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Postprandial plasma amino acid and appetite responses with ingestion of a novel salmon-derived protein peptide in healthy young adults

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Abstract

This study assessed postprandial plasma aminoacidemia, glycemia, insulinemia and appetite responses to ingestion of a novel salmon-derived protein peptide (Salmon PP) compared with milk protein isolate (Milk PI). In a randomised, participant-blind crossover design, eleven healthy adults (M = 5, F = 6; mean ± SD age: 22 ± 3 years; BMI: 24 ± 3 kg/m^2) ingested 0·3 g/kg/body mass of Salmon PP or Milk PI. Arterialised blood samples were collected whilst fasted and over a 240-min postprandial period. Appetite sensations were measured via visual analogue scales. An *ad libitum* buffet-style test meal was administered after each trial. The incremental AUC (iAUC) plasma essential amino acid (EAA) response was similar between Salmon PP and Milk PI. The iAUC plasma leucine response was significantly greater following Milk PI ingestion ($P < 0·001$), whereas temporal and iAUC plasma total amino acid ($P = 0·001$), non-essential amino acid ($P = 0·002$), glycine ($P = 0·0025$) and hydroxyproline ($P < 0·001$) responses were greater following Salmon PP ingestion. Plasma insulin increased similarly above post-absorptive values following Salmon PP and Milk PI ingestion, whilst plasma glucose was largely unaltered. Indices of appetite were similarly altered following Salmon PP and Milk PI ingestion, and total energy and macronutrient intake during the *ad libitum* meal was similar between Salmon PP and Milk PI. The postprandial plasma EAA, glycine, proline and hydroxyproline response to Salmon PP ingestion suggest this novel protein source could support muscle and possibly connective tissue adaptive remodelling, which warrants further investigation, particularly as the plasma leucine response to Salmon PP ingestion was inferior to Milk PI.

Keywords: **Marine protein: Raw materials: Peptides: Bioavailability: Energy intake**

The intake of dietary protein increases rates of muscle protein synthesis (MPS)[1,2]. The muscle anabolic response to protein ingestion is largely attributable to the postprandial rise in circulating essential amino acid (EAA) concentrations[3,4], particularly the branched-chain amino acid, leucine, as both signal and substrate for this process[5–7]. Importantly, plasma aminoacidemia following protein ingestion is contingent on the constituent amino acid profile and digestive properties[8]. Combined, these characteristics are thought to underpin the 'quality' and muscle anabolic properties of a protein source[9–12], with implications for those seeking to optimise muscle adaptive remodelling (e.g. with exercise training). Typically, animal-derived proteins contain a higher proportion of EAA and display higher rates of digestibility than most plant-based proteins[13]. However, there is a growing demand for alternative animal-derived proteins that are environmentally sustainable and efficacious for muscle anabolism[14,15].

Fish is one of the largest global proteins consumed by humans, with over 184·6 million metric tons consumed in 2022 alone[16]. Whilst fish constitutes about 17 % of global meat consumption, > 60 % of products produced from fish farms are discarded as waste[17,18]. Advances in food processing technology have made it possible to upcycle fish rest raw materials (e.g. heads, trimmings, skin, scales and backbones) into high-quality protein products and bioactive peptides for human consumption, which is both environmentally sustainable and economically efficient[18]. The content and plasma availability of EAA from fish-derived protein could support muscle anabolic processes. It was recently shown that the ingestion of a Nile tilapia-derived protein hydrolysate elicited similar plasma

Abbreviations: EAA, essential amino acids; iAUC, incremental AUC; Milk PI, milk protein isolate; MPS, muscle protein synthesis; Salmon PP, salmon-derived protein peptide; TAA, total amino acids; NEAA, non-essential amino acids.

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aminoacidaemia to a whey protein hydrolysate in young individuals following exercise[19]. Furthermore, *ex vivo* treatment of myotubes with plasma obtained after blue whiting-derived protein hydrolysate and whey protein isolate ingestion evoked similar MPS stimulation[20]. In addition to potential muscle anabolic properties, fish by-products are typically rich in connective tissue and contain a significant amount of collagen[21–23]. Collagen is rich in glycine, proline and hydroxyproline, which have purported benefits for connective tissue remodelling[24,25]. However, the processing of raw fish rest materials and the subsequent recovery and production of protein varies considerably[26–28] and influences the digestion and absorption properties of these products and their subsequent biological impact on human tissues. Hence, characterising the blood amino acid profile of emerging fish-derived proteins and peptides is crucial to understand their potential to support human muscle and connective tissue remodelling. Finally, given that the rise in circulating amino acids following protein ingestion may have implications for hunger and satiety, it is possible that the ingested protein source may influence these outcomes[29]. As such, it is important to characterise the influence of novel fish-derived proteins on indices of appetite regulation and energy intake.

The present study aimed to assess postprandial plasma aminoacidemia following the ingestion of a novel salmon-derived protein peptide (Salmon PP), compared with a high-quality reference milk protein isolate (Milk PI). Given the potential for dietary protein ingestion to influence satiety[30], we also determined the effect of Salmon PP and Milk PI on subjective indices of appetite regulation, subsequent meal energy intake and plasma insulin and glucose concentrations. We hypothesised that total and non-essential aminoacidemia would be greater in Salmon PP compared with Milk PI. Despite a lower content of EAA and leucine in Salmon PP compared with Milk PI, we hypothesised that the structural properties of Salmon PP would result in comparable essential aminoacidemia and leucinemia between supplements. Finally, we postulated that postprandial changes in appetite regulation, *ad libitum* energy intake and insulin and glucose would be similar between Salmon PP and Milk PI.

Methods

Participants

Five male and six female young healthy individuals volunteered to participate in this study (participant characteristics are presented in Table 1). Briefly, prospective participants were excluded based on the following criteria: aged < 18 or > 40 years, BMI < 18·5 or > 29·9 kg/m^2, metabolic or respiratory disease or chronic illness, habitual smoker, known allergies or intolerances to study materials and supplements, and use of medications known to affect muscle protein metabolism. Participants were informed of the study purpose, experimental procedures and potential risks associated with participating before they provided written informed consent. Participants were not informed that *ad libitum* energy intake would be assessed as part of this study, since knowledge of this measurement may have influenced dietary behaviours. Instructions given to participants prior to the *ad libitum* buffet are described below. Ethical approval was obtained by the Science, Technology, Engineering and Mathematics Ethical Review Committee at the University of Birmingham (ERN_21-1508A), and all procedures were conducted in accordance with the Declaration of Helsinki (7th edition).

Table 1. Participant characteristics

Parameter	Mean	SD
Sex (M/F)	5/6	
Age (y)	22	3
Height (cm)	175·4	10·5
Weight (kg)	74·2	17·2
BMI (kg·cm^2)	23·9	3·6
Fat mass (kg)	15·2	6·0
Body fat (%)	20·5	6·6
Fat free mass (kg)	58·8	14·3

Study design

The present study followed a randomised, participant-blind, crossover design with counterbalancing, where participants completed one preliminary visit and two experimental trials at the School of Sport, Exercise and Rehabilitation Science laboratories, University of Birmingham. The preliminary visit was conducted at least 1 week prior to the first experimental trial and involved eligibility screening, height and mass measurements, and completion of a general health history questionnaire. For each experimental trial, separated by at least 5 d, participants were asked to ingest 0·3 g/kg/body mass of Salmon PP or Milk PI. Trial order was randomised and counterbalanced between participants, to minimise any effect of trial order on study outcomes. The influence of trial order on subjective appetite perceptions and *ad libitum* energy intake confirms no influence of trial order in these outcomes. Following supplement ingestion, participants rested in the laboratory for repeat blood sampling and appetite sensation measurements over 4 hours, before consuming an *ad libitum* test meal. A schematic overview of the experimental design is presented in Fig. 1.

