Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein[1–3]

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ABSTRACT

Background: It has been suggested that a protein hydrolysate, as opposed to its intact protein, is more easily digested and absorbed from the gut, which results in greater plasma amino acid availability and a greater muscle protein synthetic response.

Objective: We aimed to compare dietary protein digestion and absorption kinetics and the subsequent muscle protein synthetic response to the ingestion of a single bolus of protein hydrolysate compared with its intact protein in vivo in humans.

Design: Ten elderly men (mean ± SEM age: 64 ± 1 y) were randomly assigned to a crossover experiment that involved 2 treatments in which the subjects consumed a 35-g bolus of specifically produced L-[1-^{13}C]phenylalanine-labeled intact casein (CAS) or hydrolyzed casein (CASH). Blood and muscle-tissue samples were collected to assess the appearance rate of dietary protein–derived phenylalanine in the circulation and subsequent muscle protein fractional synthetic rate over a 6-h postprandial period.

Results: The mean (±SEM) exogenous phenylalanine appearance rate was 27 ± 6% higher after ingestion of CASH than after ingestion of CAS ($P < 0.001$). Splanchnic extraction was significantly lower in CASH compared with CAS treatment ($P < 0.01$). Plasma amino acid concentrations increased to a greater extent (25–50%) after the ingestion of CASH than after the ingestion of CAS ($P < 0.01$). Muscle protein synthesis rates averaged 0.054 ± 0.004% and 0.068 ± 0.006%/h in the CAS and CASH treatments, respectively ($P = 0.10$).

Conclusions: Ingestion of a protein hydrolysate, as opposed to its intact protein, accelerates protein digestion and absorption from the gut, augments postprandial amino acid availability, and tends to increase the incorporation rate of dietary amino acids into skeletal muscle protein. *Am J Clin Nutr* 2009;90:106–15.

INTRODUCTION

Food intake promotes net muscle protein accretion by providing ample amino acids (AAs) as precursors for protein assembly (1). The quantity and quality of the ingested protein, ie, its digestibility and AA composition, represent important factors that modulate the anabolic response of skeletal muscle to dietary protein ingestion (2).

The ingestion of a protein hydrolysate, as opposed to its intact protein, has been proposed to facilitate protein digestion and absorption, increase plasma AA availability, and thereby augment the postprandial muscle protein synthetic response (3). A more rapid increase in circulating plasma AA concentrations has previously been reported after the ingestion of a protein hydrolysate compared with its intact protein (3). However, absolute changes in plasma AA concentrations do not necessarily represent changes in the appearance rate of exogenous (dietary) AAs (4). Although some studies have measured gastric emptying (3), nitrogen excretion (5), and gut endogenous nitrogen flow (6), direct evidence that supports the proposed differences in digestion and absorption kinetics after the ingestion of a protein hydrolysate, compared with its intact protein in vivo in humans, remains lacking. This is partly due to the restrictions set by the methodology that has been used to assess the appearance rate of AAs from the gut into the circulation. Because free AAs and protein-derived AAs exhibit a different timing and efficiency of intestinal absorption (7), simply adding labeled free AAs to a protein-containing drink does not provide an accurate measure of the digestion and absorption kinetics of the ingested dietary protein (8). To accurately assess the appearance rate of AAs derived from dietary protein, the labeled AAs need to be incorporated into the dietary protein source (7, 9). Therefore, we produced highly enriched L-[1-^{13}C]phenylalanine–labeled milk, purified the casein fraction, and enzymatically hydrolyzed part of the casein. This complex approach was required to allow true insight into the effect of different dietary protein sources on the subsequent digestion and absorption kinetics in vivo in humans.

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In this study, we hypothesize that the ingestion of a protein hydrolysate accelerates protein digestion and the absorption rate, which results in a greater increase in plasma AA availability and the muscle protein synthesis rate when compared with the ingestion of its intact protein. To test that hypothesis, elderly men were given a single bolus of specifically produced intrinsically L-[1-^{13}C]phenylalanine–labeled intact casein (CAS) or casein hydrolysate (CASH), combined with continuous intravenous L-[ring-1-$^{2}H_5$]phenylalanine, L-[1-^{13}C]leucine, and L-[ring-$^{2}H_2$]tyrosine infusion.

SUBJECTS AND METHODS

Subjects

Ten healthy, elderly, male volunteers [mean ± SEM: age: 64 ± 1 y; weight: 78.8 ± 3.1 kg; height: 1.78 ± 0.02 m; body mass index (in kg/m^2): 24.7 ± 0.7; basal glucose: 5.44 ± 0.07 mmol/L; basal insulin: 9.99 ± 1.28 mU/L; homeostasis model assessment of insulin resistance (HOMA-IR): 2.43 ± 0.32] who had no history of participating in any regular exercise program took part in this study. Subject recruitment was initiated on 26 March 2007. All subjects were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht.

Pretesting

All subjects performed an oral-glucose-tolerance test before inclusion in the study. After an overnight fast, subjects arrived at the laboratory at 0800 by car or public transportation. Body weight was measured with a digital balance with an accuracy of 0.001 kg (E1200; August Sauter GmbH, Albstadt, Germany). A catheter (Baxter BV, Utrecht, Netherlands) was inserted into an antecubital vein, and a resting blood sample was drawn after which 75 g glucose (dissolved in 250 mL water) was ingested. Thereafter, blood was sampled every 30 min until t = 120 min. Plasma glucose concentrations were measured to determine glucose intolerance and/or the presence of type 2 diabetes according to the 2006 American Diabetes Association guidelines (10).

Diet and activity before testing

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

Experiments

Each subject participated in a randomized, double-blind crossover design. All subjects were studied on 2 occasions that were separated by 14 d, in which drinks containing CAS or CASH were administered. After the ingestion of the given bolus of the test drink, plasma and muscle samples were collected during a 6-h measurement period. These experiments were designed to simultaneously assess the exogenous and endogenous rate of appearance of phenylalanine, splanchnic phenylalanine extraction, and the fractional synthetic rate (FSR) of mixed muscle protein in the vastus lateralis muscle.

