Thesis – MUCMUS Study

The effect of dietary protein on lean mass change in strength training athletes during a bulking or a cutting phase

Sander Janssen - 500619758
Jasper Beelen – 500698383
Thesis supervisors: Amely Verreijen
Robert Memelink

Version 1
Date: 15-01-2018
The effect of dietary protein on lean mass change in strength training athletes during a bulking or a cutting phase

Authors
Jasper Beelen
Jasper.beelen@hva.nl

Sander Janssen
Sander.janssen2@hva.nl

Assignment company
Hogeschool van Amsterdam
Lectoraat Gewichtsmanagement
Dr.Meurerlaan 8
1067 SM Amsterdam
020-5953400

Thesis supervisors
Amely Verreijen
a.verreijen@hva.nl

Robert Memelink
r.g.memelink@hva.nl

Bachelor Nutrition and Dietetics
January 2017
Acknowledgements

This thesis focuses on the relationship between protein intake and muscle mass change in strength training athletes while following a bulking or cutting regime. The assignment is given by Lectoraat Gewichtsmanagement of the Hogeschool van Amsterdam, under supervision of Robert Memelink.

We have chosen this subject for our thesis because of our passion for strength training and nutrition. We have learned a lot about how to do scientific research and what is needed to complete a study.

We want to thank Amely Verreijen for her help, guidance and our interesting conversations about the subject. Also we would like to thank Robert Memelink and Mariëtte van Rijmenam for their help.
Summary

**Goal:** The goal of this thesis is to study the association between protein intake and the change in lean mass during a cutting and a bulking phase. The term cutting means being in a caloric deficit for a period of time to reduce the amount of body fat and maintain as much muscle mass as possible. The term bulking is used to describe a period of time with a focus on gaining muscle mass.

**Method:** The MUCMUS study is an observational prospective study with strength athletes (aged 18+ and training at least 4 times a week). These athletes were either bulking or cutting for a period of ±10 weeks. Participants recorded their nutritional intake in a 3-day food diary and their training regimen in a 7-day training diary. Both diet and training were programmed by the athletes themselves. Both groups were measured twice; once before their period of bulking or cutting and once after ±10 weeks. Protein and energy intake was estimated by a 3-day food diary. Body composition was based on whole body lean mass (bone-free and fat-free mass), body fat mass and body fat percentage measured with a DXA-scan. Total body weight was measured with the calibrated weighing scale. The data was analysed using linear regression, with change in lean mass (kg) as the dependent variable, and protein intake (g/kg body weight) as the main determinant, with adjustments for the potential confounder caloric intake.

**Results:** The dataset, which consisted of the two visits and a complete food diary, was used for analysis. Out of the 26 participants, 13 were in the bulking phase and 13 in the cutting phase. Protein intake ranged from 1.46 to 3.57 g/kg body weight with a mean of 2.76 ± 0.70 kg. When corrected for caloric intake during the bulking phase, a significant association between protein intake and change in muscle mass (b=0.58, p=0.041) was found. This beta showed that an increase of 1 g/kg of protein leads to an increase of 0.6 kg of muscle mass. There were no significant associations in crude models without correction.

**Conclusion:** Within the small population for this study, one significant association was found between protein intake and changes in lean body mass during the bulking phase. Based on this study, no concrete recommendation can be given for strength athletes or dietitians.

**Keywords:** Protein, muscle mass, strength athletes, body composition, body fat percentage.
# Table of contents

Acknowledgements ........................................................................................................ 3

Summary .......................................................................................................................... 4

1. Introduction .................................................................................................................. 7
   1.1 Objective and research question ............................................................................. 8

2. Methods ....................................................................................................................... 9
   2.1 Study design ........................................................................................................... 9
   2.2 Study population ..................................................................................................... 9
      2.2.1 In- and exclusion criteria ............................................................................. 9
      2.2.2 Recruitment ..................................................................................................... 9
   2.3 Study Parameters ................................................................................................... 9
      2.3.1 Dietary Intake ............................................................................................... 10
      2.3.2 DXA-scan ...................................................................................................... 10
      2.3.3 Bod Pod ......................................................................................................... 10
   2.4 Statistical analysis .................................................................................................. 10
      2.4.1 Confounders ................................................................................................. 11

