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RESEARCH ARTICLE

Exercise Metabolism

Hydrolyzed collagen supplementation prior to resistance exercise augments collagen synthesis in a dose-response manner in resistance-trained, middle-aged men

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Abstract

Resistance exercise (RE) increases collagen synthesis in young and older men, whereas hydrolyzed collagen (HC) ingestion improves this response to RE in a dose-response manner in young men. However, the collagen synthesis response to RE with and without HC in middle-aged men is unknown. Eight resistance-trained men (age: 49 ± 8 yr; height: 1.78 ± 0.02 m; mass: 90 ± 4 kg) took part in this double-blind, crossover design study and undertook 4 × 10 repetitions of lower-limb RE at maximum load, after consuming 0 g, 15 g, or 30 g vitamin C-enriched HC. We analyzed venous blood samples for N-terminal propeptide of type 1 pro-collagen (PINP), β-isomerized C-terminal telopeptide of type 1 collagen (β-CTx), and 18 collagen amino acids throughout all three interventions. The serum PINP concentration × time area-under-the-curve (AUC) was higher following 30 g (169 ± 28 μg/mL × h) than 15 g (134 ± 23 μg/mL × h, $P < 0.05$) HC ingestion, and both 15 g and 30 g were higher than 0 g HC (96 ± 23 μg/mL × h, $P < 0.05$). RE with 0 g HC showed no change in serum PINP concentration. The AUCs for glycine, proline, hydroxyproline, alanine, arginine, lysine, serine, leucine, valine, and isoleucine were greater with 30 g than 15 g and 0 g HC ingestion ($P < 0.05$) and greater with 15 g than 0 g HC ingestion ($P < 0.05$). Plasma β-CTx concentration decreased after RE independently of HC dose. Our study suggests connective tissue anabolic resistance to RE in middle-aged men but ingesting 15 g HC rescues the collagen synthesis response and 30 g augments that response further. This dose response is associated with the increased bioavailability of collagen amino acids in the blood, which stimulate collagen synthesis.

NEW & NOTEWORTHY This study is the first to document the dose-response effect of hydrolyzed collagen (HC) ingestion before resistance exercise (RE) on collagen turnover in middle-aged, resistance-trained men. Strikingly, RE alone did not increase collagen synthesis (suggesting connective tissue anabolic resistance), but ingesting 15 g HC rescued the collagen synthesis response and 30 g augmented that response further. These results were associated with the increased bioavailability of collagen amino acids in the blood, which stimulate collagen synthesis.

connective tissue; glycine; hydroxyproline; proline; strength training

INTRODUCTION

Injury is a major concern for athletes of all ages, with injury rates to collagenous tissues such as muscle, ligament, and tendon being similar between young, middle-aged, and older male athletes (1). Chronic resistance exercise (RE), however, is a safe and effective method to reduce tendon injury risk (2). For example, chronic high-intensity RE causes the tendon of healthy adults to adapt by increasing its mechanical (stiffness) and morphological (size) properties (3–6), which should result in reduced tissue stress during loading (7). Furthermore, increasing tendon stiffness can improve the rate of torque development (RTD) (8), which should enhance athletic performance (9, 10) by enabling

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force to be transmitted from the muscle to the bone more efficiently (11).

The RE-induced change in tendon stiffness can be explained by an increase in tendon cross-sectional area (12), an increase in collagen fibril density (13), and an increase in collagen fibril cross-linking (14, 15). For the first two of these factors to occur, an increase in collagen synthesis is likely required for collagen content to increase (16–19). As human tendon dry weight comprises 60%–85% type I collagen (20, 21), measuring serum concentration of procollagen type I N-terminal propeptide (PINP, which is cleaved off during the maturation of procollagen to collagen) can indicate whether tendon collagen synthesis has increased following RE. Indeed, previous studies have found that PINP concentration increases following a single bout of RE (22, 23) and after 12-wk RE (24).

Given the importance of a positive net collagen turnover for RE-induced connective tissue adaptations to occur (16–19), exogenous collagen ingestion may enhance the collagen synthesis response to an acute bout of RE. Indeed, Lee et al. (23) recently showed that HC ingestion prior to high-intensity RE augmented the serum PINP concentration × time area under the curve (AUC) in a dose-response manner in resistance-trained, young men, i.e., 30 g > 15 and 0 g. This response was likely related to the higher postprandial serum concentrations of key amino acids associated with collagen composition and synthesis (e.g., glycine, proline, and hydroxyproline) in the 30 g intervention compared with the 15 g and 0 g interventions (23). However, this is the only study to date to have investigated the dose-response relationship of collagen ingestion prior to RE on collagen synthesis, and this relationship may be different in other populations. For example, it is not yet known how much collagen is necessary to maximize collagen synthesis following RE in middle-aged men.

Research typically contrasts extreme age groups to accentuate aging effects on RE and nutrition, leaving the middle-aged population (40–64 yr) often overlooked. For instance, compared with young adults (aged 18–39 yr), tendons of older adults (aged > 65 yr) show diminished extracellular matrix (ECM) gene expression in response to acute RE (18). Although older tendon properties improve with chronic RE, the rate of adaptation is slower than younger tendon (25). It is crucial, however, to examine responses in middle-aged individuals to provide meaningful exercise and nutritional recommendations for this population. Aging is known to blunt the anabolic response to RE and protein ingestion, doubling the protein intake required to optimize muscle protein synthesis after RE in older adults compared with young (26, 27). Given that collagen-rich tissues such as skin exhibit reduced collagen synthesis with age (28), it is possible that aging also alters the collagen synthesis response to exogenous collagen ingested before RE, although this remains to be explored.

