The associations of protein intake and protein distribution with muscle mass and muscle strength in community dwelling older adults aged 55 and older.

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A cross-sectional study that examines the associations of protein intake and protein distribution with muscle mass and muscle strength in community dwelling older adults aged 55 and older.

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Preface

This thesis was written by Timo de Jong and Niels Hoekman, fourth year Nutrition and Dietetics students from the Amsterdam University of Applied Sciences. In order to complete our studies, a scientific article has been written that examines the associations of protein intake and protein distribution with muscle mass and muscle strength in community dwelling older adults aged 55 and older.

The data used for this study came from the VITAMINE project, which is a randomized control trial that looks at the effect of an increased protein intake and an exercise program on the health of community dwelling older adults aged 55 and older. This study is commissioned by the Research Group Weight Management of the Amsterdam University of Applied Sciences and is led by coordinating researcher Jantine van den Helder.

We would like to use this preface to thank the people that supported us in writing our thesis. First of all, we would like to thank our tutor and mentor Jantine van den Helder, who guided us during the whole process and who was always there when help was needed. We also would like to thank our fellow students for sharing their ideas and motivating us during the writing period. Finally, we would like to thank the participants of the VITAMINE project for taking part in the study and dedicating some of their free time to scientific research.

Amsterdam, June 2018
Timo de Jong
Niels Hoekman
Abstract

Background:
Aging is accompanied by a progressive loss of muscle mass and muscle strength, also called sarcopenia. Adequate protein intake and distribution influences myofibrillar protein synthesis (MPS) and might therefore be important in the prevention of sarcopenia. Whether the daily amount and distribution of dietary protein is associated with muscle mass and muscle strength in older adults remains to be explored.

Objective:
The aim of this study was to determine whether the amount of dietary protein and distribution of protein throughout the day is associated with muscle mass and muscle strength in older adults aged 55 years and older.

Method:
This cross-sectional study included 66 men and 154 women (aged 55 to 91) from the VITAMINE study with available data including; body-composition (dual energy X-ray absorptiometry), dietary protein intake (3-day food diary) and muscle strength (handgrip strength). The coefficient of variation (CV) of protein intake at breakfast, lunch and dinner was used to calculate protein distribution across the main meals. Multivariate linear regression analysis was used to examine the associations of dietary protein intake and protein distribution with muscle mass and muscle strength.

Results:
Mean age was 72.1 ± 6.5 years, mean absolute protein intake was 77.1 ± 20.8 grams and relative intake was 1.7 ± 0.3 (g/kg BW). Distribution of protein was skewed across the day with most protein at the dinner. In this group of community dwelling older adults aged 55 and older, associations of dietary protein intake (g/kg BW) and protein distribution with skeletal muscle mass index and handgrip strength were found but these associations were not significant. Appendicular lean mass (ALM), Body Mass Index (BMI), age and gender were important confounders that influenced these associations.

Conclusion:
The results of this study suggests that more research is needed to make any recommendations about protein intake and protein distribution in the prevention of sarcopenia. Studies should be carried out to clarify the effect of protein intake and distribution on muscle mass and muscle strength. The dietician can be of worth in the prevention of sarcopenia by implementing the recommendation of 0.4 g/ kg BW per meal and collecting this data when treating community dwelling older adults.
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</table>
Introduction

The Dutch population is growing rapidly since the year 1970. This rapid growing population change is caused by the high birth rates of the 1970’s, lower birth rates in the years thereafter and the increased life expectancy. The Dutch population increased roughly by 1.2 million from the year 2000 to 2017. Besides this, the number of people aged 65 and older increased by 1 million (1). The percentage of people aged 65 or above increased from 10% in 1970 to 18% in 2015. It is expected that this percentage increases to 26.6% in 2040 (2).

The growing population of older adults will experience the aging process. Aging is accompanied by a progressive loss of muscle mass and muscle strength, also called sarcopenia (3). Loss of muscle mass is seen from the age of 40 with about 8% per decade until the age of 70, thereafter these losses increase to 15% per decade (4). Loss of muscle strength occurs from the age of 50 and declines with 1.5% annually until the age of 60. After the age of 60 losses increase to 3% each year (5). The loss in muscle mass and muscle strength results in an increase in health problems, such as a loss of muscle function, disability and frailty (6-9). Therefore, to maintain mobility and independent living for older adults, it is essential to slow down the loss of muscle mass and muscle strength (10).