3. Results ....................................................................................................................... 12
   3.1 Overview participants ............................................................................................ 12
   3.2 Effects of bulking and cutting on body weight and body composition ............... 13
   3.3 Dietary protein intake in relation to lean body mass change and total mass change ........................................................................................................................................ 16
      3.3.1 Bulking ........................................................................................................... 16
      3.3.2 Cutting ........................................................................................................... 19
      3.3.3 Overview bulking and cutting in crude and adjusted models ....................... 22

4. Discussion ................................................................................................................... 23

5. Conclusion .................................................................................................................. 25

6. Recommendation ....................................................................................................... 26

7. References ................................................................................................................. 28
1. Introduction

Strength athletes need to maintain their muscle mass and in certain instances they need to increase their muscle mass. This can be influenced by anabolic stimuli such as training and nutrition (1). For nutrition especially, protein seems an important factor (2). For muscular hypertrophy, one of the leading processes is the protein synthesis in the muscles. Protein breakdown is one of the reasons why athletes cannot infinitely gain muscle mass. Eating more protein could prevent protein breakdown in the muscles. Protein synthesis is caused by protein, to be more precise the essential amino acids like leucine which stimulate the m-TOR pathway (3). Balancing the protein synthesis and protein breakdown is important for athletes. Thus, to gain more muscle mass the athlete needs to consume a certain amount of protein (4).

By performing resistance training, athletes will induce micro-tearing in the muscle fibres, which in return are repaired by protein in the body (5). Therefore, the need for dietary protein is higher for resistance trained athletes compared to non-resistance trained individuals (6,7).

Bodybuilders can consume as much as 3 grams of protein per kilogram of body weight on training days, being on a hypocaloric diet (8). Protein researcher Stu Phillips recommends 1.8-2.7 g of protein per kg of body weight per day for strength athletes who are on a diet (9). Stu Phillips’ main belief behind the large quantities of dietary protein consumption in strength training athletes is that it is needed to generate more muscle protein. Furthermore, athletes may require more protein to aid in adaptation to the exercise stimuli.

In contrast to the recommendations of Phillips et al. (2011), others advise only up to 1.8 g/kg of body weight (10,11). In the Netherlands, the Nutritional Council has certain recommendations for protein intake based on the activity a person is participating in. According to the Nutritional Council, healthy adults have a protein requirement of 0.8 g/kg of body weight, while strength athletes have a requirement of 1.7 – 1.8 g/kg of body weight (10). This requirement is based on the increased oxidation of branched-chain amino acids, which creates a demand for protein within the body (10).

Beside the potential positive effect of higher protein intake on strength training athletes, a downside might be the burden it potentially has on the kidneys, liver and blood lipids. Higher protein consumption, however, has not shown any harmful effects on blood lipids, kidney and liver functions (12).

The MUCMUS-project has been initiated to focus on strength athletes and their protein intake because of the many variations and recommendations of it.

In practice, the range of protein consumed can vary between 0.8 g/kg to 3 g/kg of body weight (8). Most of these athletes base their diet on sources from popular fitness media, like internet forums and social media, with a wide variance in the credibility of the information that is spread.

The terms cutting and bulking are used throughout the thesis. Cutting means being in a caloric deficit for a period of time to reduce the amount of body fat and maintain as much muscle mass as possible. The term bulking is used to describe a period of time with the focus on gaining muscle mass. Both these phases are used to improve body composition.
Based on the current literature, not many recommendations have been made about the protein intake during a bulking or cutting phase.

Since there is no consensus on the recommendation for protein intake for strength athletes, this study aims to find the optimal range in the two specific phases for strength athletes.

