What is the relation between the usual protein intake and muscle mass in obese elderly (55+)?

Muscle Preservation Study

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Project number: 2012100, January, 2011
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**Thesis number**
2012100

Bachelor Nutrition & Dietetics, HvA, Amsterdam University of Applied Sciences

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Preface

This thesis is written to complete the bachelor Nutrition & Dietetics at the Hogeschool van Amsterdam, University of Applied Sciences. During 20 weeks we, and 6 other students, led the Muscle Preservation Study.

The objective of the Muscle Preservation Study is to investigate the effects of a specialized oral nutritional supplement, a protein shake, on the preservation of muscle mass in obese elderly (55+) during a weight loss program. The duration of the program is 13 weeks and consists a hypo-caloric diet and a resistance exercise program.

For our thesis we focused on the relationship between usual protein intake and muscle mass in obese elderly.

Thanks to this thesis, we obtained al lot of insight on the subject protein, muscle mass, resistance training and elderly.

We want to thank the people who helped us during our thesis:

- Amely Verreijen
- Peter Weijs
- Suzanne van der Plas
- Tarana Haarsma
- Minse de Bos-Kuil
- The 6 other students who led the Muscle Preservation Study: Joni Beintema, Astrid Bobeldijk, Nicolien Broersen, Marleen Hobijn, Floor Hogenboom and Marlou Lasschuijt.

We also want to thank Club West and the Hogeschool van Amsterdam.

Amsterdam, January 2012
Iris Bras & Marlot Wijers
Abstract

Background
Most studies show that a diet rich in protein can improve the body composition (including muscle mass) and the metabolic profile compared to a diet with lower protein content. The Dutch National Food Consumption Survey suggests that Dutch people, including elderly, consume sufficient dietary protein. Although a number of different underlying mechanisms contribute to loss of muscle mass, inadequate protein intake can accelerate this process. This may contribute to sarcopenia; the age-related loss of muscle mass, muscle strength and impaired physical functioning. A higher protein intake might be necessary to prevent these losses of muscle mass.

Methods
The primary objective of this study was to determine the relation between appendicular muscle mass and usual protein intake in obese elderly.
Secondary objective of this study was to determine whether the relation is different for animal and vegetable protein on the appendicular muscle mass.
This study included 51 subjects, 17 males and 34 females, aged 55-76 years and with a BMI between 28 kg/m² and 51 kg/m². The appendicular muscle mass was measured by using the DXA-scan type: GE Lunar Prodigy / DPX-NT and the protein intake was obtained by using the 3-day dietary record, both measurements were done at baseline. The relation between appendicular muscle mass and usual protein intake in elderly was examined by using multiple linear regression analysis adjusted for confounders. Usual protein intake was also split up in animal and vegetable protein.

Results
There was a significant relation between appendicular muscle mass and total protein intake (P=0.017), but after adjustments for body weight and gender this relation was not significant anymore. There was no significant relation between appendicular muscle mass and animal protein intake and there was a significant relation between appendicular muscle mass and vegetable protein.

Conclusion
There is a significant relation between appendicular muscle mass and the usual protein intake in obese elderly (55+), but after adjustments for body weight and gender, the relation between appendicular muscle mass and total protein intake, was not significant anymore. Results show that the relation between appendicular muscle mass and vegetable protein is significant and the relation between appendicular muscle mass and animal protein is not. Further research is needed to determine whether protein intake is of influence on appendicular muscle mass and whether there is a relation between appendicular muscle mass, animal and vegetable protein. For further research a wider protein intake is recommended.

Keywords
Protein, animal, vegetable, appendicular muscle mass, elderly, sarcopenia, overweight, obese, 3-day dietary record, DXA-scan.
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1. Introduction

Overweight and obesity are serious public health concerns in the Netherlands, because of their high prevalence. In 2009, 47% in the Dutch population was overweight (BMI ≥ 25). From this percentage 52.5% is male, of which 11.2% are obese and 41.9% is female, of which 12.4% are obese.¹

Elderly suffer the most of overweight and obesity. 58.8% of the Dutch elderly are overweight, of which 15% are obese.² Overweight, obesity and aging increase the risk of chronic diseases, including hypertension, dyslipidemia, diabetes and coronary heart disease.³,⁴ Furthermore, overweight and obesity do not only result in an increase in weight they also cause changes in the body composition, including an increase in fat mass. Aging also causes changes in the body composition, lean mass decreases during aging.