Dietary protein is crucial for the stimulation of muscle protein synthesis (MPS) and is therefore seen as an important factor in the maintenance of muscle mass and muscle strength (11,12). Former research shows that there is a relatively higher amount of protein needed to stimulate MPS in older adults compared to younger adults (13). This lower ability to stimulate MPS may contribute to the age-related decline in muscle mass and muscle strength. A previous study shows that older adults with an intake of 1.2 g/kg of protein a day lost significantly less muscle mass over an extended period of time than individuals with an intake of 0.8 g/kg (14). Therefore, the recommended daily allowance (RDA) of dietary protein of 0.8 g/kg from the Dutch dietary guidelines might not be sufficient to maintain muscle mass and muscle strength in older adults (15).

The Dutch dietary guidelines for protein intake provides no statement about an optimal protein distribution throughout the day. Most Dutch older adults have a skewed protein intake, consuming more than 50% of their dietary protein at dinner and less than 20% at breakfast (16). A maximal stimulation of MPS in older adults has been seen at 0.4 g/kg BW (body weight) per meal (17). A skewed protein intake fails to optimally stimulate MPS at breakfast and exceeds the maximum stimulation at dinner. For this reason, studies observed that a balanced protein intake throughout the day stimulates MPS more than a skewed protein intake (18,19). In contrary, other studies have seen better whole-body protein retention in individuals with a pulse protein feeding pattern compared to a spread protein feeding pattern (20,21). Therefore, whether the daily amount and distribution of dietary protein is associated with muscle mass and muscle strength remains to be explored.

The aim of this study is to determine whether the amount of dietary protein and distribution of protein throughout the day is associated with muscle mass and muscle strength in older adults aged 55 and older.
Methods

Study design
The researchers used a quantitative cross-sectional study method. This kind of study can provide valuable data from a population at any specific point in time. Data on dietary protein intake, protein distribution, muscle mass and muscle strength were required for this study. An advantage of this study design is the ability to perform tests on large populations and therefore make the data more valid and reliable for the general population.

Study population
The study population consisted of participants from the VITAMINE (ViTaL AMsterdam older adults IN thE city) study. The VITAMINE study is a randomized control trial (RCT) that investigates the effect of an increased dietary protein intake (1.2 – 1.5 g/kg BW) and increased physical activity (a hometraining program) on the health of older adults. VITAMINE selected trainers by an interview. These trainers selected the participants out of a ‘More Physical Activity for Seniors’ (Meer Bewegen voor Ouderen (MBvO) in Dutch) or a comparable exercise program. There was also a recruitment by mail, a letter and a leaflet about the study. Data from a total of 245 participants was used for this cross-sectional study.

Inclusion criteria
Subjects were included if they met the following criteria:
- Must be part of MBvO or comparable exercise program
- 55 years or older
- Willingness to take part in the study
- Written informed consent
- No conditions that may interfere with the participation in the study

Exclusion criteria
Subjects were excluded if they met the following criteria:
- Current alcohol or drug abuse
- Inability to understand the Dutch language
- Cognitive impairment (MMSE <15)
- Knee or hip surgery in the last 6 months

VITAMINE recruited students from the AUAS (Amsterdam University of Applied Sciences) to verbally explain the procedure of the study to the participants. The participants also signed an informed consent. The participants that wanted to join the program were screened by the Physical Activity Readiness Questionnaire (PARQ) and Mini-Mental State Examination (MMSE).

