Journal of Functional Foods 102 (2023) 105452

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Aminoacidemia after ingestion of protein hydrolysate produced from poultry carcasses: A comparison against whey protein in a randomized, double-blinded cross-over study in healthy young and old individuals

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ARTICLE INFO

Keywords:
Hydrolyzed protein
Amino acid kinetics
In vitro digestion
Bioavailability
Elderly
Leucine

ABSTRACT

This study investigated the aminoacidemia after ingestion of a poultry protein hydrolysate (PPH) and whey protein in healthy young and old participants. Protein-drinks were also digested using the INFOGEST static *in vitro* digestion model to simulate gastrointestinal changes in young and old adults. In fasted state, 10 young (20-40y) and 10 old (70-80y) ingested PPH or whey as a 20 g protein-drink and blood samples were collected. Plasma leucine concentration increased more when ingesting whey than PPH (young 62 ± 27 vs. 48 ± 27%, old 94 ± 57 vs. 66 ± 26%) but the peak concentration was reached faster after drinking PPH ($p < 0.05$). The *in vitro* digestion of PPH was consistent with the observed changes in plasma amino acid concentrations, but whey digestibility was lower under ageing conditions. These results show that PPH is rapidly digested and absorbed due to the hydrolysis into short peptide chains, and that healthy elderly have similar absorption as younger individuals.

1. Introduction

In order to produce sustainable but still high-quality nutritious products for humans, the meat industry is developing new products from side-streams previously used for production of animal feed. One such product is a protein hydrolysate produced from chicken (and turkey) carcasses by using a mild enzymatic protein hydrolysis process. Poultry carcasses consists of large amounts of bones and connective tissue in addition to the small amount of remaining meat (Lindberg et al., 2021). Consequently, the proportion of essential amino acids is lower in protein from poultry carcasses compared to other high-quality protein products and the bioavailability may be lower. Processing of the rest raw material with methods like e.g., enzymatic protein hydrolysis, may improve the bioavailability and the overall quality of the protein extract.

Ingestion of dietary proteins leads to an acute increase in blood concentration of amino acids, called aminoacidemia. The aminoacidemia of essential amino acids (EAA) in blood and tissue is especially important because acute changes in these amino acids influence several important biological systems, e.g. it stimulates the muscle protein synthesis (Phillips, Hill, & Atherton, 2012). Therefore, an early aminoacidemia of EAA is regarded as an indication of high efficacy for the specific protein-source ingested (Burke et al., 2012). The rate of absorption of amino acids into the blood is modulated by the degree of hydrolysis of the ingested protein (Koopman et al., 2009; Meyer, Foong, Thapar, Kritas, & Shah, 2015; Pennings et al., 2011), the overall ash content (Thuy, Lam, & Commick, 2015), and type of protein source (Paddon-Jones et al., 2015). E.g., when a normally slow digestive protein source such as casein is hydrolyzed to smaller peptides, the absorption rate may increase substantially (Koopman et al., 2009), although not reaching same absorption rate as observed with ingestion of whey in elderly (Pennings et al., 2011). The digestion and absorption of amino acids seem to be slower in elderly compared to young adults (Condino et al., 2013; Milan et al., 2015). Consequently, the use of enzymatic hydrolysis to improve digestion and absorptions rates by

Abbreviations: ANOVA, Analysis of variance; AUC, area under the curve; BCAA, Branch-chained Amino Acid; C_{max}, peak concentrations; PPH, Poultry protein hydrolysis; Dtd, Dated; EDTA, Ethylene Diamine Tetraacetic Acid; EAA, Essential Amino acid; g, gram; GC, Gas Chromatography; Mg, milligram; mL, milliliter; mM, millimolar; MPS, Muscle Protein Synthesis; mU, milliunit; $NaHCO_3$, rpm, revolutions per minute, Sodium bicarbonate; pH, power of hydrogen; SGF, gastric fluid; SIF, intestinal fluid; SIM, Selective Ion Monitoring; SSF, simulated salivary fluid; T_{max}, time to peak concentrations; U/mL, Units per milliliter; WPC80, Whey Protein Concentrate 80; μmol/l, micromole/liter.

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https://doi.org/10.1016/j.jff.2023.105452
Received 20 October 2022; Received in revised form 30 January 2023; Accepted 5 February 2023
Available online 15 February 2023

increasing the peptide content (Raastad, Vagle, & Framroze, 2017), could consequently lead to a rapid aminoacidemia, and further increase the protein source efficacy which may be especially beneficial for the older population in order to obtain a similar uptake as the young population.

The amount of ingested protein, the amino acid composition, the proportion of short peptides and the digestion and absorption rate of the amino acids are all important factors determining the final protein-source efficacy (Burke et al., 2012). For the stimulation of muscle protein synthesis, the acute increase in blood concentration of leucine (leucinemia), seems to be of special importance, as it even with small doses acts as a metabolic trigger of muscle protein synthesis, which has led to the "leucine-trigger concept" (S. M. Phillips, 2014; Wilkinson et al., 2013). Even for older individuals, ingestion of high doses of leucine (>3g) are enough to overcome anabolic resistance that follows with aging, and result in similar responses in muscle protein synthesis as younger individuals (Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2006; Landi et al., 2016; Mitchell et al., 2016). Nonetheless, it is unclear whether a protein hydrolysate made from poultry carcasses can match the large leucinemia observed after whey protein ingestion (Hamarsland et al., 2017).

The amount of essential amino acids is lower in the poultry protein hydrolysate compared to whey, with leucine and BCAA content amounting down to 50–70 % of that of whey. It is therefore questionable whether the moderate hydrolysis of poultry carcass protein improves the amino acid absorption in the elderly in such a way that it results in similar bioavailability as whey. In a different perspective, when considering the higher content of glycine in carcass hydrolysates, this could have other positive effects like preserving protein synthesis, preventing skeletal muscle waist, increasing collagen synthesis or inhibiting inflammatory cell activation (Adeva-Andany et al., 2018; Koopman, Caldow, Ham, & Lynch, 2017).

Aminoacidemia is the result of the digestion- and absorption rates of the ingested protein source, the degree of splanchnic extraction, as well as the rate of amino acid disappearance from the circulation. Because *in vivo* measures of digestion are challenging, an *in vitro* digestion model is often used as a standardized method to evaluate the protein source efficacy (Butts, Monro, & Moughan, 2012; Minekus et al., 2014). We hypothesized that having smaller peptides in the poultry protein hydrolysate would speed up digestion and lead to a faster aminoacidemia than whey; especially in elderly. The main goal of the present study was therefore to compare the aminoacidemia, and particularly leucinemia and glycinemia, after ingestion of an equal amount of protein from poultry protein hydrolysate against whey protein concentrate in healthy young and old individuals.

Further, we wanted to investigate the digestibility of the two protein sources using the INFOGEST static *in vitro* digestion model (Brodkorb et al., 2019; Minekus et al., 2014) to mimic the changes in digestive function that may occur during aging.

