Human Kinetics

ORIGINAL RESEARCH

Coingestion of Collagen With Whey Protein Prevents Postexercise Decline in Plasma Glycine Availability in Recreationally Active Men

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Whey protein ingestion during recovery from exercise increases myofibrillar but not muscle connective protein synthesis rates. It has been speculated that whey protein does not provide sufficient glycine to maximize postexercise muscle connective protein synthesis rates. In the present study, we assessed the impact of coingesting different amounts of collagen with whey protein as a nutritional strategy to increase plasma glycine availability during recovery from exercise. In a randomized, double-blind, crossover design, 14 recreationally active men (age: 26 ± 5 years; body mass index: 23.8 ± 2.1 kg·m^{-2}) ingested in total 30 g protein, provided as whey protein with 0 g (WHEY), 5 g (WC05); 10 g (WC10), and 15 g (WC15) of collagen protein immediately after a single bout of resistance exercise. Blood samples were collected frequently over 6 hr of postexercise recovery to assess postprandial plasma amino acid kinetics and availability. Protein ingestion strongly increased plasma amino acid concentrations ($p < .001$) with no differences in plasma total amino acid availability between treatments ($p > .05$). The postprandial rise in plasma leucine and essential amino acid availability was greater in WHEY compared with the WC10 and WC15 treatments ($p < .05$). Plasma glycine and nonessential amino acid concentrations declined following whey protein ingestion but increased following collagen coingestion ($p < .05$). Postprandial plasma glycine availability averaged -8.9 ± 5.8, 9.2 ± 3.7, 23.1 ± 6.5, and 39.8 ± 11.0 mmol·360 min/L in WHEY, WC05, WC10, and WC15, respectively (incremental area under curve values, $p < .05$). Coingestion of a small amount of collagen (5 g) with whey protein (25 g) is sufficient to prevent the decline in plasma glycine availability during recovery from lower body resistance-type exercise in recreationally active men.

Keywords: protein blend, connective tissue, resistance exercise, protein metabolism

Exercise increases muscle protein synthesis rates (Biolo et al., 1995). This includes substantial increases in both myofibrillar (Wilkinson et al., 2008) and muscle connective protein (Holm et al., 2010; Holwerda et al., 2021; Trommelen et al., 2020) synthesis rates. Whereas the impact of exercise on myofibrillar protein synthesis lies in conditioning the contractile protein network, the impact on muscle connective protein synthesis lies in improving the connective tissue network responsible for transferring forces generated by the contractile apparatus (Huijing, 1999).

Protein ingestion during recovery from exercise further increases postexercise muscle protein synthesis rates (Moore et al., 2009; Witard et al., 2014) and can be applied as a nutritional strategy to further augment gains in muscle mass and strength following prolonged resistance exercise training (Cermak et al., 2012). Ingestion of 20–25 g whey protein has been shown to strongly augment myofibrillar protein synthesis rates during recovery from exercise (Witard et al., 2014; Yang et al., 2012). In contrast, muscle collagen synthesis rates do not seem to be nutritionally sensitive at rest (Babraj et al., 2005; Mittendorfer et al., 2005). Furthermore, ingestion of whey or milk protein does not seem to affect postexercise muscle connective protein synthesis rates (Dideriksen et al., 2011; Holwerda et al., 2021; Trommelen et al., 2020). The proposed inability of dairy protein ingestion to further augment postexercise muscle connective protein synthesis rates may be attributed to the type of protein ingested. Prior work from our laboratory (Aussieker et al., 2023; Holwerda et al., 2017) has shown that dairy protein ingestion during recovery from exercise is accompanied by a decline in circulating glycine concentrations despite the substantial rise in circulating essential amino acids (EAA). As connective protein and its main constituent, collagen, are rich in glycine and proline (Eastoe, 1955), it has been speculated that dairy protein ingestion may provide insufficient glycine as a precursor to support a further rise in postexercise muscle connective protein synthesis rates (Alcock et al., 2019; Skov et al., 2019).

Collagen protein has a high glycine and proline content and, as such, has been advocated as a protein source that could stimulate connective tissue, skin, and bone protein synthesis rates (Alcock et al., 2019; Shaw et al., 2017; Skov et al., 2019). However, we (Aussieker et al., 2023) as well as others (Oikawa et al., 2018, 2020) have not been able to show a stimulating effect of collagen

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protein ingestion on myofibrillar or muscle connective protein synthesis rates during recovery from exercise. The latter is not surprising as collagen protein has been suggested to be a low-quality protein due to a lower EAA and leucine content (Alcock et al., 2019). It has been hypothesized that a protein blend containing both whey and collagen protein would be preferred to stimulate both myofibrillar and muscle connective protein synthesis rates by providing ample EAA, and leucine in particular, while preventing a decline in circulating plasma glycine availability.

In the present study, we recruited 15 recreationally active men to assess the impact of coingesting three different doses of collagen protein with whey protein on postprandial plasma amino acid availability during recovery from an acute bout of resistance exercise. This study determines the dosage of collagen required to prevent a postexercise decline in plasma glycine availability when coingested with whey protein.