Diet and physical activity

One week prior to the first experimental trial, participants were instructed to complete self-report weighed food diaries for the assessment of habitual macronutrient and energy intake. Further, 24 h prior to the first experimental trial, participants were instructed to complete an additional food diary and a physical activity diary. Participants were asked to replicate these diary entries 24 h prior to the second and third experimental trials. Participants were also provided with a standardised food package for consumption on the evening before each experimental trial (413 kcal, about 56 % carbohydrate, about 22 % protein and about 22 % fat). To minimise intraindividual variability in physical activity on the morning of each experimental trial, participants were asked to record their means

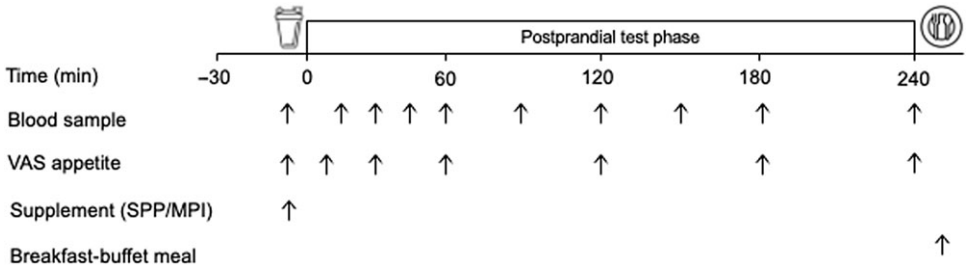

Fig. 1. Schematic of experimental trials. Trials were separated by > 5 d and involved ingestion of 0·3 g/kg/body mass salmon-derived peptide protein (Salmon PP) or milk protein isolate (Milk PI), arterialised blood sampling over 4 h and a buffet-style test meal for the assessment of *ad libitum* energy intake. VAS, visual analogue scales.

of commuting to the laboratory for their first trial and replicate this on the mornings of their subsequent experimental trials.

Experimental protocol

Participants arrived at the laboratory at about 07.00 h after an overnight fast having refrained from strenuous physical activity and abstained from alcohol consumption for the preceding 24 h period. Upon arrival, body mass and height were measured, and body composition was assessed via Bioelectrical Impedance Analysis (TANITA SC-331S). Participants then rested in a semi-recumbent position with their forearm placed under a heated blanket to arterialise venous blood. After 10 min, a cannula (BD Venflon™) connected to a three-way stopcock (BD Connecta™) was inserted antegrade into an antecubital forearm vein and 15 ml of blood sample was drawn. The cannula was then flushed with 5 ml of sterile NaCl 0·9 % (BD PosiFlush™) to maintain patency for repeated blood sampling (repeated at each blood sample). The blood sampled arm was warmed in a heated blanket to ensure blood samples were arterialised[31]. Participants were then asked to complete a series of 0–100 mm visual analogue scales to assess fasted-state appetite sensations[32]: participants marked a line through the 100 mm scale to reflect how they felt in relation to the questions at the time of assessment. Three questions from this scale: 'How hungry do you feel?', 'How full do you feel?' and 'How satisfied do you feel?' were used. Following this, participants ingested 0·3 g/kg/body mass of Salmon PP or Milk PI, according to trial order randomisation (described below). Upon consumption a timer was started, where participants were asked to consume the beverage within 3 min. To ensure all residual protein was consumed, beverage containers were rinsed with a further 200 ml which participants also consumed. Blood samples were drawn every 15 min during the first hour, and every 30 min thereafter for the 4 h postprandial period. Appetite sensations were assessed via visual analogue scales at 5 min, 30 min and then hourly following protein ingestion for the remainder of the trial. At the hourly sampling time points, visual analogue scales were completed prior to arterialised blood sampling. Water intake was permitted *ad libitum* during the first 4 h trial and was recorded to ensure replication on subsequent trials. The cannula was removed following the 4-h postprandial period and a buffet-style test meal was administered to assess *ad libitum* energy intake, after which the trial was ceased. Participants later returned to the laboratory to complete a further experimental trial, which was identical, except for the type of protein supplement they were asked to consume. At the end of this final trial, participants completed an exit questionnaire to determine the success of blinding to trial order. No adverse events were experienced by participants in either experimental treatment trial.

Supplemental beverages

The nutritional composition of the protein supplemental beverages is displayed in Table 2. Beverages were volume-matched and contained similar energy, carbohydrate, fat, and fibre. The Milk PI protein was obtained from Myprotein™ and contained about 81 g of protein per 100 g. The Salmon PP was SalMe Peptides and contained about 93·5 g of protein per 100 g. Salmon PP was made from food grade salmon raw materials, with the use of commercial food grade, non-GMO proteases in a patented process at Biomega Norway AS. After enzymatic hydrolysis, all fractions were heated to > 85°C before the water-soluble content was separated from the fat and the non-soluble fractions by centrifugal force. Thereafter, the water-soluble fraction was ultra- and nano-filtered. The retentate from the nanofiltration was further concentrated in an evaporator, before being spray-dried. The final product consisted of a mixture of peptides and free amino acids, as well as other water-soluble nutrients. Participants ingested 0·3 g/kg/body mass of protein Salmon PP or Milk PI, which equated to 22·0 ± 4·9 g (range 16·2–30·1 g) of protein for both treatments, or 23·6 ± 5·3 g and 27·2 ± 6·1 g of supplement material for Salmon PP and Milk PI, respectively. Both supplements were unflavoured, but participants were permitted a choice of strawberry, vanilla or raspberry flavour drops (Myprotein) to add to each beverage to improve palatability and promote taste-matching between Salmon PP and Milk PI (same flavour was used in both trials). Supplements were dissolved in 300 ml of cold water and the resultant beverage served in identical opaque black shaker bottles to ensure participants were blind to beverage appearance. The energy content of Milk PI and Salmon PP supplements was determined independently by bomb calorimetry (Milk PI; Impact Solutions, Livingstone, Scotland, Salmon PP; Eurofins Food and Feed, Trondheim, Norway).