The loss of lean mass is a gradual process that can begin at the age of 40-50 years.⁵ This age-related loss of muscle mass, muscle strength and muscle functioning is called sarcopenia.⁶ The loss in lean mass will eventually lead to muscle atrophy. Muscle atrophy is defined as a decrease in muscle mass, by which the muscles become smaller, thinner and weaker.⁷

Research shows that the average loss of muscle mass after the age of 50 is about 1-2% each year.⁸,⁹ Although a number of different underlying mechanisms contribute to loss of muscle mass, inadequate protein intake can accelerate this process.¹⁰ The Dutch National Food Consumption Survey (2007-2010) suggests that the average of Dutch people, including elderly (51-69 years), consume 1.0g/kg/day (female) and 1.1g/kg/day (male) of protein. This amount meets the Recommended Dietary Allowance (RDA), but a higher intake might be necessary to counteract sarcopenia.¹¹

Organs and muscles consume the most energy, so maintenance is important not only for functioning but also for losing weight and maintaining weight loss.¹² Furthermore, protein increases the thermogenesis which can contribute to weight loss. Most studies show that a hypocaloric diet rich in protein (25 en%) can improve the body composition and the metabolic profile compared to a diet with lower protein content (12 en%), during weight loss.¹³ The efficiency of the rich protein diet can be explained by the following; protein induces the thermogenesis, gives a greater feeling of satiety and maintains the fat free mass better during weight loss.¹⁴ An adult catabolizes and replaces about 3-6 g of protein/kg/day. This is called replacement synthesis, which is intensified after a meal, when there is an abundant supply of amino acids and metabolic fuels. An adult has an overall nitrogen balance, which is the average of synthesis (fed state) and catabolism (fasting state). Protein synthesis is energy expensive and the rate of synthesis is lower than the catabolism in the fasting state. There is loss of tissue protein, which provides amino acids for gluconeogenesis. This means that in the fed state the rate of protein synthesis increases, replacing the tissue that was lost. Even in severe malnutrition the catabolism rate remains the same, but the replacement synthesis drops due to low metabolic fuels.¹⁵ This means that more protein increases the synthesis and decreases the catabolism.
Weight loss is often prescribed to obese elderly, although there is reluctance to prescribing weight loss to elderly because of the fear of loss of muscle mass and impaired physical function. To prevent this loss of muscle mass, research is done to investigate the effect of protein on the preservation of muscle mass in elderly during weight loss. Research shows that a protein enriched diet is effective in the treatment of overweight and the preservation of muscle mass during weight loss. In a study done by Gordon et al., post-menopausal, obese women were examined to determine whether a hypocaloric diet (-400 kcal/day) with a higher protein intake can prevent the loss of lean mass that is commonly associated with weight loss. This is an intervention study comparing a hypocaloric diet moderately high in protein (1.2-1.5g/kg/day) to one lower in protein (0.5-0.7g/kg/day). This intervention has a duration of 20 weeks. As a result, the high protein group lost 8.4 +/- 4.5 kg and the low protein group lost 11.4 +/- 3.8 kg of body weight (P = 0.11). The low protein group lost the most weight, but also lost the most lean mass. The mean percentage lost as lean mass was respectively 17.3% +/- 27.8% and 37.5% +/- 14.6%, and this was significantly different. So it can be assumed that maintaining a higher protein intake than the RDA (0.8 g/kg/day) may reduce lean mass losses associated with voluntary weight loss in older women. This study did not include exercise. However, there are also studies that do include exercise programs. These studies show that the combination of protein and exercise give the best results in maintaining the muscle mass during weight loss in obese elderly. A study studying premenopausal overweight and obese women, during a 16 week period with a hypocaloric diet (−500 kcal/day) and exercise (−250 kcal/day). This study shows that the group with a high intake of protein (30 en%) gains lean tissue to a greater extent than the group with a lower intake of protein (15 en%). The high protein group also lost more visceral adipose tissue and trunk fat.

Animal protein provides all essential amino acids and thus is a source of high-biological-value-protein. Animal protein also contains the branched-chain amino acid leucine, which vegetable protein does not. Leucine may stimulate the muscle protein synthesis due to playing a central role in mediating mRNA translation. Elderly may consume inadequate amounts of animal protein, because of age-related factors as costs, difficulties with chewing, fear of fat and cholesterol and intolerance of certain foods. This can lead to inadequate amount of leucine. The study of Houston et al. suggests that animal protein is significant in the relation between dietary protein intake and appendicular lean mass, vegetable protein is not. The Dutch National Food Consumption Survey (2007-2010) suggests that the average of Dutch people, including elderly (51-69 years), consume 0.4g/kg/day vegetable protein and 0.6g/kg/day animal protein (female) and 0.4g/kg/day vegetable protein and 0.7g/kg/day animal protein (male).