Logistics
The logistics of the project started with a screening and a baseline measurement day. The participants arrived in a fasted state and needed to hand over a 3-day food diary, physical activity diary (PAD) and an accelerometer. The 3-day food diary was used to calculate total protein intake and the PAD was used to calculate the physical activity level (PAL). Thereafter the participants filled in a Socio-demographic characteristics form including age and gender. After filling in the form, the participants needed to change clothes for the anthropometric
tests. First, height was measured by an electronic stadiometer. Body weight (BW) was measured by a weighing scale. Body Mass Index (BMI) was calculated by squared height (m) divided by body weight (kg). Body composition was measured by the BodPod and the dual Energy X-ray Absorptiometry (DXA). Furthermore, the waist circumference, hip circumference, mid upper arm circumference, triceps skinfold, bioelectrical impedance analysis (BIA) and handgrip strength test (HGS) were measured. Breakfast was served after these tests and thereafter the following tests were conducted for:

- Cognitive functioning (Trail Making, Stroop Color Word test, Letter Fluency), physical performance (M-PPT, TUG, SPPB, 6 MWT), quality of life (RAND-36), depression (GDS) and motivation for exercise (BREQ-2)

**Measurements**

The outcome variables of this study were: muscle mass, muscle strength, dietary protein intake and protein distribution. These variables were derived from the following measurements:

- Dual Energy X-ray Absorptiometry (DXA) scan
- Handgrip strength test (HGS)
- Dietary assessment through a 3-day food diary

Muscle mass was measured by a dual Energy X-ray Absorptiometry (DXA) scan. The scan took approximately 10 minutes. The lean body mass, lean leg mass, appendicular skeletal muscle mass, regional fat mass and bone mineral density is measured by a dual energy beam that passes through the skeleton muscle. All scans were performed by a professional. DXA scans are the golden standard amongst the measuring of lean body mass (22, 23). The scan gives an accurate measurement of the total-body skeleton muscle mass (SMM) (24). Appendicular Lean Mass (ALM) was measured by the DXA scan. Skeletal Muscle Mass Index (hSMI) was calculated by dividing ALM by height square (3). hSMI was used as a dependent variable.

Muscle strength was determined by the handgrip strength (HGS) test. The HGS is an easy and low effort test and is measured with the use of a dynamometer (25,26). Handgrip strength represents the strength in the forearm and upper limb measured in a seated position by squeezing the dynamometer as hard as possible. This gives a muscle index; this muscle index is then used to identify the amount of muscle strength the participant has (27). Weaker grip strengths among the older adults are associated with disability, morbidity, and mortality. Grip strength is an important clinical assessment to identify sarcopenia and frailty in older adults (28). The HGS contained three measurements to determine muscle functioning, this was measured by the maximum strength effort of the dominant hand. HGS was used as a dependent variable.

Dietary protein intake was assessed with a 3-day food diary. A 3-day food diary has shown to be a more valid report compared to a 24-hour recall or 5-day food frequency (29,30). For a complete overview of the subjects’ nutritional intake, a total of two weekdays and one weekend day were used. The food diaries were sent to the subjects to fill in at home, including instructions about how to fill in the diaries. Food intake is divided into 6 eating
moments: ‘breakfast’, ‘morning snack’, ‘lunch’, ‘afternoon snack’, ‘dinner’ and ‘evening snack’. During the reporting period participants were instructed to follow their normal dietary habits. At the day of measurements, the 3-day food diaries were checked by Nutrition and Dietetics students and if necessary, participants were asked for additional information. Energy and dietary protein intake per day and per meal were calculated using NEVO version 2010 (Nederlandse voedingsstoffenbestand) and SPSS version 24.

All data was entered in Research Manager, an online data system. A double entry check was performed on all data. The dataset was provided by the junior researcher of coordinating of the VITAMINE project. The complete dataset was checked for potential outliers and these outliers were only excluded if they deviated more than three standard deviations from the mean. Participants with incomplete and/or unnatural 3-day food diaries (e.g., sickness) were excluded from the dataset.

**Data analysis and statistics**

The characteristics of the participants were analyzed for both men and women. Differences in characteristics were tested for significance using the independent samples t-test. The Shapiro-Wilk test was used to test for the normality of the variables. Protein distribution was calculated as a CV (Coefficient of variation). The CV is the standard deviation (SD) of the amount of dietary protein of each main meal excluding snacks, divided by the total grams of protein. Lower CV values mean that a person’s protein intake is more evenly divided throughout the day (31).

Multivariable linear regression analysis was used to examine the associations of dietary protein intake and protein distribution with muscle mass and muscle strength. Muscle mass and muscle strength were the dependent variables and dietary protein intake per kilogram BW and the distribution of protein were the independent variables.

