PROTEIN TIMING AND HEALTHY AGEING
Pulse, intermediate and spread feeding on physical performance and muscle mass

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Preface

This thesis is the end product of the bachelor in Nutrition & Dietetics at the University of Applied Sciences. For this thesis, we applied the knowledge we studied for and obtained in the last four years, and we used random controlled research and literature research. Our thesis is part of the VITAMINE study, which studies physical performance and protein intake on body composition, cognition and physical functioning of the elderly (55+ years). Over 20 weeks, we and seven other students worked on the VITAMINE study. For our thesis, we focused on the association between the timing of protein on muscle mass and physical performance. Thanks to this project, we obtained a great deal of insight on protein, muscle mass and physical performance in the elderly.

We want to thank everyone who has supported us during our graduation period. First, we would like to thank Michael Tieland for his guidance, feedback and support. We would like to thank Carliene van Dronkelaar and Jantine van den Helder for their guidance and support during the VITAMINE study. We also want to thank the other seven students in the VITAMINE study for their collaboration. We want to thank our examiner and study physician, Minse de Bos-Kuil, for reviewing our thesis. Also, we would like to thank our client Dr Ir. P.J.M. Weijs, lector, weight management, for giving us the opportunity to do this assignment.
Abstract

Background
Aging is associated with different physical changes such as lower muscle protein synthesis, loss of functional abilities, muscle weakness, and decrease in muscle mass and strength. New studies show that consuming more protein can prevent these problems. Timing is an important factor in stimulating muscle protein synthesis and maintaining muscle mass and function. This study aims to find the association of different protein timing strategies with muscle mass and physical performance in the elderly. Our research questions are:
1. What is the difference between pulse feeding, intermediate feeding and spread feeding on muscle mass (FFM appendicular) in the elderly (55+ years)?
2. What is the difference between pulse feeding, intermediate feeding and spread feeding on physical performance (PP) in the elderly (55+ years)?

Methods
This study uses the data of elderly people who are 55 years and older at the baseline of the VITAMINE study. The subjects were divided into three groups. In the pulse feeding group, 50% of the protein intake was consumed at dinner. In the spread feeding group the protein intake was evenly spread over three meals. The other subjects who fell out of the pulse and spread feeding groups were included in the intermediate group. The subjects were evaluated on lean mass (DXA-scan), physical activity (Modified Physical Performance Test) and three-day dietary records. Regression analysis was used to assess the association between the timing of protein intake and muscle mass and physical performance in older adults.

Results
The energy intake of the pulse feeding group was significantly lower compared to the intermediate group and the spread feeding group (P=0.030). However, protein intake was also lower, but not significant (P=0.118). There was no significant association between pulse, intermediate and spread feeding on muscle mass and physical performance (P=0.145, P=0.638).

Conclusion
There is no association found between pulse, intermediate and spread feeding on muscle mass and physical performance.
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2. Introduction

Since 2011, the population of the Netherlands has aged quickly. The first post-war baby-boomers reached the state pension entitlement age in 2011. In the following five years, a half million of those age 65 and older added up. This is twice as much as in the five years before 2011. In 2039, the Netherlands will have approximately 4.6 million inhabitants age 65 and older. There are not only more elderly people they are also becoming older. Half a century ago only one in 74 inhabitants were 80 years or older, whereas now, this is one in 25 inhabitants. Around 2050, 1.8 million inhabitants will be age 80 or older. This means that one in 10 people will belong to the oldest group. The growth of this group will have a great impact on the demand for care and resources needed for elderly people (1).

The physical limitations increase as people grow older (2). The causes of this decline include inadequate protein intake (3). Muscle weakness and a decrease in physical performance are consequences of the decrease in muscle mass and muscle strength (4). Severe muscle weakness in those age 65 and older causes them to become more vulnerable and less self-reliant. They become less mobile, their fall risk increases and they are less able to do household tasks or take care of themselves. On a societal level, this means high costs for care and support (4).

When people get older, protein metabolism changes (5). The digestion and absorption kinetics changes, causing proteins to be less absorbed in the elderly. As a response, muscle protein synthesis rates are lower in the elderly as compared with younger people (6). Malnutrition is also a common problem with elderly. When they consume less, there is less protein intake (7).

