present) were excluded from the region of interest. Any difficulties encountered during the anatomy tracing were flagged and discussed for interobserver consistency. The gastric contents for each timepoint were then tabulated and exported into spreadsheets in Microsoft Excel (Microsoft® Corporation). This procedure was repeated to calculate the measurement of gastric gas. The GTV was calculated by summing the gastric content and gastric gas.

Quality control analysis was performed independently by 2 researchers (SN and HS) on a subset of scans. This subset comprised 9 scans representing 3 matching timepoints (5, 60, and 180 min) across 3 participants which were selected independently by MPGB. The intraclass correlation coefficient was calculated to assess observer variability across 3 independent observers (SN, HS, LM), yielding a value of 0.92 for the image analysis of GCV.

Furthermore, alongside gastric volume measurements, the MRI images were also analyzed to quantify the relative volumes of liquid and coagulum in stomach contents following the method of Otsu [36], which has been recently applied to quantify liquid and coagulum from MRI images of milk digestion *in vitro* and *in vivo* [37,38].

Briefly, the volumes of interest manually delineated around the intragastric meal content using the software MIPAV (version 11.0.7) and converted to binary masks. Subsequently, the corresponding images and binary masks were imported into MATLAB® (version R2018a The MathWorks Inc.) to quantify the number of lighter (liquid) and darker (coagulum) voxels. Otsu's method [36] was used to divide the segmented image (image × mask) into 3 separate regions using 2 thresholds from the multithresh MATLAB function. The upper threshold was then used to split the image into liquid (above the higher threshold) and coagulum (below this higher threshold) and the number of pixels for each region calculated.

Participant and milk type were blinded for randomization to researchers during the MRI analysis.

Biochemical analyses

Venous blood samples were collected at fasting (baseline) and at regular intervals after milk consumption (i.e., 20, 30, 40, 60, 90 120, 180, 240, and 300 min) into ethylenediaminetetraacetic acid containing and P800 blood collection tubes (Becton Dickinson & Company), and plasma was immediately removed after centrifugation at 1200 × *g* for 10 min at 4°C and frozen at −20°C or −80°C prior to analysis.

Glucose, insulin, triglycerides. Plasma glucose and insulin concentrations were measured at fasting and at all collected timepoints, and triglyceride concentrations at fasting and hourly to 5 h following both milks. Plasma HDL-C, LDL-C, and total cholesterol concentrations were measured at fasting. Glucose, HDL-C, LDL-C, total cholesterol, and triglycerides concentrations were measured using a Roche Cobas c311 Autoanalyzer (Roche Diagnostics) by enzymatic colorimetric assay and insulin was measured using a Cobas E601 Autoanalyzer (Roche Diagnostics).

Amino acids. Plasma-free amino acid concentrations at fasting and at all collected timepoints following both milk ingestions were measured using ultra high-performance liquid chromatography (UHPLC) to assess 23 amino acids as described previously [30] with the following variation. A variable wavelength detector (Dionex, set at 280 nm) was added to the UHPLC system to quantify tryptophan concentration (tryptophan auto fluoresces and hence cannot be measured using fluorescence detection).

Lipidomics. Plasma lipid species were analyzed for samples collected at fasting, and 120, 180, and 240 min following both milk ingestions. Samples were extracted with chloroform:methanol, dried under N_2 (Supplemental Methods) and stored at −80°C until analysis. Prior to analysis, samples were brought to room temperature (18 ± 2°C) and reconstituted in 200 µL of butanol:methanol with 10 mM ammonium formate spiked with 5 µL of SPLASHmix deuterated standard (Avanti Lipids). Sample extracts were analyzed using a Shimadzu LCMS-9030 mass spectrometer (MS) coupled to a Shimadzu Nexera-x2 UHPLC (UHPLC-MS) system as previously described [39,40]. Raw, high-resolution LC-MS data files were processed using MS-DIAL v4.80 [41] as described in [39]. The resultant data matrix was used for downstream statistical analyses.

B vitamins. Plasma B vitamins and vitamers were analyzed at fasted baseline and hourly to 5 h after milk ingestion by UHPLC-MS/MS, as described previously [42]. Briefly, the vitamins and vitamers were measured concurrently on a panel including thiamine (B1), riboflavin (B2), nicotinamide and nicotinic acid (B3), pantothenic acid (B5), pyridoxamine, pyridoxine and 4-pyridoxic acid (B6), folic acid (B9), and trimethylamine N-oxide (TMAO; not a vitamin). Nicotinic acid, pyridoxamine, pyridoxine, and folic acid were not detected.

Appetite hormones. Plasma appetite hormones [leptin, ghrelin, glucagon-like peptide 1 (GLP-1), peptide YY (PYY)] were analyzed simultaneously using a flow cytometric multiplex array (Milliplex®MAP Kit; Human Metabolic Hormone Magnetic Bead Panel Assay; HMHMAG-34K; Merck) on a MAGPIX® Luminex and fluorescent intensity data acquisition and analysis was done using xPONENT® and Milliplex® Analyst 5.1 software (Merck), respectively, according to the manufacturer's instructions.

Questionnaires

Study data were collected and managed using REDCap (versions 9.4 through 11.2.2) electronic data capture tools hosted by the University of Auckland [43,44].

Demographic questions. Ethnicity was collected (self-report) using the categories from Statistics New Zealand Tatauranga Aotearoa Census. Participants could select >1 category from the following: New Zealand European, Māori, Samoan, Cook Islands Māori, Tongan, Niuean, Chinese, Indian, Other (with specification).

Milk consumption patterns and beliefs around dairy tolerance were collected following enrolment. Frequency of drinking milk as a standalone beverage, and participant perception of dairy or lactose intolerance were also assessed. Perceived lactose intolerance was scored as the sum of 100 mm VAS scores across abdominal cramps, rumbling, flatulence, diarrhea, and vomiting using a validated tool to screen for lactose intolerance [32].

Appetite, liking, and symptom questionnaires. A 100 mm VAS was used to assess appetite [45], liking and digestive symptom scores. The questionnaires consist of a series of VAS (100 mm), using intensity anchors (e.g., "no symptom," "the most severe symptom imaginable"). Appetite and digestive symptoms were recorded before, during, and after milk consumption, aligned with blood sampling intervals at 30 min intervals for the first 90 min, then hourly starting at 2 h for 5 h. Hedonic liking of the milks was recorded during consumption, as was perceived identity.

Body surface gastric mapping.

Gastric myoelectrical activity was measured by BSGM using a noninvasive cutaneous electrode array positioned on the abdomen as described previously [46] with modifications for concurrent assessment with MRI. The electrode array was tested for MRI safety prior to use, confirming a temperature increase <1°C over 30 min of scanning. The protocol for concurrent MRI use involved disconnection and removal of the data logger and connector clamp of the array prior to entry into the scanner. Additionally, a fabric barrier was secured between the skin and array at the data logger connection location prior to scanning.

Following abdominal skin preparation using NuPrep (NuPrep; Weaver), a 64-channel electrode array (8 × 8 electrodes; 20 mm interelectrode spacing; 196 cm^2) was placed on the anterior abdominal skin and connected to a portable data logger (Alimetry Ltd.). Passive recordings captured myoelectrical characteristics including Principal Gastric Frequency, amplitude (unadjusted for BMI), and Gastric Alimetry Rhythm Index (unadjusted for BMI). Continuous recordings were captured following the milk consumption in a semiseated position until 5 h. The data logger was disconnected during MRI scans and recordings recommenced following scan completion. Participants were allowed to perform sedentary activities during the study, although encouraged to remain in a semiseated position when possible. After 3 h, participants relocated from CAMRI to the CRU, requiring them to change clothes and walk 50 m.

