Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men

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AGING IS ACCOMPANIED by a progressive decline in skeletal muscle mass, termed sarcopenia (15). Data have been obtained to suggest that the skeletal muscle protein synthetic response to food intake is impaired in older adults (7, 12). This proposed anabolic resistance is now regarded a key factor in the etiology of sarcopenia. Consequently, we and other laboratories have been trying to define effective interventional strategies to prevent sarcopenia (20). The use of intrinsically labeled whey protein allowed us to assess the impact of the amount of whey protein ingested on subsequent muscle protein accretion without the need for extensive assumptions and extrapolations.

In the present study, 33 elderly men ingested a single bolus of 10, 20, or 35 g intrinsically labeled whey protein (n = 11/treatment). Subjects’ characteristics are presented in Table 1. None of the subjects had a history of participating in any regular exercise program. All subjects were informed on the nature and possible risk of the experimental procedures before their written informed consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht.

Participants. Thirty-three healthy, older men (73 ± 2 yr) participated in this study. Subjects were randomly assigned to ingest a single bolus of 10, 20, or 35 g intrinsically labeled whey protein (n = 11/treatment). Subjects’ characteristics are presented in Table 1. None of the subjects had a history of participating in any regular exercise program. All subjects were informed on the nature and possible risk of the experimental procedures before their written informed consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht.

Pretesting. Before selection in the study, an oral glucose tolerance test (OGTT) was performed to assess glucose tolerance and screen for type 2 diabetes prevalence according to World Health Organization criteria (2). Before the OGTT, body weight and height were assessed, and body composition was determined by DXA (Discovery A; Hologic, Bedford, MA).

Diet and activity before testing. All subjects consumed a standardized meal (32 ± 2 kJ/kg body wt, providing 55 energy/100% energy carbohydrate, 15 energy/100% energy protein, and 30 energy/100% energy fat) the evening before the experiment. All volunteers were instructed to refrain from any sort of exhaustive physical activity and...
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Fat, %</th>
<th>Lean body mass, kg</th>
<th>HbA1c, %</th>
<th>Basal glucose, mmol/L</th>
<th>Basal insulin, mU/L</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>2</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>73 ± 2</td>
<td>73 ± 2</td>
<td>73 ± 1</td>
<td></td>
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</tbody>
</table>

Table of values expressed as means ± SE; n = 11 subjects in each group. BMI, body mass index; HbA1c, hemoglobin A1c. Data were analyzed with ANOVA. No differences were observed between groups.

to keep their diet as constant as possible 3 days before the experiments.

Experimental protocol. At 8:00 A.M., following an overnight fast, subjects arrived at the laboratory by car or public transportation. A Teflon catheter was inserted in an antecubital vein for stable isotope infusion. A second Teflon catheter was inserted in a heated dorsal hand vein of the contralateral arm and placed in a hot box (60°C) for arterialized blood sampling (1). Following basal blood collection [time (t) = −240 min], the plasma phenylalanine and tyrosine pools were primed with a single intravenous dose of L-[ring-2H5]phenylalanine (2 μmol/kg) and L-[ring-2H5]tyrosine (0.775 μmol/kg), after which continuous L-[ring-2H5]phenylalanine and L-[ring-2H5]tyrosine infusion was started (0.050 ± 0.001 and 0.019 ± 0.001 μmol·kg⁻¹·min⁻¹, respectively). After resting in a supine position for 120 min, a second arterialized blood sample was drawn, and the first muscle biopsy was collected from the vastus lateralis muscle (t = −120 min), marking the start of a fasted, baseline period. During this period, additional blood samples were drawn every 30 min, and a second muscle biopsy was taken at t = 0 min. Directly following the second biopsy, subjects ingested a single bolus of 10, 20, or 35 g L-[1-13C]phenylalanine-labeled whey protein dissolved in 400 mL water, which was uniformly flavored by adding 5 mL vanilla flavor (Givaudan, Naarden, The Netherlands) per liter beverage. 400 mL water, which was uniformly flavored by adding 5 mL vanilla flavor (Givaudan, Naarden, The Netherlands) per liter beverage.