Methods

Participants

A total of 15 healthy, young men (age: 26 ± 5 years; body mass index: 23.8 ± 2.1 kg/m^2) volunteered to participate in this cross-over, double-blind, randomized controlled trial. Participants' characteristics are presented in Table 1. One subject dropped out because of scheduling conflicts, resulting in 14 participants who completed all trials. After pretesting, participants completed four test days in a randomized order during which they consumed a study beverage with either 30 g of protein in the form of only whey protein (WHEY) or as a blend of whey and collagen protein with different ratios: 25 g whey and 5 g collagen (WC05); 20 g whey and 10 g collagen (WC10); or 15 g whey and 15 g collagen (WC15). 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 and conformed to the principles outlined in the declaration of Helsinki for use of human participants and tissue. The trial was registered at the Netherlands Trial Register (NL8748) and was conducted between February 2021 and May 2021 at Maastricht University Medical Centre+, Maastricht, The Netherlands. Clinical Trial Center Maastricht independently monitored the study.

Pretesting

Participants aged 18–35 years with a body mass index >18.5 and <30.0 kg/m^2 underwent an initial screening session to assess height, body weight, and body composition via dual energy X-ray absorptiometry (Hologic, Discovery A; QDR Series). The system's software package Apex (version 4.0.2, Hologic) was used to determine whole-body lean mass, fat mass, and bone mineral content. Afterward, the one-repetition maximum (1RM) of the leg press and extension exercises (Technogym) was assessed. 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, being intolerant to the investigated proteins, participating in recreational sports activities greater than three times per week, or being completely sedentary. The pretesting and experimental trials were separated by at least 5 days. There were at least 5 days between subsequent test days.

Diet and Physical Activity

All participants refrained from strenuous physical activity and alcohol consumption and completed a food intake and physical activity record for 2 days prior to the experimental trial. The questionnaires were completed prior to the first experimental test day. In preparation for the subsequent experimental test days, participants were asked to repeat the food intake and physical activity level as noted in the record prior to test Day 1. 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 21.00 hr on the evening before each experimental test day. This prepackaged standardized meal provided 1.71 MJ, with 55% of the energy from carbohydrate, 30% energy from fat, and 15% energy from protein. With the meal, participants drank 200 ml of orange juice from concentrate, providing 0.38 MJ. Thereafter, participants remained fasted until the experimental test days.

Study Design

All participants performed four identical test days with only the study beverage being different. Participants performed a single bout of resistance exercise and immediately after completion, ingested a randomly assigned beverage (500 ml) containing either WHEY, WC05, WC10, or WC15. Whey protein isolate (Volactive Ultra Whey 90, Volac International Limited) and collagen protein hydrolysate (Bodybalance B, Gelita AG) were used as protein supplements. All beverages were flavored with vanilla flavoring (1 ml, Dr. Oetker). Amino acid profiles of the protein beverages are shown in Table 2. Upon inclusion in the study, each subject was assigned a unique randomization code (1–15). These codes were linked to the specific randomized order in which the treatments were provided. This treatment order was determined by sealed envelopes containing all possible treatment orders. The test supplements were provided in a double-blind fashion. An independent person was responsible for random assignment and preparation of the study treatment beverages, which were sequentially numbered according to subject number and the test day number. The beverages were prepared in nontransparent plastic containers and had a similar taste and smell.

Table 1   Baseline Participants' Characteristics and Average 2-Day Dietary Intake Prior to the Experimental Test Days

Age, years	26 ± 5
Height, m	1.84 ± 0.06
Weight, kg	80.9 ± 8.7
BMI, kg/m^2	23.8 ± 2.1
Lean body mass, kg	62.0 ± 7.5
Body fat, %	20.4 ± 3.9
1RM leg press, kg	228 ± 48
1RM leg extension, kg	130 ± 19
Energy, MJ/day	9.75 ± 1.69
Carbohydrate, g/day	270 ± 42
Fat, g/day	86 ± 22
Protein, g/day	103 ± 35
Protein, g·kg^{-1}·day^{-1}	1.27 ± 0.37

Note. Values represent mean ± *SD*, *n* = 14. BMI = body mass index; 1RM = one-repetition maximum.

Table 2 Amino Acid Profiles of Protein Blends (30 g of Protein)

Amino acids (g)	WHEY	WC05	WC10	WC15
Alanine	1.50	1.68	1.86	2.04
Arginine	0.63	0.89	1.15	1.41
Aspartic acid	3.30	3.04	2.78	2.52
Cysteine	0.66	0.55	0.44	0.33
Glutamic acid	5.43	5.04	4.64	4.25
Glycine	0.42	1.46	2.50	3.54
Histidine	0.51	0.48	0.44	0.41
Hydroxylysine	0.00	0.08	0.16	0.24
Hydroxyproline	0.00	0.60	1.19	1.79
Isoleucine	1.92	1.67	1.42	1.17
Leucine	3.18	2.79	2.39	2.00
Lysine	2.88	2.58	2.28	1.98
Methionine	0.66	0.60	0.53	0.47
Phenylalanine	0.90	0.86	0.81	0.77
Proline	1.65	2.01	2.37	2.73
Serine	1.38	1.31	1.24	1.17
Threonine	2.01	1.77	1.52	1.28
Tryptophan	0.42	0.35	0.28	0.21
Tyrosine	0.78	0.69	0.60	0.51
Valine	1.77	1.60	1.42	1.25
$\sum$NEAA	15.75	17.34	18.93	20.52
$\sum$EAA	14.25	12.67	11.09	9.51
$\sum$AA	30.00	30.00	30.00	30.00

Note. The amino acid content was extracted from the certificates of analysis of both the whey and collagen protein. WHEY = 30 g whey protein; WC05 = 25 g whey plus 5 g collagen; WC10 = 20 g whey plus 10 g collagen; WC15 = 15 g whey plus 15 g collagen; $\sum$NEAA = sum of total nonessential amino acids; $\sum$EAA = sum of total essential amino acids; $\sum$AA = sum of total amino acids.

