

Short Report

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Blood amino acid dynamics after ingestion of chicken-derived peptides in healthy subjects

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ABSTRACT

Background: The rate of protein digestion and amino acid (AA) absorption determines the postprandial rise in circulating AA and modulates postprandial muscle protein synthesis (MPS) rates. Furthermore, it is necessary to consider the timing of protein ingestion, along with its quantity and quality, to regulate the blood AA concentration. Chicken breasts are a popular food among athletes as they are a good source of animal protein, containing sufficient essential amino acids (EAAs) and branched-chain amino acids (BCAAs). Low-molecular-weight chicken peptides (Cpep), a novel protein supplement, were isolated from chicken breasts. Blood AA dynamics, which have a significant influence on MPS rates, were observed and compared with commercially available whey- and soy-derived protein supplements.

Objectives: We evaluated blood AA dynamics after Cpep intake compared with whey protein (WP), and soy protein (SP).

Methods: Three groups of six healthy adult men volunteers (age 39 ± 10 years) ingested 0.3 g/kg (protein/body weight) of Cpep, WP, and SP. The concentrations of AA in the plasma were measured before and after the ingestion period and their kinetics were compared.

Results: Cpep comprises free amino acids or peptides, and their average molecular weights are lower than those of WP and SP. The absorption dynamics of AA in the plasma were evaluated. After Cpep intake, EAA and BCAA concentrations peaked at 30 min and levels of EAA and BCAA were higher than those after WP and SP ingestion at 15 and 30 min, respectively. Conversely, the levels of total AA, EAA, and BCAA decreased 45 min after Cpep intake compared with WP and SP intakes. In contrast, WP and SP showed similar blood AA dynamics with a peak at 60 min.

Conclusions: Cpep is absorbed significantly faster than WP and SP, making it a useful option for efficient protein intake to maintain and increase muscle mass.

Keywords: chicken-derived peptides, blood amino acid dynamics, branched-chain amino acid, muscle protein synthesis



Chicken breast

Protein hydrolysis

Chicken peptides



Clinical study

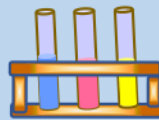
Compared with two protein supplements

Chicken peptides (Cpep)

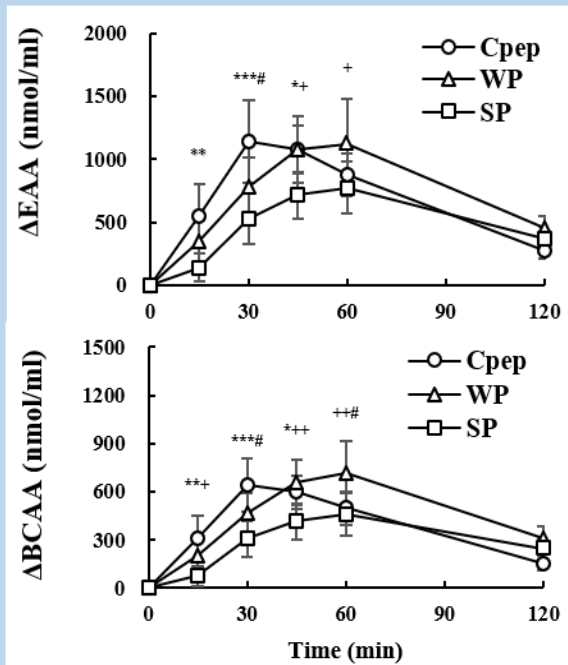
Whey protein (WP)

Soy protein (SP)

Blood amino acid dynamics



Healthy adult men (n=6)
0.3g/kg (protein/body weight)



