

# Effects of fish protein hydrolysate ingestion on postexercise aminoacidemia compared with whey protein hydrolysate in young individuals

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**Abstract:** The aminoacidemia resulting from food protein digestion in response to exercise plays an underlying role in the rate of muscle protein synthesis. Whey protein hydrolysate (WPH) has been demonstrated to cause more pronounced postexercise aminoacidemia compared with casein and soy. Although fish protein has been demonstrated to be a great source of amino acids, there is no data available providing information about the postexercise aminoacidemia after fish protein hydrolysate (FPH) intake. The present study investigated the characteristic patterns of postexercise aminoacidemia after WPH and FPH intake in nine physically active subjects (six males and three females). In a crossover, double-blind, and randomized design, all participants received oral doses of either 0.25 g/kg of FPH or WPH or placebo (PLA) immediately after a resistance exercise bout. Blood samples were taken before and at 30, 60, 90, 120 and 180 min after supplementation. There was a significant increase in plasma total amino acids (TAA), essential amino acids (EAA), branched-chain amino acids (BCAA), and leucine concentrations at 30 and 60 min after FPH supplementation, and at 30, 60, 90, and 120 min after WPH as compared to PLA. No significant differences were observed in plasma TAA, EAA, BCAA, and leucine concentrations between FPH and WPH at any time point, and there were no significant difference observed in the area under the curve for TAA, EAA, BCAA, and leucine between FPH and WPH. In conclusion, both FPH and WPH showed a rapid and pronounced postexercise aminoacidemia. FPH presented itself to be an alternative food source of rapidly digested proteins to be used after resistance exercise.

**Keywords:** aminoacidemia, exercise, fish protein, protein supplements

**Practical Application:** Fish protein hydrolysate (FPH) demonstrated a rapid and pronounced postexercise aminoacidemia. Whey protein hydrolysate showed similar effects. FPH is presented as an alternative food source of rapidly digested proteins to be consumed by the population, especially physically active individuals.

## 1. INTRODUCTION

Maintenance of muscle mass is dependent on the balance between protein synthesis and protein breakdown, so protein intake is necessary to deliver essential amino acids (EAA; Fabre et al., 2017; Reidy et al., 2014). Studies have shown that resistance exercise (RE) is a potent stimulus to increase protein synthesis and muscle mass (Fabre et al., 2017; Wernbom, Augustsson, & Thomeé, 2007). Thus, both protein intake and exercise are two of the most potent stimulators of skeletal muscle protein synthesis (Devries & Phillips, 2015; Lund et al., 2017).

The time it takes for a protein to be digested and the level of amino acid absorption play an underlying role in the rate of postexercise amino acid delivery to the muscles, which in turn impacts the overall protein synthesis (Burke et al., 2012; Reidy et al., 2013). The different sources of protein promote different

results for the delivery of amino acids in the blood, which is an important stimulus of protein synthesis (Mitchell et al., 2017). Some studies have demonstrated differences in levels, time, and sources (such as soy, egg, beef, milk and whey protein, among others) to reach maximal amino acid concentration in blood (Burke et al., 2012; Fabre et al., 2017; Melnik, 2015). Several studies have shown whey protein to be an excellent postexercise protein source when compared with other protein sources (Areta et al., 2013; Burke et al., 2012; Devries & Phillips, 2015; Mitchell et al., 2015; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009).

Tang et al. (2009) evaluated the effect of the ingestion of whey protein hydrolysate (WPH), soy protein isolate, and micellar casein on muscle protein synthesis and aminoacidemia after resistance exercises in healthy subjects. The results demonstrated that the consumption of WPH caused more pronounced aminoacidemia after 30 min of ingestion compared with casein and soy. This fact was associated with a more robust stimulus of muscle protein synthesis after WPH ingestion in response to exercise.

There has been evidence that fish protein is a great source of high-quality protein with a good proportion of EAA and positive effects on gastrointestinal absorption (Bhat, Kumar, & Bhat, 2017; Nobile et al., 2016). However, there is no data available in the literature providing information about characteristic patterns of aminoacidemia after intake of fish protein in humans. Therefore, as part of an ongoing multidisciplinary collaboration to

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promote information on the characteristic patterns of postexercise aminoacidemia after intake of protein supplements from different food matrices, the purpose of the present study was to investigate the blood amino acid response of fish protein hydrolysate (FPH) compared to WPH intake after a resistance exercise bout in physically active subjects.

## 2. MATERIAL AND METHODS

### 2.1 Participant

This study included nine physically active subjects (six males and three females aged  $27 \pm 2$  years, with a height of  $174 \pm 13$  cm, a body mass of  $74.1 \pm 9.2$  kg, with at least 3 years of RE experience). All the volunteers presented clinical and biochemical tests carried out in the last 6 months to prove their health status. Criteria of exclusion were as follows: preexisting leg injuries, previous use of supplementation (protein and amino acids supplements), or androgenic anabolic steroids ( $\leq 6$  months prior to analyses). All experimental procedures were performed in accordance with the ethical standards of the Declaration of Helsinki and approved by the institutional ethics committee of the Federal University of Rio de Janeiro, Brazil (protocol number: 53392216.0.0000.5699). All volunteers gave their written informed consent.

