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Ingestion of Wheat Protein Increases In Vivo Muscle Protein Synthesis Rates in Healthy Older Men in a Randomized Trial[1–3]

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Abstract

Background: Muscle mass maintenance is largely regulated by basal muscle protein synthesis and the capacity to stimulate muscle protein synthesis after food intake. The postprandial muscle protein synthetic response is modulated by the amount, source, and type of protein consumed. It has been suggested that plant-based proteins are less potent in stimulating postprandial muscle protein synthesis than animal-derived proteins. However, few data support this contention.

Objective: We aimed to assess postprandial plasma amino acid concentrations and muscle protein synthesis rates after the ingestion of a substantial 35-g bolus of wheat protein hydrolysate compared with casein and whey protein.

Methods: Sixty healthy older men [mean ± SEM age: 71 ± 1 y; body mass index (in kg/m^2): 25.3 ± 0.3] received a primed continuous infusion of L-[ring-$^{13}C_6$]-phenylalanine and ingested 35 g wheat protein (n = 12), 35 g wheat protein hydrolysate (WPH-35; n = 12), 35 g micellar casein (MCas-35; n = 12), 35 g whey protein (Whey-35; n = 12), or 60 g wheat protein hydrolysate (WPH-60; n = 12). Plasma and muscle samples were collected at regular intervals.

Results: The postprandial increase in plasma essential amino acid concentrations was greater after ingesting Whey-35 (2.23 ± 0.07 mM) than after MCas-35 (1.53 ± 0.08 mM) and WPH-35 (1.50 ± 0.04 mM) (P < 0.01). Myofibrillar protein synthesis rates increased after ingesting MCas-35 (P < 0.01) and were higher after ingesting MCas-35 (0.050% ± 0.005%/h) than after WPH-35 (0.032% ± 0.004%/h) (P = 0.03). The postprandial increase in plasma leucine concentrations was greater after ingesting Whey-35 than after WPH-60 (peak value: 580 ± 18 compared with 378 ± 10 μM, respectively; P < 0.01), despite similar leucine contents (4.4 g leucine). Nevertheless, the ingestion of WPH-60 increased myofibrillar protein synthesis rates above basal rates (0.049% ± 0.007%/h; P = 0.02).

Conclusions: The myofibrillar protein synthetic response to the ingestion of 35 g casein is greater than after an equal amount of wheat protein. Ingesting a larger amount of wheat protein (i.e., 60 g) substantially increases myofibrillar protein synthesis rates in healthy older men. This trial was registered at clinicaltrials.gov as NCT01952639. *J Nutr* 2016;146:1651–9.

Keywords: leucine, muscle protein synthesis, plant, wheat, whey

Introduction

The preservation of skeletal muscle mass throughout life is of key importance to maintain functional capacity and metabolic health (1, 2). Muscle mass maintenance is largely regulated by basal muscle protein synthesis rates and the ability to stimulate muscle protein synthesis after food intake (3). In particular, protein ingestion directly stimulates postprandial muscle protein synthesis rates (4–10). The muscle protein synthetic response to protein ingestion can be modulated by changing the amount, source, and type of protein consumed (11). Current research aims at identifying the characteristics of the ingested protein source that determine the magnitude of the postprandial muscle protein synthetic response to develop more effective dietary strategies that support muscle mass maintenance in health and disease.

In the US diet, plant-based proteins account for 30–50% of total dietary protein intake (12). In less-privileged countries, plant-based protein intake has been estimated to exceed 60% (13). Despite the large contribution of plant-based proteins to our diet, relatively few studies, to our knowledge, have assessed

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[3] Supplemental Table 1 and Supplemental Figures 1–8 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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the muscle protein synthetic response to the ingestion of plant-based proteins in humans (9, 10, 14–17). Nevertheless, it is generally believed that plant-based proteins are less anabolic than animal-derived proteins due to lower digestibility and deficiencies in certain essential amino acids such as leucine, lysine, and/or methionine (18). Innovations in food processing have solved many issues with regard to plant-based protein digestibility by the production of plant-based protein concentrates, isolates, and hydrolysates (19). Moreover, deficiencies in leucine, lysine, and/or methionine may exist in some, but certainly not all, plant-based proteins, because there is a great variety in the amino acid composition between various plant-based proteins (18). Most human studies performed so far have compared the muscle protein synthetic response to the ingestion of soy protein with casein (9), whey protein (9, 10), milk (17), or beef (16). Few data exist on the muscle protein synthetic response after the ingestion of other plant-based proteins.

Wheat protein is the most abundant plant-based protein in our diet and comprises ~20% of total protein intake (13). Despite the low digestibility of whole wheat (~45%), the removal of antinutritional factors (i.e., compounds that interfere with protein digestion and absorption) has resulted in purified wheat protein with a digestibility similar to that of animal-derived proteins (i.e., >90%) (19). The production of wheat protein hydrolysate has resulted in a more practical protein source to be used in various food products. However, wheat protein has a relatively low lysine and leucine content compared with isonitrogenous amounts of animal-derived proteins (20). Previous work in rodents has shown that the muscle protein synthetic response to the ingestion of a single bolus of wheat protein is lower than after the ingestion of dairy protein, and that consuming a greater amount of wheat protein can compensate for a relative lack of certain essential amino acids and allow for a greater postprandial muscle protein synthetic response (20, 21). However, this concept remains to be established in humans.

In the present study we first compared postprandial plasma amino acid profiles and the muscle protein synthetic response after the ingestion of 35 g intact or hydrolyzed wheat protein. The ingestion of 35 g wheat protein provides 2.5 g leucine, which should theoretically induce a measurable increase in postprandial muscle protein synthesis rates (22). Next, we compared the impact of ingesting 35 g wheat protein hydrolysate or the ingestion of 35 g casein and 35 g whey protein on the postprandial muscle protein synthetic response in older men. Finally, we assessed the muscle protein synthetic response to the ingestion of leucine-matched amounts of wheat protein hydrolysate compared with whey protein (i.e., 60 compared with 35 g, respectively). By using intravenous infusions of L-[ring-$^{13}C_6$]-phenylalanine and L-[ring-3,5-$^{2}H_2$]-tyrosine, we were able to evaluate whole-body amino acid kinetics as well as basal and postprandial muscle protein synthesis rates after the ingestion of plant-based and dairy proteins in vivo in humans (23). This is the first study, to our knowledge, to provide a detailed evaluation of the anabolic properties of one of the main plant-based proteins in our diet.

