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Dietary Protein Digestion and Absorption Rates and the Subsequent Postprandial Muscle Protein Synthetic Response Do Not Differ between Young and Elderly Men[1,2]

René Koopman,[3] Stéphane Walrand,[5] Milou Beelen,[3] Annemie P. Gijsen,[4] Arie K. Kies,[6] Yves Boirie,[5] Wim H. M. Saris,[4] and Luc J. C. van Loon[3,7]*

[3]Department of Human Movement Sciences; [4]Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht, Maastricht University, Maastricht 6200 MD, The Netherlands; [5]Human Nutrition Unit, Université Clermont Auvergne, Centre de Recherche en Nutrition Humaine BP 321, 63009 Clermont-Ferrand Cedex 1, France; [6]DSM Food Specialties, R&D, Biochemistry and Nutrition Department, Delft 2600 MA, The Netherlands; and [7]Top Institute Food and Nutrition, Wageningen 6700 AN, The Netherlands

Abstract

Impaired digestion and/or absorption of dietary protein lowers postprandial plasma amino acid availability and, as such, could reduce the postprandial muscle protein synthetic response in the elderly. We aimed to compare in vivo dietary protein digestion and absorption and the subsequent postprandial muscle protein synthetic response between young and elderly men. Ten elderly (64 ± 1 y) and 10 young (23 ± 1 y) healthy males consumed a single bolus of 35 g specifically produced, intrinsically L-[1-^{13}C]phenylalanine-labeled micellar casein (CAS) protein. Furthermore, primed continuous infusions with L-[ring-$^{2}H_5$]phenylalanine, L-[1-^{13}C]leucine, and L-[ring-$^{2}H_2$]tyrosine were applied and blood and muscle tissue samples were collected to assess the appearance rate of dietary protein-derived phenylalanine in the circulation and the subsequent muscle protein fractional synthetic rate over a 6-h postprandial period. Protein ingestion resulted in a rapid increase in exogenous phenylalanine appearance in both the young and elderly men. Total exogenous phenylalanine appearance rates (expressed as area under the curve) were 39 ± 3 μmol·6 h·kg^{-1} in the young men and 38 ± 2 μmol·6 h·kg^{-1} in the elderly men ($P = 0.73$). In accordance, splanchnic amino acid extraction did not differ between young (72 ± 2%) and elderly (73 ± 1%) volunteers ($P = 0.74$). Muscle protein synthesis rates, calculated from the oral tracer, were 0.063 ± 0.006 and 0.054 ± 0.004%/h in the young and elderly men, respectively, and did not differ between groups ($P = 0.27$). We conclude that protein digestion and absorption kinetics and the subsequent muscle protein synthetic response following the ingestion of a large bolus of intact CAS are not substantially impaired in healthy, elderly men. J. Nutr. 139: 1707–1713, 2009.

Introduction

Aging is associated with a gradual loss of skeletal muscle mass, often referred to as sarcopenia (1). The latter is accompanied by a reduction in strength, the loss of functional capacity, and an increased risk of developing chronic metabolic diseases like obesity and type 2 diabetes. The age-related loss of skeletal muscle mass is facilitated by a combination of factors, which include a sedentary lifestyle and a suboptimal diet (2). Sarcopenia is attributed to a disruption in the regulation of skeletal muscle protein turnover (2,3), which results in an imbalance between muscle protein synthesis and degradation. Interestingly, basal (fasting) muscle protein synthesis rates do not seem to substantially differ between the young and elderly (4–8). Therefore, research groups have started to focus on the muscle protein synthetic response to the main anabolic stimuli, i.e. food intake and physical activity. It has been well established that protein turnover in skeletal muscle tissue is highly responsive to nutrient intake in healthy, young individuals (9). Interestingly, data from recent studies suggest that the muscle protein synthetic response to the ingestion of a small amount of amino acids with (6,10) or without (11,12) carbohydrate is reduced in the elderly compared with young controls. The latter is now generally thought to represent a key factor responsible for the age-related decline in skeletal muscle mass.

One of the mechanisms that has been suggested to explain a blunted muscle protein synthetic response to protein ingestion includes impairments in dietary protein digestion and/or ab-

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* To whom correspondence should be addressed. E-mail: l.vanloon@HB.unimaas.nl.

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sorption (13). Impaired protein digestion and/or absorption might attenuate and/or reduce the appearance rate of dietary amino acids in the circulation, thereby lowering the postprandial muscle protein synthesis rate. Furthermore, amino acid uptake in the splanchnic area has been shown to be elevated in the elderly (7,13), which implies that fewer of the ingested amino acids are available for muscle protein synthesis in the elderly (13). Evidence to support the existence of differences in digestion and absorption kinetics and the subsequent muscle protein synthetic response to protein intake between young and elderly humans remains lacking. The latter is largely due to the restrictions set by the methodology as labeled amino acids need to be incorporated in the dietary protein source to accurately assess the appearance rate of amino acids derived from dietary protein (14,15). Therefore, we recently produced highly enriched L-[1-^{13}C]phenylalanine–labeled milk by infusing a large amount of L-[1-^{13}C]phenylalanine in a cow and purified the casein (CAS)[8] fraction. This complex approach was required to allow us to study the impact of protein digestion and absorption on protein metabolism in an in vivo human setting.