### 2.2 Experimental design

The study was conducted in a randomized, double-blind, crossover, and placebo (PLA)-controlled design. All subjects reported to the laboratory on four occasions, with an interval of at least 1 week between visits. The first visit was used to explain the experimental procedures, to conduct one repetition maximum (1-RM) test, and to collect baseline, blood, and blood pressure. In the second, third, and fourth visits, the participants were randomly allocated to FPH, WPH, or PLA supplementation. Blood samples were taken at the beginning of the study (before exercise and nutritional supplementation—Pre) and at 30, 60, 90, 120, and 180 min after supplementation (Figure 1). All visits were held between 07:00 and 11:00 a.m. The participants were instructed to fast for at least 8 hr and restrict physical exercise and caffeine consumption 2 days before each visit.

### 2.3 Nutritional supplementation

In a double-blind and randomized manner, all participants received oral doses of either sucralose (as PLA) or 0.25 g/kg (Jäger et al., 2017) of FPH or WPH (the dose were adjusted based on the protein content in each product) in the form of drinks diluted in 200 mL of water at room temperature immediately after RE. In order to perform the current study in a double-blind way, the participants were informed that the PLA intervention might be fish protein with different flavor, although sucralose was used as PLA (deceptive PLA administration). Thus, the participants were informed that the purpose of the study was to observe if the addition of flavor in FPH supplement could influence the aminoacidemia compared to FPH with regular flavor and WPH. At the end of the experimental procedures, all participants were informed that PLA offered was not FPH in different flavor and that this deceptive administration protocol was employed to maintain the reliability of results of the study (Bailey et al., 2015; Beedie, Stuart, Coleman, & Foad, 2006; Costa et al., 2019; Duncan, Lyons, & Hankey, 2009; Kirsch & Weixel, 1988). The FPH was prepared in our laboratory as previously described (Alvares, Conte-Junior, Pierucci, Oliveira, & Cordeiro, 2018). In brief, approximately 60 kg of tilapia (Nile tilapia—*Oreochromis niloticus*) by-products

**Table 1—Proximate composition of the FPH and WPH supplement.**

	FPH	WPH
Moisture (%)	7.13 $\pm$ 0.25	6.13 $\pm$ 0.31
Protein (%)	80.63 $\pm$ 0.97	86.47 $\pm$ 0.75
Lipids (%)	0.61 $\pm$ 0.02	0.60 $\pm$ 0.02
Carbohydrate (%)	9.90 $\pm$ 0.5	5.2 $\pm$ 0.5
Ash (%)	1.71 $\pm$ 0.03	1.61 $\pm$ 0.02

Note. The values are mean  $\pm$  standard deviation.

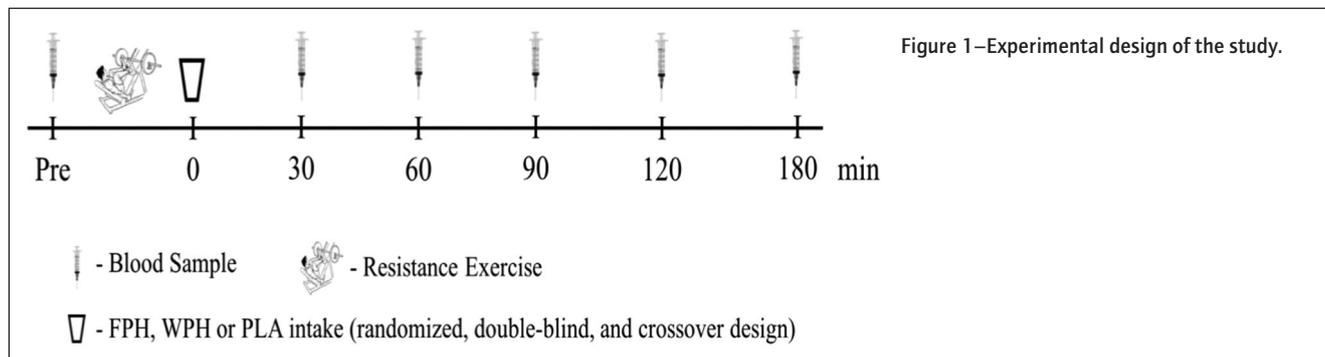
Abbreviations: FPH, fish protein hydrolyzed; WPH, whey protein hydrolyzed.