Protocol

At 0800, after an overnight fast, subjects arrived at the laboratory by car or public transportation. 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. After basal blood sample collection (t = −120 min), plasma phenylalanine, leucine, and tyrosine pools were primed with a single intravenous dose of the AA tracers L-[ring-$^{2}H_5$]phenylalanine (2 μmol/kg), L-[ring-$^{2}H_2$]tyrosine (0.775 μmol/kg), and L-[1-^{13}C]leucine (5.06 μmol/kg). Thereafter, continuous tracer infusion was started with an infusion rate of 0.046 ± 0.001 μmol · kg^{-1} · min^{-1} for L-[ring-$^{2}H_5$]phenylalanine, 0.017 ± 0.000 μmol · kg^{-1}· min^{-1} for L-[ring-$^{2}H_2$]tyrosine, and 0.110 ± 0.002 μmol · kg^{-1} · min^{-1} for L-[1-^{13}C]leucine. Thereafter, subjects rested in a supine position for 2 h, after which an arterialized blood sample and a muscle biopsy from the vastus lateralis muscle were collected (t = 0 min). Subjects then received a bolus (4.5 ml/kg) of a given test drink containing 35 g intrinsically L-[1-^{13}C]phenylalanine–labeled protein. Arterialized blood samples were collected at t = 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210, 240, 270, 300, 330, and 360 min with a second muscle biopsy taken at t = 360 min from the contralateral limb.

Blood samples were collected in EDTA-containing tubes and centrifuged at 1000 × g and 4°C for 5 min. Aliquots of plasma were frozen in liquid nitrogen and stored at –80°C. Muscle biopsies 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 (11). Muscle samples were dissected carefully and freed from any visible nonmuscle material. The muscle sample was immediately frozen in liquid nitrogen and stored at –80°C until analysis.

Preparation of intrinsically labeled protein and beverage composition

Intravenous L-[1-^{13}C]phenylalanine administration was applied in 2 cows to produce intrinsically L-[1-^{13}C]phenylalanine–labeled milk proteins. Two Holstein dairy cows [mean (±SEM) body wt (BW): 726 ± 38 kg at 26 ± 2 d of lactation] were infused with a large amount of L-[1-^{13}C]phenylalanine via the jugular vein by using a peristaltic pump at a rate of 4.16 mL/min (402 μmol phenylalanine/min) for 44–48 h. The cows were milked every 12 h during infusion and for the subsequent 6 h after cessation of infusion. Casein and whey protein were separated from the collected milk by microfiltration and ultrafiltration as described previously (8). Part of the casein fraction was enzymatically hydrolyzed by specific endopeptidases and praline-specific endoprotease (PeptoPro process) by DSM Food Specialties (Delft, Netherlands) (12). The L-[1-^{13}C]phenylalanine enrichments in the CAS and CASH proteins, which were assessed by gas chromatography-mass spectrometry after hydrolysis,

were highly enriched [29.2 and 28.9 mole percent excess (MPE), respectively]. The proteins met chemical and bacteriologic specifications for human consumption.

Subjects received a beverage volume of 350 ml to ensure a given dose of 35 g CAS or CASH. The CAS and CASH were isonitrogenous (0.070 ± 0.002 compared with 0.070 ± 0.002 g N/kg BW) and provided 142 ± 6 compared with 134 ± 6 μmol phenylalanine/kg BW, 141 ± 6 compared with 135 ± 6 μmol tyrosine/kg BW, and 322 ± 13 compared with 306 ± 13 μmol leucine/kg BW, respectively. To make the taste comparable in all treatments, beverages were uniformly flavored by adding 0.375 g sodium saccharinate, 0.9 g citric acid, and 5 ml vanilla flavor (Quest International, Naarden, Netherlands) per liter of beverage. Treatments were performed in a randomized order with test drinks being provided in a double-blind fashion.

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 AA analyses was deproteinized on ice with 10 mg dry 5-sulphosalicylic acid and mixed, and the clear supernatant was collected after centrifugation. Plasma AA concentrations were determined by HPLC after precolumn derivatization with *o*-phthaldialdehyde (13). For plasma phenylalanine, tyrosine, and leucine enrichment measurements, plasma phenylalanine, tyrosine, and leucine were derivatized to their *t*-butyldimethylsilyl derivatives, and their ^{13}C or ^{2}H enrichments were determined by electron ionization (by gas chromatography-mass spectrometry; Agilent 6890N GC/5973N MSD; 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; of masses 466, 467, 468, and 470 for unlabeled and labeled (1-^{13}C, ring-$^{2}H_2$, and ring-$^{2}H_4$) tyrosine, respectively; and of masses 302 and 303 for unlabeled and labeled leucine (14). For plasma α-ketoisocaproate (KIC) enrichment measurements, plasma KIC was derivatized to its *N*-methyl-*N*-(*Tert*-butyldimethylsilyl) trifluoroacetamide derivative, and its ^{13}C enrichment was assessed by monitoring masses 301 and 302 for unlabeled and labeled KIC, respectively (15). We applied standard regression curves in all isotopic enrichment analysis to assess linearity of the mass spectrometer and to control for the loss of tracer.

Muscle sample analyses

For measurement of L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine enrichment in the free AA pool and mixed muscle protein, 55 mg 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 (2–3 mg) was weighed, and 8 volumes (8 times dry weight of isolated muscle fibers × wet:dry ratio) of ice-cold 2% perchloric acid were added. The tissue was then homogenized and centrifuged. The supernatant was collected and processed in the same manner as the plasma samples, such that intracellular free L-[1-^{13}C]phenylalanine, L-[1-^{13}C]tyrosine, L-[ring-$^{2}H_2$]tyrosine, L-[ring-$^{2}H_4$]tyrosine, and L-[1-^{13}C]leucine enrichments could be measured by using their *t*-butyldimethylsilyl derivatives on a gas chromatography-mass spectrometer.