Cpep is absorbed more quickly than WP and SP

INTRODUCTION

The balance between the rates of protein synthesis and breakdown determines muscle mass maintenance, which is greatly influenced by dietary protein intake [1-2]. Adequate dietary protein intake is particularly desirable for athletes and athletic enthusiasts to improve their performance, or for the elderly who possess a high risk of sarcopenia and frailty [3-8]. Highly active athletes may require more protein intake to maintain and increase skeletal muscle because muscle protein breakdown increases with increased energy consumption [7-8]. Additionally, muscle protein synthesis (MPS) induced after protein intake is proportional to the amount of protein ingested [3], but the amount of protein required to induce muscle synthesis increases with age; therefore, higher protein intake is required in the elderly [3-6]. Dietary protein intake for muscle maintenance and weight gain should consider not only the quantity but also the quality and timing of intake [2,9-12]. The branched-chain amino acids (BCAAs) content in essential amino acids (EAAs) is important for protein quality, and leucine has been reported to promote MPS, in particular, by stimulating the mammalian rapamycin complex 1 (mTORC1) signaling pathway [13,14]. Furthermore, regulation of blood amino acid (AA) levels must consider quantity, quality, and the timing of protein intake because a positive correlation has been reported between BCAA levels (in particular, leucine) and MPS rate [3,13-15].

It is difficult to supply sufficient protein to satisfy these requirements through a regular diet alone. Whey protein (WP) and soy protein (SP) are

popular protein supplements and affect MPS [4,16-21]. Chicken breast is a favorable food for athletes because it is a good source of animal protein with sufficient EAA and BCAA content [21]. However, few protein supplements made from chicken breast have been found. Furthermore, protein hydrolysates containing oligopeptides are absorbed better than intact proteins and AAs [19-23]. We developed a protein supplement (Cpep), which is hydrolyzed from chicken breast, and has a molecular weight of approximately 1 kDa or less. Blood AA dynamics, which have a significant influence on MPS, were observed and compared with those of commercially available whey- and soy-derived protein supplements.

MATERIALS AND METHODS

Chemicals and reagents: All the reagents were obtained from Fujifilm Wako Pure Chemical Co. (Osaka, Japan).

Protein supplement hydrolysate: Cpep (average molecular weight 650 Da) was hydrolyzed from the chicken breast tissue. SP (average molecular weight, 18 kDa) was obtained from Fuji Oil Co., Ltd. (Osaka, Japan). WP (average molecular weight, 25 kDa) was purchased from Bayerische Milchindustrie EG (Landshut, Bayern, Germany). AA composition analysis and molecular weight distribution of each protein were analyzed using an AA analyzer (Hitachi L-8900, Japan) and HPLC (Shimadzu, LC-2030, Japan) using a Superdex 30 increase 10/300GL gel filtration column (GE Healthcare, Amersham Biosciences, Sweden).

Participants: Three groups of six healthy men volunteered to participate in this study. According to past health examinations, none of the participants had any physical or medical health complications. The participants consented to the study purpose, experimental procedures, and potential risks. This study was approved by the Institutional Ethics Board at the Research and Development Center of NH Foods Ltd. (Approval No.: 2021-01, Tsukuba, Ibaraki, Japan).

Study design: After fasting for 12 h, six healthy adult men (mean \pm standard deviation (SD), body weight 65 ± 8 kg, age 39 ± 10 years) ingested 0.3 g/kg (protein/body weight) of Cpep, WP, and SP that were dissolved in 15 mL of water per gram protein. The AA profile of each supplement is listed in Table 1. Before the participants consumed the protein beverage, a baseline sample was taken (Pre), after which they consumed the intervention beverage within < 1 min. Venous blood was collected from the cubital vein at 0, 15, 30, 45, 60, and 120 min after the ingestion of the three protein supplements. This process of overnight fasting and sequential blood draws was repeated weekly for 3 weeks to evaluate each protein source. In each experimental trial, six blood samples (approximately 7 mL) were collected in evacuated containers containing EDTA-2Na powder. Blood samples were centrifuged at $2,000 \times g$ for 10 min at 4°C to separate the plasma and stored at -80°C until further analysis. The concentrations of AA in the plasma were measured before and after the ingestion period and their kinetics were compared.

