Both basal and post-prandial muscle protein synthesis rates, following the ingestion of a leucine-enriched whey protein supplement, are not impaired in sarcopenic older males

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SUMMARY

Background: Studying the muscle protein synthetic response to food intake in elderly is important, as it aids the development of interventions to combat sarcopenia. Although sarcopenic elderly are the target group for many of these nutritional interventions, no studies have assessed basal or post-prandial muscle protein synthesis rates in this population.

Objective: To assess the basal and post-prandial muscle protein synthesis rates between healthy and sarcopenic older men.

Design: A total of 15 healthy (69 ± 1 y) and 15 sarcopenic (81 ± 1 y) older men ingested a leucine-enriched whey protein nutritional supplement containing 21 g of protein, 9 g of carbohydrate, and 3 g of fat. Stable isotope methodology combined with frequent collection of blood and muscle samples was applied to assess basal and post-prandial muscle protein fractional synthetic rates. Handgrip strength, muscle mass, and gait speed were assessed to identify sarcopenia, according to international criteria.

Results: Basal mixed muscle protein fractional synthetic rates (FSR) averaged 0.040 ± 0.005 and 0.032 ± 0.003%/h (mean ± SEM) in the sarcopenic and healthy group, respectively (P = 0.14). Following protein ingestion, FSR increased significantly to 0.055 ± 0.004 and 0.053 ± 0.004%/h in the post-prandial period in the sarcopenic (P = 0.003) and healthy groups (P < 0.001), respectively, with no differences between groups (P = 0.45). Furthermore, no differences were observed between groups in muscle protein synthesis rates during the early (0.058 ± 0.007 vs 0.060 ± 0.008%/h, sarcopenic vs healthy, respectively) and late (0.052 ± 0.004 vs 0.048 ± 0.003%/h) stages of the post-prandial period (P = 0.93 and P = 0.34, respectively).

Conclusions: Basal muscle protein synthesis rates are not lower in sarcopenic older men compared to healthy older men. The ingestion of 21 g of a leucine-enriched whey protein effectively increases muscle protein synthesis rates in both sarcopenic and healthy older men.

Public trial registry number: NTR3047.

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1. Introduction

Skeletal muscle mass, function and strength decline with an increasing age, a syndrome that has been coined sarcopenia. The decline in muscle strength and function leads to a reduced ability to perform activities of daily living, and are associated with an increased risk of adverse musculoskeletal outcomes such as falls...
and fractures [1,2]. Since sarcopenia is a strong predictor for mortality [3], it is clinically relevant to unravel the mechanisms underlying the age-related loss of skeletal muscle tissue.

Muscle mass maintenance is believed to be regulated mainly by changes in basal and post-prandial muscle protein synthesis rates (MPS). Age-related declines in basal or post-prandial muscle protein synthesis rates may be responsible for the progressive loss of skeletal muscle mass throughout the lifespan. So far, studies investigating basal muscle protein synthesis rates in older individuals have shown conflicting results. Lower basal muscle protein synthesis rates have been observed in the older populations when compared with younger populations in some studies [4–7]. In contrast, more recent work has been unable to detect significant differences in basal muscle protein synthesis rates between young and older individuals [8–12]. However, none of these studies specifically included older men and women suffering from sarcopenia. Therefore, it is likely that potential differences in basal muscle protein synthesis rates between the young and old have remained undetected, because a heterogeneous older population was selected that included many individuals who had not (yet) shown any signs of (substantial) muscle loss.

In addition to basal muscle protein synthesis, muscle maintenance is also largely determined by the muscle protein synthetic response to food intake. Because of the apparent absence of measurable differences in basal muscle protein synthesis rates between young and older populations, many research groups have shifted their focus to the muscle protein synthetic response to the main anabolic stimuli, such as food intake and physical activity. One of the primary anabolic stimuli for muscle protein synthesis is a systemic hyperaminoacidemia, resulting from the ingestion of dietary protein or essential amino acids [13–17]. A reduced sensitivity of senescent muscle to the anabolic properties of amino acid exposure has been reported by various research groups [8,10,18,19]. The post-prandial muscle protein synthetic response has been shown to be modulated by the type [20], amount [21] and total leucine content [9,22,23] of the protein ingested. Ingestion of ~20 g whey protein has been shown to increase muscle protein synthesis rates in healthy older individuals [17,24–26]. However, the post-prandial muscle protein synthetic response to protein ingestion may be blunted in the sarcopenic compared with the healthy older population. In the present study, we assessed if ingestion of a leucine-enriched whey protein can effectively increase the post-prandial muscle protein synthetic response in both healthy and sarcopenic older men.