The isolated muscle fiber mass (10–15 mg) was weighed, and 8 vol (8 × dry wt of isolated muscle fibers × wet-to-dry ratio) ice-cold 2% perchloric acid (PCA) were added. The tissue was then homogenized and centrifuged. The protein pellet was washed with three additional 1.5-mL washes of 2% PCA, dried, and hydrolyzed in 6 M HCl at 120°C for 15–18 h. The hydrolyzed protein fraction was dried under a nitrogen stream while heated to 120°C, and then 50% acetic acid solution was added and the hydrolyzed protein was passed over a Dowex exchange resin (AG 50W-X8, 100–200 mesh hydrogen form; Bio-Rad, Hercules, CA) using 2 M NH₄OH. The eluate was divided over two vials for separate measurement of both L-[1-13C]phenylalanine and L-[ring-2H5]phenylalanine enrichment in mixed-muscle protein, 55 mg of wet muscle was freeze-dried. Collagen, blood, and other nonmuscle fiber material were removed from the muscle fibers under a light microscope. The isolated muscle fiber mass (10–15 mg) was weighed, and 8 vol (8 × dry wt of isolated muscle fibers × wet-to-dry ratio) ice-cold 2% perchloric acid (PCA) were added. The tissue was then homogenized and centrifuged. The protein pellet was washed with three additional 1.5-mL washes of 2% PCA, dried, and hydrolyzed in 6 M HCl at 120°C for 15–18 h. The hydrolyzed protein fraction was dried under a nitrogen stream while heated to 120°C, and then 50% acetic acid solution was added and the hydrolyzed protein was passed over a Dowex exchange resin (AG 50W-X8, 100–200 mesh hydrogen form; Bio-Rad, Hercules, CA) using 2 M NH₄OH. The eluate was divided over two vials for separate measurement of both L-[1-13C]phenylalanine and L-[ring-2H5]phenylalanine enrichment in mixed-muscle protein as described previously (14). In short, L-[1-13C]phenylalanine and L-[ring-2H5]phenylalanine were derivatized to their N(O,S)-ethoxycarbonyl ethyl esters and MTBSTFA-phenylethynolamines, respectively (13). Thereafter, the ratios labeled/ unlabeled derivatives were determined by GC-C-IRMS (Finnigan MAT 252, Bremen, Germany) and GC-MS, respectively. Standard regression curves were applied to assess linearity of the mass spectrometer and to control for loss of tracer.

Calculations. Ingestion of L-[1-13C]phenylalanine-labeled protein, intravenous infusion of L-[ring-2H5]phenylalanine and L-[ring-2H5]tyrosine, and arterialized blood sampling were used to assess whole body protein metabolism in non-steady-state conditions. Total, exogenous, and endogenous phenylalanine rate of appearance (Ra) and plasma availability of dietary phenylalanine (i.e., fraction of dietary phenylalanine that appeared in the systemic circulation, Pheplasma) were calculated using modified Steele’s equations (6, 9). These parameters were calculated as follows:

\[
\text{Total } Ra = \frac{F - pV \cdot C(t) \cdot de_{\text{iv}} / dt}{E_{\text{sol}}(t)}
\]

(1)

\[
\text{Exo } Ra = \frac{\text{Total } Ra \cdot E_{\text{me}}(t) + pV \cdot de_{\text{pe}} / dt}{E_{\text{prot}}}
\]

(2)

\[
\text{Endo } Ra = \text{Total } Ra - \text{Exo } Ra = -F
\]

(3)

\[
\text{Pheplasma} = \frac{\Delta \text{UC}_{\text{ExoRa}}}{\text{Pheprot}} \cdot \text{BW} \cdot 100
\]

(4)

where \( F \) is the intravenous tracer infusion rate (μmol·kg⁻¹·min⁻¹), \( pV \) (0.125) is the distribution volume for phenylalanine (6), \( C(t) \) is the mean plasma phenylalanine concentration between two time points, \( de_{\text{iv}} / dt \) represents the time-dependent variations of plasma phenylalanine enrichment derived from the intravenous tracer, and \( E_{\text{sol}}(t) \) is the mean plasma phenylalanine enrichment from the intravenous tracer.