In this study, we tested the hypothesis that dietary protein digestion and absorption kinetics and the subsequent postprandial muscle protein synthetic response is impaired in vivo in healthy, elderly men. Therefore, young and elderly males were administered a single bolus of specifically produced intrinsically L-[1-^{13}C]phenylalanine–labeled intact CAS combined with continuous i.v. L-[ring-$^{2}H_5$]phenylalanine, L-[1-^{13}C]leucine, and L-[ring-$^{2}H_2$]tyrosine infusion. We directly assessed exogenous and endogenous rate of appearance (Ra) of phenylalanine, splanchnic phenylalanine extraction, and fractional synthetic rate (FSR) of mixed muscle protein following the ingestion of a single bolus of intact protein in vivo in humans.

Methods

Participants

Ten healthy elderly (64 ± 1 y) and 10 young (24 ± 1 y) male volunteers with no history of participating in any regular exercise program enrolled in the present study. Participants' characteristics are provided in **Table 1**. All men were informed about 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 participants performed an oral glucose tolerance test prior to inclusion in the study (16). Following an overnight fast, participants 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). Plasma glucose concentrations were measured to determine glucose intolerance and/or the presence of type 2 diabetes according to 2006 American Diabetes Association guidelines (17).

Diet and activity prior to testing

All participants consumed the same standardized meal [32 ± 1 kJ·kg^{-1} body weight, consisting of 55 energy% (En%) carbohydrate, 25 En% protein, and 30 En% fat] the evening prior to the experiments. All volunteers were instructed to refrain from any sort of exhaustive labor or intense exercise and to keep their diet as constant as possible 3 d prior to the experiments.

[8] Abbreviations used: AUC, area under the curve above baseline; CAS, casein; En%, energy percent; FSR, fractional synthetic rate; Ra, rate of appearance; Rd, rate of disappearance; TTR, tracer-to-tracee ratio.

TABLE 1 Participants' characteristics[1]

	Young	Elderly
Age, *y*	23 ± 1	64 ± 1
Weight, *kg*	76.8 ± 2.0	78.8 ± 3.1
Height, *m*	1.83 ± 0.02	1.78 ± 0.02
BMI, *kg/m^2*	22.9 ± 0.6	24.7 ± 0.7
Basal plasma glucose, *mmol/L*	5.17 ± 0.11	5.44 ± 0.07
Basal plasma insulin, *pmol/L*	71.32 ± 5.86	70.38 ± 8.78
HOMA-IR[2]	2.38 ± 0.23	2.43 ± 0.32

[1] Values are means ± SEM, n = 10.
[2] Homeostasis model assessment insulin resistance.

Protocol

All participants participated in a single experiment in which they ingested a single, 35-g bolus of intrinsically L-[1-^{13}C]phenylalanine–labeled micellar CAS. At 0800, after an overnight fast, participants arrived at the laboratory by car or public transportation. A Teflon catheter was inserted into an antecubital vein for stable isotope infusion. A second Teflon catheter was inserted in a heated dorsal hand vein of the contra-lateral 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 i.v. dose of the amino acid tracers {L-[ring-$^{2}H_5$]phenylalanine (2 μmol·kg^{-1}), L-[ring-$^{2}H_2$]tyrosine (0.775 μmol·kg^{-1}), and L-[1-^{13}C]leucine (5.06 μmol·kg^{-1})}. Thereafter, continuous tracer infusions were started (infusion rate of 0.046 ± 0.001 μmol·kg^{-1}·min^{-1} for L-[ring-$^{2}H_5$] phenylalanine, 0.018 ± 0.000 μmol·kg^{-1}·min^{-1} for L-[ring-$^{2}H_2$]tyrosine, and 0.111 ± 0.001 μmol·kg^{-1}·min^{-1} for L-[1-^{13}C]leucine) and participants 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). Participants then received a bolus (4.5 mL·kg^{-1}) of the test beverage containing 35 g intrinsically L-[1-^{13}C]phenylalanine–labeled intact CAS. 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 contra-lateral limb.

Blood samples were collected in 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 biopsies were obtained from the middle region of the vastus lateralis (15 cm above the patella) and ~3 cm below entry through the fascia using the percutaneous needle biopsy technique (18). 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

A specifically produced batch of intrinsically L-[1-^{13}C]phenylalanine–labeled CAS protein was prepared by the i.v. infusion of L-[1-^{13}C]phenylalanine in vivo in cows, with milk being collected during a subsequent 48-h infusion period (19). Micellar CAS and whey protein were separated from the collected milk by microfiltration and ultra-filtration as described previously (20). The L-[1-^{13}C]phenylalanine enrichment in the CAS protein was measured at 29.2 mole percent excess. The intrinsically labeled batch of protein met all chemical and bacteriological specifications for human consumption.