We selected 15 healthy and 15 sarcopenic older males to participate in an experiment where we assessed basal and post-prandial muscle protein synthesis rates. Primed continuous infusions with L-[ring-13C6]-phenylalanine were applied with the collection of blood samples and muscle tissue to assess both basal as well as post-prandial muscle protein synthesis rates following the ingestion of a supplement containing 21 g of leucine-enriched whey protein. This is the first study to investigate post-prandial muscle protein synthesis in diagnosed [27] sarcopenic older males and to compare basal and post-prandial muscle protein synthesis rates between healthy and sarcopenic older males.

2. Subjects and methods

2.1. Subjects

A total of 15 sarcopenic older men (≥65 y) and 15 healthy elderly men (≥65 y) were selected to participate in this study. Subjects responded to advertisements in newspapers and were screened for eligibility at Maastricht University, the Netherlands. We informed all subjects on the nature and possible risks of the experimental procedures before we obtained written informed consent. This study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre, the Netherlands. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization guidelines for Good Clinical Practice as appropriate for nutritional products. The Trial Registration number for this study is NTR3047.

2.2. Pre-testing

All subjects participated in a screening session to determine their eligibility for inclusion. The inclusion criteria were male sex, age 65 years or older, and a BMI from 20 through 30 kg/m². Medical history, medication use, body weight, height, and alcohol use were recorded, and glucose tolerance was assessed by a 2-h oral glucose tolerance test. A basal blood sample was drawn to determine Hba1c, calcium and CRP concentrations. Exclusion criteria included: all co-morbidities, the use of medication interacting with muscle metabolism and mobility of the limbs, co-morbidities interacting with gastric-intestinal function, inadequate glycemic control and diabetes mellitus, smoking, weight loss of more than 3 kg in the last three months, the use of protein supplements, and participation in an exercise program. In addition, the criteria derived from the European Working Group on Sarcopenia in Older People [27] and the International Working Group on Sarcopenia [28] were used to determine the presence (or absence) of sarcopenia. The presence of both low skeletal muscle mass index (SMMI,
i.e. appendicular lean mass divided by height by meters squared (kg/m²) and low muscle function (gait speed and/or grip strength) was mandatory for the diagnosis of sarcopenia. Subjects were considered to be sarcopenic with a gait speed of ≤1.0 m/s or a handgrip strength <30 kg, in combination with a SMMI <8.4 kg/m² (Fig. 1). Gait speed was determined over a 4 m interval. We assessed handgrip strength using a hydraulic handheld dynamometer (Jamar, Jackson, MI), and determined body composition by dual-energy X-ray absorptiometry (DEXA, Hologic Discovery A, Bedford, MA) [29].

2.3. Diet and activity prior to testing

The same standardized meal was consumed by all subjects the evening prior to testing, containing 2385 kJ providing 35 Energy% (En%) as carbohydrate, 49 En% as fat, and 16 En% as protein. We instructed all participants to omit any exhaustive physical activity and to keep the diet as constant as possible during three days preceding testing.

2.4. Design

After the screening session, all eligible subjects participated in a single test day during which they ingested a single bolus of a given test drink. A primed, continuous infusion of L-[ring-13C6]-phenylalanine and L-[ring-2H2]-tyrosine (Cambridge Isotopes Laboratories, Andover, MA) combined with the collection of plasma samples and muscle biopsies before and after the intake of the test drink was used to determine both basal and post-prandial MPS rates and basal whole-body protein balance.

2.5. Experimental protocol

Subjects arrived at the laboratory after an overnight fast by car or public transport in the early morning. We inserted a Teflon catheter in an antecubital vein to allow infusion of stable-isotopes. A second catheter was inserted in a vein on the hand of the contralateral arm and was placed in a hot box (60 °C) for arterialized blood sampling [30]. First, a basal plasma sample and a basal serum sample were collected (t = −240 min), after which the plasma phenylalanine and tyrosine pools were primed with a single dose of intravenously administered L-[ring-13C6]-phenylalanine (2 µmol/kg) and L-[ring-2H2]-tyrosine (0.775 µmol/kg). Thereafter, continuous tracer infusion was started with an infusion rate of 0.045 µmol/kg/min for L-[ring-13C6]-phenylalanine and 0.020 µmol/kg/min for L-[ring-2H2]-tyrosine using a calibrated infusion pump (IVAC). Subjects rested in a supine position for 90 min, after which we collected the first muscle biopsy from the vastus lateralis muscle (t = −150 min), marking the end of the pre-infusion period and the beginning of the basal period. Subsequently, arterialized blood samples were collected every 30 min, and the second muscle biopsy was collected at t = 0 min, marking the end of the basal period. Following directly after the second biopsy, a single bolus of the test drink was ingested by the subjects. The third and fourth muscle biopsy were taken at t = 120 min and at t = 300 min from the contralateral limb. Arterialized blood samples (8 mL) were taken at t = 15, 30, 45, 60, 75, 90, 105, 120, 150, 165, 180, 210, 240, 270 en 300 min, and were collected in EDTA-containing tubes. They were centrifuged at 1000 g for 10 min at 4 °C. Aliquots of plasma were frozen in liquid nitrogen and were stored at −80 °C. We obtained muscle biopsies using the percutaneous needle biopsy technique, entering the muscle ±15 cm cranial