A low protein intake and protein synthesis shows a lower muscle protein synthetic response in the elderly (6, 8). New studies show that the elderly need more protein than younger adults to support their functionality (5). Muscle protein decreases after the 30th year of life by approximately 1 percent each year (4). Increasing their protein intake is thought to help prevent age-related muscle loss and the loss of functional abilities (9).

Recent evidence indicates that in addition to total daily protein intake, the timing of protein intake is also important to stimulate muscle protein synthesis, maintain muscle mass and function in elderly (10).

Other studies have shown the difference between pulse feeding and spread feeding protein. The studies showed that a protein pulse feeding pattern is more effective than a protein spread feeding pattern on protein turnover in elderly (11-13). However, these results are adverse to those suggesting that the optimal feeding pattern is spreading four doses of 20 grams of protein across a day (14). These conflicting results between the studies could be due to age differences of the subjects in these studies. It could also be that 20 grams of protein per meal is not enough to stimulate the maximal muscle protein synthesis in the elderly.

So far, the association between protein intake per main meal on physical performance and muscle mass in the elderly has not been investigated extensively. Therefore, our main aim is to investigate the association of various protein timing strategies with muscle mass and physical performance in the elderly.
2.1 Research questions
1. What is the difference between pulse feeding, intermediate feeding and spread feeding on muscle mass (FFM appendicular) in the elderly (55+ years)?
2. What is the difference between pulse feeding, intermediate feeding and spread feeding on physical performance (PP) in the elderly (55+ years)?

2.2 Sub-questions
- What is the association between pulse feeding and muscle mass?
- What is the association between intermediate feeding and muscle mass?
- What is the association between spread feeding and muscle mass?
- What is the association between pulse feeding and physical performance?
- What is the association between intermediate feeding and physical performance?
- What is the association between spread feeding and physical performance?
3. Methods

3.1 Study design and population
This study used the participant database of the VITAMINE study. The study aimed to determine if home-based exercise, either combined with increased protein intake or not, has an effect on the physical functioning of elderly people (55+ years).

The VITAMINE study recruited the subjects through the organisation Cordaan and the ‘Meer Bewegen voor Ouderen’ (MBvO) intervention, by giving briefings and fact sheets during exercise classes of possible participants. Also, the VITAMINE study recruited people within the municipality of Amstelveen; Amstelveen-based elderly people received an invitation to participate in the study. When they decided to participate, they signed an informed consent. The study physician screened them. The study sample included vital elderly people who are 55 years or older. The elderly with kidney disease, neurological diseases (for example Parkinson's disease), people who had a knee and hip surgery in the previous six months and people with alcohol or drug abuse were excluded in this study (see Table 1 Inclusion and exclusion criteria).

Table 1 Inclusion and exclusion criteria (15-17)

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elderly from 55 years and older</td>
<td>Younger than 55 years old</td>
</tr>
<tr>
<td>Elderly with beginning functional limitations</td>
<td>Elderly with kidney disease</td>
</tr>
<tr>
<td></td>
<td>Research shows that the deterioration of renal</td>
</tr>
<tr>
<td></td>
<td>function is by a small part caused by the</td>
</tr>
<tr>
<td></td>
<td>amount of protein in our nutrition. They can't</td>
</tr>
<tr>
<td></td>
<td>be randomised in the group with protein-rich</td>
</tr>
<tr>
<td></td>
<td>advice because they have protein restriction.</td>
</tr>
<tr>
<td>Elderly who signed the VITAMINE-contract</td>
<td>Elderly with neurological diseases</td>
</tr>
<tr>
<td></td>
<td>For example Parkinson's disease, these people</td>
</tr>
<tr>
<td></td>
<td>have disorders in moving and symptoms of</td>
</tr>
<tr>
<td></td>
<td>dementia. This disease is also progressive.</td>
</tr>
<tr>
<td>Elderly who agreed to follow the protocol</td>
<td>Elderly who want to lose weight</td>
</tr>
<tr>
<td></td>
<td>The elderly who want to lose weight are also</td>
</tr>
<tr>
<td></td>
<td>excluded from this study. A lot of people who</td>
</tr>
<tr>
<td></td>
<td>are dieting have their own manner of dieting.</td>
</tr>
<tr>
<td></td>
<td>It will be likely that they will follow their</td>
</tr>
<tr>
<td></td>
<td>own diet. There is a great risk that they will</td>
</tr>
<tr>
<td></td>
<td>not follow any of the dietary advice from this</td>
</tr>
<tr>
<td></td>
<td>research. What if someone gets into a control</td>
</tr>
<tr>
<td></td>
<td>group but eats a lot of protein and does lots</td>
</tr>
<tr>
<td></td>
<td>of sports? This will give us a biased view of</td>
</tr>
<tr>
<td></td>
<td>the results and should be prevented.</td>
</tr>
<tr>
<td>Elderly who are screened by our study doctor</td>
<td>Elderly with alcohol and drugs abuse</td>
</tr>
<tr>
<td>and declared as healthy to join our research</td>
<td>To prevent dangerous situations for students if</td>
</tr>
<tr>
<td></td>
<td>they are home coaching someone, and the</td>
</tr>
<tr>
<td></td>
<td>subjects are less compliant to protocol.</td>
</tr>
</tbody>
</table>

