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ABSTRACT

Purpose: Whey protein ingestion is typically considered an optimal dietary strategy to maximize myofibrillar protein synthesis (MyoPS) following resistance exercise. While single source plant protein ingestion is typically less effective, at least partly, due to less favorable amino acid profiles, this could theoretically be overcome by blending plant-based proteins with complementary amino acid profiles. We compared the post-exercise MyoPS response following the ingestion of a novel plant-derived protein blend with an isonitrogenous bolus of whey protein. **Methods:** Ten healthy, resistance trained, young adults (male/female: 8/2; age: 26±6 y; BMI: 24±3 $kg \cdot m^{-2}$) received a primed continuous infusion of L-[*ring*-$^{2}H_5$]-phenylalanine and completed a bout of bilateral leg resistance exercise before ingesting 32 g protein from whey (WHEY) or a plant protein blend (BLEND; 39.5% pea, 39.5% brown rice, 21.0% canola) in a randomized, double-blind crossover fashion. Blood and muscle samples were collected at rest, and 2 and 4 h after exercise and protein ingestion, to assess plasma amino acid concentrations, and postabsorptive and post-exercise MyoPS rates. **Results:** Plasma essential amino acid availability over the 4 h postprandial post-exercise period was ~44% higher in WHEY compared with BLEND (P=0.04). From equivalent postabsorptive values (WHEY, 0.042±0.020%·h^{-1}; BLEND, 0.043±0.015%·h^{-1}) MyoPS rates increased following exercise and protein ingestion (time effect; P<0.001) over a 0-2 h (WHEY, 0.085±0.037%·h^{-1}; BLEND, 0.080±0.037%·h^{-1}) and 2-4 h (WHEY, 0.085±0.036%·h^{-1}; BLEND, 0.086±0.034%·h^{-1}) period, with no differences between conditions during either period or throughout the entire (0-4 h) postprandial period (time × condition interactions; all P>0.05). **Conclusions:** Ingestion of a novel plant-based protein blend stimulates post-exercise MyoPS to an equivalent extent as a whey protein,

demonstrating the utility of plant protein blends to optimize post-exercise skeletal muscle reconditioning.

Key Words: MUSCLE PROTEIN SYNTHESIS, RESISTANCE EXERCISE, SPORTS NUTRITION, STABLE ISOTOPES, WHEY PROTEIN

INTRODUCTION

Resistance exercise induced skeletal muscle recovery, adaptation, and/or hypertrophy are largely dictated by the post-exercise increment in muscle protein synthesis (MPS) rates (1, 2). Physiologically, this is achieved by each bout of resistance exercise stimulating MPS (most notably myofibrillar protein synthesis [MyoPS]) in the subsequent hours and days (~48 h) following resistance exercise (3, 4). In the absence of nutrition, however, the parallel elevation of muscle protein breakdown (MPB) rates following resistance exercise results in a negative net protein balance (3, 5, 6), ultimately limiting muscle hypertrophy. Post-exercise dietary protein ingestion augments MyoPS rates creating a net positive muscle protein balance (7, 8). Thus, optimizing the synergy between exercise and protein ingestion for stimulating MyoPS is integral in nutritionally supporting the adaptive response to prolonged training (9).

Animal-derived protein sources are generally considered more effective at stimulating post-exercise MPS rates compared with plant-based proteins (10-12). In part, this is due to animal proteins displaying greater postprandial amino acid bioavailability, often attributed to the propensity of plant-based proteins to contain higher fiber, phytonutrients, and/or other nutrients hindering gut amino acid absorption (13). However, such limitations can be overcome within the realm of sports nutrition by developing plant-based protein isolates/concentrates to be consumed as post-exercise dietary supplements. Nevertheless, whey protein is generally regarded as an optimal post-exercise dietary protein source to support post-exercise muscle reconditioning, since its ingestion has reliably been shown to stimulate MyoPS rates to a greater extent, albeit not always (14), than isonitrogenous doses of soy (10, 12) or wheat (15) protein isolates. Given efficient protein isolation techniques can largely mitigate digestion, absorption, and

bioavailability issues, the likely explanation for these findings is the more preferable amino acid composition of whey compared with plant-based proteins (16). Further, increasing societal (13) and governmental (17, 18) drives for reducing reliance on animal-derived proteins, optimizing this aspect of plant-based sports nutrition is an important academic and applied goal.

Whey protein is rich in leucine (19), the amino acid thought principally responsible for stimulating myocellular anabolic signaling pathways (20, 21). However, whey protein is also rich in other EAAs which may be of more relevance for such cell signaling pathways than previously appreciated; with recent work questioning the near exclusive role of leucine in supporting the post-exercise increase in MPS (22, 23). Further, whey protein is also high in other essential amino acids, important not only from a signaling perspective (24), but also to prevent potential substrate limitation for the continuation of elevated (> 2 h) post-exercise MyoPS rates (25, 26). Conversely, plant-based proteins are typically low(er) in key amino acids, such as leucine (e.g. hemp, wheat), isoleucine (e.g. oat, hemp), valine (e.g. hemp), methionine (e.g. soy, pea, lentil) or lysine (e.g. wheat, maize, rice) (19, 27). Recent strategies to develop optimal plant-based protein supplements have therefore focused on protein blends to overcome lower contents of key amino acids (28, 29). Accordingly, when sufficient total protein (~25-30 g), EAAs (~10 g) and leucine (~2.5 g) are consumed, such studies have shown robust increases in MPS following protein blend ingestion (29-35). However, protein blends tested to date have included animal-derived sources (30, 31, 33), have been conducted in resting muscle only which reduces the relevance to exercise (whilst also not accounting for greater systemic amino acid demand) (32-34), and/or did not compare with whey protein (29, 32-34) as an appropriate gold standard control within sports nutrition.

We composed an exclusively plant-derived protein blend with a complementary amino acid profile and no amino acid deficiencies, consisting of protein isolates from pea (high in leucine and lysine, low in methionine), brown rice (high in methionine, low in lysine), and canola (no amino acid deficiencies and with high isolation efficiency) to assess MyoPS rates following resistance exercise, and in comparison to whey protein. We hypothesized that this novel plant-based protein blend, formulated to a ratio and dose designed to deliver sufficient total protein (32 g) and leucine (2.5 g), would stimulate post-exercise MyoPS rates to the same extent as an isonitrogenous bolus of whey protein in healthy young males and females.

METHODS

Participants

Ten healthy, young adults (sex [m/f], 8/2; age: 26±6 y; body mass, 79±16 kg; BMI, 24±3 $kg \cdot m^{-2}$) volunteered to participate in this randomized, double-blind crossover design trial (Supplemental Figure 1, Supplemental Digital Content, http://links.lww.com/MSS/C984, Participant flow diagram). Participants' characteristics are displayed in Table 1. Participants were resistance trained, non-smokers, and free of any cardiorespiratory, metabolic and musculoskeletal disorders. Resistance trained was classified as performing resistance exercise ≥3 times per week, for >3 months prior to enrolling in the study. This population was selected to maximize the anabolic response to exercise given participants were familiar with optimal execution of the exercise protocol in a safe and effective manner, and to increase translational applicability of the findings. Enrolled individuals had not undergone a stable isotope infusion protocol for at least 3 months prior and were deemed healthy based on blood pressure (<140/90

mmHg), BMI (18-30 $kg \cdot m^{-2}$), and responses to a routine medical health questionnaire. Written informed consent was obtained following explanations of the experimental procedures, associated benefits, and potential risks of taking part in the study. This study is registered at clinicaltrials.gov (NCT06129513) and was approved by the University of Exeter Sport and Health Sciences Ethics Committee (22-02-02-B-02 v2) in accordance with the principles of the Declaration of Helsinki. Data collection for this study was carried out within the Nutritional Physiology Research Unit (NPRU) at the University of Exeter between March 2022 and October 2022.

Pretesting

At least one week before the first experimental trial, participants reported to the laboratory for a single pretesting visit. During this visit, body composition (via Air Displacement Plethysmography (BodPod, Life Measurement, Inc.)) was assessed, participants were familiarized with the exercise protocol to be performed during the experimental trial visits, and leg strength was assessed, as described below in *Resistance exercise protocol.*

Following pretesting, participants were randomly allocated to ingest whey (WHEY) or a plant-blend (BLEND) protein beverage in a counterbalanced order for their first and second experimental visits. Randomization was performed by an independent person using a computerized randomizer. The washout period between visits was 3-10 weeks (5.5±2.5 weeks) to allow recovery from the exercise, blood collections and muscle biopsies, and ensure local swelling and inflammation was subsided.

Diet and activity before testing

Following pretesting, all participants were instructed to refrain from any vigorous physical activity and/or exercise for 3 days prior to two experimental visits. Female participants not taking hormonal contraceptives were tested in the first 10 d of their menstrual cycle (i.e. post menses), which was assessed by self-report, to control for hormonal fluctuations (36). To control for potential variation in the participants' habitual diet, all participants were provided with an identical standardized diet on the day before each trial visit. Basal metabolic rate (BMR) was estimated using the Henry equations based on age, sex, and body mass (37). The International Physical Activity Questionnaire (IPAQ) was used to calculate a physical activity factor (38). Individual energy requirements were then calculated by multiplying the participants' BMR by their physical activity factor. An individual 1-day meal plan was designed for each participant with all food prepared, weighed, and packaged in-house in the Nutritional Physiology research kitchen facility. The participants' diet was designed to match energy expenditure (i.e. energy balance) and consisted of 1.2 g of protein per kg body mass, with 49±2% of the remaining energy being provided as carbohydrate, 34±2% as fat, and 2±0% as fiber.

