Title

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Journal

Medicine and science in sports, 57(3)

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Publication Date

2025-03-01

DOI

10.1249/MSS.0000000000003596

Peer reviewed

OPEN

Ingestion of a Whey Plus Collagen Protein Blend Increases Myofibrillar and Muscle Connective Protein Synthesis Rates

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BASIC SCIENCES

ABSTRACT

AUSSIEKER, T., J. KAISER, W. J. H. HERMANS, F. K. HENDRIKS, A. M. HOLWERDA, J. M. SENDEN, J. M. X. VAN KRANENBURG, J. P. B. GOESSENS, U. BRAUN, K. BAAR, T. SNIJDERS, and L. J. C. VAN LOON. Ingestion of a Whey Plus Collagen Protein Blend Increases Myofibrillar and Muscle Connective Protein Synthesis Rates. *Med. Sci. Sports Exerc.*, Vol. 57, No. 3, pp. 544–554, 2025. **Purpose:** Ingestion of whey protein increases myofibrillar but not muscle connective protein synthesis rates. Recently, we defined a whey and collagen protein blend (5:1 ratio) to optimize post-prandial plasma amino acid availability. Here, we assessed the ability of this blend to increase myofibrillar and muscle connective protein synthesis rates at rest and during early recovery from exercise. **Methods:** In a randomized, double-blind, parallel design, 28 men (age: 25 ± 5 yr; body mass index: 23.6 ± 2.3 kg·m^{-2}) were randomly allocated to ingest either 30 g of protein (25 g whey/5 g collagen; BLEND, $n = 14$) or a noncaloric placebo (PLA, $n = 14$) following a single session of unilateral leg resistance-type exercise. Participants received primed continuous L-[*ring*-$^{13}C_6$]-phenylalanine infusions with blood and muscle biopsy samples collection for 5 h post-prandially to assess myofibrillar and muscle connective protein synthesis rates. **Results:** Protein ingestion strongly increased plasma amino acid concentrations, including plasma leucine and glycine concentrations ($P < 0.001$), with no changes following placebo ingestion ($P > 0.05$). Post-prandial myofibrillar and muscle connective protein synthesis rates were higher in the exercised compared with the rested leg ($P < 0.001$). In addition, myofibrillar protein synthesis rates were higher in BLEND compared with PLA in both the rested (0.038 ± 0.008 and 0.031 ± 0.006%·h^{-1}, respectively; $P < 0.05$) and exercised (0.052 ± 0.011 and 0.039 ± 0.009%·h^{-1}, respectively; $P < 0.01$) leg. Muscle connective protein synthesis rates were higher in BLEND compared with PLA in the rested (0.062 ± 0.013 and 0.051 ± 0.010%·h^{-1}, respectively; $P < 0.05$), but not the exercised (0.090 ± 0.021 and 0.079 ± 0.016%·h^{-1}, respectively; $P = 0.11$) leg. **Conclusions:** Ingestion of a whey (25 g) plus collagen (5 g) protein blend increases both myofibrillar and muscle connective protein synthesis rates at rest and further increases myofibrillar but not muscle connective protein synthesis rates during recovery from exercise in recreationally active, young men. **Key Words:** CONNECTIVE TISSUE, MUSCLE REMODELING, MYOFIBRILLAR PROTEIN, RESISTANCE EXERCISE, GLYCINE, AMINO ACIDS

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Submitted for publication June 2024.
Accepted for publication October 2024.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.acsm-msse.org).

0195-9131/25/5703-0544/0
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DOI: 10.1249/MSS.0000000000003596

Exercise stimulates muscle protein synthesis, with significant increases in both myofibrillar (1,2) as well as muscle connective protein synthesis rates (3–6). The post-exercise increase in myofibrillar and muscle connective protein synthesis rates is responsible for the subsequent skeletal muscle adaptive response to exercise, resulting in the conditioning of the contractile protein and connective tissue networks, respectively (7). Consuming protein during recovery from exercise can further increase muscle protein synthesis rates (8–10), thereby supporting greater gains in muscle mass and strength following more prolonged resistance exercise training (11,12). Whereas post-exercise dairy protein ingestion has been demonstrated to further increase myofibrillar protein synthesis rates (9,13), such a stimulatory effect has not been observed for muscle connective protein synthesis rates (3,4,14–16).

Muscle connective protein, particularly collagen, is rich in glycine and proline (17). We previously hypothesized that the inability of dairy protein ingestion to further increase post-exercise

muscle connective protein synthesis rates may be attributed to insufficient post-prandial plasma glycine availability (16,18,19) (unpublished observations). Dietary collagen protein contains high levels of glycine and proline and has therefore been suggested as a preferred protein source to promote connective tissue remodeling (20–26). However, in contrast to dairy protein, collagen protein has a relative low essential amino acid content and, as such, a lower leucine content (20). This may be the reason why we (16) as well as others (27,28) were unable to detect an increase in myofibrillar protein synthesis rates following collagen protein ingestion (15,26,27). Recently, we sought to optimize post-prandial plasma amino acid profiles and improve plasma glycine availability during recovery from exercise by ingesting different protein blends containing both whey and collagen protein (19). The addition of 5 g of collagen to 25 g of whey protein was sufficient to allow a robust increase in plasma essential amino acids and leucine concentrations while also increasing post-prandial plasma glycine availability.

We hypothesized that ingestion of a whey and collagen protein blend would increase both myofibrillar and muscle connective protein synthesis rates at rest and during recovery from resistance exercise. To test our hypotheses, 28 healthy young recreationally active men ingested 30 g of a protein blend providing 25 g whey and 5 g collagen protein or a noncaloric placebo following a single bout of unilateral resistance exercise. Primed, continuous intravenous L-[*ring*-$^{13}C_6$]-phenylalanine infusions together with blood and muscle tissue samples collection were applied to assess both myofibrillar and muscle connective protein synthesis rates both at rest and during recovery from exercise.

METHODS

Participants. A total of 28 healthy, recreationally active men (age: 25 ± 5 yr; body mass index (BMI): 23.6 ± 2.3 kg·m^{-2}) volunteered to participate in this parallel-group, double-blind, randomized controlled trial. Participants' characteristics are presented in Table 1. After pretesting, participants were randomly assigned to one of two groups consuming either 30 g of a whey and collagen protein blend (25 g whey and 5 g collagen protein; BLEND; n = 14) or a noncaloric placebo (PLA; n = 14). All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre+/Maastricht University and conforms to the principles outlined in the Declaration of Helsinki for use of human participants and tissue. The trial was registered at ClinicalTrials.gov (NCT05386771) and was conducted between October 2022 and April 2023 at Maastricht University, Maastricht, the Netherlands. Clinical Trial Center Maastricht independently monitored the study.