Methods

Participants. Sixty healthy older men [mean ± SEM age: 71 ± 1 y; BMI (in kg/m^2): 25.3 ± 0.3] participated in this double-blind, parallel-group randomized trial. The trial was conducted between January 2014 and October 2014 at Maastricht University in Maastricht, Netherlands. Participants' characteristics are shown in **Table 1**. All participants were informed about the purpose of the study, experimental procedures, and possible risks before providing written consent to participate. The procedures followed were in accordance with the ethical standards of the Medical Ethics Committee of Maastricht University Medical Centre+ on human experimentation and in accordance with the Helsinki Declaration of 1975 as revised in October 2013. The trial was registered at clinicaltrials.gov (NCT01952639).

Pretesting. Volunteers between the age of 65 and 80 y and a BMI between 18.5 and 30.0 underwent a medical screening to assess their glycated hemoglobin, glucose tolerance [by a 2-h oral-glucose-tolerance test (24)], blood pressure, weight, height, and body composition (by DXA; Discovery A; Hologic). The participants were deemed healthy on the basis of their responses to a medical questionnaire and screening results. [See **Figure 1** for the CONSORT (Consolidated Standards of Reporting Trials) flow diagram.]

Study design. Participants were randomly assigned to consume 35 g wheat protein (WP-35[7]; Amygluten; Tereos; n = 12), 35 g wheat protein hydrolysate (WPH-35; Meripro; Tereos; n = 12), 35 g micellar casein (MCas-35; Refit MCI 80; Domo; n = 12), 35 g whey protein (Whey-35; Nutri Whey 800F; DMV; n = 12), or 60 g wheat protein hydrolysate (WPH-60; Meripro; n = 12). The ingestion of 35 g wheat protein provides 2.5 g leucine, which should theoretically induce a measurable increase in postprandial muscle protein synthesis rates (22). Randomization was performed by using a computerized random-number generator.

Diet and physical activity control. All of the participants were instructed to refrain from any sort of strenuous physical activity and to keep their diet as consistent as possible for 2 d before the infusion trial. On the evening before the infusion trial, all participants consumed a standardized meal (30.9 ± 0.5 kJ/kg body weight) composed of 16% of energy from protein, 33% from carbohydrate, and 51% from fat.

Infusion protocol. At 0800 h, after an overnight fast, participants arrived at the laboratory by car or public transport. A catheter was inserted into an antecubital vein for stable isotope amino acid infusion. A second catheter was inserted into a dorsal hand vein of the contralateral arm and placed in a hot box (60°C) for arterialized blood sampling (25). After taking a baseline blood sample, the plasma phenylalanine and tyrosine pools were primed with a single dose of L-[ring-$^{13}C_6$]-phenylalanine (2.1 μmol/kg) and L-[ring-3,5-$^{2}H_2$]-tyrosine (0.8 μmol/kg), after which a continuous L-[ring-$^{13}C_6$]-phenylalanine (0.048 μmol · kg^{-1} · min^{-1}) and L-[ring-3,5-$^{2}H_2$]-tyrosine (0.018 μmol · kg^{-1} · min^{-1}) intravenous infusion was initiated (t = –270 min). After resting in a supine position for 90 min, a second arterialized blood sample was drawn and a muscle biopsy sample was collected from the *vastus lateralis* of a randomly chosen leg (t = –180 min). To determine basal muscle protein synthesis rates, a second muscle biopsy sample from the same leg was collected 180 min after the first biopsy. Subsequently, participants received a drink containing WP-35 (n = 12), WPH-35 (n = 12), MCas-35 (n = 12), Whey-35 (n = 12), or WPH-60 (n = 12) (t = 0 min; **Supplemental Table 1** lists the amino acid composition of the proteins). Arterialized blood samples were collected at t = −120, −90, −60, −30, 0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min. Third and fourth muscle biopsy samples were collected from the contralateral leg at t = 120 and t = 240 min to determine postprandial muscle protein synthesis rates. Blood samples were collected in EDTA-containing tubes and centrifuged at 1000 *g* for 10 min at 4°C. Aliquots of plasma were frozen in liquid nitrogen and stored at −80°C. Biopsy samples were collected from the middle region of the *vastus lateralis*, ~15 cm above the patella and 3 cm below entry through the fascia, by using the percutaneous needle biopsy technique (26). Muscle samples were dissected carefully, freed from any visible nonmuscle material, immediately frozen in liquid nitrogen, and stored at −80°C until

[7] Abbreviations used: FSR, fractional synthetic rate; GC-MS, gas chromatography–mass spectrometry; MCas-35, 35 g micellar casein; mTORC1, mammalian target of rapamycin complex 1; Whey-35, 35 g whey protein; WP-35, 35 g wheat protein; WPH-35, 35 g wheat protein hydrolysate; WPH-60, 60 g wheat protein hydrolysate.

TABLE 1 Subject characteristics[1]

	WP-35	WPH-35	MCas-35	Whey-35	WPH-60
Age, y	68 ± 1	72 ± 2*	73 ± 1	72 ± 2	68 ± 1
Weight, kg	77.1 ± 1.7	78.6 ± 3.9	75.1 ± 2.8	79.3 ± 2.4	81.0 ± 3.0
BMI, kg/m^2	25.1 ± 0.6	25.5 ± 1.0	24.6 ± 0.5	25.2 ± 0.5	26.3 ± 0.8
Systolic BP, mm Hg	138 ± 6	140 ± 4	139 ± 5	133 ± 3	139 ± 6
Diastolic BP, mm Hg	77 ± 3	67 ± 2*	69 ± 3	69 ± 2	71 ± 3
Fat, %	22.9 ± 1.1	24.0 ± 1.0	25.1 ± 1.2	23.9 ± 0.7	25.4 ± 1.1
Appendicular lean mass, kg	25.1 ± 0.7	24.5 ± 1.0	23.0 ± 1.0	25.1 ± 0.7	25.5 ± 1.0
Lean body mass, kg	57.4 ± 1.5	56.9 ± 2.5	53.6 ± 2.0	57.8 ± 1.5	58.1 ± 2.2
Fasting glucose, mmol/L	6.0 ± 0.1	6.0 ± 0.2	6.0 ± 0.2	6.1 ± 0.1	5.8 ± 0.1
2-h glucose, mmol/L	6.5 ± 0.3	6.2 ± 0.5	6.1 ± 0.5	6.1 ± 0.4	5.5 ± 0.5
HbA1c, %	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.3 ± 0.1	5.4 ± 0.1
OGIS, mL · min^{-1} · m^{-2}	351 ± 11	357 ± 8	351 ± 23	353 ± 12	357 ± 18

