Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men[1–3]

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ABSTRACT

Background: Sarcopenia has been attributed to a diminished muscle protein synthetic response to food intake. Differences in digestion and absorption kinetics of dietary protein, its amino acid composition, or both have been suggested to modulate postprandial muscle protein accretion.

Objective: The objective was to compare protein digestion and absorption kinetics and subsequent postprandial muscle protein accretion after ingestion of whey, casein, and casein hydrolysate in healthy older adults.

Design: A total of 48 older men aged 74 ± 1 y (mean ± SEM) were randomly assigned to ingest a meal-like amount (20 g) of intrinsically L-[1-^{13}C]phenylalanine–labeled whey, casein, or casein hydrolysate. Protein ingestion was combined with continuous intravenous L-[ring-$^{2}H_5$]phenylalanine infusion to assess in vivo digestion and absorption kinetics of dietary protein. Postprandial mixed muscle protein fractional synthetic rates (FSRs) were calculated from the ingested tracer.

Results: The peak appearance rate of dietary protein–derived phenylalanine in the circulation was greater with whey and casein hydrolysate than with casein ($P < 0.05$). FSR values were higher after whey (0.15 ± 0.02%/h) than after casein (0.08 ± 0.01%/h; $P < 0.01$) and casein hydrolysate (0.10 ± 0.01%/h; $P < 0.05$) ingestion. A strong positive correlation ($r = 0.66$, $P < 0.01$) was observed between peak plasma leucine concentrations and postprandial FSR values.

Conclusions: Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. This effect is attributed to a combination of whey's faster digestion and absorption kinetics and higher leucine content. This trial was registered at clinicaltrials.gov as NCT00557388.

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INTRODUCTION

Aging is accompanied by a progressive decline in skeletal muscle mass, which is known as sarcopenia (1). Recent data suggest that the skeletal muscle protein synthetic response to food intake is impaired in older adults (2–5). This proposed anabolic resistance is now considered a key factor in the etiology of sarcopenia.

Ingestion of a meal-like amount of protein in the form of free amino acids (6, 7), milk protein (8), or beef (9) strongly stimulates skeletal muscle protein synthesis. This postprandial muscle protein synthetic response depends on the quantity, and, to some extent, the type of protein that is ingested (10). Previous work suggests that whey protein ingestion results in greater postprandial protein retention than does casein ingestion (11, 12). The greater anabolic properties of whey than of casein are mainly attributed to the faster digestion and absorption kinetics of whey, which results in a greater increase in postprandial plasma amino acid availability and thereby further stimulates muscle protein synthesis (13–16). These findings have led to the development of the "fast" and "slow" protein concept (13, 14, 17).

Besides differences in protein digestion and absorption kinetics, whey and casein also markedly differ in their amino acid composition (13, 14, 16). Whereas both proteins contain all the amino acids required to effectively stimulate muscle protein synthesis (6), whey has a considerably higher leucine content (13, 14, 16). The latter may also contribute to the proposed greater anabolic properties of whey than of casein, because leucine has been identified as the main nutritional signal responsible for stimulating postprandial muscle protein accretion (18–21). Consequently, it remains to be elucidated whether the proposed greater anabolic properties of whey than of casein protein are attributed to faster digestion and absorption kinetics or simply to differences in amino acid composition.

We recently observed that, when intact casein is hydrolyzed, in vivo protein digestion and absorption kinetics can be modulated to resemble a fast protein while the amino acid composition is retained (17). This provides us with an important tool to define the characteristics responsible for the proposed anabolic properties of whey as compared with those of casein. Consequently, our objective was to compare protein digestion

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and absorption kinetics and subsequent postprandial muscle protein accretion after the ingestion of whey, intact casein, and hydrolyzed casein in older adults. Because the metabolic fate of amino acids ingested as protein cannot be correctly assessed by oral or intravenous administration of labeled free amino acids (11, 22), we applied specifically produced intrinsically L-[1-^{13}C]phenylalanine labeled milk proteins. Labeled milk proteins were obtained by infusing cows with large quantities of L-[1-^{13}C]phenylalanine, collecting milk, purifying the casein and whey fractions, and hydrolyzing part of the casein (23).

This study provides insight into the characteristics that define the anabolic properties of different types of dietary protein under normal physiologic conditions in older adults by comparing dietary protein digestion and absorption kinetics and subsequent postprandial muscle protein accretion after the ingestion of 20 g whey, casein, and casein hydrolysate in vivo in 48 healthy older men.

SUBJECTS AND METHODS

Subjects

Forty-eight healthy older [mean (±SEM) age: 74 ± 1 y] men participated in this study. The subjects were randomly assigned to ingest a single bolus of whey, casein, or casein hydrolysate. The characteristics of the subjects are presented in **Table 1**.None of the subjects participated in any regular exercise program or had a history of type 2 diabetes, although average glycated hemoglobin concentrations of 6.1–6.2% placed them in the prediabetes category (24). All subjects were informed of 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 (Netherlands).

Pretesting

Before the subjects were selected for this study, they underwent an oral-glucose-tolerance test to assess glucose tolerance and screen for type 2 diabetes according to World Health Organization criteria (25). Before the oral-glucose-tolerance test was conducted, body weight and height were assessed, and body composition was determined by dual-energy X-ray absorptiometry (Discovery A; Hologic, Bedford, MA).