![Fig. 1. Group selection algorithm. Algorithm used to identify healthy and sarcopenic subjects, according to the criteria formulated by the European Working Group on Sarcopenia in Older People and the International Working group on Sarcopenia.](http://dx.doi.org/10.1016/j.clnu.2016.09.023)
of the patella and placing the needle ±3 cm below the fascia [31]. Muscle samples were first dissected carefully and any visible non-muscle material was removed, and were then frozen in liquid nitrogen and stored until further analysis at −80 °C.

2.6. Drinks

All subjects received a single bolus of a 21 g leucine-enriched whey protein nutritional supplement containing 3 g of total leucine, 9 g of carbohydrate and 3 g of fat with an energetic value of 628 kJ (produced by Nutricia Advanced Medical Nutrition, The Netherlands). For an overview of the composition of this supplement, see Kramer 2015 [26]. A small amount of tracer was added to the drink to prevent dilution of the L-[ring-13C6]-phenylalanine plasma enrichment.

2.7. Plasma analyses

Concentrations of plasma glucose and insulin were analyzed using commercially available kits (GLUC3, Roche; Ref: 05168791190, and Immunologic, Roche; Ref: 12017547122, respectively). High-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany) was used to determine HbA1c content in venous blood samples. To measure the plasma concentrations of all essential and non-essential amino acids, 1500 μL of 0.5 mM Tridecafluoroheptanoic acid (TDFHA) (Sigma, Zwijndrecht, The Netherlands) was mixed with 10 μL of plasma in water, and 10 μL of the internal standard solution containing stable isotope-labeled amino acids (Cambridge Isotope Laboratories, Inc., Andover, USA) was mixed in 0.1 M HCl. Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was used to determine amino acid concentrations, as described previously [32]. For plasma L-[ring-13C6]-phenylalanine and L-[ring-2H2]-tyrosine enrichment measurements, we derivatized plasma phenylalanine and tyrosine to their tert-butyldimethylsilyl (TBDMS) derivatives, and determined their 13C and/or 2H enrichments by electron ionization gas chromatography-mass spectrometry (GC–MS; Agilent 6890N GC/MS; Thermo-Finnigan, Bremen, Germany) by monitoring ion masses 44, 46, and 48. We applied standard regression curves to assess linearity of the mass spectrometer and to control for the loss of tracer.

2.9. Calculations

Infusion of L-[ring-13C6]-phenylalanine and L-[ring-2H2]-tyrosine with the collection of muscle biopsies and arterialized blood sampling were used to assess whole-body amino acid kinetics during basal state and fractional synthetic rate (FSR) of mixed muscle protein during both basal and post-prandial states.

The calculation of the whole body rate of phenylalanine appearance (Ra) and rate of phenylalanine disappearance (Rd) is summarized in the following equations:

\[ Ra = \frac{F - V}{1 + \frac{E_p}{F_p} + \frac{O}{V}} \]

\[ Rd = R_d - V \times \frac{C_2 - C_1}{t_2 - t_1} \]

In this formula, F is the intravenous tracer infusion rate (μmol/kg/min), V is the distribution volume for phenylalanine (≈0.125 L/kg), C1 and C2 are the phenylalanine concentrations (μM) at time point 1 (t1) and 2 (t2), respectively, and E1 and E2 are the plasma L-[ring-13C6]-phenylalanine enrichments at time point 1 (t1) and 2 (t2), respectively. At steady state, the phenylalanine concentrations at time point 1 and 2 are equal (C1=C2), so rate of phenylalanine appearance (Rd) and disappearance (Rd) are equal.

\[ R_d = R_d \]

The rate of phenylalanine appearance equals the breakdown and intake, and the rate of disappearance equals the synthesis and oxidation of amino acids. Thus:

\[ R\text{d} = B + I \]

\[ R\text{d} = S + O \]

Whole body protein turnover was calculated for the basal state, before intake of amino acids. Thus, intake (I) is zero and therefore changes in protein breakdown (B) equals the change in Rd.

\[ S = R_d - O \]

Whole-body oxidation (O) can be determined from the conversion (hydroxylation) of L-[ring-13C6]-phenylalanine to L-[ring-13C6]-tyrosine. The rate of hydroxylation can be calculated as follows:

\[ O = Tyr \times \frac{E_p(t)}{E_p(t) + \frac{Fp(t)}{Fp} + \frac{PheRd}{Phe}} \]

In this formula, E1 is the weighted mean plasma enrichment of L-[ring-13C6]-tyrosine during the incorporation period, Ep is the
weighted mean plasma enrichment of L-[ring-13C6]-phenylalanine (MPE) during the incorporation period, and Fp is the infusion rate of L-[ring-13C6]-phenylalanine (μmol/kg/min).