Excessive artifacts, periods of missing recordings, and sufficient data quality were assessed by 2 reviewers independently (PD, AMM); acceptance required consensus.

Statistical analysis

Statistical analyses were performed with SPSS version 27 (IBM Corporation), aside from lipidomic and BSGM analyses which were performed in R (R Development Core Team version 4.3.0) [47]. Data analysis was performed per protocol. Per protocol analysis was selected *a priori* (in lieu of intention-to-treat) given that physiological responses (the primary and key secondary outcomes) would not have been captured in the event of deviations to the protocol. Continuous data are presented as mean ± SEM unless otherwise stated. Categorical data are presented as number and percentage. Figures were generated using GraphPad Prism 9 (GraphPad Software LLC) and ggplot2 package (version 3.4.2) in R.

Values lower than the limit of quantification (appetite hormone data) were imputed at 50% of the limit of detection, where >50% of samples were detected for a participant. No other datasets were imputed. Three participants' data were excluded from derived MRI variable analysis because of the inability to fit the model ($R^2 > 0.90$) as follows: 2 participants' data resulted in a V_0 model prediction much higher than the total volume consumed (i.e., ~800–900 mL). Another failed to reach T_{50} by 3 h so derived time (T_{25}, T_{50}, and T_{75}) and GE rate at T_{50} could not be calculated. Blood samples for amino acid analysis were missing at 1 timepoint for 2 participants, so linear modeling only included $n = 18$ for

treatment × time analysis. Thiamine was not detected in 27% (65/240) of the samples, resulting in $n = 11$ for analysis. Ghrelin was below the limit of quantification in 6% (18/317) of the samples and extrapolated by Multiplex software in another 51% (162/317); 2.7% (8/301) included values were imputed, resulting in $n = 16$ for analysis. PYY was below the limit of quantification in 38% (120/317) of the samples, with 2.6% (5/189) included values imputed, resulting in $n = 9$ for analysis. BSGM data were of sufficient quality for $n = 8$ participants following PAST-M and $n = 10$ participants following UHT-M.

Gastric volume data were fitted to a previously described 5-parameter model of GE (Equation 1) [48]. Derived GE variables were calculated using a nonlinear least-square fit for of each volume–time curve (using MATLAB®, The MathWorks Inc.), and included T_{50}, T_{25}, and T_{75} in min, GE rate T_{50} in mL/min, and incremental AUC 0–180 min in mL·min. Only those data that fitted the nonlinear least-square fit model with $R^2 > 0.90$ were included for analysis, excluding 3 participants' data.

Equation 1: GE model. V: volume in mL, t: time in min, V_0: initial volume at defined time zero, f: parameter to quantify fraction of exponential decay; κ: parameter to quantify potential increase in volume from V_0; t_{empt}: time constant that quantifies the exponential decay component; G: parameter to quantify the linear effects of the late GE phase.

$$V(t) = V_0 f\left(1 + \frac{\kappa t}{t_{empt}}\right)\exp\left(\frac{-t}{t_{empt}}\right) + (1-f)(1-Gt)$$

Derived amino acid variables were calculated as the sum of plasma concentration of branched chain amino acids (BCAA), essential amino acids (EAA), non-EAA (NEAA), and total amino acids (TAA). The AUC 0–300 min was calculated using the trapezoidal method. The lipidome AUC was calculated using the R package, MESS [49] based on Kim et al. [50].

Frequency distributions were analyzed using Pearson chi-square test. Continuous variables were analyzed using Student's paired t-test or a repeated factor general linear model with fixed factors treatment and time using a Huynh–Feldt Type III sum of squares where Mauchly's sphericity failed. Sidak-adjusted post hoc tests were used for multiple comparisons. Lipidome data were analyzed (based on Kim et al. [50]) using the nparLD *f1.ld.f1* function [51] (Wald Chi-Squared test) to test for time, treatment, and treatment × time interaction effects; AUC was analyzed using the Wilcoxon signed-rank test. All lipid P values were corrected using the Benjamini–Hochberg method [52]. BSGM data were analyzed using a mixed-effects linear regression model including time, treatment, and a random effect for participant using the lme4 R package [53]. The significance level was set at $P < 0.05$.

A sample size of 20 was determined based on an 80% power (β) to detect at the 5% significance level (α) an effect size of 35% difference in GE (i.e., delayed to ~115 compared with 84 min) T_{50} between treatments. The T_{50} in healthy subjects consuming a 400 mL mixed macronutrient liquid meal is 84 ± 35 min. There are limited data upon which to estimate the treatment effect size. An effect size of 35% represents the delay observed for a 150 mL increased meal volume, or half the difference between normal digestion and functional dyspepsia [26]. Smaller effect sizes on gastric volume over time (i.e., 12%) have been observed between food structure comparisons differing in fat droplet stability [54].

Results

Subject characteristics

Of the 272 participants that were screened for eligibility, 25 were eligible for the intervention (Figure 1); of those excluded, the majority ($n = 206$) were because of the lost contact as a result of COVID-19 disruptions. Twenty subjects completed the study, 3 subjects withdrew prior to receiving any intervention, 1 subject withdrew before completing data collection during the first intervention (UHT-M), and 1 subject withdrew prior to receiving the second intervention (UHT-M).

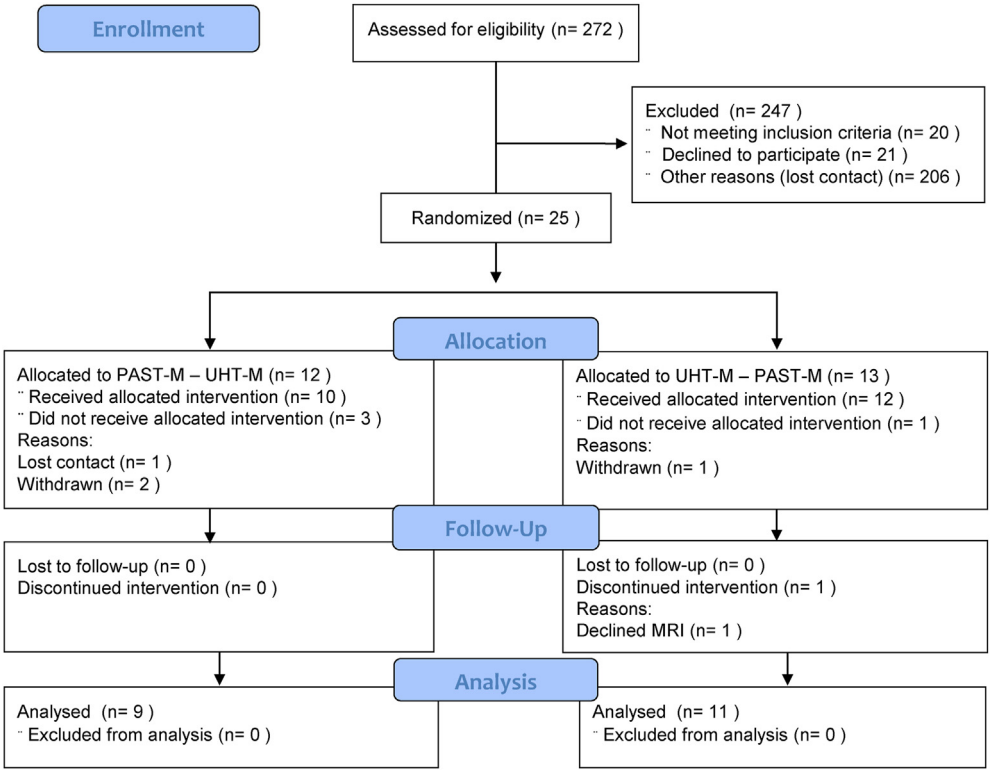

FIGURE 1. Consolidated Standards of Reporting Trial participant flow at study completion. PAST-M: pasteurized milk; UHT-M: ultra-high temperature milk.