Experimental protocol

An overview of the experimental visits is shown in Figure 1. Participants arrived at the laboratory at ~07:30 h after an overnight fast. A Teflon cannula was inserted into an antecubital vein for stable isotopically labelled amino acid infusion. After taking a venous baseline blood sample to measure background isotope enrichments, the phenylalanine pool was primed with a single intravenous dose of L-[*ring*-$^{2}H_5$]-phenylalanine (2.12 μmol·kg^{-1}). Thereafter, a continuous L-[*ring*-$^{2}H_5$]-phenylalanine intravenous tracer infusion was initiated (t = -270 min) and

maintained at a rate of 0.05 μmol·kg^{-1}·min^{-1} for the duration of the test day. After the infusion was started, a second Teflon cannula was inserted into a dorsal hand vein and placed within a heated hand unit (55°C) for the collection of repeated arterialized venous blood samples (39). Arterialized venous blood samples were collected throughout the experimental protocol at the following time points: *t* = -180, -60, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min.

After 90 min following the start of the infusion, a muscle biopsy sample was collected at *t* = -180, and again at -60 min from the same leg to determine resting and postabsorptive MyoPS rates (no weight bearing activity was permitted during this period). The order of the leg to be biopsied was randomized between participants to remove any potential order effect. All muscle biopsies were collected from the mid-region of the *M. vastus lateralis* with a modified Bergstrom suction needle under local anesthesia (2% lidocaine) (40). All biopsy samples were immediately freed from any visible blood, adipose, and connective tissue, frozen in liquid nitrogen, and stored at -80°C until subsequent analysis. At *t* = -45 min, participants executed a bout of bilateral leg resistance exercise, as described below. Upon completion of the exercise protocol, participants were taken back to the laboratory and a further muscle biopsy sample was collected from the contralateral leg to that where the initial 2 biopsies were collected from. Immediately following this biopsy participants ingested the allocated WHEY or BLEND beverage, within an allotted 5 min time period (matched from first to second visit). Consumption of the drink (*t* = 0 min) indicated commencement of the post-exercise, postprandial period and participants rested in a semi-supine position for 4 h. At *t*=5 min participants completed a 100 mm visual analogue scale (VAS) to subjectively assess protein beverage palatability with 0 ‘worst ever’ and 100 ‘best ever’, as previously described (41), and were asked to attempt to identify the protein beverage

they consumed. Palatability scores were higher for WHEY compared with BLEND (75±11 vs 51±22 mm; $P<0.01$), and protein beverage was identified correctly on 80% of occasions implying modest success of the blinding procedures. At t = 120 and 240 min, further muscle biopsies were collected (from the same leg as biopsy 3 was collected) 1-2 cm distal to the previous incision to determine postprandial and post-exercise MyoPS rates over an early and later phase.

Resistance exercise protocol

To maximize applied translation of the data, an exercise protocol was selected with ecological validity in mind but focused on the *M. quadriceps* given this was the muscle group within which MyoPS rates were determined. During the pretesting visit, 3 repetition max (RM) was assessed to estimate 1RM for safety bar squat, leg press and leg extension (42). 3RM, rather than 1RM, was selected as an accurate approach to predict 10RM while minimizing safety risks (42). Strength testing began with a brief warm-up and practice of each exercise. Thereafter, participants attempted a self-selected load for 3RM. Load was increased for each subsequent attempt with final 3RM being accepted as the last load lifted correctly before a failed attempt (±5 kg from failed attempt). 10RM was then determined as 70% of formula calculated 1RM (43). Once 3RM testing had finished, participants rested for ~5 min and were then asked to complete one set (~10 repetitions) at the calculated 70% 1RM for familiarization and verification purposes.

During the experimental trials, exercises were performed in the following order: safety bar squat, leg press and leg extension. Participants completed a brief warm-up of safety bar squat

exercise consisting of 10 repetitions at a self-selected lower load, performed as per pretesting. Thereafter, participants executed 4 sets of safety bar squat, 4 sets of leg press, and 4 sets of leg extension at their predetermined 10RM until volitional failure (8-12 repetitions), separated with 90 s rest between each set and 3 min rest between each exercise. The load was increased when participants were able to perform >12 repetitions and decreased when participants were unable to perform 8 repetitions, such that each set was carried out to voluntary exhaustion, which was substantiated using the Borg Rating of Perceived Exertion (RPE) scale (44). The load and repetition number of each exercise was noted and replicated on the subsequent experimental visit for consistency of exercise volume and intensity between trials. Verbal encouragement was provided throughout.

Protein beverage preparation

Commercially available whey protein concentrate was obtained from MyProtein™, THG plc, Manchester, UK. Selection of the individual protein isolates for the plant-blend was based on amino acid content and composition, protein content after isolation (i.e. efficiency of protein purification), and commercial availability to increase translational applicability for the resistance trained athlete. Pea protein isolate (80% protein of total mass) was produced and supplied by Direct Food Ingredients Ltd, Macclesfield, UK; brown rice protein isolate (84% protein of total mass) was produced and supplied by Nutraceuticals Group Europe Merstham, UK; canola protein isolate (86% protein of total mass) was produced and supplied by Merit Functional Foods, Winnipeg, Canada. The plant-blend was produced by mixing the freeze-dried powders at ratios (39.5% pea protein, 39.5% brown rice protein, 21.0% canola protein) designed to provide a blend rich in essential amino acids, whilst guarding against any essential (or non-essential)

amino acid deficiency according to WHO/FAO/UNU guidelines (45). All protein sources were independently analyzed (Premier Analytical Services, High Wycombe, UK) for energy, macronutrient content and amino acid composition, the details of which are presented in Table 2.

The protein drinks were prepared the evening before the experimental trials. The dried powders were dissolved in 200 mL water, blended for ~2 min, and refrigerated overnight. Drinks were enriched (1.7%) with L-[*ring*-$^{2}H_{5}$]-phenylalanine to maintain systemic isotopic steady state following protein ingestion (46). During the experimental trial, once participants had consumed the drink, an additional 50 mL of water was added to rinse the bottle and ensure that all protein had been consumed, making a total fluid volume consumed of 250 mL. Drinks were well tolerated with no adverse effects reported during or after the test day. Double blinding of the drinks was achieved by having an independent person from the research team preparing the drinks and administering them in an opaque bottle. The drinks were matched for protein content (32 g) requiring 38.8 and 38.7 g of WHEY and BLEND powders, respectively. The dose was selected to maximize the post-exercise MyoPS response in WHEY in line with currently accepted sports nutrition guidelines (9), thus to test our hypothesis and reveal parity or inferiority (compared with optimal) following BLEND ingestion.

Blood sample collection and analyses

Ten mL of arterialized venous blood was collected into a syringe at each sampling point. For each blood sample 20 μL was collected into a plastic capillary and immediately analyzed for blood glucose concentrations (Biosen C-Line GP+). A second part (5 mL) was collected in

lithium heparin-containing tubes (BD vacutainer LH; BD Diagnostics) and centrifuged immediately at 4000 rpm at 4°C for 10 min to obtain plasma samples. The plasma supernatant was then removed, aliquoted, and frozen at -80°C for subsequent analysis. The remaining blood was added to additional vacutainers (BD Vacutainer SST II tubes, BD Diagnostics), which were left to clot at room temperature for at least 30 min and then centrifuged at 4000 rpm at 4°C for 10 min to obtain blood serum. The serum supernatant was then removed, aliquoted and frozen at -80°C for subsequent analysis.

Serum insulin concentrations were analyzed using a commercially available kit (DRG Insulin ELISA, EIA-2935, DRG International Inc.). Plasma L-[*ring*-$^{2}H_5$]-phenylalanine enrichments and concentrations of phenylalanine, leucine, valine, isoleucine, lysine, histidine, glutamic acid, methionine, proline, serine, threonine, tyrosine, glycine and alanine were determined by GC-MS with electron impact ionization (Aligent) as previously described (46, 47). Briefly, to prepare samples for GC-MS, 10 µL of 2 mM norleucine was added as an internal standard to 500 µL of plasma and deproteinised on ice with 500 µL of 15% 5-sulfosalcylic acid. Samples were then vortexed and centrifuged at 4000 *g* for 10 min at 4°C. The supernatant was then loaded onto cation-exchange columns. Columns were filled with ddH_2O, followed by 6 mL 0.5 M acetic acid and then washed 5 more times with ddH_2O, with the columns allowed to drain between each step. The amino acids were then eluted with 2 mL of 6 M ammonium hydroxide (NH_4OH). The elute was dried using a Speed-Vac at 60°C and then derivative, as described before (46).