1.1 Objective and research question

This study is performed with and for strength athletes to provide more evidence for an optimal range of protein intake to promote muscle gain in the bulking phase, and to prevent muscle loss in the cutting phase. The aim is to give (sports) dietitians and athletes a more substantiated recommendation for their protein intake during a bulking or cutting phase, which can be based on either protein intake in grams (g) or protein per kilogram of body weight (g/kg).

This study focuses on the following research questions:

**What is the relation between the amount of dietary protein intake and the change in lean mass in strength training athletes* during a bulking or a cutting phase?**

*Subjects need to be at least 18 years of age and train at least four times a week for at least an hour to count as strength training athletes

The following additional questions are studied to get a better understanding about what happens to the body composition during the bulking and cutting phases, while also interpreting the results in the change of lean mass.

- How does dietary protein affect the total body mass during a bulking and cutting phase?
- How does dietary protein affect the fat mass during a bulking and cutting phase?
2. Methods

2.1 Study design

The MUCMUS study is an observational prospective study. Researchers working on this study only gathered data and did not intervene with the participants at any point during the study, since this study was purely observational.

During this study, subjects are measured just before and at the end of a bulking or cutting phase. The subject is free to choose which phase they plan to do. The measurements include the measurement of lean mass, fat mass, body weight and dietary intake.

The bulking or the cutting phase has a duration of approximately 10 weeks. During these 10 weeks the athlete trained at least 4 times a week, recorded their nutritional intake for 3 days and recorded their activity in a 7-day activity diary.

2.2 Study population

The original goal of the MUCMUS was to include at least 100 completed participants. To combat the potential drop-out of participants due to time constraints, injury, loss of communication or other reasons, it was important to recruit more than a 100 participants.

2.2.1 In- and exclusion criteria

For this study, the following eligibility criteria were applied: being a strength training athlete above the age of 18, have at least 4 training days a week and do at least an hour of strength training. Apart from these criteria, the subject needs to start in either a cutting or bulking phase.

Exclusion criteria are confirmed steroid use, which will only be tested based on inquiry and is therefore based on the honesty of the subject (no drug tests were performed). Participants were also excluded if they did not take part in the second measurement after the period of bulking or cutting, and if they had injuries which happened during the study period preventing optimal training. Each participant was informed about these criteria and signed an informed consent.

2.2.2 Recruitment

Participants were recruited in gyms surrounding Amsterdam by leaving advertisement posters in nearby gyms. Participants, who were interested in taking part, could contact the study-team by e-mail.

Participants, who met the eligibility criteria, were invited for a baseline measurement. During this baseline measurement, the participant had to confirm whether they were in the bulking or cutting phase. After approximately 10 weeks, a follow-up appointment was made to measure the difference in body composition.

2.3 Study Parameters

To answer the primary research question, the following body composition measurements were used: the Bod Pod system to measure body weight (kg), the DXA scan to measure changes in lean mass (kg), fat mass (kg) and fat mass percentage and the dietary assessment to measure protein (both in g and g/kg body weight/day) and total energy intake (kcal/day).
2.3.1 Dietary Intake

The participants were given a 3-day food diary, which is used to analyse their caloric and protein intake. Participants were instructed to document 3 days, which gave an indication of their diet during the bulking or cutting phase. Participants were instructed to preferably report 2 weekdays and 1 day in the weekend. It did not matter if they had trained on that day or not. The participants had to report their food intake and portions in grams or standard household measurements. Participants were asked to report the supplements they were using, such as protein powders, branched-chain amino acids, creatine and other fitness supplements.

Dietary intake was calculated with the NEVO-table from 2013 (13).

2.3.2 DXA-scan

Body composition is measured through a DXA-scan (Hologic, Discovery A). The DXA-scan is originally a scan to give a physician an accurate view of the bone composition of a patient. However, it can also be used calculate fat mass and (appendicular) lean mass. The scan uses 2 radiation sources which are both absorbed differently by body tissue. The detector on top detects how much radiation passes through the body. Based on the different amount of radiation a difference can be made in bone mass, fat mass and fat-free and bone-free mass (14,15).

