Relationship between Dietary Protein or Essential Amino Acid Intake and Training-Induced Muscle Hypertrophy among Older Individuals

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(Received March 15, 2017)

Summary Dietary protein intake is critical for maintaining an optimal muscle mass, especially among older individuals. Although protein supplementation during resistance training (RT) has been shown to further augment training-induced muscle mass in older individuals, the impact of daily variations in protein intake on training-induced muscle mass has not been explored thus far. Therefore, this study aimed to investigate the relationship between the dietary protein and amino acid intake and RT-induced muscle hypertrophy among older individuals. Ten healthy older men (n=10; mean age=69±2 y; body weight (BW)=61.5±2.2 kg; height=1.65±0.02 m) participated in progressive RT performed 3 times/wk for 12 wk. Body composition (using DXA) and nutritional assessments (using a 3-d dietary record) were performed before and after the training period. Leg lean mass (LLM) increased significantly (15.0±0.8 vs. 15.4±0.8 kg, p<0.05) after RT, with no change in dietary nutrient intake. The average dietary protein intake was 1.62±0.11 g/kg BW/d, while essential amino acids was 600±51 mg/kg BW/d. Although the correlation between the increase in LLM and dietary protein intake was not significant, a significant correlation was found between the increase in LLM and dietary essential amino acid (EAA) intake. Furthermore, there were significant correlations between the increase in LLM and protein as well as EAA (especially leucine) intake at breakfast among subjects with suboptimal protein intake (p<0.05). Our study findings indicate that dietary protein as well as EAA intake may be significant contributing factors in muscle hypertrophic response during RT among healthy older men.

Key Words dietary protein intake, leucine, resistance training, muscle hypertrophy

Aging-related loss of muscle mass and function has been associated with increased risk of falls and fractures and loss of independence among older individuals (1, 2). Furthermore, recent cohort studies have shown that reduced muscle mass alone is associated with an increased risk of diabetes, cardiovascular disease (3, 4), and higher mortality (5) among older individuals, highlighting the importance of skeletal muscle to the modulation of metabolism.

Protein synthesis in skeletal muscles is a nutritionally responsive process that is actively stimulated by dietary protein intake (6). Daily protein intake is essential for maintaining an optimal skeletal muscle mass (7). Traditionally, the recommended dietary allowance (RDA) has been the same for healthy adults of all age groups (i.e., 0.8 g protein/kg body weight (BW) per day) (8). However, new evidence suggests that an increased protein intake of ≥1.0 g/kg BW/d for adults older than 65 y can be beneficial (9–12). The international PROTAGE study group recommended an intake of 1.0–1.5 g protein/kg BW/d for individuals older than 65 y with or without disease, and the new Nordic Nutrition Recommendations suggest an intake of 1.2–1.4 g protein/kg BW/d, with proteins accounting for 15–20% of the total energy intake in healthy older adults (13). Furthermore, the European Society for Clinical Nutrition and Metabolism (ESPEN) recommends an intake of at least 1.0–1.2 g protein/kg BW/d for healthy older people, and an even higher intake for older individuals with acute or chronic illness (9). However, the optimal level of protein intake for older individuals remains undetermined.

Several clinical studies have investigated the impact of...
inadequate protein intake. Houston et al. reported that older individuals whose protein intake is less than the RDA had the highest lean mass loss over 3 y, whereas those whose intake was greater than the RDA showed a decrease in the loss of lean mass of more than 40% (7). Castaneda et al. compared individuals with protein intakes of 0.45 g/kg BW/d and 0.9 g/kg BW/d for 9 wk and found a decrease in lean tissue, muscle function, and immune response in the low protein intake group; conversely, lean mass, muscle, and immune function were preserved in the higher protein intake group (1-4). Therefore, it is evident that an adequate level of daily protein intake is critical for maintaining skeletal muscle mass in older populations.