The aim of this study was to investigate the effect of lower-limb RE with vitamin C-enriched HC supplementation on serum PINP concentration in resistance-trained, middle-aged men. The objective was to determine the optimal dose of HC required to stimulate maximal whole body collagen synthesis following RE in this population. We hypothesized that collagen synthesis would increase in response to RE and that this effect would be augmented in a dose-dependent manner by supplementation with vitamin C-enriched HC.

MATERIALS AND METHODS

Participants

Participants were recruited from a population of resistance-trained, middle-aged men (i.e., posters advertising the study were placed in gymnasiums of the local area, and local sports clubs were emailed with the study information, which was disseminated to members). Recruitment began in November 2019 and data collection was completed in September 2021. To be eligible to participate, volunteers had to be male, have at least 12 mo of resistance training experience (including lower-limb resistance exercise [RE] performed at least once a week), and to be free from musculoskeletal injury. Volunteers were excluded if they had a history of patellar tendon pathology, were vegan (due to the bovine source of HC), consumed nutritional supplements or medication purported to have beneficial effects on muscle-tendon properties (e.g., antioxidants, protein, etc.), had sustained a lower-limb injury in the previous six months, smoked, or were <40 or >65 yr old. The required sample size was estimated before conducting the study with G*Power software (v. 3.1.9.6, Heinrich-Heine-Universität Düsseldorf). The a priori estimation was performed using a large effect size (η_P^2 = 0.22), on the basis of the results from the study by Shaw et al. (29), which demonstrated a twofold increase in the serum PINP concentration × time AUC after exercise with 15 g compared with 5 g gelatine ingestion. A minimum of eight participants was deemed necessary to detect an effect of HC dose [one-way repeated measures analysis of variance (ANOVA); α: 0.05; power: 0.80]. To account for participants withdrawing from the study, 13 resistance-trained, middle-aged men were recruited. However, three participants withdrew prior to commencement of the first intervention, one based on recommendation from his general practitioner and two due to injuries sustained independently of the study. Two more participants completed a portion of the study in March 2020; however, due to immediate laboratory closure during the COVID-19 national lockdown restrictions in Ireland, they could not complete all interventions and were subsequently excluded from the analysis. Therefore, eight men [means ± SD: age, 49 ± 8 yr; height, 178 ± 2 cm; body mass, 90 ± 4 kg; 10 repetition maximum (10-RM) leg press, 330 ± 88 kg] were included in the final analyses after providing written informed consent prior to study commencement (Fig. 1). The study was registered at https://clinicaltrials.gov/ (identifier: NCT06236659), was approved by Liverpool John Moores University Research Ethics Committee (Approval No. 19SPS049), and complied with the Declaration of Helsinki.

Experimental Design

The study was a double-blind, repeated-measures crossover design. Participants reported to the laboratory on four separate occasions separated by 72 h. The purpose of the initial visit was to establish each participant's 10-RM on a leg press machine (Samson, New Mexico) and to familiarize them with the exercise protocol. Second, body mass and stature were measured using calibrated weighing scales (model 769, SECA, Birmingham, UK) and a wall-mounted digital stadiometer (model 264, SECA, Birmingham, UK), respectively. The order of measurements during familiarization was the same as the order they appear below. During the subsequent