The protein pellet was washed with 3 additional 1.5-ml washes of 2% perchloric acid, dried, and hydrolyzed in 6 mol/L HCl at 120°C for 15–18 h. The hydrolyzed protein fraction was dried under a nitrogen stream while heated to 120°C and 50% acetic acid solution was added to one vial, 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/L NH_4OH. Thereafter, the eluate was dried, and the purified AAs were derivatized to their *N(O,S)*-ethoxycarbonyl ethyl esters for the determination of $^{13}C/^{12}C$ ratios of muscle protein-bound phenylalanine and leucine (16). Thereafter the derivative was measured by gas chromatography-isotope ratio mass spectrometry (Finnigan MAT 252; Bremen, Germany) by using the Ultra I GC-column (no. 19091A-112; Hewlett-Packard, Palo Alto, CA) and combustion interface II and by monitoring ion masses 44, 45, and 46. By establishing the relation between the enrichment of a series of L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine standards of variable enrichment and the enrichment of the *N(O,S)*-ethoxycarbonyl ethyl esters of these standards, the muscle-protein-bound enrichment of phenylalanine and leucine was determined. We applied standard regression curves to assess the linearity of the mass spectrometer and to control for loss of the tracer. The CV for the measurement of L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine enrichment in mixed muscle protein averaged 1.0 ± 0.1% and 1.1 ± 0.1%, respectively.

Calculations

Ingestion of L-[1-^{13}C]phenylalanine-labeled protein, intravenous infusion of L-[ring-$^{2}H_5$]phenylalanine, L-[ring-$^{2}H_2$]tyrosine, and L-[1-^{13}C]leucine, and arterialized blood sampling were used to assess whole-body AA kinetics in nonsteady state conditions. Total, exogenous, and endogenous rates of appearance (R_a) and splanchnic extraction (ie, the fraction of dietary AA taken up by the gut and liver during the first pass) for phenylalanine was calculated by using modified Steele's equations (7, 15). These variables were calculated as follows:

$$\text{Total } R_a = \frac{F - pV \cdot C(t) \cdot dE_{iv}/dt}{E_{iv}t} \quad (1)$$

$$\text{Exo } R_a = \frac{\text{Total } R_a \cdot E_{po}(t) + pV \cdot dE_{po}/dt}{E_{prot}} \quad (2)$$

$$\text{Endo } R_a = \text{Total } R_a - \text{Exo } R_a - F \quad (3)$$

$$Sp = 100 \times \left(\frac{\text{Phe}_{\text{Prot}} - \text{AUC}_{\text{ExoPhe}Ra}}{\text{Phe}_{\text{Prot}}}\right) \quad (4)$$

where F is the intravenous tracer infusion rate (μmol · kg^{-1} · min^{-1}), pV (0.125) is the distribution volume for phenylalanine (17), and $C(t)$ is the mean plasma phenylalanine concentration between 2 time points. dE_{iv}/dt represents the time-dependent variations of plasma phenylalanine enrichment (expressed in the tracer:tracee ratio, or the TTR) derived from the intravenous tracer, and $E_{iv}(t)$ is the mean plasma phenylalanine enrichment from the intravenous tracer between 2 consecutive time points. Exo R_a represents the plasma entry rate of dietary phenylalanine,

$E_{po}(t)$ is the mean plasma phenylalanine enrichment for the oral tracer, dE_{po}/dt represents the time-dependent variations of plasma phenylalanine enrichment derived from the oral tracer, and E_{prot} is the L-[1-^{13}C]phenylalanine enrichment in the dietary protein. Phe_{Prot} is the amount of dietary phenylalanine ingested, $AUC_{ExoPheRa}$ represents the area under the curve (AUC) of Exo Phe R_a, which corresponds to the amount of dietary phenylalanine that appeared in the blood over a 6-h period after drink intake. To determine total leucine, R_a and R_d, calculations were performed by using both plasma leucine MPE and KIC MPE as precursors. Because the conclusions were identical whichever precursor pool was used in these calculations for whole-body fluxes, we present only the results using plasma L-[1-^{13}C]leucine.

The total rate of disappearance of phenylalanine equals the rate of phenylalanine hydroxylation and utilization for protein synthesis. These variables can be calculated as follows:

$$R_d = \text{Total } R_a - pV \times \frac{dC}{dt} \quad (5)$$

$$\text{Phe hydroxylation} = \text{Tyr } R_a \times \frac{E_p(t)}{E_t(t)} \times \frac{\text{Phe } R_d}{(F_p + \text{Phe } R_d)} \quad (6)$$

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

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

The FSR of mixed muscle protein synthesis was calculated by dividing the increment in the enrichment of the product, ie, the protein-bound L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine, by the enrichment of the precursor. Plasma L-[1-^{13}C]phenylalanine and L-[1-^{13}C]KIC enrichments were used to provide an estimate for the true FSR of mixed muscle proteins. Plasma L-[1-^{13}C]KIC was used as a precursor for the calculation of FSR instead of plasma L-[1-^{13}C]leucine enrichment because it has been shown to be more representative of the intracellular leucine enrichment (18). Muscle FSRs were calculated as follows (19):

$$FSR = \frac{\Delta Ep}{E_{\text{precursor}} \times t} \times 100 \quad (9)$$

where ΔE_p is the Δ increment of protein-bound L-[1-^{13}C]phenylalanine, L-[ring-$^{2}H_5$]phenylalanine, and L-[1-^{13}C]leucine during incorporation periods. $E_{precursor}$ is the average plasma L-[1-^{13}C]phenylalanine and L-[1-^{13}C]KIC enrichment during the time period for determination of AA incorporation (20). t indicates the time interval (h) between biopsies.

Statistics

A complete randomized design was used to assess the effect of the ingestion of intact protein (CAS) or protein hydrolysate (CASH) on plasma AA kinetics and whole-body and muscle protein synthesis rates in elderly men (n = 10). All data are expressed as means ± SEMs. Calculation of the required sample size was based on the effect size and variance observed in previous studies from our laboratory (12, 19, 21). We calculated the sample size by using the following variables: the difference in FSR >20% and a SD of 15% with a type I error of 5% and a type II error of 10%. Power calculations showed that ≥9 subjects were needed, and therefore 10 elderly men were included in this study. The plasma insulin, glucose, phenylalanine, tyrosine, and branched-chain AA (leucine, isoleucine, and valine) responses were calculated as the AUC above baseline values. A 2-factor repeated measures analysis of variance (ANOVA, general linear model) with time (df: 19) and treatment (df: 1) as factors was used to compare differences between treatments over time. In the case of significant interaction between time and treatment, a Scheffe post hoc test was applied to locate these differences. For non-time-dependent variables, a paired t test was performed to detect differences between treatments. Statistical significance was set at $P < 0.05$. All calculations were performed by using SPSS version 12.0 (SPSS Inc, Chicago, IL).