Participants received a beverage volume of 350 mL to ensure a given dose of ~35 g intact CAS. Per kg body weight, the protein provided 144 ± 4 and 142 ± 6 μmol phenylalanine, 143 ± 4 and 141 ± 6 μmol tyrosine, and 327 ± 8 μmol and 322 ± 13 leucine, in young and elderly participants, respectively (no differences were observed in amino acid administration between age groups) (P = 0.74). Beverages were flavored by adding 0.375 g sodium-saccharinate, 0.9 g citric acid, and 5 mL of vanilla flavor (Quest International) for each liter of beverage.

Plasma samples analysis

Plasma glucose (Uni Kit III, 07367204, Roche) concentrations were analyzed with the COBAS-FARA semiautomatic analyzer (Roche).

Insulin was analyzed by RIA (Insulin RIA kit, LINCO Research). Plasma amino acid concentrations were measured by HPLC after precolumn derivatization with *o*-phthaldialdehyde (21). Plasma phenylalanine, tyrosine, ketoisocaproate (KIC), and leucine enrichment were determined after derivatization (22,23) by electron ionization GC-MS (Agilent 6890N GC/5973N MSD) as previously described in detail (19). Enrichments of plasma L-[ring-$^2H_2$]tyrosine and L-[ring-$^2H_4$] tyrosine were corrected for spillover by establishing the relationship between the enrichment of a series of L-[1-^{13}C]tyrosine and L-[ring-$^2H_2$] tyrosine standards of variable enrichment and the enrichment found on [M+2] and [M+4] of the tert-butyldimethylsylil derivatives of these standards.

Muscle sample analyses

Intracellular free L-[1-^{13}C]phenylalanine, L-[1-^{13}C]tyrosine, L-[ring-$^2H_2$]tyrosine, L-[ring-$^2H_4$]tyrosine, and L-[1-^{13}C]leucine enrichments were measured using their TBDMS derivatives on a GC-MS, as described previously (19). Muscle protein-bound L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine were measured as *N(O,S)*-ethoxycarbonyl ethyl esters using GC-isotope ratio MS (19).

Calculations. Ingestion of L-[1-^{13}C]phenylalanine–labeled protein, combined with infusion of L-[ring-$^2H_5$]phenylalanine, L-[ring-$^2H_2$]tyrosine, and L-[1-^{13}C]leucine, and arterialized blood sampling were applied to assess whole-body amino acid kinetics in nonsteady state conditions. Total, exogenous, and endogenous Ra and splanchnic extraction (Sp, fraction of dietary amino acid taken up by the gut and liver during its first pass and thus not appearing in the circulation) for phenylalanine was calculated as described previously (14,22). In addition, total rate of disappearance (Rd) of phenylalanine, the rate of phenylalanine hydroxylation, and utilization for protein synthesis were calculated (19).

Fractional rate of mixed muscle protein synthesis (FSR) was calculated by dividing the increment in enrichment in the product, i.e. protein-bound L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine, by the enrichment of the precursor (19).

Statistics. The impact of the ingestion of intact protein (CAS) on plasma amino acid kinetics and whole-body and muscle protein synthesis rates was studied in young (n = 10) and elderly men (n = 10). All data were expressed as means ± SEM. Calculation of the required sample size was based on effect size and variance in previous studies from our laboratory (19,24–26). We calculated sample size using the following parameters: difference in FSR > 20%, SD of 15%, with type I error of 5% and type II error of 10%. Power calculations showed that at least 9 participants were needed and we therefore included 10 elderly men in this study. The plasma insulin, glucose, phenylalanine, tyrosine, and branched-chain amino acids (leucine, isoleucine, and valine) responses were calculated as area under the curve above baseline (AUC) values. A 2-factor repeated-measures ANOVA (general linear model) with time (degrees of freedom, 19) and age group (degrees of freedom, 1) as factors was used to compare differences between age groups over time. In case of significant interaction between time and treatment, a Scheffé post hoc test was applied to locate these differences. For nontime–dependent variables, a t test was performed to detect differences between treatments. Simple regression analysis was performed to calculate correlations between calculated FSR values using the L-[1-^{13}C]phenylalanine and L-[1-^{13}C]leucine tracer. Significance was set at $P < 0.05$. All calculations were performed using SPSS 12.0.

Results

Plasma analyses. Plasma insulin concentrations rapidly increased following the ingestion of the protein beverage in both young and elderly men (**Fig. 1**). The plasma insulin responses, expressed as AUC values, were 2.0 ± 1.9 nmol·6 h·L^{-1} in young and 3.9 ± 1.8 nmol·6 h·L^{-1} in elderly volunteers (P = 0.48). Plasma glucose responses were −2.5 ± 29.3 mmol·6 h·L^{-1} in young men and −3.2 ± 16.1 mmol·6 h·L^{-1} in elderly men and did not differ between groups (P = 0.98).