[1] Values are means ± SEMs, n = 12/group. Statistical analysis was performed on the following comparisons: *1*) WP-35 compared with WPH-35, *2*) WPH-35 compared with MCas-35 and Whey- 35, and *3*) Whey-35 compared with WPH-60. *Different from WP-35, $P < 0.05$. BP, blood pressure; HbA1c, glycated hemoglobin; MCas-35, 35 g micellar casein; OGIS, oral-glucose insulin sensitivity; Whey-35, 35 g whey protein; WP-35, 35 g wheat protein; WPH-35, 35 g wheat protein hydrolysate; WPH-60, 60 g wheat protein hydrolysate.

further analysis. For a schematic representation of the infusion protocol, see **Supplemental Figure 1**.

Plasma and muscle tissue analyses. For the determination of plasma concentrations of all essential and nonessential amino acids, 10 μL plasma was mixed with 1500 μL 0.5-mM tridecafluoroheptanoic acid (Sigma) in water and 10 μL internal standard solution containing stable isotope–labeled amino acids (Cambridge Isotopes Laboratories) in 0.1 M HCl. Amino acid concentrations were determined by using ultraperformance liquid chromatography–tandem mass spectrometry, as described previously (27). Plasma phenylalanine, tyrosine, and leucine concentrations; plasma L-[ring-$^{13}C_6$]-phenylalanine, L-[ring-$^{13}C_6$]-tyrosine, and L-[ring-3,5-$^2H_2$]-tyrosine enrichments; and muscle intracellular L-[ring-$^{13}C_6$]-phenylalanine enrichments were determined by gas chromatography–mass spectrometry (GC-MS; Agilent 7890A GC/5975C MSD; Agilent Technologies) as described in our previous work (28). Myofibrillar and mixed muscle proteins were extracted from separate pieces of muscle tissue (~60 mg) as described previously (28, 29). Myofibrillar and mixed muscle protein-bound L-[ring-$^{13}C_6$]-phenylalanine enrichments were determined by gas chromatography-combustion-isotope ratio mass spectrometry analysis as described in our previous work (30).

Calculations. Intravenous infusions of L-[ring-$^{13}C_6$]-phenylalanine and L-[ring-3,5-$^2H_2$]-tyrosine combined with arterialized blood sampling allowed us to assess whole-body amino acid kinetics in non–steady state conditions. Total rate of appearance (R_a), total rate of disappearance (R_d), and oxidation and synthesis rates were calculated by using modified Steele's equations (31, 32), as follows:

$$\text{Total } R_a = \frac{F_{\text{iv}} - \left[pV \times C(t) \times \frac{dE_{\text{iv}}}{dt}\right]}{E_{\text{iv}}(t)} \quad (1)$$

$$\text{Total } R_d = \text{Total } R_a - pV \times \frac{dC}{dt} \quad (2)$$

$$\text{Phe hydroxylation} = \text{Tyr } R_a \times \frac{E_{\text{Tyr}}(t)}{E_{\text{Phe}}(t)} \times \frac{\text{Phe } R_d}{(F_{\text{Phe}} + \text{Phe } R_d)} \quad (3)$$

$$\text{Protein synthesis} = \text{Total } R_d - \text{Phe hydroxylation} \quad (4)$$

Total R_a represents the rate at which both dietary protein–derived phenylalanine as well as phenylalanine derived from whole-body protein breakdown enters the circulation. F_{iv} is the intravenous L-[ring-$^{13}C_6$]-phenylalanine infusion rate (μmol · kg^{-1} · min^{-1}), pV (0.125 L · kg^{-1}) is the distribution volume (31), $C(t)$ is the mean plasma phenylalanine concentration between 2 consecutive time points, dE_{iv}/dt is the time-dependent variation in plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments, and $E_{\text{iv}}(t)$ is the mean plasma L-[ring-$^{13}C_6$]-phenylalanine enrichment between 2 consecutive time points. Total R_d represents the rate of phenylalanine hydroxylation (first step in phenylalanine oxidation) plus the rate of phenylalanine utilization for protein synthesis. dC/dt is the time-dependent variation in plasma phenylalanine concentrations. Tyr R_a is the total rate of tyrosine appearance based on the intravenous L-[ring-3,5-$^2H_2$]-tyrosine infusion, plasma L-[ring-3,5-$^2H_2$]-tyrosine enrichments, and plasma tyrosine concentrations; $E_{\text{Tyr}}(t)$ and $E_{\text{Phe}}(t)$ represent the mean plasma L-[ring-$^{13}C_6$]-tyrosine and L-[ring-$^{13}C_6$]-phenylalanine enrichment between 2 consecutive time points, respectively; Phe R_d is the total rate of phenylalanine disappearance; and F_{Phe} is the intravenous infusion rate of L-[ring-$^{13}C_6$]-phenylalanine (μmol · kg^{-1} · min^{-1}).

Myofibrillar and mixed muscle protein fractional synthetic rates (FSRs) were calculated by using the standard precursor-product equation, as follows:

$$FSR = \frac{\Delta E_p}{E_{\text{precursor}} \cdot t} \cdot 100 \quad (5)$$

ΔE_p is the increment in myofibrillar or mixed muscle protein–bound L-[ring-$^{13}C_6$]-phenylalanine enrichment after an incorporation period, $E_{\text{precursor}}$ is the weighted mean plasma or intracellular L-[ring-$^{13}C_6$]-phenylalanine enrichment during that incorporation period, and t is the incorporation period (h). Weighted mean plasma or intracellular enrichments were calculated by taking the average enrichment between all consecutive time points and correcting for the time between these sampling time points. The weighted mean plasma precursor pool is preferred in this setting, because the more frequent sampling time points allow for a more accurate correction of the transient changes in precursor pool enrichments over time (29). For basal FSR, muscle biopsy samples at t = −180 and 0 min were used; and for postprandial FSRs, biopsy samples at t = 0, 120, and 240 min were used.