TABLE 1. Participants' characteristics and average 2-d dietary intake before the experimental period.

	PLA (n = 14)	BLEND (n = 14)
Age (y)	24 ± 4	25 ± 6
Height (m)	1.76 ± 0.07	1.80 ± 0.07
Weight (kg)	72.4 ± 7.6	77.2 ± 9.8
BMI (kg·m^{-2})	23.4 ± 2.2	23.7 ± 2.5
Lean body mass (kg)	59.3 ± 5.3	62.6 ± 6.1
Body fat (%)	17.5 ± 5.7	18.6 ± 5.3
Rectus femoris CSA (cm^2)	13.4 ± 4.1	12.8 ± 2.2
Vastus lateralis CSA (cm^2)	32.2 ± 6.2	32.6 ± 3.4
1RM leg press (kg)	116 ± 23	130 ± 29
1RM leg extension (kg)	59 ± 13	67 ± 13
Energy (MJ·d^{-1})	9.6 ± 2.5	10.0 ± 1.8
Carbohydrate (g·d^{-1})	237 ± 86	276 ± 73
Fat (g·d^{-1})	102 ± 40	90 ± 32
Protein (g·d^{-1})	92 ± 29	104 ± 41
Protein (g·kg^{-1}·d^{-1})	1.27 ± 0.37	1.33 ± 0.46
Vitamin C (mg·d^{-1})	141 ± 63	185 ± 100
Borg scale score	16 ± 2	17 ± 2

Values represent means ± SD. Data were analyzed with independent t-tests. There were no differences between treatments.
BLEND, 25 g whey plus 5 g of collagen protein; BMI, body mass index; CSA, cross-sectional area (exercise leg only); PLA, noncaloric flavored water; 1RM, one-repetition maximum for the trained leg.

Pretesting. Participants (aged 18–35 yr with a BMI >18.5 and <30.0 kg·m^{-2}) underwent an initial screening session to assess height, body weight, and body composition (BIA, BioScan 920; Maltron International Ltd, UK). Afterward the one-repetition maximum (1RM) of both legs individually was assessed with leg press and leg extension exercises (Technogym, Rotterdam, the Netherlands). Participants were deemed healthy based on their responses to a medical questionnaire and were excluded from participation when smoking, using medication that affected protein metabolism, having any musculoskeletal diseases, or if they were intolerant to the investigated protein products. The pretesting and experimental trials were separated by at least 5 d.

Diet and physical activity. All participants refrained from strenuous physical activity and alcohol consumption and filled out food intake and physical activity questionnaires for 2 d before the experimental trial. Habitual dietary intake data were analyzed using online software available from the Dutch Health Council *(Mijn Eetmeter:* https://mijn.voedingscentrum.nl/nl/eetmeter/) and are presented in Table 1. Participants consumed the same standardized meal before 9:00 PM on the evening before the experimental trial. This prepackaged standardized meal provided 1.71 MJ, with 55% of the energy from carbohydrate, 30% energy from fat, and 15% energy from protein. In addition, participants consumed 200 mL orange juice (80 mg vitamin C) providing 0.38 MJ. Thereafter, participants remained fasted until the experimental test day.

Study design. Participants performed a unilateral leg resistance exercise session before consuming a randomly assigned beverage (300 mL) containing either 30 g of protein (25 g whey and 5 g collagen protein; BLEND) or placebo (PLA). Whey protein isolate (Volactive Ultra Whey 90; Volac International Limited, Royston, UK) and collagen protein hydrolysate (Bodybalance B; GELITA AG, Eberbach, Germany) were used. The noncaloric placebo was flavored water. Both beverages were flavored with vanilla flavoring (Dr. Oetker, Amersfoort, the Netherlands). The amino acid profile of the protein blend can be found in Supplemental Table 1 (Supplemental Digital Content, http://links.lww.com/MSS/D122) and has been published previously (19). Randomization was performed using a computerized list randomizer (http://www.randomization.com/). Participants were sequentially allocated to the treatment groups by an independent researcher, according to the

randomized list. The study beverages were prepared by a different independent researcher in nontransparent plastic containers, and both supplements had a similar taste and smell.

Experimental protocol. At ~07:45 AM, participants arrived at the laboratory in an overnight fasted state using public transport or the car (a maximum of 10-min walking was allowed). A catheter was inserted into an antecubital vein for stable isotope amino acid infusion. Subsequently, a second catheter was inserted into a dorsal hand vein of the contralateral arm for arterialized venous blood sampling. To obtain arterialized blood samples, the hand was placed in a hot box (60°C) for 10 min before blood sample collection (29). After taking a baseline blood sample ($t = -210$ min), the plasma phenylalanine pool was primed with a single intravenous dose (priming dose) of L-[ring-$^{13}C_6$]-phenylalanine (3.15 μmol·kg^{-1}, CLM-1055-MPT-PK; Cambridge Isotopes, Andover, MA) and L-[3,5-$^{2}H_2$]-tyrosine (1.20 μmol·kg^{-1}, DLM-449-MPT-PK; Cambridge Isotopes). After priming, continuous intravenous infusions of L-[ring-$^{13}C_6$]-phenylalanine (0.070 μmol·kg^{-1}·min^{-1}) and L-[3,5-$^{2}H_2$]-tyrosine (0.027 μmol·kg^{-1}·min^{-1}) were initiated and maintained using a calibrated pump (Braun, Melsungen, Germany). After resting in a supine position, at $t = -180$ min, an arterialized blood sample was obtained, and a muscle biopsy sample was collected from the vastus lateralis muscle of the resting leg to determine basal myofibrillar and muscle connective protein synthesis rates ($t = -180$–0 min). Thereafter, rectus femoris and vastus lateralis cross sectional areas were determined by ultrasound (Affinity 70G; Philips, Amsterdam, the Netherlands) with a linear array probe (eL18-4; Philips) using B-mode with a panoramic option. While maintaining in a supine position, a third and fourth arterialized blood samples were drawn ($t = -120$ min; $t = -60$ min). After resting for another 15 min ($t = -45$ min), participants initiated the unilateral leg resistance exercise intervention (described hereinafter). Immediately after the exercise intervention ($t = 0$ min), an arterialized blood sample was obtained, and a muscle biopsy sample was collected from the vastus lateralis muscle of both the rested leg and exercised leg. Subsequently, participants received a 300-mL beverage corresponding to their randomly assigned treatment allocation (BLEND, $n = 14$; PLA, $n = 14$). To minimize dilution of the steady-state plasma L-[ring-$^{13}C_6$]-phenylalanine precursor pool, 4% of the phenylalanine content was added as L-[ring-$^{13}C_6$]-phenylalanine to the BLEND beverage. Sequential arterialized blood samples were collected at $t = 30$, 60, 90, 120, 180, and 240 min throughout the postprandial period. At $t = 300$ min, an arterialized blood sample was obtained, and a muscle biopsy sample was collected from the vastus lateralis muscle of both legs to determine post-prandial myofibrillar and muscle connective protein synthesis rates ($t = 0$–300 min). When the experimental protocol was complete, the cannulas were removed, participants consumed a light meal and were monitored for ~30 min before leaving the laboratory.