2. Material and methods

2.1. Protein sources

The protein products investigated were Nor-Hydropep 90 SD, a poultry protein hydrolysate (PPH), produced and delivered by Norilia AS and a reference whey protein concentrate 80 produced by Star Nutrition (WPC80) which was purchased commercially. The amino acid composition and proximate data for the products are given in Table 1, which information was provided by Norilia for the PPH and by the commercial vendor for WPC80.

The protein content in the PPH was measured by Norilia using the Kjeldahl method (Kjeldahl, 1883). The protein proportions in the powders were 82% and 73% for PPH and WPC80, respectively, and a total dose of 24.45 g and 27.25 g were used from the respective powders to get ~ 20 g protein in each drink.

Table 1
Amino acid composition in gram and proximate data per drink in the two protein products tested in this study, and a non-statistical ratio of content in PPH in % of WPC80. Protein content is summated from the reported amino acid content.

Per serving (~20 g protein)			
Amino acid content	**PPH (g)**	**WPC80 (g)**	**PPH / WPC (%)**
Arginine	1.26	0.52	242 %
Alanine	1.64	0.98	167 %
Aspartic acid	1.64	2.04	80 %
Cysteine + cystine	0.10	0.33	30 %
Glutamine + Glutamic acid	3.06	3.41	90 %
Glycine	2.23	0.38	587 %
Histidine	0.46	0.35	131 %
Isoleucine	0.69	1.25	55 %
Leucine	1.52	2.10	72 %
Valine	0.71	1.17	61 %
Lysine	1.54	1.80	86 %
Methionine	0.46	0.44	105 %
Phenylalanine	0.69	0.68	101 %
Proline	1.27	1.20	106 %
Serine	0.71	1.06	67 %
Threonine	0.78	1.39	56 %
Tryptophan	0.10	0.38	26 %
Tyrosine	0.37	0.63	59 %
Hydroxyproline	0.85	<0.0001	–
Proximate data			
Essential amino acids (g)	6.95	9.56	73 %
Total Protein (g)	20.09	20.11	99.9 %
Free amino acids (g)	2.15	–	–
Peptide content (%)	82 %	10–15 %	
Degree of hydrolysis	18 %	–	
Collagen (g)	7.1	–	
Fat (g)	0.98	1.85	53 %
Ash (g)	1.96	1.09	180 %
Carbohydrate (g)	–	1.47	

Analysis of molecular weight distribution in PPH was performed by HPLC (1260 series HPLC, Agilent Technologies, Santa Clara, CA, USA) using size exclusion chromatography (Wang-Andersen & Haugsgjerd, 2011), which is described elsewhere (Oterhals & Samuelsen, 2015). The assumption of peptides containing<50 amino acids was used by the vendor to calculate a 10–15 % peptide content in WPC80. The same assumption was applied to the analyzes of peptides in PPH, amounting to an 82 % peptide content in PPH. The degree of protein hydrolysis in PPH was determined by using the o-phthaldialdehyde (OPA) method, as described elsewhere (Aspevik, Egede-Nissen, & Oterhals, 2016), which resulted in a 18 % degree of hydrolysis in PPH.

2.2. In vitro digestibility

The protein sources were subjected to a static *in vitro* digestion model simulating the oral-, gastric- and duodenal phases. The model was based on the INFOGEST digestion protocol with standardized electrolyte solutions for the preparation of simulated salivary fluid (SSF), gastric fluid (SGF) and intestinal fluid (SIF) (Brodkorb et al., 2019; Minekus et al., 2014). To simulate the digestive process in adults, samples (1.0 mL) were added 2 mL of SSF and kept at 37 °C for 2 min. The gastric phase was simulated by adding 4.0 mL of SGF containing pepsin (4000 U/mL) (P7000, Sigma-Aldrich Co, USA). The pH was adjusted to 3.0 before incubation in a rotary incubator (Innova® 40/40R, New Brunswick Scientific, Edison, NJ, USA) at 37 °C and 215 rpm for 120 min. To simulate the intestinal phase, samples were added 8 mL of SIF containing 0.07 mM $NaHCO_3$, porcine bile (20 mM) (B8381, Sigma-Aldrich Co, USA) and pancreatin (2.50 mg/mL) (P1750, Sigma-Aldrich Co, USA), followed by adjustment of the pH to 7 and further incubation in the rotary incubator at 37 °C and 215 rpm. This model (referred to as "young") resulted in a final trypsin activity of 10 U/ml in the intestinal phase which is less than recommended by INFOGEST (100 U/ml).

However, the lower pancreatin concentration had only minor impact on *in vitro* protein digestibility (unpublished results), while high amounts of background protein from commercial pancreatin was avoided. A similar digestion model (referred to as "old") was used to mimic the digestive process in older adults based on literature stating that the decline in digestive functions during aging includes changes in secretion and composition of saliva, less gastric fluid (higher gastric pH and reduced pepsin levels), and lowered bile and reduced levels of pancreatic enzymes (Rémond et al., 2015; Shani-Levi et al., 2017). In the present project the digestive process in older adults was simulated by lowering the level of pepsin in gastric phase to 1000 U/mL SGF while increasing the pH to 4.5 and lowering the concentrations of bile and pancreatin in SIF to 10 mM and 1.0 mg/ml SIF, respectively, resulting in a final concentration of 5 mM bile and 4 U/ml trypsin activity in intestinal phase. All *in vitro* digestion experiments were performed in triplicates and samples were withdrawn from the intestinal phase after 30 min, 80 min and 160 min for analysis of free amino acids at VITAS-Analytical Services. Blank samples (water) were included in the experiments to estimate the contribution of free amino acids from digestive fluids, and all measurements were corrected for the background (reagent control).

2.3. Feeding study design and protocol

The study had a randomized double-blinded cross-over design. All subjects met in the lab on two different test days and ingested one of the two protein drinks at each visit in a randomized order. There was a wash-out period of a minimum of 48 h between each trial. Both protein products were given as ~ 20 g protein doses dissolved in 200 mL water. In addition, the subjects drank water ad libitum after consuming the protein drinks. We included participants in the age between 20 and 40 years old for the younger group and 70–80 years old for the older group. A health screening was performed for each participant, and they were excluded if they had any conditions that could affect the digestive and absorption system which would define them as unhealthy in this context. Written informed consent was obtained from all participants before the start of the study. All procedures and methods used in this study has been evaluated by and approved by the Norwegian School of Sport Sciences Ethical Committee, reference number 159–240920, dtd 09/25/20, as well as by the Norwegian center for research data, reference number 598443, dtd 10/14/20.

A total of ten young (24–33 y) and ten old (71–80 y) healthy, physically active males and females completed the study (Table 2). At each test day, the subjects met in the lab individually planned in between 07:00 to 09:00 am after an overnight fast. After 5–10 min relaxation in the lab, the first blood sample was collected from an antecubital vein. Thereafter the protein drink was ingested. The entire drink had to be finished within 5 min (3–5 min). Thereafter blood samples were collected at 20, 30, 45, 60 and 90 min in order to follow the blood response for glucose, insulin and amino acids (Fig. 1).