In the past years a lot of protein diets have been developed. For instance the Atkins diet, developed by Dr. Robert Atkins, the South Beach diet, and the Dr. Frank diet by Dr. Frank. These diets contain a high amount of protein and a low amount of carbohydrates. They all go through different stages and in each stage more carbohydrates are introduced in the diet. There is also large evidence encouraging the efficacy of physical activity, especially resistance exercise, in maintaining or expanding muscle mass and function in elderly during weight loss.

In the past years a lot of protein diets have been developed. For instance the Atkins diet, developed by Dr. Robert Atkins, the South Beach diet, and the Dr. Frank diet by Dr. Frank. These diets contain a high amount of protein and a low amount of carbohydrates. They all go through different stages and in each stage more carbohydrates are introduced in the diet. There is also large evidence encouraging the efficacy of physical activity, especially resistance exercise, in maintaining or expanding muscle mass and function in elderly during weight loss.
A high dietary protein intake, up to 1.6 grams protein per kg body weight per day, increases muscle protein synthesis in elderly and seems to maintain fat free mass during weight loss. Several studies have shown that the particular combination of resistance exercise and high protein intake during weight loss has a beneficial effect on muscle protein synthesis.

Wolfe et al. suggests that a protein intake of 1.5g/kg/day is a reasonable RDA to optimize muscle mass, strength, health and functioning in elderly (65+). Other factors, including immune status, wound healing, blood pressure and bone health may be improved by increasing protein intake above the RDA of 0.8g/kg/day. This study did not include weight loss and exercise. However, this has not been substantiated enough to be implemented.

There are also concerns with a higher intake of protein. A rat study shows that the animals that were given a high protein consumption lived shorter than animals that were given a lower protein consumption. The study done by Durnin et al. describes which mechanisms are responsible for a shorter lifetime with high protein consumption. High protein content makes the acid load of the body higher. It is possible that the excretion of calcium in the urine increases, which also increases the risk of osteoporosis. A high protein intake can also increase the risks of having cancer and kidney failure. Evidence does not yet show that these effects occur, although it is proven to be harmful to patients with kidney failure. However this group stays below the existing RDA that is described for a healthy population. According to previous studies a protein intake up to 25 en% have no negative effects on health. That is why the upper limit in The Netherlands is set on an acceptable protein intake of 25 en% for the age range starting at 1 year old. However, the upper limit in America is set higher at 35 en%, so it is uncertain where the upper limit for protein should be set.

The main research question of this thesis is:
What is the relation between the usual protein intake and muscle mass in obese elderly (55+)?

The primary objective of this thesis is to determine the relation between appendicular muscle mass and usual protein intake in obese elderly.
The secondary objective of this thesis is to determine whether the relation is different for animal and vegetable protein on the appendicular muscle mass.

This thesis can be a good supplement for the existing literature, because there are not that many researches done on the subject of muscle preservation in elderly.
2. Methods

2.1 Introduction

To answer the thesis question the research data of the Muscle Preservation Study were used. The primary objective of the Muscle Preservation Study was to investigate the effects of a specialized oral nutritional supplement, a protein shake, on the preservation of muscle mass in obese elderly (55+) during a weight loss program. The duration of the weight loss program was 13 weeks and consisted of a hypo-caloric diet and a resistance exercise program. The subjects were randomized to get either the active product or the controlled product. Neither the researchers nor the subjects were notified which product was the active product and which product was the control product. The main parameter of the Muscle Preservation Study was the change in muscle mass. The appendicular muscle mass, that was measured by the DXA-scan in kilograms and percentage of body weight. In the Muscle Preservation Study there were also secondary parameters; changes in body composition, muscle strength, physical functioning and quality of life. This thesis only focused on the baseline data collected from the Muscle Preservation Study. The main research question of this thesis is: What is the relation between the usual protein intake and muscle mass in obese elderly (55+)? The aim of this thesis is to research if the RDA for protein (0.8g/kg/day) in elderly should be adjusted.

2.2 Subjects

The subjects had to meet the requirements of the in- and exclusion criteria from the Muscle Preservation Study.