Participants were healthy based on their clinical characteristics (Table 2). Four participants were overweight; the mean BMI of overweight participants was 26.6 ± 0.2. Participants were identified as Caucasian (57%), Asian (22%), Māori (9%), Samoan (4%), and other (9%). Although the study design was cross-over, BSGM resulted in ≤60% data loss, and limited paired comparisons ($n = 5$); the mean BMI of subjects included in BSGM analysis was 22.8 ± 1.0 and 23.4 ± 1.0 ($n = 8$ and $n = 10$, PAST-M and UHT-M, respectively).

Although participants were recruited based on self-reported habitual liquid milk consumption of ≥3 serves (250 mL each) weekly, 19 of 20 participants responded to further dairy consumption and tolerance questions. Responses for milk as a standalone beverage (as opposed to milk consumed in other forms) indicated that most subjects ($n = 13/19$) reported drinking an average of 1 glass (250 mL) of milk per day, with 8 subjects reporting >1 glass of milk per day. One subject reported drinking an average of "none."

Few participants believed they were dairy or lactose intolerant (0%, $n = 0/19$ and 16%, $n = 3/19$, respectively). Perceived lactose intolerance was below the threshold (i.e., 70 mm) for classification of lactose intolerance (40 ± 17 mm).

Of the 13 participants that reported a regular menstrual cycle, the majority were in the follicular phase (days 0–14) when consuming PAST-M ($n = 7$) and UHT-M ($n = 8$); however, only 3 participants were in the follicular phase on both occasions.

Compliance and adverse events

All subjects completed the full 500 mL of milk following each intervention. Protocol deviations were noted for the washout period between interventions because of the temporary halts to the study. The washout duration was longer than the protocol prescribed minimum (i.e., 3 d) for all subjects because of the limited availability of MRI, and longer than the prescribed maximum (i.e., 28 d) for $n = 7$ (35%) of participants, evenly distributed between intervention sequences. The mean washout period was 33 ± 8 d for all subjects, with 41 ± 14 d (PAST-M: UHT-M), and 27 ± 8 d (UHT-M: PAST-M) for each sequence, respectively. The minimum washout duration was 7 d ($n = 7$), and the maximum duration was 119 d.

One adverse event of mild and self-resolving constipation following the intervention (PAST-M) was reported.

TABLE 2
Baseline participant characteristics[1]

Measure	Value		
Age (y)	27.3	±	1.4
Ethnicity, *n* (%)			
Caucasian	13		(57)
Asian	5		(22)
Chinese	1		(4)
Indian	2		(9)
Other Asian	2		(9)
Māori	2		(9)
Samoan	1		(4)
Other	2		(9)
BMI	22.0	±	0.6
Overweight BMI[2]	26.6	±	0.2
Waist circumference (cm)	73.2	±	1.9
Glucose (mmol/L)	4.7	±	0.2
Insulin (μU/mL)	7.8	±	0.9
HDL-C (mmol/L)	1.51	±	0.10
LDL-C (mmol/L)	2.56	±	0.17
Total cholesterol (mmol/L)	4.24	±	0.21
Triglycerides (mmol/L)	0.94	±	0.06
Blood pressure (mm Hg)			
Systolic	115	±	4
Diastolic	72	±	3

[1] Values presented as mean ± SEM or count (percentage) as indicated across both assessments. Age and BMI taken on first assessment only. $N = 20$ for all measures except blood pressure ($n = 17$) and ethnicity: participants could identify with >1 ethnicity group ($n = 20$ participants; $n = 23$ ethnicity reports).

[2] Mean ± SEM BMI of $n = 4$ participants who were overweight (>25 kg/m^2).

Participant perception of milks

Subjects identified the PAST-M as pasteurized more than expected (H_0: equal frequency; χ^2 $P = 0.025$; $n = 15$ correct), but did not identify UHT-M as either pasteurized or UHT milk more than expected (χ^2 $P = 0.491$; $n = 11$ correct)—hence subjects were more likely to correctly identify the PAST-M but unable to identify the UHT-M.

Gastric contents, volumes and emptying

Gastric content visualization

Milk was easily visualized in the stomach without using any contrast agent, providing higher signal (i.e., brighter appearance) than the surrounding tissue (Figure 2A). At 5 min after ingestion, in the T2-weighted MRI images the UHT-M meal appeared homogeneous across the stomach contents, whereas PAST-M already started to show some heterogeneity consistent with an initial aggregation and curdling process. At 90 min, the stomach contents appeared more heterogeneous for both milks, with aggregates appearing darker and larger for UHT-M (Supplemental Figure 1).

Gastric volume changes over time

The GCV was greater over time for UHT-M relative to PAST-M (treatment × time interaction $P = 0.002$; Figure 2B). This difference was apparent starting from 20 min until 120 min ($P < 0.05$ each, respectively), but was no longer apparent by 180 min ($P > 0.05$). GTV was similarly greater over time for UHT-M relative to PAST-M, whereas the gastric gas volume was not different between milks ($P = 0.030$ and $P = 0.696$ each, respectively; Supplemental Figure 2).

Modeled GE rate

The primary outcome of GCV T_{50} tended to be longer for UHT-M than PAST-M (15% longer; 102 ± 7 min compared with 89 ± 8 min, UHT-M and PAST-M, respectively; $P = 0.051$; Figure 2C); this trend was significant for the GTV T_{50} ($P = 0.009$; Table 3). The GE rate at T_{50} was not different for either GCV or GTV ($P = 0.892$ and $P = 0.053$, respectively).

However, consistent with the greater GCV with UHT-M (Supplemental Figure 1), the T_{25} (GCV and GTV; $P = 0.004$ and $P = 0.006$, respectively) and T_{75} (GTV only, $P = 0.013$) were shorter following PAST-M ingestion, with a 10% lower AUC.

Gastric curd analysis

Threshold analysis (Supplemental Figure 3) showed no difference over time in the number or percentage of voxels of coagulum (darker intensity; treatment × time interaction $P = 0.174$, $P = 0.310$, respectively) or liquid (lighter intensity; treatment × time interaction $P = 0.600$, $P = 0.310$, respectively) between UHT-M and PAST-M. Irrespective of time, there was more coagulum (darker) following UHT-M than PAST-M (18,260 ± 671 compared with 16,288 ± 619 voxels; treatment effect $P = 0.015$), but no difference between milks in liquid (lighter) (10,559 ± 469 compared with 9979 ± 7128 voxels; $P = 0.092$).