Some studies suggest that in addition to the total daily intake of protein, the quantity of protein in each meal also modulates protein anabolism. Bollwein et al. found that older individuals with unevenly distributed protein intake over the course of three daily meals had a higher incidence of frailty than those with more evenly distributed protein intake (15). Mamerow et al. demonstrated that isonitrogenous and isocaloric diets with a uniform protein distribution throughout the day (approximately 30 g protein/meal) resulted in a 25% increase in the 24-h mixed muscle protein synthesis rate as compared with diets with unevenly distributed protein (approximately 10, 20, and 60 g protein/meal) (16). This implies that unevenly distributed protein intake or inadequate single protein intake may negatively impact muscle protein metabolism, even if the individuals achieve the adequate total daily protein intake. Interestingly, a recent study indicates that older individuals need to consume at least 0.61 g of protein per lean body mass (LBM) in a single meal to significantly stimulate muscle protein synthesis during the fasting state (17). The findings of these studies underscore the importance of protein intake in each meal in addition to total daily intake to maximize meal-induced muscle protein synthesis and net muscle protein balance throughout the day.

An acute bout of resistance exercise has been shown to increase muscle protein synthesis (18). Consequently, long-term resistance training (RT) has been shown to significantly increase muscle mass and function among healthy and frail older individuals, indicating the importance of resistance exercise in preventing and ameliorating age-associated loss of muscle mass (19). Furthermore, intake of protein following resistance exercise has been shown to additively increase muscle protein synthesis to a greater extent than exercise alone (20). However, long-term clinical studies on the combined effect of protein supplementation with RT reported that protein supplementation with RT did not always result in higher muscle accretion as compared with exercise alone (21, 22).

No previous study has focused on the impact of dietary protein intake—both total daily intake and in each individual meal—of older individuals on training-induced muscle hypertrophy. Therefore, the current study aimed to investigate this during 12-wk progressive RT among healthy older men.

**MATERIALS AND METHODS**

Ten healthy older men (age=69±2 y; BW=61.5±2.2 kg; height=1.65±0.02 m) participated in this study. All volunteers provided written informed consent before participating in the study. The study was approved by the Ethics Committee of Ritsumeikan University (BKC-2012-004) and was conducted in accordance with the Declaration of Helsinki. Subjects were examined by a physician to confirm they had no medical problems that might preclude participation or affect the results. No subject had performed systematic resistance exercise prior to commencement of the study, but all subjects were moderately active. Subjects were instructed to continue their normal activities of daily living and usual diets throughout the experimental period.

**Strength assessment.** One-repetition maximum (1-RM) strength tests were performed every 4 wk to adjust training intensity. Isometric (90˚ knee angle from full extended position) and isokinetic (60˚/s) peak torque in the knee extensors was assessed before and after training using a dynamometer (Biodex System 4; Biodex Medical Systems, Shirley, NY). To avoid a possible learning effect, both strength tests were performed twice at least 3 d after the first measurement. The same investigator measured strength before and after training using the same levels of vocal encouragement.

**Resistance training.** The subjects performed resistance exercise 3 times per week on alternate days for 12 wk. All training sessions were performed in the morning after breakfast (between 8 am and 10 am). Experienced trainers supervised all training sessions to ensure that proper technique was used in each exercise session. Each exercise session included 2 exercises: bilateral knee extension and bilateral knee flexion. The starting weight used during the resistance exercise portion of this study was 50% of each subject’s predetermined 1-RM for 3 sets of 14 repetitions using a weight-stack machines (Life Fitness, Rosemont, IL). The rest period between sets was 3 min. The weight was increased for each subject when a rating of perceived exertion (RPE) was 16 or less for the 14th repetition of the 3rd set. Determination of 1-RM was repeated every 4 wk during the training period to adjust the training weights to 50% of 1-RM.

**Body composition.** Body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy, GE Medical Systems, Little Chalfont, UK). Participants maintained a supine position on the DXA table with the limbs close to the body, in accordance with the manufacturer’s protocol. Lean soft tissue mass of the whole body was divided into several regions (i.e., arms, legs, and trunk).

