Collagen Peptide Supplementation during Training Does Not Further Increase Connective Tissue Protein Synthesis Rates

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ABSTRACT

KIRMSE, M., T. M. LOTTMANN, N. R. VOLK, M. DE MARÉES, A. M. HOLWERDA, L. J. C. VAN LOON, and P. PLATEN. Collagen Peptide Supplementation during Training Does Not Further Increase Connective Tissue Protein Synthesis Rates. *Med. Sci. Sports Exerc.*, Vol. 56, No. 12, pp. 2296–2304, 2024. **Introduction:** Protein supplementation increases postexercise muscle protein synthesis rates and, as such, supports exercise-induced muscle conditioning. Collagen protein has been suggested as the preferred protein source to stimulate muscle connective protein synthesis rates during recovery from exercise. Here we assessed the effects of hydrolyzed collagen peptide supplementation on both myofibrillar as well as muscle connective protein synthesis rates during 1 wk of strenuous resistance exercise training. **Methods:** In a randomized, double-blind, parallel design, 25 young men (24 ± 3 yr, 76.9 ± 6.4 kg) were selected to perform 1 wk of intense resistance–type exercise training. Subjects were randomly assigned into two groups receiving either 15 g hydrolyzed collagen peptides (COL) or a noncaloric placebo (PLA) twice daily during the intervention. Subjects were administered deuterated water (^2H_2O) daily, with blood and skeletal muscle tissue samples being collected before and after the intervention to determine daily myofibrillar and muscle connective protein synthesis rates. **Results:** Post-absorptive plasma glycine, proline, and hydroxyproline concentrations increased following collagen peptide supplementation ($P < 0.05$) and showed higher levels when compared with the placebo group ($P < 0.05$). Daily muscle connective protein synthesis rates during the intervention period exceeded myofibrillar protein synthesis rates (1.99 ± 0.38 vs 1.34 ± 0.23%·d^{-1}, respectively; $P < 0.001$). Collagen peptide supplementation did not result in higher myofibrillar or muscle connective protein synthesis rates (1.34 ± 0.19 and 1.97 ± 0.47%·d^{-1}, respectively) when compared with the placebo group (1.34 ± 0.27 and 2.00 ± 0.27%·d^{-1}, respectively; $P > 0.05$). **Conclusions:** Collagen peptide supplementation (2 × 15 g daily) does not increase myofibrillar or muscle connective protein synthesis rates during 1 wk of intense resistance exercise training in young, recreational athletes. **Key Words:** CONNECTIVE TISSUE, MYOFIBRILLAR PROTEIN, RECREATIONAL ATHLETES, LOW-QUALITY PROTEIN

Skeletal muscle tissue remodeling is driven by the balance between protein synthesis and breakdown rates, both of which occur at a rate of ~1%–2% per day (1). Resistance exercise training increases muscle protein synthesis rates, thereby increasing muscle mass and strength (2–4). The increase in muscle mass and strength is typically attributed to the increase in myofibrillar protein, which is the force-generating component of the muscle (5). However, the force generated by the contractile proteins must be transmitted through the muscle extracellular connective protein network before reaching the tendons to allow joint movement (6–8). It is apparent that the muscle connective protein network also undergoes extensive remodeling in response to acute exercise (9–12) as well as prolonged exercise training (13). In agreement, an acute bout of exercise has been reported to strongly increase both myofibrillar and muscle connective protein synthesis rates (14–17). Together, remodeling of both myofibrillar and connective proteins support the gains in muscle mass and strength following more prolonged resistance exercise training (2,4,5,7,9–11,18).

Food ingestion, and protein in particular, during recovery from exercise augments the postexercise increase in muscle protein synthesis rates (19–21). The amino acid composition of the consumed protein is considered an important factor driving the increase in postexercise muscle protein synthesis rates (21). For instance, the ingestion of proteins containing a high amount of essential amino acids, and leucine in particular, more potently increases myofibrillar protein synthesis rates when compared with proteins of a lesser quality (22). In contrast, the impact of dietary protein ingestion to increase

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muscle connective protein synthesis rates is less evident (9,17,23–27). Previous work has reported a decline in postprandial plasma glycine and proline availability following ingestion of dairy protein. This has led to the speculation that dairy proteins may not provide sufficient glycine as an amino acid precursor to support the postexercise increase in muscle connective tissue protein synthesis rates (28,29).