RESULTS

Plasma analyses

Plasma insulin concentrations increased to a greater extent in the CASH compared with the CAS treatment (**Figure 1**). Peak plasma insulin concentrations (individual peak values) averaged 50.2 ± 7.6 and 26.2 ± 3.7 mU/L in the CASH and CAS treatment, respectively ($P < 0.01$). The plasma insulin response, expressed as the AUC above baseline values, was significantly greater after the ingestion of CASH compared with CAS (Figure 1 inset; $P < 0.05$). Plasma glucose responses averaged 25.5 ± 34.4 and −3.2 ± 16.1 mmol · 6 h · L^{-1} in the CAS and CASH treatment, respectively, with no significant differences between treatments ($P = 0.46$).

Plasma phenylalanine, tyrosine, leucine, valine, and isoleucine concentrations over time are reported in **Figure 2**. Generally, plasma AA concentrations increased and remained elevated throughout the 6-h measurement period after CAS ingestion.

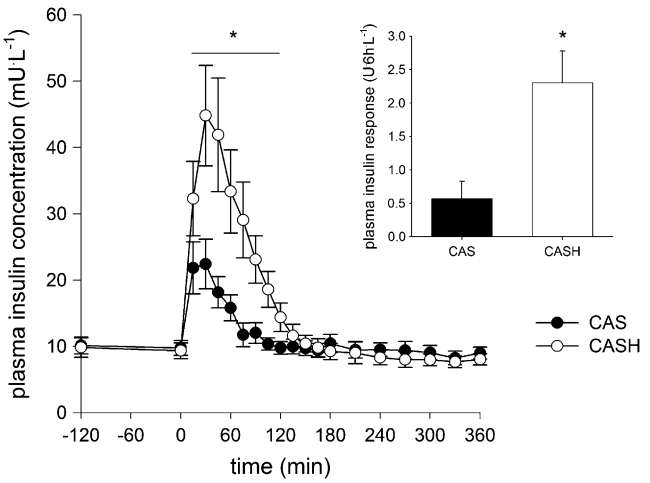

FIGURE 1. Mean (±SEM) plasma insulin concentrations (mU/ L) and insulin response (expressed as area under the curve minus baseline values) in elderly men (n = 10) after ingestion of 35 g casein (CAS) or casein hydrolysate (CASH). The horizontal line indicates the time period over which significant differences were observed between treatments. Data were analyzed with a 2-factor repeated measures ANOVA (time × treatment): time effect: $P < 0.01$; treatment effect: $P < 0.01$; interaction of time and treatment: $P < 0.01$. *Significantly different from CAS, $P < 0.05$ (paired t test).

Plasma AA concentrations increased to a greater extent after ingestion of CASH with ≈25–50% higher peak AA concentrations in the CASH compared with the CAS treatment. In contrast, 4–6 h after ingestion of the drink, plasma leucine and isoleucine concentrations were significantly lower in the CASH compared with the CAS treatment (Figure 2; $P < 0.05$). The plasma phenylalanine response averaged 6.7 ± 0.8 compared with 5.3 ± 1.5 mmol · 6 h · L^{-1} in the CASH and CAS treatments, respectively; $P = 0.25$). The plasma tyrosine response (AUC) was significantly higher in the CASH compared with the CAS treatment (18.3 ± 1.1 compared with 9.7 ± 0.8 mmol · 6 h · L^{-1}, respectively; $P < 0.01$). In addition, plasma leucine, valine, and isoleucine responses (AUC) were significantly higher in the CASH compared with the CAS treatment (42.7 ± 2.3 compared with 32.6 ± 1.8, 54.9 ± 2.9 compared with 36.7 ± 2.5, and 22.0 ± 1.2 compared with 17.7 ± 0.7 mmol · 6 h · L^{-1}, respectively; $P < 0.01$).

The time courses of the plasma L-[1-^{13}C]phenylalanine, L-[ring-$^{2}H_5$]phenylalanine, L-[1-^{13}C]leucine, L-[1-^{13}C]KIC, L-[ring-$^{2}H_2$]tyrosine, and L-[ring-$^{2}H_4$]tyrosine enrichments are shown in **Figure 3**. The plasma L-[1-^{13}C]phenylalanine enrichment (originating from the intrinsically labeled protein) quickly increased after ingestion of the test drink with higher peak values (individual peak values) observed after ingestion of CASH