Inclusion criteria:
- Age between 55 and 85 years. If women: postmenopausal
- BMI > 30 or BMI > 28 + waist circumference > 88 cm (women), > 102 cm (men)
- Ability to sign informed consent
- Willingness and ability to comply with the protocol, including:
  - Maintaining current dietary habits
  - Participation in study visits
  - Taking the study product every day
  - Ability to comply with the complete study protocol
  - Ability to understand and fill out questionnaires
- Physiotherapist’s professional view that the subject is physically fit and it is safe to participate in the resistance exercise program
Exclusion criteria:
- Any malignant disease during the last five years except for adequately treated prostate cancer without evidence of metastases, localized bladder cancer, cervical carcinoma in situ, breast cancer in situ or non-melanoma skin cancer

- Previously known:
  - Kidney failure (previous glomerular filtration rate <30 ml/min)
  - Liver failure
  - Anaemia (Haemoglobin in men <6.5 mmol/l and women <6.0 mmol/l)
  - (Chronic) inflammatory status

- Medication:
  - Corticosteroids for systemic use
  - Immunosuppressants
  - Insulin

- Dietary or lifestyle characteristics:
  - Participation in a weight loss diet three months before starting and during the study
  - Participation in a resistance exercise program three months before starting and during the study
  - Use of protein-containing or amino acid-containing nutritional supplements three months before starting and during the study
  - Current alcohol or drug abuse in opinion of the sponsor-investigator

- Indications related to interaction with the study product:
  - More than 10 µg (400 IU) of daily Vitamin D intake from medical sources
  - More than 500 mg of daily calcium intake from medical sources
  - Known allergy to milk and milk products
  - Known galactosaemia

- Sponsor-investigator’s uncertainty about the willingness or ability of the subject to comply with the protocol requirements

- Participation in any other study involving investigational or marketed products concomitantly or within four weeks prior to entry into the study
2.3 Study design

The Muscle Preservation Study was a randomized, controlled, double-blind, parallel-group study.

Subjects were randomly assigned to receive either the active product or the control product for a period of 13 weeks. Both groups followed a hypocaloric weight loss diet and participated in a resistance exercise program 3 times a week at the study center.

At baseline (week 0) and after 7 and 13 weeks the subjects visited the study center for assessments of body composition, muscle strength, physical functioning and quality of life.

Compliance, gastrointestinal tolerance, and adverse events have been checked during the 2-weekly dietary counseling sessions. During these sessions the subjects received information and tips on how to eat and live healthy.

The subject went through a screening period of 1 to 4 weeks and an intervention period (active vs. control) of 13 weeks. Therefore the study took 14 to 17 weeks for all subjects. This thesis focused on data collected at visit 1; baseline (figure 1). This means that for our thesis we excluded the hypocaloric diet, the resistance exercise program and the protein/control supplement.

2.4 Measurements and procedures

This thesis focused on the relationship between muscle mass and protein intake. The data on muscle mass were obtained by using the DXA-scan type: GE Lunar Prodigy / DPX-NT and the protein and energy intake were obtained by using the 3-day dietary record. For the measurements of the appendicular muscle mass (lean tissue mass of the arms and legs minus bone mineral content) by the DXA-scan the subjects were not allowed to eat 3 hours and drink non-caloric drinks up to 1 hour prior to the measurements. The protein and energy intake data were obtained by taking an average of the 3 days filled in by the subjects in the 3-day dietary record. The 3 days filled in by the subjects were calculated by using the Nevo tabel 2006. Other body composition data needed for this study were obtained by using air displacement
plethysmography (Bodpod). The subjects underwent 2 or 3 measurements in the Bodpod in case of a more than 150 ml difference in volume. For these measurements the subjects were not allowed to eat 5 hours and drink non-caloric drinks up to 1 hour prior to the measurements.

2.5 Statistical analysis

The statistical analysis were performed by using the SPSS Software: PASW statistics version 18. The main research question, the relation between protein intake and muscle mass, was analyzed by using the linear regression analysis. An alpha of 5% was used to determine whether the results were statistically significant or not. The normality of the outcome variable appendicular muscle mass was visually inspected by a histogram. The relation between protein intake and appendicular muscle mass appeared to be linear, visualized by using a scatter plot. Furthermore, we checked for effect modification by gender to examine whether gender effected the relation between appendicular muscle mass and total protein intake. This was tested by adding an interaction term of gender and protein intake into our model. If gender was significantly different it would be an effect modification.

Thereafter we checked for the confounders bodyweight, gender, PAL and age. We added each confounder one by one to the regression model to examine whether the bèta for protein intake changed more than 10% from the crude regression model. If there were several confounders with more than 10% change, we added the confounders with the greatest difference into our regression model. After adding this confounder we started to check the other confounders again with the new regression model until there are no confounders left with a greater difference then 10%.

This was done for the main determinant; total protein. We have also split up total protein in animal and vegetable protein and checked for the same confounders. The models for animal protein and vegetable protein were also adjusted for each other.

The amount of protein in the models will be shown in 10 grams instead of per gram. This is easier to translate into practice.
3. Results

3.1 Subjects

A total of 74 were screened in this study; 19 were excluded; 56 subjects were positively screened and randomized. Of these 56 subjects, 4 had missing values. The study included 51 subjects with complete data (Figure 2).