TABLE 1
Characteristics of subjects[1]

	Whey (n = 16)	Casein (n = 16)	Casein hydrolysate (n = 16)
Age (y)	73 ± 1	74 ± 1	74 ± 1
Weight (kg)	75.9 ± 1.5	74.9 ± 2.8	76.4 ± 1.5
BMI (kg/m^2)	25.4 ± 0.4	25.4 ± 0.6	25.0 ± 0.7
Fat (%)	21.9 ± 0.9	20.6 ± 0.9	20.6 ± 0.7
Lean body mass (kg)	59.2 ± 1.2	59.3 ± 1.9	60.7 ± 1.3
Hb A_{1c} (%)	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
$OGTT_0$ glucose (mmol/L)	5.7 ± 0.1	5.7 ± 0.1	5.8 ± 0.2
$OGTT_{120}$ glucose (mmol/L)	6.1 ± 0.3	6.2 ± 0.4	6.4 ± 0.4

[1] All values are means ± SEMs. Hb A_{1c}, glycated hemoglobin; OGTT, oral-glucose-tolerance test. $OGTT_0$, plasma glucose concentrations during basal, fasting conditions; $OGTT_{120}$, plasma glucose concentrations 120 min after ingestion of a 75-g glucose load. Data were analyzed by ANOVA. No differences were observed between groups.

Diet and activity before testing

All subjects consumed a standardized meal (32 ± 2 kJ/kg body weight) consisting of 55% of energy as carbohydrate, 15% of energy as protein, and 30% of energy as fat on the evening before the experiment began. All volunteers were instructed to refrain from any sort of exhaustive physical activity and to keep their diet as constant as possible 3 d before the experiments.

Experiments

Each subject participated in a single 8-h experiment, in which a 20-g bolus of intrinsically L-[1-^{13}C]phenylalanine–labeled protein was ingested. Subjects ingested whey, casein, or casein hydrolysate protein. After the ingestion of the dietary protein, plasma and muscle samples were collected during a 6-h postprandial period. Ingestion of labeled protein was combined with a continuous intravenous L-[ring-$^2H_5$]phenylalanine infusion to simultaneously assess exogenous and endogenous phenylalanine rates of appearance, dietary phenylalanine availability, and mixed muscle protein fractional synthetic rates (FSRs) in vivo in humans.

Protocol

At 0800, after an overnight fast, subjects arrived at the laboratory by car or public. A polytetrafluoroethylene catheter was inserted into an antecubital vein for stable-isotope infusion. A second polytetrafluoroethylene catheter was inserted into a heated dorsal hand vein of the contralateral arm and placed in a hot box (60°C) for arterialized blood sampling (26). After basal blood samples were collected (t = −120 min), the plasma phenylalanine pool was primed with a single intravenous dose of L-[ring-$^2H_5$]phenylalanine (2 μmol/kg), after which a continuous L-[ring-$^2H_5$]phenylalanine infusion was started (0.050 ± 0.001 μmol · kg^{-1} · min^{-1}). After the subjects rested in a supine position for 120 min, a second arterialized blood sample was drawn and a muscle biopsy sample was collected from the vastus lateralis muscle (t = 0 min). Subjects then received a single bolus (250 mL) of test drink containing 20 g intrinsically L-[1-^{13}C]phenylalanine–labeled whey, casein, or casein hydrolysate. Arterialized blood samples were collected at t = 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min; a second muscle biopsy sample was taken from the same incision at t = 180 min; and a third muscle biopsy sample was taken from the vastus lateralis of the contralateral limb at t = 360 min. The biopsy samples from the same incision were taken in a distal and proximal direction, respectively. Blood samples were collected into EDTA-containing tubes and centrifuged at 1000 × g for 5 min at 4°C. Aliquots of plasma were frozen in liquid nitrogen and stored at –80°C. Muscle biopsy samples were obtained 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 (27). Muscle samples were dissected carefully and freed from any visible nonmuscle material. The muscle samples were

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immediately frozen in liquid nitrogen and stored at –80°C until analyzed further.

Preparation of intrinsically labeled protein and beverage composition

Intrinsically L-[1-^{13}C]phenylalanine-labeled milk protein was obtained by infusing a Holstein cow with large quantities of L-[1-^{13}C]phenylalanine, collecting milk, and purifying the casein and whey fractions as described previously (23). Part of the casein fraction was enzymatically hydrolyzed by specific endopeptidases and proline-specific endoprotease by DSM Food Specialties (Delft, Netherlands). The L-[1-^{13}C]phenylalanine enrichments in whey, casein, and casein hydrolysate, assessed by gas chromatography–mass spectrometry (GC-MS) after hydrolysis, were 24.6, 29.4, and 29.4 mole percentage excess (MPE), respectively. The proteins met all chemical and bacteriologic specifications for human consumption. Subjects received a beverage volume of 250 mL to ensure a given dose of 20 g whey, casein, or casein hydrolysate (**Table 2**). The protein drinks provided 1.1, 1.7, and 1.7 mmol L-[1-^{13}C]phenylalanine in whey, casein, and casein hydrolysate, respectively. Drinks were uniformly flavored by adding 5 mL vanilla flavor (Givaudan, Naarden, Netherlands) per liter of beverage.

Plasma analyses

Plasma glucose (Uni Kit III, 07367204; Roche, Basel, Switzerland) concentrations were analyzed with the COBAS-FARA semiautomatic analyzer (Roche). Insulin was analyzed by radioimmunoassay (Insulin RIA kit; Linco Research Inc, St Charles, MO). Plasma (100 μL) for amino acid analyses was deproteinized on ice with 10 mg dry 5-sulfosalicylic acid and then mixed, and the clear supernatant fluid was collected after centrifugation. Plasma amino acid concentrations were measured by HPLC after precolumn derivatization with *o*-phthaldialdehyde (28). For plasma phenylalanine enrichment measurements, plasma phenylalanine was derivatized to its *t*-butyldimethyl-silyl (TBDMS) derivative, and its ^{13}C and ^{2}H enrichments were determined by electron impact ionization GC-MS (model 6890N GC/5973N MSD; Agilent, Little Falls, DE) by using selected ion monitoring of masses 336, 337, and 341 for unlabeled and labeled (1-^{13}C and ring-$^{2}H_5$) phenylalanine, respectively (29). We applied standard regression curves in all isotopic enrichment analyses to assess the linearity of the mass spectrometer and to control for the loss of tracer. Enrichments were corrected for the presence of both the ^{13}C and $^{2}H_5$ phenylalanine isotopes (30).