Statistical analysis. All of the data are expressed as means ± SEMs. Within this study we compared the following treatments: *1*) WP-35 with WPH-35, *2*) WPH-35 with MCas-35 and Whey-35, and *3*) Whey-35 with WPH-60. This allowed us to determine *1*) whether wheat protein hydrolysis affects postprandial plasma amino acid concentrations and the muscle protein synthetic response to the ingestion of wheat protein, *2*) the anabolic properties of wheat protein hydrolysate compared with both casein and whey protein, and *3*) whether the ingestion of a leucine-matched amount of wheat protein hydrolysate can compensate

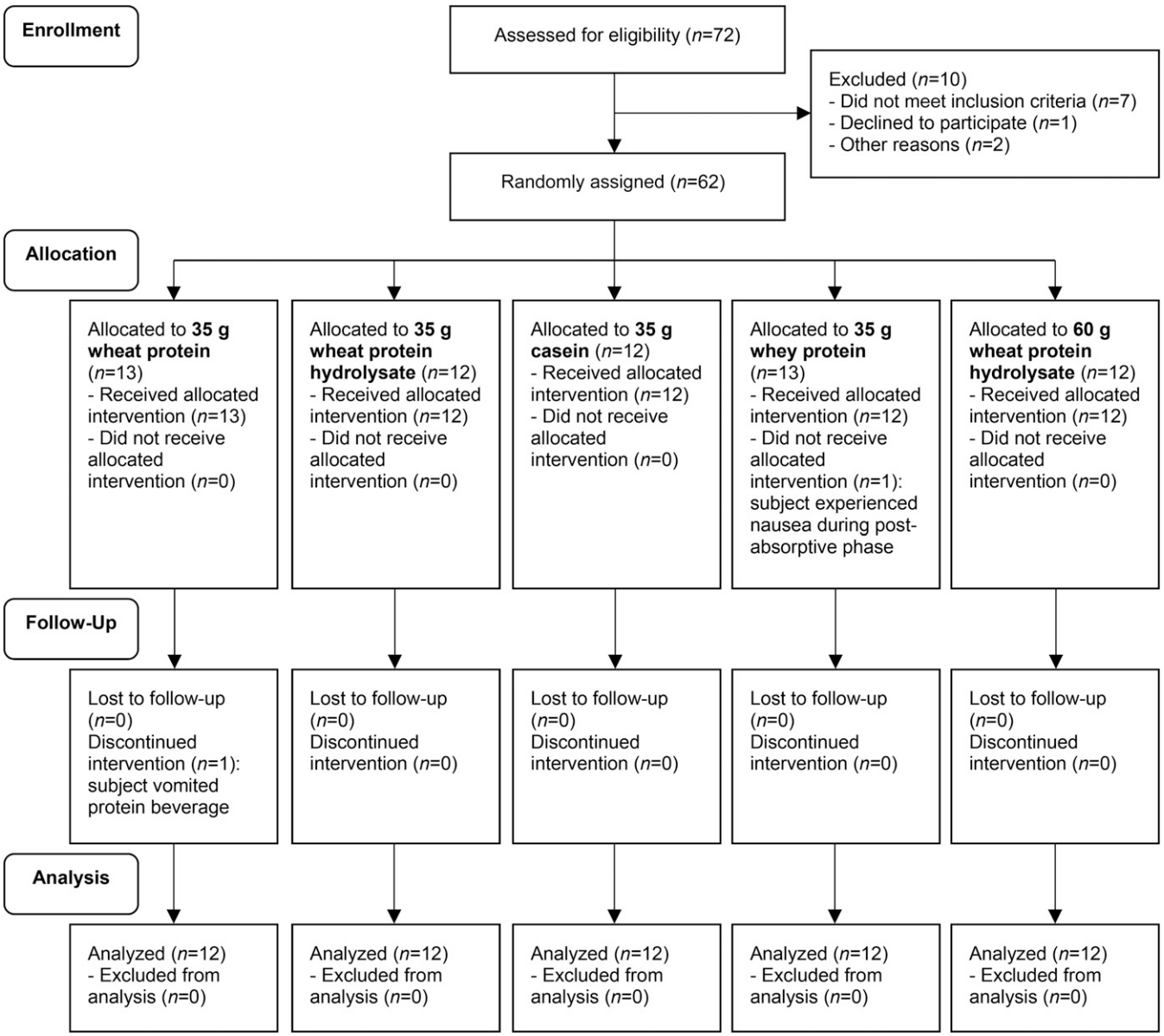

FIGURE 1 CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials.

for the (anticipated) lower anabolic properties of wheat protein hydrolysate compared with whey protein. For plasma time curves, repeated-measures ANOVA with treatment, time, and their interaction was used to identify differences between treatments over time. When significant interaction or treatment effects were observed, Tukey's post hoc analysis was performed to locate these differences. For muscle variables, ANCOVA with basal values as covariates and time and treatment as factors was used to identify differences between treatments, and repeated-measures ANOVA was used to identify differences between basal and postprandial muscle protein synthesis rates. Significance was set at $P < 0.05$. All calculations were performed by using IBM SPSS Statistics (version 21).

Results

Intact compared with hydrolyzed wheat protein. Postprandial plasma concentrations of the essential amino acids are presented in **Supplemental Figure 2**. Plasma concentrations of histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, and valine and the sum of essential amino acids increased after protein ingestion ($P < 0.001$) and did not differ between WP-35 and WPH-35 (P-interaction ≥ 0.05). Plasma methionine concentrations increased to a greater extent after the ingestion of WP-35 than after WPH-35 (P-interaction < 0.001).