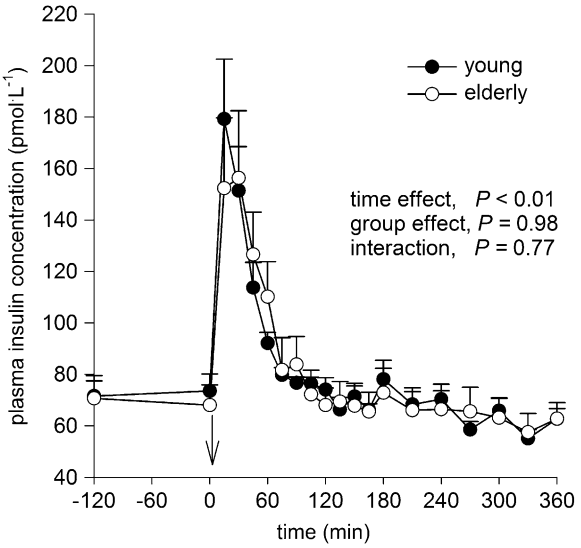

FIGURE 1 Plasma insulin concentrations and insulin responses (expressed as AUC minus baseline values) in lean young and elderly men following ingestion of 35 g CAS. Values are means + SEM, n = 10. Data were analyzed with a 2-way repeated-measures ANOVA (time × age-group). The arrow indicates the time of the protein ingestion.

Plasma phenylalanine, tyrosine, leucine, valine, and isoleucine concentrations increased in young and elderly men following the ingestion of the intact protein and remained elevated throughout the 6-h measurement period (**Fig. 2**). The plasma phenylalanine response (AUC) was 3.1 ± 0.7 mmol·6 h·L^{-1} in young men and 5.3 ± 1.5 mmol·6 h·L^{-1} in elderly men (P = 0.19). The plasma tyrosine response was higher in the elderly (9.7 ± 0.8 mmol·6 h·L^{-1}) than in the young men (6.4 ± 0.9 mmol·6 h·L^{-1}) ($P < 0.05$). The plasma leucine, valine, and isoleucine responses also were higher in the elderly than in the young men (leucine: 32.6 ± 1.8 and 23.7 ± 2.3 mmol·6 h·L^{-1}; valine: 36.7 ± 2.5 and 26.8 ± 3.5 mmol·6 h·L^{-1}; isoleucine: 17.7 ± 0.7 and 11.3 ± 1.1 mmol·6 h·L^{-1}, respectively) ($P < 0.05$).

The plasma L-[1-^{13}C]phenylalanine enrichment (exogenous tracer) quickly increased following ingestion of the test beverage in both young and elderly men, with no differences between groups over time (**Fig. 3***A*; time × age-group interaction; P = 0.44). Before the intake of the beverage (t = 0), plasma L-[ring-$^2H_5$]phenylalanine, L-[1-^{13}C]leucine, and L-[ring-$^2H_2$]tyrosine enrichments were significantly higher in the elderly compared with the young men (Fig. 3*B*,C,*E*).

Whole-body protein metabolism. Protein ingestion resulted in a rapid increase in exogenous phenylalanine appearance in both young and elderly men (**Fig. 4***A*). Total exogenous phenylalanine appearance (expressed as AUC) did not differ between groups and was 39.4 ± 3.3 μmol·6 h·kg^{-1} in young men and 38.1 ± 2.0 μmol·6 h·kg^{-1} in elderly men (P = 0.73). In addition, the calculated percentage of ingested phenylalanine taken up by the splanchnic area during its first pass (i.e. the amount of ingested phenylalanine, not appearing in plasma) did not differ between young (72.1 ± 2.2%) and elderly (73.0 ± 1.4%) volunteers (P = 0.74).

The endogenous phenylalanine appearance in plasma over 6 h was lower in the elderly (0.41 ± 0.01 μmol·kg^{-1}·min^{-1}) compared with the young participants (0.50 ± 0.01 μmol·kg^{-1}·min^{-1}) ($P < 0.01$). Total phenylalanine appearance (means over the 6 h following protein ingestion) was lower in the elderly (0.57 ± 0.01 μmol·kg^{-1}·min^{-1}) compared with the