The subjects were divided into three groups: the pulse feeding group, the intermediate group and the spread feeding group.

Figure 1 is an example of the results of the study. The pulse feeding group (blue) consumed one meal, dinner for example, where the subjects consumed 50% of the daily protein intake. The spread feeding group (green) had a protein intake of a maximum 20% difference between the main meals. The remaining test subjects were placed in the intermediate group.
3.2 Data collection
Baseline data was used for this thesis and collected on the measuring days. The data from the Modified Physical Performance Test (MPPT), the dual-energy x-ray absorptiometry (DXA-scan) and the 3-day dietary record was collected to assess muscle mass, physical functioning and protein intake.

3.3.1 Modified Physical Performance Test (MPPT test)
The MPPT test can be used as identification for mild to moderate fragility and to assess physical performance among the elderly, according to Brown, M. & Sinacore, D (2005). The result was based on the total score of nine components which were a balance test, a chair rise test, a raising a book test, a put on and take off a jacket test, a pick up a coin test, a 15-meter walking test, a 360 degrees turning test and a two stair climbing tests.

- The balance test: contained three components. First, the subject had to keep their balance by placing their feet against each other for 10 seconds. Second, the subject had to place their big toe against their heel for 10 seconds. Last, the subject had to place their feet in a straight line for 10 seconds.
- The chair rise test: was meant to see how well the subject could stand up from a chair five times without using their hands. The subjects had to get up from the seat with straight legs and sit down on the chair as fast as possible. This test was performed two times.
- The raising a book test: the subject had to raise a book above their head and put it on a closet shelf as fast as possible.
- The put on and take off a jacket test: the subject had to quickly put on and take off a jacket three times.
- The pick up a coin test: the subject had to pick up a coin as fast as possible two times.
- The 15-meter walking test: subjects had to walk a distance of 15 meters as fast as possible two times.
- The 360 degrees turning test: the subject had to make a 360 degree turn by taking steps while keeping their balance and stability. This test was performed two times.
- Stair climbing (1 stair): subjects had to walk up one stair as fast as possible.
- Stair climbing (four stairs): the subject had to walk the stairs four times up and down at normal speed.
3.3.2 The DXA-scan
The DXA-scan was used to measure the subjects' Fat Free Mass (FFM). The data from the FFM of the arms and legs was used, also known as appendicular lean mass (ALM). The DXA-scan uses dual energy X-ray to scan the whole body (18). To ensure the quality of this scan, trained researchers conducted these tests.

3.3.3 The 3-day dietary history
Dietary intake was calculated using 3-day dietary records (one weekend day and two weekdays). On the measuring day, the VITAMINE-students analysed their food diaries. The dietary records were coded according to the NEVO-table. The NEVO-table is a Dutch databank with nutrient values of most used products. All the information on the macronutrients and micronutrients are stated in the NEVO per 100 g of the product. This data was imported to Microsoft Excel for Windows 10, where formulas were used to calculate the energy and protein intake per subject and per meal.

3.4 Statistical analyses
The baseline data (n = 92) of the VITAMINE study was analysed with IBM Statistical Package for the Social Sciences (SPSS) statistics version 22. Data was expressed as mean ± standard deviation (SD). To check the data on normality, a histogram was used. The differences between the groups were investigated by the one-way ANOVA test. In addition, linear regression was used to assess the association between muscle mass, physical performance and protein timing. The independent variables were pulse feeding, intermediate feeding and spread feeding. The dependent variables were physical performance and muscle mass. Linear regression analysis was performed and adjusted for the following confounders: age, gender, energy intake and activity.