TABLE 2
Amino acid composition of the proteins[1]

	Whey	Casein	Casein hydrolysate
Alanine (g)	1.0	0.6	0.6
Arginine (g)	0.5	0.7	0.7
Aspartic acid (g)	2.3	1.3	1.3
Cysteine (g)	0.7	0.1	0.1
Glutamic acid (g)	3.2	4.1	4.1
Glycine (g)	0.4	0.3	0.3
Histidine (g)	0.4	0.5	0.5
Isoleucine (g)	1.2	1.1	1.1
Leucine (g)	2.5	1.7	1.7
Lysine (g)	2.1	1.4	1.4
Methionine (g)	0.4	0.5	0.5
Phenylalanine (g)	0.7	0.9	0.9
Proline (g)	0.7	2.1	2.1
Serine (g)	0.7	1.3	1.3
Threonine (g)	0.9	0.8	0.8
Tryptophan (g)	0.5	0.2	0.2
Tyrosine (g)	0.8	1.1	1.1
Valine (g)	1.0	1.3	1.3
Total AA (g)	20.0	20.0	20.0
Total NEAA (g)	10.7	12.1	12.1
Total EAA (g)	9.3	7.9	7.9

[1] Amounts are shown in g per 20 g protein. AA, amino acids; EAA, essential AA; NEAA, non-EAA.

Muscle analyses

For measurement of L-[1-^{13}C]phenylalanine enrichment in the tissue-free amino acid pool and 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 volumes (8× dry weight of isolated muscle fibers × wet/dry ratio) ice-cold 2% perchloric acid were added. The tissue was then homogenized and centrifuged. The supernatant fluid was collected and processed in the same manner as the plasma samples, such that tissue-free L-[1-^{13}C] phenylalanine enrichment could be measured by using its TBDMS derivative on a GC-MS.

The protein pellet was washed with 3 additional 1.5-mL washes of 2% perchloric acid, dried, and hydrolyzed in 6 mol HCl/L at 120°C for 15 to 18 h. The hydrolyzed protein fraction was dried under a nitrogen stream while heated to 120°C, 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; Biorad, Hercules, CA) by using 2 mol NH_4OH/L. The eluate was collected in a vial for the measurement of L-[1-^{13}C]phenylalanine enrichment in mixed muscle protein as described previously (17). In short, L-[1-^{13}C]phenylalanine was derivatized to its N(O,S)-ethoxycarbonyl ethyl esters (31). Thereafter, the ratios of labeled to unlabeled derivatives were determined by GC-C-IRMS (MAT 252; Finnigan, Bremen, Germany). Standard regression curves were applied to assess the linearity of the mass spectrometer and to control for the loss of tracer. The CV for the measurement of L-[1-^{13}C]phenylalanine enrichment in mixed muscle protein averaged 1.1 ± 0.1%.

Calculations

Ingestion of L-[1-^{13}C]phenylalanine protein, intravenous infusion of L-[ring-$^{2}H_5$]phenylalanine, and arterialized blood sampling were used to assess whole-body amino acid kinetics in nonsteady state conditions. Total, exogenous, and endogenous rates of appearance (R_a) and plasma availability of dietary phenylalanine (ie, fraction of dietary phenylalanine that appeared in the systemic circulation; Phe_{plasma}) were calculated by using Steele's equations with modifications (12, 22). These parameters were calculated as follows:

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$$\text{Total R}_a = \frac{F - pV \cdot C(t) \cdot \text{d}E_{iv}/\text{d}t}{E_{iv}(t)} \quad (1)$$

$$\text{ExoR}_\text{a} = \frac{\text{Total R}_\text{a} \cdot E_\text{po}(t) + pV \cdot \text{d}E_\text{po}/\text{d}t}{E_\text{prot}} \quad (2)$$

$$\text{EndoR}_\text{a} = \text{Total R}_\text{a} - \text{ExoR}_\text{a} - F \quad (3)$$

$$\text{Phe}_\text{plasma} = \left(\frac{\text{AUC}_\text{ExoRa}}{\text{Phe}_\text{Prot}}\right) \cdot 100 \quad (4)$$

where F is the intravenous tracer infusion rate (μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}), pV (0.125) is the distribution volume of phenylalanine (22), $C(t)$ is the mean plasma phenylalanine concentration between 2 time points, dE_{iv}/dt represents the time-dependent variations of plasma phenylalanine enrichment derived from the intravenous tracer, and $E_\text{iv}(t)$ is the mean plasma phenylalanine enrichment from the intravenous tracer between 2 consecutive time points. ExoR$_\text{a}$ represents the plasma entry rate of dietary phenylalanine, $E_\text{po}(t)$ is the mean plasma phenylalanine enrichment for the oral tracer, dE_{po}/dt represents the time-dependent variations in plasma phenylalanine enrichment derived from the oral tracer, E_prot is the L-[1-^{13}C]phenylalanine enrichment in the dietary protein, Phe$_\text{Prot}$ is the amount of dietary phenylalanine ingested, and AUC$_\text{ExoRa}$ represents the area under the curve (AUC) of ExoR$_\text{a}$, which corresponds to the amount of dietary phenylalanine that appeared in the blood over a 6-h period after beverage ingestion.