![Figure 2: Inclusion of subjects](image)

The subject characteristics are presented in table 1. The study population, a total of 51 subjects, had an average age of 64 years and an average BMI of 33.3. The main research question of this thesis is: what is the relation between the usual protein intake and muscle mass in obese elderly (55+). The average appendicular muscle mass in kilograms is 21.9 and the average protein intake is 78.4 grams. As seen in table 1 the males differ significantly from the females in height, weight, fat mass and in appendicular muscle mass. Other characteristics are shown in table 1 and table 2 for the diet characteristics.
Table 1: Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All Subjects (n=51)</th>
<th>Male (n=17)</th>
<th>Female (n=34)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>64 ± 5</td>
<td>65 ± 7</td>
<td>63 ± 4</td>
<td>0.094</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.8 ± 8.5</td>
<td>175.5 ± 7.0*</td>
<td>163.9 ± 6.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.5 ± 13.6</td>
<td>101.1 ± 12.4*</td>
<td>89.8 ± 12.7</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.3 ± 4.4</td>
<td>32.9 ± 3.0</td>
<td>33.5 ± 5.0</td>
<td>0.677</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>39.3 ± 9.0</td>
<td>33.9 ± 6.8*</td>
<td>42.1 ± 8.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>42.7 ± 7.8</td>
<td>33.6 ± 3.7*</td>
<td>47.2 ± 4.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Appendicular muscle mass (kg)</td>
<td>20.5 ± 4.9</td>
<td>26.4 ± 2.8*</td>
<td>17.6 ± 2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity level (kg)</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>0.991</td>
</tr>
</tbody>
</table>

*Males differ significantly from the females (P<0.05)

Table 2: Baseline diet characteristics of the study population

<table>
<thead>
<tr>
<th>Diet characteristics</th>
<th>All Subjects (n=51)</th>
<th>P5 (n=51)</th>
<th>P95 (n=51)</th>
<th>Male (n=17)</th>
<th>Female (n=34)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal)</td>
<td>1836 ± 583</td>
<td>822</td>
<td>2854</td>
<td>2014 ± 427</td>
<td>1747 ± 635</td>
<td>0.125</td>
</tr>
<tr>
<td>Protein intake (g)</td>
<td>78.1 ± 22.4</td>
<td>33.5</td>
<td>111.8</td>
<td>84.1 ± 17.9</td>
<td>75.1 ± 24.0</td>
<td>0.180</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>17.4 ± 3.1</td>
<td>11.8</td>
<td>23.0</td>
<td>17.0 ± 3.3</td>
<td>17.6 ± 2.9</td>
<td>0.554</td>
</tr>
<tr>
<td>Protein (En%)</td>
<td>0.84 ± 0.29</td>
<td>0.39</td>
<td>1.2</td>
<td>0.84 ± 0.19</td>
<td>0.84 ± 0.27</td>
<td>0.657</td>
</tr>
<tr>
<td>Animal protein (g)</td>
<td>50.1 ± 18.1</td>
<td>14.4</td>
<td>79.5</td>
<td>53.3 ± 15.7</td>
<td>48.4 ± 19.2</td>
<td>0.366</td>
</tr>
<tr>
<td>Vegetable protein (g)</td>
<td>28.0 ± 7.8</td>
<td>12.9</td>
<td>41.2</td>
<td>30.7 ± 8.4</td>
<td>26.7 ± 7.3</td>
<td>0.080</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>196 ± 53.6</td>
<td>124.2</td>
<td>296.1</td>
<td>204.8 ± 47.4</td>
<td>191.5 ± 56.7</td>
<td>0.431</td>
</tr>
<tr>
<td>Carbohydrates (En%)</td>
<td>43.1 ± 7.3</td>
<td>31.3</td>
<td>59.3</td>
<td>40.8 ± 4.6</td>
<td>44.3 ± 8.1</td>
<td>0.061</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>69.3 ± 31.5</td>
<td>19.4</td>
<td>133.3</td>
<td>75.8 ± 23.8</td>
<td>66.1 ± 34.6</td>
<td>0.304</td>
</tr>
<tr>
<td>Fat (En%)</td>
<td>32.4 ± 7.7</td>
<td>15.2</td>
<td>44.1</td>
<td>33.7 ± 6.6</td>
<td>32.1 ± 8.2</td>
<td>0.489</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>26.7 ± 13.7</td>
<td>6.9</td>
<td>56.1</td>
<td>27.5 ± 9.3</td>
<td>26.3 ± 15.5</td>
<td>0.763</td>
</tr>
<tr>
<td>Saturated fat (En%)</td>
<td>12.4 ± 3.6</td>
<td>6.3</td>
<td>19.2</td>
<td>12.2 ± 2.9</td>
<td>12.5 ± 3.9</td>
<td>0.770</td>
</tr>
</tbody>
</table>

3.2 The relation between appendicular muscle mass and total protein intake.

Figure 3 shows a significant relation between appendicular muscle mass in kilograms and total protein intake in grams. This is the unadjusted regression model for the population with a significance of P = 0.017.
Figure 3: Unadjusted linear regression model for the relation between appendicular muscle mass and total protein intake.