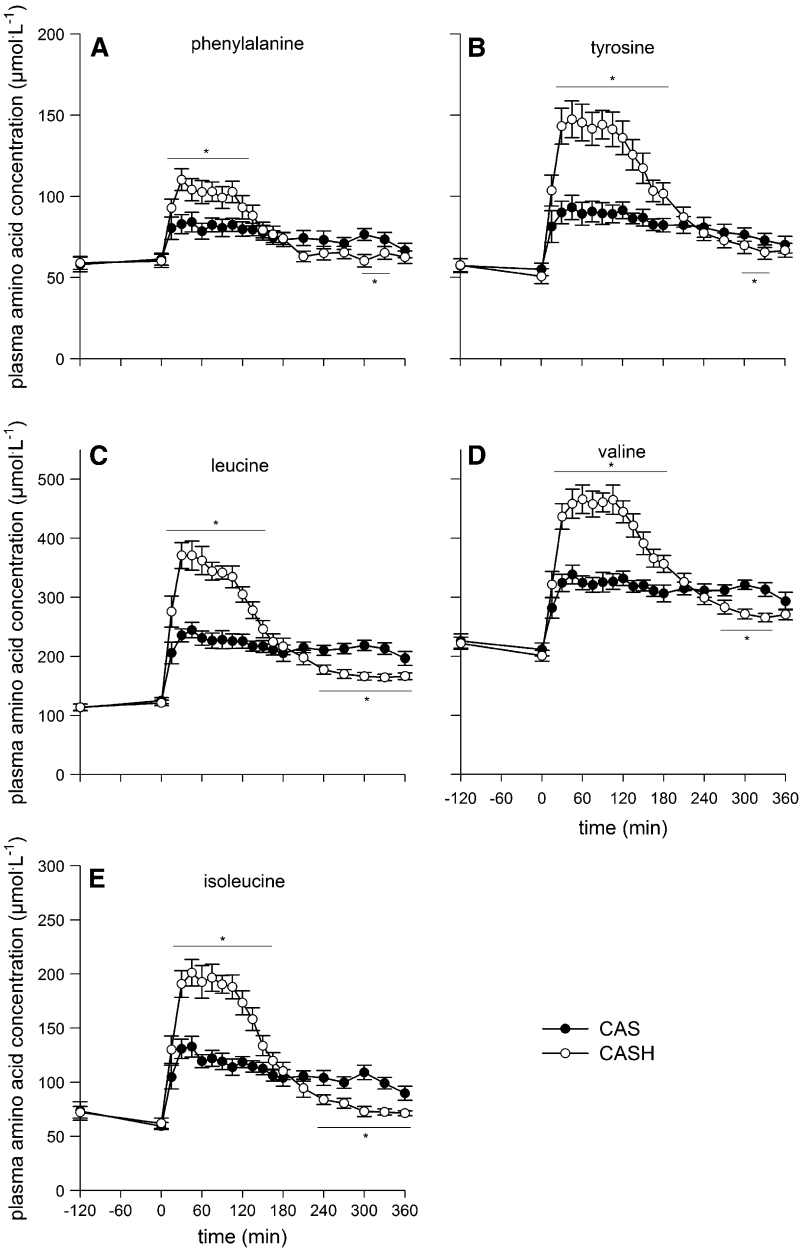

FIGURE 2. Mean (±SEM) plasma phenylalanine (A), tyrosine (B), leucine (C), valine (D), and isoleucine (E) concentrations (μmol/L) during casein (CAS) and casein hydrolysate (CASH) experiments in elderly men ($n = 10$). The horizontal lines indicate the time period over which significant differences were observed between treatments. Data were analyzed with a 2-factor ANOVA repeated measures (treatment × time). For plasma phenylalanine, tyrosine, leucine, valine, and isoleucine: time effect, $P < 0.01$; treatment effect, $P < 0.01$; interaction of time and treatment, $P < 0.0.01$. *Significantly different from the CAS treatment, $P < 0.05$ (Scheffe test).

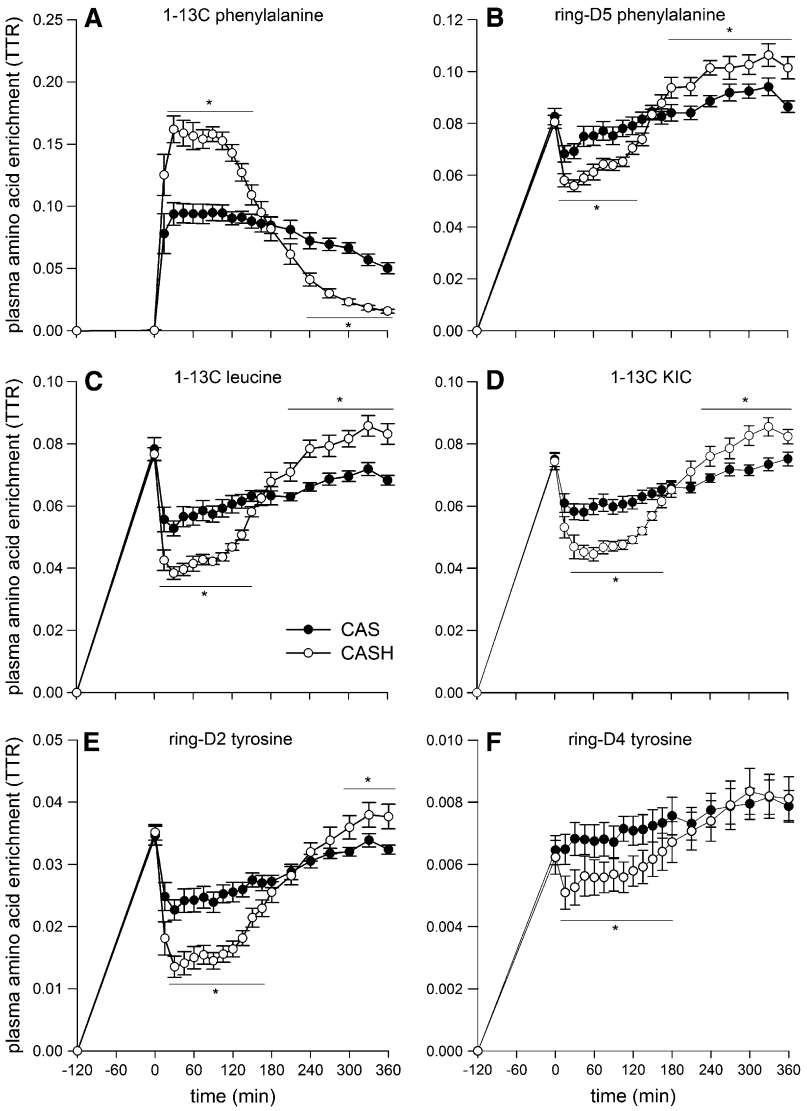

FIGURE 3. Mean (±SEM) plasma L-[1-^{13}C]phenylalanine (A), L-[ring-$^{2}H_5$]phenylalanine (B), L-[1-^{13}C]leucine (C), L-[1-^{13}C]KIC (D), L-[ring-$^{2}H_2$]tyrosine (E), and L-[ring-$^{2}H_4$]tyrosine enrichment tracer:tracee ratios (TTR) (F) during the casein (CAS) and casein hydrolysate (CASH) experiments in elderly men (n = 10). The horizontal lines indicate the time period over which significant differences were observed between treatments. Data were analyzed with ANOVA repeated measures (treatment × time). For plasma L-[1-^{13}C]phenylalanine, L-[ring-$^{2}H_5$]phenylalanine, L-[1-^{13}C]leucine, L-[1-^{13}C]KIC, L-[ring-$^{2}H_2$]tyrosine, and L-[ring-$^{2}H_4$]tyrosine enrichment: time effect, $P < 0.001$; treatment effect, $P < 0.001$; interaction of time and treatment, $P < 0.001$. *Significant differences between CAS and CASH ($P < 0.05$, Scheffe test).

compared with CAS (0.17 ± 0.01 compared with 0.12 ± 0.01 TTR; $P < 0.05$). However, plasma L-[1-^{13}C]phenylalanine enrichments were lower in CASH compared with CAS during the final 2 h of the test (Figure 3A; $P < 0.05$). Plasma L-[ring-$^{2}H_5$] phenylalanine, L-[1-^{13}C]leucine, L-[1-^{13}C]KIC, and L-[ring-$^{2}H_2$]tyrosine enrichments decreased during both treatments after ingestion of the drink. Generally, lower values were observed during the first 2–3 h after protein ingestion in the CASH compared with the CAS treatment (Figure 3, B–E; $P < 0.05$). In contrast, higher plasma enrichments were observed in the CASH compared with the CAS treatment during the final stages of the test (Figure 3, B–E; $P < 0.05$). Plasma L-[ring-$^{2}H_4$]tyrosine enrichments decreased after CASH intake only (Figure 3F; $P < 0.05$) and remained at a lower concentration during the first 3 h when compared with the ingestion of CAS. No differences in plasma L-[ring-$^{2}H_4$]tyrosine enrichments were observed between treatments during the final 3 h of the test.