The total phenylalanine rate of disappearance equals the rate of phenylalanine hydroxylation (first step in phenylalanine oxidation) and utilization for protein synthesis. This parameter is calculated as follows:

$$\text{R}_\text{d} = \text{Total R}_\text{a} - pV \cdot \frac{\text{d}C}{\text{d}t} \quad (5)$$

The FSR of mixed muscle protein synthesis was calculated by dividing the increment in enrichment in the product (ie, mixed muscle protein-bound L-[1-^{13}C]phenylalanine), by the enrichment of the precursor. Muscle tissue-free L-[1-^{13}C]phenylalanine enrichments during the decay of enrichment levels were used as a precursor to provide an estimate for the FSRs of mixed muscle proteins during nonsteady state conditions (32). Muscle FSRs were calculated as follows:

$$\text{FSR} = \frac{E_B(t) - E_B(0)}{\int_{t_0}^{t_1} E_F(t) \cdot \text{d}t} \quad (6)$$

where $E_B(t) - E_B(0)$ is the delta increment of mixed muscle protein-bound L-[1-^{13}C]phenylalanine during the 6-h incorporation period, and E_F is the mean muscle tissue-free L-[1-^{13}C]phenylalanine enrichment obtained from muscle biopsy samples at 3 and 6 h.

Statistics

All data are expressed as means ± SEMs. A two-way repeated measures analysis of variance with time and treatment as factors was used to compare differences between treatments over time in older subjects. 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, a one-factor analysis of variance with treatment as factor was used to compare differences between treatments in older subjects. Statistical significance was set at $P < 0.05$. All calculations were performed by using the SPSS 15.0.1.1 software package.

RESULTS

Plasma analyses

Plasma insulin concentrations showed a rapid, but short-lived, increase after protein ingestion in all groups (**Figure 1**). Peak plasma insulin concentrations were higher after whey and casein hydrolysate ingestion than after casein ingestion ($P < 0.05$). Plasma glucose concentrations did not change significantly after protein ingestion and averaged 5.3 ± 0.1, 5.3 ± 0.1, and 5.2 ± 0.1 mmol/L, respectively. Plasma phenylalanine, leucine, and total essential amino acid (EAA) concentrations over time are illustrated in **Figure 2**. After dietary protein ingestion, a rapid increase in plasma EAA concentrations was observed in all groups, with higher peak amino acid concentrations after whey and casein hydrolysate than after casein ingestion ($P < 0.05$). Peak plasma phenylalanine and leucine concentrations were significantly higher after casein hydrolysate than after casein ingestion ($P < 0.05$). In addition, peak plasma leucine concentrations were higher after whey than after casein hydrolysate ingestion ($P < 0.01$).

The time courses of plasma L-[1-^{13}C]phenylalanine and L-[ring-^{2}H$_5$]phenylalanine enrichments are illustrated in **Figure 3**. Plasma L-[1-^{13}C]phenylalanine enrichment (ingested tracer) increased after ingestion of the test drink, with higher peak values observed after ingestion of casein hydrolysate than after ingestion of whey and casein ($P < 0.01$). However, plasma

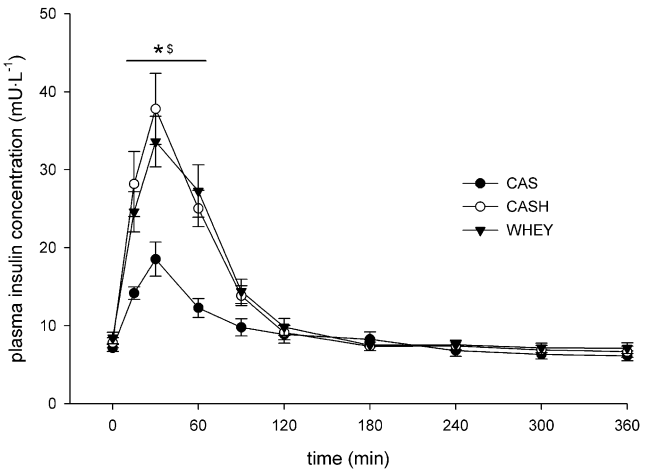

FIGURE 1. Mean (±SEM) plasma insulin concentrations after ingestion of casein (CAS; n = 16), casein hydrolysate (CASH; n = 16), and whey (WHEY; n = 16). Data were analyzed with repeated-measures ANOVA (time × treatment). In case of a significant interaction, a Bonferroni post hoc test was applied to locate the differences. Time effect, $P < 0.001$; treatment effect, $P < 0.01$; time × treatment interaction, $P < 0.001$. *WHEY significantly different from CAS, $P < 0.05$. $CASH significantly different from CAS, $P < 0.05$.

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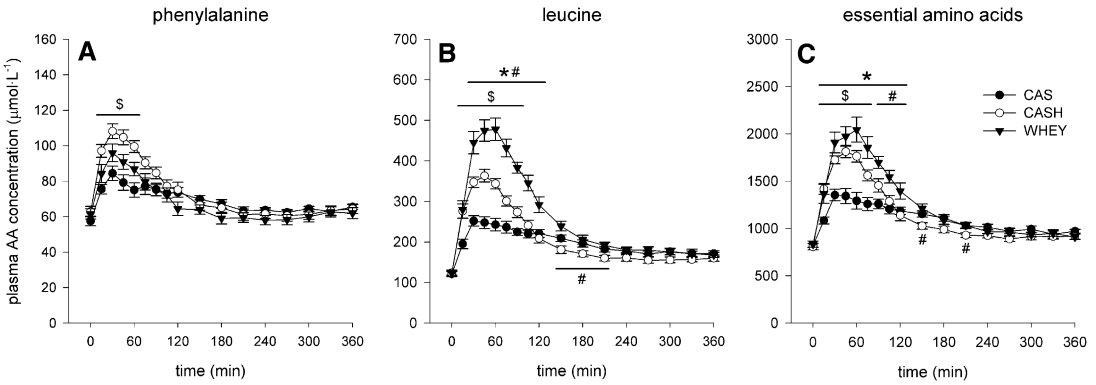