Meléndez-Hevia et al. (30) further suggest that the availability of glycine, relative to the demand, is generally insufficient due to our current dietary habits. Collagen, the primary component of connective protein, is notably rich in glycine (30%) and proline (13%) (31,32). It has, therefore, been argued that ingestion of glycine- and proline-rich collagen peptides may enhance the availability of specific nonessential amino acids that are necessary for connective tissue protein synthesis, potentially yielding a favorable impact on synthesis itself (28,33). However, we and others have recently reported that collagen-derived protein supplementation does not seem to further stimulate postexercise connective tissue protein synthesis rates when compared with whey protein supplementation (34,35). However, without the inclusion of a non-nitrogenous control condition, it remains unclear whether collagen peptide supplementation can further increase muscle connective protein synthesis rates and, as such, supports muscle conditioning during exercise training. Moreover, it remains unclear whether an intense exercise training regimen spanning several days could further increase the demands for connective tissue-specific amino acids, such as those mentioned previously.

In the present study, our hypothesis posited that supplementing with collagen peptides throughout 7 consecutive days of rigorous exercise would augment the availability of specific nonessential amino acids, thereby facilitating enhanced endogenous synthesis of connective tissue proteins. To test our hypotheses, we recruited healthy, young adults to ingest either collagen peptides or a non-nitrogenous placebo twice daily during 7 d of intensive resistance exercise training, which included concentric and ample eccentric exercise, such as drop jumps and rope skipping. We combined deuterium oxide (^2H_2O) administration with muscle biopsy collection to quantify both myofibrillar and muscle connective tissue protein synthesis rates throughout the intervention period.

METHODS

Participants. Thirty-two healthy male participants (age: 24.5 ± 2.9 yr; body mass index: 23.5 ± 2.0 kg·m^{-2}) were recruited to participate in this study. Before inclusion, all participants displayed the ability to correctly perform a barbell back squat at a weight of at least 100% of their body weight. Participants were excluded if they followed a vegetarian or vegan diet, a progressive resistance exercise training program, and/or if they ingested nutritional supplements. All participants were informed verbally and in writing about the purpose, procedures, and risks of the study before obtaining their written consent. This investigation was approved by the local Ethics Committee of the Faculty of Sport Science of the Ruhr University Bochum, Germany (EKS V 05/2020) and has been conducted in accordance with the latest version of the Declaration of Helsinki.

Study overview. An overview of the study protocol is displayed in Figure 1. The study was performed in a randomized, double-blind, placebo-controlled fashion. In short, the study consisted of an 8-d exercise intervention period. The experimental test days included a baseline testing day ($t = -10$ d), a deuterium oxide dosing day ($t = -1$ d), and pre- ($t = 0$ d) and post-training ($t = 8$ d) biopsy sampling days. Participants were randomly assigned into one of two treatment groups, which were matched for squat one-repetition maximum (1RM) relative to their fat-free body mass. The exercise intervention consisted of daily training involving the barbell squat, bench press, deadlift, and bent-over row exercises, drop jump movements, and rope skipping. During the training intervention, participants were randomly assigned to consume either 15 g collagen peptides or 15 g of a noncaloric placebo twice daily.

Baseline testing. On the baseline testing day ($t = -10$ d), participants visited the laboratory in a fasted and rested state.

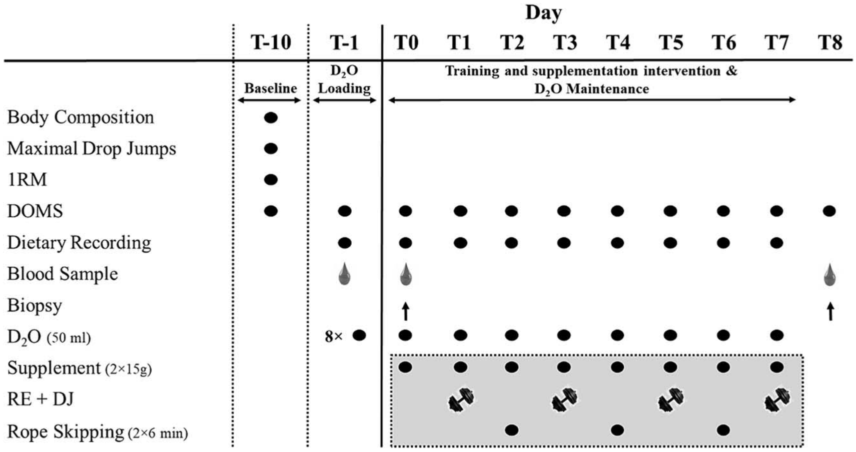

FIGURE 1—Overview of the study protocol. The dark-gray section shows the supplementation and exercise intervention. *T*, time point (shown in days); D_2O, deuterated water; DOMS, delayed onset of muscle soreness; RE, resistance exercise; DJ, drop jump.