**Table 2—Amino acid profile of the fish protein hydrolysate (FPH) and whey protein hydrolysate (WPH).**

Amino acid (mg/g)	FPH	WPH
Alanine	18.23 $\pm$ 3.21	14.24 $\pm$ 1.86
Arginine	33.34 $\pm$ 6.54	11.02 $\pm$ 0.85
Aspartic acid	135.45 $\pm$ 19.70	173.52 $\pm$ 14.19
Glutamic acid	191.24 $\pm$ 30.79	223.78 $\pm$ 23.23
Glycine	38.08 $\pm$ 6.69	17.64 $\pm$ 1.50
Histidine	80.47 $\pm$ 8.07	68.17 $\pm$ 1.77
Isoleucine	20.87 $\pm$ 4.58	45.75 $\pm$ 7.41
Leucine	33.78 $\pm$ 6.41	49.43 $\pm$ 5.74
Lysine	35.07 $\pm$ 6.16	40.48 $\pm$ 5.07
Methionine	29.77 $\pm$ 1.93	18.44 $\pm$ 1.96
Phenylalanine	41.28 $\pm$ 7.12	55.72 $\pm$ 8.92
Serine	55.28 $\pm$ 9.32	55.68 $\pm$ 6.21
Threonine	66.30 $\pm$ 12.25	115.83 $\pm$ 13.37
Tryptophan	2.57 $\pm$ 0.44	1.49 $\pm$ 0.38
Valine	37.31 $\pm$ 3.67	63.26 $\pm$ 2.32
TAA	819.02 $\pm$ 124.10	954.40 $\pm$ 94.80
EAA	347.40 $\pm$ 48.23	458.60 $\pm$ 42.20
BCAA	91.96 $\pm$ 14.48	158.40 $\pm$ 14.60

Note. The values are mean  $\pm$  standard deviation. FPH, fish protein hydrolyzed; WPH, whey protein hydrolyzed; TAA, total amino acids; EAA, essential amino acids; BCAA, branched-chain amino acids.

(dark muscle, frame, skin, bones, guts, and fins) were acquired from the Regional Cooperative of Fish farmers in Cachoeira de Macacu, Rio de Janeiro, Brazil. All the tilapia by-products were acquired from three different batches (20 kg each batch). The fish mince was dried in stove at 180 °C for 100 min, and then the mass formed during this process was milled in a multiprocessor for 2 min. Subsequently, a second heating (200 °C for 40 min) and milling procedures were repeated to obtain the final flour. The fish flour was mixed with distilled water (1:2) and adjusted to pH 7.5 with 1 M potassium hydroxide. The hydrolysis process was done in a water bath (Dubnoff orbital – NT 230, Nova Técnica, Brazil) setup at 55 °C. The enzymatic hydrolysis was started by adding 2% of 2.4 L Alcalase enzyme (Novozymes, Novo Alle, DK-2880 Bagsvaerd, Denmark). After 4 hr of hydrolysis, the enzyme was inactivated by heating at 90 °C for 15 min in a water bath. The mixture was then centrifuged at  $8,000 \times g$  for 10 min and the supernatant was collected and filtered in 0.45  $\mu$ m cellulose filter. The FPH was passed into a spray-drier (mini spray dryer B-290 advanced, Büchi Labortechnik AG, Switzerland) and the resulting powder was used for supplementation. The WPH (Dymatize ISO 100 hydrolyzed 100%) was purchased from a dietary supplements store. Both FPH and WPH were analyzed for proximate composition, according to AOAC method (AOAC, 2012), and total amino acids (TAA) by using a high-performance liquid chromatography (HPLC) system, according to Gatti, Gioia, Leoni, and Andreani (2010), which are summarized in the Tables 1 and 2, respectively.



## 2.4 Plasma amino acids analysis

Blood was drawn from antecubital veins and collected in Ethylenediaminetetraacetic acid (EDTA)-containing tubes, and then immediately centrifuged at  $3,000 \times g$  for 10 min at  $4^\circ\text{C}$  in order to separate the plasma, before storing it at  $-20^\circ\text{C}$  for later analysis. Plasma amino acids were analyzed as previously described by Gatti et al. (2010) with modifications in the sample deproteinization step. In brief, 50  $\mu\text{L}$  of plasma was mixed with 50  $\mu\text{L}$  of 1.5 M perchloric acid (v/v) to remove proteins. After 2 min at room temperature, 0.750 mL  $\text{H}_2\text{O}$  and 25  $\mu\text{L}$  potassium carbonate were added. The tubes were centrifuged at  $10,000 \times g$  for 2 min. The sample (50  $\mu\text{L}$ ) was mixed with 50  $\mu\text{L}$  of the  $\text{H}_2\text{O}$  and 40  $\mu\text{L}$  of the 2,5-dimethyl-1H-pyrrole-3,4-dicarbaldehyde reagent solution (v/v) for 10 min. A total of 360  $\mu\text{L}$  of mobile phase (0.05 M triethylammonium phosphate buffer) was added to the derivatized solution, which was immediately analyzed by HPLC. The HPLC device was equipped with a 3- $\mu\text{m}$  reversed-phase C18 column ACE (250 mm  $\times$  4.6 mm, i.d.) guarded by a 5- $\mu\text{m}$  reversed-phase C18 guard column Ascentis (20 mm  $\times$  4.6 mm; i.d.) and a photodiode array detector model RF-10AXL (Shimadzu) monitoring absorbance at 320 nm.