After examining for effect modification by gender we concluded that gender was not an effect modifier. The effect of gender on the relation between appendicular muscle mass and protein intake was not significant. Thus, the study population was not split up in male and female, due to the small study population. Even though this is seen regularly in scientific researches. Bodyweight and gender appeared to confound the relation between appendicular muscle mass and protein intake the most. Therefore these variables were included into the regression model. (Figure 4)

\[ \text{Appendicular muscle mass} = \beta_0 + \beta_1 \times \text{total protein} + \beta_2 \times \text{body weight} + \beta_3 \times \text{gender} \]

Figure 4: Adjusted regression formula total protein.

3.3 The relation between appendicular muscle mass and animal and vegetable protein intake.

Figure 7 shows that the relation between appendicular muscle mass and animal protein was not significant for the total population (\( P = 0.090 \)). Figure 8 shows that the relation between appendicular muscle mass and vegetable protein was significant for the total population. Even though females were significant (\( P = 0.016 \)) and males were not (\( P = 0.215 \)) in the relation between appendicular muscle mass and vegetable protein, we did not split up the population, because gender was no effect modifier (\( P = 0.700 \)).
Figure 5: Unadjusted linear regression model for the relation between appendicular muscle mass and animal protein intake.

Figure 6: Unadjusted linear regression model for the relation between appendicular muscle mass and vegetable protein intake.

As we analyzed the relation between appendicular muscle mass and animal protein, there was no effect modification by gender. Body weight and gender appeared to confound this relation the most. Therefore these variables were included into the regression model. (Figure 7)

\[ \text{Appendicular muscle mass} = \beta_0 + \beta_1 \times \text{animal protein} + \beta_2 \times \text{body weight} + \beta_3 \times \text{gender} \]

Figure 7: Adjusted regression formula for animal protein.

We also analyzed the relation between appendicular muscle mass and vegetable protein. As seen in the relation between appendicular muscle mass and animal protein, there was also no effect modification by gender. Body weight and gender appeared to confound this relation. Therefore these variables were included into the regression model. (Figure 8)

\[ \text{Appendicular muscle mass} = \beta_0 + \beta_1 \times \text{vegetable protein} + \beta_2 \times \text{body weight} + \beta_3 \times \text{gender} \]

Figure 8: Adjusted regression formula for vegetable protein.
Table 2 shows the unadjusted and adjusted regression models for the relation between appendicular muscle mass and protein intake at baseline. As seen in the table, the more confounders were added to the regression model the greater $R^2$ becomes. $R^2$ is a statistical term to show how much of the variation in appendicular muscle mass is explained by protein intake (Table 2).

Table 3: Adjusted regression models and standard error (SE).

<table>
<thead>
<tr>
<th>Appendicular Muscle Mass (kg) (n = 51)</th>
<th>$\beta$ (SE)</th>
<th>95% CI •</th>
<th>$P$ value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein (10g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: unadjusted</td>
<td>0.737 (0.297)</td>
<td>(0.140; 1.335)</td>
<td>0.017*</td>
<td>0.112</td>
</tr>
<tr>
<td>Model 2: model 1 + adjusted for body weight and gender</td>
<td>0.190 (0.148)</td>
<td>(-0.107; 0.487)</td>
<td>0.205</td>
<td>0.819</td>
</tr>
<tr>
<td><strong>Animal protein (10g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: Unadjusted</td>
<td>0.656 (0.379)</td>
<td>(13.205; 21.303)</td>
<td>0.090</td>
<td>0.058</td>
</tr>
<tr>
<td>Model 1a: Adjusted for vegetable protein</td>
<td>0.255 (0.393)</td>
<td>(-0.535; 1.045)</td>
<td>0.519</td>
<td>0.169</td>
</tr>
<tr>
<td>Model 2: adjusted for vegetable and body weight and gender</td>
<td>-0.003 (0.187)</td>
<td>(-0.380; 0.373)</td>
<td>0.987</td>
<td>0.829</td>
</tr>
<tr>
<td><strong>Vegetable protein (10g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: Unadjusted</td>
<td>2.550 (0.829)</td>
<td>(0.884; 4.215)</td>
<td>0.003*</td>
<td>0.162</td>
</tr>
<tr>
<td>Model 1b: Adjusted for animal protein</td>
<td>2.312 (0.911)</td>
<td>(0.481; 4.143)</td>
<td>0.014*</td>
<td>0.169</td>
</tr>
<tr>
<td>Model 2: Adjusted for animal and body weight and gender</td>
<td>0.863 (0.437)</td>
<td>(-0.017; 1.743)</td>
<td>0.054**</td>
<td>0.829</td>
</tr>
</tbody>
</table>