Fractional synthesis rate (FSR) of mixed muscle protein was calculated by dividing the increment in enrichment of the product, i.e. protein-bound L-[ring-13C6]-phenylalanine, by the enrichment of the precursor, i.e. plasma L-[ring-13C6]-phenylalanine enrichment. Muscle FSR was calculated as follows:

$$\text{FSR} = \frac{\Delta E_p}{E_{\text{precursor}} \times t} \times 100$$

In this formula, $\Delta E_p$ is the increment in protein-bound L-[ring-13C6]-phenylalanine after an incorporation period, $E_{\text{precursor}}$ is the weighted mean plasma L-[ring-13C6]-phenylalanine enrichment (TTR) during that incorporation period, $t$ indicates the incorporation period (h) between biopsies, and the factor 100 is needed to express the FSR in percent per hour (%/h). For basal FSR, muscle biopsies at $t = -2.5$ and $0$ h were used, and for post-prandial FSR, muscle biopsies at $t = 0, 2$ and $5$ h were used.

### 2.10. Statistics

Sample size calculation for the part of the study in which study groups healthy elderly men resulted in a number of 15 subjects per group [26]. Therefore, also 15 sarcopenic subjects were included in the study.

All data are expressed as mean ± standard error (SEM). Subjects’ characteristics were compared between groups using a two-sample t-test, nonparametric Wilcoxon rank sum test, or Fisher’s Exact test where appropriate. The concentrations of the plasma total amino acids (AA), essential AA (EAA), phenylalanine, and leucine at different time points were compared between groups using a mixed model for repeated measures (MMRM) with "group", "time", and their interaction as fixed effects, and "subject" as a random effect, and using the baseline value as covariate. In addition, peak concentrations and incremental area under the curve above baseline values (iAUC) of plasma total AA, EAA, phenylalanine and leucine were calculated and were compared between groups using ANCOVA with “group” as factor and using the baseline value as covariate. Insulin and glucose responses and plasma L-[ring-13C6]-phenylalanine enrichments were analyzed in the same way as the amino acid concentrations.

Whole-body phenylalanine kinetics were analyzed using unpaired two-sample t-tests to determine differences between the two groups. The post-prandial muscle fractional synthesis rates (FSR), which was the primary outcome measure, and the post-prandial muscle enrichments were analyzed using ANCOVA with baseline as covariate to determine differences with basal FSR within study groups. Basal FSR and muscle enrichment were compared between groups using two-sample t-tests. For the comparison of FSR and muscle enrichments between groups, an ANCOVA was used combining the post-prandial time points/periods as depending variables, with baseline as covariate and with “group” and “time” as factor. Statistical significance was

### Table 1

<table>
<thead>
<tr>
<th>Subjects’ characteristics</th>
<th>Healthy</th>
<th>Sarcopenic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>69 ± 1</td>
<td>81 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77 ± 0.02</td>
<td>1.72 ± 0.02</td>
<td>0.09</td>
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<tr>
<td>Weight (kg)</td>
<td>79.5 ± 1.9</td>
<td>74.1 ± 2.7</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 ± 0.3</td>
<td>25.1 ± 0.6</td>
<td>0.65</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>61.1 ± 1.5</td>
<td>54.2 ± 1.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>20.2 ± 0.8</td>
<td>23.5 ± 1.2</td>
<td>0.03</td>
</tr>
<tr>
<td>ALM (kg)</td>
<td>26.8 ± 0.7</td>
<td>22.5 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SMMI (kg/m²)</td>
<td>2.6 ± 0.1</td>
<td>7.6 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td>43.4 ± 1.9</td>
<td>26.1 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gait speed (m/sec)</td>
<td>1.3 ± 0.4</td>
<td>0.7 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total balance score (0–4)</td>
<td>3.9 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to complete 5 chair stands (s)</td>
<td>10.0 ± 0.3</td>
<td>18.3 ± 1.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Total SPPB score (0–12)</td>
<td>11.8 ± 0.1</td>
<td>6.1 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.3 ± 0.2</td>
<td>5.6 ± 0.2</td>
<td>0.17</td>
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<tr>
<td>2-h glucose (mmol/L)</td>
<td>5.7 ± 0.3</td>
<td>6.8 ± 0.4</td>
<td>0.06</td>
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<td>Glycated haemoglobin (%)</td>
<td>5.7 ± 0.1</td>
<td>5.7 ± 0.1</td>
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<tr>
<td>HOMA-IR index</td>
<td>2.37 ± 0.51</td>
<td>2.49 ± 0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>OGIS index</td>
<td>324 ± 22</td>
<td>336 ± 15</td>
<td>0.63</td>
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<tr>
<td>Baseline hsCRP level (mg/L)</td>
<td>1.2 ± 0.2</td>
<td>4.6 ± 1.1</td>
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<tr>
<td>Baseline Calcidiol level (ng/mL)</td>
<td>68.3 ± 6</td>
<td>58.8 ± 8</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Abbreviations: BMI, Body Mass Index; ALM, Appendicular Lean Mass; SMMI, Skeletal Muscle Mass Index; SPPB, Short Physical Performance Battery; HOMA-IR, Homeostatic Model Assessment — Insulin Resistance; OGIS, Oral Glucose Insulin Sensitivity. Data were analyzed using Two-sample t-test, Non-parametric Wilcoxon rank sum test, or Fisher’s Exact test. Characteristics were tested as potential confounders, which did not reveal any significant effect.