Supplemental Figure 3 shows plasma leucine and phenylalanine concentrations as well as L-[ring-$^{13}C_6$]-phenylalanine enrichments as measured by GC-MS for the calculation of whole-body amino acid kinetics. Plasma leucine and phenylalanine concentrations increased after protein ingestion to a similar extent in both groups (P-interaction ≥ 0.05). Plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments transiently declined after protein intake ($P < 0.001$), with no differences between WP-35 and WPH-35 ($P = 0.46$).

Total phenylalanine appearance rates increased after the ingestion of both intact and hydrolyzed wheat protein ($P < 0.001$), with no differences between treatments (P-interaction = 0.32; **Figure 2**A). The postprandial muscle protein synthetic response as assessed over the entire 0- to 4-h postprandial

period did not differ between WP-35 and WPH-35 (P = 0.65; Figure 2B).

Wheat protein hydrolysate compared with dairy protein. The postprandial increase in plasma essential amino acid and leucine concentrations was greater after the ingestion of Whey-35 than after WPH-35 and MCas-35 (P-interaction < 0.001; **Figure 3**A, B). Moreover, MCas-35 ingestion resulted in more prolonged hyperaminoacidemia than did WPH-35 ingestion (P-interaction < 0.001). Postprandial plasma lysine and methionine concentrations were different between all 3 treatments and were higher after Whey-35 ingestion and lower after the ingestion of WPH-35 (P-interaction < 0.001; Figure 3C, D). A complete overview of all essential amino acid concentrations is presented in **Supplemental Figure 4**.

Supplemental Figure 5 shows plasma leucine and phenylalanine concentrations as well as L-[ring-$^{13}C_6$]-phenylalanine enrichments as measured by GC-MS for the calculation of whole-body amino acid kinetics. Plasma leucine concentrations increased to a greater extent after the ingestion of Whey-35 than after MCas-35 and WPH-35 (P-interaction < 0.001). The ingestion of WPH-35 resulted in a slightly greater but more transient increase in plasma leucine concentrations than did MCas-35 ingestion (P-interaction < 0.001). Postprandial plasma phenylalanine concentrations were significantly higher after WPH-35 ingestion than after Whey-35 (P-interaction < 0.001). MCas-35 ingestion resulted in a slightly lower but more

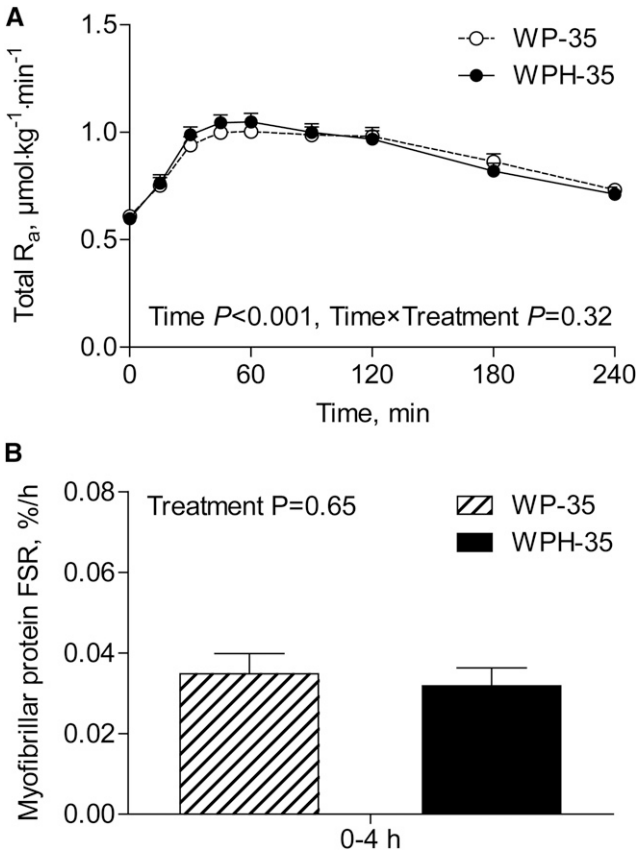

FIGURE 2 Whole-body total R_a (A) and myofibrillar protein FSRs calculated on the basis of the plasma precursor pool (B) over the entire (0–4 h) postprandial period after the ingestion of WP-35 or WPH-35 in healthy older men. Values are means ± SEMs, n = 12/group. FSR, fractional synthetic rate; R_a, rate of appearance; WP-35, 35 g wheat protein; WPH-35, 35 g wheat protein hydrolysate.