Experimental Protocol

At ~07:45 hr, participants arrived at the laboratory in the overnight fasted state. A catheter was inserted into a dorsal hand vein for arterialized venous blood sampling. To obtain arterialized blood samples, the hand was placed in a hot box (60 °C) for 10 min prior to blood sample collection. After taking a baseline blood sample ($t = -45$ min), participants initiated the resistance exercise intervention (described later). Immediately after cessation of the exercise intervention ($t = 0$ min), an arterialized blood sample was obtained. Subsequently, participants received a 500-ml beverage corresponding to their randomly assigned treatment allocation. During the postprandial period, participants were only allowed to consume water. Further arterialized blood samples were then collected at $t = 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300,$ and 360 min in the postprandial period. Blood samples were collected into ethylenediamine tetraacetic acid–containing tubes and centrifuged at $1,000g$ for 15 min at 4 °C. Aliquots of plasma were frozen in liquid nitrogen and stored at −80 °C. When the experimental protocol was complete, the cannula was removed, and participants ate and were assessed for ~30 min before leaving the laboratory.

Resistance Exercise Session

All participants followed the same exercise protocol on all four test days, which consisted of a 5-min warm-up on a cycle ergometer followed by the leg press and leg extension exercise. The first set for both exercises was a warm-up set of 10 repetitions at 40% 1RM. The next three sets consisted of eight 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 of the leg press exercise were finished, participants continued with the leg extension exercise.

Plasma Analyses

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 ultraperformance liquid chromatograph mass spectrometry (ACQUITY UPLC H-Class with QDa; Waters). Fifty microliters of blood plasma was deproteinized using 100 µl of 10% sulfosalicylic acid with 50 µM of MSK-A2 internal standard (Cambridge Isotope Laboratories). Subsequently, 50 µl of ultrapure demineralized water was added, and samples were centrifuged (15 min at 14,000 RPM). After centrifugation, 10 µl of supernatant was added to 70 µl of Borate reaction buffer (Waters). 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 ultraperformance liquid chromatograph mass spectrometry.

Statistical Analysis

All data are expressed as mean ± *SD*. Time-dependent variables (i.e., plasma glucose, insulin, and amino acid concentrations) were analyzed by a two-factor repeated-measures analysis of variance with time as a within-participants factor and treatment group as a between-participants factor. The analysis was carried out for the period right before protein or placebo ingestion ($t = 0$ min) until the end of the experimental trial ($t = 360$ min). Values of $t = -45$ were not statistically different from $t = 0$; therefore, only the latter are reported. In case of a significant interaction effect, individual time points were analyzed using a one-way analysis of variance with the time points as the dependent variable and treatment as the independent variable. Trapezoidal rule adjusted to baseline concentrations ($t = 0$ min) was applied to calculate the incremental area under curve (iAUC) of the amino acid concentrations. Nontime-dependent variables (i.e., iAUC) were compared between treatment groups using a one-way analysis of variance. Statistical significance was set at $p < .05$. Bonferroni-corrected post hoc comparisons were performed where appropriate. All calculations were performed using SPSS (version 25.0).

Results

Plasma Glucose and Insulin Concentrations

Plasma glucose concentrations decreased after protein ingestion (main effect of time: $p < .001$; Figure 1A). Plasma insulin concentration increased after ingestion of the protein beverages (time effect: $p < .001$; Figure 1B). No group differences were detected for plasma glucose and insulin concentrations (main effect of treatment: $p > .05$).

Plasma Amino Acid Availability

Results for all measured amino acids are visualized in a heat map showing the fold change in plasma amino acid concentration
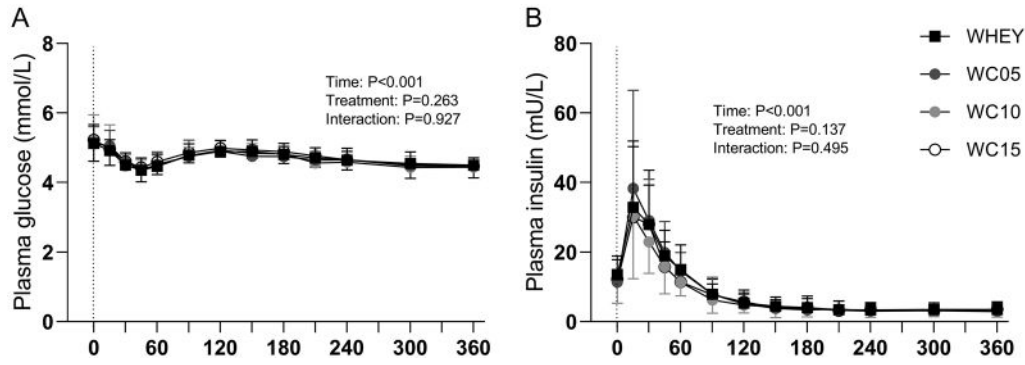
Figure 1 — Plasma glucose (A) and insulin (B) concentrations following protein ingestion during recovery from a single bout of resistance exercise (t = 0–360 min). The dotted line represents the ingestion of the protein. Values represent mean ± *SD*, n = 14. Data were analyzed by two-factor repeated-measures analysis of variance. Bonferroni post hoc testing was used to detect differences between groups. WHEY = 30 g whey protein; WC05 = 25 g whey plus 5 g collagen protein; WC10 = 20 g whey plus 10 g collagen protein; WC15 = 15 g whey plus 15 g collagen protein.

