Postprandial Plasma Amino Acid Responses Between Standard Whey Protein Isolate and Whey Protein Isolate Plus Novel Technology

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ABSTRACT

BACKGROUND: Muscle mass is an important determinant of metabolic health and physical function. It has previously been demonstrated that the postprandial rise in circulating essential amino acids (EAA) acts as the main stimulus for muscle protein synthesis (MPS). This study investigated postprandial plasma amino acid (AA) responses of 2 different forms of whey protein isolate (WPI) with iso-caloric and iso-nitrogenous profiles to investigate plasma concentrations of EAA.

METHODS: In all, 12 healthy men (n = 12) between 19 and 32 years of age were recruited for a randomized, cross-over design, which involved consumption of protein supplements on 2 testing days separated by a 6-day washout period between conditions. On each testing day, subjects consumed either 29.6 g of WPI or WPI + io (whey protein isolate plus Ingredient Optimized Protein®) mixed with 236 mL of water. Plasma EAA and branch chain amino acid (BCAA) concentrations were assessed from whole body donated by subjects at pre-consumption and 30, 60, 90, 120, and 180 minutes post consumption.

RESULTS: Plasma levels of total EAA concentration was significantly greater in WPI + io at 30, 60, 90, and 120 minutes post consumption ($P < .01$, $P < .001$, $P < .01$, and $P < .01$, respectively). Plasma levels of total BCAA concentration was significantly greater in WPI + io at 30, 60, 90, and 120 minutes post consumption ($P < .01$, $P < .001$, $P < .01$, and $P < .05$, respectively) compared with WPI. For leucine, only WPI + io had elevated levels compared with pre-test at 90 minutes post consumption ($P < .001$).

DISCUSSION: Both conditions significantly elevated EAA, BCAA, and leucine from basal levels. However, we conclude that the consumption of the treated WPI significantly raises plasma EAA, BCAA, and leucine to a greater extent compared with WPI with no treatment. Thus, supplementation with WPI that has undergone Ingredient Optimized® technology may be highly beneficial for those who partake in regular exercise, elderly individuals, or those affected by a reduced sensitivity to amino acids.

KEYWORDS: Amino Acids, Whey Proteins, Leucine, Bioavailability, Postprandial Period

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Introduction

Muscle mass is an important determinant of metabolic health and physical function.[1] As such, muscle mass profiles have been indicated to positively impact performance in athletic events[2] and activities of daily living,[3,4] muscle strength,[5] and quality of life.[6] Thus, the maintenance or addition of muscle mass is highly sought. The loss of muscle mass and function is underpinned by increased rates of muscle protein breakdown or a reduced muscle protein synthetic response to protein feeding.[7] However, the ability of protein to impact muscle health is highly dependent on (1) the amount of protein ingested,[8,9] (2) the quality (ie amino acid composition) of the protein source,[10] and (3) absorption kinetics of amino acids.[11-13]

Increases in muscle protein synthesis (MPS) have been attributed to the postprandial rise in circulating essential amino acids (EAA).[14] Consequently, protein quality has been defined as the capacity of a protein to provide EAA.[15] However, a subgroup of EAA known as branched chain amino acids (BCAA; valine, isoleucine, and leucine) have also been shown to be important regulators of protein anabolism.[16] Leucine, in particular, has been suggested to be a direct indicator of protein quality as it has demonstrated the ability to independently stimulate MPS protein synthesis alone.[17,18] Therefore, BCAA and leucine content of protein sources should be recognized when considering protein quality. Whey protein (WP) is considered to be a high-quality protein source due to its high concentrations of EAA, BCAA, and leucine.[11,13,19] As a result, WP has become a popular supplement among those looking to maintain or increase muscle mass.[20]

Recently, the use of atmospheric plasma has been implemented in powdered whey protein isolate (WPI). Plasma-altered WPI powders have exhibited increased surface area[21] as well as positive impacts to solubility and dispersibility,[22] which serve as potential benefits for beverage production. Plasma modification has further been shown to alter the taste and perceived mixability of powdered protein.[23] Furthermore, plasma modification has demonstrated ability to alter protein structure in such a way to expose the hydrophobic pockets of a protein.[24] These structural

Table 1. Subject characteristics.