## 2.5 Exercise protocol

The present study was conducted using a resistance exercise test of Leg Press  $45^\circ$  (Movement<sup>®</sup>) and Leg Extension (Movement<sup>®</sup>). The 1-RM was estimated according to Sugiura, Hatanaka, Arai, Sakurai, and Kanada (2016). After warm-up, the volunteers performed the leg press exercise. Afterward, the knee extension exercise was done. Three sets of 10 to 12 RM of Leg Press  $45^\circ$  and Leg Extension exercise were done. The interval between sets and exercises was 2 min. The nutritional supplementation was offered immediately after RE to simulate the habitual routine of physically active subjects that practice this modality of exercise.

## 2.6 Statistical analysis

An a priori sample-size calculation (compute required sample size—given  $\alpha$ , power, and effect size) was conducted (G\*Power v. 3.1.9.2) for an F test (ANOVA: repeated measures, within-between interaction for six-time points). On the basis of a statistical power ( $1 - \beta$ ) of .80, a moderate effect size ( $f = .25$ ), correlation among repeated measures of 0.5, nonsphericity correction  $\epsilon$  of 1, and an overall level of significance of .05, at least eight participants (submitted to all three treatments in a crossover fashion, totalizing 24 samples) would be needed to detect statistical significance. A medium effect size (Cohen's  $f = .25$  for ANOVA test) was chosen according to the recommendation to estimate the sample size, taking into account the ethical aspects (to avoid using a too large sample size in human studies) and effectiveness of

the nutritional intervention (Cunningham & McCrum-Gardner, 2007; Sullivan & Feinn, 2012). Additionally, the determination of the effect size for aminoacidemia after FPH and WPH ingestion was based on Cohen's  $d$ , because Cohen's  $f$  identifies the magnitude of the differences among all groups (FPH, WPH, and PLA), but does not identify differences between specific groups (that is, FPH vs. WPH). Thus, the magnitude of the nutritional intervention on aminoacidemia was calculated by Cohen's  $d$ , with values  $<0.2$  considered trivial, 0.2 to  $<0.5$  small effect, 0.5 to  $<0.8$  moderate effect, and  $\geq 0.8$  large effect ( $d = 2f$  or, conversely,  $f = 1/2d$ ; Cunningham & McCrum-Gardner, 2007; Grove & Cipher, 2016).

The normality, sphericity, and homogeneity of variances of the data were examined with the Shapiro–Wilk, Mauchly, and Levene tests, respectively. To identify differences in plasma TAA, EAA, branched-chain amino acids (BCAA), and leucine concentrations between FPH, WPH, and PLA, a two-way ANOVA with repeated measures ( $3 \times 7$ ; treatment  $\times$  time) was used. Area under the curve from 0 to 180 min ( $\text{AUC}_t$ ) was calculated with PKSolver add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. To identify differences in the  $\text{AUC}_t$  among PLA, WPH and FPH, a repeated one-way ANOVA measure was used. When a significant  $F$ -value was found, an additional post hoc test with Bonferroni was performed. For all variables, when sphericity was violated, a Greenhouse–Geisser correction was used. Statistical significance was set at the .05 level of confidence. All analyses were performed using a commercially available statistical package (IBM SPSS Statistics version 23 for Mac, Chicago, IL) and the results were expressed as the mean  $\pm$  standard deviation ( $SD$ ).

## 3. RESULTS

All nine subjects completed the study. The plasma TAA, EAA, BCAA, and leucine concentrations over 180 min after a single dose of FPH, WPH, and PLA in response to RE are shown in the Figures 2, 3, 4, and 5, respectively. There was a significant interaction effect regarding time (Pre, 30, 60, 90, 120, and 180 min) per supplementation (FPH vs. WPH vs. PLA) for TAA ( $P < 0.0001$ ), EAA ( $P < 0.0001$ ), BCAA ( $P < 0.0001$ ), and leucine ( $P < 0.0001$ ). There was a main effect regarding time for TAA ( $P < 0.0001$ ), EAA ( $P < 0.0001$ ), BCAA ( $P < 0.0001$ ), and leucine ( $P < 0.0001$ ). Post hoc analysis revealed a significant increase in TAA at 30 min ( $P < 0.0001$ ) and 60 min ( $P = 0.002$ ) after FPH supplementation, and at 30 min ( $P < 0.0001$ ), 60 min ( $P < 0.0001$ ), 90 min ( $P = 0.001$ ), and 120 min ( $P = 0.021$ ) after WPH as compared to PLA (Figure 2). Regarding EAA, post hoc analysis revealed a significant increase at 30 min ( $P = 0.047$ ) and 60 min ( $P = 0.033$ ) after FPH supplementation, and at 30 min