Blood and muscle tissue sampling. Blood samples were collected into EDTA-containing tubes and centrifuged at 1000*g* for 15 min at 4°C. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C. Muscle biopsy samples were collected using a 5-mm Bergström needle custom-adapted for manual suction. Samples were obtained from separate incisions from the middle region of the vastus lateralis, ~15 cm above the patella and ~3 cm below entry through the fascia, under 1% xylocaine local anesthesia with adrenaline (1:100,000). Muscle samples were freed from any visible non-muscle material, immediately frozen in liquid nitrogen, and stored at −80°C until further processing.

Resistance exercise session. All participants followed the same unilateral resistance exercise protocol that consisted of five sets on the leg-press and leg-extension machines (Technogym, Rotterdam, the Netherlands). The selection of the exercised versus resting leg was randomized. The first set for both exercises was a warm-up set for 10 repetitions at 40% 1RM. The next three sets were 8–10 repetitions at 80% 1RM. The last set was performed at 80% 1RM until failure. Resting periods of 2 min were allowed between all sets. After all sets at the leg press exercise were finished, participants continued with the leg extension exercise. Rating of perceived exertion was evaluated by the Borg scale (6–20; Table 1).

Plasma analysis. Plasma glucose and insulin concentrations were analyzed using commercially available kits (GLUC3, Roche, Ref: 05168791 190, and Immunologic, Roche, Ref: 12017547 122, respectively). Quantification of plasma amino acid concentrations was performed using ultra-performance liquid chromatograph mass spectrometry (UPLC-MS; ACQUITY UPLC H-Class with QDa; Waters, Saint-Quentin, France). A total of 50 μL of blood plasma was deproteinized using 100 μL of 10% SSA with 50 μM of MSK-A2 internal standard (Cambridge Isotope Laboratories, Andover, MA). Subsequently, 50 μL of ultra-pure demineralized water was added, and samples were centrifuged (15 min at 21,000*g*). After centrifugation, 10 μL of supernatant was added to 70 μL of Borate reaction buffer (Waters, Saint-Quentin, France). In addition, 20 μL of AccQ-Tag derivatizing reagent solution (Waters) was added after which the solution was heated to 55°C for 10 min. An aliquot of 1 μL was injected and measured using UPLC-MS. Plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments were determined by UPLC-MS. For this, plasma phenylalanine was derivatized to its 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate derivative, and enrichments were determined by UPLC-MS by using mass detection of masses 336, 342, and 346 for unlabeled and ($^{13}C_6$ and $^{13}C_9$-^{15}N)-labeled phenylalanine, respectively. We applied standard calibration curves in all isotopic enrichment analyses to assess the linearity of the mass spectrometer and to control for the loss of tracer.

Muscle tissue analysis. Muscle connective and myofibrillar protein-enriched fractions were isolated from ~100 mg of wet muscle tissue by hand homogenizing on ice using a pestle in an extraction buffer (10 μL·mg^{-1}; buffer recipe: 2.29 g sucrose, 0.606 g Tris, 0.373 g KCl, and 0.372 g EDTA in 100 mL of ddH_2O (pH 7.4) and right before use 1 tablet cOmplete™ mini and 1 tablet PhosSTOP™ (Roche Holding AG, Switzerland) per 10 mL of buffer was added). The samples were spun for 15 min at 700*g* and 4°C. The supernatant was transferred to a separate tube for Western blot analysis. The

pellet was washed with 400 µL of extraction buffer before vortexing and centrifugation at 700*g* and 4°C for 10 min. The supernatant was removed, and the pellet was washed with 500 µL ddH_2O before vortexing and centrifugation at 700*g* and 4°C for 10 min. The supernatant was removed, and 1 mL of homogenization buffer (buffer recipe: 0.242 g Tris, 0.877 g NaCl, 0.074 g EDTA, 8.558 g sucrose, and 0.5 mL Triton X-100 in 100 mL of ddH_2O (pH 7.4)) was added, and the material was suspended by vortexing before transferring into microtubes containing 1.4-mm ceramic beads and Lysing Matrix D (MP Biomedicals, Irvine, CA). The microtubes were vigorously shaken four times for 45 s at 5.5 $m \cdot s^{-1}$ (FastPrep-24 5G, MP Biomedicals) to mechanically lyse the protein network. Samples were then left to rest at 4°C for 3 h before centrifugation at 700*g* and 4°C for 20 min, discarding the supernatant and adding 1 mL of homogenization buffer. The microtubes were shaken for 40 s at 5.5 $m \cdot s^{-1}$ before centrifugation at 700*g* and 4°C for 20 min. The supernatant was discarded, and 1 mL of KCl buffer (buffer recipe: 5.22 g KCl and 2.66 g sodium pyrophosphate in 100 mL ddH_2O) was added to the pellets before being vortexed and left to rest overnight at 4°C. The next morning, samples were vortexed and centrifuged at 1600*g* for 20 min at 4°C where the supernatant was used for myofibrillar protein isolation and the pellet for muscle connective protein isolation.

For the myofibrillar isolation, the supernatant was transferred to a separate tube. Then, 3.4 mL EtOH 100% was added, samples were vortexed, left for 2 h at 4°C, and then centrifuged at 1600 *g*, for 20 min at 4°C. The supernatant was discarded, and 70% EtOH was added to the pellet, vortexed, and centrifuged again at 1600*g*, for 20 min at 4°C. The supernatant was again discarded, and the remaining pellet was suspended in 2 mL of 6 M HCl in glass screw-cap tubes and left to hydrolyze overnight at 110°C.

For the connective protein isolation, the pellet, containing both immature and mature connective proteins, was mixed with 1 mL KCl buffer and left for 2 h at 4°C. The samples were vortexed and centrifuged at 1600*g* for 20 min at 4°C, and the supernatant was discarded. To the pellet, 1 mL ddH2O was added, vortexed, left for 2 h at 4°C, and then centrifuged at 1600*g*, for 20 min at 4°C. The supernatant was removed, and the remaining pellet was suspended in 1 mL of 6 M HCl in glass screw-cap tubes and left to hydrolyze overnight at 110°C.

