Protein Supplementation at Breakfast and Lunch for 24 Weeks beyond Habitual Intakes Increases Whole-Body Lean Tissue Mass in Healthy Older Adults1–3

Catherine Norton,4,5 Clodagh Toomey,4,5 William G McCormack,4,5 Peter Francis,4,5 Jean Saunders,6 Emmet Kerin,4 and Philip Jakeman4,5*

4Human Science Research Unit, Center for Interventions in Inflammation, Infection, and Immunity, 5Food for Health Ireland, and 6Statistical Consultancy Unit, University of Limerick, Limerick, Ireland

Abstract

Background: Key areas of research on the preservation of lean tissue mass (LTM) during aging are determinations of the protein requirement and optimal protein intake at meals.

Objective: The aim of this study was to determine the effect of protein supplementation at breakfast and lunch for 24 wk beyond habitual intakes on whole-body LTM in healthy adults aged 50–70 y.

Methods: In a single-blinded, randomized, controlled design, 60 healthy older men and women (aged 61 ± 5 y) with a body mass index (in kg/m²) of 25.8 ± 3.6 consumed either 0.165 g/kg body mass of a milk-based protein matrix (PRO) or an isoenergetic, nonnitrogenous maltodextrin control (CON) at breakfast and midday meals, the lower protein–containing meals of the day, for 24 wk. Dual-energy X-ray absorptiometry was used to measure the change in LTM.

Results: After the intervention, protein intake in the PRO group increased from 0.23 ± 0.1 to 0.40 ± 0.1 g/kg for breakfast and from 0.31 ± 0.2 to 0.47 ± 0.2 g/kg for the midday meal. In response, LTM increased by 0.45 (95% CI: 0.06, 0.83) kg in the PRO group compared with a decrease of 0.16 (95% CI: −0.49, 0.17) kg in the CON group (P = 0.006). Appendicular LTM accounted for the majority of the difference in LTM, increasing by 0.27 (95% CI: 0.05, 0.48) kg in the PRO group compared with no change in the CON group (P = 0.002).

Conclusions: Protein supplementation at breakfast and lunch for 24 wk in healthy older adults resulted in a positive (+0.6 kg) difference in LTM compared with an isoenergetic, nonnitrogenous maltodextrin control. These observations suggest that an optimized and balanced distribution of meal protein intakes could be beneficial in the preservation of lean tissue mass in the elderly. This trial was registered at clinicaltrials.gov as NCT02529124.

Keywords: healthy aging, sarcopenia, lean tissue mass, meal level protein intake, milk protein matrix

Introduction

The postmaturation decline in lean tissue mass (LTM)7 is an insidious process with an estimated loss rate of 1–2%/y from the age of 50 y (1–4). Principal to the preservation of LTM is the maintenance of skeletal muscle mass for metabolic health and to defer the onset of sarcopenia and frailty. A number of underlying mechanisms contribute; however, to date, the main nutritional focus in relation to the preservation of LTM in aging has been protein requirement (5–10) and the age-specific change in muscle protein synthesis (MPS) in response to protein and amino acid intake (11–16). The current RDA for protein in healthy men and nonpregnant adult women of all ages is 0.8 g ⋅ kg⁻¹ ⋅ d⁻¹ of high-quality (i.e., high in essential amino acids) protein. On the basis of nitrogen-balance studies, the RDA for protein is nominally 2 SDs above the Estimated Average Requirement (0.66 g ⋅ kg⁻¹ ⋅ d⁻¹) and reflects the average minimal amount of protein (nitrogen) intake to balance nitrogen excretion and prevent a progressive loss of body protein (17). However, achieving nitrogen balance may be an inadequate outcome with respect to the age-related decline of LTM. Recent consensus statements suggest that a protein intake above the

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3 Supplemental Tables 1–4 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
4 Abbreviations used: ALTM, appendicular lean tissue mass; BM, body mass; CON, maltodextrin control; LTM, lean tissue mass; MPS, muscle protein synthesis; PRO, milk-based protein matrix.
5 To whom correspondence should be addressed. E-mail: phil.jakeman@ul.ie.