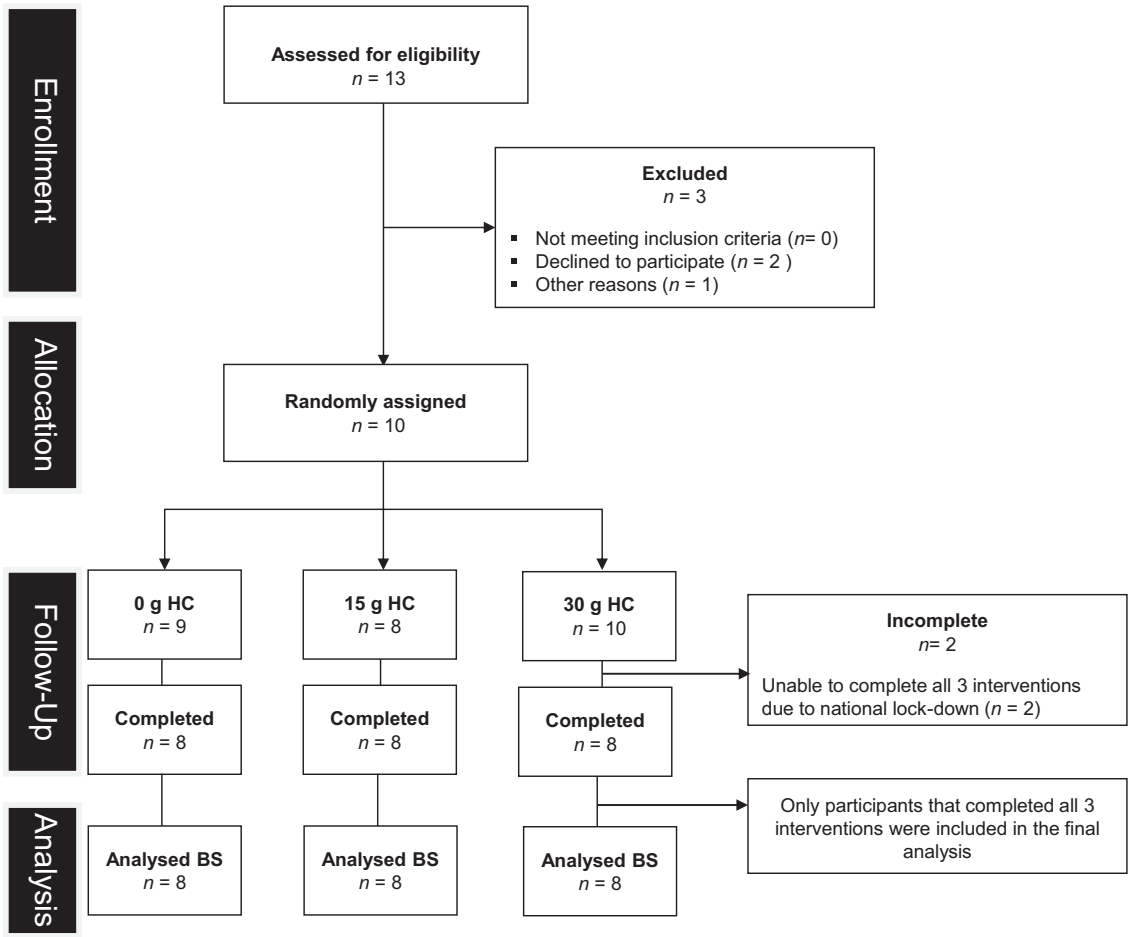

Figure 1. CONSORT diagram. BS, blood sample; HC, hydrolyzed collagen intervention.

three visits, participants reported to the laboratory at 0700 after a 10-h overnight fast and ingested either 0, 15, or 30 g vitamin C-enriched hydrolyzed collagen (HC) supplement in a randomized order, prior to performing four sets of 10-RM on the leg press machine. This was followed by 6-h fasted, passive (seated) rest. Venous blood samples were collected at regular intervals for the duration of each intervention (Fig. 2), and serum/plasma samples were analyzed for the concentration of procollagen type I N-terminal propeptide (PINP, a marker of collagen synthesis), 18 collagen amino acids, and β-isomerized C-terminal telopeptide (β-CTx, a marker of collagen breakdown).

Leg Press 10 Repetition Maximum Protocol

Following a brief dynamic warm-up, comprising 5-min submaximal cycling and 5-min full body dynamic stretching, participants performed 2–4 sets of leg press RE (Samson, New Mexico) with no additional load to determine their

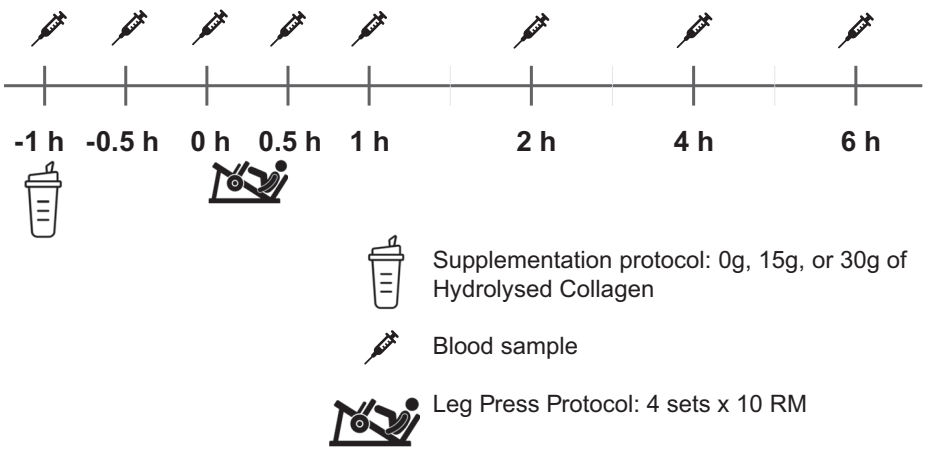

Figure 2. Schematic timeline of data collection.

individual foot placement and range of motion on the machine. The 10-RM was determined during four stages: participants first performed 1–2 sets of 5–10 repetitions at an estimated 50%–70% of their perceived 10-RM. Following this, the load was increased by 5–20 kg on each subsequent set for a total of 4–6 attempts, separated by 3 min rest until the participant could only perform 10 repetitions or fewer. The final load, for which the participant successfully completed 10 repetitions, was deemed to be their 10-RM.

Dietary Control Measures

Habitual dietary behavior of all participants was measured to ensure participants maintained their normal diet throughout the three nutritional interventions and to ensure the same meal was consumed on the night prior to each intervention. To provide an estimate of habitual macronutrient and energy intake, participants were required to complete a dietary log of all food and beverages consumed on 2 weekdays and 1 weekend day (e.g., Thursday, Friday, and Saturday) during the week prior to the first intervention (Table 1). Instructions and reference guides to assist in estimating portion sizes were provided. Estimates of nutrient and energy intake were assessed using nutritional software (Nutritics v5.80, Nutritics LTD, Dublin, Ireland). Before each intervention, participants were instructed to abstain from caffeine and alcohol in the preceding 24 h and to finish consuming their final meal no later than 2100 in the evening. In between interventions, participants were asked to continue monitoring their food intake and to inform the researchers of any deviation from their food diaries. Electronic reminders to maintain the same intakes were sent to each participant on the days between interventions, where they were also asked to confirm their compliance. A final electronic reminder was sent to each participant at 2000 the night before each intervention, stating that they should consume the same final meal and begin fasting from 2100. Participants were instructed to fast overnight (water could be consumed ad libitum) and report to the laboratory at 0700 the following morning.