Following hydrolyzation of the isolated myofibrillar and connective protein fractions, the free amino acids were then dissolved in 25% acetic acid solution, passed over cation exchange AG 50 W-X8 resin columns (mesh size: 100–200, ionic form: hydrogen; Bio-Rad Laboratories, Hercules, CA), washed 5 times with water, and finally eluted with 2 M NH4OH. To determine myofibrillar and connective protein L-[ring-$^{13}C_6$]-phenylalanine enrichments by GC-IRMS analysis, the purified amino acids were converted into *N*-ethoxycarbonyl ethyl ester derivatives with ethyl chloroformate. The samples were measured using a gas chromatography–isotope ratio mass spectrometer (Finnigan MAT 252; Thermo Fisher Scientific, Bremen, Germany) equipped with an Ultra I GC-column (no. 19091A-112; Hewlett-Packard, Palo Alto, CA) and combustion interface II (GC-C-IRMS). Ion masses 44, 45, and 46 were monitored for ^{13}C phenylalanine. By establishing the relationship between the enrichment of a series of L-[ring-$^{13}C_6$]-phenylalanine, standards of variable enrichment, and the enrichment of the *N*(O,S)-ethoxycarbonyl ethyl esters of these standards, the muscle-protein-bound enrichment of phenylalanine was determined.

Calculations. The fractional synthetic rates (FSR) of myofibrillar and muscle connective protein were calculated by dividing the increments in myofibrillar and muscle connective protein enrichment by weighted mean precursor (plasma) amino acid tracer enrichment. Consequently, myofibrillar and muscle connective protein FSRs were calculated as follows:

$$\mathrm{FSR}\ (\% \cdot \mathrm{h}^{-1}) = \left(\frac{E_{m2} - E_{m1}}{E_{\mathrm{precursor}} \times t}\right) \times 100\% \qquad (1)$$

E_{m1} and E_{m2} represent protein-bound L-[ring-$^{13}C_6$]-phenylalanine, $E_{\mathrm{precursor}}$ represents the average plasma free L-[ring-$^{13}C_6$]-phenylalanine enrichment during the tracer incorporation period, and t indicates the time interval (h) between biopsies.

Statistical analysis. An *a priori* sample size calculation was performed with differences in postprandial muscle connective protein synthesis rates between the groups as the primary outcome measure. A minimum sample size of 14 participants per treatment was calculated using a power of 80%, a significance level of 0.05, an SD of 0.0125%·h^{-1}, and a difference in muscle connective protein synthesis rates of 0.014%·h^{-1} between treatments as based on our data of a previous publication (16). Baseline characteristics and dietary intake between groups were compared using an independent *t*-test. The trapezoidal rule adjusted to baseline concentration ($t = 0$) was applied to calculate the incremental area under curve (iAUC) of the amino acid concentrations. Time-dependent variables (i.e., plasma glucose, insulin, amino acid, and concentrations) were analyzed by a repeated-measures ANOVA with time as a within-subject factor and treatment group as a between-subject factor. The analysis was carried out for the period starting at the time of protein or placebo ingestion ($t = 0$ min) until the end of the experimental trial ($t = 300$ min). In case of a significant interaction effect, individual time points were analyzed using independent-samples *t*-tests. Plasma phenylalanine enrichments were analyzed by a repeated-measures ANOVA with time as a within-subject factor and treatment group as a between-subject factor for $t = -180$ until 300 min. Non–time-dependent variables (i.e., basal and post-exercise myofibrillar and muscle connective protein and iAUC) were compared between treatment groups using a paired-samples *t*-test. A secondary statistical analysis was performed on myofibrillar and muscle connective protein FSR in a condition-dependent manner with post-absorptive FSR and post-prandial FSR using a two-factor repeated-measures ANOVA with condition (post-absorptive, rested and exercised) as a within-subject factor and treatment group (BLEND and PLA) as a between-subject factor. Bonferroni-corrected *post hoc* comparisons were performed where appropriate. Statistical significance was set at $P < 0.05$. All data in text and figures are expressed as mean ± SD. All

calculations were performed using SPSS 27.0 (SPSS Inc., Chicago, IL).

RESULTS

Participants' characteristics and habitual dietary intake. There were no significant differences in the participants' characteristics between the treatment groups (Table 1). Similarly, there were no differences in habitual dietary intake and exercise-related ratings of perceived exertion (Borg) between treatment groups (Table 1). Dietary vitamin C intake of the 2 d before the experimental test day averaged 141 ± 63 and 163 ± 85 mg·d^{-1} in BLEND and PLA, respectively (main effect of treatment, $P > 0.05$). All participants ingested amounts of vitamin C above the Recommended Dietary Allowance of 90 mg·d^{-1} for men (30).

Plasma glucose and insulin concentrations. Plasma glucose concentrations declined over time ($P < 0.05$), with no differences between treatments (Fig. 1A). BLEND ingestion resulted in significant increases in circulating insulin concentrations, with values exceeding those observed in the placebo treatment at $t = 30–90$ min (time–treatment group interaction, $P < 0.001$; Fig. 1B).

Plasma amino acid concentrations. Results for all measured amino acids are visualized in a heat map showing the fold-change in plasma amino acid concentrations following test drink ingestion when compared with baseline $t = 0$ min (Fig. 2). BLEND ingestion increased plasma amino acid concentrations compared with PLA, with increases in plasma amino acid availability in BLEND (as represented by the iAUC, $P < 0.05$; data not shown). Significant *time × treatment group interactions* were observed for all plasma amino acid concentrations (all $P < 0.001$). Plasma EAA concentrations were higher at time points $t = 30–180$ min in BLEND compared with PLA ($P < 0.05$, Fig. 3A/4B). Plasma nonessential amino acid and proline concentrations were higher at time points $t = 30–120$ min in BLEND compared with PLA ($P < 0.05$, Fig. 3B/E). Plasma leucine concentrations were higher at time points $t = 30–240$ min in BLEND compared with PLA ($P < 0.05$, Fig. 3C). Plasma glycine concentrations were higher at time points $t = 30–90$ min in BLEND compared with PLA ($P < 0.05$, Fig. 3D). Plasma hydroxyproline and hydroxylysine concentrations were higher at time points $t = 30–300$ and $t = 30–180$ min in BLEND when compared with PLA, respectively ($P < 0.05$, Fig. 4).

Stable isotope tracer analyses. Analysis of plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments revealed a significant *time–treatment group interaction* effect ($P < 0.001$; Fig. 5). During the early post-prandial phase ($t = 30–90$ min), plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments were lower in BLEND compared with PLA ($P < 0.05$). However, time-weighted plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments over the entire 5 h post-prandial period did not differ between groups ($P > 0.05$).