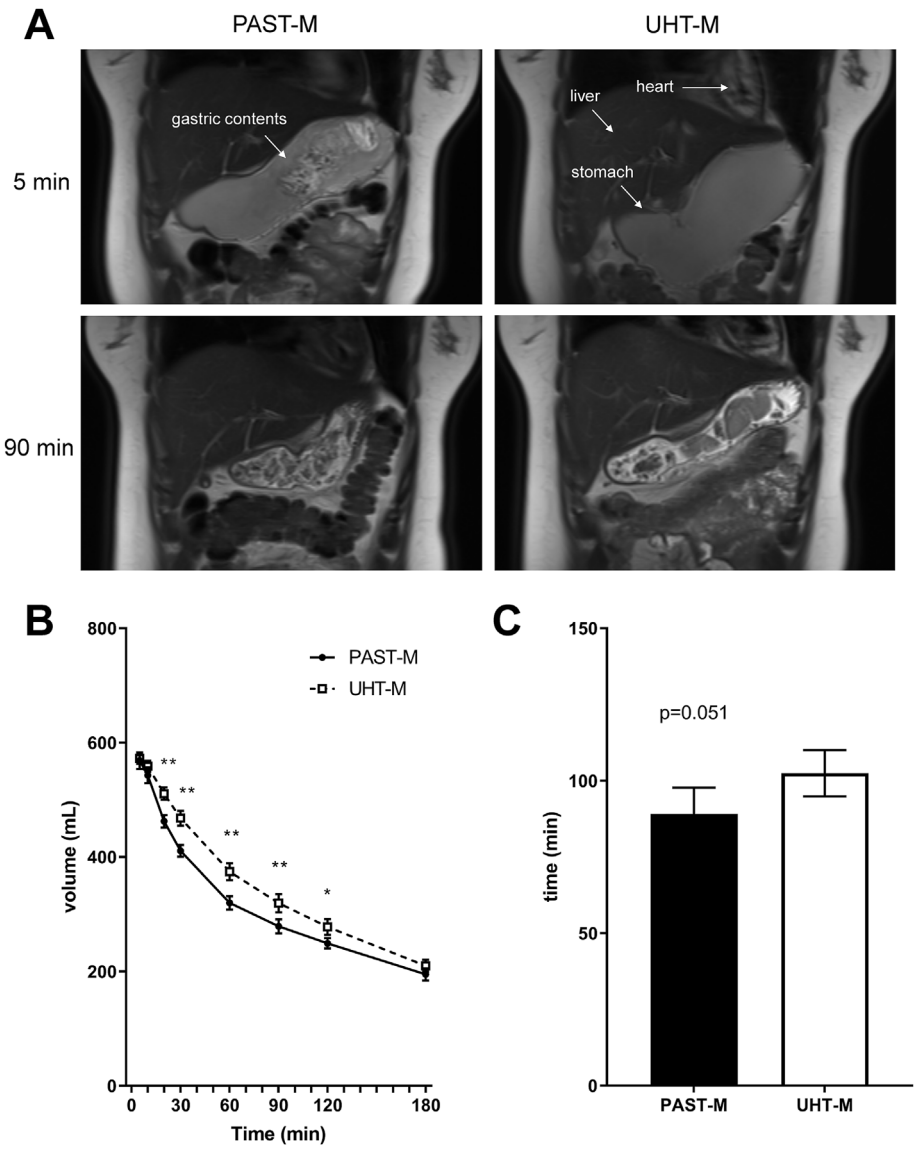

FIGURE 2. (A) Representative T2-weighted coronal image of gastric contents at 5 and 90 min, (B) gastric content volume (GCV) over 3 h, and (C) the emptying half-time of GCV (T_{50}) for PAST-M and UHT-M. Data are presented as mean ± SEM for PAST-M (black) and UHT-M (white); ($n = 19$) for GCV; ($n = 17$) for GCV T_{50}. There was an interaction (treatment × time) for gastric volume ($P = 0.002$). *$P < 0.05$, **$P < 0.01$ between milks at specified timepoint, respectively. Interactions were analyzed by a general linear model with Sidak corrected post hoc adjustment. PAST-M, pasteurized milk; UHT-M, ultra-high temperature milk.

Plasma amino acids

The UHT-M resulted in a greater TAA response than PAST-M (AUC, 114,146 ± 8065 compared with 83,011 ± 13,533 μmol·min·L^{-1} $P = 0.033$; Table 4), driven by EAA, BCAA, and NEAA (treatment × time interaction $P < 0.001$, $P < 0.001$, and $P = 0.011$, respectively; Figure 3A–D), which were all higher following UHT-M consumption. For UHT-M, TAA, and NEAA concentrations continued to increase from 20 to 60 min ($P < 0.001$), whereas for PAST-M they did not (Figure 3A and D). The maximum concentration (C_{max}) of BCAA response was higher following UHT-M than PAST-M (597 ± 17 compared with 557 ± 15 μmol/L $P = 0.022$), whereas the C_{max} of EAA, TAA, and NEAA response did not differ (1314 ± 31 compared with 1248 ± 39 μmol/L $P = 0.069$, 3280 ± 77 compared with 3225 ± 100 μmol/L $P = 0.550$ and 1833 ± 55 compared with 1841 ± 62 μmol/L $P = 0.803$, UHT-M compared with PAST-M, respectively).

All the individual amino acids which responded differently between milks were found in higher postprandial concentrations between 40 and 180 min with UHT-M relative to PAST-M. This included all BCAA, arginine, lysine, phenylalanine ($P < 0.001$ each; Figure 3E) and tyrosine ($P < 0.001$), of which only the BCAA and tyrosine had a greater AUC with UHT-M ($P < 0.05$ each; Table 4). Although glutamine, serine, and ornithine responses also differed between milks over time ($P = 0.020$, $P = 0.003$, and $P = 0.017$, respectively), concentrations were not different at any specific timepoint nor were there differences in AUC (Table 4).

Clinical biochemistry

The total triglyceride, glucose, and insulin responses following milk ingestion did not differ between milks (treatment × time interaction $P = 0.150$, $P = 0.542$, and $P = 0.495$, respectively; Supplemental Figure 2).

TABLE 3
Gastric content and total volume modeled parameters following UHT-M and PAST-M

Parameter[1]	PAST-M				UHT-M				*P* value[2]
	Mean		SEM	95% CI	Mean		SEM	95% CI	
GCV									
AUC (mL·min)	56,272	±	1910	(52,421, 60,124)	61,603	±	2262	(57,042, 66,165)	0.006
T_{25} (min)	32	±	4	(26, 41)	42	±	3	(37, 53)	0.004
T_{50} (min)	89	±	8	(75, 120)	102	±	7	(90, 125)	0.051
T_{75} (min)	221	±	5	(212, 233)	229	±	4	(223, 237)	0.133
Gastric emptying rate at T_{50} (mL/min)	1.6	±	0.2	(1.2, 2.0)	1.6	±	0.1	(1.4, 1.8)	0.892
GTV									
AUC (mL·min)	61,922	±	2048	(57,791, 66,052)	69,577	±	3050	(63,425, 75,729)	0.003
T_{25} (min)	26	±	2	(22, 32)	39	±	3	(33, 53)	0.006
T_{50} (min)	78	±	8	(65, 109)	105	±	8	(92, 126)	0.009
T_{75} (min)	215	±	7	(202, 231)	236	±	2	(232, 240)	0.013
Gastric emptying rate at T_{50} (mL/min)	2.3	±	0.3	(1.6, 2.8)	1.6	±	0.1	(1.4, 2.0)	0.053

Abbreviations: AUC 0–180 min; CI, confidence interval; GCV, gastric content volume; GTV, gastric total (content + gas) volume; PAST-M: pasteurized milk; T_{25}, time to empty 25% of stomach contents; T_{50}, emptying half-time of stomach contents; T_{75}, time to empty 75% of stomach contents; UHT-M: ultra-high temperature milk.
[1] Values presented as means ± SEM (95% CI); $n = 18$ for AUC, $n = 17$ for all other parameters.
[2] Significance analyzed by Student's *t*-test.