**Dietary assessments.** Nutritional assessments were performed before and during the training period.

Energy and nutrient intakes were measured by 3-d dietary records and photographic records. The subjects completed dietary records, either consecutively or non-consecutively, for 2 d during weekdays and one during weekends. The total amount of each nutrient was aver-
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All foods and beverages were weighed separately on a scale (2-kg kitchen scales KD-812; Tanita, Tokyo, Japan) before cooking. The subjects used a disposable camera (27 shots; Fuji Film, Tokyo, Japan) to take photographs of meals before and after eating. A dietitian used these photographs to complete any missing data and to resolve any discrepancies or obtain further information when necessary. Nutrient intakes were calculated by Excel Eiyokun (version 6, Kenpakusha Co., Tokyo, Japan) according to the Standard Table of Foods Composition in Japan 2010.

Blood analysis. Fasting blood samples were obtained to avoid any effects of food consumption. On arrival at the laboratory, venous blood from the antecubital vein was collected after 30 min of rest, and blood sampling was performed before and after the intervention period. Serum and plasma samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was immediately transferred to polypropylene tubes and stored at −80°C until analysis. Fasting serum concentrations of total cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride levels were determined using standard enzymatic techniques (Medic, Inc., Shiga, Japan). Plasma glucose concentrations were analyzed by the hexokinase G6PDH/UV method (Medic, Inc.). The blood glycated hemoglobin (HbA1c) content was analyzed using the latex agglutination method (Medic, Inc.).

Assessment of daily activity. Daily physical activities were evaluated using a triaxial accelerometer (Active Style Pro; Omron Healthcare, Kyoto, Japan). All subjects were asked to wear a triaxial accelerometer for 1 wk. The amount of physical activity during this week was used to calculate the average daily physical activity level.

Statistical analysis. All values are expressed as means±standard error (SE), unless indicated otherwise. SPSS version 19 (SPSS Inc., Chicago, IL) was used for statistical analysis. Differences in essential amino acids and protein intake at breakfast, lunch, and dinner were analyzed using one-way ANOVA with the Bonferroni post hoc test when appropriate. Differences between pre- and post-training values were assessed by a paired t-test. Relationships between the protein or essential amino acids, and the daily intake value.

Table 1. Nutritional intake of subjects before (pre) and after (post) the training period.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
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<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>2,657±211</td>
<td>2,419±215</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>81±8</td>
<td>64±8</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>346±30</td>
<td>333±27</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>109±10</td>
<td>102±9</td>
</tr>
<tr>
<td>(g/kg BW/d)</td>
<td>1.76±0.13</td>
<td>1.62±0.11</td>
</tr>
<tr>
<td>(g/kg LBM/d)</td>
<td>2.30±0.17</td>
<td>2.12±0.14</td>
</tr>
<tr>
<td>EAA (mg/d)</td>
<td>41,069±4,108</td>
<td>37,883±3,928</td>
</tr>
<tr>
<td>(mg/kg BW/d)</td>
<td>660±56</td>
<td>600±51</td>
</tr>
<tr>
<td>(mg/kg LBM/d)</td>
<td>866±73</td>
<td>785±64</td>
</tr>
<tr>
<td>Leucine (mg/d)</td>
<td>8,113±130</td>
<td>7,460±746</td>
</tr>
<tr>
<td>(mg/kg BW/d)</td>
<td>130±10</td>
<td>118±10</td>
</tr>
<tr>
<td>(mg/kg LBM/d)</td>
<td>171±13</td>
<td>155±12</td>
</tr>
</tbody>
</table>

All values represent means±SE (n=10). No difference was found between the pre- and post-training values. EAA, essential amino acids.

Table 2. Protein, essential amino acids and leucine intake for each meal (breakfast, lunch, and dinner) during the training period.