Blood sampling and analysis

Arterialised blood samples were collected into tubes containing anti-coagulant K^2EDTA (BD Vacutainer®) and were placed on

Table 2. Supplement composition

Energy and macronutrients (per 100 g of protein)	Salmon-derived protein peptide	Milk protein isolate
Energy (kcal)	495·8	481·3
Fat	< 0·5 g	1·4 g
Of which saturates	< 0·5 g	1·2 g
Carbohydrates	n.d.	5·4 g
Of which sugars	< 0·04	4·4 g
Protein	93·5 g	81 g
Salt	0·6 g	0·2 g
Aspartic acid	9·40	7·40
Glutamic acid	14·50	9·10
Serine	4·60	5·30
Glycine	15·00	1·70
Histidine	2·00	2·30
Arginine	7·00	3·70
Threonine	3·90	3·80
Alanine	7·80	3·00
Proline	7·30	9·90
Tyrosine	1·70	4·60
Valine	3·50	6·00
Methionine	2·60	2·60
Isoleucine	2·70	5·60
Leucine	4·80	9·80
Phenylalanine	2·60	4·70
Lysine	7·40	8·70
Tryptophan	0·81	1·70
Hydroxyproline	4·00	–
Total cysteine/cystine	0·49	0·90
∑ TAA	**98·10**	**90·80**
∑ EAA	**30·31**	**45·20**
∑ NEAA	**67·79**	**45·60**

n.d, none detected; ∑ TAA, summed total of total amino acids; ∑ EAA, summed total of essential amino acids; ∑ NEAA, summed total of non-essential amino acids. Bold font highlights summed totals from the data presented in normal font.

ice for 30 min before centrifugation at 4000 *g* for 10 min at 4°C. Plasma was aliquoted in duplicate and immediately transferred to −80°C for storage until further analysis. Analysis of plasma amino acid concentrations was conducted using EZ: faast procedure according to the manufacturer's instructions. In brief, 50 µl of plasma was combined with 50 µl of EZ: faast internal AA standard and 50 µl of DDH_2O in a sample preparation vial. Samples were transferred slowly (1 min) through a sorbent tip attached to a 1·5 ml syringe. Then, 200 µl of wash solution (1-propanol and H_2O) was added to sample preparation vials and transferred slowly through the sorbent tips into the syringe barrel. Liquid accumulated in the syringe barrel was then discarded. Then, 200 µl of freshly prepared elution medium (a NaOH-based solution) was added to the sample preparation vial. Using a 0·6 ml syringe, the eluting medium was transferred slowly through sorbent particles to the filter plug in the sorbent tip. After, the liquid and particles were ejected from the syringe into the sample preparation vial; this step repeated further two times. Then, 50 ml of derivatisation solution (a mixture of CHCl3, 2, 2, 4 trimethylpentane and propylchloroformate) was added to the sample preparation vial and vortexed for 10 s to emulsify. To separate the emulsion into two layers, 100 µl of acid solution (HCl-based) was added. The upper layer (containing derivatised AA) was transferred to an autosampler vial and analysed via Gas Chromatography (Agilent 6890W) fitted with a Flame Ionization Detector.

Plasma glucose concentrations were measured in duplicate using an automated analyser (Rx Daytona, Randox Laboratories). Plasma concentrations of insulin were measured in duplicate using ELISA kits (Mercodia), according to manufacturer instructions, where all samples for a participant were measured on the same plate or run.

Energy intake

Within-laboratory energy intake was assessed at each trial by provision of a buffet-style *ad libitum* test meal comprising water, cornflakes, semi-skimmed milk, white bread, margarine, raspberry jam, strawberry yogurt pot, bananas, apples, breakfast bars, scotch pancakes, baked beans, Cheddar cheese and porridge. To prevent any influence of external cues on eating behaviour, participants consumed the meal in isolation and were instructed to refrain from using their mobile phones throughout. Participants were instructed to 'help themselves to the food items' and to 'eat as much or as little' as they liked until comfortably full. Food items were weighed by the researcher before and after the test meal, and the weighted difference in food was recorded. Water intake was permitted *ad libitum* during the test meal. Both within-laboratory and habitual energy intakes were calculated from self-reported diet diaries using the following energetic values for each macronutrient: carbohydrate 3·75 kcal/g, fat 8·94 kcal/g and protein 4·02 kcal/g (Elia & Cummings, 2007).

Statistical analysis

A minimum sample size of 11 was calculated in order to detect an effect size of 0·95 with 80 % power (G * Power 3.1.9.7), based on the effect size in similar studies of postprandial aminoacidemia with ingestion of supplemental protein sources in healthy young individuals[33–35]. Descriptive statistics were calculated using Microsoft Excel. Incremental AUC (iAUC) for postprandial metabolite and hormonal responses were calculated with the trapezoid method using the Time Series Response Analyser (Narang et al., 2020). Figures were produced and statistical analysis was performed in GraphPad Prism, where statistical significance was accepted at $P \leq 0{\cdot}05$. Time-dependent variables were analysed using two-way repeated-measures ANOVA, or mixed-effects models (depending on missing data points) with *post hoc* Bonferroni correction. Time-independent variables were analysed using one-way repeated-measures ANOVA, or mixed-effects models (depending on missing data points) with *post hoc* Bonferroni correction. Data are presented as mean ± SD for tables and mean ± SEM for figures.

Results

Standardisation and blinding

Protein beverages were correctly identified on 54 % of occasions, where five of eleven participants failed to identify a single beverage correctly. Trial order was correctly identified by only six of eleven participants. The Salmon PP beverage was correctly identified on six occasions and the Milk PI beverage on six occasions.

Plasma glucose and insulin concentrations

Plasma glucose concentrations were modestly altered following protein ingestion (time effect; $P = 0{\cdot}0123$: Fig. 2(a)). Plasma glucose concentration was increased above post-absorptive values at 15 min following Salmon PP ingestion only ($P = 0{\cdot}0123$). There were no statistically significant differences in plasma glucose concentrations between Salmon PP and Milk PI at any time point (trial effect: $P = 0{\cdot}992$; interaction effect: $P = 0{\cdot}918$). Plasma insulin concentrations increased following protein ingestion (time effect; $P < 0{\cdot}001$: Fig. 2(b)). Plasma insulin concentrations were increased above post-absorptive values at 30 and 60 min after ingestion of Salmon PP and Milk PI ($P < 0{\cdot}05$ for all), returning to post-absorptive values by 90 min post-ingestion. There were no significant differences in plasma insulin concentration between Salmon PP and Milk PI at any time point (trial effect: $P = 0{\cdot}607$; interaction effect: $P = 0{\cdot}664$).

Plasma total, essential and non-essential amino acid concentrations

Plasma total amino acid (TAA) concentrations increased following protein ingestion (time effect: $P < 0{\cdot}001$; Fig. 3(a)), with a main effect of trial and an interaction effect detected ($P < 0{\cdot}001$ for both). Plasma TAA concentrations were increased above post-absorptive values from 15 to 120 min following ingestion of Salmon PP and Milk PI ($P < 0{\cdot}05$ for all) and 150 min following ingestion of Salmon PP only ($P = 0{\cdot}021$). Plasma TAA concentrations were significantly greater following Salmon PP compared with Milk PI at 30, 45, 60 and 90 min post-ingestion ($P < 0{\cdot}05$ for all). Peak TAA concentration was significantly greater for SPI compared with Milk PI (4841 ± 237 *v.* 3755 ± 159 μmol/l, respectively; $P = 0{\cdot}0017$). Time-to-peak TAA concentration did not differ between Salmon PP and Milk PI (46·4 ± 3·6 *v.* 54·6 ± 5·96 min, respectively; $P = 0{\cdot}17$). A significant main effect of trial was detected for plasma TAA iAUC over the 240-min postprandial phase, which was greater in Salmon PP compared with Milk PI ($P = 0{\cdot}001$; Fig. 3(b)).