TABLE 4
Plasma amino acid AUC following UHT-M and PAST-M

Amino acid[1]	PAST-M				UHT-M				*P* value[2]
	Mean		SEM	95% CI	Mean		SEM	95% CI	
Alanine	7148	±	2406	(2433, 11,863)	8973	±	1537	(5960, 11,986)	0.379
Arginine	1573	±	555	(485, 2661)	2510	±	552	(1428, 3591)	0.220
Asparagine	2156	±	377	(1417, 2895)	2400	±	149	(2108, 2691)	0.494
Aspartic acid	–68	±	44	(–154, 19)	5	±	49	(–91, 101)	0.320
Citrulline	–1262	±	167	(–1589, –934)	–1240	±	191	(–1614, –867)	0.918
Glutamic acid	7	±	580	(–1130, 1143)	1064	±	473	(137, 1991)	0.131
Glutamine	16,227	±	2070	(12,169, 20,285)	19,421	±	1676	(16,136, 22,705)	0.191
Glycine	–1603	±	822	(–3213, 7)	–3070	±	813	(–4663, –1476)	0.101
Histidine	1335	±	376	(599, 2071)	1985	±	343	(1313, 2656)	0.188
Hydroxyproline	–104	±	55	(–213, 5)	–212	±	50	(–310, –115)	0.059
Isoleucine	4654	±	716	(3251, 6057)	6855	±	553	(5771, 7939)	0.013
Leucine	8406	±	1077	(6296, 10,517)	12,109	±	965	(10,217, 14,000)	0.007
Lysine	11,481	±	1384	(8768, 14,194)	13,505	±	1368	(10,824, 16,187)	0.203
Methionine	898	±	198	(511, 1286)	1303	±	159	(992, 1615)	0.043
Ornithine	2049	±	347	(1369, 2729)	2943	±	405	(2149, 3737)	0.059
Phenylalanine	1158	±	341	(489, 1826)	2106	±	223	(1669, 2542)	0.023
Proline	15,361	±	1524	(12,375, 18,348)	20,127	±	971	(18,224, 22,030)	0.002
Serine	3489	±	660	(2196, 4783)	3278	±	354	(2585, 3971)	0.756
Taurine	–2632	±	757	(–4116, –1148)	–1194	±	806	(–2773, 385)	0.184
Threonine	3550	±	822	(1939, 5162)	4047	±	488	(3090, 5004)	0.498
Tryptophan	–154	±	446	(–1027, 720)	509	±	311	(–100, 1118)	0.254
Tyrosine	1957	±	527	(924, 2991)	3582	±	329	(2938, 4227)	0.003
Valine	7383	±	1270	(4895, 9872)	13,141	±	1020	(11,143, 15,140)	0.001
Pooled[3]									
TAA	83,011	±	13,533	(56,487, 109,534)	114,146	±	8065	(98,338, 129,953)	0.033
BCAA	20,443	±	2978	(14,606, 26,281)	32,105	±	2442	(27,318, 36,892)	0.003
EAA	40,285	±	6165	(28,202, 52,368)	58,069	±	4135	(49,966, 66,173)	0.016
NEAA	44,675	±	7277	(30,413, 58,937)	55,780	±	3915	(48,105, 63,454)	0.107

Abbreviations: CI, confidence interval; PAST-M: pasteurized milk; UHT-M: ultra-high temperature milk.
[1] Values presented as means ± SEM (95 % CI) in μmol·min·L^{-1}; $n = 20$.
[2] Significance analyzed by Student's *t*-test.
[3] TAA, total amino acids: all measured amino acids; BCAA, branched chain amino acids: isoleucine, leucine, valine; EAA, essential amino acids: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine; NEAA, nonessential amino acids: alanine, arginine, asparagine, aspartic acid, citrulline, glutamic acid, glutamine, glycine, proline, serine, tyrosine.

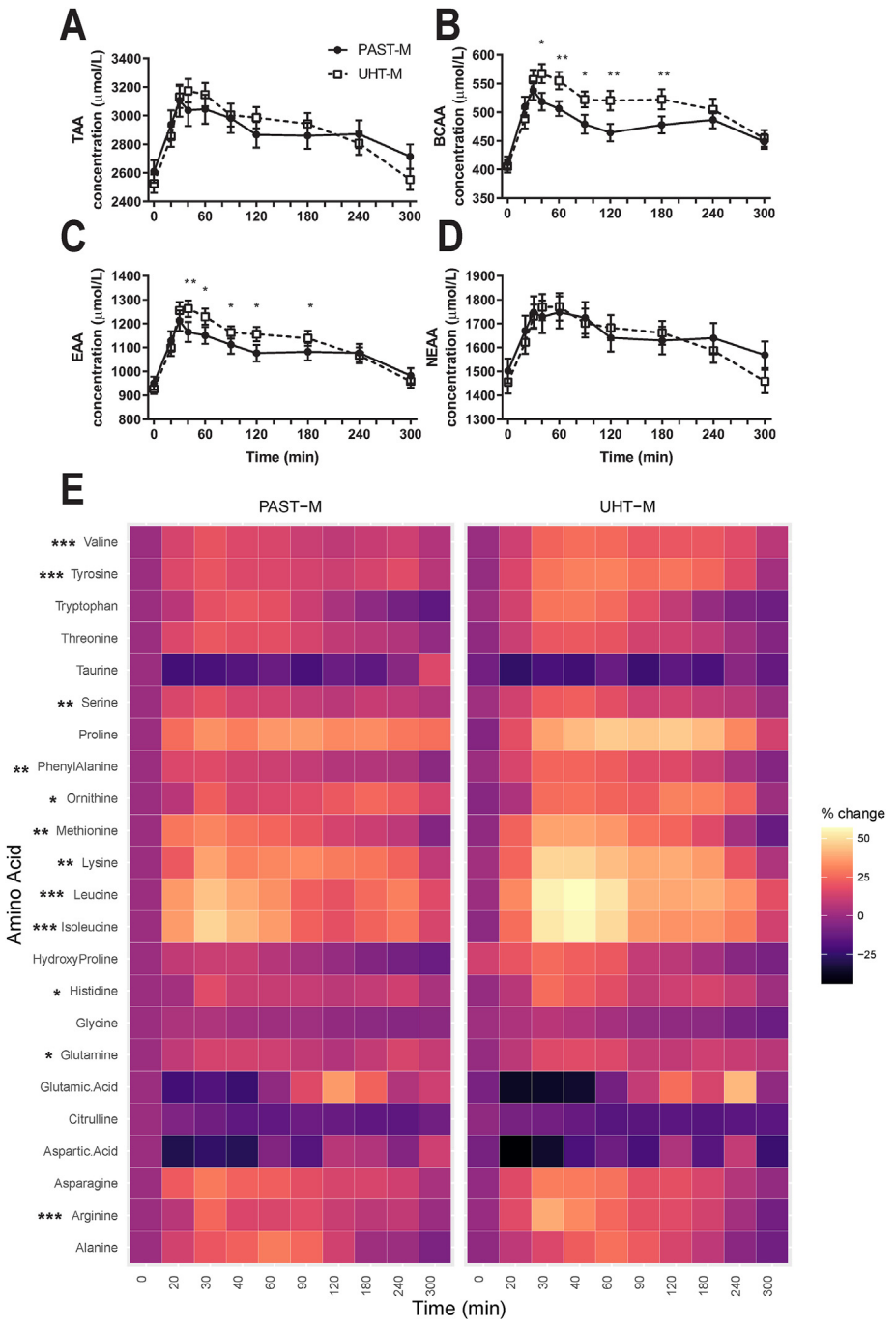

FIGURE 3. Pooled plasma amino acid concentrations for PAST-M (black) and UHT-M (white; A–D) and heatmap of postprandial changes in individual amino acids (E). Panel A–D: (A) TAA: total amino acids; (B) BCAA: branched chain amino acids; (C) EAA: essential amino acids; (D) NEAA: nonessential amino acids. Data are presented as mean ± SEM in μmol/L ($n = 18$). There was an interaction (treatment × time) for TAA, BCAA, EAA, and NEAA ($P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.011$, respectively). (E) Values are presented as mean fold % changes relative to concentrations at PAST-M fasting (0 min); *$P < 0.05$, **$P < 0.01$, ***$P < 0.01$ denotes interaction time × milk with postprandial UHT-M abundance greater than PAST-M. Interactions were analyzed by general linear model with Sidak corrected post hoc adjustment. PAST-M, pasteurized milk; UHT-M, ultra-high temperature milk.