Myofibrillar protein synthesis. Post-absorptive myofibrillar protein synthesis rates averaged 0.028 ± 0.013 and 0.023 ± 0.010%·h^{-1} in BLEND and PLA, respectively, with no differences between groups (main effect of treatment, $P > 0.05$; Fig. 6). Post-prandial myofibrillar protein synthesis rates in the rested leg over the 5 h period were significantly higher in BLEND compared with PLA at 0.038 ± 0.008 and 0.031 ± 0.006%·h^{-1}, respectively (main effect of treatment, $P < 0.05$; Fig. 6). Post-prandial myofibrillar protein synthesis rates in the exercised leg over the 5 h period were significantly higher in BLEND compared with PLA at 0.052 ± 0.011 and 0.039 ± 0.009%·h^{-1}, respectively (main effect of treatment, $P < 0.05$; Fig. 6). Time-dependent analysis revealed higher myofibrillar protein synthesis rates for the post-prandial rested and exercised compared with the post-absorptive condition, and higher rates for the exercised compared with the rested condition (main effects of condition $P < 0.001$).

Muscle connective protein synthesis. Post-absorptive muscle connective protein synthesis rates averaged 0.050 ± 0.018 and 0.042 ± 0.013%·h^{-1} in BLEND and PLA, respectively, with no differences between groups (main effect of treatment, $P > 0.05$; Fig. 7). Post-prandial muscle connective protein synthesis rates in the rested leg over the 5 h period were significantly higher in BLEND compared with PLA at 0.062 ± 0.013 and 0.051 ± 0.010%·h^{-1}, respectively (main effect of treatment, $P < 0.05$; Fig. 7). Post-prandial muscle connective protein

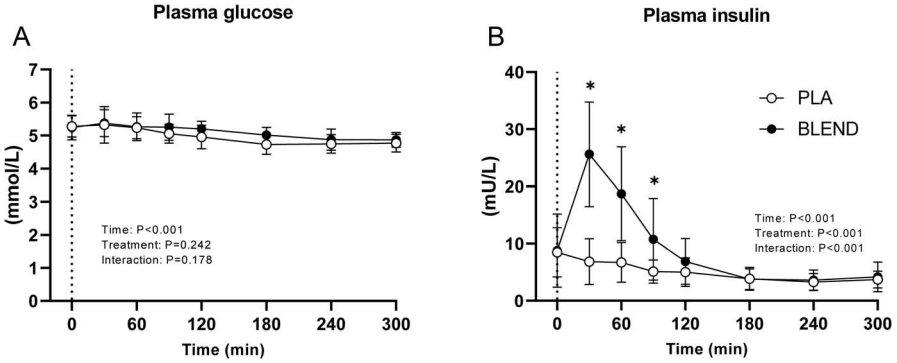

FIGURE 1—Plasma glucose (A) and insulin (B) concentrations following test drink ingestion during recovery from a single bout of unilateral leg resistance exercise ($t = 0–300$ min). The dotted line represents the ingestion of the test drink. Values represent means ± SD; $n = 14$ per group. Data were analyzed by two-factor repeated-measures ANOVA. Bonferroni *post hoc* testing was used to detect differences between groups. *Significant treatment difference within the time point, $P < 0.05$. BLEND, 25 g whey plus 5 g collagen protein; PLA, placebo (water).

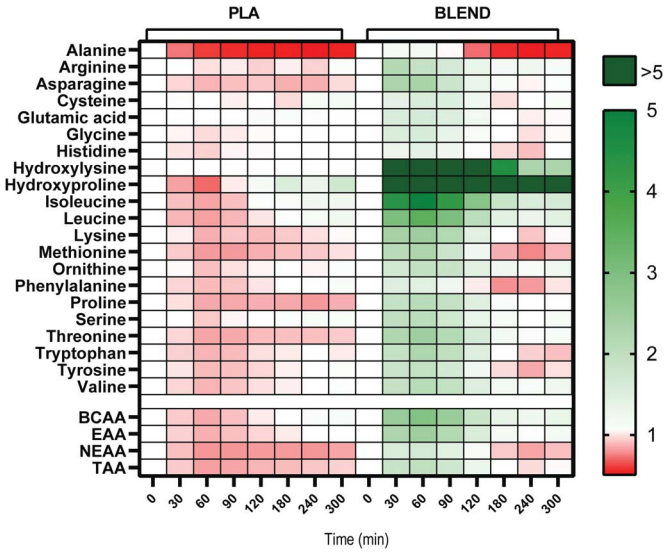

FIGURE 2—Heat map of fold changes in plasma amino acid concentrations during the experimental test day after the test drink ingestion during recovery from a single bout of unilateral leg resistance exercise. BCAA, branched-chain amino acids; BLEND, 25 g whey plus 5 g collagen protein (n = 14); EAA, essential amino acids; NEAA, non-essential amino acids; PLA, placebo (water) (n = 14); TAA, total amino acids. For hydroxyproline and hydroxylysine, values under the detection limit were set to 0. Values of t = 0 were set to 1.

synthesis rates in the exercised leg over the 5-h period averaged 0.090 ± 0.021 and 0.079 ± 0.016%·h^{-1} in BLEND and PLA, respectively, with no statistical difference between groups (main effect of treatment, P = 0.11; Fig. 7). Time-dependent analysis revealed higher muscle connective protein synthesis rates for the post-prandial rested and exercised compared with the post-absorptive condition, and higher rates for the exercised compared with the rested condition (main effects of condition, P < 0.001).

DISCUSSION

In the present study, we demonstrated that the ingestion of a protein blend combining whey and collagen protein strongly increased plasma amino acid concentrations during recovery from a single bout of unilateral resistance exercise. Ingestion of the protein blend increased both myofibrillar and muscle connective protein synthesis rates in the rested leg. Following exercise, protein blend ingestion increased myofibrillar but not muscle connective protein synthesis rates in the exercised leg.

In our study, the ingestion of the protein blend combining both whey and collagen protein resulted in a rapid rise in circulating plasma amino acid concentrations (Figs. 2–4). The post-prandial rise in circulating amino acids represented the amino acid composition of the ingested protein blend (16,20). These data confirm our previous observations of post-prandial plasma amino acid responses following the ingestion of whey protein combined with different doses of collagen protein during recovery from exercise (19). The rapid rise in circulating plasma EAA and leucine concentrations following protein ingestion (Fig. 3A/C) suggests efficient digestion and absorption of the whey protein fraction of the protein blend (9,16,31,32). The marked rise in plasma hydroxyproline and hydroxylysine concentrations (Fig. 4A/B) shows that also the collagen protein fraction in the blend was rapidly digested and absorbed, which agrees with prior work from our laboratory (16) as well as others (20,22,23). In line, we observed robust increases in circulating plasma glycine concentrations following ingestion of the protein blend (Fig. 3D). This is in contrast to the decline in plasma glycine availability that is typically observed following the ingestion of dairy protein both at rest (18,20) as well as during recovery from exercise (16,19). We hypothesized that the post-prandial increase in EAA concentrations (and leucine in particular) combined with the absence of a decline in plasma glycine availability increases both myofibrillar and muscle connective protein synthesis rates.