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between two consecutive time points. Exo \( R_d \) represents the plasma entry rate of dietary phenylalanine, \( E_{\text{p}}(t) \) is the mean plasma phenylalanine enrichment for the oral tracer, \( dE_{\text{p}}/dt \) represents the time-dependent variations of plasma phenylalanine (Phe\(_{\text{plasma}}\)) enrichment derived from the oral tracer, and \( E_{\text{precursor}} \) is the L-[\( ^{1-13}C \)]phenylalanine enrichment in the dietary protein. Endo \( R_a \) is endogenous phenylalanine appearance rate. Phe\(_{\text{oral}}\) is the amount of dietary phenylalanine ingested, and \( \text{AUC}_{\text{ExoRa}} \) represents the area under the curve (AUC) of phenylalanine appearance rate. Phe\(_{\text{oral}}\) is the amount of dietary phenylalanine that appeared in the blood over the 4-h period following drink intake. BW represents the subjects’ body weight in kilograms.

Total rate of disappearance of phenylalanine (\( R_d \)) equals the rate of phenylalanine (PHE)-to-tyrosine (TYR) conversion (first step in phenylalanine oxidation) and utilization for protein synthesis. These parameters can be calculated as follows:

\[
\text{Total } R_d = \text{Total } R_a - pV \cdot \frac{dC}{dt} \quad (5)
\]

\[
\text{PHE-to-TYR conversion} = \frac{\text{Tyr } R_a \cdot E_{\text{p}}(t)}{E_{\text{p}}(t)} \cdot \frac{\text{Phe } R_d}{(F_p + \text{Phe } R_d)} \quad (6)
\]

\[
\text{Protein synthesis} = \text{Total } R_d - \text{Phe hydroxylation} \quad (7)
\]

\[
\text{Phe net balance} = \text{Protein synthesis} - \text{Endo } R_a \quad (8)
\]

where \( \text{Phe } R_d \) and \( \text{Tyr } R_a \) are the flux rates for phenylalanine and tyrosine, respectively, \( E_{\text{p}}(t) \) and \( E_{\text{p}}(t) \) are the mean plasma enrichments of L-[\( ^{ring-2H4} \)]tyrosine and L-[\( ^{ring-2H5} \)]phenylalanine, respectively, and \( F_p \) is the infusion rate of the phenylalanine tracer.

The fractional rate of mixed-muscle protein synthesis [fractional synthetic rate (FSR)] was calculated in percent per hour by the precursor-product method (27):

\[
\text{FSR} = \frac{\Delta E_p}{E_{\text{precursor}}} \cdot 100 \quad (9)
\]

where \( \Delta E_p \) is the delta increment of muscle protein-bound L-[\( ^{ring-2H5} \)]phenylalanine during the incorporation period, \( E_{\text{precursor}} \) is the average plasma L-[\( ^{ring-2H5} \)]phenylalanine enrichment during the time period for determination of amino acid incorporation, and \( t \) indicates the time interval (h) between biopsies.

Statistics. All data are expressed as means \( \pm \) SE. A two-way repeated-measures ANOVA with time and treatment as factors was used to compare differences between treatments over time. In case of a significant interaction between time and treatment, a Bonferroni post hoc test was applied to locate these differences. For non-time-dependent variables, one-way ANOVA with treatment as factor was used to compare differences between treatments. Statistical significance was set at \( P < 0.05 \). All calculations were performed using the SPSS 15.0.1.1 software package.