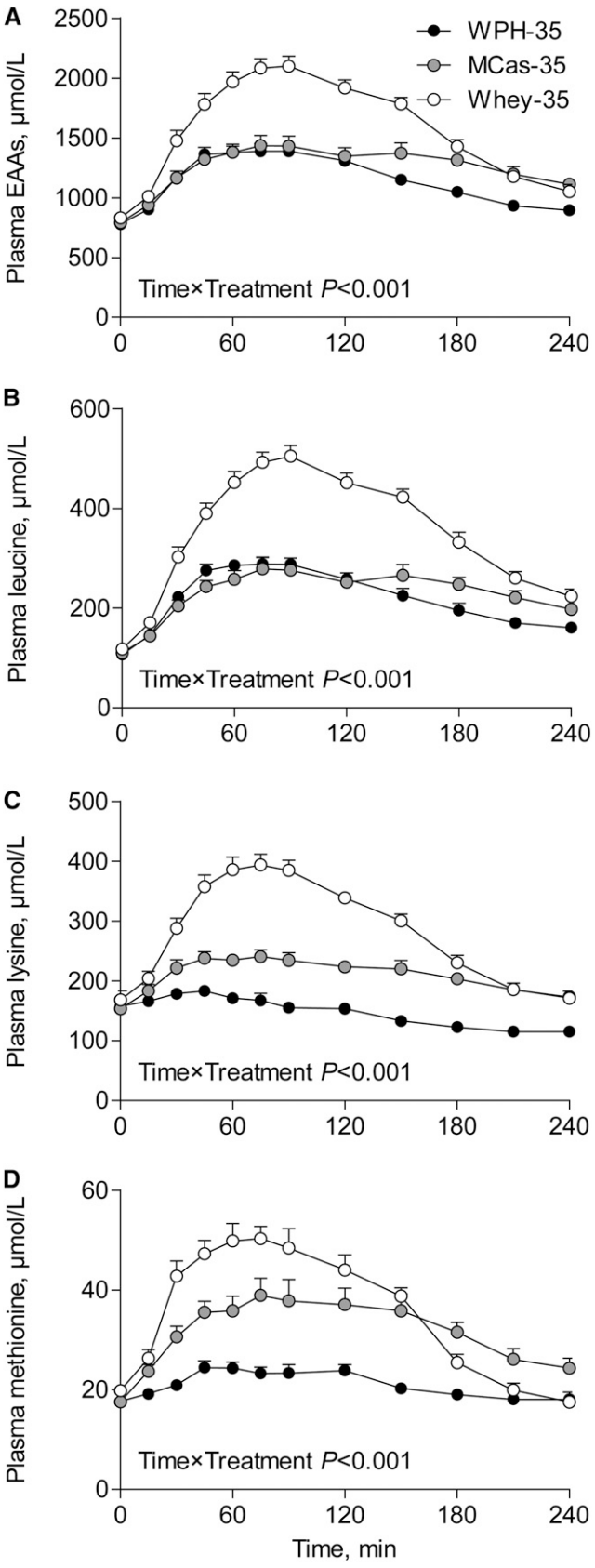

FIGURE 3 Plasma EAA (A), leucine (B), lysine (C), and methionine (D) concentrations after the ingestion of WPH-35, MCas-35, or Whey-35 in healthy older men. Values are means ± SEMs, n = 12/group. EAA, essential amino acid; MCas-35, 35 g casein; Whey-35, 35 g whey protein; WPH-35, 35 g wheat protein hydrolysate.

prolonged increase in plasma phenylalanine concentrations than did Whey-35 ingestion (P-interaction < 0.001). The dilution in plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments was reflective of the increase in plasma phenylalanine concentrations, with the greatest dilution after the ingestion of WPH-35 compared with MCas-35 and Whey-35 (P-interaction < 0.001).

Whole-body phenylalanine kinetics are shown in **Supplemental Figure 6**. Total phenylalanine appearance rates, total phenylalanine disappearance rates, and phenylalanine utilization for protein synthesis increased after protein ingestion, with a greater increase after WPH-35, a moderate increase after Whey-35, and a lower but more prolonged elevation after MCas-35 ingestion (P-interaction < 0.001). Phenylalanine oxidation was higher after the ingestion of WPH-35, which is likely due to a higher phenylalanine content and/or suboptimal amino acid composition of wheat protein compared with dairy protein (18). Phenylalanine oxidation rates reached similar peak values after the ingestion of MCas-35 and Whey-35 and remained elevated for a longer time period after MCas-35 ingestion (P-interaction = 0.001).

Myofibrillar protein synthesis rates calculated on the basis of the plasma precursor pool (**Figure 4**) increased from basal rates after the ingestion of MCas-35 when assessed over the late (2–4 h) and entire (0–4 h) postprandial period (time P = 0.007 and P = 0.008, respectively). The ingestion of Whey-35 or WPH-35 did not significantly stimulate muscle protein synthesis rates above basal values (P = 0.12 and P = 0.25, respectively). Postprandial myofibrillar protein synthesis rates were higher after the ingestion of MCas-35 than after WPH-35 when assessed over the early (0–2 h) and entire (0–4 h) postprandial period (treatment P = 0.027 and P = 0.011, respectively). Similar responses were observed by using the mixed muscle protein fraction and when muscle protein synthesis rates were calculated on the basis of the intracellular precursor pool (data not shown).

Leucine-matched amounts of whey protein compared with wheat protein hydrolysate. The postprandial increase in plasma concentrations of isoleucine, leucine, lysine, methionine, threonine, tryptophan, and valine and the sum of essential amino acids was greater after ingesting Whey-35 than after ingesting WPH-60 (P-interaction < 0.001; **Supplemental Figure 7**). Plasma histidine and phenylalanine concentrations increased to a greater extent after the ingestion of WPH-60 than after Whey-35 (P-interaction < 0.001). **Figure 5** shows plasma leucine and phenylalanine concentrations as well as L-[ring-$^{13}C_6$]-phenylalanine enrichments as measured by GC-MS for the calculation of whole-body amino acid kinetics. Despite equal leucine content, plasma leucine concentrations increased to a greater extent after the ingestion of Whey-35 than after WPH-60 (P-interaction < 0.001). Plasma phenylalanine concentrations increased to a greater extent and remained elevated for a more prolonged period after the ingestion of WPH-60 than after Whey-35 (P-interaction < 0.001). The postprandial

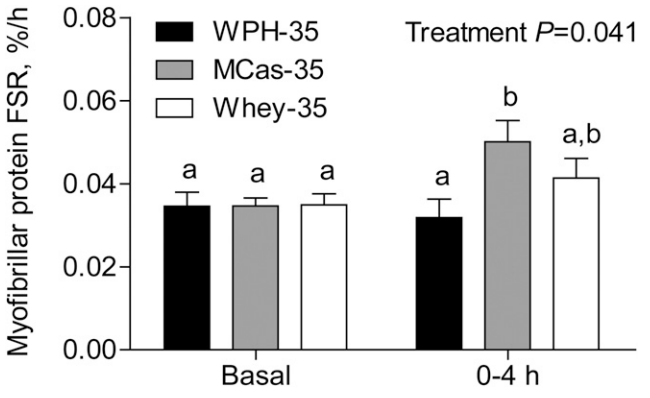

FIGURE 4 Myofibrillar protein FSRs, calculated on the basis of the plasma precursor pool, during the fasting state (Basal) and over the entire (0–4 h) postprandial period after the ingestion of WPH-35, MCas-35, or Whey-35 in healthy older men. Values are means ± SEMs, n = 12/group. Labeled bars without a common letter differ, P < 0.05. FSR, fractional synthetic rate; MCas-35, 35 g casein; Whey-35, 35 g whey protein; WPH-35, 35 g wheat protein hydrolysate.