Plasma lipidome

A total of 213 lipid species were detected in plasma following milk ingestion, of which 68 species exhibited a significant time response, 165 exhibited AUC values significantly different from 0, and 67 were significant for both tests in ≥1 treatment group ($P < 0.05$).

Postprandial responses differed between UHT-M and PAST-M for 78 lipids (treatment × time interaction $P < 0.05$; Figure 4, Supplemental Tables 1 and 2) and 42 lipids differed between milk types irrespective of postprandial timepoint (main treatment effect $P < 0.05$; Figure 4, Supplemental Table 1). Triacylglyceride (TG) species with significant treatment × time interaction exhibited a more rapid increase in relative intensity following ingestion of UHT-M compared with PAST-M, with maximum relative abundance being reached after 120–180 min, after which a decrease in plasma lipid concentration

could be observed (Supplemental Figure 4). Although the increase in TG abundance was slower with PAST-M, abundance at 240 min often exceeded the maximum abundance observed for UHT-M (Supplemental Figure 4). Shorter chain length, saturated TGs exhibited the greatest postprandial increase in relative intensity (log fold-change >400%; Figure 4). TGs with high fold-change generally comprised 10:0, 12:0, 14:0, 16:0, and 17:0 fatty acids, as well as 18:1 and 18:2 in some cases. For TGs with only a main treatment effect ($P < 0.05$), final abundance (i.e., 240 min) was higher for UHT-M, whereas the time profile was generally similar between milk type. TGs comprised only of saturated fatty acids were not represented in this group, with each containing ≥1 of 15:1, 17:1, 17:2, 18:2, 18:3, 19:1, or 19:2.

Changes in non-TG lipids with significant treatment × time interactions were less pronounced, generally with a fold-change of <50% (Figure 4). The greatest postprandial changes were observed for lysophosphatidylcholine 15:2, phosphatidylcholine (PC) 30:0, phosphatidylethanolamine (PE) 36:4, and PE 38:5 (Supplemental Figure 4). PC 30:0 and PE 38:5 were unable to be annotated to the fatty acid level, and as such are only putatively characterized. As with the TGs, the ingestion of UHT-M resulted in higher relative levels, although this was less pronounced than that observed for TGs.

Plasma B vitamins

There were no differences in the postprandial response of any B vitamins or vitamers between milk heat treatments (treatment × time interaction $P = 0.087$ thiamine, $P = 0.305$ riboflavin, $P = 0.209$ nicotinamide, $P = 0.686$ pantothenic acid, $P = 0.106$ 4-pyridoxic acid and $P = 0.741$ TMAO; Supplemental Figure 5). However, UHT-M resulted in a greater AUC for plasma riboflavin and pantothenic acid (3074 ± 680 compared with 2684 ± 680 nmol·min·L^{-1} $P < 0.001$ and 2815 ± 1563 compared with 1675 ± 1790 nmol·min·L^{-1} $P = 0.018$ each, UHT-M compared with PAST-M, respectively; Table 5) whereas PAST-M resulted in a greater AUC for plasma 4-pyridoxic acid (than UHT-M (-2209 ± 543 compared with -2807 ± 860 nmol·min·L^{-1} $P = 0.029$).

Plasma appetite hormones

Plasma leptin, ghrelin, and PYY responses were not different between milk heat treatments ($P = 0.722$, $P = 0.188$, and $P = 0.440$, respectively; Supplemental Figure 6). Plasma GLP-1 concentrations were higher at 90 min following UHT-M than PAST-M (treatment × time interaction $P = 0.011$; Supplemental Figure 6).

Symptom and appetite scores

No digestive symptom or appetite scores differed between milk heat treatments following ingestion (Supplemental Table 3).

Body surface gastric mapping

The necessity to pause BSGM readings during MRI scans resulted in missing periods of data collected because of the protocol, variable delays to the recommencement of recordings during reader attachment, and contributed to artifacts generated because of movement. Nevertheless, of the 20 participants, 100% ($n = 20$) and 90% ($n = 18$) had BSGM performed at 1 or 2 interventions, respectively, with 19 records for each milk type. Of these, only 40 and 50% of captured data was

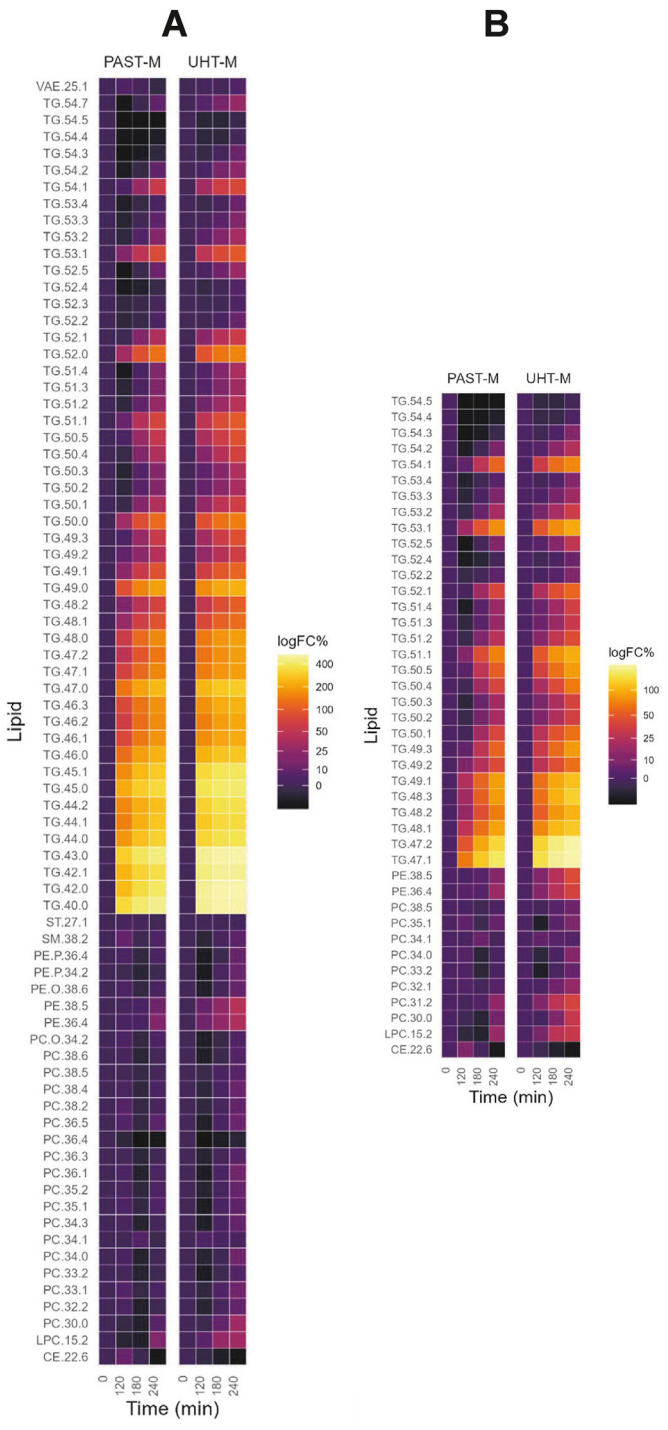

FIGURE 4. Heatmap of statistically significant postprandial changes in individual lipids based on milk × time (A) or milk (B) effect. Values are presented as mean log fold-change percentage over time (logFC%) relative to concentrations at fasting (0 min); $n = 20$. CE, cholesterol ester; LPC, lysophosphatidylcholine; PAST-M, pasteurized milk; PC, phosphatidylcholine; PC-O, ether-linked phosphatidylcholine; PE, phosphatidylethanolamine PE-P, ether-linked phosphatidylethanolamine; SM, sphingomyelin; ST, sterol; TG, triacylglyceride; UHT-M, ultra-high temperature milk; VAE, vitamin A fatty acid ester.