• Confidence interval for bèta
* Significant (P<0.05)
** Borderline significant

There was a significant relation between appendicular muscle mass and total protein intake, 11.2% of the variance ($R^2$) in appendicular muscle mass was explained by total protein intake. After adjustments for body weight and gender into the model, this relation became nonsignificant. 81.9% of the variance is explained by body weight, gender and protein.

There was no significant relation between appendicular muscle mass and animal protein intake. Even after adjustments for vegetable protein this relation remains nonsignificant with a weak $R^2$ (16.9%). After adjustments for vegetable protein, body weight and gender the relation was also not significant, but the $R^2$ was strong (82.9%).

The unadjusted model for vegetable protein was significant and after adjustments for animal protein the relation stays significant with a weak $R^2$, respectively 16.2% and 16.9%. After the
unadjusted model was adjusted for animal, body weight and gender the relation became borderline significant, but the variance in appendicular muscle mass for 82.9% explained by vegetable protein intake.
4. Discussion

4.1 Research question and results

The main research question of this thesis is to determine the relation between appendicular muscle mass and usual protein intake in obese elderly (55+). The results show that there seems to be a relation between appendicular muscle mass and total protein intake, but that this relation becomes nonsignificant after adjustments for body weight and gender are made. The hypothesis of the current study is that the muscle mass in elderly significantly increases when the dietary intake of protein increases. The hypothesis cannot be fully confirmed. Previous observational studies show mixed results on dietary protein intake and body composition. Baumgartnet et al.\textsuperscript{36} and Mitchel et al.\textsuperscript{37} show that protein intake is not associated with muscle mass. Both suggest that physical activity is of a greater influence on muscle mass. Breen et al.\textsuperscript{5} and Cuthbertson et al.\textsuperscript{38} suggest that elderly require higher quantities of protein intake to stimulate muscle protein synthesis. This is due to the fact that elderly may be less able to efficiently utilize amino acids for muscle protein synthesis. In particular Breen\textsuperscript{5} shows that a dietary plan including at least 20 grams of high quality protein per meal is necessary to promote muscle protein synthesis and thereby muscle mass. In this thesis we did not look at this aspect and the range of protein intake of the participant was also not wide enough.

Secondary objective of this thesis is to determine whether the relation is different for animal and vegetable protein on the appendicular muscle mass. Our thesis shows that vegetable protein is of greater influence on the appendicular muscle mass than animal protein. It is striking to see that vegetable protein is of greater influence than animal protein, because most studies suggest that animal protein, a source of high-biological-value protein, is more of influence on muscle mass than vegetable protein.\textsuperscript{22,39,40} Animal protein contains the branched-chain amino acids leucine. Leucine may stimulate the muscle protein synthesis due to playing a central role in mediating mRNA translation, but Breen\textsuperscript{5} also suggests that there are other essential amino acids required to facilitate the anabolic actions of leucine.

The current RDA for dietary protein intake is 0.8g/kg/day. There is some question regarding whether the RDA is sufficient or not. Some studies suggest that the RDA is too low,\textsuperscript{21,23,41} were others maintain the current RDA.\textsuperscript{42,43} Wolfe et al.\textsuperscript{30} suggests that a protein intake of 1.5g/kg/day is a reasonable RDA. We cannot make any suggestions regarding the RDA, because the results of our study, in the relation between appendicular muscle mass and protein intake are significant, but become insignificant after adjustments are made. Furthermore, our study results are based on elderly who do not consume very high amounts of protein a day. The range (P5-P95) of the protein intake of this study is 0.4 to 1.2g/day with a mean amount of 0.8g/per day. So we cannot analyze whether a high amount of protein has a positive effect on appendicular muscle mass.