following test drink ingestion when compared with baseline (t = 0 min set to value 1; Figure 2).

Plasma amino acid concentrations and 6-hr postprandial plasma amino acid availability are shown in Figures 3 and 4. Following protein ingestion, plasma total amino acid (TAA) concentrations increased ($p < .001$; Figure 3A). No differences in plasma TAA iAUC were observed between the groups ($p > .05$; Figure 3B).

Plasma EAA and leucine concentrations increased after protein ingestion (main effect of time: $p < .001$). Significant *time × treatment group interactions* were observed for plasma EAA and leucine ($p < .001$), with group differences at time points t = 30 min until t = 180 min and t = 30 min until t = 240 min, respectively (all $p < .05$, Figures 3C and 4A). Maximum plasma EAA and leucine concentrations were higher in WHEY compared with WC10 and WC15, with WC05 showing significantly higher values than WC15 (all $p < .05$), whereas no difference between WHEY and WC05 was detected ($p > .05$). The time to reach peak plasma concentrations did not differ for EAA and leucine between groups ($p > .05$). The plasma EAA and leucine iAUC showed the highest values for WHEY and the lowest values for WC15 ($p < .05$, Figures 3D and 4B).

Plasma nonessential amino acids (NEAA), glycine, and proline concentrations increased after protein ingestion (main effect of time: $p < .001$). Plasma hydroxyproline concentrations increased in all groups except following WHEY (time effect: $p < .001$). Significant *time × treatment group interactions* were observed for plasma NEAA, glycine, proline, and hydroxyproline concentrations ($p < .001$), with group differences at time points t = 30 until t = 360 min, t = 15 until t = 360 min, t = 30 until t = 120 min, and t = 15 until t = 360 min, respectively ($p < .05$, Figures 3E, 4C, 4E, and 4G). Peak plasma NEAA concentrations were higher in WC15 compared with WHEY, WC05, and WC10 ($p < .05$). Peak plasma proline concentrations were higher in WC15 compared with WHEY ($p < .05$). For glycine and hydroxyproline, a clear dose–response relationship of peak plasma concentration was detected, with WC15 showing the highest values and WHEY showing the lowest values ($p < .001$). The time to reach the peak plasma concentrations was not different for NEAA, glycine, proline, and hydroxyproline ($p > .05$). The plasma NEAA, glycine, proline, and hydroxyproline iAUC showed the highest values for WC15 and the lowest values for WHEY, with values for WC05 and WC10 being intermediate ($p < .05$, Figures 3F and 4D–4H). The iAUC of glycine resulted in negative values in WHEY (Figure 4D).

Discussion

In the present study, we observed that differences in postprandial plasma amino acid concentrations correspond well with the amino acid composition of different whey plus collagen protein blends. Ingestion of 30 g whey protein immediately after exercise resulted in a decline in postprandial plasma glycine availability during the acute recovery period. Coingesting 5 g collagen with 25 g whey protein was sufficient to prevent the decline in plasma glycine availability and allowed maintenance of circulating plasma glycine concentrations above baseline values during recovery from a single bout of resistance exercise.