FIGURE 2. Mean (±SEM) plasma phenylalanine (A), leucine (B), and essential amino acid (C) concentrations after ingestion of casein (CAS; n = 16), casein hydrolysate (CASH; n = 16), and whey (WHEY; n = 16). Data were analyzed with repeated-measures ANOVA (time × treatment). In case of a significant interaction, a Bonferroni post hoc test was applied to locate the differences. Plasma phenylalanine concentration: time effect, $P < 0.001$; treatment effect, $P = 0.12$; time × treatment interaction, $P < 0.05$. Plasma leucine concentration: time effect, $P < 0.001$; treatment effect, $P < 0.001$; time × treatment interaction, $P < 0.001$. Plasma essential amino acid (AA) concentration: time effect, $P < 0.001$; treatment effect, $P < 0.001$; time × treatment interaction, $P < 0.001$. *WHEY significantly different from CAS, $P < 0.05$. $^{\$}$CASH significantly different from CAS, $P < 0.05$. $^{\#}$WHEY significantly different from CASH, $P < 0.05$.

L-[1-^{13}C]phenylalanine enrichments were higher in casein than in casein hydrolysate and whey during the later stages of the experimental period ($P < 0.01$). Plasma L-[ring-$^{2}H_5$]phenylalanine enrichment (infused tracer) decreased during all treatments after dietary protein ingestion, and the lowest values were observed during the early stages after casein hydrolysate ingestion ($P < 0.05$). After the initial decrease, plasma L-[ring-$^{2}H_5$]phenylalanine enrichments increased over time, and higher values were observed after whey and casein hydrolysate than after casein ingestion ($P < 0.05$).

Whole-body phenylalanine kinetics

Whole-body phenylalanine kinetics are presented in **Figure 4**. Ingestion of the intrinsically labeled dietary protein resulted in a rapid rise in exogenous phenylalanine appearance rates (Figure 4A), and higher peak values were observed after whey and casein hydrolysate than after casein ingestion ($P < 0.05$). In contrast, during the later stages of the postprandial period, exogenous phenylalanine appearance rates were higher after casein than after whey and casein hydrolysate ingestion ($P < 0.01$). Total exogenous phenylalanine appearance rates (expressed as AUC over 6 h) were expressed as a fraction of the total amount of phenylalanine that was ingested (Equation *4*). The fraction of dietary phenylalanine that appeared in the systemic circulation during the 6 h postprandial period was higher after whey (58 ± 1%) than after casein (53 ± 1%; $P < 0.01$) and casein hydrolysate (55 ± 1%; $P < 0.05$) ingestion. However, no differences were observed between whey and casein hydrolysate ingestion during the first few hours (0–2 h) after protein ingestion (41 ± 1% and 39 ± 1%, respectively). Ingestion of whey and casein hydrolysate resulted in a much higher plasma availability of dietary phenylalanine than did ingestion of casein (25 ± 1%; $P < 0.001$) during this early postprandial phase (0–2 h).

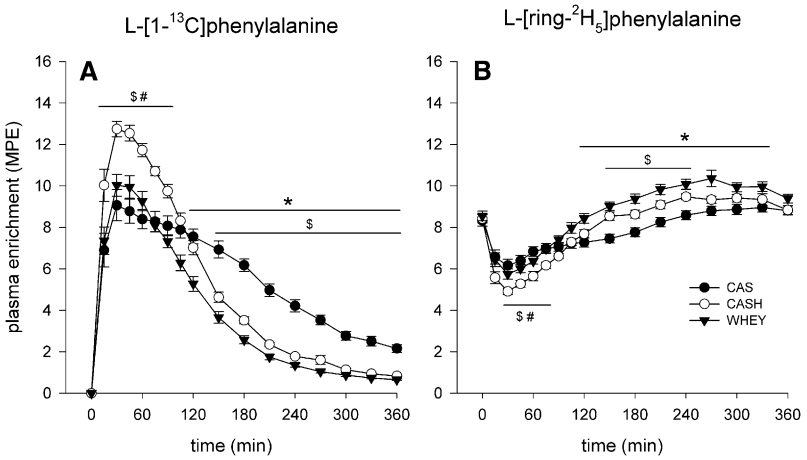

FIGURE 3. Mean (±SEM) plasma L-[1-^{13}C]phenylalanine (A) and L-[ring-$^{2}H_5$]phenylalanine (B) enrichments expressed as mole percentage excess (MPE) after ingestion of casein (CAS; n = 16), casein hydrolysate (CASH; n = 16), and whey (WHEY; n = 16). Data were analyzed with repeated-measures ANOVA (time × treatment). In case of a significant interaction, a Bonferroni post hoc test was applied to locate the differences. Plasma L-[1-^{13}C]phenylalanine enrichment: time effect, $P < 0.001$; treatment effect, $P < 0.001$; time × treatment interaction, $P < 0.001$. Plasma L-[ring-$^{2}H_5$]phenylalanine enrichment: time effect, $P < 0.001$; treatment effect, $P < 0.01$; time × treatment interaction, $P < 0.001$. *WHEY significantly different from CAS, $P < 0.05$. $^{\$}$CASH significantly different from CAS, $P < 0.05$. $^{\#}$WHEY significantly different from CASH, $P < 0.05$.