The change in total lean mass (kg) has been measured over a 10 week period with an initial measurement and a second measurement 10 weeks later.

The DXA-scan gives information about the body composition of different body compartments. A distinct difference in fat mass, fat-free and bone mass can been seen in the results of the scan. The choice to use whole body lean mass instead of appendicular mass is made because in strength sports a lot of lean mass is gained in the torso. Choosing only the appendicular mass would be disregarding this gained muscle mass.

2.3.3 Bod Pod

Body composition and lung volume were measured through the Bod Pod (Life Measurement, Inc) which is an air displacement plethysmograph. The Bod Pod works through a measurement of body volume. Using this data in combination with the weight of a subject, it calculates the density of the body, which is the fat mass and lean mass (14,15). The only data being used from the Bod Pod is the total body weight, since the scale is calibrated with calibrated weights before each measurement (16,17).

2.4 Statistical analysis

The recorded data was stored in predetermined Excel-files while also using a double data-entry method. This method was used to prevent mistakes when recording the collected data. Any discrepancies between the data entries would be noticed. These Excel-files were made by previous students working in the MUCMUS-project. The data were entered anonymously: the subjects were coded with a tracking number in the dataset. The statistical analyses have been done by using IBM statistics SPSS version 23.

To answer the research questions, linear regression analyses were run for protein intake and changes in lean mass with the amount of protein (g) and (g/kg of body weight) as the main determinants and for fat mass as the main outcome variable. This was mainly done to verify whether the participant was in the bulking or cutting phase. This, however, was also done in
order to get a complete picture of how the protein intake could possibly affect different aspects of the body composition.

Furthermore, subject characteristics of the bulking and the cutting phase were compared by an independent samples t-test for the continuous variables and by a chi-square test for the nominal variables.

### 2.4.1 Confounders

The caloric intake is expected to be the main confounder in this study. It is suspected that due to the subjects being free in their diet, the caloric intake will be too diverse and will influence the lean mass. In a cutting phase, the caloric deficit can vary too much. It can either be 200 kcal or 1500 kcal which can lead to not enough weight loss on one end and on the other it can lead to the catabolism of muscle tissue. In the bulking phase, there is a possibility that the surplus is not sufficient enough to lead to any significant increase in muscle mass, which will result in non-significant data (18).

Gender is expected to either be an effect modifier or a confounder due to a difference in the speed of muscular hypertrophy between male and female participants. However, based on the literature, the effects of protein intake on lean muscle mass change will differ between genders. But they will not be directly affected by it as factor, thus it is less likely that gender is a confounder. In this study, gender was not taken into account due to the sample size being too small when the participants were split in male and female.
3. Results

3.1 Overview participants

In Table 1 an overview is given for the characteristics of the participants.

Table 1. Baseline characteristics of subjects in the MUCMUS study in either the bulking or the cutting phase

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bulking phase (n = 13)</th>
<th>Cutting phase (n =13)</th>
<th>p-value(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n male (% male)</td>
<td>10 (77.0)</td>
<td>8 (62.0)</td>
<td>0.395</td>
</tr>
<tr>
<td>Age, y</td>
<td>23.5 ± 3.6</td>
<td>26.9 ± 5.1</td>
<td>0.042</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>77.60 ± 10.36</td>
<td>81.95 ± 12.79</td>
<td>0.350</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>62.17 ± 10.50</td>
<td>63.36 ± 12.30</td>
<td>0.792</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>14.57 ± 1.87</td>
<td>18.26 ± 4.24</td>
<td>0.008</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>18.62 ± 3.47</td>
<td>21.95 ± 5.29</td>
<td>0.069</td>
</tr>
<tr>
<td>Years of training experience</td>
<td>4.6 ± 2.7</td>
<td>7.3 ± 3.8</td>
<td>0.048</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>2956 ± 818</td>
<td>2508 ± 784</td>
<td>0.167</td>
</tr>
<tr>
<td>Protein intake, g/day</td>
<td>214 ± 63</td>
<td>192.4 ± 84</td>
<td>0.467</td>
</tr>
<tr>
<td>Protein intake, g/kg BW/day</td>
<td>2.76 ± 0.70</td>
<td>2.30 ± 0.88</td>
<td>0.157</td>
</tr>
<tr>
<td>Protein intake, g/kg FFM/day</td>
<td>3.46 ± 0.89</td>
<td>2.94 ± 0.97</td>
<td>0.170</td>
</tr>
</tbody>
</table>