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During the test day, measurements, including height, body mass, fat mass, fat-free mass, maximal drop jump height, and 1RM of the four barbell exercises, were performed. Fat mass and fat-free mass were measured using a bioelectrical impedance analysis system (InBody 770; JP Global Markets GmbH, Eschborn, Germany) (36,37). Participants then ate breakfast before performing standardized drop jump testing on a contact platform (Haynl-Elektronik GmbH, Schönebeck, Germany) to determine flight time and estimate maximal drop jump height (38,39). 1RM strength testing for the squat, bench press, deadlift, and bent-over row exercises were performed as previously described (40,41).

Deuterium oxide dosing protocol. The deuterium oxide dosing protocol consisted of a dosing day followed by maintenance days throughout the intervention period. During the dosing day ($t = -1$ d), participants ingested 400 mL of 70% deuterium oxide (Cambridge Isotopes Laboratories, Andover, MA) separated in eight doses of 50 mL between 8:30 AM and 6:30 PM, as previously described (42). During the maintenance days, participants ingested one 50-mL dose before sleep to maintain body water deuterium enrichment. No adverse effects were reported from deuterium oxide dosing throughout the study.

Supplementation and dietary intake recording. During the 8-d intervention period, participants were randomly assigned to ingest either 15 g hydrolyzed collagen peptides (COL; BODYBALANCE®; Gelita AG, Eberbach, Germany (43)) or 15 g of a noncaloric placebo (PLA; Silica, SIPERNAT 22 S; Evonik Industries, Essen, Germany) two times per day. One dose was consumed directly after training, and the other dose was consumed every night before sleep. The intervention was performed in a double-blinded fashion whereby an independent researcher performed treatment randomization and beverage preparation. Subjects received labeled packages containing dried beverage contents, which were dissolved in 250 mL orange juice (hohes C; Eckes-Granini Deutschland GmbH, Nieder-Olm, Germany) to ensure sufficient vitamin C intake (a cofactor involved in collagen formation (44,45)). Participants were encouraged to maintain their regular dietary habits during the experimental period and recorded their dietary intake using Myfitnesspal (46) from $t = -1$ d until $t = 7$ d.

Training intervention. The exercise training intervention included multijoint barbell movements, drop jump movements, and rope skipping. The training program included concentric and a large proportion of eccentric contractions, because these have been shown to robustly stimulate muscle connective protein synthesis rates (47–49). The barbell exercises and drop-jump exercises were performed within the same sessions, between 10:00 AM and 2:00 PM on $t = 1$, 3, 5, and 7 d. Each session consisted of a warm-up (planks, push-ups, and body weight squats) followed by squat, bench press, deadlift, bent-over row, and drop jump movements performed in a randomized order. The barbell movements consisted of a warm-up set of 10 repetitions at 50% of 1RM, followed by three sets of 10 repetitions at 70% of 1RM separated by 150 s of rest between sets. When all repetitions were completed with adequate technique, the training weight was increased on subsequent sessions by 2.5 kg in bench press and bent over row and by 5 kg in squat and deadlift in each barbell exercise. The drop jump movements consisted of four sets of 10 drop jumps from a 40-cm box and were performed on a contact plate to monitor jumping height. Participants were instructed to keep their hands on their hips and to jump as high as possible on each jump. On $t = 2$, 4, and 6 d, participants performed two sessions of 6 min of continuous rope skipping, at 10:00 AM and 4:00 PM. Each rope skipping session was supervised by means of a virtual video call. Participants were instructed to abstain from other sporting or exhaustive activities throughout the investigation period.

Perception of muscle soreness. Perception of muscle soreness was assessed using a 100-mm visual analog scale (no pain = 0 to severe pain = 100). Participants completed the visual analog scale on $t = -10$ d (baseline) and on each morning after waking up during the intervention (50).