RESULTS

Plasma analyses. Plasma insulin concentrations showed a rapid, but short-lived, increase following whey protein ingestion in all groups (Fig. 1A). Peak plasma insulin concentrations were higher following ingestion of 35 g compared with 10 and 20 g whey protein (\( P < 0.05 \)). Plasma glucose concentrations did not change over time or between treatments and averaged 5.2 \( \pm \) 0.1, 5.1 \( \pm \) 0.1, and 5.1 \( \pm \) 0.1 mmol/l in the 10-, 20-, and 35-g experiment, respectively (Fig. 1B).

Plasma phenylalanine, leucine, and essential amino acid (EAA) concentrations over time are illustrated in Fig. 2. Following whey protein ingestion, a rapid increase in plasma EAA concentrations was observed in all groups, with the lowest and highest concentrations following ingestion of 10 and 35 g whey protein, respectively (\( P < 0.01 \)). Plasma phenylalanine concentrations were significantly higher following ingestion of 20 and 35 g compared with the ingestion of 10 g whey protein (\( P < 0.05 \)). The lowest and highest (peak) plasma leucine concentrations were observed in the 10- and 35-g experiment, respectively (\( P < 0.01 \)).

The time course of plasma L-[\( ^{1-13}C \)]phenylalanine and L-[\( ^{ring-2H2} \)]phenylalanine enrichments is illustrated in Fig. 3. Plasma L-[\( ^{1-13}C \)]phenylalanine enrichments (ingested tracer) do not change over time or between treatments and averaged 2.2 \( \pm \) 0.3, 3.4 on July 7, 2017 http://ajpendo.physiology.org/ Downloaded from

![Fig. 1. Plasma insulin (A; mU/l) and glucose (B; mmol/l) concentrations following ingestion of 10 (n = 11), 20 (n = 11), and 35 (n = 11) g whey protein. Values represent means \( \pm \) SE. Data were analyzed with repeated-measures ANOVA (time \( \times \) treatment). In case of a significant interaction, a Bonferroni post hoc test was applied to locate these differences. Time effect, \( P < 0.001 \); treatment effect, \( P < 0.05 \); time \( \times \) treatment interaction, \( P < 0.01 \). *35 g significantly higher compared with 10 g, \( P < 0.05 \).](http://ajpendo.physiology.org/Downloadedfrom)
rapidly increased after ingestion of the test drinks, with the lowest and highest enrichments following ingestion of 10 (n = 11), 20 (n = 11), and 35 (n = 11) g whey protein, respectively (P < 0.01). The plasma L-[ring-2H5]phenylalanine enrichment (infused tracer) decreased following whey protein ingestion in all groups, with lower values observed during the early stages following ingestion of 20 and 35 g compared with 10 g whey protein (P < 0.05). Following this initial decrease, plasma L-[ring-2H5]phenylalanine enrichments slowly returned to baseline levels, with higher values following ingestion of 10 g compared with 20 and 35 g whey protein (P < 0.05).

Whole body protein metabolism. Whole body protein metabolism over time is presented in Fig. 4. Ingestion of the labeled whey protein resulted in a rapid rise in exogenous phenylalanine appearance rate (Fig. 4A), with the lowest and highest (peak) values observed following ingestion of 10 and 35 g whey protein, respectively (P < 0.05). Total exogenous phenylalanine appearance, expressed as AUC over 4 h, was
calculated as a fraction of the total amount of phenylalanine that was ingested (\( \text{Eq. 4} \)). The fraction of dietary phenylalanine that appeared in the systemic circulation during the 4-h postprandial period was 61/1100% and 35/1100% following ingestion of 10, 20, and 35 g whey protein and did not differ among treatments. Endogenous phenylalanine appearance rates decreased following whey protein ingestion and did not differ among treatments, although a trend \( (P = 0.08) \) toward lower values following 35 g compared with 10 g was observed at \( t = 180–240 \) min (Fig. 4B). Total phenylalanine disappearance rate (Fig. 4C) equals the rate of phenylalanine-to-tyrosine conversion, which is the first step in phenylalanine oxidation (Fig. 4D), and the utilization for protein synthesis. Phenylalanine-to-tyrosine conversion rates directly increased following whey protein ingestion in all treatments and returned to baseline values by the end of the 4-h period. The highest amount \( (35 \text{ g}) \) showed greater values at \( t = 45–120 \) and \( t = 90–120 \) min compared with the ingestion of 10 and 20 g whey protein \( (P < 0.01) \), respectively.