Skeletal muscle tissue analysis

Muscle biopsy tissue samples were analyzed for myofibrillar protein-bound L-[*ring*-$^{2}H_5$]-phenylalanine, determined by GC-MS, as previously described (46). To summarize, 40-50 mg of frozen muscle tissue were homogenized by a mechanical tissue grinder in a homogenization buffer (in mM: 50 TRIS·HCL pH 7.4, 1 EDTA, 1 EGTA, 10 ß-glycerophosphate salt, 50 NaF and 0.5 activated Na_3VO_4) with a complete protease inhibitor cocktail tablet (1 tablet per 50 mL of buffer). The homogenate was centrifuged at 2200 *g* for 10 min at 4°C. The supernatant was discarded, and the remaining pellet was washed in 500 µL of homogenization buffer and centrifuged again at 700 *g* for 10 min at 4°C, and the remaining supernatant was discarded. The remaining protein portion (myofibrillar and collagen) was then solubilized in 750 µL of 0.3 M sodium hydroxide, heated at 50 °C for 30 min and centrifuged at 10,000 *g* for 5 min at 4°C. The consequent supernatant (myofibrillar fraction) was then aliquoted into a new 2 mL Eppendorf and precipitated in 500 µL of 1 M perchloric acid. These samples were centrifuged at 700 *g* for 10 min at 4°C, and the resultant supernatant was discarded. The remaining myofibrillar pellet was washed in 1 mL of 70% ethanol and centrifuged at 700 g for 5 min at 4°C before the ethanol was removed. This step was repeated once more before the amino acids were then hydrolyzed by adding 2 mL of 6 M hydrochloric acid and heating at 110°C for 24 h.

Once hydrolyzed, the amino acids were then dried on a heating block at 110°C for 24 h. Samples were then reconstituted in 1.5 mL of 25% acetic acid and pipetted into the cation-exchange column. The Eppendorf was rinsed with another 1.5 mL of 25% acetic acid. The columns were eluted with 2 mL of 6 M NH_4OH into a 2 mL Eppendorf, and the eluate was dried in a Speed-Vac at 60°C. Samples were cleaned by adding 1 mL of ddH2O and 1 mL of 0.1 %

formic acid in acetonitrile and centrifuged at 10,000 *g* for 3 min at 4°C. The supernatant was aliquoted into a new Eppendorf and dried in a Speed-Vac at 80°C.

To derivative the muscle sample, 50 µL of MTBSTFA +1% tertbutyl-dimethylchlorosilane and 50 µL of acetonitrile were added to the dry samples, vortexed and heated at 95°C for 45 min. The samples were analyzed by GC-MS (7890 GC coupled with a 5975 MSD, Agilent Technologies) in triplicate using electron impact ionization and selected ion monitoring for the measurement of isotope ratios. One µl of the sample was injected in splitless mode (injector temperature: 280°C). Peaks were resolved using an HP5-MS 30 m × 0.25 mm ID × 0·25 µm capillary column (Agilent). Helium was used as the carrier gas at 1.2 mL/min constant flow rate. The temperature ramp was set from 80–245°C at 11°C/min, then to 280°C at 40°C/min. Selected ion recording conditions were used to monitor fragments m/z 237 and 239, respectively, for the m + 3 and m + 5 fragments of phenylalanine-bound protein and m/z 336 and 341, respectively, for the m + 0 and m + 5 fragments of the phenylalanine-free fraction. A single linear standard curve from mixtures of known m + 5/m + 0 ratios for L-[*ring*-$^{2}H_{5}$]-phenylalanine was used to determine the enrichments of the protein-bound samples using the m + 5/m + 3 ratio.

Calculations

The fractional synthetic rate (FSR) of myofibrillar muscle protein was calculated using the precursor-product equation (47), as follows:

$$FSR\ (\% \cdot h^{-1}) = \frac{\Delta E\mathrm{p}}{E\mathrm{precursor}\ \cdot t} \cdot 100$$

Where ΔEp is the increment in protein-bound L-[*ring*-$^2H_5$]-phenylalanine in myofibrillar muscle protein between 2 biopsies, $E_{precursor}$ is the weighted mean L-[*ring*-$^2H_5$]-phenylalanine enrichment in the plasma over time, and t indicates the tracer incorporation time (h) between muscle biopsies.

Total postprandial glucose, insulin and amino acid availabilities were calculated as incremental AUC (iAUC) using the trapezoid rule, with baseline set as $t = 0$.

Statistical analyses

Based on previous research (48, 49), a 2-sided power analysis revealed a sample size of 10 participants was sufficient to detect a clinically relevant difference in postprandial, post-exercise FSR of 0.01±0.009%·h^{-1} (or ~30% when expressed as a relative difference) between conditions (WHEY vs BLEND) as the primary outcome measure (P<0.05, 80% power, effect size 0.9; G∗power version 3.1.9.7). Secondary outcomes included serum insulin and plasma amino acid responses and remaining outcomes were classified as tertiary. Differences in time-dependent serum insulin concentrations, plasma amino acid concentrations, plasma L-[*ring*-$^2H_5$]-phenylalanine enrichments, muscle L-[*ring*-$^2H_5$]-phenylalanine enrichments, and myofibrillar FSR were analyzed using two-way (time × condition) repeated measures ANOVA. When significant interactions were observed, Bonferroni *post-hoc* tests were performed to locate individual differences. Differences in exercise volume, background L-[*ring*-$^2H_5$]-phenylalanine enrichments, peak blood analyte concentrations, time-to-reach blood peak analyte concentrations, and iAUC values were analyzed using paired t tests. Data were tested for sphericity, and where violations occurred the Greenhouse-Geisser correction was automatically

applied. Statistical significance was set at $P<0.05$. Statistical analyses were performed using GraphPad Prism version 9.5.0. All data are expressed as means ± SD.

RESULTS

Exercise characteristics

Total volume (repetitions × load) performed was matched between trials and therefore did not differ between conditions for safety bar squat (WHEY, 789±325; BLEND, 791±322 kg), leg press (WHEY, 1355±718; BLEND, 1357±716 kg), leg extension (WHEY, 776±338; BLEND, 775±337 kg), or across the full exercise protocol (WHEY, 11,682±5,392; BLEND, 11,692±5,372 kg) (all $P>0.05$). Average RPE scores across sets reported were equivalent between conditions for safety bar squat (WHEY, 15±2; BLEND, 15±2), leg press (WHEY, 16±2; BLEND, 17±2), leg extension (WHEY, 17±2; BLEND, 18±2), and across the entire exercise protocol (WHEY, 16±2; BLEND, 17±1) (all $P>0.05$).

Serum insulin concentrations

Serum insulin responses are presented in Figure 2. From similar postabsorptive concentrations, serum insulin concentrations increased (time effect; $P<0.001$) initially following exercise ($P<0.001$), and further following protein ingestion ($P<0.001$), but no differences in these increases between conditions were observed (time × condition interaction; $P=0.67$). The rise was transient (remaining elevated from t=0-120 min) before returning to postabsorptive levels in both groups. Consequently, serum insulin availability over the 4 h postprandial period, as expressed by iAUC, was modestly negative and similar between conditions ($P=0.36$).

Plasma amino acid concentrations

The samples from one participant were excluded from the plasma amino acid analyses due to analytical technical issues. Therefore, all data presented for plasma amino acid responses are for n=9. Plasma total (TAA), essential (EAA), and non-essential (NEAA) amino acid concentrations are depicted in Figure 3. From equivalent postabsorptive concentrations, plasma TAA, EAA and NEAA concentrations increased following protein ingestion (time effects; all P<0.001), but to different extents between conditions for TAA and EAA only (time × condition interactions; both P<0.01). Plasma EAA concentrations increased more rapidly following ingestion of WHEY compared with BLEND (time-to-peak; 35±11 vs 63±22 min; P=0.01) and peak concentrations were ~32% greater for WHEY compared with BLEND (2019±509 vs 1469±247 μmol·L^{-1}; P=0.02). Postprandial plasma EAA availability over the 4 h postprandial period, expressed by iAUC, was ~44% higher for WHEY compared with BLEND (P<0.05). Postprandial peak plasma TAA and NEAA concentrations and iAUCs did not differ between WHEY and BLEND (all P>0.05).

Plasma leucine, lysine, and methionine concentrations increased following protein ingestion (time effects; all P<0.001), but to differing degrees between conditions (time × condition interactions; all P<0.001) (Figure 4). Postprandial peak plasma leucine, lysine, and methionine concentrations were ~39%, ~37%, and ~39% higher for WHEY compared with BLEND, respectively (all P<0.05), but were reached earlier for leucine and methionine only (both P<0.05). Total plasma availability over the 4 h postprandial period was greater following WHEY compared with BLEND ingestion for leucine concentrations (P=0.02), and a trend was observed for lysine (P=0.07) and methionine (P=0.05) concentrations.