VARIABLE	MEAN ± *SD*
Age (years)	25 ± 4
Height (cm)	181.3 ± 6.7
Fat mass (kg)	18.18 ± 6.83
Fat-free mass (kg)	73.70 ± 7.0
Total mass (kg)	91.88 ± 12.60
Body fat (%)	19.3 ± 4.5

alterations have been confirmed by using a protein thermal shift, which showed an improved ability for dye to bind to the protein.[25] Improving protein powder's hydrophobicity enhances enzymatic degradation and ultimately promotes increased digestibility, as has been demonstrated using other protein modification methods.[26]

To date, no study has looked at the aggregate improvements of atmospheric plasma and protein on the blood plasma amino acid response. Therefore, the purpose of this study was to investigate the postprandial plasma amino acid (AA) responses of 2 different forms of whey protein isolate (WPI) with iso-caloric and isonitrogenous profiles. In a randomized, crossover trial, one serving of WPI and one serving of WPI + treatment of atmospheric plasma (WPI + io) were used to investigate postprandial response of EAA, BCAA, and leucine plasma concentrations.

Methods

Study population

In all, 12 healthy men between 19 and 32 years of age who regularly engage in whole body resistance training (2-3 days/week) were recruited and considered eligible for the study. Subjects were screened to ensure that they met and would adhere to the following criteria prior to entry into the study: (1) maintain a diet consisting of 15%-20% protein, 45%-55% carbohydrate, and 25%-30% fat according to a 3-day dietary food recall; (2) not taking performance-enhancing supplements for the previous 6 weeks; (3) non-smokers; (4) not taking amino acid supplements; (5) not using anabolic or catabolic hormones; (6) not on medication or supplements known to influence any of the variables measured in the study; and (7) free of metabolic diseases. Written informed consent was obtained from all study participants, and the protocol was approved by an external Institutional Review Board (IntegReview IRB, Austin, TX; Protocol #1101). Characteristics of the subject pool are presented in Table 1. Anthropometric data were assessed from a whole-body, dual-energy X-ray absorptiometry scan (DXA; Hologic Inc., Bedford, MA, USA).

Study design and protocol

The cross-over study (n = 12) involved consumption of protein supplements on 2 testing days separated by a 6-day washout period between conditions to evaluate plasma amino acid profiles following ingestion of supplement shakes. The investigated supplements (Corr-Jensen Inc., Denver, CO, USA) were WPI and WPI + treatment (WPI + io [ioProtein®]). Both WPI and WPI + io were sourced from a single 1 kg container to ensure that both conditions were from the same supplier, batch, had the same production date, and were stored in the same manner. On preparation of samples, half was designated for WPI and half was treated (WPI + io). The treatment condition was exposed to cold atmospheric plasma to incite functional and structural changes in the protein peptide to more readily expose binding sites for enzymatic cleavage.

A total of 12 subjects reported to the laboratory between 07:00 and 08:00 after an overnight fast (⩾10 hours), and a catheter (Introcan® Safety IV Catheter; Braun Medical Inc., Bethlehem, PA, USA) was inserted into an antecubital vein and a resting blood sample was drawn at time zero (pre). Immediately thereafter, subjects ingested a bolus of 29.6 g of one of the testing conditions mixed with 236 mL of water. Following ingestion of the supplement, subjects were not allowed to consume any food products until the 3-hour time course was completed. In addition, subjects were not allowed to consume water 1 hour before or 1 hour after consumption of investigational product. Serial blood samples were collected at time 0 (immediately prior to ingestion of the protein) and at 30, 60, 90, 120, and 180 minutes after consuming the study product. Immediately after collection, blood samples were stored in ice and centrifuged for 15 minutes at 2500×*g* at 4°C. The resulting plasma was stored at −80°C until analysis of amino acid concentration. This process of overnight fasting, consumption of test shake (ie 29.6 g of either WPI or WPI + io mixed with 236 mL of water), and sequential blood draws were applied to both testing days.