- Age: muscle mass and physical performance reduces by aging (9, 19,20).
- Gender: muscle mass and physical activity is different in men and women (20-22).
- Energy intake: a higher energy intake leads to a higher protein intake (23, 24).
- Activity: physical activity improves physical performance and muscle mass (24-26).

The SPSS syntax is used and the significance is set at p-value lower than P ≤0.05.
4. Results

4.1 Characteristics of the subjects

In this research, 104 subjects were screened and 12 subjects were excluded due to missing data. In total, there were 92 subjects included. In the pulse feeding group (N=22), there were three men and 19 women. In the intermediate group (N=50) there were 14 men and 36 women. The spread feeding group (N=20) counted three men and 17 women. In figure 1, the distribution of the groups is displayed.

![Figure 1 Distribution of the groups](image)

Table 2 displays the characteristics of the subjects. The average age of the subjects was 71.7 years. In the pulse feeding group, the mean age was 71.5 years; for the intermediate group this was 72.1 years, and for the spread feeding group the mean age was 71.8 years. The average height of the subjects was 166 cm. In the pulse feeding group, this was 164 cm, for the intermediate group this was 168 cm, and for the spread feeding group, this was 165 cm. The mean weight of the subjects was 74 kg. In the pulse feeding group, this was 71 kg, the intermediate group 75 kg, and for the spread feeding group 74 kg. This makes the average BMI of the subjects 26.7 kg/m². The pulse feeding group had a mean BMI of 26.5 kg/m², the average BMI of the intermediate group was 26.5 kg/m² and the spread feeding group had a BMI of 27.3 kg/m².

Table 2: Characteristics of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pulse feeding (N=22) Mean ±SD</th>
<th>Intermediate (N=50) Mean ±SD</th>
<th>Spread feeding (N=20) Mean ±SD</th>
<th>Mean ±SD</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n)</td>
<td>3</td>
<td>14</td>
<td>3</td>
<td>20 (21.7%)*</td>
<td>0.288</td>
</tr>
<tr>
<td>Women (n)</td>
<td>19</td>
<td>36</td>
<td>17</td>
<td>72 (78.3%)*</td>
<td>0.288</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.5 ± 8.36</td>
<td>72.1 ± 7.19</td>
<td>71.8 ± 6.49</td>
<td>71.9 ± 7.27</td>
<td>0.933</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1.64 ± 0.07</td>
<td>1.68 ± 0.08</td>
<td>1.65 ± 0.075</td>
<td>1.66 ± 0.08</td>
<td>0.143</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 ± 10.5</td>
<td>75 ± 13.58</td>
<td>74 ± 18.9</td>
<td>73.6 ± 14.2</td>
<td>0.613</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 ± 3.79</td>
<td>26.5 ± 4.01</td>
<td>27.3 ± 6.93</td>
<td>26.7 ± 4.7</td>
<td>0.785</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>18.9 ± 3.18</td>
<td>20.8 ± 4.33</td>
<td>18.5 ± 3.86</td>
<td>20.1 ± 4.04</td>
<td>0.145</td>
</tr>
<tr>
<td>MPPT (Total score)</td>
<td>33.82 ± 2.72</td>
<td>33.1 ± 3.66</td>
<td>33.7 ± 3.16</td>
<td>33.4 ± 3.33</td>
<td>0.638</td>
</tr>
</tbody>
</table>

Values are mean ±SD = Standard deviation
BMI = Body Mass Index
FFM = Fat Free Mass
*percentage men and women in the study
**ANOVA one way test was run over the variable and the difference between groups
4.2 Protein intake

In table 3, the nutritional intake of the subjects is displayed. The average energy intake of the pulse feeding group was 1604 kcal, which is significantly lower than the intermediate group (1965 kcal) and the spread feeding group (1856 kcal). The average energy intake of the pulse feeding group was 361 kcal lower than the intermediate group and 252 kcal less than the spread feeding group (P= 0.030).

The pulse feeding group had a mean consumption of 68.6 g protein, the intermediate group consumed 80 g and the spread feeding group consumed 75.1 g protein each day. The calculated protein intake in energy percent was 18.1% for the pulse feeding group, 16.5% for the intermediate group and 16.6% for the spread feeding group.