In agreement with our hypothesis, ingestion of the protein blend, providing 25 g whey plus 5 g collagen protein, significantly increased both myofibrillar and muscle connective protein synthesis rates throughout the 5 h post-prandial period (Figs. 6 and 7, respectively). The observed increase in myofibrillar protein synthesis rates following ingestion of the protein blend is in agreement with previous work (9,13,32,33) showing increases in myofibrillar protein synthesis rates following the ingestion of 20–30 g whey protein. We extend on the results of these prior reports, with the observation that ingestion of a protein blend combining whey plus collagen protein can also increase muscle connective protein synthesis rates (Fig. 7). Previous work has failed to detect a stimulating effect of (dairy) protein ingestion on muscle connective protein synthesis rates (3–5,14–16,34,35). Consequently, it has been suggested that muscle connective proteins are either less susceptible to the anabolic signal provided by (dairy) protein derived amino acids or that a post-prandial stimulatory response is more delayed for muscle connective protein synthesis rates (36). We previously speculated that the inability of (dairy) protein ingestion to increase muscle connective protein synthesis rate may be attributed to the post-prandial decline in plasma

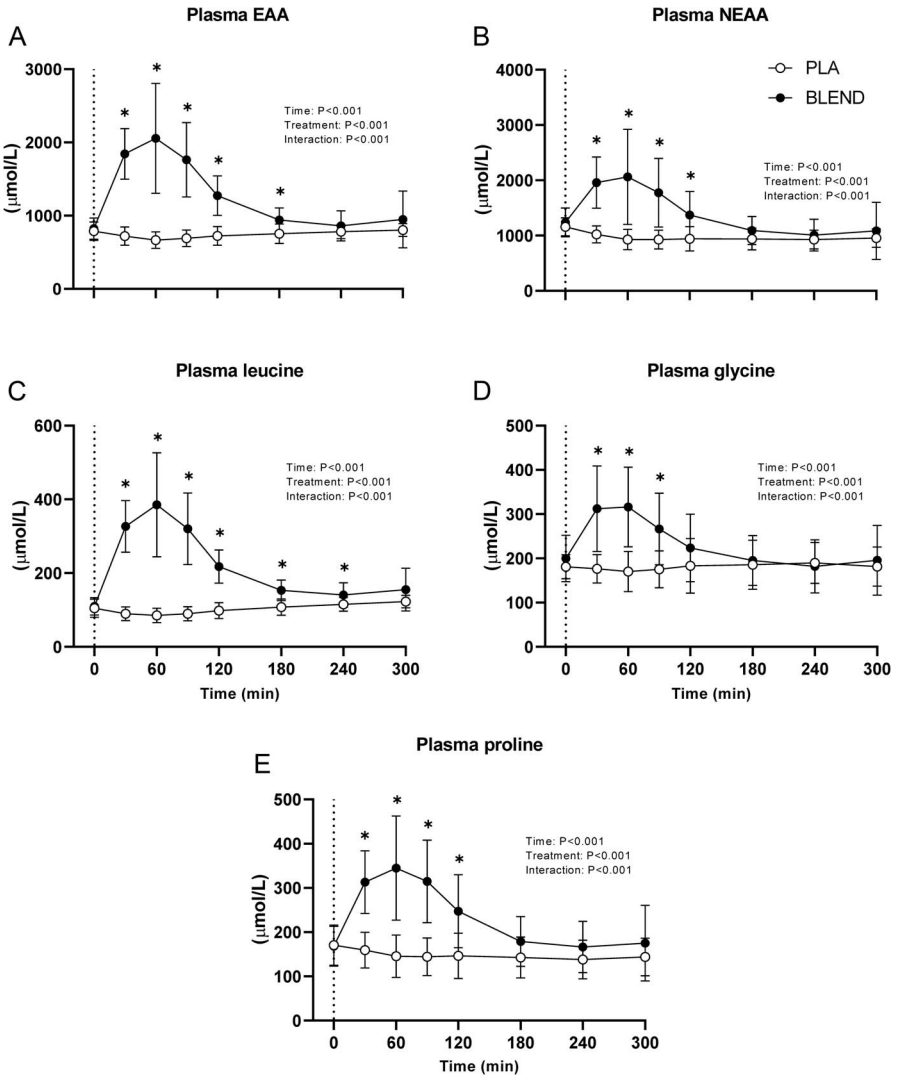

FIGURE 3—Post-prandial plasma amino acid concentrations following whey plus collagen protein or placebo ingestion during recovery from a single bout of unilateral leg resistance exercise (t = 0–300 min). Data are displayed for EAA (A), NEAA (B), leucine (C), glycine (D), and proline (E). The dotted line within the graphs represents the ingestion of the test drink. Values represent means ± SD; n = 14 per group. Data for plasma amino acid concentrations were analyzed by a two-factor repeated-measures ANOVA. Bonferroni *post hoc* testing was used to detect differences between groups. *Significant treatment difference within the time point, $P < 0.05$. BLEND, 25 g whey plus 5 g collagen protein; PLA, placebo (water). EAA, essential amino acids; NEAA, non-essential amino acids.

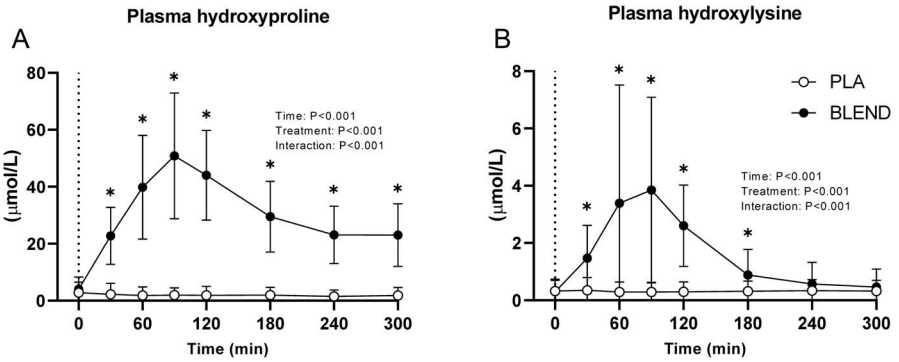

FIGURE 4—Post-prandial plasma hydroxyproline and hydroxylysine concentrations following whey plus collagen protein or placebo ingestion as a proxy for collagen protein digestion and amino acid absorption (t = 0–300 min). Data are displayed for hydroxyproline (A) and hydroxylysine (B). The dotted line within the graphs represents the ingestion of the test drink. Values represent means ± SD; n = 14 per group. Data for plasma amino acid concentrations were analyzed by a two-factor repeated-measures ANOVA. Bonferroni *post hoc* testing was used to detect differences between groups. *Significant treatment difference within the time point, $P < 0.05$. BLEND, 25 g whey plus 5 g collagen protein; PLA, placebo (water).