Plasma non-essential amino acid (NEAA) concentrations increased following protein ingestion (time effect: $P < 0{\cdot}001$; Fig. 3(c)), with a main effect of trial ($P < 0{\cdot}0029$) and an interaction effect detected ($P < 0{\cdot}001$ for both). Plasma NEAA concentrations were increased above post-absorptive values from 15 to 120 min following ingestion of Salmon PP ($P < 0{\cdot}001$ for all), whereas plasma NEAA concentrations were increased above post-absorptive values at 30–90 min post-ingestion of Milk PI ($P < 0{\cdot}05$ for both). Plasma NEAA concentrations were significantly greater following Salmon PP compared with Milk PI at 15–120 min post-ingestion ($P < 0{\cdot}05$ for all). Peak NEAA concentration was significantly greater for Salmon PP compared with Milk PI (3441 ± 189 *v.* 2403 ± 143 μmol/l, respectively; $P = 0{\cdot}0010$). Time-to-peak TAA concentration did not differ between Salmon PP and Milk PI (45·0 ± 3·5 *v.* 53·2 ± 6·7 min, respectively; $P = 0{\cdot}258$). A significant main effect of trial was detected for plasma NEAA iAUC over the 240-min postprandial phase, which was greater in Salmon PP compared with Milk PI ($P < 0{\cdot}001$; Fig. 3(d)).

Plasma EAA concentrations increased following protein ingestion (time effect: $P < 0{\cdot}001$; Fig. 3(e)), with no significant differences between trials (trial effect: $P = 0{\cdot}525$; interaction effect: $P = 0{\cdot}770$. Plasma EAA concentrations were increased above post-absorptive values from 15 to 150 min following ingestion of Salmon PP and Milk PI ($P < 0{\cdot}05$ for all). Peak EAA concentration was not significantly different between Salmon PP and Milk PI (1466 ± 90 *v.* 1430 ± 41 μmol/l, respectively; $P = 0{\cdot}588$). Time-to-peak EAA concentration was not significantly different between Salmon PP and Milk PI (50·5 ± 7·1 *v.* 51·8 ± 4·6 min, respectively; $P = 0{\cdot}870$). Plasma EAA iAUC over the 240-min postprandial phase was not significantly different between Salmon PP and Milk PI ($P = 0{\cdot}912$; Fig. 3(f)).

Plasma leucine concentrations increased following protein ingestion (time effect: $P < 0{\cdot}001$; Fig. 3(g)), with a main effect of trial ($P = 0{\cdot}0124$) and an interaction effect detected ($P < 0{\cdot}001$). Plasma leucine concentrations were increased above post-absorptive values from 15 to 150 min following ingestion of Milk PI ($P < 0{\cdot}05$ for all), whereas plasma leucine was increased from 15 to 60 min post-ingestion of Salmon PP ($P < 0{\cdot}05$ for all). Plasma leucine concentrations were significantly greater following Milk PI compared with Salmon PP at 60–150 min post-ingestion ($P < 0{\cdot}05$ for all). Peak leucine concentration was not significantly different between Salmon PP and Milk PI (264 ± 14 *v.* 273 ± 10 μmol/l, respectively; $P = 0{\cdot}525$). Time-to-peak leucine concentration was significantly different between

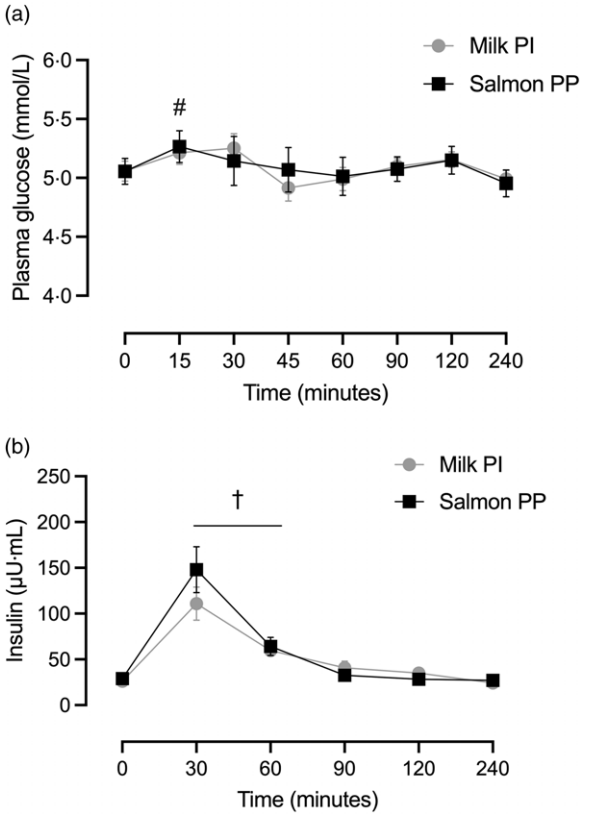

Fig. 2. Postprandial plasma insulin (a) and glucose (b) concentrations following ingestion of salmon-derived peptide protein (Salmon PP; black) and milk protein isolate (Milk PI; grey) in young healthy adults. *n* 11. Data are presented as mean ± SEM. *A statistically significant difference from 0-min fasted-state time point for both groups ($P < 0{\cdot}05$). #A statistically significant difference from 0-min fasted-state time point for Salmon PP only ($P < 0{\cdot}05$).