Blood samples were drawn into one serum and one EDTA Vacutainer tube at each collection. EDTA tubes were centrifuged right after blood samples were drawn whereas the serum clotted in room temperature for 30–40 min before centrifugation. Centrifugation was done at 3400 rpm for 10 min at 20 °C. After centrifugation, the serum tubes were analyzed

Table 2
Subject characteristics given as mean ± standard deviation. * = significant difference between young and old ($p < 0.01$).

Subjects	Young	Old
N=	10 (m = 7, f = 3)	10 (m = 7, f = 3)
Age (y)	28.1 ± 3.1	75.6 ± 3.4
Height (m)	1.80 ± 0.10	1.79 ± 0.10
Body mass (kg)	74.5 ± 7.6	75.1 ± 11.2
BMI (kg/m^2)	22.9 ± 1.3	23.4 ± 11.2
Cardio exercise (min/week)	232 ± 17	82 ± 73*
Strength exercise (min/week)	112 ± 133	79 ± 50

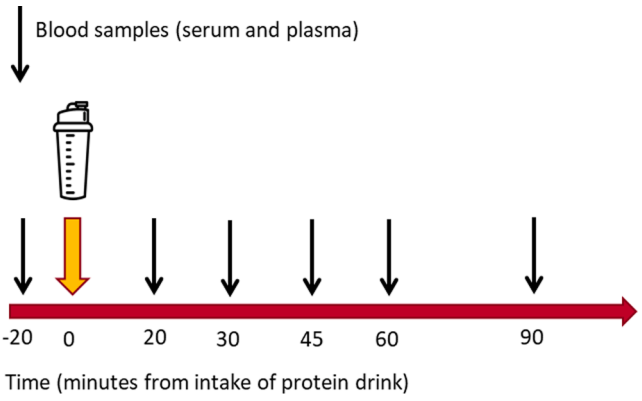

Fig. 1. Timeline showing the timing of the blood samples after ingestion of protein drinks. The sample protocol was repeated for both protein drinks on separate days with a washout period of minimum 48 h between trials.

for insulin and glucose at Fürst laboratories. The plasma from the EDTA tubes were transferred into new tubes and frozen at −20 °C for later analysis. When the data collection was completed, the plasma tubes were analyzed for amino acids profile at VITAS-Analytical Services.

2.4. Analyzes

Blood serum was analyzed at Fürst laboratories (Oslo, Norway) for insulin concentration (ADVIA Centaur® XPT Insulin-analysis, Siemens Healthcare Diagnostics, Germany) with measurement range 0.5–300 mU/L and for glucose concentration (ADVIA Chemistry XPT® GLUH_c Assay, Siemens Healthcare Diagnostics, Germany) with measurement range 0.2–38.9 mmol/l.

The following amino acids were analyzed in plasma and *in vitro* gastrointestinal juice at VITAS-Analytical Services (Oslo, Norway); histidine, isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, tryptophan, alanine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine and tyrosine. Ten µl plasma sample was diluted with water and propanol. A mix of stable isotope labeled amino acids was added and used as internal standards. Samples were derivatized using propyl chloroformate and extracted into isooctane before analysis by GC-MS. Instrumental analysis was performed on an Agilent 6890 GC system with a split/split-less injector and a 5973 N mass selective detector operated in SIM mode (Agilent Technologies, Palo Alto, CA, USA). Separation of amino acids were performed on a Zebron ZB-AAA analytical column (Phenomenex, Torrance, CA, USA). Calibration was performed using five-point calibration curves for each analyte.

2.5. Statistical analyzes

All statistical analyzes were completed in Prism 8 (GraphPad Prism, version 8.2.1 (441), GraphPad Software Inc., San Diego, CA, USA).

To analyze *in vitro* concentrations of amino acids, unpaired Welch's *t*-test was used to compare differences in the area under the curve (AUC) between the "young" and "old" *in vitro* digestion condition.

Two-way repeated measures ANOVA with Bonferroni post hoc test was used to analyze the *in vivo* time change in blood concentrations for the younger and older group following ingestion of either PPH or WPC80, whether there was a different relative time change between the younger and older group after drinking either protein drinks and to evaluate differences in blood concentrations between protein drinks for both groups. Furthermore, we used the two-way ANOVA to analyze differences between protein drinks for the younger and older group separately, and between groups for each protein drink separately, in peak concentrations (C_{max}), time to peak concentrations (T_{max}), and AUC.

The *in vitro* digested and the *in vivo* measured amino acid

concentrations were not compared directly using statistical methods.

3. Results

All factor and interaction effects from the comparisons in glucose, insulin and plasma concentrations, as well as the p-values from comparisons in *in vitro* digesta, are shown in Table 3

3.1. Changes in glucose and insulin concentrations

There was a time effect ($p < 0.001$, Fig. 2C-D) in glucose concentration after ingestion of the two protein drinks. Glucose concentration was reduced after 45 min for the younger group (CHP: −13 ± 0%; WPC80: −21 ± 14%; $p < 0.001$) and the older group (CHP: −11 ± 0%; WPC80: −10 ± 14%; $p < 0.01$) and was back to baseline after 90 min for both groups. The younger group had an interaction effect between protein drink and time ($p < 0.05$), with a significant larger reduction in glucose concentration 45 and 60 min after WPC80 ingestion ($p < 0.05$). There was no difference in glucose concentration between young and old participants after ingestion of either protein drink.

The changes in insulin concentration resulted in a time effect ($p < 0.001$) and interaction effect between protein source and time ($p < 0.05$) for both groups, and for protein source for the older group ($p < 0.001$). Insulin serum concentration was increased for the younger group 20 min after ingestion, 234 ± 130% of PPH ($p < 0.001$) and 305 ± 177% for WPC80 ($p < 0.001$), without any significant difference between the protein drinks (Fig. 2A). The increase in insulin concentration for the younger group was back to baseline 45 min after drinking PPH and 90 min after drinking WPC80. There was a 443 ± 117 % increase in insulin concentration for the older group 20 min after drinking WPC80 ($p < 0.01$) which returned to baseline after 60 min (Fig. 2B). There was no change in insulin concentration for the older group when drinking PPH, which led to a significant lower response than WPC80 at 20, 30, 45 and 60 min after ingestion ($p < 0.05$). An age effect and interaction effect between age and time was found when drinking WPC80 ($p < 0.01$), where post hoc test resulted in significantly higher increase in insulin concentration for the older group 60 and 90 min after drinking WPC80 compared to the younger group ($p < 0.05$).

3.2. In vitro digestion model

In vitro digestion of WPC80 resulted in 46% and 38% higher AUC concentration in the young model compared to the old model for leucine (Fig. 3D, $p < 0.001$) and phenylalanine (Fig. 4D, $p < 0.001$), respectively. Higher AUC concentrations were also observed in the young compared to old model when digesting WPC80 for the concentration of lysine, methionine, threonine, tryptophan, glutamic acid, tyrosine, as well as total BCAA (Supplementary Fig. 5D), total EAA (Supplementary Figure 6D) and TAA (Supplementary Figure 7D). The old model resulted in higher AUC concentrations for glycine (Supplementary Fig. 2G), histidine, aspartic acid and proline compared to the young model.