Fig. 2. Plasma glucose and insulin concentrations. Mean (±SEM) plasma glucose (A) and insulin (B) concentrations (mmol/L and mU/L, respectively) in healthy (n = 15) and sarcopenic (n = 15) subjects during the fasting period and after ingestion of 21 g of leucine-enriched whey protein supplement. Comparisons between groups at specific time points were done using mixed model repeated measures (MMRM) analyses; no significant differences between groups. Glucose: iAUC P = 0.15, peak-value P = 0.38; Insulin: iAUC P = 0.74, peak-value P = 0.77 (all ANCOVA).

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3. Results

3.1. Participants

A total of 15 healthy, non-sarcopenic, elderly men (age: 69 ± 1 y; body weight: 79.5 ± 1.9 kg; BMI: 25.5 ± 0.3 kg/m²) and 15 sarcopenic elderly men (age: 81 ± 1 y; body weight: 74.1 ± 2.7 kg; BMI: 25.1 ± 0.6 kg/m²) were included and participated in the experiment between December 2011 and April 2013. Participants’ characteristics are shown in Table 1. The group of sarcopenic elderly differed significantly from the group of healthy elderly regarding the physical tests (SPPB and handgrip strength) and DEXA results, which confirms that the selection of subjects was done adequately. Age, alcohol consumption, BMI, lean body mass, skeletal muscle mass, weight, hsCRP and calcidiol were tested as possible founders, but did not significantly influence the results of the between-group analyses of the FSR outcome parameters. There were no dropouts during the study.

3.2. Safety and tolerance

No gastro-intestinal complaints were observed after intake of the drinks in any of the two groups. The recorded adverse events did not differ between the groups and were not considered to be related to the use of the study product. No serious adverse events occurred during the execution of this study.

3.3. Plasma glucose and insulin

Plasma glucose and insulin concentrations in the healthy and sarcopenic group are displayed in Fig. 2. For glucose, a significant time effect was observed in both groups (MMRM model, P < 0.0001), without a group effect or group x time effect (P = 0.11 and P = 0.92, respectively). No significant differences in glucose concentrations were seen between the groups at any time point. No difference in glucose peak concentration or incremental area under the curve (iAUC) above baseline levels was seen between the healthy and sarcopenic group (7.1 ± 0.2 and 7.0 ± 0.2 mmol/L, ANCOVA P = 0.38, and 92 ± 12 and 117 ± 10 mmol/L/5 h, P = 0.15, respectively). Plasma insulin concentrations also significantly increased after ingestion of the supplement (MMRM model, significant time effect (P < 0.0001), no interaction (P = 0.92) effect, no group effect (P = 0.78)). Again, no significant differences in insulin concentrations were seen between the groups at any time point. Furthermore, no difference was seen between the healthy and sarcopenic group in terms of insulin peak concentration or iAUC (63 ± 13 and 57 ± 9 mU/L, P = 0.77 and 3455 ± 776 and 3475 ± 623 mU/L/5 h, P = 0.74, respectively).