Protein ingestion during recovery from exercise is a common strategy to support skeletal muscle reconditioning by providing amino acids as precursors for de novo muscle protein synthesis (Pennings et al., 2011; Trommelen et al., 2016). In the present study, we showed a substantial increase in circulating plasma amino acid concentrations following ingestion of 30 g protein (Figure 2). Ingestion of the different protein blends resulted in distinct postprandial plasma amino acid responses. As reported previously (Alcock et al., 2019; Gorissen et al., 2016), postprandial plasma amino acid concentrations highly depend on the amino acid profile of the protein(s) provided. In the present study, we assessed the postprandial plasma amino acid profile following ingestion of, in total, 30 g protein provided as whey protein with 0 g (WHEY), 5 g (WC05), 10 g (WC10), and 15 g (WC15) of collagen protein immediately after a single bout of resistance exercise. Ingestion of 30 g whey protein (WHEY) resulted in a rapid increase in circulating EAA and NEAA concentrations, with high plasma leucine concentrations in particular (Figures 2–4). These data are in line with previous work from our group (Aussieker et al., 2023; Gorissen et al., 2016) as well as others (Tang et al., 2009; Yang et al., 2012) and show the impact of ingesting a high-quality protein source, such as whey, on postprandial plasma amino acid concentrations during recovery from exercise. It has been well established that the postprandial increase in plasma EAA concentrations, and plasma leucine in particular, following ingestion of 20–30 g whey protein strongly increases myofibrillar protein synthesis rates during recovery from exercise (Churchward-Venne et al., 2013; Witard et al., 2014; Yang et al., 2012). However, whey protein ingestion during recovery from exercise does not seem to augment muscle connective protein synthesis rates (Aussieker et al., 2023; Mikkelsen et al., 2015). We (Aussieker et al., 2023) and others (Alcock et al., 2019; Shaw et al., 2017) have speculated that this
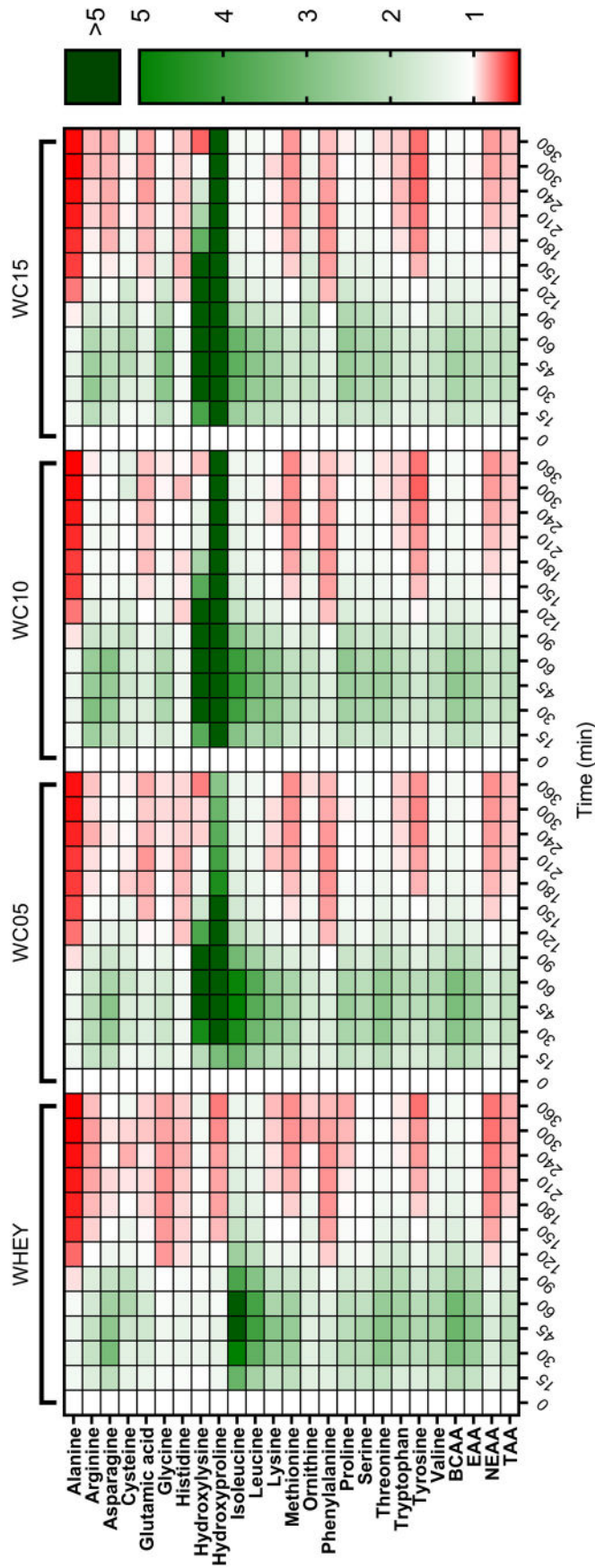
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Figure 2 — Heat map of fold changes in plasma amino acid concentrations during the experimental test days after the protein blend ingestion during recovery from a single bout of resistance exercise. For hydroxyproline and hydroxylysine, values under the detection limit were set to 0. Values of $t = 0$ were set to 1. WHEY = 0 g whey protein; WC05 = 25 g whey protein; WC10 = 20 g whey plus 10 g collagen protein; WC15 = 15 g whey plus 15 g collagen protein, $n = 14$; TAA = total amino acids; EAA = essential amino acids; BCAA = branched-chain amino acids; NEAA = nonessential amino acids.
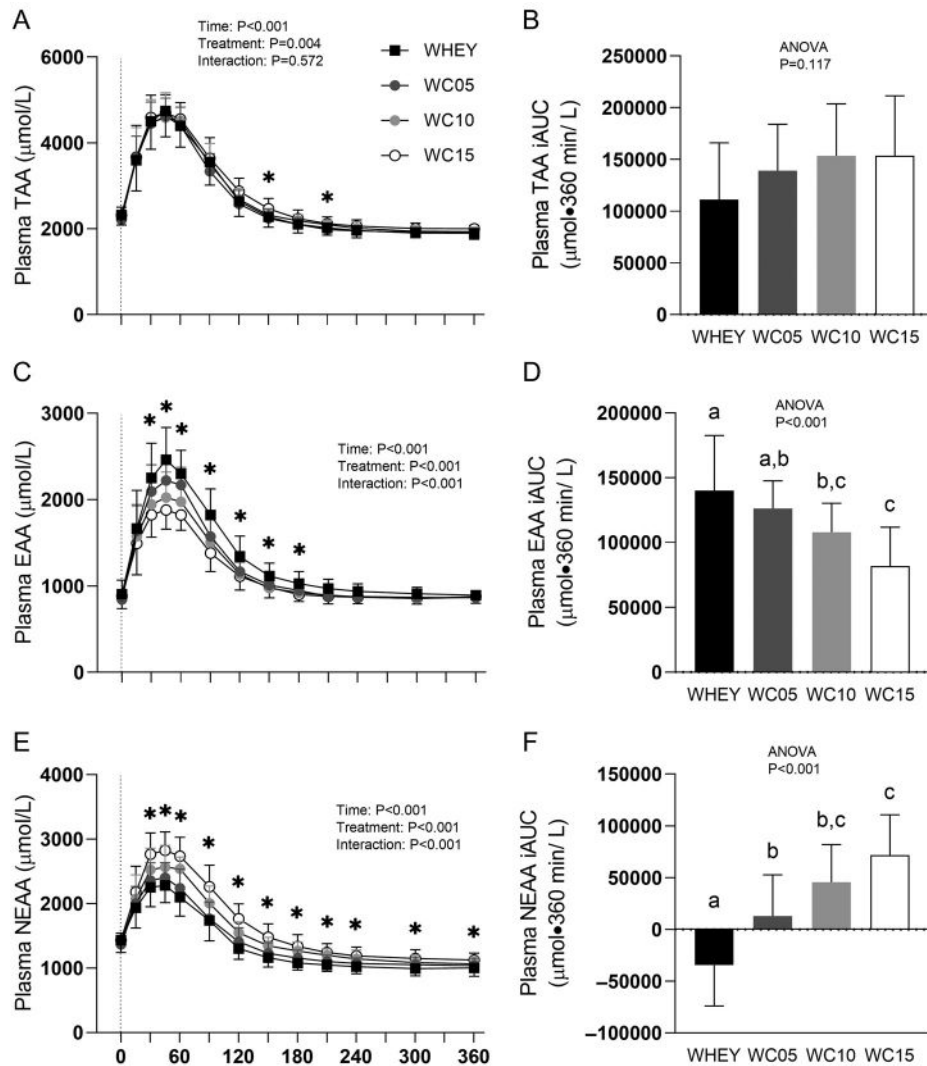
Figure 3 — Plasma amino acid concentrations following protein ingestion during recovery from a single bout of resistance exercise ($t = 0$–360 min) in the left column and iAUC for the postprandial period in the right column. Data are displayed for TAA (A, B), EAA (C, D), and NEAA (E, F). The dotted line within the left column graphs represents the ingestion of the test drink. Values represent means ± SD, $n = 14$. Data for plasma amino acid concentrations were analyzed by two-factor repeated-measures analysis of variance. Data for iAUC were analyzed by a one-way analysis of variance. Bonferroni post hoc testing was used to detect differences between groups. *Significant group difference within the time point, $p < .05$. Treatments without a common letter differ, $p < .05$. WHEY = 30 g whey protein; WC05 = 25 g whey plus 5 g collagen protein; WC10 = 20 g whey plus 10 g collagen protein; WC15 = 15 g whey plus 15 g collagen protein; TAA = total amino acids; EAA = essential amino acids; NEAA = nonessential amino acids; iAUC = incremental area under the curve.