Additionally, gender was examined as possible effect modifier by adding an interaction term to the raw regression model. The variable was accepted as an effect modifier when the significance was $p < 0.05$ for the interaction term. Besides this, confounders were found using the “change-in-estimate” criterion. This criterion compares the estimated measure of association before and after adjusting for confounding. If the difference in the regression co-efficient ($\beta$) $\geq 10\%$, a confounder is present. Confounders were found by a hierarchical multiple regression strategy. With this strategy a confounder is added to the model one by one. The confounder corresponding with the highest percentage stays in the model and the other confounders will be tested in the new adjusted model. Potential confounders were protein/kg BW, age, total caloric intake, Body Mass Index (BMI), Appendicular Lean Mass (ALM) and gender (32,33).

For the statistical analyses, SPSS version 24.0 (IBM) was used and the level of significance for all statistical tests was set at $p < 0.05$. 
Results

Demographic characteristics

A total of 220 participants (154 women and 66 men) were included in the analysis. Data of 25 participants were excluded from the analysis because of invalid and missing data. Participants’ ages varied from 55 to 91 years and were not different between men (72.0 ± 6.5 y) and women (72.1 ± 6.4 y). No significant interaction was found between gender and the dependent variables. Handgrip strength scores were significantly higher in men (43.6 ± 10.2 kg) than in women (26.2 ± 6.9 kg) (p< 0.001). Skeletal muscle mass index was significantly lower in women (7.0 ± 0.8 kg/m²) than in men (8.4 ± 1.0 kg/m²) (p=0.043). Remarkably, according to the cutoff point from the Rosetta study (1986 – 1992) seven men and two women were defined having sarcopenia. BMI, PAL and total calorie intake did not differ between men and women.

Table 1.
Characteristics of the total population from the VITAME (VITal AMsterdam older adults IN thE city) study

<table>
<thead>
<tr>
<th></th>
<th>All (n = 220)</th>
<th>Men (n = 66)</th>
<th>Women (n = 154)</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72.1 ± 6.5</td>
<td>72.0 ± 6.5</td>
<td>72.1 ± 6.4</td>
<td>0.637</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
<td>0.694</td>
</tr>
<tr>
<td>Primary, n (%)</td>
<td>3 (1.4)</td>
<td>0 (0)</td>
<td>3 (2)</td>
<td>0.021*</td>
</tr>
<tr>
<td>Secondary, n (%)</td>
<td>46 (20.9)</td>
<td>11 (16.7)</td>
<td>35 (22.7)</td>
<td>0.035*</td>
</tr>
<tr>
<td>MBO, n (%)</td>
<td>48 (21.8)</td>
<td>14 (21.2)</td>
<td>34 (22.1)</td>
<td>0.776</td>
</tr>
<tr>
<td>HBO, n (%)</td>
<td>86 (39.1)</td>
<td>24 (10.9)</td>
<td>62 (40.3)</td>
<td>0.253</td>
</tr>
<tr>
<td>WO, n (%)</td>
<td>37 (16.8)</td>
<td>17 (25.8)</td>
<td>20 (13)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sarcopenia, n (%)</td>
<td>9 (4.1)</td>
<td>7 (10.6)</td>
<td>2 (1.3)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Dietary intake