\(^1\)Data are presented as means ± SD or as number (percentage).

As seen in the table, there is a 50/50 split in participants, with a population of 13 in each group.

Noteworthy characteristics are the lean mass and fat percentages. Both groups have comparable scores on average. Three characteristics have a significant difference between the two groups namely: age, fat mass (kg) and years of training experience (p<0.05). With ‘years of training experience’ close to having no significant difference (p=0.048). Both groups seem in line with expectations; with the bulking group having lower fat percentages and the cutting group higher ones. The bulking group also has a higher average caloric intake.
3.2 Effects of bulking and cutting on body weight and body composition.

Figure 1 presents the average body weight change in both the bulking and cutting phase in kg/week scale. This is done to correct for the difference in length of each participant’s respective phase.

For the bulking, the left diagram, a total body mass change of 0.14 ± 0.56 kg per week is shown. On average, 0.10 ± 0.55 of lean mass was gained of the total mass and 0.04 ± 0.16 kg of fat was gained. This confirmed that, on average, our participants followed a typical bulking phase. More muscle mass was gained with slight amounts of fat mass. In the bulking phase, no significant changes have been found for total, lean or fat mass (p>0.05).

While no significant changes in fat mass can be positive during a bulking phase, having no significant changes in total or lean mass could be an indication that these participants did not do the bulk correctly. This is because during a bulking phase, lean mass should increase with a minimal increase in fat mass.

For the cutting, the right diagram, a total body mass change of -0.12 ± 0.29 kg per week is shown. On average, 0.00 ± 0.21 of lean mass was gained of the total mass and -0.12 ± 0.18 kg of fat was gained. This can confirm that, on average, our participants entered a typical cutting phase. Fat mass was lost, while even a small amount of lean mass was gained. For cutting, no significant changes were found in total or lean mass (p>0.05). However, for fat mass, there was a significant change (p<0.05).

No significant changes in lean mass with a significant change in fat mass could be an indication that the cutting phase was done as it should be, meaning maximum fat loss and minimum lean mass loss. The non-significant changes in total mass are not necessarily a good or a bad thing during the cutting phase as this is decided solely by the goal of the participant.

![Figure 1. Average changes in body composition in participants in the MUCMUS study in kg/week.](image)

On an individual basis, a lot more variance is shown between participants in each group, see Figure 2.
In the bulking phase, some of the participants barely gained any lean mass. While, others lost lean mass or gained mostly fat. With one participant losing a large amount of lean mass while also gaining fat mass.

As seen in Figure 3, in the cutting phase a similar effect is shown with participants actually gaining a lot of muscle mass, while losing fat. One of the participants gained both fat and lean mass. While another participant gained fat and lost lean mass. Some of the participants lost both fat mass and lean mass.
There is no clear association between the amounts of protein and the way people react to the cutting or bulking phase. Some of the participants, who have nearly the same amount of protein intake, still differ a lot in their body composition changes.
3.3 Dietary protein intake in relation to lean body mass change and total mass change

To study the relation between protein intake and change in muscle mass, a linear regression analysis was performed with the protein intake as the main determinant and lean mass, fat mass and total body weight as outcome variables. All body mass changes are specified in kg per week to equal out differences in bulking or cutting periods.