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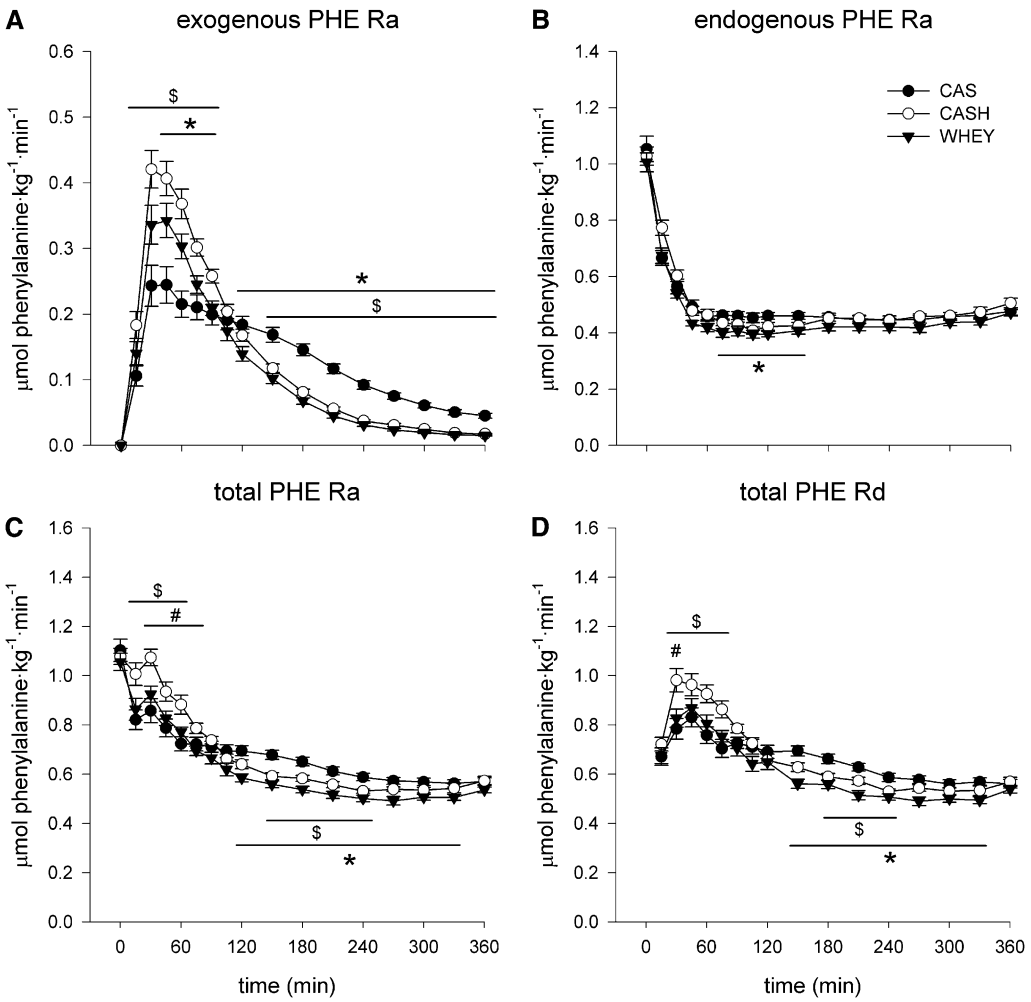

FIGURE 4. Mean (±SEM) whole-body phenylalanine (PHE) kinetics expressed per kg body weight. Exogenous PHE rate of appearance (Ra; A), endogenous PHE R_a (B), total PHE R_a (C), and total PHE rate of disappearance (D; Rd) after ingestion of casein (CAS; n = 16), casein hydrolysate (CASH; n = 16), and whey (WHEY; n = 16). Data were analyzed with repeated-measures ANOVA (time × treatment). In case of a significant interaction, a Bonferroni post hoc test was applied to locate the differences. Exogenous PHE R_a: time effect, $P < 0.001$; treatment effect, $P < 0.001$; time × treatment interaction, $P < 0.001$. Endogenous PHE R_a: time effect, $P < 0.001$; treatment effect, $P = 0.07$; time × treatment interaction, $P < 0.05$. Total PHE R_a: time effect, $P < 0.001$; treatment effect, $P = 0.30$; time × treatment interaction, $P < 0.001$. Total PHE R_d: time effect, $P < 0.001$; treatment effect, $P = 0.26$; time × treatment interaction, $P < 0.001$. *WHEY significantly different from CAS, $P < 0.05$. $^{\$}$CASH significantly different from CAS, $P < 0.05$. $^{\#}$WHEY significantly different from CASH, $P < 0.05$.

Endogenous phenylalanine appearance rates rapidly decreased after protein ingestion, and lower values were observed after ingestion of whey than after ingestion of casein (t = 75–150 min; Figure 4B; $P < 0.05$). Total phenylalanine appearance and disappearance rates (Figures 4C and 4D) were higher during the initial stages after ingestion of casein hydrolysate than after ingestion of casein ($P < 0.05$), whereas rates were higher during the later stages after casein than after whey and casein hydrolysate ingestion ($P < 0.05$).

Muscle tracer analysis

Tissue-free L-[1-^{13}C]phenylalanine enrichments (MPE), determined in muscle samples collected 0, 3, and 6 h after protein ingestion were 0.11 ± 0.03, 2.59 ± 0.17, and 0.61 ± 0.05 for whey; 0.15 ± 0.07, 4.97 ± 0.36, and 1.64 ± 0.13 for casein; and 0.13 ± 0.08, 3.78 ± 0.25, and 0.84 ± 0.08 for casein hydrolysate. Tissue-free enrichments changed significantly over time ($P < 0.001$) and differed between treatments ($P < 0.001$). Protein-bound L-[1-^{13}C]phenylalanine enrichments at 3 and 6 h into the postprandial period were 0.010 ± 0.001 and 0.017 ± 0.002 MPE after whey ingestion, 0.012 ± 0.001 and 0.019 ± 0.002 MPE after casein ingestion, and 0.012 ± 0.001 and 0.017 ± 0.002 MPE after casein hydrolysate ingestion. Protein-bound phenylalanine enrichments increased over time ($P < 0.001$) and did not differ significantly between treatments.