<table>
<thead>
<tr>
<th></th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>27.5±5.8*</td>
<td>26.5±3.4**</td>
<td>45.7±4.3</td>
</tr>
<tr>
<td>(g/kg BW)</td>
<td>0.43±0.09*</td>
<td>0.43±0.06**</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>(g/kg LBM)</td>
<td>0.55±0.10*</td>
<td>0.57±0.08**</td>
<td>0.95±0.08</td>
</tr>
<tr>
<td>EAA (mg)</td>
<td>10,269±2.279</td>
<td>9,535±1.458*</td>
<td>17,218±2.113</td>
</tr>
<tr>
<td>(mg/kg BW)</td>
<td>161±34*</td>
<td>154±23*</td>
<td>272±28</td>
</tr>
<tr>
<td>(mg/kg LBM)</td>
<td>206±40*</td>
<td>204±32*</td>
<td>358±38</td>
</tr>
<tr>
<td>Leucine (mg)</td>
<td>2,108±470</td>
<td>1,897±2.80*</td>
<td>3,304±3.84</td>
</tr>
<tr>
<td>(mg/kg BW)</td>
<td>33±7</td>
<td>31±4*</td>
<td>52±5</td>
</tr>
<tr>
<td>(mg/kg LBM)</td>
<td>42±8</td>
<td>40±6*</td>
<td>69±7</td>
</tr>
</tbody>
</table>

All values represent means±SE (n=10). *Significantly different from dinner (p<0.05); **Significantly different from dinner (p<0.01). kg BW, kg per body weight; kg LBM, per kg lean body mass. EAA, essential amino acids.
amino acid intake and changes in leg lean mass were determined using Pearson’s correlation coefficients. The level of statistical significance was set to \( p < 0.05 \).

### RESULTS

**Physical activity**

There were no significant differences in the physical activity of subjects before or during the training period (630±39 kcal/d and 728±88 kcal/d respectively).

**Daily nutritional intake**

Nutritional intake of the subjects before and during the intervention period demonstrated no significant change in total energy or nutrient composition in response to RT (Table 1).

**Protein, essential amino acids and leucine distribution throughout the day**

The average protein, essential amino acids and leucine intake for each meal (breakfast, lunch, and dinner) were calculated separately (Table 2). Protein intake at dinner was significantly higher than that at breakfast (\( p < 0.05 \)) and lunch (\( p < 0.01 \)) (Table 2). Furthermore, intake of essential amino acids at dinner was significantly higher than that at breakfast and lunch (\( p < 0.05 \)). Similarly, leucine intake at dinner was significantly higher than that at breakfast and lunch (\( p < 0.05 \)). Although leucine intake at breakfast was over 50% lower than that at dinner, this difference was not significant (Table 2).

**Blood parameters**

Basal concentrations of plasma triglycerides, total cholesterol, HDL cholesterol, blood glucose, and HbA1c before and after the training period showed no significant change in response to RT (Table 3).

**Strength and muscle mass**

After the training period, there was a significant increase in isometric knee extension and flexion strength (Table 4; \( p < 0.05 \)). Similarly, both isokinetic knee extension and flexion strength increased significantly in response to training (\( p < 0.05 \)). One-repetition maximum (1-RM) of both knee extension and flexion was also significantly improved (22.9±3.8% and 24.0±4.6%, respectively; \( p < 0.05 \)). Whole body fat-free mass and leg lean mass increased significantly after the training period (Table 5; \( p < 0.05 \)).
Correlations between protein or essential amino acid intake and training-induced changes in lean mass

When comparing nutrient intake and training-induced changes in leg lean mass, we noted a non-significant correlation between daily protein intake and change in leg lean mass \( (p=0.06 \text{ and } 0.09 \text{ for g/d and g/kg BW/d of protein intake, respectively; Table 6A, Fig. 1A}) \). In contrast, significant correlations were noted between intake of essential amino acids and changes in lean mass \( (p<0.05; \text{Table 6B, Figs. 2A and 3A}) \). In addition, no significant relationship was detected when the daily protein and many amino acids intake was expressed in g/kg LBM/d or mg/kg LBM/d (data not shown).