Supplementation with Vitamin C-Enriched Collagen Hydrolysate

The supplement beverages were made by a laboratory technician (independent to this study), using opaque bottles to ensure that both the researchers and participants remained blinded. The supplement in each of the three interventions contained either 0, 15, or 30 g unflavored hydrolyzed collagen (HC) powder (Collagen Protein, MyProtein, Manchester, UK), together with 50 mg vitamin C powder (Holland and Barrett, Dublin, Ireland) and 400 mL water. To ensure the supplement in each of the three interventions remained isocaloric, 30.5 g and 15.3 g maltodextrin (MyProtein, Manchester, UK) were added to the 0 g and 15 g doses of HC, respectively. To match each supplement for taste, 3 g noncaloric sweetener (Pure Via 100% Xylitol, Merisant UK Ltd., Buckinghamshire, UK) was added to the 0 g dose, and 4 g was added to both the 15 g and 30 g HC doses.

Table 1. *Mean daily energy, macronutrient, and vitamin C intake for all participants (n = 8)*

Energy, kcal/day	2,378 ± 757
Carbohydrate, g/day	242 ± 48
Carbohydrate, g/kg/day	2.7 ± 0.5
Protein, g/day	153 ± 95
Protein, g/kg/day	1.7 ± 1.1
Fat, g/day	81 ± 40
Fat, g/kg/day	0.9 ± 0.4
Vitamin C, mg/day	127 ± 105

Values represent means ± SD.

Resistance Exercise Intervention and Blood Sampling

Upon arrival at the laboratory, participants rested for 15 min before an indwelling cannula (BD Nexiva, 22 G, BD Medical, Berkshire, UK) was inserted into an antecubital vein to allow for repeated blood sampling. A baseline blood sample was collected (−1 h) into 5 mL serum and plasma separating tubes (BD Medical, Berkshire, UK), and the participant was instructed to consume the entire contents of the supplement within 5 min of that blood sample being taken. After ingestion, participants rested for 1 h while additional blood samples were drawn at two more time points (−0.5 h and 0 h). Participants then completed a standardized warm-up, comprising dynamic stretching and two ascending warm-up sets at 50%–80% 10-RM on the leg press machine (Samson, New Mexico). The RE intervention comprised 4 sets of 10 repetitions at 90%–100% 10-RM, with each set separated by 2 min rest. Having completed the RE, participants were required to remain in a rested state in the laboratory for the subsequent 6 h while refraining from eating and drinking, apart from ingesting water ad libitum. Blood samples were drawn at eight time points over the 7-h period (−1 h, −0.5 h, 0 h, +0.5 h, +1 h, +2 h, +4 h, +6 h; Fig. 2). The cannula was flushed with 5 mL saline solution every 30 min to prevent clotting. Whole blood was collected in 5-mL serum-separating tubes (BD Medical, Berkshire, UK) at all time points and allowed to clot for at least 30 min. At time points −1 h, +0.5 h, + 2 h, and +6 h, additional whole blood samples were collected in 5-mL plasma-separating tubes (BD Medical, Berkshire, UK). Whole blood samples were centrifuged at 2,000 revolutions per minute (rpm) for 10 min, before being aliquoted into 1.5-mL Eppendorf tubes and stored at −70°C until subsequent analysis.

Blood Analyses

The methods for measuring markers of collagen synthesis and breakdown and concentrations of circulating collagen amino acids have been described in detail previously (23) and are therefore described here in brief. PINP analyses were performed at South East Technological University, whereas β-CTX and amino acid profile analyses were performed at the Bioanalytical Facility, University of East Anglia.

PINP and β-CTx

Serum samples at rest prior to HC ingestion, 0.5 h post RE, 1 h post RE, 2 h post RE, 4 h post RE, and 6 h post RE were used to measure serum PINP concentrations using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Cloud-Clone Corp, Wuhan, China). The intra-assay coefficient of variation (CV) was <10% and the interassay CV was <12%, with a detection range of 2.47–200 μg/L and sensitivity of <0.91 μg/L. The

ELISA absorbance readings were performed at 450 nm, using a VersaMax microplate reader (Molecular Devices Corporation, Sunnyvale, CA). The concentration × time total area under the curve (AUC) for PINP and amino acids (see below) were calculated using Prism software (v. 9.4.1, GraphPad Inc., San Diego, CA). EDTA plasma concentrations of β-CTx were measured using electrochemiluminescence immunoassay on a Cobas e601 analyzer (Roche Diagnostics, Germany). The interassay coefficient of variation (CV) for β-CTx was ≤ 3% between 0.2 and 1.5 μg/L with the sensitivity of 0.01 μg/L.

Amino Acid Profile by LC-MS/MS Analysis

Eighteen amino acids associated with collagen composition (glycine, proline, hydroxyproline, glutamic acid, alanine, arginine, aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, histidine, tyrosine, methionine, and glutamine) were measured simultaneously using anionic ion-pair reverse phase liquid chromatography tandem mass spectrometry system following derivatization of the amino acid with *n*-butanol hydrogen chloride. The assay range was 0–2,000 nmol/L for alanine, glutamine, glutamic acid, glycine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine, whereas for arginine, aspartic acid, histidine, hydroxyproline, isoleucine and methionine the assay range was 0–500 nmol/L. Interassay precision CV for all amino acids studied was between 3.3% and 10.3%.

Statistical Analysis

All statistical analyses were conducted while researchers were still blinded to participant information and supplement dose. Data were analyzed using GraphPad Prism, v. 9 (San Diego, CA) and are presented as means ± SD unless otherwise stated. Serum amino acid concentrations were analyzed using two-factor (time × supplement dose) repeated-measures ANOVAs. The same analyses were performed on total PINP concentrations over time, and Tukey's post hoc test was computed for post hoc comparisons. The concentration × time total area under the curve (AUC) was computed for serum PINP and for each amino acid and compared between interventions using a one-way repeated-measures ANOVA with Tukey's post hoc analyses performed for multiple pairwise comparisons.