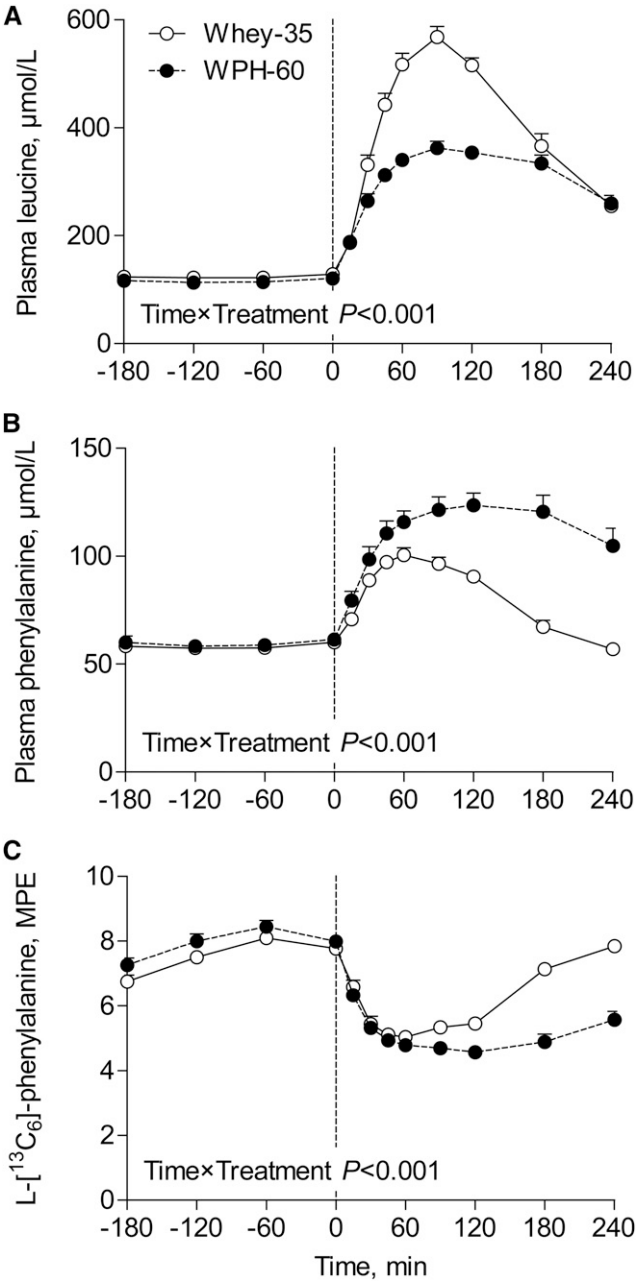

FIGURE 5 Plasma leucine (A) and phenylalanine (B) concentrations and L-[ring-$^{13}C_6$]-phenylalanine (C) enrichments (MPE) during the fasting state and after the ingestion of Whey-35 or a leucine-matched amount (i.e., 60 g) of WPH-60 in healthy older men. Values are means ± SEMs, n = 12/group. MPE, mole percent excess; Whey-35, 35 g whey protein; WPH-60, 60 g wheat protein hydrolysate.

dilution in plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments was reflective of the increase in plasma phenylalanine concentrations, with a more prolonged dilution after WPH-60 ingestion (P-interaction < 0.001).

Total phenylalanine appearance rates, total phenylalanine disappearance rates, and phenylalanine utilization for protein synthesis increased after the ingestion of both Whey-35 and WPH-60, with a more prolonged elevation after WPH-60 ingestion (P-interaction < 0.001; **Supplemental Figure 8**). Phenylalanine oxidation increased to a greater extent and remained elevated for a longer time period after the ingestion of WPH-60 than after Whey-35 (P-interaction < 0.001).

Myofibrillar protein synthesis rates calculated on the basis of the plasma precursor pool (**Figure 6**) increased from basal rates after the ingestion of WPH-60 when assessed over the late (2–4 h) and entire (0–4 h) postprandial period (time $P < 0.001$ and $P = 0.017$, respectively) and did not increase from basal rates after the ingestion of Whey-35 (time $P = 0.11$). Similar responses were observed by using the mixed muscle protein fraction and when muscle protein synthesis rates were calculated on the basis of the intracellular precursor pool (data not shown).

Discussion

In the current study, we assessed the postprandial response to the ingestion of intact compared with hydrolyzed wheat protein. The ingestion of 35 g intact or hydrolyzed wheat protein was followed by a rapid increase in circulating essential amino acid concentrations, with no differences between protein forms (Supplemental Figure 2). Accordingly, postprandial muscle protein synthesis rates did not differ after the ingestion of intact compared with hydrolyzed wheat protein (Figure 2). Because no differences were evident between the postprandial responses to the ingestion of intact and hydrolyzed wheat protein, and solubility is much higher for the protein hydrolysate, it would generally be preferable to apply a wheat protein hydrolysate in the development of (liquid) nutritional supplements.

Plant-based proteins generally exhibit lower digestibility than animal-derived proteins (33). As such, less of the dietary protein is effectively digested and absorbed, resulting in lower postprandial availability of dietary protein–derived amino acids as precursors for de novo muscle protein synthesis (34). However, once freed from antinutritional compounds that interfere with protein digestion and absorption, purified plant-based proteins are likely to possess digestion and absorption kinetics that are not different from animal-derived proteins (19, 33). Here, we assessed postprandial amino acid profiles after the ingestion of purified wheat protein hydrolysate compared with casein and whey protein. The ingestion of WPH-35, MCas-35, and Whey-35 rapidly increased plasma essential amino acid concentrations (Figure 3). The ingestion of wheat protein hydrolysate resulted in postprandial peak essential amino acid concentrations similar to casein ingestion, despite the lower essential amino acid content of the ingested wheat protein hydrolysate. However, casein ingestion resulted in a more prolonged elevation of circulating essential amino acid concentrations compared with the ingestion of the same amount of wheat protein hydrolysate (Figure 3). The ingestion of whey protein, compared with wheat protein hydrolysate and casein, resulted in a more prominent postprandial increase in plasma essential amino acid concentrations. Plasma lysine and methionine concentrations increased only marginally after the ingestion of wheat protein hydrolysate compared with casein and whey protein, which is in agreement with the lower lysine and methionine contents in wheat protein hydrolysate (1.5% and 0.6%, respectively) compared with casein (7.6% and 2.1%, respectively) and whey protein (10.1% and 2.0%, respectively). These data imply that wheat protein hydrolysate is well digested and absorbed, with a substantial postprandial increase in plasma amino acid availability. Despite this rapid postprandial increase in circulating amino acid concentrations, we observed no significant increase in muscle protein synthesis rates after the ingestion of WPH-35 (4% ± 17%; $P = 0.25$) and an intermediate increase in muscle protein synthesis rates after the ingestion of Whey-35 (33% ± 24%; $P = 0.12$; Figure 4). In contrast, the ingestion of an equal amount of casein resulted in a 48% ± 16% increase in muscle protein synthesis rates compared with basal values ($P = 0.011$; Figure 4). The absence of a measurable increase in muscle protein synthesis rates after the ingestion of Whey-35 was surprising because we previously observed a significant 44% and 38% increase in muscle protein synthesis rates after the ingestion of whey protein in old (8) and young (35) men, respectively.