<table>
<thead>
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<th>Men (n = 66)</th>
<th>Women (n = 154)</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/d</td>
<td>1866 ± 491</td>
<td>2078 ± 506</td>
<td>1774 ± 456</td>
<td>0.333</td>
</tr>
<tr>
<td>Protein (g / kg / BW)</td>
<td>1.07 ± 0.30</td>
<td>1.06 ± 0.30</td>
<td>1.07 ± 0.30</td>
<td>0.927</td>
</tr>
<tr>
<td>Protein total (g)</td>
<td>77.1 ± 20.8</td>
<td>84.8 ± 22.1</td>
<td>73.8 ± 19.4</td>
<td>0.259</td>
</tr>
<tr>
<td>Breakfast (g)</td>
<td>13.0 ± 6.6</td>
<td>14.2 ± 7.4</td>
<td>12.5 ± 6.5</td>
<td>0.079</td>
</tr>
<tr>
<td>Morning snack (g)</td>
<td>3.8 ± 4.3</td>
<td>3.6 ± 4.4</td>
<td>3.8 ± 4.3</td>
<td>0.855</td>
</tr>
<tr>
<td>Lunch (g)</td>
<td>18.4 ± 8.2</td>
<td>20.0 ± 9.2</td>
<td>17.6 ± 7.6</td>
<td>0.067</td>
</tr>
<tr>
<td>Afternoon snack (g)</td>
<td>6.3 ± 6.3</td>
<td>6.9 ± 8.1</td>
<td>6.0 ± 5.4</td>
<td>0.054</td>
</tr>
<tr>
<td>Dinner (g)</td>
<td>34.0 ± 11.9</td>
<td>37.6 ± 13.09</td>
<td>32.4 ± 11.0</td>
<td>0.050*</td>
</tr>
<tr>
<td>Evening snack (g)</td>
<td>4.9 ± 5.0</td>
<td>6.0 ± 5.8</td>
<td>4.5 ± 4.6</td>
<td>0.037*</td>
</tr>
<tr>
<td>Protein CV</td>
<td>0.57 ± 0.23</td>
<td>0.57 ± 0.21</td>
<td>0.56 ± 0.24</td>
<td>0.310</td>
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Body composition

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<th>Men (n = 66)</th>
<th>Women (n = 154)</th>
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</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>73.6 ± 14.0</td>
<td>81.4 ± 13.0</td>
<td>70.3 ± 13.0</td>
<td>0.408</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 4.2</td>
<td>25.6 ± 3.4</td>
<td>26.0 ± 4.5</td>
<td>0.100</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>24.5 ± 7.5</td>
<td>21.1 ± 5.1</td>
<td>26.0 ± 8.0</td>
<td>0.049*</td>
</tr>
<tr>
<td>ALM (kg)</td>
<td>21.1 ± 4.7</td>
<td>26.5 ± 3.9</td>
<td>18.8 ± 2.6</td>
<td>0.001*</td>
</tr>
<tr>
<td>SMI (kg/m²)</td>
<td>7.4 ± 1.1</td>
<td>8.4 ± 1.0</td>
<td>7.0 ± 0.8</td>
<td>0.043*</td>
</tr>
<tr>
<td>Max. HGS (kg)</td>
<td>31.4 ± 11.3</td>
<td>43.6 ± 10.2</td>
<td>26.2 ± 6.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>PAL</td>
<td>1.5 ± 0.15</td>
<td>1.5 ± 0.17</td>
<td>1.5 ± 0.13</td>
<td>0.016*</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs; MBO, practical education; HBO, higher education; WO, scientific; BW, body weight; CV, coefficient of variation; BMI, body mass index; ALM, appendicular lean mass; SMI, skeletal muscle index; HGS, handgrip strength; PAL, Physical activity level.

2 ± SD below reference population of the Rosetta Study (1986 – 1992) is used as cut off point for sarcopenia.

3 P values for the difference between men and women analyzed by independent t test.
Protein intake
Average dietary protein intake of the study population was 77.1 ± 20.8 g. Slightly higher dietary protein intake was seen in men (84.8 ± 22.1 g) compared to women (73.8 ± 19.4 g). However, protein per kilogram of body weight was almost even in both men and women (1.06 ± 0.30 and 1.07 ± 0.30). In total, 67.5% of the study population reached or exceeded the RDA of 0.8 g/kg BW. The amount of dietary protein per meal and per day is shown in figure 1. As expected the dinner meal contributed the most to the total protein intake. The least amount of protein was consumed during breakfast in both men and women. The mean amount of protein at dinner was significantly (37.6 ± 13.09 g) higher in men than in women (32.4 ± 11.0 g) (p=0.05).

![Figure 1. Mean ± SD protein intake per meal and snacks (left y axis) and total protein intake per day (right y axis) in men and women.](image-url)
Protein distribution
The distribution of dietary protein was skewed across the day with the largest amount of protein consumed at dinner and the smallest amount at breakfast. The dinner meal accounted for more than 44.2% and the breakfast meal accounted for 17.2% of the total protein intake. The CV values ranged from 0.05 to 1.13 and the average CV was 0.57, which did not differ between men and women. Figure 2 shows dietary protein distribution between different quintiles of CV values, whereby Q1 reflects a protein intake of 9, 14 and 43 g/meal, and a Q2 reflects a protein intake of 17, 21 and 26 g/meal.