Blood and muscle tissue sampling. Venous blood samples were collected in an overnight fasted state in EDTA containing tubes at the mornings of $t = -1$, 0, and 8 d. After centrifugation, aliquots of 500 μL of plasma were frozen in liquid nitrogen and stored at −80°C until further analysis. Muscle tissue samples were collected on $t = 0$ and 8 d using a 5-mm Bergstrøm needle under vacuum from the *m. vastus lateralis muscle* of the right leg (51). All samples were freed from visible nonmuscular tissue and immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

Plasma amino acid and muscle protein analyses. The measurement of plasma amino acid concentrations was performed using ultraperformance liquid chromatography–mass spectrometry (MS) as described previously (10). The measurement of plasma free [^{2}H]-alanine enrichment was performed using gas chromatography–mass spectrometry (GC-MS) as previously described (52). Muscle myofibrillar and connective proteins were isolated before measuring [^{2}H]-alanine enrichments as previously described (10,53). Briefly, ~60 mg of wet muscle tissue was homogenized in a standard extraction buffer (10 μL/mg), and the myofibrillar and connective proteins were isolated using a combination of vigorous mechanical homogenization (FastPrep-24 5G; MP Biomedicals, Irvine, CA) and connective protein precipitation using a KCl buffer. Myofibrillar and connective protein fractions were isolated and separately hydrolyzed overnight at 110°C. The free amino acids were then purified and converted into their *N*(O,*S*)-ethoxycarbonyl ethyl ester derivatives before measuring [^{2}H]-alanine enrichments on the Gas Chromatography Combustion Isotope Ratio Mass Spectrometer (GC-C-IRMS) as described previously (42,54). Ion masses 2 and 3 were monitored to determine the ^{2}H/^{1}H ratios of muscle protein bound alanine. A series of known standards were applied to assess linearity of the mass spectrometer and to control for the loss of tracer.

Calculations. The fractional synthetic rates (FSR) of myofibrillar and connective proteins were calculated by dividing the increment in myofibrillar and connective tissue protein

TABLE 1. Baseline parameters recorded at t = −10 d with Welch's t-test results (P value).

Baseline Parameters	PLA (n = 12)	COL (n = 13)	Mean Dif. (95% CI)	P
Age (yr)	23.7 ± 2.8	25.1 ± 3.0	−1.4 (−3.8 to 0.9)	0.222
Height (m)	1.81 ± 0.05	1.82 ± 0.05	−0.01 (−0.05 to 0.03)	0.597
Bodyweight (kg)	76.0 ± 5.3	77.8 ± 7.3	−1.8 (−7.1 to 3.4)	0.485
Body fat mass (kg)	10.5 ± 4.7	10.4 ± 2.5	0.1 (−3.1 to 3.3)	0.941
Fat-free mass (kg)	65.5 ± 4.5	67.4 ± 7.3	−1.9 (−6.9 to 3.1)	0.433
1RM squat (kg)	110 ± 20	111 ± 19	−2 (−18 to 15)	0.844
1RM deadlift (kg)	130 ± 14	130 ± 22	0 (−16 to 15)	0.935
1RM bench press (kg)	81 ± 12	77 ± 16	4 (−8 to 16)	0.526
1RM bent over row (kg)	90 ± 10	89 ± 13	1 (−9 to 11)	0.834
Drop jump max (cm)	35.1 ± 6.1	31.1 ± 3.3	4.1 (−0.6 to 8.9)	0.083

Data presented as means ± SD, mean differences (PLA–COL), and 95% CI.

[^{2}H]-alanine enrichments by the average plasma free [^{2}H]-alanine enrichment over the intervention period. Consequently, muscle myofibrillar and connective protein FSRs were calculated as follows:

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

E_{m1} and E_{m2} represent the myofibrillar or connective protein-bound [^{2}H]-alanine enrichments at t = 0 and t = 8 d, respectively. $E_{\mathrm{precursor}}$ represents the average plasma free [^{2}H]-alanine enrichment during the tracer incorporation period, and t indicates the time interval (in days) between biopsies.

Statistics. All data are expressed as mean ± SD and 95% confidence intervals (CI). Data were analyzed using commercially available software (GraphPad Prism 9.2.0, San Diego, CA). All data were tested and confirmed for normality using Shapiro–Wilk test. Participants' characteristics, external training volume, baseline testing results and dietary intake, and myofibrillar and connective protein fractional synthesis rates were compared using Welch's t-test for independent groups. We opted for the Welch's t-test due to the disparate sizes of the final groups, making it impractical to make a clear assumption regarding homogeneity of variance (55). Perception of muscle soreness and amino acid concentrations were compared using a two-factor repeated-measures ANOVA with time as a within-participants factor and group as a between-participants factor. Upon a significant finding, Bonferroni-corrected *post hoc* comparisons were performed to detect differences between the groups and/or time points. Statistical significance was set at P < 0.05.