3.3.1 Bulking

![Graph showing lean mass changes in kg/week versus protein in g/kg body weight in bulking group]

Figure 4. Lean mass changes in kg/week versus protein in g/kg body weight in bulking group
Figure 5. Fat mass changes in kg/week versus protein in g/kg body weight in bulking group
Figure 6. Total body mass changes in kg/week versus protein in g/kg body weight in bulking group.

In Figure 4, 5 and 6, the correlation is shown between different kinds of body mass and protein intake in g/kg for the bulking phase. First for total body mass, then fat mass and last lean mass as the main determinant.

For lean mass, an increase of 0.43 kg for each g/kg protein consumed is shown in Figure 4.

For fat mass, an increase of 0.08 kg for each g/kg protein consumed is shown in Figure 5. For total body mass, an increase of 0.52 kg for each g/kg protein consumed is shown in Figure 6.
Thus, it can be confirmed that the protein intake does have the highest association with the lean mass.

3.3.2 Cutting

![Graph showing lean mass changes in kg/week versus protein in g/kg body weight in cutting group](image)

Figure 7. Lean mass changes in kg/week versus protein in g/kg body weight in cutting group
Figure 8. Fat mass changes in kg/week versus protein in g/kg body weight in cutting group

\[ y = 0.01x - 0.133 \]

\[ p = 0.931 \]

\[ r^2 = 0.001 \]
In Figure 7, 8 and 9, the association is shown between different kinds of body mass and protein intake in g/kg for the cutting phase. For lean mass, a change of -0.04 kg for each g/kg protein consumed is shown in Figure 7. For fat mass, an increase of 0.01 kg for each g/kg protein consumed is shown in Figure 8. For total body mass, a change of -0.03 kg for each g/kg protein consumed is shown in Figure 9.

Thus, from the results it can be concluded that there is a negative association between protein intake and lean mass during the cutting phase and a positive association between protein intake and fat mass. This is noteworthy considering the goal of the cutting phase.
3.3.3 Overview bulking and cutting in crude and adjusted models

Table 2. Association between protein intake and lean mass change/week during a period of bulking or cutting, crude models and models adjusted for potential confounder caloric intake

<table>
<thead>
<tr>
<th></th>
<th>Baseline Phase</th>
<th>P</th>
<th>Adjusted model for caloric intake with outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main determinant protein intake in grams/kg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1(^1)</td>
<td>0.43 (0.197)</td>
<td>0.052</td>
<td>-0.035 (0.073)</td>
</tr>
<tr>
<td>Model 2(^2)</td>
<td>0.58 (0.248)</td>
<td>0.041</td>
<td>0.042 (0.112)</td>
</tr>
<tr>
<td><strong>Main determinant protein intake in grams</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1(^1)</td>
<td>0.00 (0.00)</td>
<td>0.148</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Model 2(^2)</td>
<td>0.00 (0.00)</td>
<td>0.093</td>
<td>0.00 (0.00)</td>
</tr>
</tbody>
</table>

1. Crude Model with outcome
2. Adjusted model for caloric intake with outcome

As seen in Table 2, there is no significant association found between protein intake in g/kg body weight and lean mass changes. However, when corrected for caloric intake, there is a significant association protein intake in g/kg body weight and lean mass changes during the bulk phase. (P<0.05)

Table 2 displays no significant association even when corrected.
4. Discussion

The main objective of this thesis was to study the association of dietary protein intake on the changes in lean mass during either a bulking or cutting phase. While there have been a few studies on the topics of dietary protein intake and muscle mass, not many studies look at the changes in body composition during these phases (6,8,10,18,19). In the fitness community, a lot of people tend to cycle these two phases, so their importance for bodybuilders and other strength athletes can be considered high.