TABLE 5
Plasma B vitamin incremental AUC following UHT-M and PAST-M

B vitamin[1]	PAST-M				UHT-M				*P* value[2]
	Mean		SEM	(95% CI)	Mean		SEM	(95% CI)	
4-Pyridoxic acid	−2209	±	543	(–3274, –1145)	–2807	±	860	(–4492, –1121)	0.029
Nicotinamide	−1047	±	12,101	(–24,765, 22,670)	10,240	±	10,976	(–11,272, 31,751)	0.578
Pantothenic acid	1675	±	1790	(–1834, 5184)	2815	±	1563	(–248, 5878)	0.018
Riboflavin	2684	±	680	(1351, 4018)	3074	±	680	(1742, 4406)	<0.001
Thiamine	−56	±	83	(–220, 107)	–54	±	49	(–150, 41)	0.784
TMAO	−3110	±	1266	(–5591, –629)	–2324	±	1193	(–4663, 15)	0.493

Abbreviations: CI, confidence interval; PAST-M: pasteurized milk; TMAO, trimethylamine N-oxide; UHT-M: ultra-high temperature milk.
[1] Values presented as means ± SEM (95% CI) in nmol·min·L^{-1}; $n = 20$.
[2] Significance analyzed by Student's *t*-test.

sufficient quality for analysis ($n = 8$ PAST-M and $n = 10$ UHT-M, respectively); this included only 5 subjects' paired assessments, and a mean of 4 of 6 timepoints included for unadjusted amplitude and rhythm index. Only 5 records for each milk included the first hour. Data for Principal Gastric Frequency were available for $n = 3$ and $n = 7$ (PAST-M and UHT-M, respectively) and a mean of 2 of 6 timepoints.

None of the measured metrics [Principal Gastric Frequency, amplitude (unadjusted for BMI), Gastric Alimetry Rhythm Index (unadjusted for BMI)] were different between milk types ($P = 0.640$, $P = 0.917$, $P = 0.578$, each, respectively, Table 6).

The unadjusted amplitude did not change over time ($P > 0.05$). The unadjusted Gastric Alimetry Rhythm Index was higher at 2–3 h than between 0 and 1 h, irrespective of milk heat treatment (0.13 ± 0.02 arbitrary units $P = 0.074$). The Principal Gastric Frequency decreased relative to the 1–2 h timepoint at all other postmeal timepoints ($P < 0.05$ each, respectively).

Discussion

We investigated whether faster GE with UHT-M explained the greater circulating amino acid uptake relative to PAST-M previously observed [11]. Contrarily, PAST-M emptied more quickly than UHT-M, particularly during early digestion. Despite higher gastric volumes with UHT-M, aminoacidemia was heightened relative to PAST-M [11], aligning with in vitro gastric digestion and rat model [6] evidence of faster gastric protein release. UHT-M elevated several circulating lipid species and B vitamins. Appetite (subjective or hormonal) or gastric myoelectrical activity did not differ. Nutrients are more readily available from UHT-M than PAST-M. Overall GCV of a complex dairy matrix does not directly predict circulating nutrient appearance.

Gastric contents emptied more slowly with UHT-M. The effect size of UHT-M GCV T_{50} was only 15%, yet power was estimated on a 35% expected difference. This implies that a more severe heat treatment has a smaller effect than a 30% larger meal (i.e., effect size ~35%) [26] or the delay caused by a stable emulsion (i.e., effect size ~18%) [54]. Yet, whereas the smaller-than-predicted effect on GCV T_{50} implies insufficient power, the T_{25} effect was 31%, showing an expected magnitude of difference that was earlier, and indeed opposite, than anticipated. Physiologically, this effect may be relevant for nutrient availability [55] or appetite regulation [54], although neither outcome corresponded with delayed GE. Indeed, aminoacidemia AUC was greater with UHT-M

TABLE 6
Body surface gastric mapping parameters of unadjusted amplitude, unadjusted Gastric Alimetry Rhythm Index and Principal Gastric Frequency over 5 h and overall following PAST-M and UHT-M ingestion

Measure[1]	Time	PAST-M				UHT-M				*P* value[2]
		Mean		SEM	(95% CI)	Mean		SEM	(95% CI)	
Unadjusted amplitude (μV)	0–1 h	42.28	±	1.42	(39.5, 45.05)	39.24	±	6.48	(26.54, 51.93)	0.917
	1–2 h	39.90	±	6.71	(26.75, 53.05)	53.47	±	11.20	(31.52, 75.42)	
	2–3 h	38.86	±	8.23	(22.73, 54.98)	38.67	±	8.70	(21.61, 55.73)	
	3–4 h	41.72	±	15.36	(11.63, 71.82)	44.17	±	8.66	(27.2, 61.14)	
	4–5 h	45.55	±	13.86	(18.37, 72.72)	38.51	±	8.72	(21.43, 55.59)	
	Overall	37.68	±	10.01	(18.06, 57.3)	40.04	±	5.55	(29.17, 50.92)	
Unadjusted Gastric Alimetry Rhythm Index (AU)	0–1 h	0.10	±	0.01	(0.08, 0.13)	0.10	±	0.01	(0.07, 0.12)	0.578
	1–2 h	0.11	±	0.01	(0.08, 0.13)	0.12	±	0.02	(0.08, 0.17)	
	2–3 h	0.12	±	0.02	(0.09, 0.15)	0.13	±	0.01	(0.11, 0.16)	
	3–4 h	0.10	±	0.02	(0.06, 0.13)	0.11	±	0.01	(0.09, 0.13)	
	4–5 h	0.11	±	0.02	(0.08, 0.14)	0.09	±	0.01	(0.08, 0.11)	
	Overall	0.10	±	0.01	(0.08, 0.13)	0.11	±	0.01	(0.09, 0.13)	
Principal gastric frequency (cpm)[3]	0–1 h	—			(2.59, 3.13)	—			(2.94, 3.19)	0.640
	1–2 h	2.86	±	0.14	(2.83, 2.97)	3.06	±	0.06	(2.84, 3.13)	
	2–3 h	2.90	±	0.04	(0, 0)	2.98	±	0.07	(2.79, 3.19)	
	3–4 h	2.93	±	0.00	(0, 0)	2.99	±	0.10	(0, 0)	
	4–5 h	2.85	±	0.00	(0, 0)	2.83	±	0.00	(0, 0)	
	Overall	2.93	±	0.00	(0, 0)	3.06	±	0.00	(0, 0)	

Abbreviations: AU, arbitrary unit; CI, confidence interval; PAST-M: pasteurized milk; UHT-M: ultra-high temperature milk.
[1] Data presented as mean ± SEM (95% CI); $n = 8$ PAST-M and $n = 10$ UHT-M, respectively.
[2] Significance between milks over time analyzed by mixed effect linear regression model.
[3] Principal Gastric Frequency data were not available for 0–1 h and insufficient overall to perform statistical analysis.

than PAST-M across BCAA (57%), EAA and leucine (44%), evidence of faster protein digestion from UHT milk [6], greater N retention [11], and a higher plasma EAA trend [15]. UHT-M may offer clinical advantages over PAST-M, for example, stimulating skeletal muscle protein synthesis. The substantial AUC effect of UHT-M aligns with the effect of doubling whey dose on increasing plasma leucine AUC (44%), and the corresponding intramuscular leucine AUC (95%) and myofibrillar fractional synthetic rate (20%) [56]. Gastric myoelectrical, hormonal, glycemic, triacylglyceridemic, and perceived appetite responses were similar between milks. Plasma GLP-1 was transiently elevated following UHT-M but this was independent of other appetite or insulinotropic responses suggesting negligible heat treatment effects on acute metabolic responses or appetite regulation. Overall, dairy heat treatment may be an effective modifier of dairy matrix and physiological responses to optimize nutritional impacts through digestive kinetics and enhanced circulating nutrient availability.