Table 3: The energy and protein intake of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pulse feeding (N=22) Mean ±SD</th>
<th>Intermediate (N=50) Mean ±SD</th>
<th>Spread feeding (N=20) Mean ±SD</th>
<th>Mean ±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (Kcal/day)</td>
<td>1604 ± 583.3</td>
<td>1965 ± 521</td>
<td>1856 ± 442.3</td>
<td>1851 ± 534.2</td>
<td>0.030</td>
</tr>
<tr>
<td>Protein intake (Gram/day)</td>
<td>68.6 ± 21.1</td>
<td>80 ± 22.6</td>
<td>75.1 ± 18.8</td>
<td>76.1 ± 21.6</td>
<td>0.118</td>
</tr>
<tr>
<td>Protein intake (En% of total)</td>
<td>18.1 ± 5.3</td>
<td>16.5 ± 3.4</td>
<td>16.6 ± 4.6</td>
<td>16.9 ± 4.2</td>
<td>0.308</td>
</tr>
</tbody>
</table>

Values are mean ±SD = Standard deviation
En% = energy percentage

Table 4 displays the timing of the protein intake per group separated over three meals. Markedly, for breakfast, the pulse feeding group consumed ±10 g protein less than the spread feeding group. For lunch, the pulse feeding group consumed less protein (12.33 g protein) than the intermediate group (19.31 g protein) and the spread feeding group (21.16 g protein). The highest protein intake at dinner was found in the pulse group (40.26 g of proteins) compared to the intermediate group (33.01 g of proteins) and spread feeding group (24.8 g of proteins).

Table 4: Timing of protein intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Diner</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pulse</td>
<td>Intermediate</td>
<td>Spread</td>
</tr>
<tr>
<td>Protein intake (g/meal)</td>
<td>8.45 ± 5.99</td>
<td>12.59 ± 7.10</td>
<td>18.28 ± 7.03</td>
</tr>
<tr>
<td>Protein intake (En% of total)</td>
<td>2.75 ± 2.19</td>
<td>2.62 ± 1.47</td>
<td>4.07 ± 1.78</td>
</tr>
</tbody>
</table>

Values are mean ±SD = Standard deviation
En% = energy percentage
*P≤0.05 = significant
4.3 Body composition
In table 2, the average fat-free mass (FFM) was 18.8 kg. The mean of the pulse feeding group was 18.9 kg, the intermediate group was 20.8 kg, and the spread feeding group was 18.5 kg.

In table 5, the association between FFM and pulse feeding is displayed. The protein intake of the pulse feeding group was associated with FFM (P = 0.032). Adjusting the regression analysis for confounders showed no significant association between FFM and pulse feeding (Beta = 0.049, P = 0.180).

**Table 5: Results between FFM and pulse feeding**

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.075</td>
<td>0.007 - 0.143</td>
<td>0.032</td>
</tr>
<tr>
<td>Model I*</td>
<td>0.027</td>
<td>-0.034 - 0.089</td>
<td>0.363</td>
</tr>
<tr>
<td>Model II**</td>
<td>0.049</td>
<td>-0.025 - 0.123</td>
<td>0.180</td>
</tr>
</tbody>
</table>

*Model I = crude + adjusted for age + gender
**Model II = model II + adjusted for energy intake + physical activity

In table 6, the association between the FFM and intermediate feeding is displayed. The protein intake of the intermediate group had a non-significant association with FFM (B = 0.021, P = 0.485).

**Table 6: Results between FFM and intermediate feeding**

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.006</td>
<td>-0.050 - 0.062</td>
<td>0.829</td>
</tr>
<tr>
<td>Model I*</td>
<td>0.020</td>
<td>-0.015 - 0.055</td>
<td>0.256</td>
</tr>
<tr>
<td>Model II**</td>
<td>0.021</td>
<td>-0.039 - 0.081</td>
<td>0.485</td>
</tr>
</tbody>
</table>

*Model I = crude + adjusted for age + gender
**Model II = model II + adjusted for energy intake + physical activity

In table 7, the result between FFM and spread feeding is displayed. The Crude model adjusted for age and gender had a significant association between FFM and spread feeding (P=0.003, P=0.020). However, adjusted for energy intake and physical activity, the association between FFM and spread feeding was non-significant (B = 0.066, P = 0.214).