RESULTS

Baseline testing and participant characteristics. Thirty-two young men were recruited to participate in this study. Four subjects were excluded due to insufficient quantity muscle tissue sampling at t = 0 d. Two subjects dropped out after baseline testing due to concerns about the biopsy sampling procedure. One subject discontinued participation after t = 3 d due to anxiety about the posttraining biopsy sampling. A total of 25 participants completed the study and were included in the statistical analysis. Participants' group-specific characteristics and measured baseline body composition, strength, and jumping performance are presented in Table 1. No significant differences were recorded for any parameter between the groups.

External training volume and dietary recordings. The included participants completed all training sessions and exercises. No differences in training volume and drop jump heights during training were observed between groups (Table 2, P > 0.05, respectively). No differences in daily energy and macronutrient intake were found throughout the experimental protocol from t = −1 to t = 7 d between the groups (Table 2, P > 0.05, respectively).

Perception of muscle soreness. Delayed-onset muscle soreness increased during the training period (main effect for time, P < 0.001) with no differences detected between groups (main effect for group, P = 0.870; group–time, P = 0.999). Specifically, perceived muscle soreness was increased from baseline at t = 2 d (COL: 49.8 ± 24.5 mm, PLA: 51.7 ± 27.5 mm) and remained elevated until t = 8 d (all P < 0.001).

Plasma amino acid concentrations. Fasting plasma ΣEAA concentrations were higher in PLA compared with COL at t = 0 d (P = 0.017). No differences in plasma ΣEAA concentrations were observed between groups at t = 8 d (P > 0.999). Plasma ΣEAA concentration decreased in both groups over time (P < 0.001) and to a greater extent in PLA versus COL (P = 0.040). ΣNEAA concentration, excluding hydroxyproline, in plasma did not exhibit significant differences between the groups at any time point (t = 0 d: P = 0.618; t = 8 d: P = 0.865), nor within groups between time points (COL: P = 0.941; PLA: P = 0.084). Plasma glycine concentrations were not different between groups at t = 0 d (P > 0.999) or at t = 8 d (P = 0.097). Plasma glycine concentration increased over time in COL (P < 0.001), with no changes

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TABLE 2. Total external training volume, means of daily drop jump height, and means of daily macronutrient and energy intake recorded during the intervention period (t = 0–7 d) with Welch's t-test results (P value).

	During Intervention	PLA (n = 12)	COL (n = 13)	Mean Dif. (95% CI)	P
External Training Volume	Squat (kg)	11,013 ± 2061	10,993 ± 1570	19 (−1516 to 1555)	0.979
	Deadlift (kg)	12,434 ± 1850	13,021 ± 2458	−587 (−2382 to 1208)	0.505
	Bench press (kg)	7867 ± 1414	7727 ± 1498	140 (−1065 to 1345)	0.812
	Bent over row (kg)	9262 ± 1288	9084 ± 1522	178 (−986 to 1342)	0.756
	Ø DJ height (cm)	33.9 ± 6.9	33.2 ± 4.7	0.79 (−4.25 to 5.82)	0.748
Mean energy and macronutrient intake	Total energy (kcal·d^{-1})[a]	2443 ± 379	2421 ± 432	22 (−313 to 358)	0.892
	Carbohydrate (kcal·d^{-1})	1303 ± 223	1350 ± 286	−47 (−259 to 164)	0.648
	Fat (kcal·d^{-1})	690 ± 204	623 ± 191	66 (−97 to 230)	0.411
	Protein (kcal·d^{-1})[a]	450 ± 73	447 ± 117	3 (−77 to 83)	0.935
	Protein (g·kg^{-1}·d^{-1})[a]	1.47 ± 0.25	1.41 ± 0.31	0.05 (−0.18 to 0.29)	0.645

Data presented as means ± SD, mean differences (PLA–COL), and 95% CI.
[a] The supplemented hydrolyzed collagen peptides of COL were excluded for comparisons.