Association between dietary protein intake and muscle mass
Table 2 shows the multivariable linear regression results, examining the association between dietary protein intake (g/kg BW) and muscle mass. After correction for confounders, no significant association was found between total protein intake (g/kg BW) and skeletal muscle mass index (β.adjusted = .203, 95% CI: -.144, .550, p= 0.249). However, the added confounders BMI and Gender were both significant and have therefore a meaningful value on the observed association between protein intake (g/kg BW) and skeletal muscle mass index.

Table 2. Multivariable linear regression association between protein intake (g/kg BW) and skeletal muscle mass index.

<table>
<thead>
<tr>
<th>Model</th>
<th>β</th>
<th>Sig.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein/kg</td>
<td>-7.02</td>
<td>.005</td>
</tr>
<tr>
<td>2</td>
<td>Protein/kg</td>
<td>.203</td>
<td>.249</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>.170</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Energy (kcal a day)</td>
<td>-3.306</td>
<td>.753</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-1.478</td>
<td>.000</td>
</tr>
</tbody>
</table>

BW, body weight; BMI, body mass index; kcal, kilocalories.
**Association between dietary protein intake and muscle strength**

Table 3 shows the multivariable linear regression results, examining the association between protein intake (g/kg BW) and muscle strength. After correction for confounders, no significant association was found between total protein intake (g/kg BW) and handgrip strength (βadj = .526, 95% CI: -2.801, 3.853, p = 0.756). However, the added confounders ALM, BMI, Age and Gender were all significant and have therefore a meaningful value on the observed association between protein intake (g/kg BW) and handgrip strength.

Table 3. Multivariable linear regression association between protein intake (g/kg BW) and handgrip strength.

<table>
<thead>
<tr>
<th>Model</th>
<th>B</th>
<th>Sig.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein/kg</td>
<td>-.762</td>
<td>.770</td>
</tr>
<tr>
<td></td>
<td>ALM</td>
<td>1.291</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>-.570</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-.359</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-7.200</td>
<td>.000</td>
</tr>
</tbody>
</table>

BMI, body mass index.

**Association between protein distribution and muscle mass**

Table 4 shows the multivariable linear regression results, examining the association between protein distribution and muscle mass. After correction for confounders, no significant association was found between protein distribution and skeletal muscle mass index (βadj = .044, 95% CI: -.275, .362, p = 0.788). Nonetheless, the added confounders BMI and Gender were both significant and therefore have a meaningful value on the observed association between protein distribution and skeletal muscle mass index.

Table 4. Multivariable linear regression association between protein distribution and skeletal muscle mass index.

<table>
<thead>
<tr>
<th>Model</th>
<th>B</th>
<th>Sig.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein CV</td>
<td>-.196</td>
<td>.542</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>.165</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-1.464</td>
<td>.000</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; BMI, body mass index.
Association between protein distribution and muscle strength

Table 5 shows the multivariable linear regression results, examining the association between protein distribution and muscle strength. After correction for confounders, no significant association was found between protein distribution and handgrip strength ($\beta$ adjusted = -0.547, 95% CI: -4.548, 3.454, p = 0.788). Nonetheless, the added confounders ALM, BMI, Age and Gender were all significant and have therefore a meaningful value on the observed association between protein distribution and handgrip strength.

Table 5. Multivariable linear regression association between protein distribution and handgrip strength.