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RDA (i.e., between 1.0 and 1.5 g·kg\(^{-1}\)·d\(^{-1}\)) may be of benefit to the preservation of LTM in healthy adults (9, 10).

Guideline recommendations for daily protein intake make no reference to meal-level protein intake or the optimal distribution of protein intake throughout the day. Estimates of meal-level dietary protein intake in the United States (17) and Europe (7) find the lower protein–containing meal (normally breakfast, ~8–15 g) to be suboptimal with respect to stimulation of MPS, with only the higher protein–containing meal (normally dinner, ~30–40 g protein) reaching a protein intake that is optimal for postprandial MPS (9, 14). Working within the current recommended guidelines for daily protein intake but redistributing the meal-level intake of protein across breakfast, lunch, and dinner may offer a strategy to optimize net protein balance. In support of this strategy, isoenergetic and isonitrogenous diets consumed evenly (~30 g protein/meal) throughout the day resulted in a 25% greater 24-h mixed-muscle protein fractional synthesis rate compared with a skewed (~10, 20, and 60 g protein/meal) daily protein intake (18). Independent of the daily amount, the source of protein intake can modulate the rate of MPS. Milk protein consists of ~80% casein and ~20% whey. Casein and whey are considered to be of high quality due to their high relative essential amino acid composition, rate of digestion and absorption, and bioactivity, which can be enhanced by partial hydrolysis (19, 20). The protein synthetic response to postprandial hyperaminoacidemia (21) and other bioactive stimuli provided by protein ingestion (20) is impaired in the skeletal muscle of older adults (12), particularly after a low (<30 g/meal) protein intake (5, 9) or when the dose of essential amino acids, leucine in particular, is low (11–14, 22). These findings suggest that a higher amount of protein intake per meal, ~0.40 g/kg, would be optimal for skeletal muscle MPS and to retain LTM in older adults.

To test this hypothesis, the present study sought to provide a high-quality protein supplement for 24 wk beyond habitual intakes to healthy, independent-living older adults. Supplemental protein, equivalent to 0.33 g protein/kg per day, was consumed in 2 equal parts with the lower protein–containing meals of the day (i.e., breakfast and lunch). The primary outcome was the change in LTM measured by DXA.

**Methods**

**Subject recruitment and progression.** All of the participants were informed of the purpose of the study and all known risks before providing written, informed consent. The study was approved by the Faculty of Education and Health Sciences Research Ethics Committee (EHSREC010/45), University of Limerick. All procedures were carried out in accordance with the ethical standards outlined in the most recent version of the Declaration of Helsinki. The trial was registered at clinicaltrials.gov as NCT02529124.

Figure 1 shows a flowchart of the progress from recruitment through completion at 24 wk. A convenience sample of healthy men and women (aged 50–70 y) from the University of Limerick’s Body Composition Study were invited by e-mail or word-of-mouth to participate in the study. After written, informed consent 178 volunteers were assessed for eligibility, submitted to a clinical examination, and provided a dietary record to a qualified dietitian. Those defined as healthy, free-living, fully mobile, and independent-living and with no indication of lactose intolerance or adverse reaction to dairy-based foods were invited to participate. Twenty subjects were excluded after screening (musculoskeletal issues: n = 6; medication: n = 6; celiac disease: n = 4; impaired fasting blood glucose concentration: n = 3; cancer in the previous 2 y, n = 1; and cardiopulmonary abnormality: n = 1) and 18 declined to participate further. Participants were then randomly assigned to receive a supplement containing either a milk-based protein matrix (PRO) or an isoenergetic,
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Table 1: Energy and Macronutrient Intakes in Older Adults in the CON and PRO Groups at Baseline and During the 24-wk Intervention