Whole-body protein metabolism

Ingestion of the intrinsically labeled protein in the CASH and CAS treatments resulted in a rapid increase in the exogenous phenylalanine appearance rate (**Figure 4**A), with significantly higher peak phenylalanine appearance rates (individual peak values) observed in the CASH compared with the CAS treatment (0.35 ± 0.03 compared with 0.18 ± 0.01 μmol phenylalanine · kg^{-1} · min^{-1}, respectively; $P < 0.001$). In addition, total exogenous phenylalanine appearance (expressed as AUC over 6 h) was 27 ± 6% (range: 8–60%) higher in the CASH compared with the CAS treatment ($P < 0.001$). In addition, the calculated percentage of ingested phenylalanine taken up by the splanchnic area during its first pass (ie, the amount of ingested phenylalanine not appearing in plasma) was significantly lower in the CASH compared with the CAS treatment

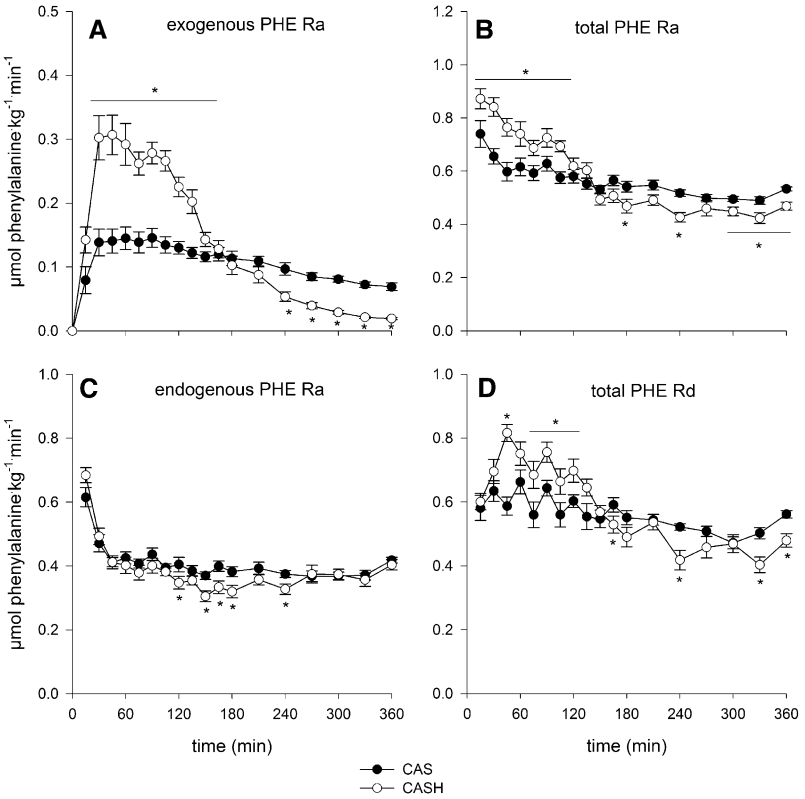

FIGURE 4. Mean (±SEM) rate of exogenous (A), total (B), and endogenous (C) phenylalanine (PHE) appearance in plasma (Ra) and of total phenylalanine disappearance (Rd) from plasma (D) in μmol · kg^{-1} · min^{-1} during the casein (CAS) and casein hydrolysate (CASH) experiments in elderly men (n = 10). The horizontal lines indicate the time period over which significant differences were observed between treatments. Data were analyzed with repeated-measures ANOVA (treatment × time). Exogenous Ra: time effect, $P < 0.001$; treatment effect, $P < 0.001$; interaction of time and treatment, $P < 0.001$. Total Ra: time effect, $P < 0.05$; treatment effect, $P < 0.001$; interaction of time and treatment, $P < 0.001$. Endogenous Ra: time effect, $P = 0.06$; treatment effect, $P < 0.001$; interaction of time and treatment, $P < 0.001$. Total Rd: time effect, $P < 0.05$; treatment effect, $P < 0.001$; interaction of time and treatment, $P < 0.001$. *Significant differences between CAS and CASH ($P < 0.05$, Scheffe test).

(66.1 ± 1.2% compared with 73.0 ± 1.4%, respectively; $P <$ 0.01). Total (exogenous and endogenous) phenylalanine appearance rates were significantly higher during the first 105 min after protein ingestion in the CASH compared with the CAS treatment (peak rates averaged 0.92 ± 0.03 compared with 0.79 ± 0.04 μmol phenylalanine · kg^{-1} · min^{-1}, respectively; $P < 0.05$).

Total phenylalanine appearance rates decreased to a greater extent over time during the CASH compared with the CAS treatment. As a result, mean total phenylalanine appearance in plasma, measured over the entire 6-h period, did not differ between treatments (P = 0.52). Endogenous phenylalanine appearance rates rapidly declined after protein ingestion in both the CASH and CAS treatments (Figure 4C). The average endogenous phenylalanine appearance in plasma over 6 h tended to be lower in the CASH compared with the CAS treatment (0.39 ± 0.01 compared with 0.41 ± 0.01 μmol phenylalanine · kg · min^{-1}, respectively; P = 0.058).