The *in vitro* digestion of PPH resulted in higher AUC concentration in the old, compared to the young (Table 3), model for isoleucine (19%, $p < 0.05$), valine (15%, $p < 0.05$), histidine (35%, $p < 0.01$), tryptophan (26%, $p < 0.05$), tyrosine (19%, $p < 0.05$) and glutamic acid (19%, $p < 0.05$). The *in vitro* digestion showed no differences between the young and old model for isoleucine, valine, alanine, cysteine and serine when digesting WPC80, and for leucine, lysine, methionine, threonine, phenylalanine, alanine, aspartic acid, cysteine, proline, serine, glycine, BCAA, EAA and TAA when digesting PPH (Fig. 3C,D; Fig. 4C,D; Supplementary Fig. 2G; Supplementary Fig. 5C,D; Supplementary Figure 6C,D; Supplementary Figure 7C,D; Table 3).

3.3. Changes in plasma amino acid concentration

Ingestion of both protein drinks resulted in increased blood plasma concentration of leucine (PPH: young 48 ± 27%, old 66 ± 26%; WPC80: young 62 ± 27%, old 94 ± 57%; Fig. 5A, B; $p < 0.001$). Intake of WPC80 increased the leucine concentration more than PPH at 30, 45, 60 and 90 min after intake ($p < 0.001$). Compared to PPH, intake of WPC80 further resulted in a higher leucine C_{max} (Supplementary Fig. 2A; $p < 0.001$), higher leucine C_{max} in % of baseline (Supplementary Fig. 2D; $p < 0.001$), a slower T_{max} leucine concentration (Supplementary Fig. 4A; $p < 0.05$) and a greater area under the curve (Fig. 3A, D; $p < 0.0001$) for both groups). The same pattern was observed for BCAA, EAA and TAA (Supplementary Figs. 3 and 4).

The opposite pattern was observed for the plasma glycine concentration, where an increase in glycine was seen 20 min after ingestion of PPH for both groups (young: 18 ± 15%, Fig. 5C, $p < 0.01$; Old: 23 ± 17%, Fig. 5D, $p < 0.001$), whereas there was no change after ingestion of WPC80. The increase in glycine concentration after intake of PPH caused a significant different change between the two protein drinks at all time points ($p < 0.05$). Compared to WPC80, plasma glycine concentration after intake of PPH resulted in higher C_{max}, higher C_{max} in % of baseline and a greater AUC (Supplementary Fig. 2C, E, G; $p < 0.001$) for both groups.

Ingestion of both protein drinks resulted in increased blood plasma concentration of phenylalanine (PPH: young 19 ± 9%, old 24 ± 21%; WPC80: young 21 ± 13%, old 29 ± 16%; $p < 0.01$). Intake of WPC80 increased the phenylalanine concentration more than PPH at 30, 45, 60 and 90 min for the older group, and at 30 min for the younger group, after intake ($p < 0.01$). Compared to PPH, plasma phenylalanine concentrations after intake of WPC80 had a higher C_{max} (Supplementary Fig. 2B; $p < 0.05$) and higher C_{max} in % of baseline (Supplementary Fig. 2E; $p < 0.05$) for the older group and a greater AUC (Fig. 4A, B; $p < 0.01$) for the younger and older group (Supplementary Fig. 2B, E and Fig. 4A, B).

For the remaining amino acids, we observed in general that the change in blood concentration reflected the amino acid content of the two protein drinks apart from alanine and cysteine, having similar peak concentration for both groups after intake of both protein drinks, and for aspartic acid and glutamic acid, having higher peak concentration for the younger group after intake of PPH (Supplementary Table 1). Compared to PPH, intake of WPC80 resulted in higher plasma Cmax and AUC in isoleucine ($p < 0.001$), valine ($p < 0.001$), lysine ($p < 0.05$), methionine ($p < 0.01$), threonine ($p < 0.001$), tryptophan ($p < 0.001$), and tyrosine ($p < 0.01$) for both groups and aspartic acid for the older group ($p < 0.001$), higher AUC for serine ($p < 0.05$), aspartic acid ($p < 0.001$) and glutamic acid ($p < 0.01$) in the younger group , and no difference between protein drinks in alanine, cysteine, histidine and proline for both groups.

When drinking WPC80, the relative change in leucine and BCAA concentration was significantly higher for the old, compared to the younger, group after 45, 60 and 90 min ($p < 0.05$) and the change in EAA concentration was significantly higher for the old, compared to the younger, group after 45 and 90 min ($p < 0.05$). There was no different C_{max}, T_{max} or AUC between the younger and older group, but we observed a significantly higher leucine ($p < 0.001$), BCAA ($p < 0.001$) and EAA ($p < 0.01$) C_{max} in % from baseline for the old, compared to the younger, group after ingesting WPC80.

For the remaining amino acids we observed a higher plasma C_{max} for isoleucine ($p < 0.01$), higher plasma C_{max} in % from baseline for isoleucine ($p < 0.01$), valine ($p < 0.01$), methionine ($p < 0.05$), threonine ($p < 0.05$), aspartic acid ($p < 0.01$) and proline ($p < 0.01$), faster T_{max} for histidine ($p < 0.01$) and higher plasma AUC for isoleucine ($p < 0.05$) for the old, compared to the younger, group when ingesting WPC80, as well as a faster T_{max} for aspartic acid when ingesting PPH ($p < 0.05$). When ingesting either WPC80 or PPH, the younger group had higher plasma C_{max} and AUC in histidine compared to the older group ($p < 0.001$).

Table 3
Statistical analyzes of changes in plasma and *in vitro* digesta amino acid concentration. Factor and interactions effect are shown from two-way ANOVA used for the *in vivo* comparisons and p-values from the Welsh's t-tests are shown for the *in vitro* comparisons. Statistical p-values; *=p < 0.05, **=p < 0.01, ***p < 0.001.