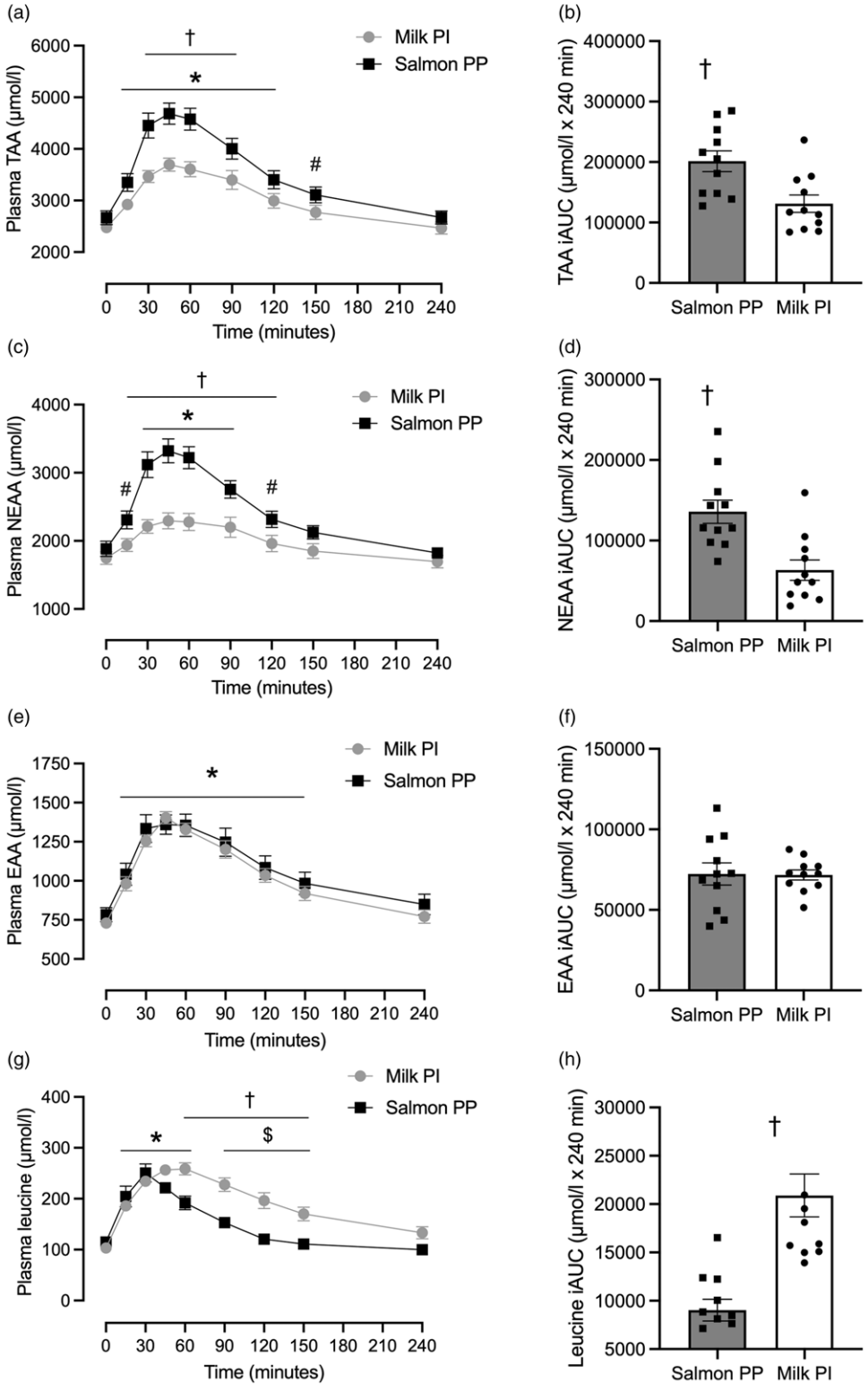

Fig. 3. Postprandial plasma amino acid responses to ingestion of salmon-derived peptide protein (Salmon PP; black) and milk protein isolate (Milk PI; grey) in young healthy adults. Time course and incremental AUC (iAUC) of plasma total amino acids (TAA; a, b), non-essential amino acids (NEAA; c, d), essential amino acids (EAA; e, f) and leucine (g, h) concentrations for n 11. Data are presented as mean ± SEM and individual values. †A statistically significant difference between Salmon PP and Milk PI ($P < 0{\cdot}05$). *A statistically significant difference from 0-min fasted-state time point for both groups ($P < 0{\cdot}05$). #A statistically significant difference from 0-min fasted-state time point for Salmon PP only ($P < 0{\cdot}05$). $A statistically significant difference from 0-min fasted-state time point for Milk PI only ($P < 0{\cdot}05$). EAA is the sum of histidine, threonine, lysine, methionine, valine, isoleucine, leucine and phenylalanine. NEAA is the sum of alanine, arginine, asparagine, citrulline, cysteine, glutamine, glutamic acid, glycine, ornithine, proline, taurine and tyrosine. iAUC, incremental AUC.

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Salmon PP and Milk PI (34·1 ± 3·0 *v.* 51·8 ± 5·6 min, respectively; $P = 0·011$). A significant main effect of trial was detected for plasma leucine iAUC, which was greater in Milk PI compared with Salmon PP ($P < 0·001$; Fig. 3(h)).

Plasma glycine proline and hydroxyproline concentrations

Plasma glycine concentrations increased following protein ingestion (time effect: $P < 0·001$; Fig. 4(a)), with a main effect of trial ($P < 0·001$) and an interaction effect detected ($P < 0·001$). Plasma glycine concentrations were increased above post-absorptive values from 15 to 90 min following ingestion of Salmon PP ($P < 0·05$ for all), whereas plasma glycine was increased only at 60 min post-ingestion of Milk PI ($P = 0·0475$). Plasma glycine concentrations were significantly greater following Salmon PP compared with Milk PI from 30 to 150 min post-ingestion ($P < 0·05$ for all). Peak glycine concentration was significantly greater for Salmon PP compared with Milk PI (479 ± 45 *v.* 281 ± 15 μmol/l, respectively; $P = 0016$). Time-to-peak glycine concentration did not differ between Salmon PP and Milk PI (47·7 ± 2·0 *v.* 49·1 ± 5·1 min, respectively; $P = 0·724$). A significant main effect of trial was detected for plasma glycine iAUC, which was greater in Salmon PP compared with Milk PI ($P = 0·0025$; Fig. 4(b)).

Plasma hydroxyproline concentrations increased following protein ingestion (time effect: $P < 0·001$; Fig. 4(c)), with a main effect of trial and an interaction effect detected ($P < 0·001$ for both). Plasma hydroxyproline concentrations were increased above post-absorptive values from 30 to 240 min following ingestion of Salmon PP ($P < 0·001$ for all), whereas plasma hydroxyproline was increased at 45 min post-ingestion of Milk PI ($P = 0·044$). Plasma hydroxyproline concentrations were significantly greater following Salmon PP compared with Milk PI at 30–240 min post-ingestion ($P < 0·01$ for all). Peak hydroxyproline concentration was significantly greater for Salmon PP compared with Milk PI (74·9 ± 6·6 *v.* 28·3 ± 2·6 μmol/l, respectively; $P < 0·001$). Time-to-peak plasma hydroxyproline concentration did not differ between Salmon PP and Milk PI (68·2 ± 9·3 *v.* 55·0 ± 3·5 min, respectively; $P = 0·563$). A significant main effect of trial was detected for plasma hydroxyproline iAUC, which was greater in Salmon PP compared with Milk PI ($P < 0·001$; Fig. 4(d)).

Plasma proline concentrations increased following protein ingestion (time effect: $P < 0·001$; Fig. 4(e)), with a main effect of trial ($P = 0·0412$) and an interaction effect ($P = 0·001$) detected. Plasma proline concentrations were increased above post-absorptive values from 15 to 120 min following ingestion of Salmon PP ($P < 0·05$ for all) and from 15 to 150 min post-ingestion of Milk PI ($P < 0·05$ for all). Plasma proline concentrations were significantly greater following Salmon PP compared with Milk PI from 30–60 min post-ingestion ($P < 0·05$ for all). Peak proline concentration was significantly greater for Salmon PP compared with Milk PI (469 ± 29 *v.* 335 ± 25 μmol/l, respectively; $P < 0·001$). Time-to-peak plasma proline concentration did not differ between Salmon PP and Milk PI (40·9 ± 4·4 *v.* 49·1 ± 5·01 min, respectively; $P = 0·192$). Plasma proline iAUC over the 240-min postprandial phase was not significantly different between Salmon PP and Milk PI ($P = 0·401$; Fig. 4(f)).