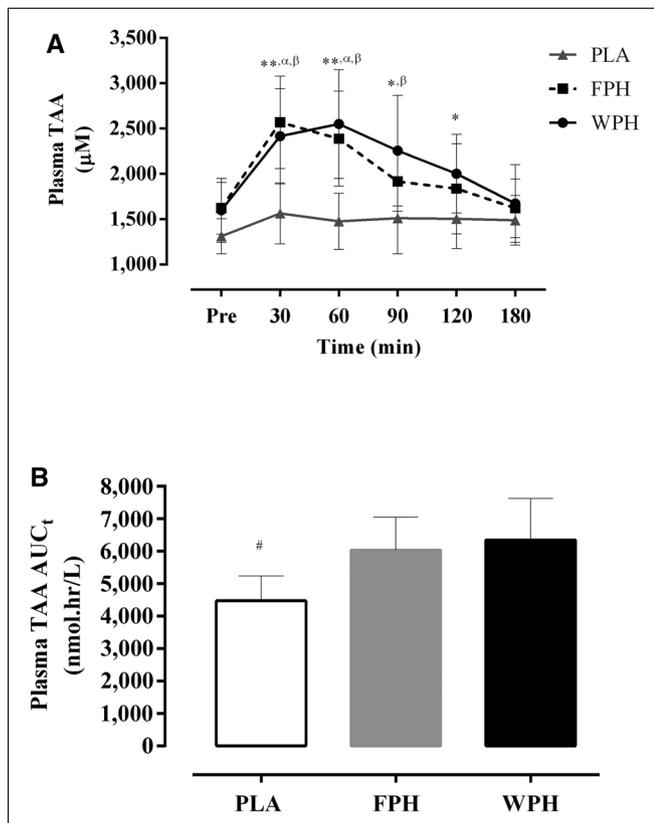


Figure 2—Plasma TAA concentrations (A) and total area under the curve (AUC<sub>t</sub>) (B) during the 180 min postsupplementation period. Abbreviations: TAA, total amino acids; PLA, placebo; FPH, fish protein hydrolyzed; WPH, whey protein hydrolyzed. \*\*Significantly different from Pre for FPH and WPH; \*significantly different from Pre for WPH only; <sup>α</sup>significantly different from PLA for FPH; <sup>β</sup>significantly different from PLA for WPH; <sup>#</sup>significantly different from FPH and WPH.

( $P = 0.013$ ), 60 min ( $P = 0.002$ ), 90 min ( $P = 0.032$ ), and 120 min ( $P = 0.021$ ) after WPH as compared to PLA (Figure 3). Regarding BCAA, post hoc analysis revealed a significant increase at 30 min ( $P < 0.0001$ ) and 60 min ( $P = 0.005$ ) after FPH supplementation, and at 30 min ( $P < 0.0001$ ), 60 min ( $P < 0.0001$ ), 90 min ( $P < 0.0001$ ), and 120 min ( $P = 0.009$ ) after WPH as compared to PLA (Figure 4). Regarding leucine, post hoc analysis revealed a significant increase in leucine at 30 min ( $P = 0.001$ ) and 60 min ( $P = 0.026$ ) after FPH supplementation, and at 30 min ( $P < 0.0001$ ), 60 min ( $P = 0.001$ ), 90 min ( $P = 0.002$ ), and 120 min ( $P = 0.045$ ) after WPH as compared to PLA (Figure 5). Individual average variation in TAA, EAA, BCAA, and Leucine total area under the curve (AUC<sub>t</sub>) for PLA versus FPH versus WPH are shown in the Figure 6.

Additionally, the effect size for TAA between FPH and WPH at pre ( $d = 0.073$ ), 30 min ( $d = 0.296$ ), 60 min ( $d = 0.285$ ), 90 min ( $d = 0.693$ ), 120 min ( $d = 0.354$ ), and 180 min ( $d = 0.137$ ) was determined. The effect size for EAA between FPH and WPH at pre ( $d = 0.034$ ), 30 min ( $d = 0.240$ ), 60 min ( $d = 0.502$ ), 90 min ( $d = 0.966$ ), 120 min ( $d = 0.434$ ), and 180 min ( $d = 0.175$ ) was determined. The effect size for BCAA between FPH and WPH at pre ( $d = 0.140$ ), 30 min ( $d = .229$ ), 60 min ( $d = .783$ ), 90 min ( $d = 1.198$ ), 120 min ( $d = 0.644$ ), and 180 min ( $d = 0.505$ ) was determined. The effect size for leucine between FPH and WPH at pre ( $d = 0.033$ ), 30 min ( $d = 0.215$ ), 60 min ( $d = 0.609$ ), 90 min ( $d = 1.000$ ), 120 min ( $d = 0.529$ ), and 180 min ( $d = 0.543$ ) was