<table>
<thead>
<tr>
<th>Model</th>
<th>Protein CV</th>
<th>$\beta$</th>
<th>Sig.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein CV</td>
<td>0.593</td>
<td>.859</td>
<td>27.088</td>
</tr>
<tr>
<td>2</td>
<td>Protein CV</td>
<td>-0.547</td>
<td>.788</td>
<td>-4.548</td>
</tr>
<tr>
<td></td>
<td>ALM</td>
<td>1.291</td>
<td>.000</td>
<td>0.873</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>-0.587</td>
<td>.000</td>
<td>-0.880</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.358</td>
<td>.000</td>
<td>-0.510</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>-7.200</td>
<td>.000</td>
<td>-11.048</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; ALM, appendicular lean mass; BMI, body mass index.
Discussion

The aim of this study was to determine whether the amount of dietary protein and the distribution of protein throughout the day is associated with muscle mass and muscle strength in older adults aged 55 and older. In our regression model, protein intake and protein distribution were associated with skeletal muscle mass index (hSMI) and handgrip strength (HGS) in older adults aged 55 and older, however these associations were not significant.

Several studies have investigated the association of the amount of dietary protein with muscle mass and muscle strength, because protein intake has a direct effect on protein turnover and induces a muscle protein synthesis response (11, 34-36). In these studies, either a significant positive or no significant association was found. In line with our results, a recent cross-sectional study showed no significant association between total protein intake with skeletal muscle mass index and handgrip strength in older adults (mean age 77 y) (34). This outcome supports our results found in older adults. In contrary, recent studies did show a significant positive association of total protein intake with muscle mass and muscle strength. In these studies, protein intakes higher than the RDA of 0.8 g/kg BW were associated with higher lean body mass (LBM) and a better preservation of muscle mass in older adults (36-40).

Differences in these outcomes might be explained by study designs, population characteristics, confounding variables, assessment methods and outcome measurements. For example, in our study a 3-day food diary was used to assess dietary protein intake and handgrip strength was measured to assess muscle strength. In comparison, Sahni et al. used a semi quantitative food frequency questionnaire (FFQ) to assess dietary protein intake and used quadriceps strength to assess muscle strength (36).

Protein distribution of the population used in this study was skewed towards the dinner meal. This is in line with results from Tieland et al. were an uneven protein distribution was found among Dutch older adults. In our study Individual protein distribution varied greatly. This is confirmed by the large variation in the CV values (0.05 – 1.13). The lowest quintile of protein distribution reflected a protein intake of 9, 14 and 43 g/meal (n=54), whereas the highest quintile reflected a protein intake of 17, 21 and 26 g/meal (n=54).

In our regression model, protein distribution over the main meals was not significantly associated with muscle mass and muscle strength. This is in line with a recent cross-sectional study that examined the association of protein distribution with muscle mass and muscle strength (34). This study of Gingrich et al. found that a more even distributed dietary protein intake and the number of meals providing ≥0.4 g protein/kg body weight were not significantly associated with the muscle mass and muscle strength in older adults. Similar to our results, the skeletal muscle mass index and handgrip strength were used to assess muscle mass and muscle strength. In addition, in a study with a different design, Kim et al. found in their RCT that a more even distributed protein intake had no effect on muscle mass and muscle strength in older adults (41).
In contrary to our findings, protein distribution was independently associated with greater muscle mass and muscle strength in free living older adults (mean age 74 y) in two recent longitudinal studies (32,33). In these studies, done by Farsijani et al., a more even distribution of protein intake, independent of the total quantity, was significantly associated with higher muscle strength and higher ALM through the entire follow-up period. However, rates of declines in muscle strength and ALM in both sexes were not associated with evenly distributed protein intakes. In comparison to our cross-sectional study Farsijani et al. used different methods to assess muscle strength (hand, and leg strengths) and muscle mass (ALM).

Possible reasons that the distribution of dietary protein was not significantly associated with muscle mass and muscle strength in our cross-sectional study might be that the population of our study did not reach the suggested amount of dietary protein at each meal. The suggested quantity of protein to reach maximal stimulation of MPS in older adults is an intake of 0.4 g/kg or 30g of protein per meal (based on average body weight: 74 kg). In our study, 81% of the participants did not reach the optimal protein intake of 0.4 g/kg for at least two of their meals. These findings are in line with a recent study from Cardon-Thomas et al. this study looked at the 0.4 g/kg BW threshold in older adults and found that 79% of the participants consumed less than the 0.4 g/kg BW threshold for at least two of their meals (42). However, the recommended protein intake of 0.4 g/kg BW per meal to optimal stimulate MPS is based on a study conducted under laboratory conditions where healthy participants only consumed high quality protein sources (13). It remains unclear if this recommendation is also optimal under real life circumstances.