Appetite and energy intake

Total energy, relative macronutrient intake and water intake over 24 h prior to Salmon PP and Milk PI trials was similar and did not differ from habitual values (Table 3), with the exception of water intake being lower in Salmon PP compared with habitual values ($P = 0·032$). Subjective ratings of post-absorptive and postprandial appetite sensations during Salmon PP and Milk PI trials are displayed in Fig. 5(a)–(c). There were no significant differences in the ratings of fullness, hunger or satisfaction between Salmon PP and Milk PI at any time point. Fullness increased above fasted values at 5 and 30 min post-protein for Salmon PP and Milk PI ($P < 0·05$ at all time points for both) and decreased below fasted values at 240 min post-protein ($P = 0·017$ and 0·010 for Salmon PP and Milk PI, respectively). Hunger increased above fasted values at 180 and 240 min post-protein for Salmon PP ($P < 0·001$ at both time points) and 120–240 min post-protein for Milk PI ($P < 0·001$ at all time points). Satisfaction increased above fasted values at 5 min post-protein for Salmon PP and Milk PI ($P = 0·024$ and 0·007, respectively) and decreased below fasted values at 180 and 240 min post-protein for Salmon PP ($P = 0·028$ and 0·034, respectively) and only 240 min post-protein for Milk PI ($P = 0·013$). Fullness, hunger and satisfaction iAUC were not significantly different between trials ($P > 0·05$ for all, data not reported). *Ad libitum* energy intake during the buffet-style breakfast meal did not differ between trials (Fig. 5(d); $P = 0·910$), nor did the relative consumption of carbohydrate, fat or protein ($P > 0·05$ for all; Table 3 and Fig. 5(e)). Trial order did not affect temporal or composite ratings of fullness, hunger or satisfaction ($P > 0·05$ for all), nor did trial order affect *ad libitum* energy intake with the breakfast meal ($P = 0·673$).

No adverse events were reported during the trial.

Discussion

The present study is the first to characterise the blood amino acid and appetite response to a novel protein peptide derived from salmon by-products (Salmon PP) in young healthy adults. Our findings show that ingestion of 0·3 g/kg/body mass of protein from Salmon PP, sufficient for maximal postprandial MPS stimulation in young adults[36,37], resulted in higher postprandial plasma TAA concentrations and equivalent EAA concentrations to the same relative protein dose from Milk PI. The rise in plasma leucine was more rapid and transient, and overall leucine exposure is lower, in Salmon PP compared with Milk PI. Plasma glycine, proline and hydroxyproline concentrations were robustly increased after Salmon PP ingestion only. Ingestion of Salmon PP and Milk PI transiently altered self-reported hunger and appetite sensations, and glucose and insulin concentrations to a similar extent. Collectively, these data suggest that the postprandial amino acid blood profile following Salmon PP ingestion has the potential to support the remodelling of skeletal muscle and connective tissue.

There is growing interest in the development of alternative protein sources that are both environmentally sustainable and high-quality with respect to the profile and postprandial bioavailability of amino acids for human tissue remodelling[38]. The amount of rest raw material from the fishing industry is

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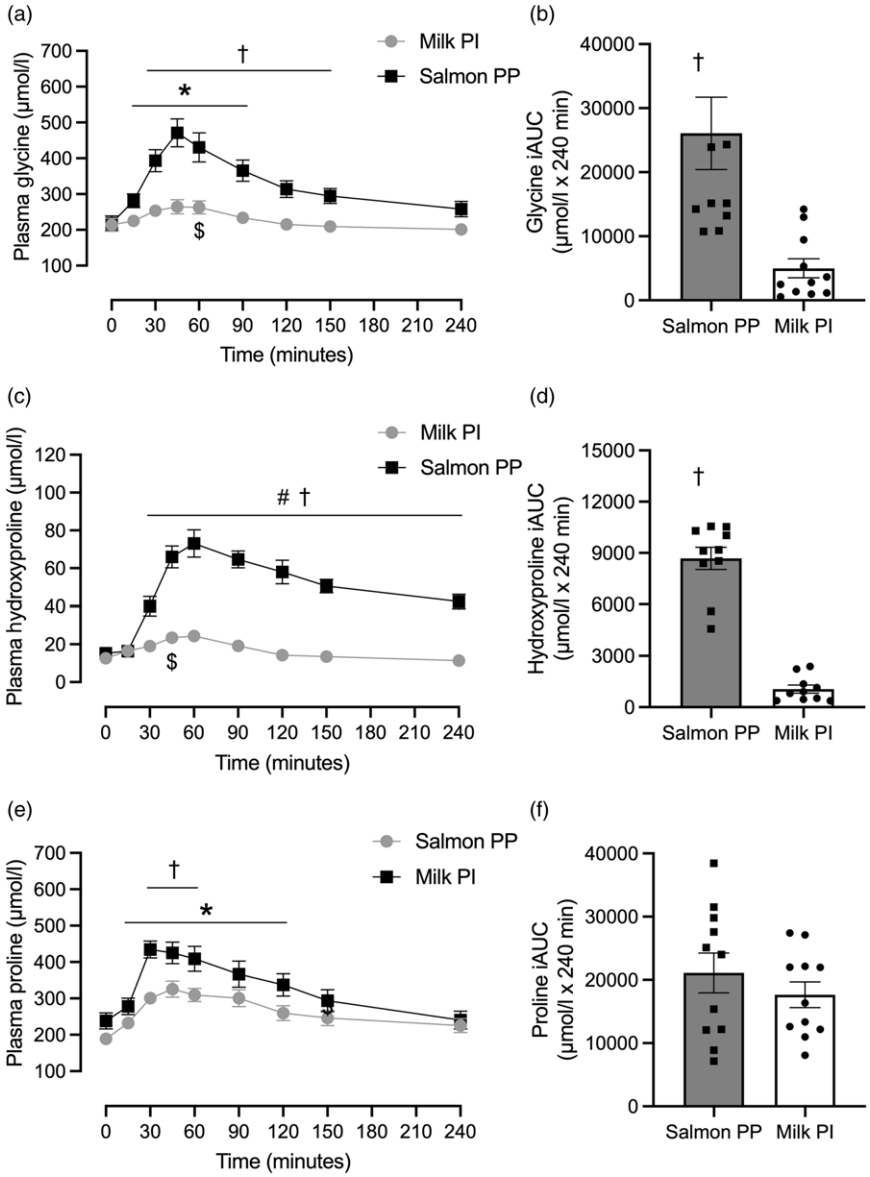

Fig. 4. Postprandial plasma amino acid responses to ingestion of salmon-derived peptide protein (Salmon PP; black) and milk protein isolate (Milk PI; grey) in young healthy adults. Time course and incremental AUC (iAUC) of plasma glycine (a), (b), hydroxyproline (c), (d) and proline (e), (f) concentrations for *n* 11 (*n* 10 for proline iAUC due to missing data for one participant). Data are presented as mean ± SEM and individual values. †A statistically significant difference between Salmon PP and Milk PI ($P < 0{\cdot}05$).

estimated to be about two-thirds of the overall amount of fish, causing a huge economic and environmental concern[18]. It is now possible to produce materials with high added nutritional value from these fish rest raw materials, creating a sustainable strategy towards a circular bioeconomy[18]. Fish-derived proteins and peptides have the potential to support muscle and connective tissue adaptive remodelling through their content and postprandial blood profile of EAA, glycine, proline and hydroxyproline[24]. To date, there is limited information on the plasma amino acid response to ingestion of protein sources produced from fish rest raw material, which may vary considerably based on the source and processing of fish and the procedures used for protein recovery[27]. In the present study, the temporal and iAUC TAA response over a 4-h postprandial period was greater in Salmon PP than Milk PI, whereas the temporal and iAUC plasma EAA response was equivalent between supplements. Given that total protein provided was equal, and the EAA content was about 1·5-fold greater in Milk PI compared with Salmon PP, we speculate that the rapid digestive properties of Salmon PP may explain the comparable EAA and greater TAA blood profile. Indeed, the production of Salmon PP involved enzymatic hydrolysis, the peptides from which contain hydroxyproline and proline, proposed to resist peptidase action to be absorbed intact[39]. Extending on these findings, oro-ileal assays and intrinsically labelled protein methods could provide insight into the 'true' protein digestibility and amino acid bioavailability of Salmon PP and novel marine-derived proteins more generally.