<table>
<thead>
<tr>
<th></th>
<th>CON Baseline</th>
<th>CON Intervention</th>
<th>PRO Baseline</th>
<th>PRO Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/d</td>
<td>1890 ± 380</td>
<td>2006 ± 384</td>
<td>2079 ± 576</td>
<td>2190 ± 578</td>
</tr>
<tr>
<td>Carbohydrate, %TEI</td>
<td>44 ± 8</td>
<td>47 ± 7</td>
<td>48 ± 9</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>Fat, %TEI</td>
<td>34 ± 6</td>
<td>32 ± 6</td>
<td>31 ± 6</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>Protein, %TEI</td>
<td>19 ± 3</td>
<td>17 ± 3</td>
<td>16 ± 3</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>Protein, g·d⁻¹</td>
<td>86 ± 16</td>
<td>86 ± 16</td>
<td>83 ± 19</td>
<td>106 ± 20</td>
</tr>
<tr>
<td>Protein, g·kg⁻¹·d⁻¹</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SDs; n = 29 (24 women and 5 men; CON group) and n = 32 (22 women and 9 men; PRO group). CON, maltodextrin control; PRO, milk protein matrix; %TEI, percentage of total energy intake excluding alcohol.

Dietary intake. An estimate of participants’ dietary intake was undertaken within a 4-d period before commencement of supplementation. After comprehensive instruction and mentoring by a qualified dietician, participants recorded food and fluid intake for 4 consecutive days (2 weekdays and 2 weekend days) by using an estimated food intake record. The dietician provided oral and written instruction on recording food type, quantity, cooking method, and meal time. During follow-up and subsequent visits, the dietician checked the diary for completeness and obtained any additional information required to improve the accuracy of the dietary record. Food intake data were coded and subsequently analyzed by proprietary software (WISP V4; Tinuviel Software). Modifications were made to the food-composition database to include recipes of composite dishes, nutritional supplements, generic Irish foods that were commonly consumed, and new foods on the market. All previous modifications to the food-composition database were also checked and updated from current manufacturers’ instructions as necessary. To remove some of the bias introduced by misreporting of food records, under-reporters [i.e., reported energy intake to basal metabolic rate ratio of <1.1 (23)] were excluded from further analysis. Meal-level analysis of protein intake and amino acids was extracted for breakfast, midday (lunch), evening (dinner), and snack consumption.

Clinical biochemistry. Blood samples were obtained by venipuncture of an antecubital vein. Serum and plasma were separated by centrifugation at 10,000 × g at 4°C for 5 min and frozen at −78°C until analysis. Plasma insulin (catalog no. 12017547122) and serum total 25-hydroxyvitamin D (catalog no. 06506780160) were measured on a Roche Cobas e411 autoanalyzer (Roche Diagnostics). Plasma glucose (catalog no. 442640) and serum calcium (catalog no. A28937), albumin (catalog no. 442765), urea (catalog no. 442820), cholesterol (catalog no. 467825), TGs (catalog no. 445850), HDL cholesterol (catalog no. 650207), LDL cholesterol (catalog no. 969706), and creatinine (catalog no. A60298) were measured on a UniCel DxC 800 autoanalyzer (Beckman Coulter). The interassay CV was <5% for all analytes other than vitamin D, total cholesterol (6.9%), and LDL cholesterol (6.0%).

Anthropometric and body-composition measurements. Height was measured to the nearest 0.1 cm by using a stadiometer (Seca) and BM was measured to the nearest 0.1 kg (MC-180MA; Tanita UK Ltd.). Whole-body compositional analysis was performed using DXA as per the recommendations of the International Society of Clinical Densitometry (24). A Lunar iDXA scanner with enCORE version 14.1 software (GE Healthcare) was used to capture and analyze whole-body scans. Calibration by means of a phantom block was performed daily. To standardize test conditions and tissue hydration, participants were instructed to refrain from strenuous exercise in the 12-h period before testing and to attend after an overnight fast. Participants consumed 500 mL water 1 h before the scan and were instructed to void and defecate, if required, immediately before measurement. The enCORE software provided first-pass segmentation of the total body into arm, leg, and trunk regions of interest. A qualified DXA technician checked for correct anatomic alignment and made manual adjustments where necessary. Whole-body and stature-normalized (kg/m²) indexes of body fat and LTM were determined. The precision (root mean square CV) for repeated measurement of LTM was 0.55% and 1.2% for body fat mass.