In our study neither protein quantity nor distribution had a significant association with muscle mass and muscle strength. Factors other than nutrition might play a larger role in maintaining muscle mass and muscle strength with advanced age. Resistance exercise (RE) is shown to be an effective intervention to increase LBM in older adults and it might be conceivable that RE could serve as an effective strategy in the prevention of sarcopenia (43). In addition, the reduction of sedentary time is also associated with a reduced loss of muscle mass with advanced age (44). Also, because of the previous mentioned studies associating protein intake and protein distribution with muscle mass and muscle strength, older adults might benefit from a higher protein intake and a more even distribution across meals. Therefore, future research should be conducted to clarify the associations of protein intake and distribution with muscle mass and muscle strength in community dwelling older adults.

First option for future research is that a population with frail and sarcopenic characteristics (e.g., low muscle mass and performance decrease) should be used to see if the possible effect of a higher protein intake and a more even distribution on muscle mass and muscle strength is generalizable towards the larger population of community dwelling older adults. Second, the protein digestibility-corrected amino acid score (PDCAAS) should be taken in account. This score represents the amount of amino acids that become available for skeletal muscle and may therefore influence the anabolic effect of protein. Third, the recommendation of 0.4 g/kg BW protein per meal to optimally stimulate protein synthesis has to be confirmed under real life circumstances, whereby differences in protein sources and sex specific differences in protein metabolism are also accounted for (45,46). The dietician can be of worth by implementing the recommendation of 0.4 g/kg BW per meal
when treating community dwelling older adults in the prevention of sarcopenia. This data can be used to confirm if this amount is appropriate for the age related decline in muscle mass and muscle strength. This practical data might in turn play a role in the adjustment and creation of dietary guidelines for protein intake and protein distribution respectively.

This study had multiple strengths. First, protein distribution is calculated using CV values. This makes the variable continuous and relative, which is superior to categorical variables. Second, the DXA scan was used. The DXA scan is a precise and accessible technique to measure body composition. Third, procedures were performed by trained personnel who conducted the standardized tests. Fourth, a relatively large sample size was used for this study. However, this study also had some limitations. First, as shown in table 1 the participants of this study were healthy community dwelling older adults, which is seen by their relatively young age and high PAL values. Therefore, the results of this study might not be generalizable to the larger population of community dwelling older adults. Different outcomes might be found when using a population which also includes frail and/or sarcopenic characteristics (e.g., low muscle mass, performance decrease). Our population characteristics reduces the ability to compare our results with high quality studies. This number is limited, because of the fact that most studies do not include healthy older adults. Second, the skeletal muscle mass index and hand grip strength do not cover all aspects of muscle health. Third, we did not account for the quality of protein consumed, which may have an influence on MPS and in turn on muscle mass.
Conclusion

Total protein intake and protein distribution showed no significant associations with muscle mass and muscle strength in community dwelling older adults aged 55 and older. These results contradict the common belief that higher protein intakes and a more even distribution is associated with greater muscle mass and muscle strength. However, future studies should be conducted before making any dietary recommendations. These studies should take account for protein quality and sex-specificity in protein metabolism. The dietician can be of worth in the prevention of sarcopenia by implementing the recommendation of 0.4 g/kg BW per meal and collecting this data when treating community dwelling older adults.
References


(43) Deutz, Nicolaas E.P.; Bauer, Jürgen M.; Barazzoni, Rocco; Boirie, Yves; Bosy-Westphal, Anja; Cederholm, Tommy; Cruz-Jentoft, Alfons; Krznarić, Zeljko; Nair, K.Sreekumar; Singer, Pierre; Teta, Daniel; Tipton, Kevin; Calder, Philip C. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. Clinical Nutrition 2014;33(6):929-936.

(44) Shad, Brandon J.; Wallis, Gareth; Loon, Luc J.; van Thompson, Janice L. Exercise Prescription for the Older Population: The Interactions Between Physical Activity, Sedentary Time, and Adequate Nutrition in Maintaining Musculoskeletal Health. Maturitas 2016;93:78-82.