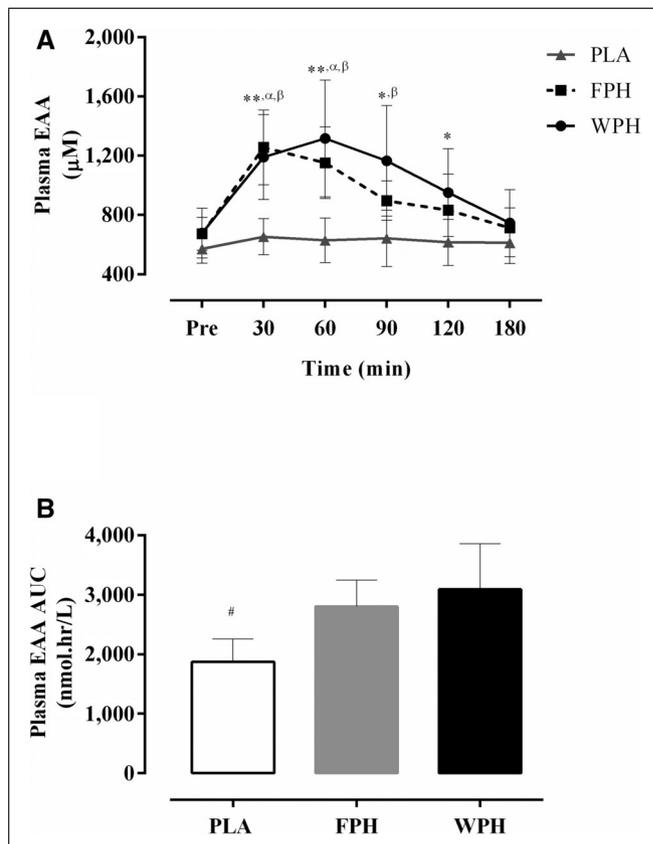


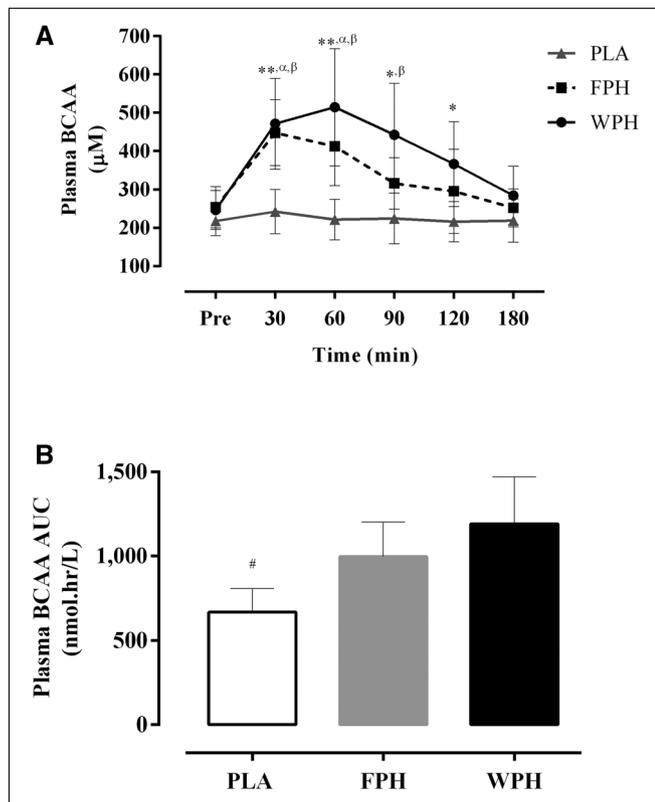
Figure 3—Plasma EAA concentrations (A) and total area under the curve (AUC<sub>t</sub>) (B) during the 180 min postsupplementation period. Abbreviations: EAA, essential amino acids; PLA, placebo; FPH, fish protein hydrolyzed; WPH, whey protein hydrolyzed. \*\*Significantly different from Pre for FPH and WPH; \*significantly different from Pre for WPH only; <sup>α</sup>significantly different from PLA for FPH; <sup>β</sup>significantly different from PLA for WPH; <sup>#</sup>significantly different from FPH and WPH.

determined. The effect size for AUC between FPH and WPH to TAA ( $d = 0.274$ ), to EAA ( $d = 0.461$ ), to BCAA ( $d = 0.656$ ), and to leucine ( $d = 0.656$ ) was determined.

#### 4. DISCUSSION

To our knowledge, this was the first study to provide evidence of characteristic patterns of postexercise aminoacidemia after intake of a protein supplement from fish (FPH) in comparison to a protein supplement from milk (WPH). A rapid and pronounced hyperaminoacidemia after FPH and WPH intake was observed. Plasma TAA, EAA, BCAA, and leucine concentrations peaked at 30 min after FPH intake, whereas the peak occurred at 60 min after WPH.

It has been suggested that the resulting aminoacidemia from protein food digestion is an independent variable that affects muscle protein synthesis (West et al., 2011). Previous studies have shown that achieving rapid and pronounced plasma concentration of amino acids (that is, EAA and leucine) is associated with increased rates of muscle protein synthesis after resistance exercise (West et al., 2011). Boirie et al. (1997) observed a rapid postexercise aminoacidemia and leucinemia after WPH ingestion as compared to casein protein. In others words, the ingestion of a rapidly digested whey protein, compared with slowly digested casein, results in a rapid transient aminoacidemia of greater amplitude than does a gradual prolonged aminoacidemia with