Whole body protein metabolism expressed as AUC in the basal period and postprandial period is presented in Fig. 5. Phenylalanine released into the circulation from whole body protein breakdown, expressed as AUC of Endo Ra, decreased in all groups to the same extent following protein ingestion compared with basal values \( (P < 0.01) \). Phenylalanine used for whole body protein synthesis, expressed as AUC of total Rd,

\[
\begin{align*}
\text{AUC}_{\text{Endo Ra}} & = \int \text{Endo Ra} \, dt \\
\text{AUC}_{\text{total Rd}} & = \int \text{total Rd} \, dt \\
\end{align*}
\]

In case of a significant interaction, a Bonferroni post hoc test was applied to locate these differences. Exogenous phenylalanine appearance rates: time effect, \( P < 0.001 \); treatment effect, \( P < 0.001 \); time \( \times \) treatment interaction, \( P < 0.001 \). Endogenous phenylalanine appearance rates: time effect, \( P < 0.001 \); treatment effect, \( P = 0.34 \); time \( \times \) treatment interaction, \( P < 0.05 \). Total phenylalanine disappearance rates: time effect, \( P < 0.001 \); treatment effect, \( P < 0.001 \); time \( \times \) treatment interaction, \( P < 0.001 \). Phenylalanine-to-tyrosine conversion rates: time effect, \( P < 0.001 \); treatment effect, \( P < 0.001 \); time \( \times \) treatment interaction, \( P < 0.001 \). *35 g significantly different compared with 10 g, \( P < 0.05 \); $35 g significantly different compared with 20 g, \( P < 0.05 \); #20 g significantly different compared with 10 g, \( P < 0.05 \).
minus phenylalanine-to-tyrosine conversion rate, increased in all groups following protein ingestion compared with basal values ($P < 0.05$). Whole body protein synthesis was higher following ingestion of 20 and 35 g compared with 10 g whey protein ($P < 0.01$). Protein oxidation, expressed as AUC of phenylalanine-to-tyrosine conversion rate, increased in all groups following protein ingestion compared with basal values ($P < 0.01$). Protein oxidation was higher following ingestion of 35 g compared with 10 g ($P < 0.01$). Whole body net protein balance increased in all groups following protein ingestion compared with basal values ($P < 0.01$), with the lowest and highest values observed following ingestion of 10 and 35 g, respectively ($P < 0.01$).

**Muscle tracer analysis.** The increment in muscle protein-bound $\text{L-[ring-}^{2}\text{H}_{4}\text{]}$phenylalanine enrichment during the basal period (between the first and the second biopsy) was $0.0059 \pm 0.0005$, $0.0059 \pm 0.0005$, and $0.0065 \pm 0.0006$ MPE in the groups that ingested 10, 20, and 35 g whey protein, respectively, and did not differ among treatments. The increment in muscle protein-bound $\text{L-[ring-}^{2}\text{H}_{4}\text{]}$phenylalanine enrichments in the postprandial period (between the second and third biopsy) was $0.0102 \pm 0.0010$, $0.0119 \pm 0.0012$, and $0.0136 \pm 0.0009$ MPE in the 10-, 20-, and 35-g experiment, respectively ($P = 0.08$). The increment in muscle protein-bound $\text{L-[1}\text{-}^{13}\text{C]}$phenylalanine enrichments in the postprandial period differed substantially among experiments ($P < 0.01$; Fig. 6) and was higher following ingestion of 35 g compared with 10 ($P < 0.01$) or 20 ($P < 0.05$) g whey protein.