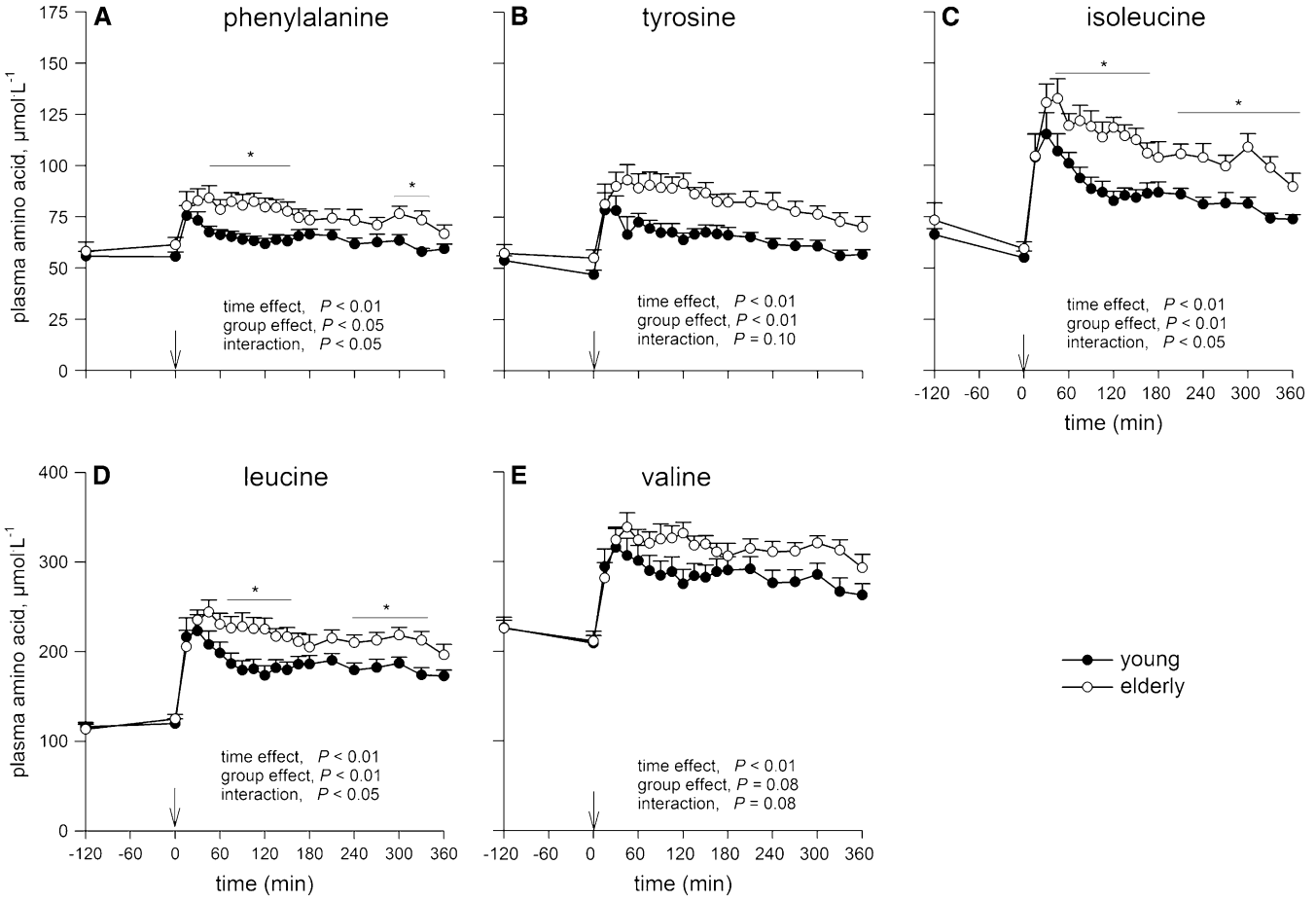

FIGURE 2 Plasma phenylalanine (*A*), tyrosine (*B*), isoleucine (*C*), leucine (*D*), and valine (*E*) concentrations following CAS ingestion in lean young and elderly men. Values are means + SEM, $n = 10$. Data were analyzed with a 2-way repeated-measures ANOVA (time × age-group). *Age groups differ at these times, $P < 0.05$ (*t* test). The arrow indicates the time of the protein ingestion.

young men (0.65 ± 0.02 μmol·kg^{-1}·min^{-1}) ($P < 0.01$). The phenylalanine disappearance (Fig. 4*D*) from plasma over the 6-h period following ingestion of the protein was lower in the elderly (0.65 ± 0.02 μmol·kg^{-1}·min^{-1}) compared with the young men (0.56 ± 0.01 μmol·kg^{-1}·min^{-1}) ($P < 0.01$). In addition, phenylalanine hydroxylation following protein ingestion was higher in the young (0.075 ± 0.004 μmol·kg^{-1}·min^{-1}) compared with the elderly men (0.053 ± 0.004 μmol·kg^{-1}·min^{-1}) ($P < 0.01$). Despite the fact that a greater proportion of the phenylalanine that disappeared from the circulation was converted to tyrosine in the young (11.5 ± 0.5%) compared with the elderly men (9.4 ± 0.6%) ($P < 0.01$), mean whole-body protein synthesis was higher in the young (0.57 ± 0.02 μmol phenylalanine·kg^{-1}·min^{-1}) compared with the elderly men (0.51 ± 0.01 μmol phenylalanine·kg^{-1}·min^{-1}) ($P < 0.01$). However, total net protein balance over the 6-h period following protein ingestion did not different between groups and was 34.3 ± 2.1 μmol phenylalanine·6 h·kg^{-1} in young men and 28.7 ± 3.0 μmol phenylalanine·6 h·kg^{-1} in elderly men ($P = 0.14$). Using L-[1-^{13}C]leucine or k-[1-^{13}C]KIC as a tracer, we observed similar changes in Ra and Rd over time in young and elderly men as determined using phenylalanine as a tracer (data not shown).