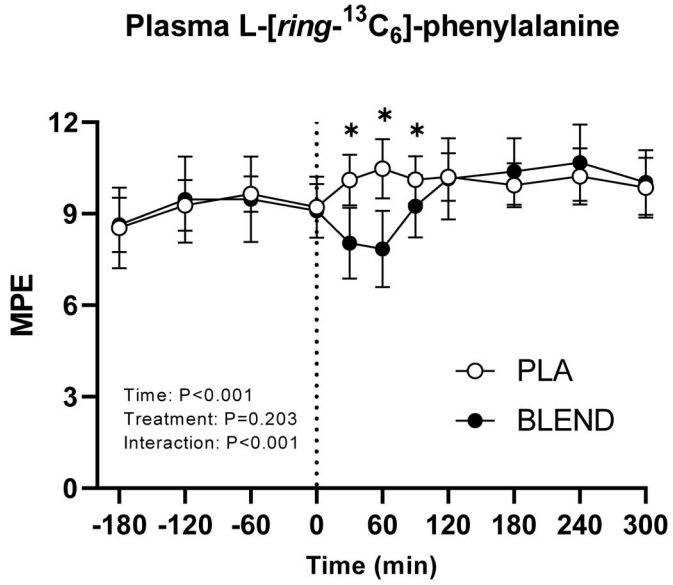

FIGURE 5—Plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments (MPE) before (t = −180 to 0 min) and after whey plus collagen protein or placebo ingestion during recovery from a single bout of unilateral leg resistance exercise (t = 0–300 min). The dotted line represents the ingestion of the test drink. Values represent means ± SD; n = 14 per group. Data were analyzed by a two-factor repeated-measures ANOVA. Bonferroni *post hoc* testing was used to detect differences between groups. *Significant treatment difference within the time point, P < 0.05. BLEND, 25 g whey plus 5 g collagen protein; PLA, placebo (water).

glycine availability (3,4). Our data tend to support the proposed importance of dietary protein–derived glycine (37–39), although we can only speculate on the properties of the protein blend to stimulate connective protein synthesis rates in the rested leg (40). In short, we show that ingestion of a protein blend combining whey with collagen protein (30 g, ratio of 5:1) can increase both myofibrillar and muscle connective protein synthesis rates. The latter provides leads for the development of (more) effective interventional strategies to support healthy aging, injury prevention, rehabilitation, and performance (26,41–44).

Resistance-type exercise increased both myofibrillar and muscle connective protein synthesis rates, which was evident from the higher protein synthesis rates observed in the exercised when compared with the rested leg (Figs. 6 and 7). The stimulating effect of exercise on both myofibrillar and

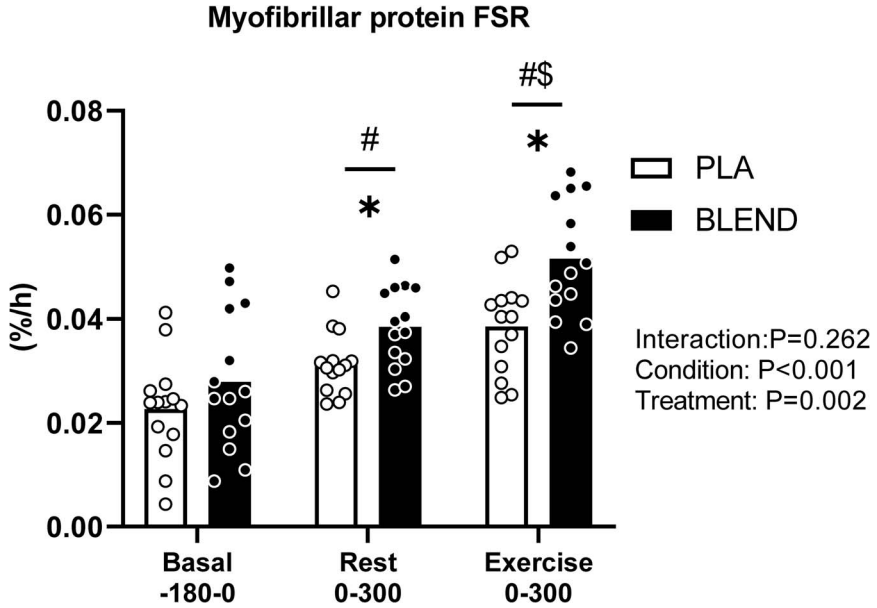

FIGURE 6—Fractional myofibrillar protein synthesis rates (%·h^{-1}) during the post-absorptive period (basal; t = −180 to 0 min), after whey plus collagen protein or placebo ingestion in rest and during recovery from a single bout of unilateral leg resistance exercise (t = 0–300 min). Bars represent means and circles represent individual values; n = 14 per group. Data within one condition have been analyzed by independent-samples t-tests. Time-course data were analyzed by a two-factor repeated-measures ANOVA. Bonferroni *post hoc* testing was used to detect differences between groups. *Significant treatment difference within the condition, P < 0.05. #Significant difference compared with the basal condition, P < 0.05. $Significant difference compared with the rested condition, P < 0.05. BLEND, 25 g whey plus 5 g collagen protein; PLA, placebo (water).