RESULTS

Effects of Collagen Supplementation Combined with Resistance Exercise on Collagen Synthesis

Serum PINP concentration at baseline did not differ between interventions (0 g: 11.7 ± 9.3 μg/L; 15 g: 13.3 ± 7.3 μg/L; 30 g: 11.3 ± 8.7 μg/L; $F_{2,9} = 0.126$, $P = 0.882$). There was a main effect of time ($F_{7,49} = 6.51$, $P < 0.001$) and dose ($F_{2,14} = 18.3$, $P < 0.001$). There was also a dose × time interaction effect ($F_{14,98} = 3.54$, $P < 0.001$), as there were no changes across time in the 0 g HC intervention, but there were changes in serum PINP concentration during the 15 g and 30 g HC interventions. Serum PINP concentration was higher at all time points after RE during the 30 g HC intervention compared with the 0 g intervention (Fig. 3*A*, $P < 0.05$), whereas 15 g was higher than 0 g at + 0.5 h and 1 h only ($P < 0.05$). In both the 15 g and 30 g HC interventions, PINP concentrations peaked at 2 h after RE. Peak PINP values were 70% and 146% higher than 0 g at 2 h after exercise in the 15 g and 30 g HC interventions, respectively. Compared with 0 g HC, the PINP concentration × time total AUC (Fig. 3*B*) was 1.4 times greater in the 15 g HC intervention ($P = 0.024$) and 1.8 times greater in the 30 g HC intervention ($P < 0.001$). Finally, the AUC was 1.2-fold greater in the 30 g compared with the 15 g HC intervention ($P = 0.033$).

Effects of Collagen Supplementation Combined with Resistance Exercise on Collagen Breakdown

Plasma β-CTx concentration did not differ at baseline between the three interventions ($P < 0.0001$; Fig. 4). There was a main effect of time ($F_{1,8} = 16.4$, $P = 0.004$), i.e., during all three interventions, plasma β-CTx decreased immediately after RE and remained lower for the duration of the interventions. In addition, at 6 h after exercise (the final sample time point in each intervention), β-CTx concentration was greater than at + 0.5 h ($P < 0.001$) and + 2 h ($P < 0.001$), but lower than baseline ($P < 0.001$). There was no main effect of dose ($F_{2,13} = 0.911$, $P = 0.421$) and there was no dose × time interaction ($F_{3,24} = 0.675$, $P = 0.595$) on plasma β-CTx concentration. Finally, there was no difference in the β-CTx concentration × time total AUC between interventions ($F_{2,14} = 1.24$, $P = 0.320$; Fig. 4*B*).

Serum Amino Acid Profile

Figure 5 displays the serum concentrations of 18 amino acids that constitute type I collagen over the 7-h period after ingestion of 0, 15, or 30 g HC. There were main effects of time for glycine, proline, hydroxyproline, glutamic acid, alanine, arginine, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, tyrosine, and methionine ($P < 0.05$). There were main effects of dose for glycine, proline, hydroxyproline, alanine, arginine, lysine, serine, leucine, valine, and isoleucine, with 15 and 30 g HC displaying greater concentrations of these amino acids than 0 g HC ($P < 0.05$) and 30 g HC displaying greater concentrations than 15 g HC ($P < 0.05$). There were dose × time interactions for all but three amino acids (aspartic acid, histidine, and glutamine).

DISCUSSION

This is the first study to demonstrate that whole body collagen synthesis increases in response to resistance exercise (RE) in combination with the ingestion of vitamin C-enriched hydrolyzed collagen (HC) in resistance-trained, middle-aged men. Our data support our hypothesis, as the nutritional supplementation (both 15 and 30 g HC) was sufficient to increase serum PINP concentration, and the PINP concentration × time AUC was more than RE alone in a 6-h period after RE. Interestingly, the 30 g HC intervention led to a ~27% greater PINP total AUC compared with the 15 g HC intervention, which led to a ~39% greater total AUC than the 0 g HC intervention. This HC dose-response effect on whole body collagen synthesis was reflected by a similar HC dose-response effect on serum collagen amino acid availability. These data therefore show that high-intensity lower-limb RE alone is insufficient to stimulate collagen synthesis in

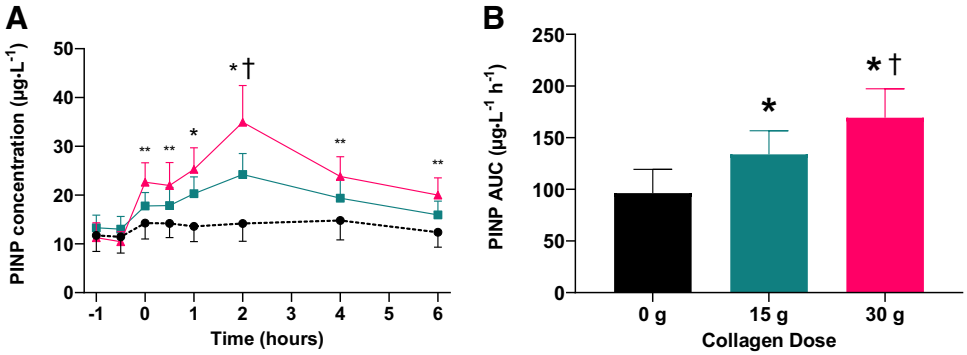

Figure 3. *A*: mean ± SE serum N-terminal propeptide of type 1 pro-collagen (PINP) concentration over time ($n = 8$). Supplementation was provided at exercise −1 h (0 g black circles, 15 g green squares, 30 g pink triangles). Resistance exercise was completed between 0 h and + 0.5 h; *15 g and 30 g greater than 0 g; †30 g greater than 15 g; **30 g, not 15 g, greater than 0 g. *B*: the serum PINP concentration × time total area under the curve (AUC) in response to resistance exercise combined with 0 g, 15 g, and 30 g hydrolyzed collagen supplementation ($n = 8$). Data are mean ± SE. *Greater than 0 g; †30 g greater than 15 g ($P < 0.05$).

resistance-trained, middle-aged men. However, supplementing RE in this population with either 15 or 30 g HC (enriched with vitamin C) rescues this apparent anabolic resistance to RE, probably by collagen amino acids stimulating collagen synthesis independently of RE.