Muscle analysis. Basal free L-[1-^{13}C]phenylalanine, L-[1-^{13}C]leucine, L-[1-^{13}C]tyrosine, and L-[ring-$^{2}H_2$]tyrosine enrichments determined in the muscle biopsies collected prior to the ingestion of the test beverage did not differ between young and elderly men. Free muscle L-[1-^{13}C]leucine, L-[1-^{13}C]tyrosine, and L-[ring-$^{2}H_2$]tyrosine enrichments had increased over time but did not differ between groups. Muscle free L-[ring-$^{2}H_4$]tyrosine enrichment (TTR) tended to be higher in the young (0.0080 ± 0.0008) compared with the elderly (0.0059 ± 0.0006) ($P = 0.06$).

The increase in protein-bound L-[1-^{13}C]phenylalanine enrichment (TTR) did not differ between age groups and was 0.00027 ± 0.00003 in the young men and 0.00025 ± 0.00002 in elderly men ($P = 0.73$). The increase in protein-bound L-[1-^{13}C]leucine enrichment (TTR) was 0.00021 ± 0.00002 in young men and 0.00020 ± 0.00002 in elderly men ($P = 0.64$).

Mixed muscle protein synthesis rates. Mixed muscle protein FSR, with the mean plasma L-[1-^{13}C]phenylalanine enrichment as precursor, did not differ between young (0.054 ± 0.004%/h) and elderly men (0.063 ± 0.006%/h) ($P = 0.27$). Using the L-[1-^{13}C]leucine tracer, FSR was 0.052 ± 0.005%/h in young men and 0.064 ± 0.007%/h in elderly participants ($P = 0.19$). FSR values calculated from the oral L-[1-^{13}C]phenylalanine tracer correlated with those calculated using the i.v. L-[1-^{13}C]leucine tracer ($r = 0.80$; $P < 0.01$).

Discussion

In this study, we assessed dietary protein digestion and absorption and the subsequent muscle protein synthetic response to the ingestion of a single bolus of intact CAS protein in vivo in