Mixed muscle protein synthesis rates

Mixed muscle protein synthesis rates, expressed as FSRs with tissue-free L-[1-^{13}C]phenylalanine enrichment as precursor, are presented in **Figure 5**. FSR values were higher after whey ingestion (0.15 ± 0.02%/h) than after both casein (0.08 ± 0.01%/h; $P < 0.01$) and casein hydrolysate (0.10 ± 0.01%/h; $P < 0.05$). When plasma L-[1-^{13}C]phenylalanine enrichment was used as

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precursor, calculated FSR values were lower, but showed a similar intervention effect (data not shown).

Correlations

Postprandial FSR values showed a significant positive correlation with the delta peak increase in plasma EAA concentrations ($r = 0.55$, $P < 0.01$). The postprandial rise in plasma leucine concentrations showed the strongest correlation with postprandial FSR values ($r = 0.66$, $P < 0.01$). However, significant correlations were also observed for the rise in plasma isoleucine ($r = 0.60$, $P < 0.01$), tryptophan ($r = 0.45$, $P < 0.01$), threonine ($r = 0.44$, $P < 0.01$), lysine ($r = 0.43$, $P < 0.01$), and valine ($r = 0.39$, $P < 0.05$) concentrations, whereas the rise in phenylalanine ($r = 0.26$, $P = 0.10$) and methionine ($r = 0.20$, $P = 0.21$) concentrations did not show a significant correlation. Correlations between postprandial FSR values and the delta peak increase in plasma EAA (A) and leucine (B) concentrations are illustrated in **Figure 6**.

DISCUSSION

In the current study, we compared dietary protein digestion and absorption kinetics and subsequent muscle protein accretion after the ingestion of 20 g whey, casein, and casein hydrolysate in healthy older men. Intrinsically, L-[1-^{13}C]phenylalanine milk proteins were ingested, in combination with continuous intravenous infusion of L-[ring-$^{2}H_5$]phenylalanine, to allow insight into the metabolic fate of dietary protein-derived amino acids after protein ingestion. Protein-derived phenylalanine appeared more rapidly in the circulation after whey and casein hydrolysate ingestion than after casein ingestion. Whey protein ingestion resulted in greater mixed muscle protein FSRs than did casein and casein hydrolysate administration. Postprandial FSR values correlated strongly with the rise in plasma leucine concentration.

In older adults, ingestion of whey protein has been suggested to effectively increase postprandial protein retention when compared with casein ingestion (11, 12). Much of this effect can be attributed to apparent differences in digestion and absorption kinetics between these proteins (13, 14). We observed a more rapid rise in plasma phenylalanine, leucine, and total EAA concentrations after whey than after casein ingestion (Figure 2). In addition, a more rapid rise in plasma L-[1-^{13}C]phenylalanine enrichment was observed after whey than after casein ingestion (Figure 3). In agreement, whole-body tracer kinetics showed a much greater appearance rate of protein-derived phenylalanine in the circulation during the first 2 h after whey than after casein ingestion (Figure 4A). These findings agree with previous work that identified whey as a fast and casein as a slow digestible protein (13, 14).

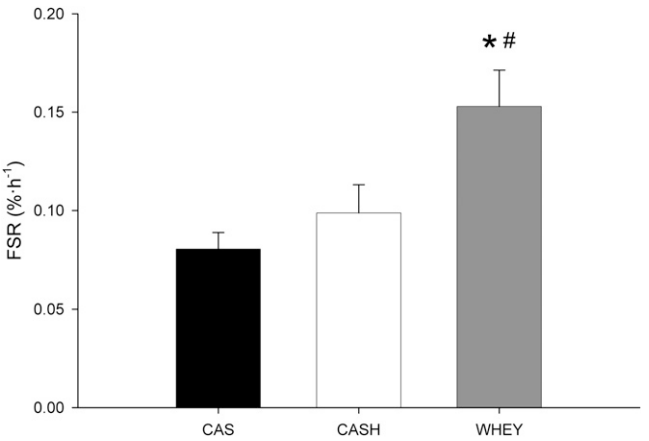

FIGURE 5. Mean (±SEM) mixed muscle protein fractional synthetic rates (FSR), with tissue-free L-[1-^{13}C]phenylalanine enrichments as precursor, after ingestion of casein (CAS; $n = 16$), casein hydrolysate (CASH; $n = 16$), and whey (WHEY; $n = 16$). Data were analyzed with ANOVA with Bonferroni correction. *WHEY significantly different from CAS, $P < 0.01$. #WHEY significantly different from CASH, $P < 0.05$.

Besides differences in digestion and absorption kinetics, whey and casein also differ markedly in their amino acid composition (Table 2). Consequently, the proposed greater anabolic properties of whey than of casein could be attributed to differences in digestion and absorption kinetics as well as to differences in amino acid composition between these proteins. In trying to define the characteristics responsible for the proposed greater anabolic properties of whey than of casein, we hydrolyzed part of the intrinsically labeled casein to obtain a casein hydrolysate. The latter provided us with an important tool to investigate the effect of differences in amino acid composition and digestion and absorption kinetics on postprandial muscle protein synthesis, because a casein hydrolysate has digestion and absorption kinetics similar to those of whey protein (13, 17) while retaining its unique amino acid composition (Table 2). In accordance, we observed similar dietary protein-derived phenylalanine appearance rates after ingestion of whey and casein hydrolysate (Figure 4A). Furthermore, the amount of dietary phenylalanine that appeared in the circulation directly after protein intake (0–2 h) did not differ after whey and casein hydrolysate ingestion and was higher than that after casein ingestion. However, plasma dietary phenylalanine availability over the total 6-h postprandial period was higher after whey (58 ± 1%) than after casein hydrolysate (55 ± 1%; $P < 0.05$) and casein (53 ± 1%; $P < 0.01$) ingestion. The latter finding implies that more dietary amino acids became available after whey than after casein hydrolysate and casein ingestion.