Statistical analysis. Baseline body-composition and clinical biochemical data were checked for normality of distribution by using the Shapiro-Wilk test and expressed as means ± SDs for normally distributed variables and medians (IQRs) for skewed variables. An independent t test or Mann-Whitney U test was used to compare differences between groups at baseline. The treatment effect was calculated as the change in DXA-derived body composition from baseline to 24 wk and presented as means (95% CIs). These data were found to be normally distributed and analyzed by univariate ANOVA with treatment (CON compared with PRO group) and sex (men compared with women) as fixed factors. To determine the influence of baseline BM and LTM on the outcome, data were analyzed by ANCOVA with group and sex as fixed factors and baseline value of the dependent variable (LTM or BM) as the covariate. Dietary intake data are reported as means ± SDs. Statistical analysis was performed by using PASW Statistics 18.0 for Windows (SPSS, Inc.). Significance (2-tailed) was set at P < 0.05 for all analyses.

Results
The data presented relate to the 60 healthy older adults (31 in the PRO and 29 in the CON group) who completed the study to week 24. Clinical biochemistry confirmed the general health of the subjects (Supplemental Table 2), which did not change in response to the intervention at week 24.

Analysis of the 4-d estimate of dietary intake is provided in Table 1. There was nothing remarkable in the dietary intake and no difference was observed in the percentage of energy intake of...
carbohydrate, fat, or protein between CON and PRO groups. Intakes corresponded to current Institute of Medicine’s recommendations (25) and were comparable to mean daily intakes from the Irish National Adult Nutrition Survey (23). Ninety-four percent of subjects reported a dietary protein intake of ≥0.8 g · kg⁻¹ · d⁻¹, and 50% achieved a protein intake of ≥1.2 g · kg⁻¹ · d⁻¹. Breakfast and midday meals were identified as the lower protein-containing (Supplemental Table 3) and lower leucine-containing (Supplemental Table 4) meals of the day. Supplementation in the PRO group achieved a balance of ~30% of daily protein (≥0.4 g/kg) intake per meal and increased energy intake by 112 kcal/d for the PRO group and by 117 kcal/d for the CON group (Table 1).

Anthropometric and body-composition values did not differ between CON and PRO groups at baseline (Table 2). The appendicular LTM (ALTM) index was characterized by reference to young adults of the same sex and ethnic group (26). All participants exceeded the ALTM index criterion value for age-related sarcopenia (27). The net change in BM and composition after the intervention was calculated by subtracting the baseline value from the value at week 24. The net change in BM (ΔBM) was positive in the PRO group and negative in the CON group, resulting in a difference in ΔBM between the groups of 0.9 kg at week 24 (Table 3). Similar to ΔBM, the net change in LTM (ΔLTM) was positive in the PRO group and negative in the CON group. The difference in ALTM between the groups was 0.6 kg at week 24. ΔLTM was greater in men than in women (treatment × sex interaction, P = 0.013). Covariate analysis confirmed that neither LTM nor BM at baseline exerted a significant influence on the change in LTM. No difference in the net change in body fat mass between the groups was observed.

### Discussion

The study hypothesis was developed from recent evidence indicating that 25–30 g high-quality protein is required per meal to optimally stimulate lean tissue protein synthesis (8, 14, 28) and that an even distribution of protein intake throughout the day may act as effective countermeasures to the age-related loss of LTM (29). To our knowledge the present study is the first longitudinal, randomized, controlled trial to test the validity of this hypothesis in healthy older adults.