Preparation of plasma samples

For amino acid assessment, 900 µL serum was added to 90 µL of a 50% trichloroacetic acid (TCA) solution, vortexed, and snap frozen in liquid nitrogen and stored at −80°C until analysis. Sample preparation for liquid chromatography with tandem mass spectrometry (LC-MS/MS) analyses was as follows: an aliquot of 25 µL of the TCA deproteinized serum was pipetted into a glass vial (1 mL) and diluted with 125 µL water. A solid-phase extraction tip was attached to a 1.5 mL syringe and the diluted serum sample was pulled slowly through the SPE tip by moving the piston of the syringe. Thereafter, 200 µL of the washing solution (solution 2) was added to the glass vial and pulled through the SPE tip to remove urea and other matrix components. The syringe was removed leaving the SPE tip inside the glass vial. Then, 100 µL of a freshly prepared elution/extraction (solution 3A/3B: 3/2% (v/v)) buffer solution was pipetted into the vial, a syringe of 0.6 mL was attached to the pipette tip, and the piston was pulled up approximately 1 cm and attached to the SPE tip. The elution/extraction buffer was drawn into the SPE tip, and the solid-phase sorbent

material was then expelled into the glass vial by pushing the piston down. This step was repeated until all SPE sorbent material from the pipette tip was expelled.

Derivatization agent (solution 4:50 μL) was added and the vial was vortexed vigorously for 10 seconds. The derivatization agent was allowed to react for 1 minutes, and 100 μL of organic extraction solvent (solution 5: propyl chloroformate in chloroform) was added and the vial was vortexed vigorously for 20 seconds and was allowed to stand for 1 minutes for organic solvent phase separation. In case the organic solvent phase separation was not complete, 100 μL of a saturated sodium chloride solution was added, and the glass vial was vortexed one more time for 10 seconds, and after 1 minute of organic solvent phase separation, an aliquot of 100 μL of the upper organic phase was transferred into a new glass vial, and the organic solvent was evaporated until dryness at ambient temperature with high-purity nitrogen gas. The residue was dissolved with 100 μL of a mixture of methanol/water (62/38) and pipetted into a plastic spring-loaded micro-insert and placed into an autosampler vial with a septum cap.

Liquid chromatography with tandem mass spectrometry

The liquid chromatographic separation was performed on the Phenomenex LC AAA-MS column (250 mm × 3 mm ID, 4 μm particles) using a Waters 2695 HPLC system (Milford, MA, USA) with integrated autosampler and sample chiller set at a temperature of 10°C and a Waters column heater module controlled by a Waters temperature control module (TCM). Separation of the amino acid derivatives was achieved by gradient elution using the following gradient: 0 minutes 38% A, 13 minutes 17% A, 13.01 minutes 38% A, and 20 minutes 38% A at a flow of 0.5 mL/min at a column temperature of 35°C. Eluent A consisted of 100% water containing 10 mM ammonium formate and B consisted of 100% methanol. The sample injection volume was 10 μL.

A Thermo Quest TSQ triple quadrupole mass spectrometer (San Jose, CA, USA) equipped with an API 2 electro spray ionization (ESI) probe was employed for analyses of the amino acid enrichments. The mass spectrometer conditions were 1 second scan time; heated capillary temperature: 325°C; spray voltage: 4.5 kV; conversion dynode: 15 kV; electron multiplier voltage: 1600 V; and sheath gas pressure: 0.62 MPa. Collision-induced dissociation (CID) spectra of the (stable isotope labeled) amino acid derivatives were obtained at an argon collision cell pressure of 8.27×10^{-6} MPa.