				Glucose	Insulin	Leucine	Isoleucine	Valine	BCAA	Histidine	Lysine	Methionine	Threonine	Tryptophan	Phenylalanine	EAA	Alanine	Asparticacid	Cysteine	Glutamicacid	Proline	Serine	Tyrosine	Glycine	TAA
In vivo	Young	Time		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0.808	***	***	***	***	***	***
		Protein source		*	*	***	***	***	***	0.889	**	**	**	***	**	***	0.609	**	0.459 t	***	*	*	**	***	***
		Time × Protein source		*	*	***	***	***	***	0.080	***	***	***	***	***	***	*	***	0.785	*	*	*	***	***	***
	Old	Time		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0.683	***	***	***	***	***	***
		Protein source		0.708	*	***	***	***	***	0.778	**	**	***	***	**	***	0.878	***	0.193	0.203	0.582	0.297	**	***	***
		Time × Protein source		0.141	*	***	***	***	***	*	***	***	***	***	***	***	**	***	0.190	0.107	***	**	***	***	***
	PPH vs WPC80	Young % change	Time	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0.632	***	***	***	***	***	***
			Protein source	0.173	0.335	***	***	***	***	0.614	***	**	***	***	**	***	0.429	***	0.836	0.506	0.2563	**	***	***	**
			Time × Protein source	*	0.251	***	***	***	***	0.104	***	**	***	***	***	***	*	***	0.640	*	0.055	*	***	***	***
		Old % change	Time	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0.624	***	***	***	***	***	***
			Protein source	0.510	***	***	***	***	***	0.646	**	**	***	***	**	***	0.231	***	**	0.923	**	*	***	***	**
			Time × Protein source	0.177	***	***	***	***	***	*	***	**	***	***	***	***	**	***	0.326	0.168	*	**	***	***	***
	Young vs Old	PPH % change	Time × Age	0.902	0.882	*	0.092	0.383	0.152	0.493	0.414	0.633	0.375	0.419	0.451	0.254	0.755	0.257	0.870	0.150	*	0.774	0.646	0.681	0.254
			Time	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0.298	***	***	***	***	***	***
			Age	0.877	0.865	*	*	0.084	*	0.663	0.072	0.140	0.085	0.313	0.509	0.057	0.892	0.893	0.234	0.996	*	0.966	0.221	0.852	0.057
		WPC80 % change	Time × Age	0.089	***	***	**	***	***	0.483	0.076	0.088	0.057	0.26	*	**	0.967	**	0.786	0.774	***	0.917	*	0.72	**
			Time	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0.324	***	***	***	***	***	***
			Age	0.146	**	**	*	**	**	0.682	*	*	0.062	0.318	*	**	0.949	*	0.376	0.591	**	0.911	0.071	0.711	**
		C_{max}	Protein × Age			0.885	*	0.553	0.100	0.757	0.294	0.746	0.236	0.541	0.462	0.161	0.887	0.120	0.337	0.655	0.735	0.881	0.984	0.360	0.839
			Protein			***	***	***	***	0.483	***	***	***	***	**	***	0.627	***	0.920	*	*	*	***	***	***
			Age			0.968	*	0.716	0.445	***	0.248	0.223	0.685	0.495	0.958	0.593	0.125	0.835	0.224	0.904	0.424	0.600	0.058	0.107	0.322
		C_{max} % change	Protein × Age			*	0.057	*	*	0.944	0.356	0.250	0.186	0.439	0.168	0.076	0.765	*	0.068	0.567	0.072	0.864	0.107	0.740	0.631
			Protein			***	***	***	***	0.512	***	***	***	***	**	***	0.205	***	0.186	0.809	*	**	***	***	**
			Age			0.001	**	*	**	0.918	*	0.052	*	0.242	0.149	*	0.739	0.056	0.849	0.225	0.010	0.854	0.141	0.790	0.481
		T_{max}	Protein × Age			0.905	0.919	0.066	0.562	0.173	0.749	0.331	0.499	0.808	0.668	0.548	0.882	0.194	0.694	0.873	0.251	0.166	*	0.147	0.331
			Protein			***	***	***	***	*	***	***	0.086	***	*	***	**	0.055	0.287	0.873	0.131	0.282	***	0.063	***
			Age			0.184	0.331	0.954	0.412	*	0.408	>0.999	0.455	0.864	>0.999	0.591	0.423	*	0.867	0.180	0.395	0.069	0.825	0.616	0.424
		AUC	Protein × Age			0.172	*	0.248	0.362	0.749	0.957	0.613	0.577	0.711	0.854	0.578	0.637	0.154	0.960	0.146	0.300	0.379	0.508	*	0.390
			Protein			***	***	***	***	0.810	***	***	***	***	***	***	0.616	***	0.118	***	*	**	***	***	***
			Age			0.675	0.088	0.385	0.876	***	0.281	0.268	0.753	0.352	0.744	0.903	0.091	0.185	0.053	0.324	0.297	0.621	0.077	0.085	0.154
In vitro	Young vs Old	PPH WPC80	AUC AUC			0.151 **	* 0.082	* 0.612	0.069 *	** *	0.053 ***	0.978 *	0.117 ***	* *	0.620 **	0.075 **	0.142 0.664	0.180 **	0.153 0.072	* *	0.864 *	0.080 0.221	* *	0.078 *	0.055 **

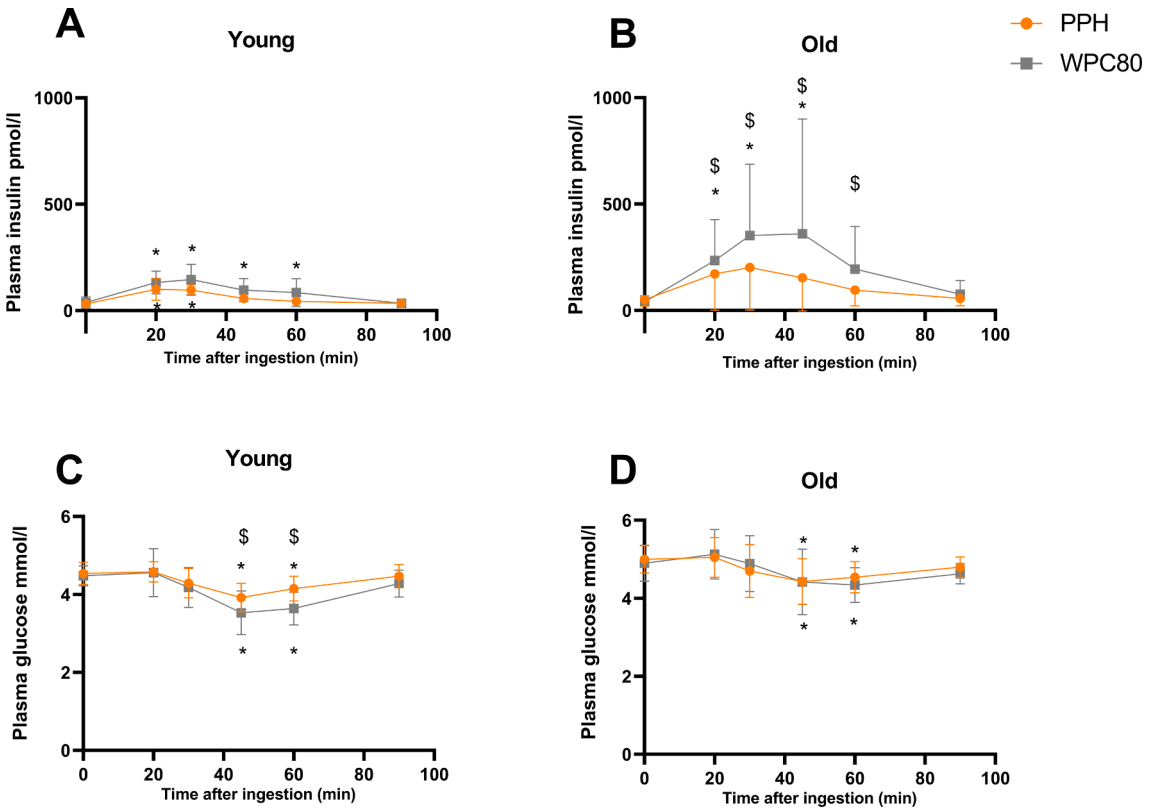

Fig. 2. The insulin and glucose response in absolute values after intake of PPH or WPC80 for the younger (A and C) and older (B and D) group. *= Significantly different from baseline ($p < 0.05$); $= significantly different relative change between protein drinks ($p < 0.05$).