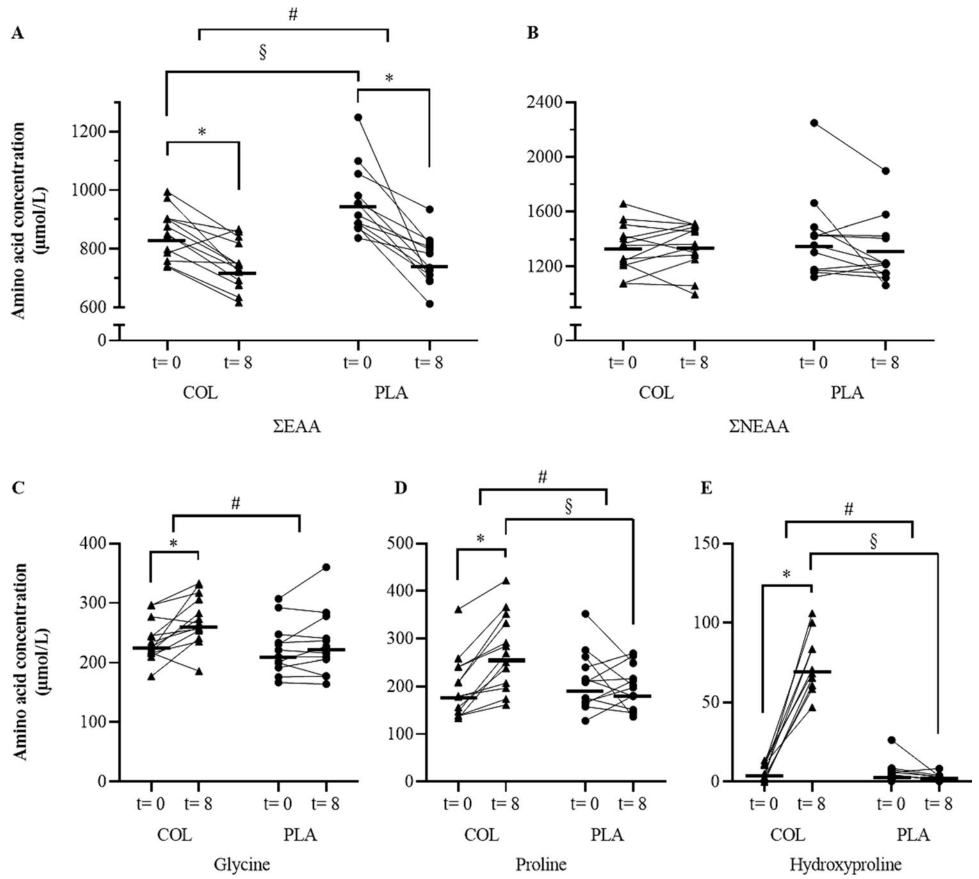

FIGURE 2—Fasting plasma ΣEAA (A), ΣNEAA (B), glycine (C), proline (D), and hydroxyproline (E) concentrations before and after the intervention period in the COL (triangles) and PLA (circles) supplemented groups. The dark horizontal lines show the mean values per group and time point. #Significant interaction effect (ANOVA: time–group; $P < 0.05$). §Significant difference between the groups at either day $t = 0$ or $t = 8$ ($P < 0.05$). *Significant difference compared with day $t = 0$ within the respective group ($P < 0.05$).

observed in PLA ($P = 0.711$). Plasma proline concentrations did not differ between groups at $t = 0$ d ($P > 0.999$). Plasma proline concentrations were higher in COL compared with PLA at $t = 8$ d ($P = 0.029$). Plasma proline concentrations increased over time in COL ($P < 0.001$), with no changes in PLA ($P = 0.812$). Plasma hydroxyproline concentrations did not differ between groups at $t = 0$ d ($P > 0.999$). Plasma hydroxyproline concentrations were higher in COL compared with PLA at $t = 8$ d ($P < 0.001$). Plasma hydroxyproline concentrations increased over time in COL ($P < 0.001$), with no changes observed in PLA ($P = 0.854$). A graphical representation of ΣEAA, ΣNEAA, glycine, proline, and hydroxyproline is provided in Figure 2. Data of all single amino acids are shown in the Supplemental Table (Supplemental Digital Content, http://links.lww.com/MSS/A1).

Myofibrillar and connective tissue protein synthesis rates. No differences were observed in MyoPS (COL: $1.34 \pm 0.19\%\cdot d^{-1}$; PLA: $1.34 \pm 0.27\%\cdot d^{-1}$; $P = 0.933$) and ConPS (COL: $1.97 \pm 0.47\%\cdot d^{-1}$; PLA: $2.00 \pm 0.27\%\cdot d^{-1}$, $P = 0.821$) between groups (Fig. 3).

DISCUSSION

The present study assessed the impact of daily hydrolyzed collagen peptide supplementation on myofibrillar and connective protein synthesis rates during 1 wk of whole-body resistance-type exercises, drop jumps, and rope skipping. In contrast to our hypotheses, we found that hydrolyzed collagen peptide supplementation did not further increase connective tissue protein synthesis. Myofibrillar protein synthesis was also not further increased by the hydrolyzed collagen peptides during the training period compared with noncaloric placebo supplementation, which was consistent with our hypothesis.