Remaining individual plasma amino acid responses are depicted in Supplemental Figure 2 (Supplemental Digital Content, http://links.lww.com/MSS/C984, Time course and iAUC of plasma alanine, glutamic acid, glycine, histidine, isoleucine, phenylalanine, proline, serine, threonine, tyrosine, and valine concentrations, during the final hour of the postabsorptive and 4 h postprandial period in healthy young adults). Remaining plasma amino acid concentrations increased following protein ingestion (time effects; all $P<0.001$), and to different extent between conditions (time × condition interactions; all $P<0.05$) except for alanine, glutamic acid, histidine and proline (all $P>0.05$). Postprandial plasma availabilities were equivalent between conditions for remaining amino acids except for isoleucine and threonine (both WHEY>BLEND; $P<0.05$), as well as glycine and phenylalanine (both BLEND>WHEY; $P<0.05$).

Plasma and skeletal muscle tracer enrichments

Plasma phenylalanine concentrations are presented for $n=9$, and plasma and muscle L-[*ring*-$^{2}H_5$]-phenylalanine enrichments are presented for $n=10$. In the postabsorptive period, plasma L-[*ring*-$^{2}H_5$]-phenylalanine enrichments averaged 6.0±0.2 and 6.2±0.1 MPE in WHEY and BLEND, respectively, with no differences between conditions ($P>0.05$). Plasma L-[*ring*-$^{2}H_5$]-phenylalanine enrichments changed over time (time effect; $P<0.001$) and to a different extent between conditions (time × condition interaction; $P=0.004$). Specifically, plasma L-[*ring*-$^{2}H_5$]-phenylalanine enrichments decreased following protein ingestion in WHEY from $t=30$-90 min ($P<0.05$) but remained unchanged in BLEND ($P>0.05$), but no individual differences between conditions were observed at any time point ($P>0.05$) (Figure 5). Weighted mean plasma L-[*ring*-$^{2}H_5$]-phenylalanine enrichments (used as the precursor for calculation of myofibrillar FSRs) were equivalent between conditions during the early (WHEY, 5.2±0.4; BLEND, 5.3±1.0

MPE; *P*=0.96) and total (WHEY, 5.9±0.5; BLEND, 5.6±0.8; *P*=0.24) postprandial period, but were higher in WHEY compared with BLEND during the late postprandial period (6.3±0.6 vs 5.8±0.8 MPE; *P*=0.02). Myofibrillar protein-bound L-[*ring*-$^{2}H_{5}$]-phenylalanine enrichment data are presented in the Supplemental Digital Content (Supplemental Digital Content, http://links.lww.com/MSS/C984).

Myofibrillar protein synthesis rates

Myofibrillar FSRs calculated using the plasma precursor pool are depicted in Figure 6. From equivalent postabsorptive, resting values between conditions (*P*=0.92), myofibrillar FSRs roughly doubled during the initial 2 h post-exercise postprandial period: from 0.042±0.020 to 0.085±0.037%·h^{-1} and from 0.043±0.015 to 0.080±0.037%·h^{-1} following ingestion of WHEY and BLEND, respectively (time effect; *P*<0.001). Myofibrillar FSRs remained elevated compared with basal during the late (2-4 h) phase of the post-exercise postprandial period (WHEY, 0.085±0.036%·h^{-1}; BLEND, 0.086±0.034%·h^{-1}; *P*<0.001), with no further rise compared with the early phase (*P*=0.79) nor any differences between conditions at either time point (time × condition interaction; *P*=0.93). Accordingly, myofibrillar FSRs were elevated above basal values throughout the entire 4 h postprandial post-exercise period (WHEY, 0.084±0.020%·h^{-1}; BLEND, 0.082±0.031%·h^{-1}; time effect; *P*<0.001) and to an equivalent extent between conditions (time × condition interaction; *P*=0.80).

DISCUSSION

In the present study we assessed the post-resistance exercise myofibrillar protein synthetic (MyoPS) response following the ingestion of a novel plant-based protein blend

compared with an isonitrogenous bolus of whey protein in resistance trained young adults. The present data support our hypothesis insofar as a carefully designed pea, brown rice, and canola protein blend, providing an optimal dose of protein (>30 g) and leucine (2.5 g), and no essential amino acid deficiencies, ingested immediately post-exercise supported MyoPS rates to an equivalent extent as whey protein. Further, this was the case over a relatively acute (0-2 h) and more prolonged (up to 4 h) post-exercise period, implying an optimal anabolic response in each condition and therefore high applied translational value.

Lower amounts of key essential amino acids (EAAs), in particular leucine, lysine and methionine, have been suggested to impair optimal post-exercise MyoPS following plant protein ingestion (19, 50). Blending different plant-derived protein sources to provide an amino acid profile more similar to animal protein is increasingly argued as a strategy to maximize the muscle reconditioning response to plant protein ingestion following resistance exercise (28). In the present study, we formulated a novel exclusively plant-based protein blend, consisting of pea (high in leucine and lysine, but low in methionine), brown rice (high in methionine, low in lysine), and canola protein (no amino acid deficiencies and highly efficiently isolated (i.e. 86% protein of total mass) to allow high protein content of the blend) isolates in a ~2:2:1 ratio to achieve a balanced amino acid profile more reminiscent of animal protein. Accordingly, and in contrary to other plant-blend protein concentrates/isolates (33, 34), EAA (37.5% of total protein), BCAA (16.6%), and individual amino acid contents, including leucine (7.8%), methionine (1.9%) and lysine (5.9%), were compliant with the WHO/FAO/UNU guidelines (45). Moreover, by providing 2.5 g leucine per bolus of dietary protein, we also argued that its ingestion post-exercise would theoretically maximize the anabolic response.

Execution of resistance exercise followed by protein consumption roughly doubled (~100% and ~91% increase in whey and blend conditions, respectively) MyoPS rates compared with resting postabsorptive rates over the entire 4 h recovery period (Figure 6). This rise is quantitatively in line with previous work aiming to nutritionally maximize MyoPS following bilateral resistance exercise (48, 49, 51). Indeed, by selecting whey protein (10, 26, 52) as a control condition and providing an optimal total protein and leucine dose (53, 54), paired with an ecologically valid exercise protocol designed to maximally stimulate a large muscle tissue mass (55), it is reasonable to assume that this achieved an optimal anabolic stimulus. Therefore, we propose that, for the first time, optimal post-exercise and postprandial MPS rates were likely achieved with an entirely plant-based protein isolate. Previous work has reported robust increases in MPS following (animal/plant) blend protein isolate ingestion when compared with animal-protein controls (29-35). However, in contrast to the present work, these studies have not been exclusively plant-based blends (30, 31, 33, 35), have been conducted in rested muscle tissue only (32-34) (thereby not recruiting a large amount of muscle mass during exercise which exacerbates amino acid demand (55)), or have not compared to whey as a gold standard isolate (29), all of which limit the broader application to optimizing approaches in vegan sports nutrition, at least from a protein supplementation perspective.

In concert with its favorable amino acid composition (27), the potent anabolic potential of whey protein has also been attributed to its rapid digestion and subsequent amino acid absorption kinetics (56). It has therefore been argued that whey may be preferred for stimulating rapid (but perhaps more transient) rises in MPS compared with ‘slower’ proteins (10, 57, 58), which may be of particular relevance within sports nutrition, especially for resistance trained athletes, where

rapid and maximal post-exercise MPS responses, after every bout, are warranted to optimize efficient adaptation. To this end, we also determined the impact of protein ingestion on *temporal* post-exercise MPS rates, encompassing an immediate 0-2 h and more sustained 2-4 h period within the composite 4 h postprandial period. We also selected plant proteins within our blend associated with high efficiency isolations (i.e. ~80%, 84%, and 86% of total mass for pea, brown rice, and canola, respectively) to maximize the removal of nutrients which may slow the rate of amino acid entry into the circulation (59). Despite whey protein still showing evidence of being more rapidly digested and absorbed, and facilitating an overall greater postprandial systemic amino acid availability (Figures 3, 4), the plant-blend ingestion still achieved comparable post-exercise MyoPS rates even when accounting for the higher resolution captured within temporal measurements.

The divergent circulating amino acid response following whey and plant-blend protein ingestion deserves further consideration. To some extent, most of these differences were likely a by-product of our experimental design; that is providing the two protein sources as isonitrogenous boluses but with differing individual amino acid contents. For example, EAA, leucine, lysine, and methionine contents were ~17%, ~25%, ~48%, and ~15% higher in whey compared with the blend, which, post-ingestion, translated into quantitatively greater peak concentrations (~32-39%) and total postprandial availabilities (~44-90%) (Figure 3, 4). However, it is unlikely that differences in amino acid content are fully explanatory, given the skewed responses observed for specific amino acids. For instance, the blend contained only 0.1 g (~15%) less methionine than whey protein, but total postprandial methionine availability was ~90% lower upon blend compared with whey protein ingestion. Interestingly, this is in

agreement with similar studies reporting lower availability of total and specific amino acids (most notably methionine or lysine) following concentrated/isolated plant/blend protein ingestion compared with isonitrogenous animal-derived proteins (11, 29-31, 33, 34). Given the similar purity of the two protein sources provided (both within the present and previously cited studies), the reasons for the disparate responses are not clear, though could be attributed to amino acid profiles of plant-based proteins having more of a propensity towards splanchnic extraction (60), oxidation (i.e. ureagenesis) (12, 61) and/or certain amino acids (e.g. methionine; (29)) being less bio-accessible if still contained within any aspect of their original food matrix (i.e. imperfect isolation methods despite our selection only of sources where such methods have been well established (62)). Given the deliberately balanced amino acid profile of our blends, we consider the structural complexities of the multiple plant protein sources used in the present study as well as the purification the most likely explanation. For example, our ~83% blend isolate still leaves scope for antinutritional compounds typically found in plant-proteins (e.g. phytic acid in pea and brown rice (63, 64) or glucosinolates in canola (65)) to still be present and capable of impairing or attenuating digestion and absorption (59).