**Table 7: Results between FFM and spread feeding**

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.127</td>
<td>0.048 - 0.207</td>
<td>0.003</td>
</tr>
<tr>
<td>Model I*</td>
<td>0.107</td>
<td>0.019 - 0.194</td>
<td>0.020</td>
</tr>
<tr>
<td>Model II**</td>
<td>0.066</td>
<td>-0.43 - 0.175</td>
<td>0.214</td>
</tr>
</tbody>
</table>

*Model I = crude + adjusted for age + gender
**Model II = model II + adjusted for energy intake + physical activity

The FFM had a non-significant association between the pulse group, intermediate group and the spread group according to the ANOVA Post-Hoc Bonferroni test (P = 0.145). The difference between the FFM of the pulse group and the intermediate group was not significant (P = 0.206). Also, the intermediate group compared to the spread group was non-significant (P = 0.644). In conclusion, no association between FFM was detected in the pulse and spread feeding group (P = 1.000).
4.4 Physical performance
The average score of the MPPT was 33.39 points. For the pulse group the average score was 33.82 points, the intermediate group had a mean score of 33.1 points and for the spread group this was 33.7 points. Additionally, the average scores of the groups were quite similar. The ANOVA Post-Hoc Bonferroni test displayed a non-significant association between pulse group, intermediate group and spread group on physical performance (P = 0.638). See table 2.

In table 8, the results between physical performance and pulse feeding are displayed. The crude model had a positive regression line (B = 0.010) after adjusting the confounders: the Beta gives a negative regression line (B = 0.000, B = -0.029). However, the association between physical performance and pulse feeding is non-significant (P = 0.514).

**Table 8: Results between physical performance and pulse feeding**

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.010</td>
<td>-0.050 - 0.070</td>
<td>0.721</td>
</tr>
<tr>
<td>Model I*</td>
<td>0.000</td>
<td>-0.069 - 0.070</td>
<td>0.992</td>
</tr>
<tr>
<td>Model II**</td>
<td>-0.029</td>
<td>-0.122 - 0.064</td>
<td>0.514</td>
</tr>
</tbody>
</table>

*Model I = crude + adjusted for age + gender  
**Model II = model II + adjusted for energy intake + physical activity

In table 9, the results between physical performance and the intermediate group are displayed. The physical performance had a non-significant association with the intermediate group (P = 0.161).

**Table 9: Results between physical performance and intermediate feeding**

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>-0.007</td>
<td>-0.054 - 0.040</td>
<td>0.751</td>
</tr>
<tr>
<td>Model I*</td>
<td>0.017</td>
<td>-0.024 - 0.059</td>
<td>0.405</td>
</tr>
<tr>
<td>Model II**</td>
<td>-0.034</td>
<td>-0.083 - 0.014</td>
<td>0.161</td>
</tr>
</tbody>
</table>

*Model I = crude + adjusted for age + gender  
**Model II = model II + adjusted for energy intake + physical activity

Table 10 displays the results between physical performance and the spread group. Physical performance and the spread group had a positive regression line (B = 0.016). However, there is a non-significant association (P = 0.733).

**Table 10: Results between physical performance and spread feeding**

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>-0.018</td>
<td>-0.100 - 0.065</td>
<td>0.660</td>
</tr>
<tr>
<td>Model I*</td>
<td>-0.010</td>
<td>-0.096 - 0.076</td>
<td>0.809</td>
</tr>
<tr>
<td>Model II**</td>
<td>0.016</td>
<td>-0.085 - 0.118</td>
<td>0.733</td>
</tr>
</tbody>
</table>

*Model I = crude + adjusted for age + gender  
**Model II = model II + adjusted for energy intake + physical activity
5. Discussion

The present study aims to study the relation between pulse, intermediate and spread feeding on muscle mass and physical performance. Within our study, there was no significant association between pulse, intermediate or spread feeding on muscle mass and physical performance in elderly people.

The present study has some strengths and weaknesses. A strength of the present study is the usage of DXA. Although some other measurement methods such as Magnetic Resonance Imaging (MRI) and computed tomography (CT) are considered as more accurate, the DXA has been addressed as a valid measure to assess fat-free mass. In addition, MRI and CT have some disadvantages. The MRI-scan is high in costs, can be claustrophobic for test subjects and subjects can be concerned about the radiation exposure (27). One study, which looked at spread and pulse feeding in hospitalised elderly patients, used a DXA-scan combined with BIA (28). However, studies show a high correlation between DXA, air displacement plethysmography (BODPOD) and bioelectrical impedance (BIA) (29). This correlation makes the use of BODPOD and BIA unnecessary. Therefore, DXA is an accurate measurement.