Peak plasma phenylalanine disappearance and phenylalanine hydroxylation rates (individual peak values) were significantly higher in the CASH compared with the CAS treatment (0.85 ± 0.03 compared with 0.73 ± 0.03 and 0.16 ± 0.03 compared with 0.09 ± 0.01 μmol phenylalanine · kg^{-1} · min^{-1}, respectively; $P < 0.05$). Phenylalanine disappearance and hydroxylation rates decreased to a greater extent over time in the CASH compared with the CAS treatment. As a result, average total phenylalanine disappearance in plasma over the entire 6-h measuring period did not differ between treatments (P = 0.43). On average, phenylalanine hydroxylation tended to be higher during the CASH compared with the CAS treatment (0.065 ± 0.008 compared with 0.053 ± 0.004 μmol phenylalanine · kg^{-1} · min^{-1}, respectively; P = 0.10). Average whole-body protein synthesis did not differ between treatments and averaged 0.51 ± 0.01 and 0.51 ± 0.01 μmol phenylalanine · kg^{-1} · min^{-1} in the CASH and CAS treatments, respectively (P = 0.78). Total net protein balance (AUC synthesis minus AUC endogenous R_a) over the 6-h period after protein ingestion tended to be higher in the CASH compared with the CAS treatment (40.6 ± 3.4 compared with 34.3 ± 2.1 μmol phenylalanine · 6 h · kg^{-1}, respectively; P = 0.08).

By using [1-^{13}C]leucine as an additional intravenous tracer, we observed similar changes in R_a and R_d over time between the CASH and CAS treatments when compared with phenylalanine tracer kinetics (data not shown). Peak leucine R_a and R_d (individual peak values) were significantly higher in CASH compared with CAS treatments (R_a: 3.26 ± 0.12 compared with 2.43 ± 0.13 μmol leucine · kg^{-1} · min^{-1}, respectively; R_d: 2.93 ± 0.10 compared with 2.25 ± 0.07 μmol leucine · kg^{-1} · min^{-1}, respectively; $P < 0.01$). Average total leucine R_a and R_d over the entire 6-h period was 7 ± 1% and 8 ± 2% higher in the CASH compared with the CAS treatment, respectively ($P < 0.05$).

Muscle analysis

No differences were observed in basal free L-[1-^{13}C]phenylalanine, L-[1-^{13}C]leucine, L-[1-^{13}C]tyrosine, and L-[ring-$^{2}H_2$]tyrosine enrichment that was determined in the muscle biopsies collected before the ingestion of the test drink between treatments. Free muscle L-[1-^{13}C]leucine, L-[1-^{13}C]tyrosine, and L-[ring-$^{2}H_2$]tyrosine enrichments increased over time. However, no differences were observed in free AA enrichment in the biopsy samples collected 6 h after the ingestion of the protein drink between treatments. A significant time × treatment interaction was observed for free muscle L-[1-^{13}C]phenylalanine enrichment ($P < 0.01$). Six hours after protein intake, muscle free L-[1-^{13}C]phenylalanine enrichment was significantly lower in the CASH compared with the CAS experiment, ie, 0.0133 ± 0.0011 compared with 0.03283 ± 0.0035 TTR, respectively ($P < 0.001$).

The increase in protein-bound L-[1-^{13}C]phenylalanine enrichment tended to be higher in the CASH compared with the CAS treatment (0.00035 ± 0.00011 compared with 0.00025 ± 0.00002 TTR, respectively; $P = 0.07$). The increase in protein-bound L-[1-^{13}C]leucine enrichment averaged 0.00020 ± 0.00002 compared with 0.00023 ± 0.00002 TTR in the CAS and the CASH treatment, respectively ($P = 0.35$).

Mixed muscle protein synthesis rates

Mixed muscle protein FSRs, with the mean plasma L-[1-^{13}C]phenylalanine enrichment as a precursor (**Figure 5**A), tended to be higher (33 ± 16%; $P = 0.10$) in the CASH compared with the CAS treatment. By using the L-[1-^{13}C]leucine tracer, FSR values were similar, and no significant differences were observed between the CASH compared with the CAS treatment (Figure 5B, $P = 0.35$). A significant positive correlation was observed between FSR values calculated by using L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine as tracers ($r = 0.71$, $P < 0.01$).

DISCUSSION

In this study, we assessed dietary protein digestion and absorption kinetics and the subsequent muscle protein synthetic response to the ingestion of a single bolus of protein hydrolysate compared with ingestion of its intact protein in vivo in healthy, elderly men. The men were studied by using specifically produced intrinsically L-[1-^{13}C]phenylalanine–labeled intact (CAS) and hydrolyzed (CASH) casein. This is the first study to show that ingestion of a casein hydrolysate, as opposed to its intact protein, accelerates the appearance rate of dietary phenylalanine in the circulation, lowers splanchnic phenylalanine extraction, increases postprandial plasma amino acid availability, and tends to augment subsequent muscle protein synthesis in vivo in humans.

The rate of dietary protein digestion and absorption and the subsequent splanchnic amino acid extraction determine postprandial amino acid delivery to the periphery (9). The availability of dietary amino acids has been shown to be an important regulator of postprandial muscle protein metabolism (22–25). To allow the assessment of dietary protein digestion and absorption, and the subsequent postprandial skeletal muscle protein synthetic response in vivo in humans, we applied specifically produced intrinsically L-[1-^{13}C]phenylalanine-labeled casein. It has been speculated that enzymatic predigestion of a protein source can be applied to modulate its in vivo digestion and absorption kinetics (3). In accordance, in this study we observed a greater increase in plasma amino acid concentrations after ingestion of the hydrolyzed casein (CASH) when compared with its intact protein CAS (Figure 2). These observations are in line with Calbet et al (3), who reported higher peak plasma AA concentrations after intragastric administration of hydrolyzed casein when compared with its intact protein. We extend these findings by directly measuring the true plasma appearance rate of dietary phenylalanine after ingestion of both the intact and hydrolyzed intrinsically labeled L-[1-^{13}C]phenylalanine casein (Figure 4). The exogenous phenylalanine appearance rate increased to a greater extent after ingestion of the hydrolysate when compared with the intact protein (Figure 4). During the 6-h postprandial period, ≈25% more dietary phenylalanine appeared in the circulation after ingestion of the hydrolysate when compared with the intact protein. Consequently, this study shows that a hydrolyzed protein is more rapidly digested and absorbed, which results in a greater AA delivery to the periphery in vivo in elderly men. In addition, we show that ≈70% of the ingested phenylalanine does not appear in the circulation within a 6-h postprandial period. This finding is in line with previous work in pigs showing that, although ≈90% of the dietary phenylalanine is absorbed, the splanchnic area extracts ≈50% to sustain its functional mass (4). Interestingly, the percentage of the AAs extracted within the splanchnic area varies between different