**Mixed-muscle protein synthesis rates.** Mixed-muscle protein synthesis rates are expressed as FSR and presented in Table 2. In the fasted, basal period, FSR values did not differ among treatments. In contrast, FSR values were significantly different among treatments in the postprandial period ($P < 0.01$). Ingestion of 35 g resulted in significantly higher FSR values when compared with basal values ($P < 0.05$) or ingestion of 10 g whey protein ($P < 0.05$).

### Table 2. Mixed-muscle protein FSR

<table>
<thead>
<tr>
<th>Whey Protein, g</th>
<th>FSR Basal, %/h</th>
<th>FSR Postprandial, %/h</th>
<th>Difference from basal, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.035 ± 0.002</td>
<td>0.037 ± 0.003</td>
<td>−1 ± 14</td>
</tr>
<tr>
<td>20</td>
<td>0.029 ± 0.004</td>
<td>0.041 ± 0.004</td>
<td>16 ± 13</td>
</tr>
<tr>
<td>35</td>
<td>0.052 ± 0.004*</td>
<td>0.052 ± 0.004*</td>
<td>44 ± 16*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; $n = 11$ subjects in each group. Mixed-muscle protein fractional synthetic rates (FSR) in the fasted, basal period and following ingestion of 10, 20, and 35 g whey protein. Data were analyzed with ANOVA. *35 g significantly different compared with 10 g, $P < 0.05$. ‡Significantly different compared with basal, $P < 0.05$.

**DISCUSSION**

In the present study, intrinsically $\text{L-[1}\text{-}^{13}\text{C]}$phenylalanine-labeled whey protein was used to assess digestion and absorption kinetics of whey protein and its subsequent incorporation in newly synthesized muscle protein in vivo in older males. The present study shows that ingestion of 35 g whey protein results in greater amino acid absorption and subsequent stimulation of de novo muscle protein synthesis compared with the ingestion of 10 or 20 g whey protein in healthy, older men.

Whey protein ingestion has been shown to effectively promote postprandial muscle protein accretion in older adults (19). However, information on the impact of the amount of whey protein ingested by older adults on protein digestion, amino acid absorption, and postprandial muscle protein accretion has not been established. Following the ingestion of 10, 20, and 35 g intrinsically $\text{L-[1}\text{-}^{13}\text{C]}$phenylalanine-labeled whey protein, a rapid increase in plasma insulin concentrations (Fig. 1A), plasma amino acid concentrations (Fig. 2), and plasma $\text{L-[1}\text{-}^{13}\text{C]}$phenylalanine enrichments (Fig. 3A) was found, with the lowest and highest values observed following ingestion of 10 and 35 g, respectively. By combining plasma phenylalanine concentrations and tracer enrichments, the fraction of dietary phenylalanine that appeared in the circulation was calculated (Eq. 4). These fractions were $61 \pm 1$, $63 \pm 3$, and $59 \pm 2\%$ in
the groups that received 10, 20, and 35 g whey protein, respectively, and did not differ among treatments. Previous work has suggested that first-pass clearance of amino acids by visceral tissues increases with increasing amounts of protein consumed (26). In agreement, when expressed as absolute amounts, 4 ± 1, 8 ± 1, and 14 ± 1 g whey protein-derived amino acids were retained in the gut and did not appear in the circulation during the 4-h postprandial period after ingesting 10, 20, and 35 g whey protein, respectively. Despite greater retention in the gut, more amino acids became available in the circulation following ingestion of 35 g whey protein compared with the ingestion of 10 or 20 g whey protein in older men.