A wider implication of the divergent plasma amino acid responses following whey and plant-blend protein ingestion relates to the proposed regulation of MPS by plasma leucine (i.e. the leucine threshold/trigger concepts). The present data join a growing body of work which fail to confirm the directional (or at least quantitative) association between the speed, magnitude, and/or overall postprandial leucine response and the stimulation of (post-exercise) MPS (22). Interestingly, the lack of support for the ‘leucine trigger hypothesis’ has, to date, mainly come via studies that have used protein-rich whole foods (29, 46, 48, 49, 66-68), in contrast to the

present study which provided isolated protein sources. We are not the first, however, to show lesser systemic leucine (and other EAAs) responses in tandem with comparable postprandial (and post-exercise) stimulations of MPS following concentrated/isolated plant vs animal protein ingestion, with >25 g of potato (69), pea (29) and soy (14) protein as examples. Collectively, therefore, it may be considered that if sufficient leucine is provided within a protein source (in line with the 'leucine threshold' hypothesis (70)) to provoke a systemic rise in leucine concentrations, the exact circulating levels are perhaps lower than assumed and/or of less(er) consequence for (post-exercise) muscle anabolism (22). Whether the same is true for (all) other (essential) amino acids, either limiting as signal or substrate, remains to be elucidated. Furthermore, whether or not parity exists between well-considered plant-based protein blends and whey protein in their capacity to optimize (post-exercise) muscle reconditioning at lower ('suboptimal') protein or leucine doses, following prolonged supplementation, and/or in compromised adults displaying a reduced anabolic sensitivity to protein ingestion (71) also requires further investigation.

CONCLUSIONS

In conclusion, ingestion of a novel plant-based protein blend stimulates post-resistance exercise MyoPS rates, and to an equivalent extent as an isonitrogenous bolus of whey protein. These data imply that carefully considered approaches to plant-based sports nutrition can deliver optimal post-exercise muscle reconditioning and adaptive responses to resistance exercise training.

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Conflict of Interest

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Author contributions

A.J.M., J.P.M., C.L.E., M.A.H., F.B.S., and B.T.W. conceived and designed the research; I.v.d.H., A.J.M., S.W., and B.T.W. performed experiments; I.v.d.H., D.R.A., and A.J.M. analyzed data; I.v.d.H., F.B.S., and B.T.W. interpreted results of experiments; I.v.d.H. prepared figures; I.v.d.H. and B.T.W. drafted manuscript; I.v.d.H., A.J.M., A.J.M., F.B.S. and B.T.W. edited and revised manuscript; S.W. was responsible for randomization and preparation of the

experimental beverages; I.v.d.H., A.J.M., S.W., J.M., C.L.E., M.A.H., D.R.A., A.J.M., F.B.S., and B.T.W. approved final version of manuscript.

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FIGURE LEGENDS

Figure 1. Schematic representation of the experimental visits.

Figure 2. Time course (A) and iAUC (B) of serum insulin concentrations during the final hour of the postabsorptive (time course only) and 4 h postprandial period in healthy young adults. The dashed vertical line represents the ingestion of 32 g protein from whey (WHEY; *n*=10) or plant-blend (BLEND; *n*=10), following a bout of bilateral resistance exercise. Values are means ± SD. Time course data were analyzed using a two-way repeated-measures ANOVA (time × condition), with Bonferroni *post hoc* tests applied to detect individual differences. iAUC data were analyzed using a paired *t*-test. iAUC, incremental area under the curve.

Figure 3. Time course and iAUC of plasma TAA (A, B), EAA (C, D), and NEAA (E, F) concentrations, during the final hour of the postabsorptive (time course only) and 4 h postprandial period in healthy young adults. The dashed vertical line represents the ingestion of 32 g protein from whey (WHEY; *n*=9) or plant-blend (BLEND; *n*=9), following a bout of bilateral resistance exercise. Values are means ± SD. Time course data were analyzed using a two-way repeated-measures ANOVA (time × condition), with Bonferroni *post hoc* tests applied to detect individual differences. iAUC data were analyzed using a paired *t*-test. *, significant difference between conditions ($P<0.05$). EAA, essential amino acid; iAUC, incremental area under the curve; NEAA, non-essential amino acids; TAA, total amino acids.

Figure 4. Time course and iAUC of plasma leucine (A, B), lysine (C, D), and methionine (E, F) concentrations, during the final hour of the postabsorptive (time course only) and 4 h postprandial period in healthy young adults. The dashed vertical line represents the ingestion of 32 g protein from whey (WHEY; *n*=9) or plant-blend (BLEND; *n*=9), following a bout of bilateral resistance exercise. Values are means ± SD. Time course data were analyzed using a two-way repeated-measures ANOVA (time × condition), with Bonferroni *post hoc* tests applied to detect individual differences. iAUC data were analyzed using a paired *t*-test. *, significant difference between conditions (P<0.05). iAUC, incremental area under the curve.

Figure 5. Time course of plasma phenylalanine concentrations (A) and plasma L-[*ring*-$^{2}H_{5}$]-phenylalanine enrichments during the postabsorptive and 4 h postprandial period in healthy young adults. The dashed vertical line represents the ingestion of 32 g protein from whey (WHEY; *n*=10) or plant-blend (BLEND; *n*=10), following a bout of bilateral resistance exercise. Values are means ± SD. Data were analyzed using a two-way repeated-measures ANOVA (time × condition), with Bonferroni *post hoc* tests applied to detect individual differences. *, significant difference between conditions (P<0.05). MPE, mole % excess.

Figure 6. Myofibrillar FSR calculated using the plasma L-[*ring*-$^{2}H_{5}$]-phenylalanine precursor pool for the basal (postabsorptive) temporal postprandial (0-2 h and 2-4 h) (A), and total 4 h postprandial period (B) in healthy young adults. Postprandial state represents a 4 h period following ingestion of 32 g protein from whey (WHEY; *n*=10) or plant-blend (BLEND; *n*=10) and a bout of bilateral resistance exercise. Values are means ± SD. Data were analyzed using a

two-way repeated-measures ANOVA (time × condition). *, significant difference from basal (P<0.05). FSR, fractional synthetic rate.