could be attributed to the lack of sufficient glycine and proline in dairy protein to provide sufficient amino acid precursors to support the postexercise increase in muscle connective protein synthesis rates. Consequently, it has been proposed that the ingestion of a blend of high-quality whey protein with collagen protein may be a preferred nutritional strategy to provide ample EAA without compromising glycine and/or proline availability. Here, we addressed the impact of coingesting three different doses of collagen protein on postprandial plasma amino acid availability during recovery from exercise (Figures 2–4).

Coingestion of collagen was followed by rapid collagen digestion and amino acid absorption, as evident from the rapid postprandial rise in circulating hydroxyproline (Figure 4G). Postprandial plasma hydroxyproline concentrations showed a strong dose-dependent increase following the ingestion of the three different doses of collagen (Figure 4H). As more collagen was

included in the 30 g protein blend, postprandial increases in total EAA concentrations were attenuated, and plasma NEAA levels were further increased (Figures 3C–3F). Such differences may compromise the anabolic properties of the protein blend as the postprandial rise in plasma (essential) amino acid availability can modulate the anabolic potential of a protein or protein blend (van Vliet et al., 2015; World Health Organization, 2007). Despite WHEY containing 11.7% more EAA than WC05, the plasma EAA availabilities over the entire postprandial period did not significantly differ from each other. The protein blends containing 10 and 15 g collagen provided ~25% and 40% less EAA than WHEY, resulting in significantly lower plasma EAA responses (Figures 3C and 3D).