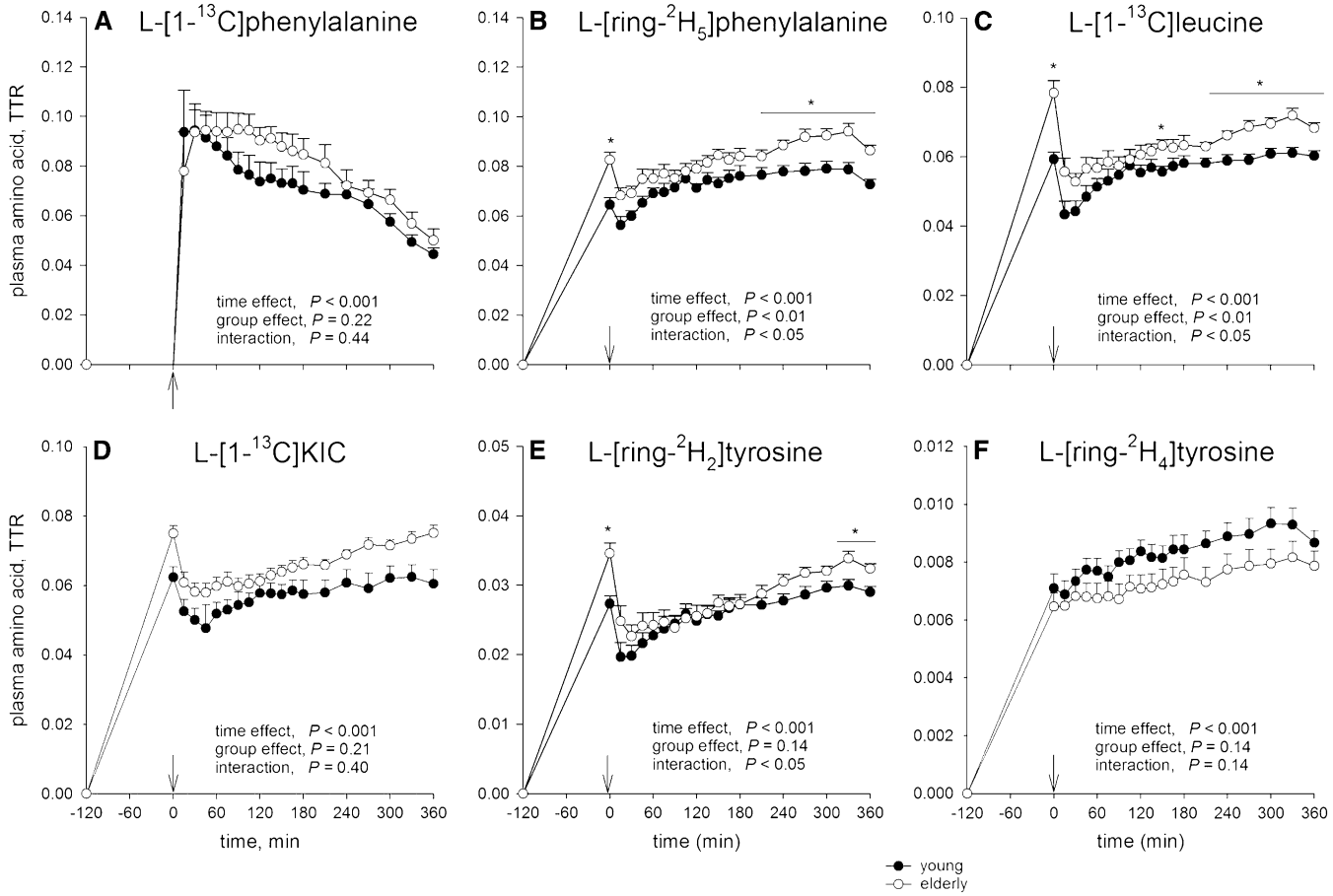

FIGURE 3 Plasma L-[1-^{13}C]phenylalanine (*A*), L-[ring-$^{2}H_5$]phenylalanine (*B*), L-[1-^{13}C]leucine (*C*), L-[1-^{13}C]KIC (*D*), L-[ring-$^{2}H_2$]tyrosine (*E*), and L-[ring-$^{2}H_4$]tyrosine enrichment (*F*) (TTR) following intact CAS ingestion in young and elderly men. Values are means + SEM, $n = 10$. Data were analyzed with repeated-measures ANOVA (age-group × time). *Age groups differ at these times, $P < 0.05$ (Scheffé test). The arrow indicates the time of the protein ingestion.

healthy young and elderly males. The latter was studied by using specifically produced intrinsically L-[1-^{13}C]phenylalanine–labeled micellar CAS. This is the first study to show that protein digestion and absorption efficiency and the subsequent anabolic response to the ingestion of 35 g of intact protein is not impaired in healthy, elderly men.

We measured the appearance rate of labeled phenylalanine, derived from the ingestion of dietary protein, into the circulation in vivo in humans. The use of intrinsically L-[1-^{13}C]phenylalanine–labeled intact CAS enables us to study the proposed differences in in vivo dietary protein digestion/absorption, splanchnic amino acid extraction, and the subsequent muscle protein synthetic response between young and elderly men. Following CAS ingestion, we observed a greater increase in plasma amino acid concentrations in elderly men compared with the young men. In general, peak plasma concentrations were ~10–15% higher in the elderly compared with the young men (Fig. 2). These observations are consistent with previously published data from our laboratory (25) and are indicative of a reduced whole-body amino acid flux in elderly humans (3, 25) (Fig. 4) and/or reduced distribution volume for specific amino acids (27). However, changes in plasma amino acid profiles and whole-body phenylalanine Ra and Rd do not necessarily represent the appearance rate of dietary protein-derived phenylalanine following the digestion and/or absorption of the ingested CAS. Therefore, we assessed the appearance rate of [1-^{13}C]phenylalanine derived from the ingested intrinsically labeled CAS. Exogenous phenylalanine appearance rate rapidly increased following ingestion of the protein in both the young and elderly, with no significant differences between groups in peak appearance rates (Fig. 4). In addition, the groups did not differ in the total amount of dietary phenylalanine that appeared in the circulation following ingestion of the intact protein. These observations are consistent with previously published data (22) and clearly show that in vivo dietary protein digestion and absorption are not impaired in healthy, elderly men following ingestion of 35 g of intact CAS.

In this study, ~70% of the ingested phenylalanine does not to appear in the circulation within the 6-h postprandial period. The latter is consistent 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 (28). Interestingly, the percentage of the amino acids extracted within the splanchnic area seems to vary among different amino acids and depends on the amount, quality, and digestibility of the dietary protein source (29) and the coingestion of other macronutrients (30,31). It has previously been suggested that splanchnic extraction of dietary amino acids is greater in the elderly, which would attenuate amino acid delivery to the periphery and, as such, contribute to the loss of muscle mass with aging (13). Previous studies have reported greater splanchnic phenylalanine (7) and leucine (13) extraction following the continuous ingestion of small boluses of protein or amino acids. In contrast, others have not observed such differences in