In our study design, we chose to use high-intensity leg press RE to stimulate the quadriceps muscle-tendon unit (MTU), as this mode of RE is considered a primary stimulus to enhance the material and mechanical properties of human patellar tendon (3). There are no other studies, to our knowledge, that have described the collagen synthesis response to RE in middle-aged men. Surprisingly, we observed no increase in collagen synthesis following RE alone, which was unexpected, given various types of exercise, including running, skipping, and RE, have all been shown to increase collagen synthesis in young men and women independently of nutritional supplementation (23, 29–31). In the time since our study was initiated, 12 high-intensity sets of RE have been shown to increase the fractional synthetic rate (FSR) of muscle connective tissue protein in older men (32). Furthermore, the collagen FSR of older tendon has very recently been shown to increase after 4-wk moderate-intensity leg press RE using 12 weekly sets of leg press RE (25). Taken together, these findings imply that a greater volume (i.e., greater number of sets) may be required to maximize the serum PINP response in middle-aged men. However, Lee et al. (23) observed an increase in whole body collagen synthesis (also measured via serum PINP concentration) following RE alone in young men, using a very similar RE protocol to that used in our study. Thus, our data suggest a blunted collagen synthesis response to RE alone in middle-aged men for a given volume and relative intensity of RE. Nevertheless, the addition of an HC supplement (containing at least 15 g HC) appears to "rescue" this blunted collagen synthesis to RE in this population.

There is a parallel body of research describing the interaction between RE, dietary protein supplementation, and muscle protein synthesis (MPS) responses (33). The term "anabolic resistance" describes how older skeletal muscle is not as responsive as young muscle to RE (34). The provision of additional whey protein can assist in mitigating this issue and recovering MPS rates after RE in older tissues, but the dose required can be double that required to maximize MPS in young (26). At least 15 g HC supplementation rescued the collagen synthesis response in our study, and the advantage observed during the 30 g intervention may be indicative of a need for higher doses as age increases. This is likely given that in young men, collagen synthesis increased after RE alone and increased further with ingestion of 30 g HC but with no benefit of a 15-g dose (23). It is interesting that just 15 g HC was sufficient to augment collagen synthesis in middle-aged men, which suggests that the collagen synthesis response may already be close to maximized by RE alone in younger populations. Furthermore, aging is associated with

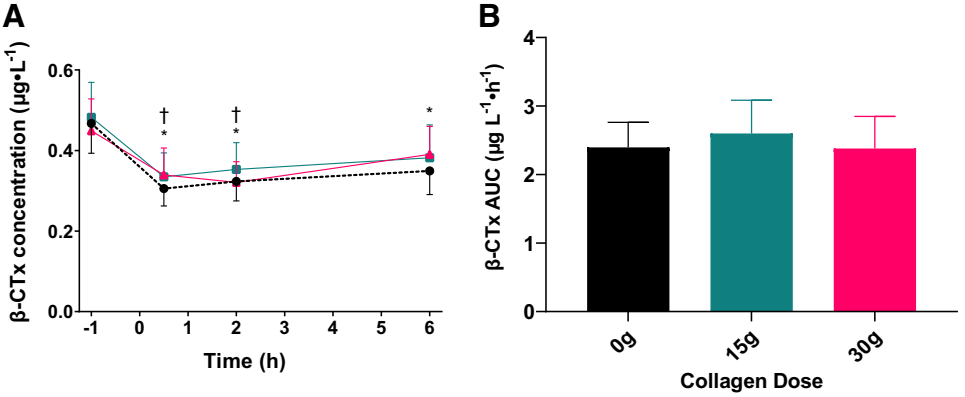

Figure 4. *A*: mean ± SE plasma β-isomerized C-terminal telopeptide of type 1 collagen (β-CTx) concentration over time ($n = 8$). Supplementation was provided at exercise −1 h (0 g black circles, 15 g green squares, 30 g pink triangles). Resistance exercise was completed between 0 h and + 0.5 h. *Lower than −1 h; †lower than −1 h and + 6 h ($P < 0.05$). *B*: plasma β-CTx concentration × time total area under the curve (AUC) in response to resistance exercise combined with 0 g, 15 g, or 30 g hydrolyzed collagen supplementation ($n = 8$). Data are mean ± SE.