Exogenous phenylalanine rates of appearance increased rapidly following whey protein ingestion in all groups, with peak values being reached within 30–60 min (Fig. 4A). No other macronutrients were included because we aimed to assess the postprandial response of different amounts of whey protein without possible confounding effects of carbohydrate and/or fat intake on protein digestion and absorption kinetics (10, 17). Consequently, nonisocaloric protein drinks were compared, and, as a result, different insulin responses were observed between experiments (Fig. 1A). Differences in the insulin response are unlikely to have modulated the observed muscle protein synthetic response because increases in plasma EAA concentrations, and not insulin per se, are responsible for stimulating postprandial muscle protein synthesis (5, 16). Circulating insulin levels are regarded permissive rather than modulatory with concentrations of ∼10–15 mU/l being required to allow a maximal muscle protein synthetic response (5, 7, 11, 16). In the present study, plasma insulin levels exceeded these concentrations in all treatments (Fig. 1A). In agreement, the observed plasma insulin levels were also sufficient to maximize the inhibition of protein breakdown in all treatments (Fig. 5).

Muscle protein FSR following whey protein ingestion were calculated under non-steady-state conditions, which may underestimate (peak) postprandial muscle protein synthesis rates (Table 2). Because we were more interested in an aggregated 4-h postprandial response, we also assessed the metabolic fate of whey protein-derived amino acids in de novo muscle protein (Fig. 6). Only ingestion of 35 g whey protein significantly increased muscle protein synthesis rates compared with baseline values. The latter seems to be at odds with the whole body kinetics (Fig. 5), showing a positive whole body protein balance in all treatments. However, whole body protein kinetics do not necessarily represent skeletal muscle tissue, since splanchic tissues and other organs contribute largely to postprandial protein metabolism (26).

The present study shows that ingestion of 35 g whey protein results in greater amino acid absorption and subsequent use for de novo muscle protein synthesis compared with the ingestion of 10 or 20 g whey protein. With habitual protein ingestion in a single meal varying between 10 g (breakfast) and 35 g (dinner) in institutionalized and independently living elderly (24), it has been suggested that increasing the amount of protein at breakfast and/or lunch may represent an effective dietary strategy to stimulate postprandial muscle protein accretion and, as such, improve muscle mass preservation in older adults. It should be noted that the dose-response relationship is likely specific for more rapidly digestible protein sources. Previous work from our laboratory suggests that ingesting greater amounts of more slowly digestible protein does not result in greater amino acid absorption (14, 19). In agreement, we observed a dose-response effect between the amount of protein ingested and subsequent amino acid absorption rates following ingestion of a casein hydrolysate but not intact casein. Besides protein digestion and absorption kinetics, amino acid composition as well as coingestion of other macronutrients are likely to modulate the muscle protein synthetic response following meal ingestion. Nonetheless, the presented work clearly underlines the impact of the amount of protein ingested on subsequent amino acid availability and muscle protein synthesis.

In conclusion, the ingestion of 35 g whey protein results in more amino acids being absorbed and subsequently used for de novo muscle protein synthesis compared with the ingestion of 10 or 20 g whey protein. These observations imply that anabolic resistance to food intake in older adults can, at least partly, be compensated for by ingesting a greater amount of whey protein.

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DISCLOSURES

This trial was registered at clinicaltrials.gov as NCT00557388 and was not funded by external sources.

AUTHOR CONTRIBUTIONS

Author contributions: B.P. and L.J.v.L. conception and design of research; B.P., B.B.G., A.d.L., and A.H.Z. performed experiments; B.P., A.P.G., and J.M.S. analyzed data; B.P. and L.J.v.L. interpreted results of experiments; B.P. prepared figures; B.P. drafted manuscript; B.P. and L.J.v.L. edited and revised manuscript; B.P., B.B.G., and L.J.v.L. approved final version of manuscript.

REFERENCES