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Table 3. Dietary intake analysis

	Habitual		Pre-Salmon PP		Pre-Milk PI				
Dietary intake	Mean	SD	Mean	SD	Mean	SD	Salmon PP *v.* Milk PI	Hab *v.* Salmon PP	Hab *v.* Milk PI
Energy intake (kcal)	2549	649	2035	940	2155	732	$P = 0{\cdot}880$	$P = 0{\cdot}127$	$P = 0{\cdot}358$
Carbohydrates (g/kg/body mass)	3·94	1·19	3·66	1·50	3·79	1·42	$P = 0{\cdot}936$	$P = 0{\cdot}743$	$P = 0{\cdot}920$
Protein (g/kg/body mass)	1·68	0·44	1·30	0·66	1·34	0·73	$P = 0{\cdot}993$	$P = 0{\cdot}592$	$P = 0{\cdot}661$
Fat (g/kg/body mass)	1·35	0·43	0·95	0·56	0·98	0·44	$P = 0{\cdot}999$	$P = 0{\cdot}558$	$P = 0{\cdot}598$
Water (ml)	1652	710	1074	528	1311	1089	$P = 0{\cdot}686$	$P = 0{\cdot}032^{*}$	$P = 0{\cdot}417$
Dietary intake (buffet meal)			Salmon PP		Milk PI		Salmon PP *v.* Milk PI		
Energy intake (kcal)	–		1229	656	1201	393	$P = 0{\cdot}122$	–	–
Carbohydrates (g/kg/body mass)	–		2·48	1·12	2·34	0·52	$P = 0{\cdot}424$	–	–
Protein (g/kg/body mass)	–		0·60	0·24	0·59	0·19	$P = 0{\cdot}960$	–	–
Fat (g/kg/body mass)	–		0·46	0·25	0·51	0·24	$P = 0{\cdot}763$	–	–

Salmon PP, Salmon-derived peptide protein; Milk PI, milk protein isolate.
Data are presented as mean ± SD. Intake of macronutrients is expressed relative to participant body mass. A statistically significant difference in dietary variable from habitual values ($P < 0{\cdot}05$).

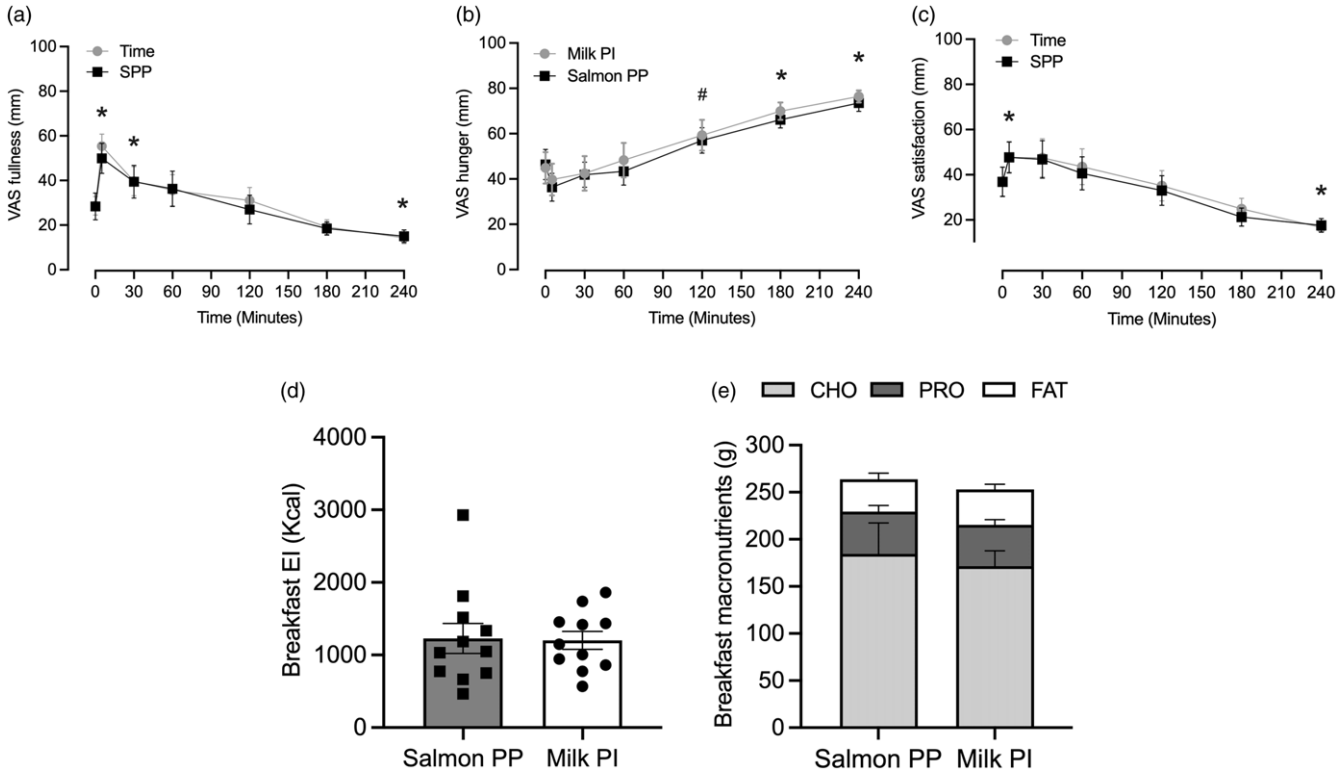

Fig. 5. Postprandial perceptions of fullness (a), hunger (b) and satisfaction (c) following ingestion of salmon-derived peptide protein (Salmon PP; black) and milk protein isolate (Milk PI; grey), and *ad libitum* total energy (d) and macronutrient (e) intake at the breakfast test meal (consumed 240 mins after Salmon PP/Milk PI ingestion) in young healthy adults for *n* 11. Data are presented as mean ± SEM and individual values (for breakfast energy intake only). *A statistically significant difference from 0-min fasted-state time point ($P < 0{\cdot}05$). #A statistically significant difference from 0-min fasted-state time point for Salmon PP only ($P < 0{\cdot}05$). $A statistically significant difference from 0-min fasted-state time point for Milk PI only ($P < 0{\cdot}05$). VAS, visual analogue scales.