As measured by a 4-d estimate of food intake, the elderly participants in this study were considered protein sufficient (9, 10). Protein supplementation at breakfast and lunch for 24 wk beyond habitual intakes in these participants may not, therefore, appear to be warranted. However, consistent with meal-level protein intakes in the elderly (7, 17), lower protein intakes were evident at breakfast (−15 g) and midday meals (−20 g), with the majority of protein intake occurring at the evening meal (−40 g). Protein intake was, therefore, considered suboptimal for postprandial protein synthesis for 2 of the 3 main meals of the day.

To effect the required change in meal-level protein intake we chose to provide a high-quality protein supplement to the lower protein-containing meals of the day rather than attempt a redistribution of habitual intakes. Participants’ compliance and subjective rating of the supplementation regimen lend support to this approach. However, it was not possible to confirm that dietary supplementation had been attained without compensatory adjustment of dietary intakes.

As a result of the intervention, protein intakes in the PRO group increased to ≥0.4 g/kg per meal. This amount of protein is considered within the range (±2 SDs) of the “optimal” protein intake (0.4 ± 0.14 g/kg) to stimulate MPS in the elderly (22). Leucine intakes at breakfast and midday and evening meals increased to 1.8, 1.9, and 1.5 g, respectively, and were closer to the 3 g required to maximal stimulate MPS in older adults (29). These data support an augmented, postprandial rate of protein synthesis (30) in the PRO group compared with the CON group.

It is difficult to accurately predict the change in BM that would result from a 6-mo increase in energy intake of ~100 kcal/d, accumulating to 16.8 Mcal at week 24. The measured outcome was a net increase in BM of 0.7 kg (~1%) in the PRO group compared with a net decrease of −0.22 kg in the CON group (Table 3). Assuming a metabolizable energy density of ~7.7 kcal/kg for subjects with ~25 kg body fat (31), the BM equivalent to the increased energy intake would be ~2.2 kg. That this did not occur suggests that participants modified their habitual intake and/or energy expenditure over the course of the intervention, and to a greater extent in the CON than in the PRO group.

Body-composition analysis revealed no difference in net body fat mass but a significant increase in net lean tissue mass (ΔLTM) in the PRO compared with the CON group. The difference in

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>PRO</th>
<th>P²</th>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.5 ± 5.8</td>
<td>62.2 ± 4.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.66 (1.61, 1.68)</td>
<td>1.63 (1.57, 1.68)</td>
<td>0.22</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>71.9 ± 12.4</td>
<td>70.5 ± 11.7</td>
<td>0.67</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.9 ± 4.1</td>
<td>25.7 ± 3.1</td>
<td>0.88</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>35.2 ± 8.3</td>
<td>34.2 ± 8.1</td>
<td>0.63</td>
</tr>
<tr>
<td>Body fat mass, kg</td>
<td>25.7 ± 8.5</td>
<td>24.2 ± 7.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Lean tissue mass, kg</td>
<td>40.2 (36.6, 43.8)</td>
<td>40.1 (31.5, 48.8)</td>
<td>0.50</td>
</tr>
<tr>
<td>Lean tissue mass index, kg/m²</td>
<td>15.1 (14.0, 15.6)</td>
<td>14.9 (13.1, 16.6)</td>
<td>0.83</td>
</tr>
<tr>
<td>Appendicular lean tissue mass, kg</td>
<td>17.9 (16.2, 19.8)</td>
<td>17.7 (13.3, 22.1)</td>
<td>0.53</td>
</tr>
<tr>
<td>Appendicular lean tissue mass index, kg/m²</td>
<td>6.6 (6.1, 7.2)</td>
<td>7.0 (6.0, 8.0)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs or medians (IQRs); n = 29 (24 women and 5 men; CON group) and n = 31 (22 women and 9 men; PRO group). No significant differences were observed between groups. CON, maltodextrin control; PRO, milk protein matrix.

2 P values for the difference between PRO and CON groups analyzed by independent t test or Mann-Whitney U test.
The final content. All authors read and approved the final manuscript. Data; and PJ wrote the manuscript and had primary responsibility for the ESPEN Expert Group. Clin Nutr 2014;33:929–36.


References