3.4. Plasma amino acids

The time course of plasma phenylalanine (A), leucine (B), total essential amino acid (EAA; C), and total amino acid (AA; D)
concentrations over time are depicted in Fig. 3. Total plasma amino acid concentrations increased after ingestion of the supplement in both groups (MMRM model, time effect P < 0.001, group effect P = 0.08, interaction effect P = 0.91). No difference in peak concentration of any of the amino acids was seen between the two groups. Plasma phenylalanine concentrations were higher in the sarcopenic group during a part of the post-prandial period, i.e. at time point t = 45, 60, 75, 90, 135 and 150 min (MMRM, P < 0.05, Fig. 3A). Leucine concentrations were higher in the sarcopenic group for time points t = 75 until t = 105 min and t = 135 (P < 0.05; Fig. 3B). Total essential amino acid concentrations were higher in the sarcopenic group at t = 75, t = 90 and t = 135 min (MMRM, P < 0.05, Fig. 3C). In addition, higher iAUC were seen for plasma phenylalanine, leucine and EAA concentrations in the sarcopenic group when compared with the healthy group (ANOVA, 3.1 ± 0.3 vs 2.1 ± 0.1 mmol/L/5 h, P = 0.006; 51.2 ± 2.3 vs 42.5 ± 1.7 mmol/L/5 h, P = 0.01; and 161.4 ± 8.4 vs 136.0 ± 7.0 mmol/L/5 h, P = 0.03, respectively). Total plasma amino acid concentration only differed between the groups at t = 90 min (Fig. 3D).

3.5. Plasma L-[ring-13C6]-phenylalanine

Figure 4 shows the plasma L-[ring-13C6]-phenylalanine enrichments. No significant differences in L-[ring-13C6]-phenylalanine enrichments were seen between the groups at any time point. After ingestion of the supplement, a small decrease in L-[ring-13C6]-phenylalanine enrichments was observed in both groups.

3.6. Whole-body phenylalanine kinetics

During the basal period, whole-body protein breakdown measured by phenylalanine appearance averaged 38.1 ± 0.7 and 36.0 ± 0.8 μmol/kg/h in the healthy and sarcopenic group, respectively, without significant differences between the groups (t-test, P = 0.06). Whole-body protein synthesis measured by phenylalanine disappearance corrected for amino acid oxidation neither differed between the groups (t-test, P = 0.13), with an average rate of 35.0 ± 0.7 and 33.4 ± 0.7 μmol/kg/h, respectively. However, whole-body phenylalanine hydroxylation did show a significant group effect (t-test, P < 0.01, 3.3 ± 0.2 vs 2.5 ± 0.2 μmol/kg/h, in the healthy and sarcopenic group, respectively). In total, basal whole-body net protein balance was negative in both groups, but significantly higher in the sarcopenic elderly (−2.0 ± 0.2 μmol/kg/h) when compared with the healthy elderly (−2.6 ± 0.2 μmol/kg/h) (t-test, P < 0.01).

3.7. Muscle tracer analyses

Muscle tissue-free and protein-bound L-[ring-13C6]-phenylalanine enrichments are presented in Table 2. In the two groups, a significant rise in muscle tissue-free L-[ring-13C6]-phenylalanine was observed at both 2 h and 5 h following ingestion of the supplement compared with basal values (ANOVA, P < 0.0001 and P < 0.001 in the healthy and sarcopenic group, respectively). Muscle tissue-free L-[ring-13C6]-phenylalanine enrichments were significantly higher in the sarcopenic elderly group at baseline in comparison with the healthy elderly group (t-test, P < 0.001), while there was no difference between groups at 2 and 5 h (ANOVA, P = 0.68 and P = 0.25, respectively). Mixed muscle protein-bound L-[ring-13C6]-phenylalanine enrichments showed a significant rise in both groups at 2 h and 5 h compared with basal values (ANOVA, P < 0.0001 for both time points for each group). There were no statistical differences between the two groups at any specific time point.

3.8. Mixed-muscle protein synthesis rates

Mixed-muscle protein synthesis rates, expressed as fractional synthesis rates (FSR) with plasma L-[ring-13C6]-phenylalanine enrichments as precursor, are shown in Fig. 5. FSR values were calculated for the basal period (−2.5−0 h), the early post-prandial period (0−2 h), the late post-prandial period (2−5 h), and the cumulative post-prandial period (0−5 h). A significant rise in mixed-muscle FSR was seen in both healthy and sarcopenic elderly in the cumulative post-prandial period (ANOVA, P < 0.001 and

Table 2

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Healthy elderly</th>
<th>Sarcopenic elderly</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle tissue-free enrichments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.49 ± 0.20</td>
<td>5.54 ± 0.19</td>
<td>&lt;0.0011</td>
</tr>
<tr>
<td>120</td>
<td>5.44 ± 0.25*</td>
<td>5.92 ± 0.21*</td>
<td>0.0824</td>
</tr>
<tr>
<td>300</td>
<td>5.21 ± 0.15*</td>
<td>6.07 ± 0.16*</td>
<td>0.2523</td>
</tr>
<tr>
<td><strong>Muscle protein-bound enrichments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.0058 ± 0.0005</td>
<td>0.0072 ± 0.0009</td>
<td>0.1815</td>
</tr>
<tr>
<td>120</td>
<td>0.0150 ± 0.0013*</td>
<td>0.0164 ± 0.0008*</td>
<td>0.5524</td>
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<tr>
<td>300</td>
<td>0.0268 ± 0.0014*</td>
<td>0.0295 ± 0.0015*</td>
<td>0.2524</td>
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</table>