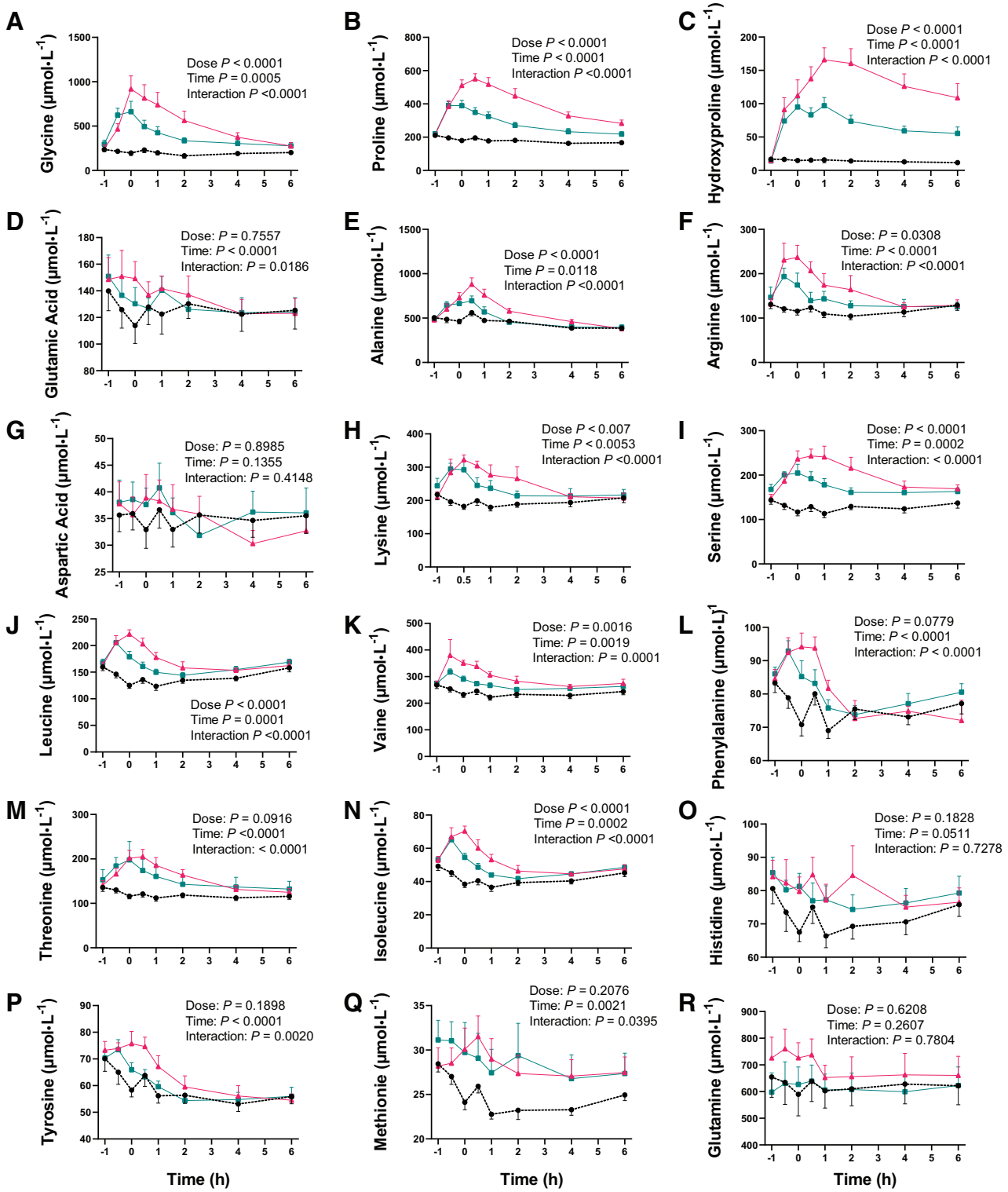

Figure 5. Mean ± SE serum concentration of 18 amino acids (glycine, *A*; proline, *B*; hydroxyproline, *C*; glutamic acid, *D*; alanine, *E*; arginine, *F*; aspartic acid, *G*; lysine, *H*; serine, *I*; leucine, *J*; valine, *K*; phenylalanine, *L*; threonine, *M*; isoleucine, *N*; histidine, *O*; tyrosine, *P*; methionine, *Q*; and glutamine, *R*) across time after ingestion of 0 g (black circles), 15 g (green squares), and 30 g (pink triangles) of hydrolyzed collagen. Hydrolyzed collagen ingestion took place at −1 h, and resistance exercise began at 0 h.

reduced collagen synthesis (at rest) in men (35), which may help explain the importance of exogenous HC supplementation, even in relatively small amounts, to augment collagen synthesis in middle-aged men following RE. It is noteworthy, however, that peak serum PINP concentration (which occurred after RE) and total PINP AUC were lower in middle-aged men and more comparable with the preexercise levels observed in young men (23), further suggesting a blunted

response of older connective tissue to HC ingestion and RE. It remains to be seen whether ingestion of >30 g HC would further recover the post-RE collagen synthesis response in middle-aged men.

Compared with fasted levels in the current study, there was a 2- to 2.9-fold increase in peak PINP concentration following RE with HC ingestion. In addition, we have shown that collagen feeding alone increases collagen synthesis, albeit for a short duration and to a much lesser extent than during the post-RE period. The increases in serum PINP concentration in response to both exogenous HC supplementation and RE correspond to the dose-response elevation (30 > 15 > 0 g) in serum amino acid concentration (Fig. 5). The key amino acids found in collagen (e.g., glycine, proline, and hydroxyproline) peaked in our serum samples between 1 and 2 h after ingestion of the supplement (Fig. 5), which is in agreement with previous studies in young healthy men and women (23, 29, 36–38). There are several mechanisms by which exogenous HC ingestion may have promoted collagen synthesis. First, although glycine is a nonessential amino acid, the amount synthesized per day in humans may be insufficient for optimal collagen synthesis (39) and the abundance of dietary glycine supplied by collagen supplements (and confirmed by our amino acid profiles) may make up for this shortcoming. Second, in a bovine model, 10 g/day of dietary glycine increased collagen synthesis in articular chondrocytes, which are specialized cells in the ECM of cartilage (40), suggesting a collagen synthesis-stimulating role of glycine. In addition, proline and hydroxyproline have been shown in vitro to regulate transforming growth factor-β (TGF-β) in fibroblasts, leading to downstream phosphorylation of protein kinase B (Akt) and mammalian target of rapamycin complex I (mTORCI), the key regulatory pathway leading to collagen protein synthesis (41). Due to the greater postexercise responses in our data (compared with the preexercise postprandial period), it is conceivable that the increased levels of collagen synthesis in the 15 g and 30 g HC interventions were the results of cumulative signaling from both the RE-induced mechanical loading (19) and amino acid-induced stimulation of growth factors (41) within the ECM of target connective tissue (e.g., in the MTU).