In this study, we showed that the muscle protein synthetic response after the ingestion of wheat protein hydrolysate is lower than after the ingestion of casein. It seems likely that this is attributable to differences in amino acid composition, with the essential amino acid and leucine contents being lower in wheat protein hydrolysate (~10 and 2.5 g/35 g protein, respectively) than in casein (~15 and 3.2 g/35 g protein, respectively). In the current study, participants consumed a substantial 35-g wheat protein dose providing 2.5 g leucine, which has been suggested by the PROT-AGE study group to be sufficient to stimulate muscle protein synthesis (22). Clearly, the leucine content of the protein source or the postprandial increase in circulating leucine concentrations are not the only factors responsible for determining the postprandial increase in muscle protein synthesis rates. We hypothesized that ingesting a greater dose of wheat protein hydrolysate, matched for the amount of leucine present in 35 g whey protein, would result in a similar postprandial increase in plasma amino acid concentrations and muscle protein synthesis rates as observed after the ingestion of the Whey-35. Despite an equal leucine content in the WPH-60 and Whey-35 bolus (both 4.4 g leucine), we observed that plasma leucine concentrations increased to a greater extent after the ingestion of the Whey-35 than after WPH-60 (Figure 5).

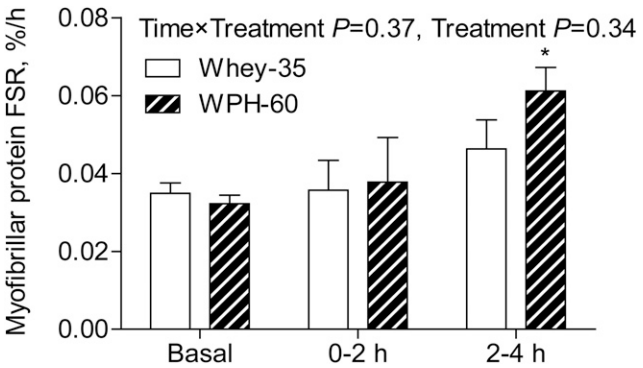

FIGURE 6 Myofibrillar protein FSRs, calculated on the basis of the plasma precursor pool, during the fasting state (Basal) and over the early (0–2 h) and late (2–4 h) postprandial period after the ingestion of Whey-35 or a leucine-matched amount (i.e., 60 g) of WPH-60 in healthy older men. Values are means ± SEMs, n = 12/group. *Different from Basal, $P < 0.05$. FSR, fractional synthetic rate; Whey-35, 35 g whey protein; WPH-60, 60 g wheat protein hydrolysate.

Nevertheless, the more sustained appearance of amino acids into the circulation after the ingestion of WPH-60 than after Whey-35 resulted in a greater stimulation of postprandial muscle protein synthesis rates. We (28, 36) and others (37) previously observed a delayed increase in muscle protein synthesis rates in older compared with younger individuals. A more sustained provision of amino acids may facilitate the delayed postprandial increase in muscle protein synthesis in older individuals, resulting in a greater postprandial muscle protein synthetic response after the ingestion of WPH-60 than after Whey-35. These data provide evidence that both the type and amount of protein consumed define the postprandial muscle protein synthetic response, and that the amount consumed can be modified to match the anabolic properties of a certain protein source. Certainly, leucine plays a key role in the initiation of muscle protein synthesis through mammalian target of rapamycin complex 1 (mTORC1) signaling. However, with all of the treatments providing ≥2.5 g leucine, all treatments may have exceeded the leucine threshold for assembly of the initiation complex, eliminating leucine as the key factor determining the postprandial increase in muscle protein synthesis rates.

Dairy proteins are very potent for the stimulation of muscle protein synthesis due to their high digestibility and high leucine content, but they are relatively expensive. From a global sustainability and economic standpoint, there is an increasing interest in the application of plant-based proteins (18). The muscle protein synthetic response to the ingestion of plant-based protein is generally deemed inferior to that of dairy protein ingestion (9, 10, 16, 17, 20). Although this does not necessarily apply to all plant-based proteins (18), we confirm that the ingestion of WPH-35 induces a lower postprandial muscle protein synthetic response than does the ingestion of the same amount of casein. However, the lesser postprandial muscle protein synthetic response may be compensated for by increasing the amount of protein ingested (Figure 6). This study provides proof-of-concept that the ingestion of WPH-60 stimulates muscle protein synthesis. The ingestion of a bolus of 60 g protein does not represent a practical dietary strategy to stimulate muscle protein synthesis. Therefore, a more practical, cost-effective, and sustainable strategy may be to fortify plant-based protein sources with dairy protein to increase the anabolic properties of lower protein doses. Recently, Reidy et al. (38, 39) showed that the ingestion of a soy-dairy protein blend stimulated postexercise muscle protein synthesis rates to a similar extent as a bolus of whey protein containing an equal essential amino acid content.

We conclude that the postprandial muscle protein synthetic response to the ingestion of 35 g casein is greater than the response to the ingestion of the same amount of wheat protein. The ingestion of a larger amount of wheat protein (i.e., 60 g) substantially increases myofibrillar protein synthesis rates in healthy older men. These data provide useful information when developing or optimizing food product formulations combining wheat or other plant-based proteins with dairy proteins to stimulate muscle protein synthesis rates and to support muscle mass maintenance.

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