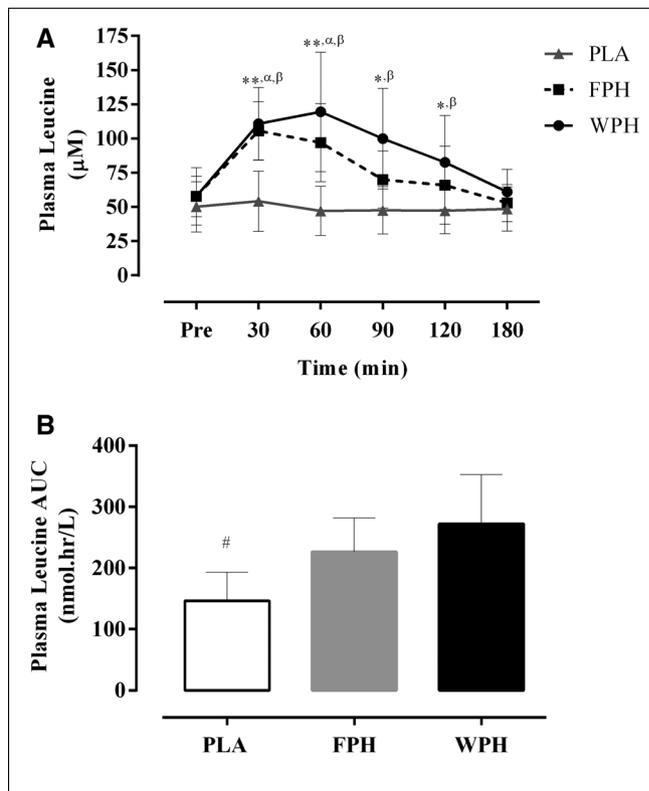


**Figure 4**—Plasma BCAA concentrations (A) and total area under the curve (AUC<sub>t</sub>) (B) during the 180 min postsupplementation period. Abbreviations: BCAA, branched-chain amino acids; PLA, placebo; FPH, fish protein hydrolyzed; WPH, whey protein hydrolyzed. \*\*Significantly different from Pre for FPH and WPH; \*significantly different from Pre for WPH only; <sup>α</sup>significantly different from PLA for FPH; <sup>β</sup>significantly different from PLA for WPH; <sup>#</sup>significantly different from FPH and WPH.

casein that influences whole-body and skeletal muscle protein synthesis after exercise. In the present study, the plasma peak of TAA, EAA, BCAA, and leucine was at 30 min after FPH and 60 min after WPH, suggesting that FPH promotes rapid and pronounced postexercise aminoacidemia, although differences in peak concentrations and AUC<sub>t</sub> of TAA, EAA, BCAA, and leucine were not significantly different between FPH and WPH.

There is evidence showing higher peak values for TAA, EAA, BCAA, and leucine after WPH than the other protein sources (for example, soy and casein; Tang et al., 2009), suggesting that whey protein is a useful supplement for individuals engaged in RE in order to reach muscle hypertrophy. Our data demonstrated that plasma TAA, EAA, BCAA, and leucine were not significantly different after WPH and FPH supplementation, indicating that FPH ingestion seems to induce an increase in plasma amino acids in a way similar to WPH, which is a well-known protein supplement that increases plasma amino acids bioavailability (Areta et al., 2013; Burke et al., 2012; Devries & Phillips, 2015; Fabre et al., 2017; Mitchell et al., 2015; Tang et al., 2009; Wu, 2009).

Although no statistical differences in plasma TAA, EAA, BCAA, and leucine were observed when comparing WPH to FPH, a moderate-to-large effect size for TAA, EAA, BCAA, and leucine was observed over postingestion time (from 30 to 180 min) for WPH, suggesting that WPH might be somewhat more effective than FPH in terms of postingestion plasma amino acids delivery. It is important to point out that the protein content in each supplement was adjusted for the participant's weight before its inges-



**Figure 5**—Plasma leucine concentrations (A) and total area under the curve (AUC<sub>t</sub>) (B) during the 180 min postsupplementation period. Abbreviations: PLA, placebo; FPH, fish protein hydrolyzed; WPH, whey protein hydrolyzed. \*\*Significantly different from Pre for FPH and WPH; \*significantly different from Pre for WPH only; <sup>α</sup>significantly different from PLA for FPH; <sup>β</sup>significantly different from PLA for WPH; <sup>#</sup>significantly different from FPH and WPH.

tion and FPH contained less TAA (~14%), EAA (~24%), BCAA (~41%), and leucine (~31%) than WPH supplement, which may explain the moderate-to-large effect size observed in plasma amino acids (TAA, EAA, BCAA, and leucine concentration) over the time after WPH as compared to FPH. Furthermore, it is noteworthy that although the current study showed similar postexercise aminoacidemia after FPH or WPH intake, the data should be interpreted with caution as the FPH was prepared in our laboratory (enzymatic hydrolysis) and the WPH was acquired commercially (chemical hydrolysis).

It has been demonstrated that diet, metabolism, sex, age, lifestyle, and genetic factors (Cynober, 2002; Schmidt et al., 2016) may influence the concentrations of plasma amino acids. Furthermore, the intestinal microbiota may also influence the plasma amino acids, because the small intestine is involved in protein digestibility as well as amino acid absorption (Dai, Wu, & Zhu, 2011; Jandhyala et al., 2015). A previous study has demonstrated that plasma amino acids may vary intraindividually from 9.5% to 46.4% (Corte & Venta, 2010) after protein supplement intake, which aligns with our findings (Hamarsland et al., 2017; Tang et al., 2009). However, it is important to point out that, in both studies (Tang et al., 2009 and Hamarsland et al., 2017), the dosage of the protein supplements was adjusted according to the EAA content of the supplement, which may increase the individual variation as compared to ingestion of the same amount of protein kilogram of body weight as administered in the present study.