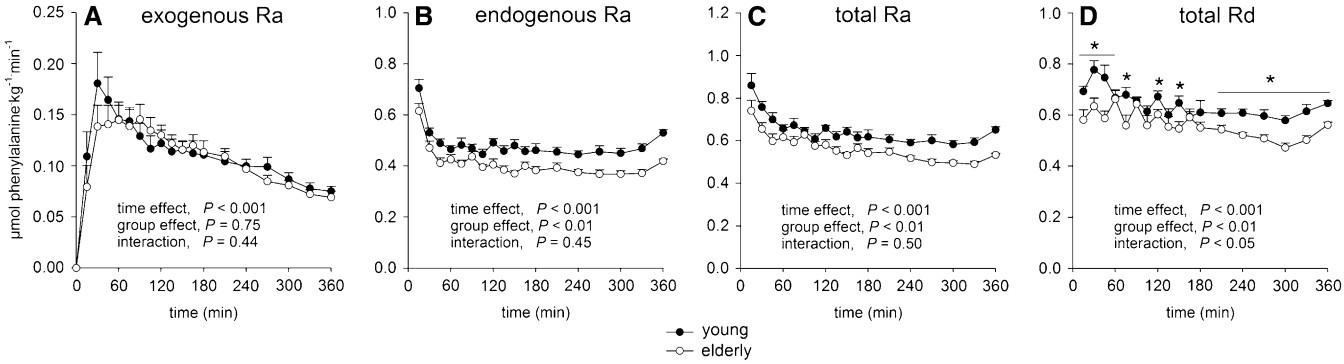

FIGURE 4 The rate of exogenous (*A*), endogenous (*B*), and total (*C*) phenylalanine (PHE) appearance in plasma (Ra) and total phenylalanine disappearance (Rd) from plasma (*D*) following intact CAS ingestion in young and elderly men. Values are means + SEM, n = 10. Data were analyzed with repeated-measures ANOVA (age-group × time). *Age groups differ at these times, $P < 0.05$ (Scheffé test).

splanchnic amino acid extraction between young and old people (22). Here, we found similar splanchnic phenylalanine extraction rates following ingestion of a single bolus of intact protein in young (72 ± 2%) and elderly (73 ± 1%) males ($P = 0.74$). The latter indicates that splanchnic amino acid extraction following ingestion of a large bolus of intact protein is also not impaired in healthy, elderly men.

Whole-body protein turnover rates are lower in the fasting state (3), following protein intake (13,22), and/or after physical activity (25) in the elderly compared with young adults. In accordance, we observed 10–15% lower whole-body phenylalanine appearance rates in the elderly (Fig. 4*C*). Following the ingestion of the intact protein, protein breakdown rates (endogenous phenylalanine release) declined to a similar extent in both groups (Fig. 4*B*). However, total and endogenous phenylalanine release remained lower in the elderly men. In addition to the measurement of total, exogenous, and endogenous phenylalanine appearance rates, we also measured the plasma phenylalanine disappearance and hydroxylation rates. The latter 2 were used to calculate postprandial whole-body protein synthesis rates. Phenylalanine disappearance and hydroxylation rates following protein ingestion were significantly lower in the elderly compared with the young men. These observations are consistent with reports of higher leucine oxidation rates following protein ingestion in young than in elderly men (13). Despite the fact that a greater proportion of the phenylalanine that disappeared from the circulation was converted to tyrosine, whole-body protein synthesis rates were higher in the young compared with the elderly men ($P < 0.01$). However, total net protein balance over the 6-h postprandial period did not differ between groups ($P = 0.14$). The latter suggests that the whole-body anabolic response to the ingestion of a single bolus of intact protein is not impaired in healthy, elderly men.

There is considerable discrepancy in the literature concerning the proposed blunted postprandial muscle protein synthetic response in elderly humans (32). Several studies have reported significantly lower muscle protein synthesis rates following food intake in elderly compared with young adults (6,11,12), whereas others have reported no differences in the anabolic response between age groups (5,7,33). Therefore, we determined the incorporation rate of L-[1-^{13}C]phenylalanine (from the ingested intrinsically labeled dietary protein) and L-[1-^{13}C]leucine (from the continuous i.v. tracer infusion) into the protein pool in skeletal muscle tissue collected prior to and 6 h after protein ingestion. The net increase in L-[1-^{13}C]phenylalanine enrichment in skeletal muscle tissue protein did not differ between young and elderly men ($P = 0.73$), which indicates that a similar amount of dietary amino acids were incorporated in skeletal muscle protein in the 2 groups. In accordance, the FSR did not differ between groups ($P = 0.27$), which was verified using muscle protein FSR calculations based on the i.v. L-[1-^{13}C]leucine administration. The apparent discrepancy between our findings and some (11,12), but not all, studies (5,7,33) may be attributed to differences in the mode of amino acid administration, i.e. single bolus (5,11,12,33) compared with multiple boluses (7,25); the source or type of amino acids ingested, i.e. essential amino acids (5,11,12,34), mixed amino acids (7), hydrolyzed milk proteins (25), or beef (33); the amount of amino acids that were provided (5–7,11,12,25,33); and the time period (6 vs. 3 h in other studies) during which the postprandial protein synthetic response was assessed (11,12).

This study provides a direct in vivo assessment of the digestion absorption kinetics and subsequent muscle protein synthetic response in vivo in humans. We show that protein synthesis is not reduced in elderly men following the intake of a relatively large amount of dietary protein. More research is warranted to assess the potential differences in the postprandial muscle protein synthetic response to the ingestion of smaller, more meal-like amounts of intact protein (~20 g) in young and elderly men. However, measuring the incorporation rate of labeled amino acids derived from even smaller amounts of intrinsically labeled dietary protein will be methodologically challenging.

In conclusion, dietary protein digestion and absorption kinetics and the subsequent muscle protein synthetic response following the ingestion of a large bolus of intact CAS protein is not impaired in vivo in healthy, elderly men.

Acknowledgments

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