Leucine has been identified as the key regulator in promoting myofibrillar protein synthesis rates (Churchward-Venne et al., 2012, 2013). A leucine trigger hypothesis has been proposed,
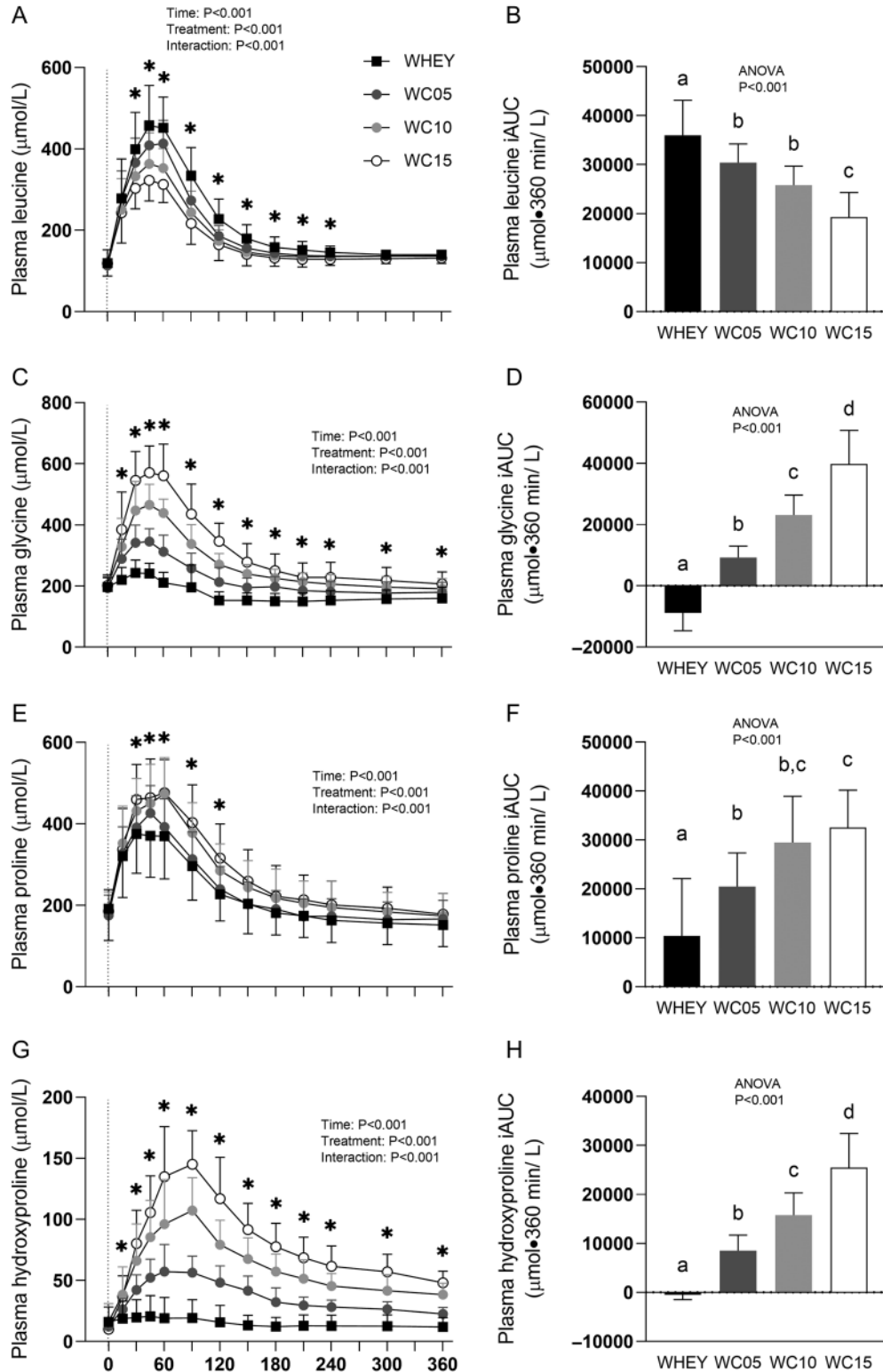
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Figure 4 — Plasma leucine, glycine, proline, and hydroxyproline concentrations following protein ingestion during recovery from a single bout of resistance exercise ($t = 0$–360 min) in the left column and iAUC for the postprandial period in the right column in young men and women. Data are displayed for leucine (A, B), glycine (C, D), proline (E, F), and hydroxyproline (G, H). The dotted line within the left column graphs represents the ingestion of the protein. Values represent mean ± *SD*, $n = 14$. Data for plasma amino acid concentrations were analyzed by two-factor repeated-measures analysis of variance. Data for iAUC were analyzed by a one-way analysis of variance. Bonferroni post hoc testing was used to detect differences between groups. *Significant group difference within the time point, $p < .05$. Treatments without a common letter differ, $p < .05$. WHEY = 30 g whey protein; WC05 = 25 g whey plus 5 g collagen protein; WC10 = 20 g whey plus 10 g collagen protein; WC15 = 15 g whey plus 15 g collagen protein; iAUC = incremental area under the curve.

implying that a certain amount of leucine needs to be ingested to stimulate muscle protein synthesis rates (Zaromskyte et al., 2021). However, such a leucine threshold is likely less relevant in our postexercise recovery setting as we provided more than ~2 g leucine in all provided protein blends, which is considered the minimal effective dose (Table 2). Plasma leucine availability over the 6-hr postprandial time period was significantly higher in WHEY compared with WC05, WC10, and WC15 ($15 \pm 19\%$, $32 \pm 27\%$, and $61 \pm 14\%$ higher in WHEY compared with the collagen blends, respectively, Figure 3B). However, peak plasma leucine concentrations did not significantly differ between WHEY and WC05 (487 ± 93 vs. 428 ± 58 µmol/L, respectively, Figure 3A).