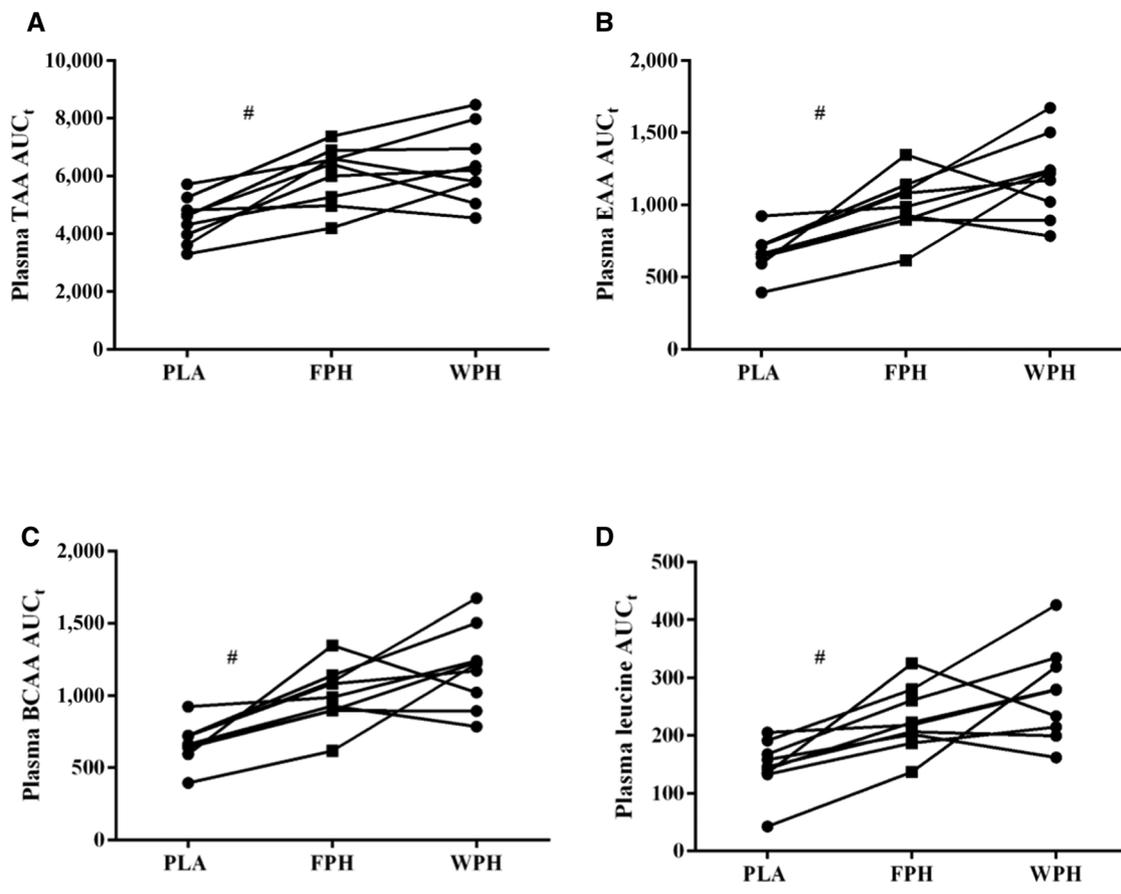


Figure 6—Individual average variation TAA total area under the curve ( $AUC_t$ ) for PLA versus FPH versus WPH (A); individual average variation EAA  $AUC_t$  for PLA versus FPH versus WPH (B); individual average variation BCAA  $AUC_t$  for PLA versus FPH versus WPH (C); individual average variation leucine  $AUC_t$  for PLA versus FPH versus WPH (D). Abbreviations: EAA, essential amino acids; PLA, placebo; FPH, fish protein hydrolyzed; TAA, total amino acids; WPH, whey protein hydrolyzed. #Significantly different from FPH and WPH.

The findings of the present study are valuable because the rapid delivery of EAA to the muscle cells can enhance the stimulus for protein synthesis, which is crucial for those who practice RE. Thus, it is important to evaluate the effect of alternative protein sources on postexercise aminoacidemia in order to increase the current pool of knowledge, and thus to help professionals to delineate an effective nutritional strategy. Taken together, these findings suggest that FPH contains a good proportion of EAA, which may be useful in increasing the pool of amino acids in the plasma after a RE session.

## 5. Conclusions

In conclusion, both fish protein and whey protein supplements have been shown to deliver rapid and pronounced postexercise aminoacidemia. FPH can be considered a valuable and alternative food source of rapidly digested proteins to be used after resistance exercise to provide an anabolic advantage that may lead to greater muscle hypertrophy.

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## AUTHOR CONTRIBUTIONS

E.M.C., O.C.V., M.V.S., and G.V.O. contributed to volunteers' recruitment and amino acids analysis. E.M.C. and T.S.A. contributed substantially to data acquisition, statistical analysis, and data interpretation and also they were the manuscript writer. All authors read and approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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