Correlations between protein or essential amino acid intake and training-induced muscle hypertrophy

No significant correlation was observed between protein or essential amino acid intake of any meal (breakfast, lunch, or dinner) and changes in leg lean mass \( (n=10) \). However, significant correlations between both protein intake and essential amino acid intake at breakfast and changes in leg lean mass were observed when the cut-off point of protein intake for maximal meal-induced muscle protein synthesis was set at 0.61 g/kg LBM/meal \( (n=7) \), and subjects who exceeded the cut-off point were excluded \( (n=3) \) (Table 7, Figs. 1B and 2B) \( (17) \). Among all the essential amino acids, leucine intake at breakfast showed the strongest correlation.
with change in leg lean mass ($p \leq 0.01$; Table 7, Fig. 3B). However, no significant relationship between protein or essential amino acid intake at lunch and change in leg lean mass was found. None of the subjects was taking below 0.61 g/kg LBM/meal of protein at dinner.

**DISCUSSION**

In response to progressive RT, healthy older men showed significantly increased strength and muscle mass within a 12-wk period. Furthermore, we report for the first time that training-induced increase in muscle...
mass was significantly correlated with daily protein and essential amino acid intake, underscoring the importance of dietary nutrient intake during training periods for older men.

In the current study, subjects were trained at 50% of their maximal strength, which is lower in intensity than the recommendation put forth by the American College of Sports Medicine (23). RT at high intensity (70–85% 1-RM) typically induces a significant increase in muscle mass and function for both frail and healthy older individuals (19, 24, 25). However, several studies have also reported a significant increase in muscle mass with exercises at an intensity below 70% 1-RM as indicated by significant increase in muscle thickness (26) or fiber cross sectional area (27). In the current study, leg lean mass increased significantly after the 12-wk training period by an average of 2.9% (0.7–6.1% increase in leg lean mass). This increase is similar to a 3% increase reported by Leenders et al., in which healthy men exercised using leg press and leg extension machines (4 sets of 8 repetitions) at a higher exercise intensity (75–80% 1-RM) for 24 wk (22). Similarly, Taaffe et al. investigated the effect of low intensity (40% of 1-RM for 14 repetitions) and high intensity (80% of 1-RM for seven repetitions) RT using leg press, knee extension, and knee flexion exercises. Three times a week for 52 wk among healthy older women (27). Exercise intensity and repetitions were designed such that the exercise volume (weight multiplied by repetition) was identical between groups. After the training period, a significant increase in type I fiber area (p<0.05) was observed for both groups while the increase in type II fiber area was not statistically significant in either group. However, there was no statistically significant difference in fiber areas between groups, indicating that low intensity exercise was as effective as high intensity exercise in eliciting muscle hypertrophy among older individuals (27). Accordingly, the exercise intensity used in the current study was appropriate to induce muscle hypertrophy among older men.

The increase in leg lean mass was accompanied by a substantial increase in leg muscle strength. The increase in 1-RM leg extension strength in the current study (approximately 23%) was comparable to that achieved with higher exercise intensity (75–80% 1-RM: approximately 27% increase in 1-RM strength) in a previous study by Verdijk et al. (21). In a study by Vincent et al. (28), healthy older men and women trained for 24 wk at either low intensity (50% 1-RM) or high intensity (80% 1-RM) using 12 weight machines including leg press, leg extension and leg curl. Each exercise was performed for one set of 13 repetitions (50% 1-RM) or 8 repetitions (80% 1-RM) giving equivalent exercise volumes (weight×repetitions). After the training period, 1-RM significantly increased for all exercises tested and the increase in total strength was similar between the two groups (17.2% and 17.8% for high and low intensity, respectively). Similarly, in our previous study, when healthy older men performed RT for 12 wk at 70% 1-RM (leg extension and flexion exercises; 3 sets of 10 repetitions), the increase in isokinetic and isometric leg extension strength was not significantly higher than that observed using a comparable exercise volume in the current study (p>0.10; data not shown) (29). Accordingly, low intensity resistance exercise is an effective training intensity for individuals with specific physical limitations, and for those who are apprehensive regarding their ability to undertake strength training.

No significant changes in blood parameters, including basal glucose, triglyceride, or cholesterol levels, were observed in response to RT. Our results agree with those of previous studies that indicated no changes in lipid markers after progressive RT in older women for 16 wk (30) or middle-aged men for 20 wk (31). However, they run contrary to other studies that have demonstrated improved insulin sensitivity (32) and reduced total cholesterol level (22) after RT in older women and men. The results of this study cannot clarify the discrepancy between glucose and lipid markers and this requires further investigation.