The present exercise protocol involved a large volume of shortening and lengthening contractions by way of barbell and plyometric exercises as well as rope skipping. The lengthening and plyometric components of the program were chosen as they have been shown to have a potent capacity to stimulate myofibrillar and connective protein synthesis rates (16,56). Furthermore, the combination of different exercise movements is reflective of training regimens commonly applied by athletes (i.e., during preseason training). The application of deuterium oxide allowed for the measurement of protein synthesis rates over the 8-d protocol, which allowed the integration of the anabolic responses from the different exercise movements performed on consecutive days. We confirmed full compliance with the program allowing all subjects to reach the intended high training volume, without any differences between the collagen and placebo groups (Table 2).

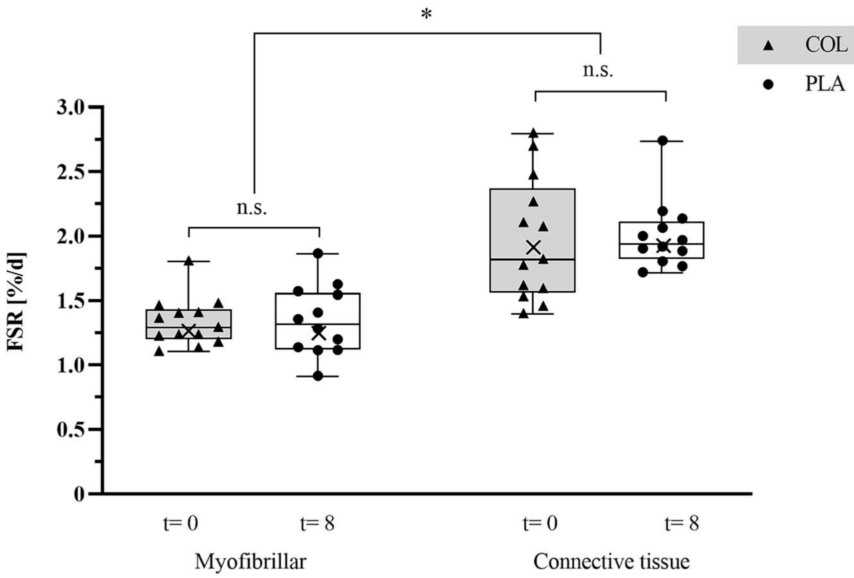

FIGURE 3—Daily myofibrillar protein and muscle connective protein fractional synthesis rates (%·d^{-1}) during 8 d of collagen peptide or placebo supplementation. Box and whisker plots were used to display the data with median (line), mean (cross), interquartile range (box), and minimum and maximum values (tails). Data were analyzed with an independent *t*-test. Neither myofibrillar (*P* = 0.932) nor connective tissue (*P* = 0.821) protein synthesis rates showed differences between groups. *Significant difference between myofibrillar and connective protein FSR (*P* < 0.001). n.s., not significantly different.

Furthermore, the high training intensity was confirmed by the elevated delayed onset of muscle soreness (DOMS) scores observed throughout the intervention period irrespective of treatment group (Fig. 3) (57).

Protein ingestion has been well established to further increase myofibrillar protein synthesis rates during recovery from exercise (58–60). The stimulatory properties of dietary protein ingestion have been attributed to the rise in circulating essential amino acid concentrations (61), with leucine known to be particularly anabolic (62,63). Here, we observed no differences between plasma essential amino acid (Fig. 2) or leucine concentrations (Online Resource 1) between collagen and placebo supplementation at t = 8 d. In contrast, we observed that collagen supplementation resulted in greater plasma glycine, proline, and hydroxyproline concentrations when compared with placebo supplementation at t = 8 d (Fig. 2). Our observations in the more prolonged setting aligns well with our recently published data (35) and those of others (28), demonstrating that single-bolus collagen ingestion robustly increases plasma glycine, proline and hydroxyproline concentrations and only marginally increases leucine concentrations over the postprandial period. Combined, these data seem to reflect adequate digestion and absorption of collagen protein based on its relative abundance in glycine (~25%), proline (~13%), hydroxyproline (~13%) contents, and sparsity of leucine (~3%) content (27,35). In agreement with our hypothesis, we observed that collagen supplementation did not further increase daily myofibrillar protein synthesis rates during intensive resistance-type exercise training (Fig. 3). Our findings over an 8-d exercise protocol extend upon our recent work in the acute setting demonstrating no impact of ingesting 30 g collagen on myofibrillar protein synthesis rates during 5 h of recovery from a barbell squatting session (35). The absence of an anabolic effect of collagen ingestion is likely attributed to its low essential amino acid and leucine contents. For reference, several studies have demonstrated that the ingestion of 2–3 g leucine within a meal potently increases postexercise myofibrillar protein synthesis rates in both the acute setting (62–64) and over several days (65). In contrast, the 15 g collagen protein provided here contains merely 0.4 g leucine. Based on the present findings and those of Aussieker et al. (35), it appears that collagen protein should not be regarded as a preferred supplemental protein source to enhance skeletal muscle mass gain following prolonged exercise training. However, despite the absence of greater myofibrillar protein synthesis rates, collagen supplementation has previously been reported to further increase skeletal muscle mass and strength gains during more prolonged resistance-type exercise training (43,66). The discrepancy between studies remains unclear, although it should be noted that the muscle mass and strength gains reported in one of the existing long-term training studies (43) are far above what has been reported in several comparable studies evaluating the impact of protein supplementation (67). Clearly, further investigation is needed to establish whether collagen supplementation promotes the skeletal muscle adaptive responses to prolonged resistance exercise training.