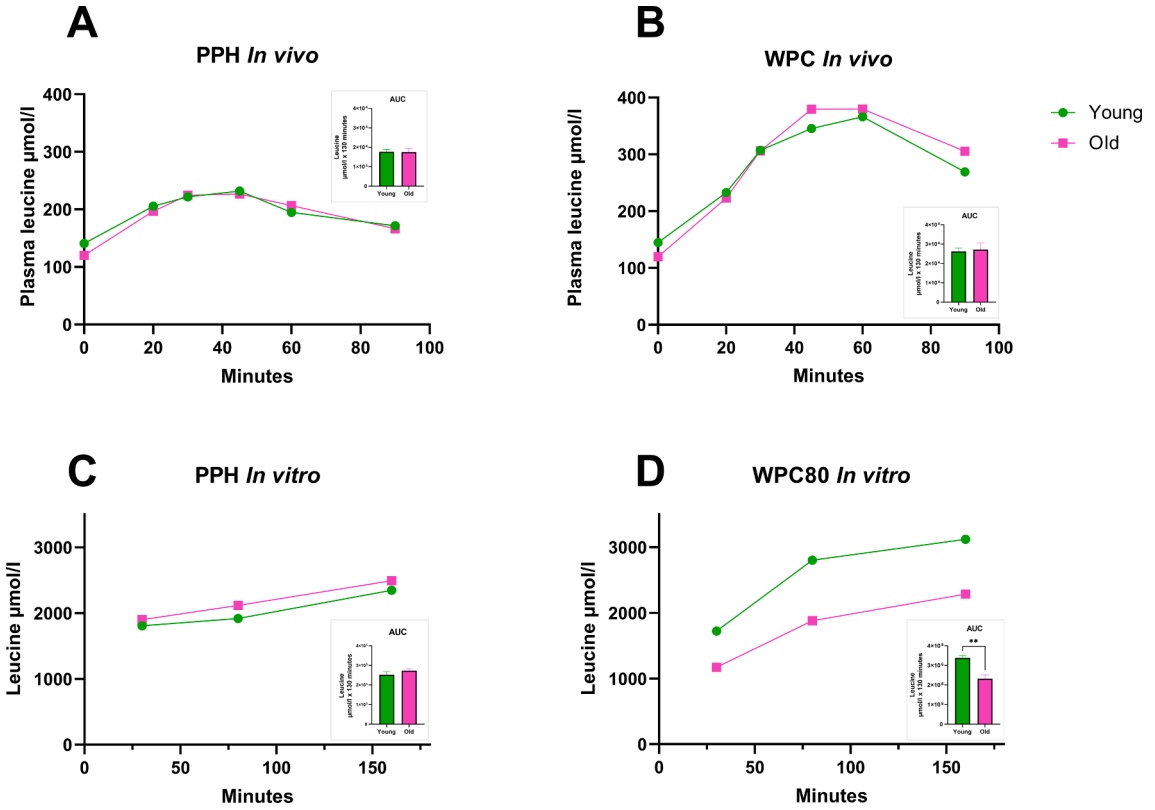

Fig. 3. The leucine response in plasma (µmol/l) and *in vitro* digested samples (µmol/l digesta) for PPH (A and C) and WPC (B and D) in absolute values and AUC. ** = significantly different between groups ($p < 0.01$).

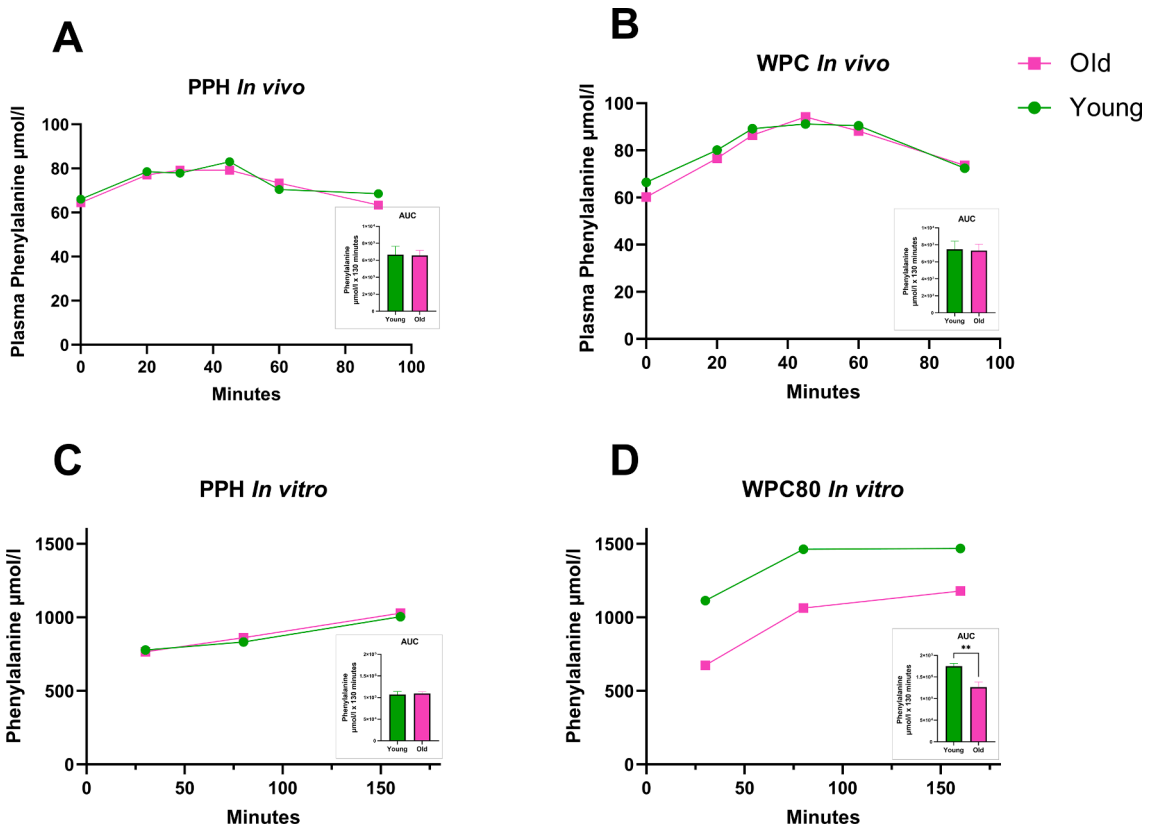

Fig. 4. The phenylalanine response in plasma (µmol/l) and *in vitro* digested samples (µmol/l digesta) for PPH (A and C) and WPC (B and D) in absolute values and AUC. ** = significantly different between groups ($p < 0.01$).