The two main amino acids in connective tissue are glycine and proline, with glycine residing at every third position of the α chains of collagen (Eastoe, 1955). Although whey protein provides moderate amounts of proline, glycine is only present in very limited amounts. Notably, we observed a decline in postprandial plasma glycine availability following the ingestion of whey protein during recovery from an acute bout of resistance exercise (Figure 4D). We have observed this previously and speculated that such a decline in plasma glycine availability may compromise the capacity to further increase muscle connective protein synthesis rates during recovery from exercise (Aussieker et al., 2023). This could explain previous observations from our laboratory (Aussieker et al., 2023) as well as others (Dideriksen et al., 2011; Mikkelsen et al., 2015; Oikawa et al., 2020) that dairy protein ingestion increases myofibrillar but not muscle connective protein synthesis rates during recovery from a single bout of resistance-type exercise. Interestingly, it has been suggested that the supply of glycine through endogenous pathways and dietary intake is insufficient for adequate collagen synthesis (Meléndez-Hevia et al., 2009; Yu et al., 1985). Furthermore, glycine has been shown to have a protective effect in muscle wasting (reviewed by Koopman et al. [2017]), may play an important role in muscle regeneration (Gheller et al., 2021), and is required for the synthesis of creatine, heme, glutathione, purine, and collagen (Adeva-Andany et al., 2018; Alves et al., 2019). A decline in circulating plasma glycine does not necessarily imply that the decline in plasma glycine availability compromises the skeletal muscle adaptive response to exercise. However, the observation that the postprandial plasma concentrations and availability declined despite the ingestion of 30 g whey protein (providing 0.42 g glycine) implies an increased demand for glycine during recovery from exercise. In support, we have previously observed substantial increases in muscle connective protein synthesis rates during recovery from exercise (Aussieker et al., 2023; Holwerda et al., 2021; Trommelen et al., 2020). Furthermore, using intrinsically L-[1-^{13}C]-phenylalanine- and L-[1-^{13}C]-leucine-labeled milk protein, we were able to show that these dietary protein-derived amino acids were used for de novo muscle connective protein synthesis (Holwerda et al., 2021; Trommelen et al., 2020). Therefore, we speculate that postexercise muscle connective protein conditioning may be restricted under conditions in which insufficient amounts of exogenous glycine are provided (Trommelen et al., 2023). The impact of protein ingestion on postexercise muscle connective protein synthesis may be dependent on the amount of protein-derived precursors and the time during which they are made available. We studied the impact of collagen coingestion with whey as a means to improve postprandial plasma glycine availability. Collagen protein is a good and easily accessible source of glycine, which has about 14.5 times more glycine than whey

protein. Coingestion of collagen with whey protein strongly increased plasma glycine concentrations (Figure 4C), resulting in positive postprandial plasma glycine availability (Figure 4D). Interestingly, coingestion of only a small amount of 5 g collagen (WC05) was sufficient to prevent the postprandial decline in plasma glycine availability without a relevant decrease in either the plasma EAA or leucine response when compared with the ingestion of whey protein only.

During recovery from resistance exercise, the two main structural components in skeletal muscle tissue that will undergo reconditioning are myofibrillar and muscle connective proteins (Miller et al., 2005; Wilkinson et al., 2008). Therefore, dietary protein should be targeted to deliver amino acid precursors to support the postexercise remodeling in both myofibrillar and muscle connective proteins. In that context, protein blends with collagen have been proposed to support those adaptations due to their unique amino acid profile (Deane et al., 2020). As the suggested lower quality of collagen protein when compared with dairy protein could compromise the capacity of such a protein blend to stimulate postexercise muscle protein synthesis, we sought to establish the impact of coingesting different doses of collagen with whey protein during recovery from exercise. Here, we observed that merely 5 g of collagen protein is already sufficient to prevent a decline in postprandial plasma glycine availability during recovery from lower body resistance-type exercise. Such an amount could provide sufficient glycine to cover the increased precursor demands during the postexercise increase in muscle connective protein synthesis rates and could be combined with 20–25 g of whey protein to maximize postexercise myofibrillar protein synthesis rates. However, more work will be needed to establish whether the ingestion of 5 g collagen with 25 g whey protein will further increase myofibrillar as well as muscle connective protein synthesis rates during recovery from an acute bout of resistance exercise.

In conclusion, the coingestion of a small amount of collagen (5 g) with whey protein (25 g) is sufficient to prevent the decline in plasma glycine availability during recovery from a single bout of resistance exercise in healthy, young men. Future work is required to determine whether a protein blend combining 25 g whey plus up to 5 g collagen protein may provide sufficient amino acid precursors to support an increase in both myofibrillar as well as connective protein synthesis rates during recovery from exercise.

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