Statistical analysis

Results were obtained for plasma concentrations and were EAAs (valine, leucine, isoleucine, threonine, methionine, tryptophan, phenylalanine, and lysine), BCAAs (valine, leucine, and isoleucine), and leucine alone. Classification of amino acid essentiality is in accordance to previous literature.[27] Two-way repeated measures analysis of variance (ANOVA) was used to assess plasma amino acid concentrations assuming condition (WPI and WPI + io) and time (0 minutes [pre], 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 180 minutes post supplementation) as fixed factors. Whenever a significant *F*-value was obtained, a post hoc test with a Tukey adjustment was performed for multiple-comparison purposes. Incremental area under the curve (iAUC) was calculated by subtracting the baseline (ie pre) concentration form each subsequent timepoint (ie 30, 60, 90, 120, and 180 minutes) and then applying the linear trapezoidal rule to the resulting concentration. The total iAUC of the 2 conditions were analyzed by paired-sample t-test. The significance level was previously set at $P < .05$. Results are expressed as mean ± standard error.

Results

Plasma amino acid concentrations

The results for plasma AA concentrations are displayed in Figure 1, and the raw data are represented in Table 2. Both WPI and WPI + io stimulated a significant rise in EAA, BCAA, and leucine concentration by 30 minutes post ingestion ($P < .001$); however, WPI + io demonstrated a more pronounced aminoacidemia than WPI ($P < .001$). For plasma EAA, WPI + io demonstrated significantly higher concentrations than WPI at 30, 60, 90 ($P < .001$), and 120 minutes post ingestion ($P < .01$). In addition, WPI + io produced significantly higher BCAA concentrations at 60 and 90 minutes post ingestion and higher leucine concentration at 60 minutes post ingestion compared with WPI ($P < .001$). The iAUC for plasma EAA, BCAA, and leucine concentration was significantly greater in WPI + io by 55.2%, 52.6%, and 50.8%, respectively ($P < .001$; Table 3).

Discussion

This study investigated postprandial plasma concentration of EAA, BCAA, and leucine in response to ingestion of 2 isocaloric and iso-nitrogenous WPI supplements in healthy men. Despite similar rates of AA appearance in plasma, WPI + io demonstrated significantly greater EAA, BCAA, and leucine availability compared with WPI as measured by plasma concentrations responses and iAUC. In addition, both conditions significantly elevated plasma EAA, BCAA, and leucine concentrations at 30 and 60 minutes. However, only in WPI + io were EAA and BCAA concentrations significantly elevated at the 90 and 120 minutes post consumption time points, respectively. Leucine concentration for WPI + io remained significantly elevated at the 90 minutes post consumption. In addition, plasma levels of total EAA and BCAA concentration were significantly greater in WPI + io at 30, 60, 90, and 120 minutes post ingestion compared with WPI.

This study is in agreement with previous literature that the ingestion of a fast absorbing, high-quality protein source