Before and after the training period, no significant change in energy intake was observed. Furthermore, no significant change in macronutrient intake, including protein, carbohydrate, or fat intake, was observed. Previous studies have also indicated that RT does not impact the total energy intake among older individuals (21, 22, 33–35). In the current study, the average daily protein intake was 1.62 g/kg BW/d, which was substantially higher than the RDA (0.8 g/kg BW/d). A previous study has also indicated higher daily protein intakes than the RDA among healthy older Japanese men and women (1.27 and 1.31 g/kg BW/d respectively) (36).

Recently, an association between protein intake and physical performance was described in the Hertfordshire Cohort Study (37). This cross-sectional analysis reported an association between a higher percentage of energy from protein intake and a faster 3-mile walking speed in community-dwelling women. In the InCHIANTI study (38), a cross-sectional study that involved more than 800 older Italians, the frailty risk was twice as high in the lowest quintile of protein intake as compared with that in the quintiles of higher protein intake (36). Furthermore, Sahni et al. reported in a cross-sectional study of 2,600 older men and women in the Framingham Offspring Cohort in Massachusetts that participants in the highest quartile of protein intake demonstrated higher leg muscle mass compared with those in the lowest quartile of protein intake (39). The findings of these studies together indicate the importance of levels of dietary protein intake in maintaining muscle mass and function.

We observed only a non-significant trend for a correlation between daily protein intake and changes in lean mass when the former was expressed in overall weight or in relative to body weight. On the other hand, daily intake of essential amino acids was significantly correlated with lean mass increase in healthy older individuals. The importance of essential amino acids and leucine on muscle protein anabolism has been reported previously. Volpi et al. have compared the protein metabolism after the ingestion of 18 g essential amino acids
with 40 g balanced amino acids (18 g essential amino acids +22 g non-essential amino acids) in older men and women. They demonstrated that acute ingestion of amino acids caused a significant but similar increase in muscle protein synthesis between groups indicating that essential amino acids, but not non-essential amino acids, are primarily responsible for amino acid-induced augmentation in muscle protein anabolism in older subjects (11). Among the essential amino acids, the branched-chain amino acid leucine has been shown to independently stimulate mTORC1 signaling and subsequent muscle protein synthesis in vivo and in vitro (40). It is well established that a combination of resistance exercise and leucine-rich protein, including milk protein and egg protein, increases muscle protein synthesis more significantly than resistance exercise alone in a fasted state (41). Several studies have demonstrated a dose response relationship between the amount of whey protein consumed after exercise and exercise-induced increase in muscle protein synthesis (20). Furthermore, the intake of whey protein after resistance exercise induces higher muscle protein synthesis than isonitrogenous soy protein, which contains less leucine and essential amino acids per gram of protein as compared with whey protein (42). Within EAA, we observed a significant correlation between daily leucine intake and training-induced increases in lean mass. However, we also observed significant correlations between daily intake of other single amino acids (including lysine, methionine and threonine) and changes in lean mass. Although WHO/FAO/UNU has reported the minimum level of dietary amino acids intake necessary to maintain body protein mass (43), to our knowledge, other than those studies on branched-chain amino acids (44), no previous study has investigated the effect of individual amino acid intake on muscle protein synthesis. A previous animal study has indicated that while hypoaaminoacidemia blunted the basal muscle protein synthesis, addition of leucine alone did not restore the basal muscle protein synthesis (45). Although this study indicates the importance of all amino acids in maintaining muscle protein synthesis, further studies are necessary to clarify the anabolic effect of a single amino acid, other than branched amino acids, on muscle protein metabolism.