Notwithstanding, given the prominence of EAA for postprandial MPS stimulation and the potency of Milk PI on MPS stimulation in younger adults[40,41], it is intuitive that the blood EAA profile achieved with Salmon PP ingestion would elicit a robust and perhaps maximal MPS response[42–44]. In support of this notion, Lees and colleagues[20] reported similar MPS stimulation in myotubes following *ex vivo* treatment with postprandial plasma obtained after ingestion of 0·33 g/kg/body mass blue-whiting and whey protein hydrolysates, despite lower plasma EAA and leucine with blue-whiting protein.

Leucine is known to upregulate intracellular signalling intermediates in the mechanistic target of rapamycin pathway

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for MPS stimulation[45]. Hence, the postprandial MPS response to protein ingestion has been largely attributed to the ingested dose and blood profile of leucine, such as the peak magnitude, rate of rise and total availability of plasma leucine[46–48]. In the present study, despite a comparable plasma EAA response between supplements, the iAUC plasma leucine response to Salmon PP was lower than Milk PI ingestion. The discrepant plasma EAA and leucine iAUC responses may be due to the large > 2-fold difference in leucine content between Salmon PP and Milk PI, which was greater in magnitude than the difference in summed EAA (about 1·5-fold) between supplements. This discrepancy may also be explained by specific differences in the splanchnic extraction of divergent peptides between Salmon PP and Milk PI. Irrespective, the peak magnitude of plasma leucine did not differ between supplements, and the rate of rise (or time-to-peak) was more rapid in Salmon PP. Interestingly, recent evidence suggests that although the blood leucine profile may be a determinant of the MPS response to isolated protein sources, this may only be pertinent in older adults[48,49]. Moreover, a maximal postprandial MPS response to protein ingestion appears to be achievable in young individuals irrespective of leucine content of a protein source, or blood profile upon ingestion[49]. Thus, whilst leucine content and ensuing blood variables may need to surpass a given threshold to maximise postprandial MPS stimulation, this may be lower (or saturated earlier) than first thought, particularly if abundant EAA are available to support MPS[42,44]. As such, in healthy younger adults under non-exercised conditions, we suggest the EAA response to isolated supplemental Salmon PP and Milk PI would be the primary determinant for postprandial MPS, although this requires further investigation.

Fish rest raw materials used in protein production (e.g. heads, trimmings, skin, scales and backbones) are typically rich in connective tissue and contain a significant amount of collagen that can vary amongst fish species[22,24]. Whilst high-quality, rapidly digestible proteins are generally preferable for maximal MPS stimulation, initial studies suggest that connective tissue protein synthesis remains largely unaltered[50,51]. This is an important consideration as muscle connective tissue (extracellular matrix) plays a crucial role in contractile force transmission to tendon and bone[24] and is in a constant state of turnover that could be influenced by nutritional factors. Fish-derived proteins contain collagen, which is a rich source of glycine, proline and hydroxyproline with purported anabolic properties for connective tissue remodelling[52,53]. In the present study, the peak magnitude and total availability (iAUC) of glycine, proline and hydroxyproline were markedly increased following Salmon PP ingestion, and very low or completely absent following Milk PI ingestion. These findings are in agreement with earlier observations that oral ingestion of fish-derived collagen hydrolysates results in a dose-dependent rapid increase in hydroxyproline-containing peptide concentrations in plasma[54]. The present study also expands on earlier characterisations of postprandial amino acid availability following fish-derived protein ingestion, where the blood responses of these NEAA were not considered[20]. The combined temporal and iAUC blood amino acid concentrations, and specific glycine, proline and hydroxyproline profiles reported herein, position Salmon PP as a novel sustainable ingredient with the potential to support muscle and connective tissue remodelling. In light of recent work demonstrating no effect of pure collagen supplementation (very low EAA content) on muscle connective tissue protein synthesis during acute recovery from resistance exercise[55], an important next step is to understand how Salmon PP ingestion might influence these parameters.

An increase in postprandial plasma amino acid concentrations has been linked to increased satiety with protein ingestion (through alterations in appetite hormones)[56]. In animals, leucine can stimulate satiety and reduce food intake via a central mechanism[57]. Therefore, differences in postprandial plasma TAA and leucine concentration between Salmon PP and Milk PI ingestion may influence satiety and subsequent energy intake, as shown in comparisons between other isolated protein sources with divergent AA profiles[56,58,59]. However, despite differences in TAA and leucine blood profiles with Salmon PP and Milk PI, changes in perceived appetite sensations and subsequent *ad libitum* energy (and macronutrient) intake were indistinguishable between Salmon PP and Milk PI, along with comparable insulin and glucose responses. The findings show that beverage blinding was successful and peak perceived appetite ratings were evenly balanced across trials (data not reported), suggesting that correct beverage identification on had little impact on subjective ratings. Congruent with these observations, others have failed to detect differences in perceived appetite or *ad libitum* energy intake between protein sources that elicit divergent postprandial plasma total, EAA and/or leucine profiles[60,61]. We acknowledge that by assessing *ad libitum* energy intake 240 min after protein ingestion, when high hunger levels had been reached, potential differences between protein sources may have been missed. Finally, the appetite-lowering effect of acute protein intake may differ in longer-term regimens, where changes in energy intake are multifaceted[62]. Therefore, the potential long-term influence of Salmon PP on appetite and energy intake warrants further investigation.

Whilst present study is the first to demonstrate blood aminoacidemia and appetite responses to a novel fish-derived protein peptide, there are several limitations that should be acknowledged. First, we were unable to analyse concentrations of appetite regulatory hormones to determine the mechanisms of Salmon PP- and Milk PI-induced satiety. This is important as the appetite-regulatory hormonal responses to ingestion of different protein sources are unclear and there is a reported discordance between appetite-regulatory hormone concentrations in blood and energy intake in humans[29]. Second, in female participants, we did not monitor menstrual cycle phase, nor were trials scheduled to fall on the same menstrual cycle phase. Given that menstrual cycle phase has been suggested to influence subjective ratings of appetite and energy intake[63], consideration of this variable is necessary in future studies. Similarly, it possible that the palatability of treatments may have influenced appetite outcomes[64], although this was not measured. However, our blinding protocol was successful, and treatments were indistinguishable, (described in results) suggesting there was no consistent and detectable fish aftertaste with Salmon PP that would influence appetite regulation and energy intake.

In conclusion, the findings of the present study suggest that the content and bioavailability of plasma EAA from a novel

protein peptide developed from fresh salmon rest raw materials was equivalent to a high-quality Milk PI and has the potential to support skeletal muscle adaptive remodelling. This warrants further investigation as the net plasma leucine response to Salmon PP was lower than Milk PI, which may limit the capacity for maximal postprandial MPS stimulation in some scenarios. Additionally, the ingestion of Salmon PP only resulted in a robust increase in plasma glycine, proline and hydroxyproline, which may have implications for the adaptive remodelling of connective tissue. Finally, Salmon PP ingestion resulted in similar transient alterations in appetite sensations and *ad libitum* energy intake to Milk PI and may be a suitable protein source to support appetite regulation.

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