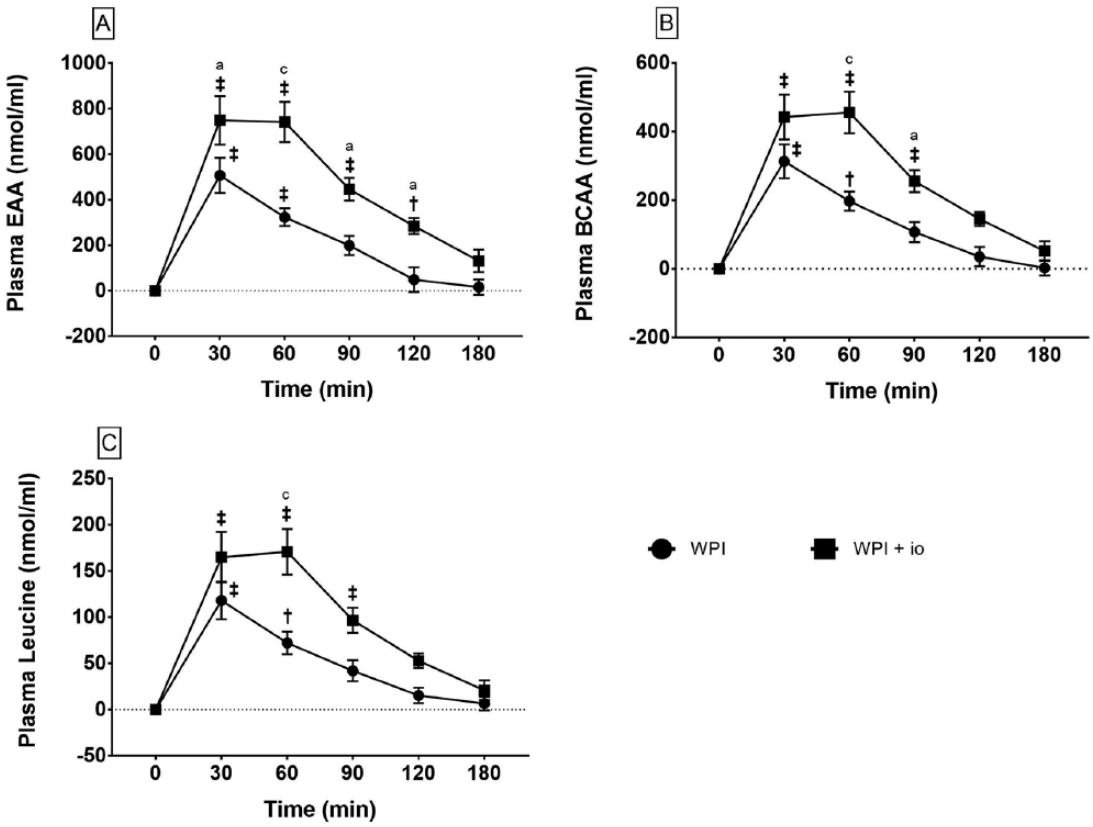

Figure 1. 3-hour time course response of plasma cocentrations of (a) EAA, (b) BCAA, and (c) leucine. BCAA indicates branch chain amino acid; EAA, essential amino acids; a, b, and c indicate difference in A versus B condition at a given time point ($P < .05$, $P < .01$, $P < .001$); † and ‡ indicate difference from 0 min ($P < .01$, $P < .001$).

Table 2. Plasma concentrations of leucine, ΣBCAA, and ΣEAA for WPI and WPI + io.

	PRE	30 MINUTES	60 MINUTES	90 MINUTES	120 MINUTES	180 MINUTES
Leucine (nmol/mL)						
WPI	121.13 ± 7.92	239.24 21.22$^{\#}$	193.23 ± 16.39$^{\wedge}$	163.01 ± 12.58$^{\#}$	136.48 ± 11.33	127.74 ± 8.33
WPI + io	139.29 ± 13.05	304.19 ± 25.46$^{\#,a}$	310.03 ± 22.07$^{\#,c}$	235.95 ± 15.40$^{\#,b}$	192.08 ± 13.22	160.13 ± 7.57
ΣBCAA (nmol/mL)						
WPI	411.08 ± 26.30	724.34 ± 58.34$^{\#}$	608.32 ± 44.17$^{\#}$	518.08 ± 31.65	446.33 ± 34.39	413.70 ± 24.52
WPI + io	476.98 ± 43.94	919.04 ± 58.22$^{\#,c}$	932.51 ± 53.44$^{\#,c}$	732.56 ± 46.88$^{\#,b}$	621.88 ± 43.90^{*,a}	528.47 ± 23.48
ΣEAA (nmol/mL)						
WPI	788.77 ± 37.14	1295.61 ± 95.70$^{\#}$	1111.57 ± 55.75$^{\#}$	987.09 ± 38.09	837.52 ± 47.65	804.57 ± 31.21
WPI + io	902.35 ± 76.95	1650.96 ± 100.05$^{\#,b}$	1643.44 ± 78.88$^{\#,c}$	1347.98 ± 83.08$^{\#,b}$	1186.24 ± 88.04$^{\wedge,b}$	1033.14 ± 44.69