Recent studies have indicated that as well as total protein intake throughout the day, protein distribution in each meal has a significant impact on protein accretion and muscle function among older individuals. In a recent cross-sectional study of an elderly population, Bollwein et al. found that although frail, pre-frail, and non-frail individuals showed no differences in absolute or relative protein consumption, and the intake of all subjects exceeded the RDA, non-frail subjects had a more uniform protein distribution pattern across all daily meals, whereas frail and pre-frail participants concentrated their protein consumption during the midday meal, with a lower intake at breakfast (15). Furthermore, Moore et al. indicated that older individuals require 0.61 g/kg LBM/d of protein per meal to maximally stimulate muscle protein synthesis (17). This indicates that protein intake should be distributed equally among three meals and each meal should contain at least 0.61 g/kg LBM of high quality protein to facilitate optimal muscle protein synthesis. Although our subjects were consuming protein at levels higher than the RDA, protein content was not distributed equally between meals, with protein intake at dinner significantly higher than that at breakfast or lunch. Furthermore, breakfast and lunch protein intake were below the recommended threshold of 0.61 g/kg LBM, indicating a suboptimal protein intake for maximal muscle protein synthesis in those subjects. Interestingly, when statistical analysis was performed for those subjects with suboptimal protein intake (n=7), there were significant correlations between changes in leg lean mass and protein or essential amino acids (especially leucine) intake at breakfast (Figs. 1B, 2B, and 3B). These data suggest suboptimal protein intake during breakfast may negatively affect the hypertrophic response to RT. In contrast, although protein and essential amino acids intake were significantly lower at lunch than those at dinner, we did not observe a significant correlation between changes in leg lean mass and protein or amino acids intake at lunch. The mechanism for this discrepancy is not clear from current results. Since the breakfast is consumed after the longest fasting period of the day (i.e. overnight fast), ingestion of an ample amount of protein is critical to suppress the fasting-induced muscle protein breakdown and to stimulate muscle protein synthesis. In addition, all subjects trained in the morning after breakfast. Although it has been demonstrated that protein supplementation increases exercise-induced muscle protein synthesis as a function of increased protein intake (11), recent meta-analysis indicate that protein timing did not influence hypertrophy (46). Further studies are warranted to investigate the influence of the amount of protein and essential amino acids within an individual meal on training-induced muscle hypertrophy.

Our nutritional analysis was conducted by recording individual meals by photography and by weighing each ingredient before cooking. Reference values for food ingredients in standard tables of food composition are based on the amino acid analysis using chromatography. Therefore, we were able to analyze the amino acid composition of individual meals in this study. Although we did not assess snacks between meals, a cross-sectional study in healthy older adults reported that snacks only contributed to 1.4% and 2.3% of the total protein intake in men and women respectively (47).

One major limitation of this study was the small number of subjects studied. In particular, the intake of leucine at breakfast and change in leg lean mass should be interpreted with caution. Although the cut-off point for maximal meal-induced muscle protein synthesis is feasible to determine suboptimal protein intake, those subjects who were excluded from the calculation did not demonstrate any higher increase in leg lean mass. Future studies using a larger number of subjects would allow greater statistical power when assessing the rela-
relationship between dietary nutrient intake and training-induced muscle hypertrophy.

In summary, the current study indicates that dietary protein and essential amino acid intake are significant contributing factors in the muscle hypertrophic response to RT among older men. Larger clinical studies are warranted to further investigate the role of dietary protein and leucine intake on training-induced muscle hypertrophy among different populations, including frail individuals.

Grants
This work was supported by JSPS KAKENHI Grant Nos. 25282200 and 25560379 to S. Fujita. This work was also supported by the Japanese Council for Science, Technology and Innovation, SIP (Project ID 14533567), Technologies for Creating Next-generation Agriculture, Forestry and Fisheries (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).

Author contributions
N.Y. and S.F. conceived and designed this research; N.Y., K.S., R.O., T.K., T.H., and S.F. performed the experiments; N.Y. analyzed the data; N.Y. and S.F. interpreted the results of the experiments; N.Y. prepared the figures; N.Y. and S.F. drafted the manuscript; N.Y., K.S., R.O., T.K., T.H., and S.F. approved the final version of the manuscript; N.Y., K.S., R.O., T.K., T.H., and S.F. edited and revised the manuscript.

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