Supplemental Digital Content

SDC 1: Supplemental Digital Content.docx

Figure 1

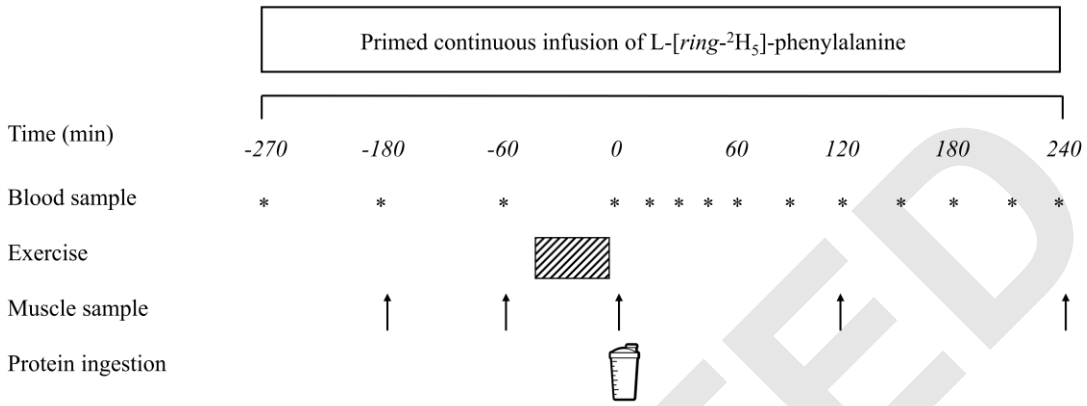


Figure 2

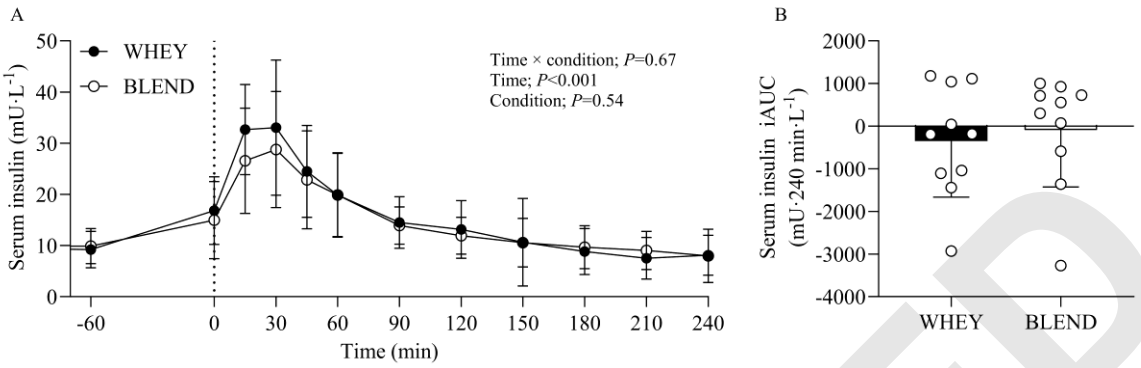


Figure 3

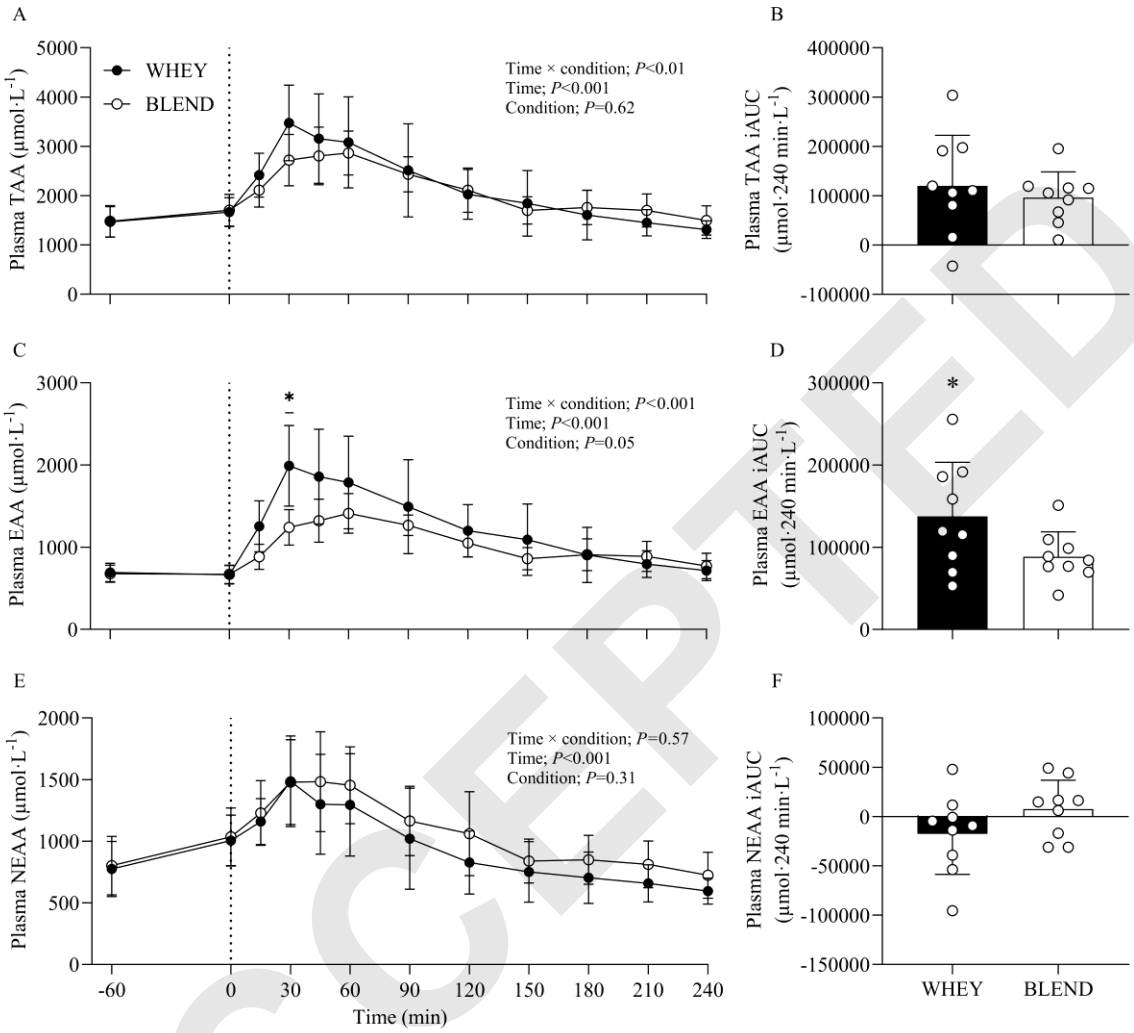


Figure 4

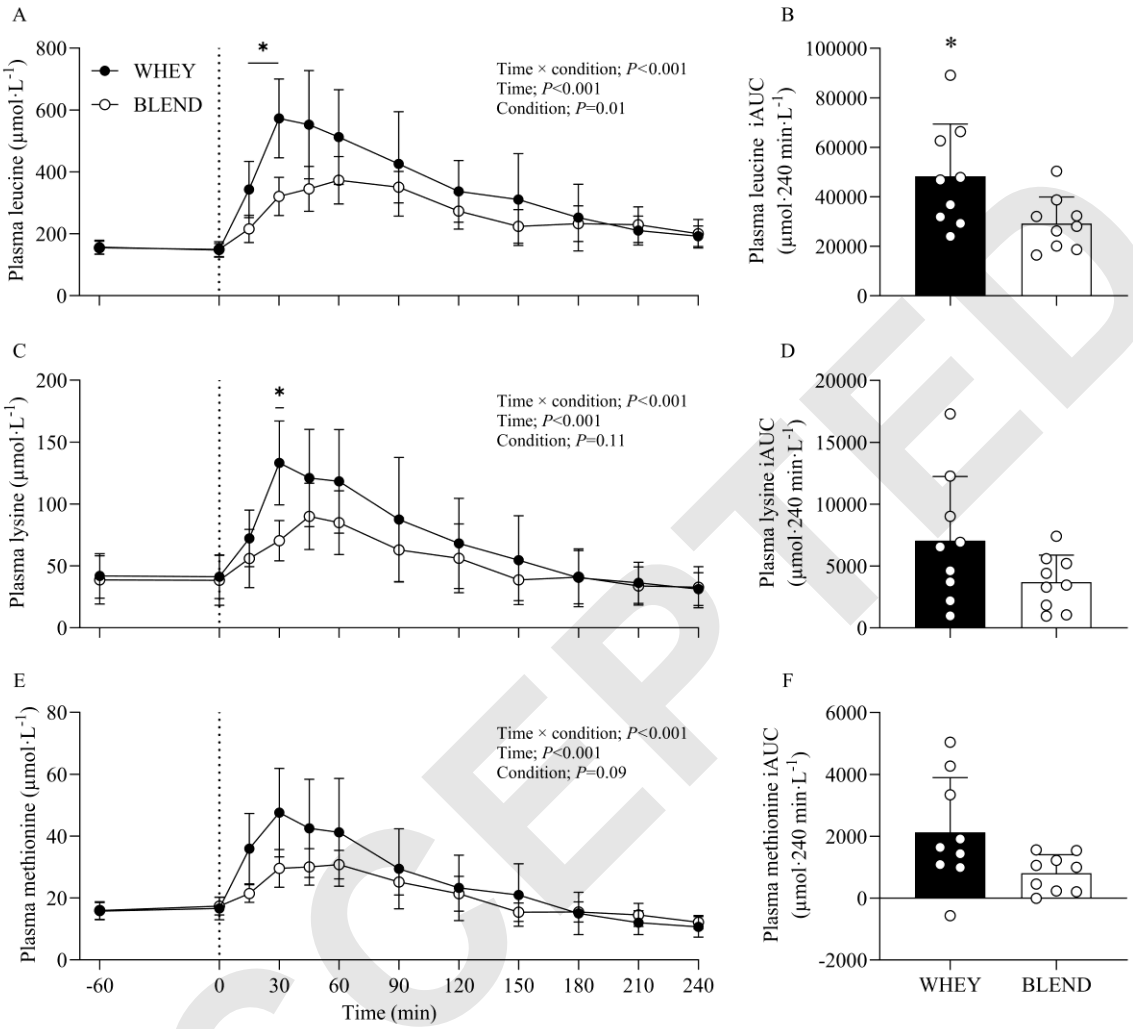


Figure 5

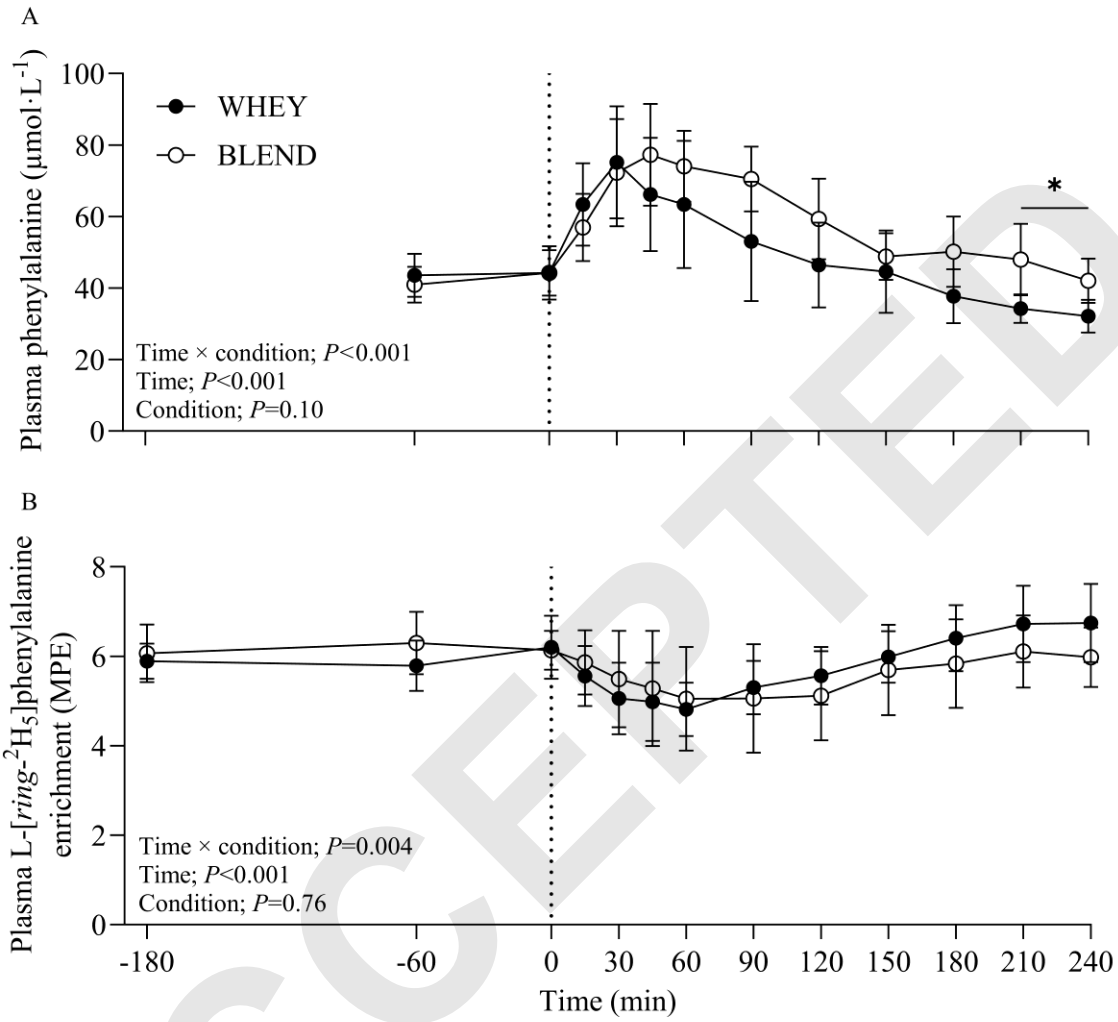


Figure 6

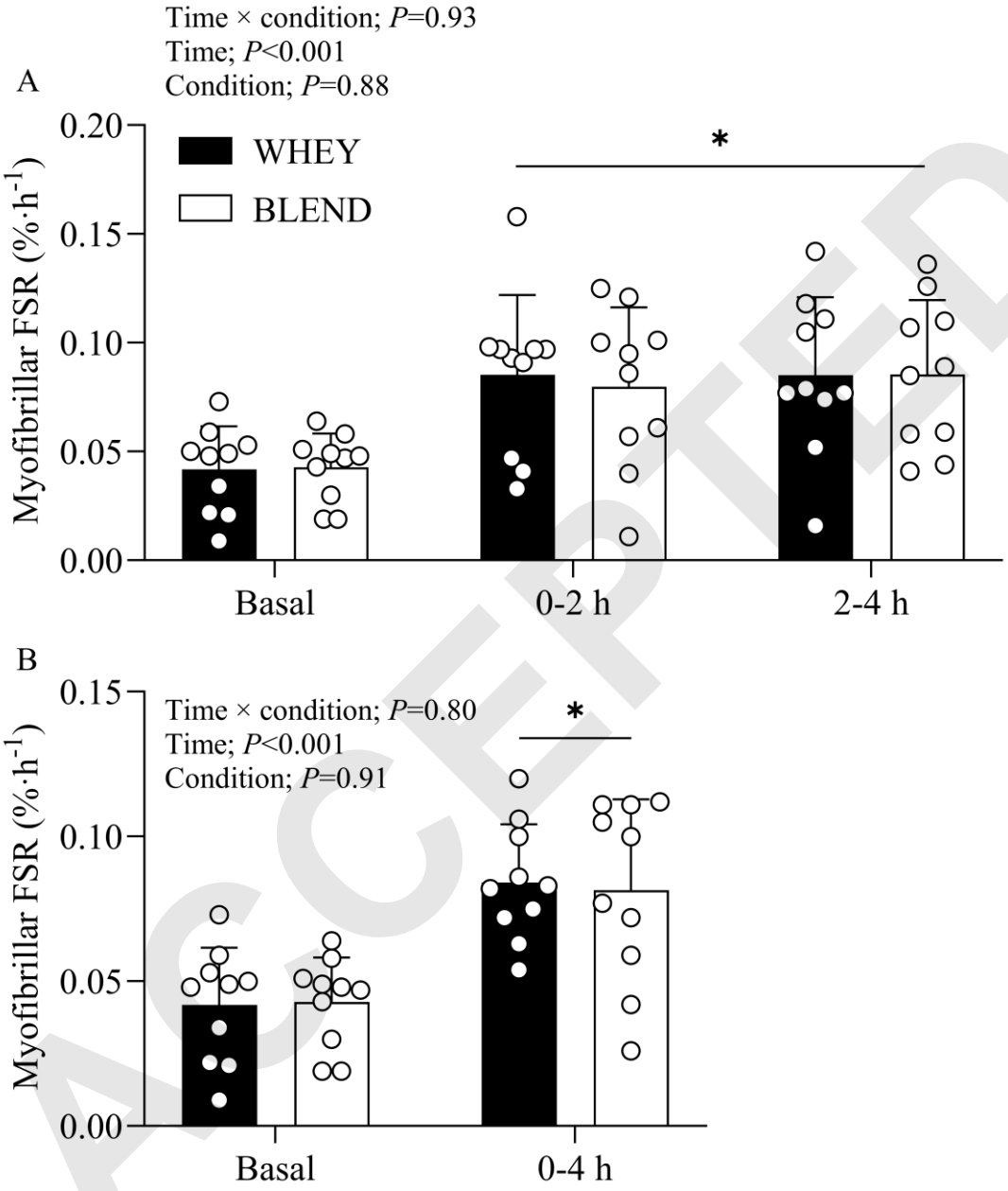


Table 1. Participants' characteristics

	Value
Sex (m/f)	8/2
Age (y)	26 ± 6
Body mass (kg)	79 ± 16
Height (cm)	178 ± 10
BMI (kg/m^2)	24 ± 3
Lean mass (kg)	64 ± 14
Body fat (%)	13 ± 7
Systolic BP (mmHg)	126 ± 39
Diastolic BP (mmHg)	71 ± 6
Safety bar squat 1RM (kg)	104 ± 40
Leg press 1RM (kg)	209 ± 82
Leg extension 1RM (kg)	127 ± 42

Values represent mean±SD. BP, blood pressure; 1RM, one repetition maximum.

Table 2. Nutritional composition of the experimental beverages (38.8 g whey and 38.7 g plant-blend (39.5% pea, 39.5% brown rice, 21.0% canola)).