Abbreviations: BCAA, branch chain amino acid; EAA, essential amino acids; WPI, whey protein isolate; WPI + io, whey protein isolate plus Ingredient Optimized Protein®.
Data are expressed as mean ± standard error.
a, b, c Significant differences between conditions at a given time point ($P < .05$, $P < .01$, and $P < .001$).
*, ^, # Difference from PRE $P < .05$, $P < .01$, and $P < .001$.

results in a robust rise in plasma amino acid levels in as early as 30 minutes.[13,28] For instance, Burke et al[28] demonstrated that postprandial AA levels of a fast absorbing protein (ie skim milk) peaked at 50 minutes, compared with 100 minutes for slower digesting proteins. In addition, the authors of this study found marked differences in plasma AA availability, despite both conditions being iso-nitrogenous, thus suggesting a difference in absorption kinetics between

Table 3. Plasma leucine, BCAA, and EAA expressed as iAUC.

	WPI	WPI + IO
iAUC × 10^2 (min nmol/mL)		
Leucine	78.52 ± 0.31	159.72 ± 0.47*
ΣBCAA	201.89 ± 0.74	426.61 ± 1.23*
ΣEAA	335.07 ± 1.16	747.58 ± 2.03*

Abbreviations: BCAA, branch chain amino acid; EAA, essential amino acids; iAUC, incremental area under the curve; WPI, whey protein isolate; WPI + io, whey protein isolate plus Ingredient Optimized Protein®.
Data are expressed as mean ± standard error.
*Significantly greater than WPI ($P < .001$).

two forms of fast absorbing, iso-caloric and iso-nitrogenous protein sources.

Both WPI and WPI + io of this study were sourced from the same product batch and therefore had similar AA profiles. However, the rise in EAA, BCAA, and leucine was of greater amplitude and lasted longer in WPI + io compared with WPI. These differences in circulating amino acids are likely due to WPI + io being treated with cold atmospheric plasma to provoke structural changes in protein peptides to more readily expose binding sites for enzymatic cleavage. This treatment has been shown to expose hydrophobic pockets of protein and increase protein surface hydrophobicity by as much as 20%.[24] Bioavailability of a protein depends on its ability to cross the intestinal mucosa and enter systemic circulation.[29] Proteins with a more hydrophobic surface can permeate the epithelial barrier with more efficiency than proteins with a hydrophilic surface,[30] thereby increasing bioavailability. Furthermore, it has been demonstrated that increasing hydrophobicity of WPI enhances enzymatic degradation, ultimately promoting greater bioavailability.[26]

Previous research showing divergent MPS responses to the same dose of protein suggest that the speed at which AA appear in the blood, and the leucine content of the protein, are the primary factors that determine the magnitude of the MPS response.[31,32] Furthermore, in a rested state, plasma availability of leucine may be more important than the rate of aminoacidemia in determining the MPS response.[8,33] As such, the similar rapid aminoacidemia resulting from both conditions may be more relevant to post exercise feeding, in which the muscle is sensitized to protein feeding.[11,32]

Conclusions

In summary, we report that the ingestion of the treated WPI significantly raises plasma EAA, BCAA, and leucine compared with WPI with no treatment. Furthermore, the rise in EAA, BCAA, and leucine was extended to a larger degree in the treated group compared with the non-treated group. Thus, the technical application of treating WPI with plasma surface modification to further expose hydrophobic pockets and increase enzymatic degradation appears to promote greater concentrations of circulating EAA, BCAA, and leucine. Future research should consider larger sample sizes to denote greater levels of significance between conditions. Furthermore, a similar design could be carried out in an exercising, not resting, condition to investigate the impact of these different proteins on aminoacidemia.

Author Contributions

JMW and RPL were involved in conceptualizing the study and structuring the study design. MHS and MWS were involved in carrying out study procedures and data collection. MHS performed statistical analysis of study data. All authors assisted in writing the manuscript for submission and approved the final version of the manuscript. The results provided in this manuscript do not constitute endorsement of the product by the authors.

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