The test subjects had to complete a 3-day dietary record. Even though completing a 7-day dietary record would give a better impression, three days are generally accepted as valid to assess protein intake. In addition, 3-day food records may result in a better motivation for test subjects (30, 31). One study used the 3-day food records to estimate energy and protein intake (32). In contrast, the health ABC study used 24-hour recalls in collecting data about their nutritional intake by interviewing the subjects (30). This is more accurate, as the food questionnaires are interviewed. However, this gives us a limited insight into food habits, protein and energy intake. Overall, a 3-day food record is a better measurement. At last, inquiries on the food diaries can be taken on the measuring day.

Despite these strengths, a few limitations should be addressed. One limitation is the physical performance measure, the MPPT. The MPPT has a maximum score, which makes it impossible to see maximum physical performance. This causes the scores of the elderly to be closer together. A test like the 6-minute walk test has no maximum score and can, therefore, provide a fairer outcome. However, the 6-minute walk test does not measure physical functioning very precisely and is, therefore, a less accurate way of measuring. Other studies have used the Short Physical Performance Battery (SPPB). This study had a significant association between physical performance and protein intake (33). The MPPT and SPPB both have maximum scores. However, the MPPT has more components, which makes the MPPT a more accurate measurement for physical performance. Also, the time of the MPPT is noted. This allows time to be calculated, which has no maximum. In our thesis, the time records are not used. In a new study, the time could be used instead of the score.

Another limitation might be the population size. In our study, we measured 92 elderly people. In comparison, our study used a small population – other studies included 304 and 2,066 test subjects (8, 34). A literature study with 2,066 subjects gave significant results; the protein intake was associated with changes in lean mass (8). However, this study In addition, a large population could give a more accurate outcome. However, a large population is more time consuming and higher in costs. Therefore, a small population could be an advantage.
The majority of our sample population is considered generally active and fit. In the overall population, fitness in the elderly can differ from very fit to very fragile. The fitness of the elderly could give a distorted view of the older population. The fitness of the elderly is linked to the measurements of the MPPT-test. As a result, the average scores of the MPPT are almost at its highest. This causes the scores of the elderly to be close to each other, which make it difficult to see an association between pulse, intermediate or spread feeding on physical performance. To get a more accurate outcome on the elderly population, the study should include more variety among elderly people, for example, fragile elderly people.

We are the first study to investigate pulse, intermediate and spread feeding in association with muscle mass and physical performance. The main research questions of this thesis were to determine the association between spread, intermediate and pulse feeding on appendicular muscle mass and physical functioning in elderly (55+ years). Our crude model shows an association between pulse feeding and spread feeding on muscle mass. However, corrected for age, gender, energy intake and physical activity, this association becomes non-significant. Other associations were already non-significant from the crude model. The hypothesis in this thesis was that pulse feeding will ensure significantly increased muscle mass and physical functioning. This hypothesis cannot be accepted. So, the general belief that pulse feeding would be beneficial to support muscle growth in the elderly should be reconsidered in a randomised controlled trial (RCT), as our study was a cross-sectional study of which no causal effect can be drawn.

Recently, multiple studies have indicated that 25–30 g of a high-quality protein is necessary to reach the point of maximal stimulation of muscle protein syntheses in the elderly (35). This could be the reason for muscle mass growth, in pulse feeding, in multiple studies. However, the average energy intake of our pulse feeding group was significantly lower than the intermediate and spread feeding group. A lower energy intake could indicate a lower protein intake. This could be the reason for a non-significant outcome of our pulse feeding group. The elderly might need more protein per meal to get a better muscle protein synthesis and therefore gain a greater muscle growth (36-37). If this is the case, spread feeding with a minimum protein intake of 25-30 g a meal could be most effective for muscle mass growth (35).