Mean (±SEM) muscle tissue-free and muscle protein-bound L-[ring-13C6]-phenylalanine enrichments (MPE) during the fasting period (t = 0 h) and post-prandial period (t = 2 and t = 5 h) following ingestion of 21 g of leucine-enriched whey protein supplement in healthy (n = 15) and sarcopenic (n = 15) subjects. Data were analyzed to compare the post-prandial enrichments between groups using Two-sample t-test, and ANCOVA with basal values as covariate. Comparisons within the groups between the post-prandial time point and basal values were done using ANCOVA. *Significant increase compared with basal (t = 0 h) muscle enrichment.
P = 0.003, respectively, Fig. 5A). Furthermore, mixed-muscle FSR was significantly higher than basal FSR in the healthy and sarcopenic group in both the early (ANOVA, P = 0.009 and P = 0.004, respectively) and late (P < 0.001 and P = 0.008, respectively) post-prandial period. No significant difference was observed for basal FSR between the two groups (t-test, 0.032 ± 0.003 and 0.040 ± 0.005%/h, in the healthy and sarcopenic group, respectively, P = 0.14; Fig. 5B). Both the early and late post-prandial muscle protein synthesis rates did not differ significantly between the two groups (ANOVA, 0.060 ± 0.008 vs 0.058 ± 0.007%/h and 0.048 ± 0.003 vs 0.052 ± 0.004%/h; P = 0.93 and P = 0.34, respectively; Fig. 5B). Likewise, no significant difference was observed for the cumulative post-prandial FSR between the healthy and sarcopenic group (ANOVA, 0.053 ± 0.004 and 0.055 ± 0.004%/h, respectively, P = 0.45; Fig. 5B). Similar results were obtained using the muscle tissue-free L-[ring-13C6]-phenylalanine enrichments as precursor pool (data not shown).

4. Discussion

We demonstrated that muscle protein synthesis rates in sarcopenic older men were effectively increased by the ingestion of 21 g of a leucine-enriched whey protein. Basal muscle protein synthesis rates and post-prandial muscle protein synthesis rates did not differ between sarcopenic and healthy older men.

Older individuals with diagnosed sarcopenia have lost substantial amounts of skeletal muscle mass and strength when compared with their healthy older controls. We hypothesized that this loss of muscle mass is attributed to a decline in basal muscle protein synthesis rates and/or a blunted muscle protein synthetic response to feeding. We selected a group of 15 sarcopenic older males (81 ± 1 y) from a total of 161 potential subjects who responded to advertisements placed in local newspapers aimed at elderly males who had been experiencing signs of muscle loss and an incipient decline in mobility. Sarcopenia was diagnosed using the criteria as formulated by the EWGSOP [27] and the International Working Group on Sarcopenia [28] in an algorithm including gait speed, handgrip strength and skeletal muscle mass index (Fig. 1). The presence of both low SMMI (<8.4 kg/m²) and low muscle function (gait speed <1.0 m/s and/or grip strength <30 kg) was required for the diagnosis of sarcopenia. A control group of 15 healthy older males (69 ± 1 y) were recruited from a pool of independently living elderly who responded to an advertisement in the local newspaper. As expected, the sarcopenic elderly showed significantly lower skeletal muscle mass when compared with the healthy controls (Table 1).

Previous work has suggested lower basal muscle protein synthesis rates in older compared with younger individuals as a rationale for the age-related loss of skeletal muscle mass and strength [4–7]. We hypothesized that basal muscle protein synthesis rates are reduced in sarcopenic when compared with healthy older men. However, our data showed no significant differences in basal muscle protein synthesis rates between groups (0.040 ± 0.005 vs 0.032 ± 0.003%/h in the sarcopenic and healthy group, respectively; P = 0.14, Fig. 5B). In fact, the basal muscle protein synthesis rates tended to be higher as opposed to lower in the sarcopenic older subjects compared with the healthy controls. These data tend to be in line with more recent reports showing no detectable differences in basal muscle protein synthesis rates between the young and elderly [8,10,11] and the observation of rather higher than lower basal muscle protein synthesis rates in the older compared with the young [34]. Consequently, a structural decline in basal muscle protein synthesis rate does not seem to be the underlying mechanism of the difference in skeletal muscle mass between sarcopenic and non-sarcopenic elderly.