In the postprandial phase, a rapid increase in plasma EAA concentrations was observed in all groups, and peak plasma EAA concentrations were higher after whey and casein hydrolysate than after casein ingestion (Figure 2C). Specific differences in plasma EAA concentrations after protein ingestion were observed when individual EAAs were assessed. In particular, peak plasma leucine concentrations differed significantly between treatments, and the highest and lowest peak concentrations were observed after whey and casein ingestion, respectively (Figure 2B). Because the rate of appearance of dietary amino acids from the gut was greater after casein hydrolysate than after casein ingestion (Figure 4A), it is evident that the greater rise in plasma leucine concentration after casein hydrolysate administration (Figure 2B) was entirely attributed to its accelerated digestion and absorption kinetics. However, despite similar protein digestion and absorption kinetics after whey and casein hydrolysate ingestion, a greater rise in plasma leucine concentration was observed after whey than after casein hydrolysate ingestion. The latter finding was largely attributed to the greater leucine content of whey (12.5%) than of casein hydrolysate (8.5%). This difference might be of substantial relevance, because the

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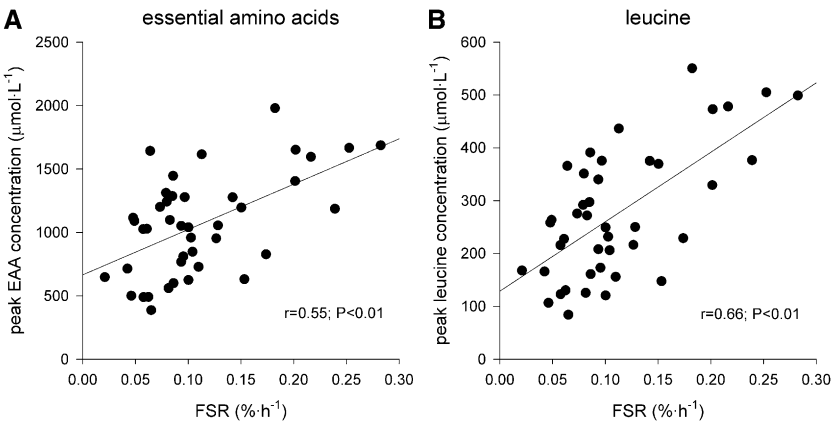

FIGURE 6. Correlations between postprandial mixed muscle protein fractional synthetic rates (FSR) and delta peak plasma essential amino acid (EAA; A) and leucine (B) concentrations. EAA ($r = 0.55$, $P < 0.01$) and leucine ($r = 0.66$, $P < 0.01$). Data were analyzed with 2-tailed tests of significance by using Pearson's correlation coefficients ($n = 48$).

postprandial rise in plasma leucine concentration has been proposed to be responsible for the increase in mixed muscle protein synthetic rate (18–21).

To evaluate the postprandial muscle protein synthetic response to the ingestion of the different proteins sources, mixed muscle protein FSRs were calculated on the basis of the ingested L-[1-^{13}C]phenylalanine tracer. This allowed us to compare the capacity of the different protein sources to stimulate de novo postprandial muscle protein accretion. Mixed muscle protein FSR values assessed after whey ingestion were higher when compared with casein ($P < 0.01$) and casein hydrolysate ($P < 0.05$; Figure 5). These FSR values clearly showed that whey ingestion stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men.

In this study, we found significant correlations between the postprandial rise (delta peak) in plasma EAA concentrations and FSR values (Figure 6A). The postprandial rise in plasma leucine concentrations showed the strongest positive correlation ($r = 0.66$; $P < 0.01$) with postprandial FSR values (Figure 6B). This seems to be in line with the suggestion that leucine forms a key factor regulating postprandial muscle protein synthesis (18–21). We speculated that the postprandial rise in plasma EAA concentration, particularly leucine, defines the subsequent postprandial rate of muscle protein synthesis. In the current study, we observed a greater rise in plasma leucine concentrations after whey than after casein and casein hydrolysate ingestion. This difference was attributed to faster digestion and absorption kinetics and to the higher leucine content of the protein, which resulted in peak plasma leucine concentrations that were considerably higher after whey than after casein and casein hydrolysate ingestion (526 ± 21, 282 ± 13, and 381 ± 14 μmol/L, respectively).

The current study was the first to compare in vivo dietary protein digestion and absorption kinetics and subsequent postprandial muscle protein accretion after the ingestion of a meal-like amount of whey, intact casein, and hydrolyzed casein in healthy older men. Whey protein is more effective than casein and casein hydrolysate at promoting postprandial muscle protein accretion in healthy older men. The greater muscle protein synthetic response to whey ingestion is likely attributable to both its faster digestion and absorption kinetics and higher leucine content, which thereby further increases the postprandial rise in plasma leucine concentrations. These data are of great clinical relevance to the determination of more effective nutritional strategies to attenuate age-related losses of muscle mass.

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The authors' responsibilities were as follows—BP and LJCvL: designed the study; BP: organized and carried out the clinical experiments with the assistance of JMGS; JMGS and APG: performed the stable-isotope analyses; BP: performed the statistical analysis of the data and wrote the manuscript together with LJCvL and YB; and HK: provided medical assistance. None of the authors had a personal or financial conflict of interest.

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