Slower GE with UHT-M compared with PAST-M contrasts with *in vitro* and animal (rat [6] and pig [24]) studies of gastric dynamics, which suggest faster protein emptying with UHT-M. Parameters describing protein digestion differ across *in vitro*, *in vivo* animal, and human studies; along with differences in study design and the complexity of dairy matrices [2,8] *in vitro–in vivo* correlations may be limited [57]. Gastric volume by MRI does not capture curd weight, a key measure in previous nonhuman milk heat treatment studies. *In vitro*, faster protein digestion with UHT-M was described as lower curd weight and greater protein hydrolysis by 220 min [6]. In an *in vivo* rat model, wet and dried curd weight were greater at 30–120 min for UHT-M than PAST-M, but by 240 min, were equal, interpreted as faster emptying of UHT-M [6]. A higher wet weight early in UHT-M digestion supports curd moisture content influencing stomach volume and aligns with observations here of greater early UHT-M gastric volumes. However, in pigs, UHT-M showed faster dry matter and protein emptying [24], but the total stomach content weight was equal to PAST-M (Ahlborn et al., unpublished). More rapid dry matter and protein emptying was mirrored in curd but not liquid gastric contents [24]. Digestion models predicted protein dynamics but not physiological explanations and implications, emphasizing requirements for human research assessing food structure impacts on health outcomes.

Although UHT-M GCV was larger for longer, the rapid aminoacidemia suggests that proteins or nutrients not restricted within the (predominately casein) curd were released more quickly (i.e., whey: β-lactoglobulin [16]), without complete curd breakdown. *In vitro* [6] and animal models [6,24] showed faster solid curd emptying [6,24] and protein hydrolysis [6] with UHT-M: β-lactoglobulin is rapidly hydrolyzed [6] facilitated by heat denaturation [58], and the softer coagulum [7,24] has decreased pepsin resistance [7]. Whey elicits faster aminoacidemia than casein [59]. The rapid circulating appearance of riboflavin, pantothenic acid and milk-derived lipids [12,60–62] with UHT-M similarly supports rapid liquid phase GE [63–65]. The looser, moisture-retaining UHT curd [6] would explain greater retained gastric volume while simultaneously enhancing protein hydrolysis enzyme diffusion pathways. The inversely correlated gastric volume and circulating nutrient appearance implies that the composition of digesta phases is a critical determinant of digestion.

Within the complex milk matrix, with dynamic solid and liquid constituents and nutrient composition during digestion [2], overall gastric volume insufficiently predicted circulating nutrient delivery. Hence, although GE using MRI is accurate and validated [66], gastric volume does not describe small intestinal nutrient delivery rate, limiting predictions of nutritional and metabolic consequences. Correlation between gastric behavior and nutritional consequences should therefore not be assumed, particularly for complex food matrices. MRI can also discriminate gastric contents beyond volume [67], for example visual variation of compositionally different gastric contents [67], for example, emulsions [54,68] or viscosity [69]. Here, stomach intensity was homogeneous (i.e., gray) immediately postprandially, shifting (by 90 min) to distinct heterogeneous fat/water (i.e., white) weighted regions, with differing visual distribution between UHT-M and PAST-M [36]. Different gastric curd fat and protein distributions have been described between UHT-M and PAST-M using confocal imaging in vitro [6]. Here, thresholding of the curd, using methods by Otsu [36] found no response difference in curd and liquid distribution between UHT-M and PAST-M, but showed UHT-M was darker overall. Although this may suggest that more of the stomach volume consisted of curd with UHT-M, it is unclear whether the curd structure also differed. Novel techniques, which are not limited by choice of threshold settings, are being developed to map gastric content using MRI [70] to quantify and qualify coagulum through texture metrics [38,71] or even protein hydrolysis degree [37]. Such valuable tools to non-invasively describe digesta dynamics should be considered in future studies, ensuring inclusion of optimized scanning parameters.

This is the first study describing concurrent, although not simultaneous, gastric myoelectrical activity with MRI. Concurrent MRI procedures deviated from the standard Gastric Alimetry protocol [72], which impaired data quality, generating artifacts and collection gaps during the most dynamic period of gastric activity [73]. Yet, despite low Gastric Alimetry Rhythm Index [73], Principal Gastric Frequency and amplitude [73] confirmed higher activity during 1–2 h postmeal, aligning with normative values [73]. Data limitations or a narrow activity range in this healthy population [73,74] may have contributed to no detected differences.

This study has some limitations. The generalizability of the findings may be limited for demographics other than young healthy females, because both BMI and sex can affect digestion. Overweight contributes to faster meal responses [73]; here, 4 participants were overweight (BMI 26.6 $\pm$ 0.2), which may have increased average meal response. Females have higher gastric amplitude and frequency [73]; therefore, the results may be different in males. Another limitation was that the menstrual stage was uncontrolled and most participants' phases differed between visits. Slower GE has been reported for follicular [75] and luteal [76] phases, although some studies indicate no impact of the menstrual cycle [29,76,77]. Yet, uncontrolled menstrual phase and wider BMI range may have improved finding generalizability. Elderly populations may respond differently given altered protein digestion or metabolism [78]; previously, UHT-M and PAST-M elicited similar aminoacidemia [15] although [75] milk processing variability may contribute to inconsistencies [15].

No fasting MRI scan was performed; hence, fasting gastric secretions or compliance were not assessed. At 5 min, GCV exceeded milk volume (i.e., 570 mL); variation in gastric conditions affects digestion [79,80] potentially impacting coagulation [6]. Supine posture may reduce GE and aminoacidemia [81]; real-world settings may exaggerate physiological outcomes. Variable, often long, washouts possibly increased interindividual variation because of changing health status (e.g., BMI, hormones, lifestyle). Milk batches, seasonality, and nutrient profiles varied, potentially impacting findings while improving generalizability to commercially available milk. Meal volume [82] and caloric load [83] influence GE; although UHT-M had slightly higher caloric (0.7%), protein (6%), and fatty acid content, these are unlikely to sufficiently explain greater nutrient responses.

In conclusion, GE was slower after UHT-M than PAST-M, contrasting with previous *in vitro* and *in vivo* reports. Yet the resulting greater aminoacidemia and circulating lipid profiles following UHT-M align with previous findings, supporting greater circulating nutrient availability. Gastric volume differences neither explained nor impacted appetite and metabolic responses. The inverse relationship between volume and circulating nutrient availability highlights the importance of intragastric dynamics and the complexity of dairy curd formation impacting nutrient associations with liquid or solid digesta emptying. Caution is required in assuming circulating nutrient delivery from complex food matrices based on overall gastric dynamics or extrapolating physiological impacts of foods from non-human models.

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Author contributions

The authors' contributions were as follows – AMM, MPGB, WCM, NCR, RFM: designed the research; AMM, MPGB, SC, SN, SC, PD, AAG, KF, DB, PS, AS, RFM: conducted the research; CLH, LM, PD, AAG, GO: provided essential materials; AMM, CLH, LM, SN, HS, SC, PD, AAG, KF, DB, SR: analyzed data; AMM, MPGB, NCR, SN, DB, RFM: wrote the article; AMM: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript, code book, and analytical code will be made available upon request pending application and approval.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.03.002.

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