Plasma hydrolyzed amino acid concentrations: The plasma was thawed and filtered using 0.5 mL 3 K

Amicon Ultra centrifugal filter units (Merck Millipore Ltd., Cork, Ireland). The supernatant was hydrolyzed in aqueous 6 N hydrochloric acid for 24 h at 110°C . After hydrolysis, the hydrolysate was dried using a rotary vacuum evaporator at 50°C and then dissolved in lithium citrate buffer (pH 2.98, JEOL Ltd, Tokyo, Japan). The concentrations of each AA in plasma were analyzed using a high-speed amino acid analyzer and MCI buffer™ kits (Hitachi High-Tech Fielding Co., Tokyo, Japan), and the total amino acids (TAAs) content, EAAs, and BCAAs (leucine, isoleucine, and valine) were evaluated.

Statistical analysis: All data are expressed as the mean \pm SD. Differences in the mean values among the experimental groups were analyzed by two-way repeated measures analysis of variance and Bonferroni/Dunn post-hoc test. Differences were considered statistically significant at $p < 0.05$, 0.01, and 0.001.

RESULTS

Composition of test supplement: WP and SP each contained over 78% protein (% of dry weight), whereas Cpep had a higher protein content (98%) than WP and SP (Table 1). Cpep is composed of free amino acids (FAAs) or peptides, and its average molecular weight is smaller than WP (25 kDa) and SP (18 kDa, data not shown). The AA compositions of the Cpep, WP, and SP are listed in Table 1. The EAA content of Cpep and WP was approximately 43.6 and 46.1 (g/100 g of protein), respectively, which was higher than that of SP (37.5). The compositional evaluation revealed similar levels of BCAA for Cpep (19.1) and SP (18.5). In contrast, the WP content was approximately 23.2%.

Table 1. Amino acid composition of protein supplements

	Cpep	WP	SP
Total protein (% of dry weight)	98.3	78.7	78.8
NEAA (g/100 g of protein)			
Alanine	6.5	5.1	4.4
Arginine	7.0	2.4	7.6
Aspartic acid	10.5	11.0	11.9
Cystine	0.5	2.3	1.0
Glutamic acid	16.5	17.7	19.6
Glycine	4.3	1.8	4.3
Proline	3.7	5.8	5.1
Serine	4.2	5.0	5.1
Tyrosine	3.4	2.8	3.5
EAA (g/100 g of protein)			
Histidine	3.0	1.7	2.5
Isoleucine	5.0	6.4	5.0
Leucine	8.7	10.9	8.3
Lysine	10.1	9.0	6.4
Methionine	2.7	2.1	0.7
Phenylalanine	3.8	3.3	5.4
Threonine	5.0	6.9	4.0
Valine	5.4	5.9	5.2
Total NEAAs (g/100 g of protein)	56.4	53.9	62.5
Total EAAs (g/100 g of protein)	43.6	46.1	37.5
Total BCAAs (g/100 g of protein)	19.1	23.2	18.5

NEAA, nonessential amino acid; EAA, essential amino acid; BCAA, branched-chain amino acids