According to the literature, the elderly could benefit more from consuming rapidly absorbed protein, unlike younger people who benefit more from a slow digested dietary protein. These results may appear to be consistent with the outcome of spread and pulse feeding studies because ‘fast’ protein could be seen as similar to pulse feeding. ‘Fast’ protein stimulates amino acid oxidation and protein synthesis, this can be absorbed in a shorter period of time and therefore cause a pulse effect (37). This could cause the positive effect of rapidly absorbed protein. However, some studies indicate that the positive effect of pulse feeding cannot be seen in younger adults (10-12). Ageing reduces the skeletal muscle mass and function, this is explainable by lower muscle protein synthesis rates in the elderly (6). Therefore, the elderly may need more rapidly absorbed protein and more protein at once (38). More research is needed to determine if rapidly or slowly digested protein has an association with muscle mass and physical performance in the elderly.

Other studies show that timing of pulse feeding during the day is essential (28). Those with evening consumption had a significantly higher leg lean mass than those with afternoon protein consumption (28). In our study, all the pulse feeders ate their pulse meal in the evening. However, there is no significant outcome between pulse feeding and muscle mass and physical performance. However, age should be included. Age was not considered in the study. As seen earlier, age can give a different outcome. It may therefore be that the timing of pulse feeding has no effect on the elderly, which is seen in our study. Though there may be other indications that do not result in a significant outcome of our study. The fitness of the elderly, for example. More research should be done with elderly people.
Animal-based proteins may be better than plant-based sources as they contain more essential amino acids. Animal-based proteins supply all nine of the essential amino acids, while plant-based protein contains fewer essential amino acids. These sources are lower in lysine and/or methionine than the animal-based proteins. This study concludes that a variation in vegetables with higher concentrations of lysine and methionine is important to a “complete” essential amino acids’ profile (39). However, this study did not evaluate the protein source. The results in our study were close. This could be due to the fact that all the elderly had greater animal-based protein consumption. We didn’t examine the main protein source of the elderly and can therefore make no conclusions. In future research, timing and protein source should both be studied (39).

According to literature, a pulse feeding pattern is more common in fit elderly people. The study looked at fragility of elderly and the timing of protein intake (40). The subjects in our study were generally fit; however, only 25% of the subjects consumed a pulse meal. These results appear to be inconsistent with literature. According to the study on the fragility of the elderly, there should be more pulse feeders in our research. However, the fitness of the elderly may not only be related to timing of protein intake but also to physical activity. A number of studies show that physical activity in the elderly is associated with muscle mass (24, 25)). Also, this could have an association with physical performance. A pulse feeding diet could be the best diet for the elderly to gain muscle mass and physical performance. Yet physical activity could be just as effective as the timing of the protein intake. More research should be done to determine if physical activity and pulse feeding has an equivalent association with muscle mass and physical performance.

The results of protein pulse, intermediate or spread feeding were non-significant in our study. There have been few studies investigating the timing of protein intake on physical functioning. As described above, a minimum of 25-30 g protein could have a positive association with muscle mass. In another study on obese elderly people, it is seen that physical functioning improved at 30 g of protein each meal (41). However, the results cannot be compared due to the differences in design between studies. Still, these data show a possible benefit of the timing of protein on physical functioning and are in conflict with our data. Therefore, more research with similar designs is needed to show the effects of protein timing on performance. Also, the subjects were measured at baseline; therefore, the protein intake could not be controlled. The subjects filled in their dietary history according to their own diet. Therefore, the pulse, intermediate and spread feeding groups were not even. When there is more data available from visit three (the second measuring day), where some of the subjects had protein information, those data should be used.
6. Conclusion and recommendations

The main conclusion of the study is that pulse, intermediate and spread feeding does not have a significant association with muscle mass and physical performance in the elderly (55+ years).

As different studies have found results in the timing on protein intake, it could indicate that there is an association with muscle mass and physical performance.

To form a better conclusion, our research should have included a larger study sample of a minimum of 200 subjects. Also, the subjects should have a greater variety in fitness. Contacting the municipality for help to recruit the elderly by address could randomise the subjects. MPPT should be used to measure physical functioning. However, the measured time of each component should be used instead of the score, which is given for time. At last, protein intake of the subjects should be monitored to ensure all groups consume the same total daily protein. This prevents an incorrect outcome due to lower protein intake in one of the groups – in our case, the pulse feeding group.

Dieticians need to focus on protein intake, but currently no evidence for pulse, intermediate or spread feeding was found. Still, they should focus on a greater amount of protein intake for the elderly. Also, this could lead to fewer costs for care and support. In today's society where there are more elderly than young adults and the population continues to become older, this could be beneficial for society.
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