Although glycine and proline are considered nonessential amino acids, some have proposed that dietary intake and endogenous production may not suffice to meet the increased demands of various metabolic processes, including elevated connective protein synthesis following exercise (30). As such, our primary hypothesis was that the greater glycine and proline provision following collagen supplementation would promote a further increase in muscle connective protein synthesis rates throughout an intensive daily exercise training protocol. However, despite greater glycine and proline availability, we were unable to detect greater connective protein synthesis rates following collagen supplementation when compared with placebo supplementation (Fig. 3). This finding aligns with most (10,17,24,25,34,68), but not all (9) studies evaluating the (more) acute impact of protein ingestion on connective protein synthesis rates *in vivo* in humans. Notably, Holm et al. (9) showed that whey ingestion further increased connective protein synthesis rates during the later (>3 h) stages of postexercise recovery. As such, it would seem as though a potential connective protein synthetic response to protein supplementation may be more easily detectable over a more prolonged measurement period (10,68). Recently, Oikawa et al. (34) applied deuterium oxide and reported no differences between whey and collagen peptide supplementation over a comparable 6-d period of exercise training. In the present study, the use of deuterium oxide and comparison between collagen and (non-nitrogenous) placebo supplementation allows us to extend upon these findings and conclude that collagen supplementation does not increase muscle connective protein synthesis rates over several days of intensive exercise training *in vivo* in humans. Taken together, it appears as muscle connective protein remodeling is not sensitive to an increase in amino acid precursors following collagen protein supplementation.

In the present study, we evaluated the impact of collagen supplementation on connective tissue remodeling within skeletal muscle tissue. Skeletal muscle tissue contains relatively low collagen content (~4% of dry mass) (69) when compared with other collagenous musculoskeletal tissues, such as ligament and tendon, which contain up to 85% collagen (70). We could speculate that the greater glycine and proline requirements following muscle connective tissue conditioning during intense resistance training are not limited by habitual protein intake. In this study, male volunteers consumed $>1.4 \pm 0.3$ g protein per kilogram bodyweight per day, well above protein requirements set by the World Health Organization ($0.8\ \mathrm{g \cdot kg^{-1} \cdot d^{-1}}$) as well as habitual protein intake in more sedentary males (71). It could be hypothesized that the greater protein intake in these recreational athletes was well above requirements to provide ample amino acid precursors to maximize both myofibrillar and muscle connective protein conditioning during intense exercise training. In contrast to the hypothesis proposed by Meléndez-Hevia et al. (30), the habitual protein intake of our participants seems to provide sufficient glycine and proline and, as such, does not compromise daily myofibrillar or muscle connective protein synthesis during intense exercise training without dietary constraints. Although collagen supplementation does not seem to provide any benefit to the skeletal muscle adaptive response to intense resistance exercise training, it could still be hypothesized that glycine and proline provision could limit adaptive responses in nonmuscle tissue. With their higher collagen contents, bone, cartilage, tendons, and ligaments would presumably require large amounts of glycine and proline to support increased synthesis rates following exercise (72). As such, future work should evaluate the impact of collagen supplementation to enhance connective protein synthesis of other musculoskeletal tissues (72).

CONCLUSIONS

In conclusion, collagen peptide supplementation (2×15 g daily) does not increase myofibrillar or muscle connective protein synthesis rates during 1 wk of intense resistance exercise training in young, recreational athletes.

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