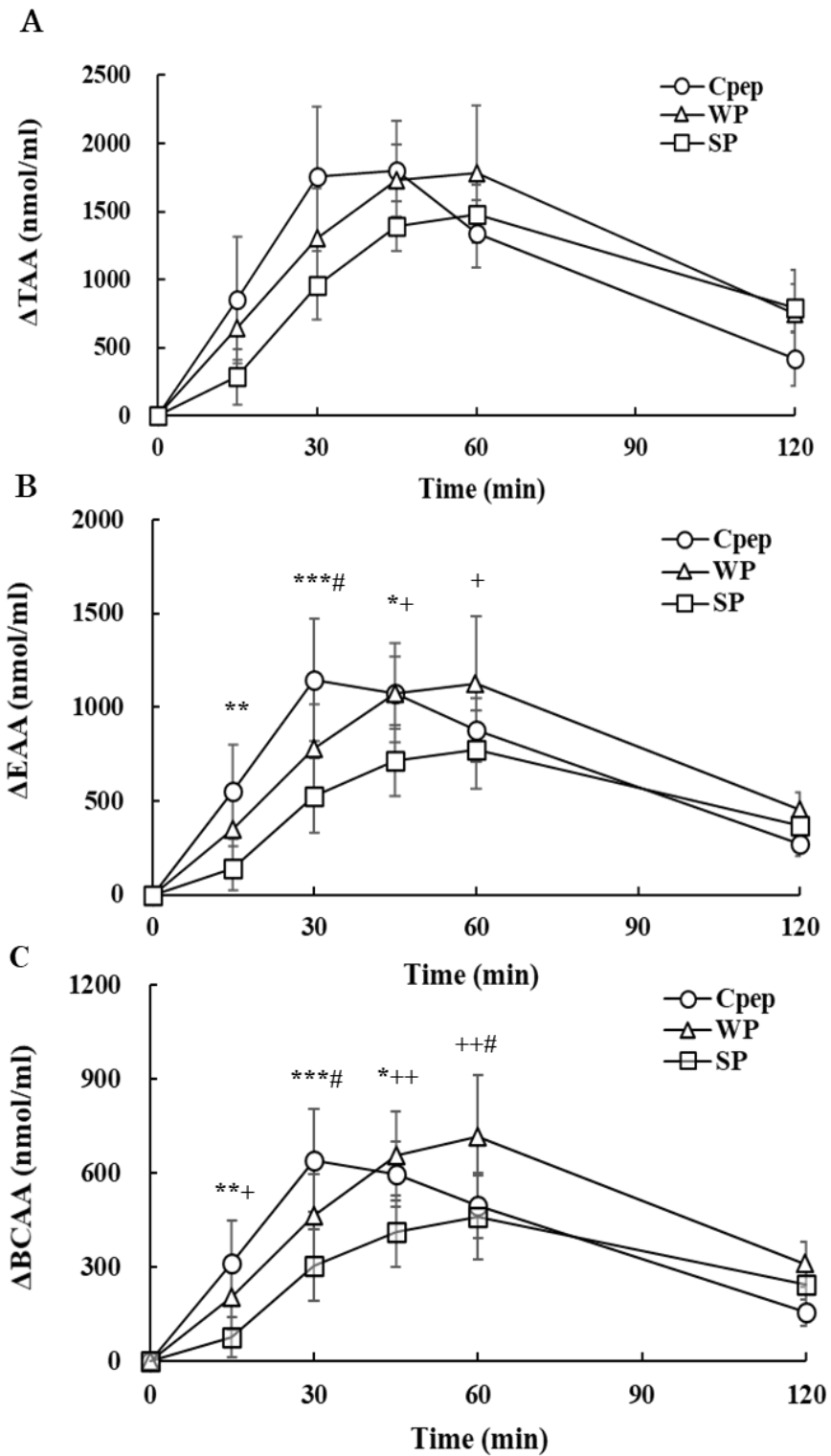


Figure 1. Plasma-hydrolyzed amino acid concentrations of TAA (A), EAA (B), and BCAA (C) were measured at 0, 15, 30, 45, 60 and 120 min after the ingestion of the three protein supplements. All values of concentration change ($\Delta V = V_{post} - V_0$) are mean \pm SD. n = 6. * Cpep vs SP (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). # Cpep vs WP (# $p < 0.05$), + WP vs SP (+ $p < 0.05$, ++ $p < 0.01$).