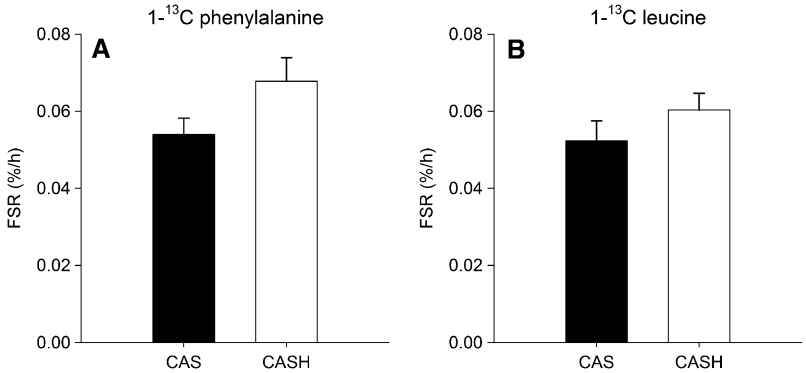

FIGURE 5. Mean (±SEM) fractional synthetic rate (FSR) of mixed muscle protein after the ingestion of intact casein (CAS) or hydrolyzed casein (CASH) in elderly men ($n = 10$) by using plasma L-[1-^{13}C]phenylalanine (A) and L-[1-^{13}C]leucine enrichment (B) as precursors. Data were analyzed with a paired t test. No significant differences were observed between treatments.

amino acids and seems to depend on the amount, quality, and digestibility of the dietary protein source (26) and on the coingestion of other macronutrients (27, 28). Previously published data from human studies suggest that when protein or AAs are ingested in small boluses over a prolonged period of time, ≈50% of dietary phenylalanine (29) and leucine (30) is extracted by the splanchnic area in elderly men. In this study, we show that the percentage of the ingested phenylalanine that does not appear in plasma is significantly (≈10%) lower after the ingestion of a single bolus of casein hydrolysate when compared with its intact protein (66 ± 1% compared with 73 ± 1%, respectively; $P < 0.01$). Consequently, hydrolyzed casein provides a protein source that is more rapidly digested and absorbed in vivo in humans, which improves postprandial plasma AA availability.

It has been reported that greater postprandial plasma AA availability will compensate for an attenuated postprandial muscle protein synthetic response in the elderly and augment net muscle protein accretion (15). In this study, we observed that whole-body protein breakdown rates tended to be further lowered after ingestion of the protein hydrolysate compared with the intact protein ($P = 0.058$), which may be due to the greater insulin release that was observed after protein hydrolysate ingestion (31, 32). Elevated insulin concentrations have been shown to inhibit proteolysis (31, 33, 34), stimulate AA uptake (35), and/or augment muscle protein synthesis (35, 36). Some groups propose that insulin is rather permissive instead of modulatory and that plasma insulin concentrations of ≈10–15 μU/mL are already sufficient to allow a maximal muscle protein synthetic response (37, 38). In contrast, it is also suggested that postprandial increases in circulating insulin concentrations are instrumental in stimulating skeletal muscle blood flow and thereby augment AA delivery to the muscle (39, 40). Consequently, both the increase in postprandial plasma AA availability and the greater plasma insulin response after CASH compared with CAS ingestion (during the initial 3-h postprandial period) might enhance postprandial muscle protein anabolism.

We used the plasma phenylalanine rate of disappearance and hydroxylation to calculate postprandial whole-body protein synthesis rates. Over the entire 6-h period, whole-body protein synthesis rates did not differ between treatments ($P = 0.78$). Whole-body net protein balance (AUC synthesis minus AUC endogenous R_a) tended to be higher in the CASH compared with the CAS treatment ($P = 0.08$). This result indicates that the intake of a protein hydrolysate, as opposed to its intact protein, further stimulates the anabolic response to food intake mainly by inhibiting whole-body protein breakdown. However, postprandial whole-body protein synthesis and breakdown rates do not necessarily reflect changes on a muscle-tissue level (19). Therefore, we also determined the incorporation rate of L-[1-^{13}C]phenylalanine (from the intrinsically labeled dietary protein) into the muscle protein pool in skeletal muscle-tissue samples, which tended to be greater after the ingestion of casein hydrolysate (0.00035 ± 0.00011) when compared with ingestion of the intact protein (0.00025 ± 0.00002; $P = 0.07$). As a result, observed FSR values tended to be ≈30% higher over the 6-h period after the ingestion of the casein hydrolysate compared with the ingestion of the intact protein ($P = 0.10$). Similar differences were observed when calculating FSR on the basis of intravenous L-[1-^{13}C]leucine administration. However, due to large intersubject variability, no significant differences in the muscle protein synthetic response to protein ingestion were observed between treatments (Figure 5). This may be due to the timing of the collection of muscle-tissue samples (22). On the basis of whole-body phenylalanine flux data and circulating plasma amino acid and insulin concentrations, it could be speculated that net muscle protein accretion was greater during the first 3 h after CASH ingestion when compared with CAS. This might explain why differences in the observed FSR values did not reach statistical significance when assessed over the entire 6-h period. Future studies should consider differentiating the muscle protein synthetic response to dietary protein intake during the acute (<3 h) from that during the more prolonged (>3 h) postprandial period. Another factor that may explain the lack of statistical difference in FSR values after CAS and CASH ingestion is the ingestion of a relatively large amount of dietary protein in the present study. A bolus of 35 g dietary protein may have been more than sufficient to maximize the postprandial muscle protein synthetic response (38, 41, 42). More research is warranted to assess the potential differences in the postprandial muscle protein synthetic response to the ingestion of smaller, meal-like amounts of hydrolyzed compared with intact protein (≈20 g). However, measuring the incorporation rate of labeled AAs derived from even smaller amounts of intrinsically labeled dietary protein will be methodologically challenging.

In conclusion, ingestion of a protein hydrolysate, as opposed to its intact protein, accelerates protein digestion and absorption from the intestine, lowers splanchnic AA, extraction, augments postprandial plasma AA availability, and tends to increase the incorporation of dietary AAs into mixed muscle protein in vivo in elderly men.

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