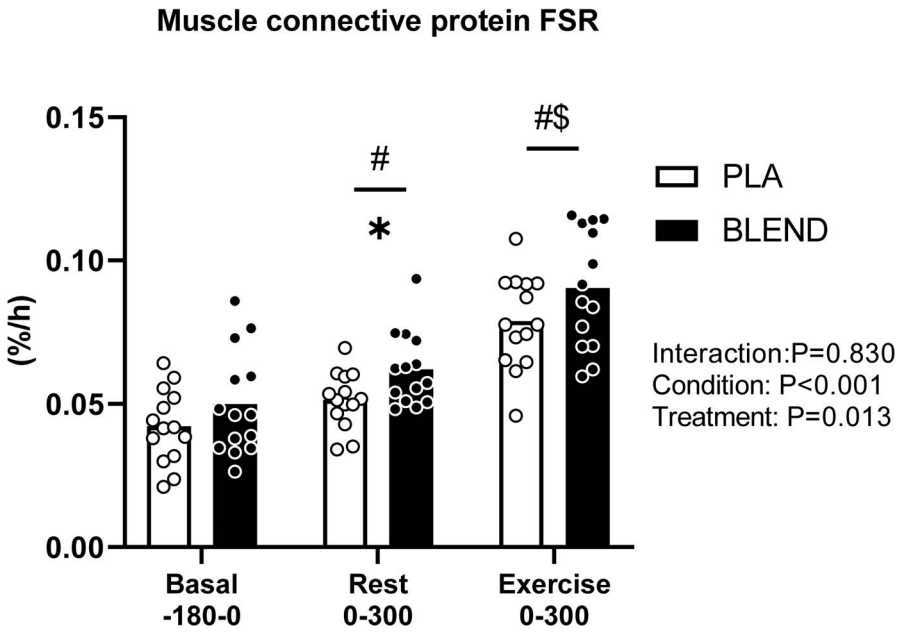

FIGURE 7—Fractional muscle connective protein synthesis rates (%·h^{-1}) during the post-absorptive period (basal; $t = -180$ to 0 min), after whey plus collagen protein or placebo ingestion in rest and during recovery from a single bout of unilateral leg resistance exercise ($t = 0$–300 min). Bars represent means, and circles represent individual values; $n = 14$ per group. Data within one condition have been analyzed by independent-samples *t*-tests. Time-course data were analyzed by a two-factor repeated-measures ANOVA. Bonferroni *post hoc* testing was used to detect differences between groups. *Significant treatment difference within the condition, $P < 0.05$. #Significant difference compared with the basal condition, $P < 0.05$. $Significant difference compared with the rested condition, $P < 0.05$. BLEND, 25 g whey plus 5 g collagen protein; PLA, placebo (water).

muscle connective protein synthesis has been reported several times by our laboratory (3,4,16,45) as well as numerous others (1,2,5,15,32,35,36,46,47). It has been well established that (dairy) protein ingestion during recovery from exercise further increases muscle protein synthesis rates (9,32,48,49). In agreement, we observed higher myofibrillar protein synthesis rates during recovery from exercise following ingestion of the whey plus collagen protein blend (Fig. 6). In contrast to data showing greater post-exercise increases in myofibrillar protein synthesis rates following protein ingestion, few data are available on the impact of post-exercise protein ingestion on muscle connective protein synthesis rates. Most (3–5,14–16,34,35) but not all (40,50) studies report that protein ingestion during recovery from exercise does not further increase muscle connective protein synthesis rates. Whereas most of these data are based on studies that applied dairy protein (3,4,14,15,35), more recent work from our laboratory (16) as well as others (27) have shown that ingestion of dietary collagen also does not further increase muscle connective protein synthesis rates during the early stages of post-exercise recovery.

As dairy protein does not provide much glycine (16,20), we as well as others have speculated that collagen protein may be a good source of glycine to support muscle connective protein conditioning. The role of glycine has been a subject of interest given its abundance in bodily collagen (17) and its relative insufficiency in most dietary proteins (20,51). It has been suggested that glycine availability may be restrictive to support connective tissue remodeling (39). However, in contrast, others described how glycine could rarely (if ever) become limiting for synthesis of even glycine-rich proteins (38,52–54). In the present study, we observed a post-prandial increase in plasma glycine availability following ingestion of the protein blend. This is quite different from previous observations showing a decline in plasma glycine availability following post-exercise dairy protein ingestion (16,19,20). However, the greater post-prandial plasma glycine availability did not further increase post-exercise muscle connective protein synthesis rates. Whether this is attributed to the relative short timeline of a 5 h post-prandial period (36,40) or that nutritional modulation is not effective beyond the stimulating effect of exercise remains unclear. It is interesting to note that even in the control treatment (placebo), a large increase in muscle connective protein synthesis rate was observed in the exercised compared with the resting leg, without the apparent need for greater circulating plasma glycine and proline concentrations (Fig. 7). Therefore, these data imply that endogenous release of glycine and proline is sufficient to provide ample amino acid precursors to support the post-exercise increase in muscle connective protein synthesis rates during recovery from exercise.

Leucine is well documented for its important role in stimulating myofibrillar protein synthesis rates (33,55). Furthermore, leucine has been shown to stimulate protein synthesis rates in tissues other than muscle (56,57). Here we observed positive moderate correlations between the plasma availability (iAUC) of leucine and both myofibrillar and muscle connective protein synthesis rates in both the rested ($r = 0.374$, $P = 0.0498$ and $r = 0.421$, $P = 0.026$, respectively) and exercised ($r = 0.632$, $P < 0.001$ and $r = 0.421$, $P = 0.026$, respectively) leg. These findings support the concept that leucine not only serves as a trigger for myofibrillar protein

synthesis but may also impact muscle connective protein synthesis. Therefore, ingestion of leucine or a leucine-rich protein blend may support remodeling of both the contractile as well as connective protein network.

Our data suggest that plasma glycine availability does not compromise muscle connective protein synthesis rates during the early stages of post-exercise recovery. However, it should be noted that other muscoskeletal tissues, such as ligaments, tendons, cartilage, and bone, are composed of a greater percentage of collagen (~75%–95%) when compared with muscle tissue (<5%). The adaptive response of such collagenous tissues to exercise training or orthopedic surgery may, be more dependent on dietary glycine provision (58,59). Therefore, future studies should also address the dietary amino acid requirements of musculoskeletal tissues other than only muscle to optimize their conditioning. However, such studies will likely be restricted to presurgical interventions to assess the impact of nutritional interventions to modulate connective tissue protein synthesis rates in such collagen-rich tissues.

CONCLUSIONS

In conclusion, ingestion of a protein blend providing 25 g whey plus 5 g collagen increases both myofibrillar and muscle connective protein synthesis rates at rest. Ingestion of such a whey plus collagen protein blend further increases myofibrillar but not muscle connective protein synthesis rates during the early stages of post-exercise recovery.

The authors thank Lisa Kuin for her medical assistance, and Annemarie P. Gijssen, Wendy E. Sluijsmans, and Hasibe Aydeniz for their analytical support. They also extend their gratitude to all study participants for their time and commitment. This study was funded by a public–private cooperation between Maastricht University, the Netherlands, GELITA AG, Germany, and TKI Health–Holland, the Netherlands. L.J.C. v. L. and his laboratory have received research grants, consulting fees, speaking honoraria, or a combination of these for research on the impact of exercise and nutrition on muscle metabolism, which include research funding from companies that produce collagen such as GELITA AG and PB Leiner. A full overview on research funding is provided at https://www.maastrichtuniversity.nl/l.vanloon. U. B. is an employee of GELITA AG. K. B. has received has also received grants, consulting fees, speaking honoraria, and donations from nutritional companies such as PepsiCo, Bergstrom Nutrition, Ynsect, and GelTor to study the effect of dietary collagen on endogenous collagen synthesis. T. S. was supported by a research grant from GELITA AG to perform studies on collagen protein ingestion and post-exercise recovery. T. A., J. K., W. J. H. H., F. K. H., A. M. H., J. M. S., J. M. X. v. K. and J. P. G. G. report no conflicts of interest related to this work. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine. This trial was registered at ClinicalTrials.gov (NCT05386771).

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