Plasma hydrolyzed amino acid analyses: The concentrations of plasma-hydrolyzed AAs were analyzed, and EAA, BCAA, and TAA were evaluated. After Cpep intake, EAA and BCAA concentrations peaked at 30 min and levels of EAA and BCAA were higher than those after WP and SP ingestion at 15 and 30 min (Figure 1B, 1C). Conversely, the levels of EAA and BCAA decreased 45 min after Cpep intake (Figure 1B, 1C). In contrast, WP and SP showed similar absorption dynamics, with a peak at 60 min (Figure 1B, 1C). TAA concentration after ingestion of Cpep tended, however, not significantly, to be absorbed more quickly than WP and SP (Figure 1A). Similar results were obtained for plasma FAA concentrations (data not shown).

DISCUSSION

The AA content of Cpep was similar in both EAA of WP and BCAA of SP, reflecting the AA content of each protein source. Regarding the blood AA dynamics, TAA, EAA, and BCAA concentrations peaked 30 min after Cpep intake, and the levels of EAA and BCAA were higher than those of WP and SP ingestion at 15 and 30 min. Cpep has an average molecular weight of 650 Da and a high oligopeptide content and was considered rapidly absorbed via the peptide transporter PepT1. Conversely, the levels of TAA, EAA, and BCAA decreased 45 min after Cpep intake compared to WP and SP, which may reflect differences in molecular weight. In contrast, WP and SP showed similar blood AA dynamics, with a peak at 60 min, regardless of their different molecular weights. In addition, the concentrations of EAA and BCAA after WP intake were higher than those in SP, which possibly reflects the composition of individual AA originally present in the protein. As

seen in the AA composition table, WP contained more EAA and BCAA (especially leucine and isoleucine) than SP. Furthermore, when the blood AA dynamics of each AA were examined individually, it was observed that Cpep was generally absorbed faster than WP and SP (data not shown). The absorption of lysine ($p < 0.05$), arginine ($p < 0.05$), and valine ($p < 0.05$) contained in Cpep was significantly faster than that of AA contained in WP and SP. This is reflected in the size of the peptide composition of each AA, the ease of peptide absorption by the peptide transporter PepT1, and the AA content.

As mentioned above, it is necessary to consider the blood AA concentration in MPS [4-8]. Resistance exercise can activate mTORC1 and increase the rate of MPS, which is effective in maintaining and increasing muscle mass not only in athletes but also in the elderly [7,8,13,14,19]. Since the rate of MPS is the highest immediately after resistance exercise and decreases over time, protein intake immediately after exercise has the most synergistic effect [24-25]. In these situations, Cpep is considered effective because it rapidly increases the concentrations of EAA and BCAA in the blood. However, because the disappearance was also rapid, it was considered desirable to use Cpep in combination with a regular diet or other protein supplements.

In Japan, the Foods with Function Claims (FFC) system was introduced in April 2015. These foods, which can achieve the specified health effects, are allowed to be labeled as functional foods [26]. With a global fitness boom and an aging population, functional foods for muscle maintenance, growth, and promotion of good health have become a necessity [7,8,13,14,19]. Responding to an increasingly aging population in Japan, a growing

number of food items with health claims such as “maintains muscle mass and strength” or “improves walking ability” are being labeled under the FFC system [27]. Further, we would like to elucidate the involvement of Cpep and its active ingredients in muscle hypertrophy with the aim of utilizing the FFC system, which is not limited to protein supplementation.

CONCLUSIONS

As a novel protein supplement, Cpep is absorbed significantly faster than WP and SP, which is associated with the increased content of low-molecular-weight peptides and FAAs. Cpep is a useful option for efficient protein intake to maintain and increase muscle mass. Further research is required to investigate its effects on MPS.

List of Abbreviations: Cpep: chicken-derived peptides, WP: whey protein, SP: soy protein, AA: amino acid, TAA: total amino acid, EAA: essential amino acid, BCAA: branched-chain amino acid, FAA: free amino acid, MPS: muscle protein synthesis, mTORC1: mammalian rapamycin complex 1, SD: standard deviation, FFC: Foods with Function Claims.

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Competing Interests: There are no conflicts of interest to declare.

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