4. Discussion

This study investigated the differences in aminoacidemia following ingestion of hydrolyzed poultry protein (PPH) and whey protein (WPC80) in healthy young and old individuals. Here we show that ingestion of PPH, compared to WPC80, leads to a lower increase in blood concentrations of leucine, BCAA or EAA and a greater increase in blood concentration of glycine. We further show a faster time to peak in leucine concentration after ingestion of PPH compared to WPC80. Surprisingly, ingestion of WPC80 led to higher relative increase in plasma concentration and a greater relative increase in peak concentration in leucine, BCAA and EAA, for the older compared to the younger adults. As a contrast, the *in vitro* digestion of WPC80 showed lower aera under the curve in leucine, BCAA and EAA in the old compared to the young model,

WPC80 resulted in a higher increase in EAA, and especially for BCAA including leucine, in both young and old adults compared to PPH, whereas drinking PPH led to a higher glycinemia. This was not surprising, given that PPH had a lower content of leucine (72% of WPC80) and the other BCAAs, and a higher glycine content (587% of WPC80). Because the degree of hydrolysis of the ingested protein has been shown to improve the digestion and thereby the rate of absorption of amino acids into the blood (Koopman et al., 2009; Meyer et al., 2015; Pennings et al., 2011), we hypothesized a fast aminoacidemia after PPH ingestion. The large proportion of small peptides in PPH should in theory increase the sum of recognition sites of digestive enzymes compared to WPC80, but we were not able to estimate this due to limited information on peptide characteristics. The smaller peptides in PPH did not lead to substantially faster absolute rise in blood amino acid concentrations than WPC80, neither in the old nor younger group, but the relative rise in amino acid concentration was somewhat faster with PPH. Therefore, The small difference between protein drinks in the rate of aminoacidemia was, however, not surprising given the fact that whey protein is well-known as a "fast" protein (Boirie et al., 1997).

In general, the changes in blood concentrations of essential amino acids and glycine reflected, non-statistically, the content of the specific amino acids in the protein drink. For leucine, the content in PPH was 72% of that in WPC80 and the peak leucine concentration after intake of CHP was 65% and 59% of that observed after intake of WPC80 for the younger and older adults respectively. This is in line in with the results from Raastad and colleagues' (2017) study, where a similar protein hydrolysate produced from salmon carcass was compared to a whey protein concentrate. Because of the higher leucine content in WPC80, the absolute rise in leucine concentration was higher than after ingestion of PPH, but the rise in leucine concentration 20 min after intake of PPH was, in percent of the WPC80 increase, 74% for the younger adults (increase of 65 vs 88 µmol/l for PPH and WPC80) and 75% for the elderly (increase of 77 vs 103 µmol/l for PPH and WPC80). Further, time to peak leucin concentration after ingestion of PPH was ~ 18 min faster for both groups compared to WPC80. These findings support a relatively faster absorption of leucine from PPH compared with WPC80, which also was reported in the study comparing salmon hydrolysate to WPC80 (Raastad et al., 2017). A more efficient uptake after drinking PPH could further be argued by looking at the threonine and tryptophan concentrations, where the content in PPH was 56% and 26% respectively, of that in WPC80. When drinking PPH, the peak concentration for the groups combined was, of that in WPC80, ~72% of threonine and ~ 60% for tryptophan. On the other side, we observed that the peak concentration of phenylalanine, with similar content in the two protein drinks, was ~ 10% lower for the groups combined when drinking PPH compared to WPC80.

Ingestion of whey has been reported to give a slower and lower aminoacidemia in elderly compared to young adults, due to increased retention in the splanchnic tissues in elderly (Dangin et al., 2003; Milan

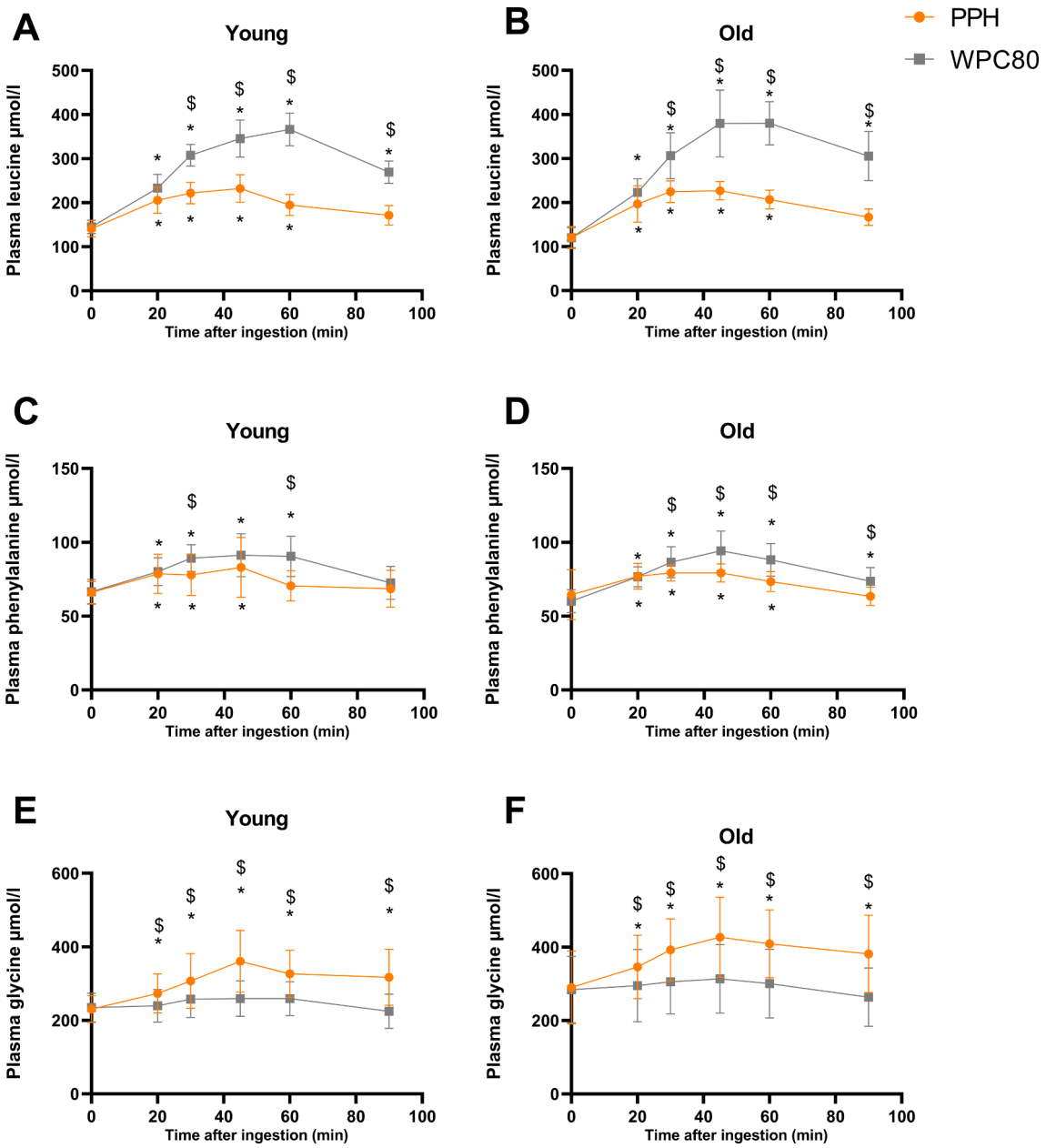

Fig. 5. The plasma leucine, phenylalanine and glycine response in absolute values after intake of PPH or WPC80 for the younger (A, C and E) and older (B, D and F) group. *= Significantly different from baseline ($p < 0.05$); $= significantly different relative change between protein drinks ($p < 0.05$).