In addition to basal muscle protein synthesis rates, post-prandial stimulation of muscle protein synthesis has been identified as an important component in the regulation of muscle mass maintenance. It has been well established that the older population shows an impaired muscle protein synthetic response to anabolic stimuli such as food intake and physical activity [8,18,35,36]. The mechanisms responsible for this anabolic resistance may be multifactorial and may include impairments in protein digestion and amino acid absorption [37], increased extraction of plasma amino acids into the splanchnic tissues [15], reduced amino acid delivery to skeletal muscle tissue [38], reduced amino acid uptake in muscle [39], and impaired intramuscular anabolic signaling [8]. As sarcopenic elderly have experienced loss of skeletal muscle mass and strength, we hypothesized that these sarcopenic elderly show an attenuated muscle protein synthetic response to feeding when compared with healthy controls. Following ingestion of the 21 g bolus of leucine-enriched whey protein supplement, we observed a rapid post-prandial rise in plasma amino acid and insulin.
concentrations in both groups (Figs. 2B and 3A–D). This resulted in 21 g of a leucine-enriched whey protein supplement, are not impaired in sarcopenic older males, Clinical Nutrition (2016), http://dx.doi.org/10.1016/

during the early (0–2 h), late (2–5 h) or entire 5 h post-prandial phase between groups (P = 0.93, P = 0.34, and P = 0.45, respectively, Fig. 5B). This shows that there are no impairments in the post-prandial muscle protein synthetic response to the ingestion of a 21 g bolus of leucine-enriched whey protein in sarcopenic elderly compared with healthy controls. This demonstrates that even in sarcopenic elderly senescent muscle seems to maintain its capacity to respond to the ingestion of a bolus of whey protein fortified with free leucine.

The present study expands upon previous work aiming to elucidate the changes in basal and post-prandial muscle protein synthesis rates associated with aging, by comparing both basal and post-prandial muscle protein synthesis rates between healthy and sarcopenic older men. Despite the substantial differences in muscle mass and strength between the sarcopenic elderly and their healthy controls, we could not detect any differences in basal or post-prandial muscle protein synthesis rates between groups. This raises the question what (other) mechanisms may be responsible for the substantial loss of skeletal muscle mass in the sarcopenic geriatric population. We have recently reported substantial muscle loss after merely 5–14 days of muscle disuse, attributed to both a decline in basal muscle protein synthesis and impairments in the anabolic response to feeding [40]. This work, as well as work from others [41–43], suggests that muscle mass loss with aging may be largely attributed to muscle loss experienced during short, successive periods of bed rest following injury or disease, characterized by muscle disuse and malnutrition. This catabolic crisis theory [44] implies that the substantial loss of muscle mass and strength experienced during such episodes is not regained during recovery from injury or disease in the older population, resulting in a progressive loss of muscle mass throughout the later stages of the lifespan. In support of this theory, the medical history of our sarcopenic elderly showed multiple reports of hospitalization and surgery over (at least) the preceding 5 years with multiple comorbidities. We believe more focus is needed to address the impact of such periods of accelerated muscle loss following injury, surgery or disease on the development of sarcopenia in the older population.

In the present study, we compared the post-prandial muscle protein synthetic response following the ingestion of a 21 g of a leucine-enriched whey protein nutritional supplement between healthy and sarcopenic older men. Ingestion of 20 g whey protein has previously been shown to represent an effective anabolic stimulus in the older population [24] and was, therefore, applied in this study. In agreement, a ~35–65% increase in muscle protein synthesis rate was observed following protein ingestion in the older volunteers included in the present study. The absence of any structural differences in the post-prandial muscle protein synthetic response to feeding between the healthy and sarcopenic elderly indicates that there are no substantial impairments in anabolic sensitivity in sarcopenic elderly to the anabolic stimulus provided in the study (Fig. 5B). However, we cannot rule out that some level of anabolic resistance may be evident following ingestion of small (6 g), more meal-like amounts of dietary protein in sarcopenic versus healthy elderly. Nonetheless, our data clearly show that there is still remarkable responsiveness to proper anabolic stimuli in the compromised older sarcopenic patient, providing important leads to targeted nutritional intervention and dietary support.

In conclusion, basal muscle protein synthesis rates are not reduced in sarcopenic older men compared to healthy older men. The ingestion of 21 g of a leucine-enriched whey protein effectively increases muscle protein synthesis rates in healthy as well as sarcopenic older men.

Conflicts of interest

None of the authors had a personal or financial conflict of interest.

Statement of authorship

IFK, HMH, SV, YCL and LJClvL designed the research; IFK, HMH, IK, JMS, JvK, JB, and APG conducted the research; IFK and LBV analyzed data; IFK, LBV, MP, and LJClvL wrote the paper. All authors read and approved the final manuscript.

Disclosure summary

TI Food and Nutrition, a public–private partnership on precompetitive research in food and nutrition, funded this project. The researchers are responsible for the study design, data collection and analysis, decision to publish, and preparation of the manuscript. The industrial partners have contributed to the project through regular discussion.

Disclaimer

None.

Sources of support

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References