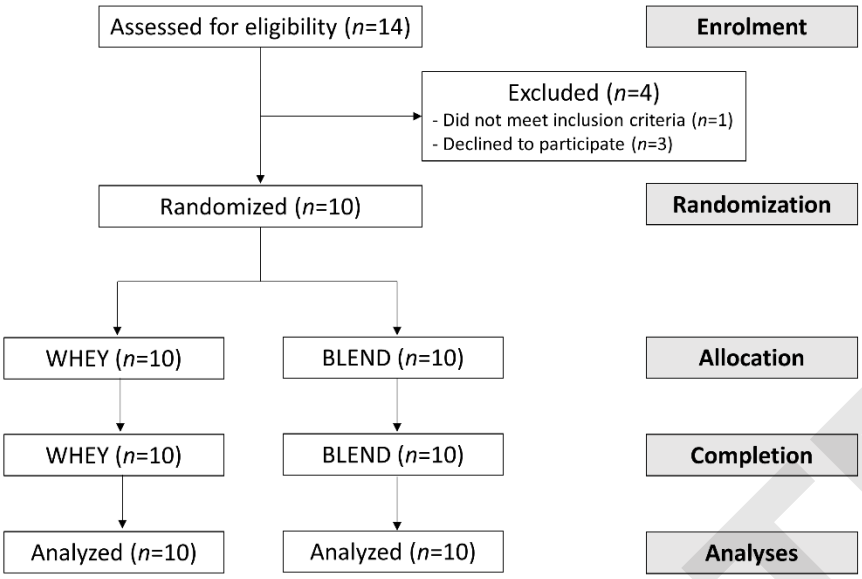
	WHEY	BLEND
Macronutrients and energy		
Protein (g)	32.0	32.0
Carbohydrate (g)	0.6	0.1
Fat (g)	2.5	3.1
Fiber (g)	2.5	2.9
Energy (kcal)	158	151
Energy (kJ)	666	637
Amino acid content (g)		
Alanine	1.6	1.5
Arginine	0.8	2.7
Aspartic acid	3.6	3.1
Cysteine	0.7	0.6
Glutamic acid	5.5	6.0
Glycine	0.6	1.4
Histidine	0.5	0.9
Isoleucine	1.6	1.2
Leucine	3.2	2.5
Lysine	3.1	1.9
Methionine	0.7	0.6
Phenylalanine	1.0	1.6
Proline	2.0	1.6
Serine	1.8	1.7
Threonine	2.1	1.2
Tryptophan	0.7	0.4
Tyrosine	1.0	1.3
Valine	1.6	1.6
EAA	14.5	12.0
NEAA	17.5	20.1

Protein content (g) is calculated from the sum of the amino acids measured after complete hydrolysis. BLEND, plant-blend protein; EAA, essential amino acids; NEAA, non-essential amino acids; TAA, total amino acids; WHEY, whey protein.