et al., 2015; Mitchell et al., 2016; Paddon-Jones et al., 2004). It was therefore surprising to observe that ingestion of both PPH and WPC80 resulted mostly in similar time changes in amino acid concentrations for both young and old adults. Further, we even observed a higher increase in leucine, BCAA and EAA plasma concentrations for the old, compared to the younger group, 45 min after ingestion of WPC80. This was also the case in percent change from baseline in peak leucine, BCAA and EAA concentration for the elderly. There are no indications that elderly are able to absorb more of the ingested protein into plasma compared to the younger adults, so other explanations are more likely (Boirie, Gachon, & Beaufrère, 1997; Gorissen et al., 2020). Smaller blood volume and a slower rate of disappearance of amino acids from blood plasma to other tissue, such as muscle, are possible explanations for the higher amino acids concentrations observed 45 min after ingestion of WPC80 in the elderly. The elderly also reached their peak insulin concentration later compared to the younger adults after ingestion of WPC80, which could be an age-related decline in insulins effect on microvascular flow causing a poorer amino acid transportation (Burd et al., 2009). However, the elderly reached peak concentration faster than the younger adults in histidine, when drinking WPC80, and aspartic acid, when drinking PPH. We did also see a similar rate in the rise of leucine, BCAA and EAA concentration for the younger and older adults after ingestion of PPH. Consequently, it could be discussed whether the smaller peptides in PPH contributes to a removal of any difference in the rate of digestion and absorption between young adults and elderly. We did, however, not measure *in vivo* digestion, intestinal absorption, or the uptake from blood to muscle and other tissues directly, which prevents us from evaluating how these different processes influenced the accumulation of amino acids in the blood. Consequently, we cannot conclude on the cause for the higher leucine, BCAA and EAA concentrations observed in the elderly in our study. In any case, the intracellular changes in amino acid concentrations are likely the direct stimulators of the initiation of MPS and any anabolic responses in muscle can therefore not be extrapolated from the measurements of aminoacidemia in this

study.

Compared to young, the digestion and absorption of amino acids seem to be slower in elderly (Condino et al., 2013; Milan et al., 2015), which was supported by our *in vitro* results. One way to overcome the difference in digestion and absorption could be to modulate the protein source in order to improve the digestion and absorption rate, and the use of mild hydrolysis in the PPH production was meant to improve digestion and absorptions rates, especially in the older population, by increasing the peptide content. In line with this expectation, we did observe similar *in vitro* digestion rates in the old and young model when PPH was digested. Results from the *in vitro* digestion experiments did in general support the *in vivo* data, except for the digestibility of WPC80, which was significantly reduced when simulating the digestive process in older adults. As shown in Figs. 4 and 5, the release of leucine and phenylalanine to the gastrointestinal juice was lower in the elderly model. This was not in line with the *in vivo* measurements which showed few differences in the rise in amino acid concentrations between young and old adults after ingestion of WPC80. The leucine content differed greatly in the two protein drinks, but the phenylalanine did not, arguing that the amino acid content was not a factor causing the differences observed between the young and old *in vitro* digestion results of WPC80.

The discrepancy between *in vivo* and *in vitro* measurements for WPC80 could be related to the static digestion models used in the present study. The models use constant ratios of meal to digestive fluids and a constant pH for each step of digestion, which may not be suitable for simulating differences in digestion kinetics (Brodkorb et al., 2019). Further, the model simulating the digestive process in older adults used rather low levels of enzymes that may be more relevant in the later stages of aging when malnutrition due to poor digestibility is more prevalent (Maître et al., 2021). Various gastrointestinal models simulating the digestive process in elderly have been published indicating that protein digestibility is diminished or delayed, however lack of harmonization of methodology does not allow definite conclusions to be drawn (Makran et al., 2022). Accurate information about the composition of gastrointestinal fluids is scarce and further experiments are needed to obtain a protocol that is well adjusted to elderly. Although we did not measure the *in vivo* digestion rate, the results from the aminoacidemia indicated that the digestive capacity of WPC80 in elderly was underestimated in the *in vitro* model. This could potentially be attributed to reports of a slower rate of disappearance in elderly (Dangin et al., 2003), which could result in similar increase in plasma amino acid concentrations for young and old adults, even if the elderly had reduced digestive capacity. Another potential explanation for the discrepancy between the *in vivo* and *in vitro* results in our study could be related to our generally active elderly group. Consequently, the *in vitro* model may better reflect digestive capacity in sedentary elderly if physical activity and physical fitness can have a positive impact on the gastrointestinal tract.

5. Conclusion

The increase in blood amino acid concentrations reflected the content in the protein sources, which consequently led to a larger increase in blood concentrations of the most important amino acids for muscle protein synthesis when ingesting WPC80 compared to PPH. However, ingestion of PPH showed a faster time to peak leucine concentration than WPC80. Therefore, moderate hydrolysis increasing the number of smaller peptides can provide a faster absorption, which is in line with our hypothesis. Interestingly, our results display few differences between young and old adults regardless of protein source. This indicates that digestion and absorption of the protein sources tested in this study were not impaired in our group of elderly. The training status of our older subjects might have impacted these results, emphasizing exercise as an important tool to recover any age differences. The discrepancy between poorer *in vitro* digestibility of WPC80 in the old, compared to young, adult model and no difference in amino acid concentrations between old and young adults *in vivo*, indicates that the *in vitro* model underestimated the protein digestion rate in a group of healthy elderly that perform regular exercise. Future research will benefit from inclusion of both active and sedentary elderly as well as looking at intracellular muscle changes and training adaptation.

Ethical statement

Ethical approval for the involvement of human subjects in this study has been evaluated by and approved by the Norwegian School of Sport Sciences Ethical Committee, reference number 159–240920, dtd 09/25/20, as well as by the Norwegian center for research data, reference number 598443, dtd 10/14/20.

CRediT authorship contribution statement

Elise Lander: Writing – original draft, Writing – review & editing, Validation, Data curation, Formal analysis, Visualization, Investigation, Methodology, Project administration. **Bente Kirkhus:** Writing – review & editing, Methodology. **Diana Lindberg:** Writing – review & editing, Methodology, Investigation. **Truls Raastad:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The main financial support was given by the Research Council of Norway through the project «Next generation tailor-made chicken products» (grant no. 269261). Financial support was further given from the Norwegian Fund for Research Fees for Agricultural Products through the projects “Foodsmack” (grant no. 262308) and “SunnMat” (grand no. 262300), from the Research Council of Norway through projects “Notably” (grant no. 280709) and the research infrastructure “FoodPilotPlant Norway” (grant no. 296083), from the RFF Oslofjordfondet through the project “Chickenlysis” (grant no. 235839), and lastly, internal financing from Nofima AS through the project “Peptek” are greatly acknowledged. The authors want to thank Silje Bergum at Nofima, Norway, for technical contribution. The Nor-Hydropep 90 SD was kindly provided by Norilia, Norway,

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105452.

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