We measured plasma β-CTx concentration as a marker of whole body collagen breakdown and observed a sustained decrease after supplement ingestion (regardless of HC dose) and RE, suggesting a positive net collagen turnover following RE with or without HC ingestion. In a recent study, Aussieker et al. (37) found that ingestion of both 30 g whey protein and 30 g HC with RE appeared to inhibit whole body collagen breakdown (i.e., serum CTX-I concentration decreased) 2 h after RE in young adults. However, this was not the case when water alone was ingested with RE, where serum CTX-I concentration remained unaltered throughout the intervention. This may suggest a positive effect of protein ingestion (regardless of protein type) on whole body collagen breakdown were it not for the fact that plasma β-CTx concentration decreased in our 0 g HC intervention. In our study, we calorie-matched both the 0 g and 15 g HC beverages to the energy of the 30 g HC supplement; thus, all of our RE interventions took place with the same energy availability. It is likely, therefore, that the inhibited collagen breakdown observed in young adults (37) and in our study of middle-aged men was the result of calorie intake, regardless of macronutrient composition, which is in line with previous work by Henriksen et al. (42). Furthermore, given that plasma β-CTx concentration decreased 90 min after supplement ingestion (time: 0830) in our study and remained lower than fasted concentrations (time: 0700) for the remainder of all three interventions (Fig. 4), it is unlikely that the decrease we observed in plasma β-CTx concentration was associated with diurnal variation, as previously suggested (43).

It has previously been shown that procollagen type I C-terminal propeptide concentration (another marker of collagen synthesis) and Achilles tendon collagen content (estimated from echo intensity) simultaneously increased after 2 mo of resistance training in healthy young men, which corresponded to an increase in Achilles tendon stiffness (44). This suggests that enhanced tendon collagen synthesis and content are required to improve the mechanical properties of the MTU in response to progressive overload. Thus, given our findings, we can speculate that repeated stimulation (through RE and 30 g HC supplementation) would result in greater increases in tendon size and stiffness in middle-aged men over a prolonged period. Emerging data in young men (45, 46) and young women (47, 48) suggest positive outcomes in certain morphological and mechanical tendon properties when HC is ingested in combination with resistance training in young populations. However, it is not yet known whether similar outcomes would be found in middle-aged men and women. One previous study, however, did show that daily supplementation with 10 g collagen for 12 wk did not increase serum PINP concentration in middle-aged men (49). However, it is unclear whether increasing the dose of collagen and/or supplementing in combination with RE would alter this response.

Limitations

Human tendon (30) and serum (22) PINP concentration are known to increase after an acute bout of exercise, and serum PINP remains elevated for up to four days after RE in humans (50), which is in accordance with the elevated collagen fractional synthetic rate in skeletal muscle and tendon following RE (51). Thus, although we did not measure collagen synthesis directly within the MTU, measuring a biomarker of collagen synthesis in the form of PINP, which is cleaved off during the maturation of procollagen to collagen (52), is a reliable alternative. Finally, this study included solely male participants and, considering the potential effects of estrogen on collagen synthesis (53), it is not known whether we would have seen different results in resistance-trained, middle-aged, pre- or postmenopausal women.

Conclusions

Our data show that the combination of high-intensity, lower-limb resistance exercise and hydrolyzed collagen supplementation increases collagen synthesis in middle-aged, resistance-trained men in a dose-response manner, i.e., 30 g > 15 g > 0 g. Crucially, this important finding was reflected in a collagen dose-response effect on the bioavailability of collagen amino acids known to independently stimulate collagen synthesis. Given the lack of response to resistance exercise

alone, our findings suggest the sensitivity of collagen rich tissues, such as tendon and ligament, to resistance exercise may be reduced in middle age, which can be recovered with the ingestion of hydrolyzed collagen.

IRB STATEMENT

The study was registered at https://clinicaltrials.gov/ (identifier: NCT06236659), was approved by Liverpool John Moores University Research Ethics Committee (Approval Number: 19SPS049) and complied with the Declaration of Helsinki. All participants provided written informed consent prior to study commencement.

DATA AVAILABILITY

Data described in the article, code book, and analytic code will be made available upon request from the corresponding author pending application and approval.

SUPPLEMENTAL MATERIAL

Supplemental Table S1 DOI. https://doi.org/10.6084/m9.figshare.26947501.

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DISCLAIMERS

The content is solely the authors' responsibility and does not necessarily represent the official views of the authors' affiliations.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

C.D.N. and R.M.E. conceived and designed research; C.D.N. performed experiments; C.D.N., J.C.Y.T., J.D., R.D., and W.D.F. analyzed data; C.D.N. and R.M.E. interpreted results of experiments; C.D.N. prepared figures; C.D.N. drafted manuscript; C.D.N. and R.M.E. edited and revised manuscript; C.D.N., J.C.Y.T., J.D., R.D., W.D.F., K.E., C.E.S., and R.M.E. approved final version of manuscript.

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