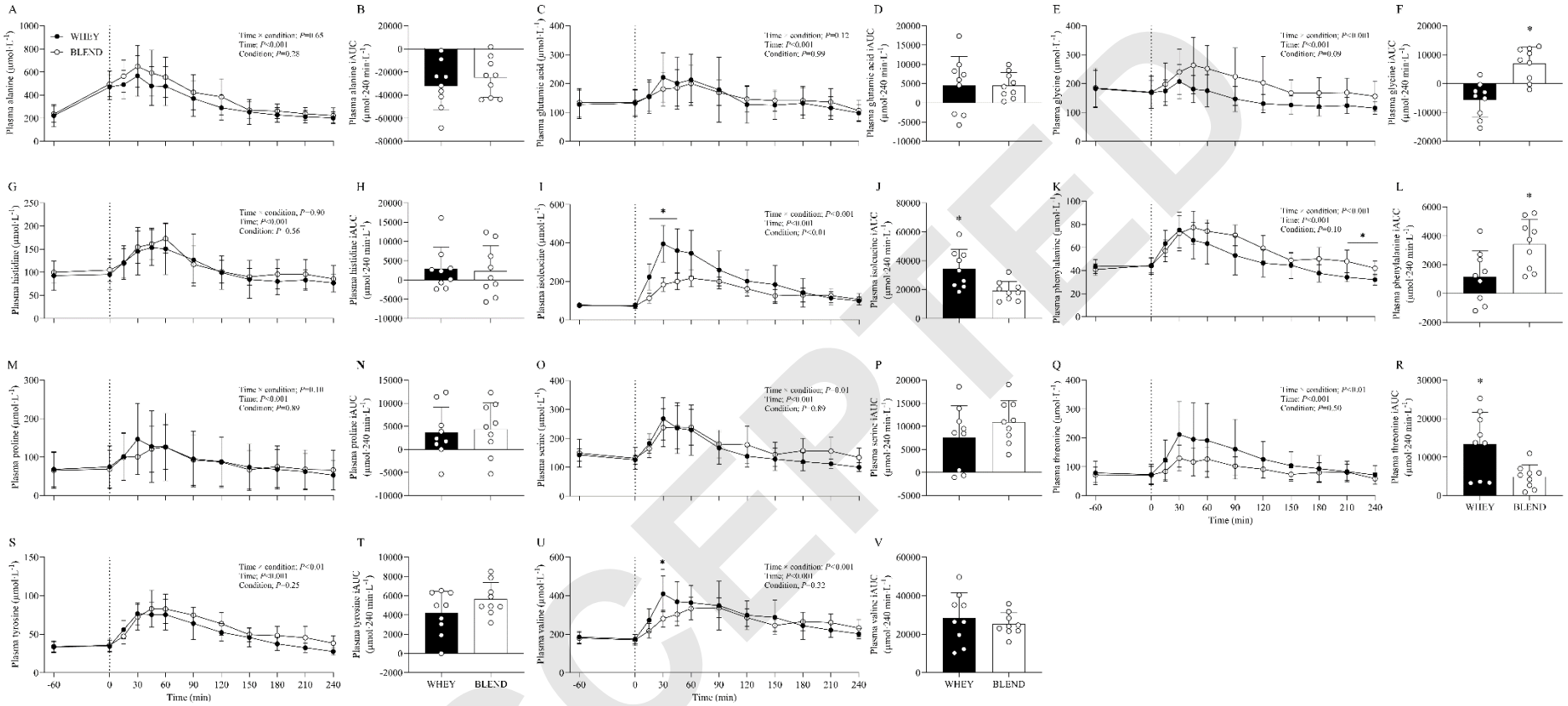
Supplemental Digital Content

Supplementary data – myofibrillar protein-bound L-[*ring*-$^{2}H_5$]-phenylalanine enrichment data

Myofibrillar protein-bound L-[*ring*-$^{2}H_5$]-phenylalanine enrichments were equivalent between conditions at baseline (P=0.12) but increased during the postabsorptive period (time effect; P<0.001), and to the same extent between conditions (time × condition interaction; P=0.77) (WHEY, from 0.051±0.015 to 0.056±0.014 MPE; BLEND, from 0.031±0.023 to 0.037±0.023 MPE). Myofibrillar protein-bound L-[*ring*-$^{2}H_5$]-phenylalanine enrichments increased in the biopsies collected after exercise (time effect; P<0.001) but to equivalent extents between conditions (time × condition interaction; P=0.71) (WHEY, to 0.061±0.016 MPE; BLEND, to 0.041±0.023 MPE). Following protein ingestion myofibrillar protein-bound L-[*ring*-$^{2}H_5$]-phenylalanine enrichments continued to increase at 2 h (WHEY, to 0.070±0.017 MPE; BLEND, from to 0.049±0.023 MPE) and 4 h (WHEY, to 0.082±0.018 MPE; BLEND, to 0.059±0.023 MPE) (time effect; P<0.001) with no differences between conditions at any time point (time × condition interaction; P=0.53).



Supplemental Figure 1. Participant flow diagram.


A
WHEY
BLEND
Time × condition; P=0.65
Time; P<0.001
Condition; P=0.28
Plasma alanine (μmol·L⁻¹)
B
Plasma alanine iAUC (μmol·240 min·L⁻¹)
C
Time × condition; P=0.12
Time; P<0.001
Condition; P=0.59
Plasma glutamic acid (μmol·L⁻¹)
D
Plasma glutamic acid iAUC (μmol·240 min·L⁻¹)
E
Time × condition; P<0.001
Time; P<0.001
Condition; P=0.09
Plasma glycine (μmol·L⁻¹)
F
Plasma glycine iAUC (μmol·240 min·L⁻¹)
G
Time × condition, P=0.90
Time; P<0.001
Condition; P=0.56
Plasma histidine (μmol·L⁻¹)
H
Plasma histidine iAUC (μmol·240 min·L⁻¹)
I
Time × condition; P<0.001
Time; P<0.001
Condition; P<0.01
Plasma isoleucine (μmol·L⁻¹)
J
Plasma isoleucine iAUC (μmol·240 min·L⁻¹)
K
Time × condition; P<0.001
Time; P<0.001
Condition; P=0.10
Plasma phenylalanine (μmol·L⁻¹)
L
Plasma phenylalanine iAUC (μmol·240 min·L⁻¹)
M
Time × condition; P=0.10
Time; P<0.001
Condition; P=0.89
Plasma proline (μmol·L⁻¹)
N
Plasma proline iAUC (μmol·240 min·L⁻¹)
O
Time × condition; P=0.01
Time; P<0.001
Condition; P=0.89
Plasma serine (μmol·L⁻¹)
P
Plasma serine iAUC (μmol·240 min·L⁻¹)
Q
Time × condition; P<0.01
Time; P<0.001
Condition; P=0.50
Plasma threonine (μmol·L⁻¹)
Time (min)
R
Plasma threonine iAUC (μmol·240 min·L⁻¹)
WHEY
BLEND
S
Time × condition; P<0.01
Time; P<0.001
Condition; P=0.25
Plasma tyrosine (μmol·L⁻¹)
Time (min)
T
Plasma tyrosine iAUC (μmol·240 min·L⁻¹)
WHEY
BLEND
U
Time × condition; P<0.001
Time; P<0.001
Condition; P=0.22
Plasma valine (μmol·L⁻¹)
Time (min)
V
Plasma valine iAUC (μmol·240 min·L⁻¹)
WHEY
BLEND

Supplemental Figure 2. Time course and iAUC of plasma alanine (A, B), glutamic acid (C, D), glycine (E, F), histidine (G, H), isoleucine (I, J), phenylalanine (K, L), proline (M, N), serine (O, P), threonine (Q, R), tyrosine (S, T), and valine (U, V) concentrations, during the final hour of the postabsorptive (time course only) and 4 h postprandial period in healthy young adults. The dashed vertical line represents the ingestion of 32 g protein from whey (WHEY; n=9) or plant-blend (BLEND; n=9), following a bout of bilateral resistance exercise. Values are means ± SD. Time course data were analyzed using a two-way repeated-measures ANOVA (time × condition), with Bonferroni *post hoc* tests applied to detect individual differences. iAUC data were analyzed using a paired *t*-test. *, significant difference between conditions (P<0.05). iAUC, incremental area under the curve.