4.2 Other factors of influence on the results.

Body weight and gender are added to the regression model, because they are the biggest confounders. Besides body weight and gender, there are other factors that influence the relation
between appendicular muscle mass and protein intake. Age and PAL appear to confound, but because of the small population we do not include these two into the regression model. We also decided not to examine the relation between appendicular muscle mass and ethnicity because besides Caucasians our study population only included 4 Suriname, 1 Iranian and 1 Antillean. However studies do indicate that black people have more muscle mass than Caucasians.\(^{44}\)

### 4.3 Strength and limitation

This study has its strengths and its limitations. One of the limitations of the study is that the participants might not have completed the 3-day dietary record correctly. It is known that the 3-day dietary record can be underreported.\(^{45}\) In this study we examine the difference between the total energy expenditure and the reported amount of energy obtained from the 3-day dietary record. We examine whether the participants have under- or overreported their intake during filling in the 3-day dietary record. The Resting Energy Expenditure (REE) is the energy needed in rest, the PAL is the energy expended per day. The total amount of energy needed per day, can be calculated by multiplying REE and PAL. This total amount of energy should approximately be equal with the 3-day dietary record. If not, the subjects could be under- or over reporting. However, the PAL can also be filled in incorrectly. For reliable results, we took a range of 10% for the accepted difference between the calculated total amount of energy per day and the amount of energy from the 3-day dietary record. From the 51 included subjects 5 have missing values (n= 4 no PAL, n= 1 no REE). 9 have filled in their 3-dietary record correctly, 33 underreported and 4 overreported their 3-day dietary record.

Some of the products written in the 3-day dietary record are also not specified enough. For example, some people wrote down ‘meat’, but do not specify what kind of meat and how it is prepared. Furthermore, the participants do not always write down the amount of grams, so it has to be estimated. This results in the next limitation. Sixteen different students have transferred the 3-day dietary record into the database. Even though all students have the same training on how to fill in the 3-day dietary record into the database, there is a possibility of inconsistency in the interpretation of the students.

Another limitation of the study is the possible generalization of the results. This is due to the fact that this thesis has a small number of participants that have enrolled in the study (n=51). The study has also the additional limitation that it has a small age range (55-77 years). Therefore, the results of this study cannot be generalized for the total population.

Besides limitations, this study has also its strengths. One of the strengths is the measurements of muscle mass by using DXA-scan. This is supported by the study from Kim J. et al.\(^{46}\), which suggests that DXA-scan models can provide reliable and accurate estimates of total-body skeletal muscle mass in adult populations.

We cannot establish a relation between appendicular muscle mass and protein intake in elderly (55+). Thus, dietary protein should be further investigated for its potential to increase muscle mass in elderly.
5. Conclusion

There is a significant relation between appendicular muscle mass and the usual protein intake in obese elderly (55+), but after adjustments for body weight and gender, the relation between appendicular muscle mass and total protein intake, is not significant. The results of this study show that gender is not an effect modifier. In the present study, body weight and gender confound the relation between appendicular muscle mass and usual protein intake. Animal protein and vegetable protein were not only confounded for body weight and gender in the relation with appendicular muscle mass, but are also confounded for each other.

Results show that the relation between appendicular muscle mass and vegetable protein is significant and the relation between appendicular muscle mass and animal protein is not. There is no explanation found why vegetable protein is significant and animal protein is not significant in the relation with appendicular muscle mass.

Further research is needed to determine whether protein intake is of influence on appendicular muscle mass and whether there is a relation between appendicular muscle mass, animal and vegetable protein.

5.1 Recommendations

Using the data of the present study we can conclude that there is a relation between appendicular muscle mass and usual protein intake, but that after adjustments this relation seems to becomes unstable. Thus a hard recommendation on protein intake cannot be made. We recommend that further research is needed to determine whether protein intake is of influence on appendicular muscle mass. In the present study total protein intake is split up in animal protein and vegetable protein. The results strikingly show that vegetable protein is of greater influence on appendicular muscle mass than the animal protein. Further research should be done to examine what the relation is between appendicular muscle mass, animal protein and vegetable protein.

Furthermore, the range (P5-P95) of the protein intake of this study is 0.4 to 1.2g/per day, and the mean amount is 0.8g/per day, meaning that the range of the variance as well as the amount are not very large. Research should be done in elderly (55+) who consume a larger amount (1.5g/per day or higher) of protein per day, but also with a greater range of protein intake per day to examine whether there is a relation between appendicular muscle mass and a larger amount of protein. The study results are also based on an evenly divided protein intake throughout the day. It should be examined whether there is a relation between appendicular muscle mass and a higher amount of protein taken at once per meal. For instance an amount of 20 grams protein at once per meal, which is equal to 65 grams of prepared chicken, to promote muscle protein and thereby appendicular muscle mass.
6. References