A concrete comparison between this study and other studies is hard to make, since few studies have the observational nature that this study has. This means that most studies have had a specific amount of protein being consumed during the study. This study did not have a set amount of consumed protein while other studies did have a set amount of protein. Usually high protein is compared in two groups, for example the study done by Antonio et al. In this particular study there were two groups, one consuming 2.6 g/kg of body weight and the other consuming 3.3 g/kg of body weight (20). However, no significant changes were found in body composition after a four week period between the two groups. This may indicate that at higher amounts of protein intake no increased effect in muscular hypertrophy can be found. Furthermore, no negative side effects were found regarding the kidneys, liver and blood lipids (12).

Participants taking part in the MUCMUS-study had a very diverse range of protein intake. Therefore it was hard to compare it to current literature. Thus, this makes the MUCMUS-study a good preliminary study and further research is required, using the limitations and data found in this study as a guideline to set up a more specific approach. This study, based on a relatively small sample size, shows a significant association between protein intake in g/kg body weight and lean body mass changes. This is however only during the bulking phase. The adjusted model seems to be more relevant over the crude model, since energy intake is a relevant confounder in this study.

Since lean mass change has been used as the primary outcome variable, this can be the reason that no significant association has been found in the cutting phase with minimal change in lean mass being a goal in the cutting phase. No significant changes are actually positive. Thus, it can be said that protein intake is less important than expected in a cutting phase.

Besides the large variation in protein intake, there is also a large variation in training style, eating style, training frequency and macronutrient distribution. With no clear guideline for the participants to follow and with every participant being able to choose their own approach, this gives rise to a possibility of more diversity in results. This can also be found in the current results with people taking in similar amounts of dietary protein, but having completely different results. Therefore, for future research a stricter approach in study design would be recommended.

This is also an argument for the question whether the bulking and cutting phases were done as they should be. As seen in the results the differences were small and mostly not significant.
Limitations:

There are many factors which influence the relation of protein intake and change in lean mass, thus are limiting for this study. The largest factor is the use of anabolic steroids in the bodybuilding and fitness community. According to a study done by Gwezdik among a group of 281 (64.6%) professional athletes concerned with fitness, bodybuilding and powerlifting up 30% have admitted to use anabolic steroids (21). Steroids, or AAS (Anabolic Androgenic Steroids), increase protein synthesis and other processes, which in turn increase the amount of lean mass that can be gained (22). They do also limit the amount of lean mass breakdown and increase the amount of fat loss during a cutting phase (22). This makes it a strong confounding factor during the bulking phase. However, in our study, there is no way to check if someone is currently using or has used AAS. There is, however, one way to test this, which is by doing a blood test. But, as this would be invasive, it is not used in the study. To test this thoroughly, multiple samples would be needed during different stages of the study. On top of this, this would only enable a test for current use, not previous use or short term use between tests.

Therefore, in further studies, groups should be made instead of excluding the participants which have used or are currently using AAS. This could also provide more data on the effects of AAS on protein synthesis.

Another limiting factor is the starting points regarding the training experience of many of the athletes and genetic potential. Muscle size is limited by myonuclear domain in the muscles. These nuclei have control over a given area inside the muscle. The nuclei have a limit to the amount of muscular fibres they can control. To continue muscular hypertrophy more nuclei are needed, which in turn come from satellite cell pools surrounding the muscle fibres (23,24). According to this, training experience and genetic potential are interfering with the relation between protein intake and lean mass change.

Due to small sample size, the amount of confounders that the data can be corrected for, are also limited. Thus, only a correction for caloric intake has been done. As for gender, which was suspected as either a confounder or effect-modifier, no correction or splitting of the analyses was done. Furthermore, splitting up the analyses would make the population too small on both sides. Correcting for it as a confounder would not be correct due to the correction for caloric intake and the sample size being only 13 in both groups.
5. Conclusion

Only one significant association was found between dietary protein intake in g/kg lean mass and bodyweight changes during either the bulking or the cutting phase. During the bulking phase, when corrected for caloric intake a significant association is found.

Since the sample size is small and since there are more confounding factors that could not be taken into account, this conclusion is still preliminary and more research should be performed to give a clearer insight into the relation between protein intake and lean mass changes in a phase of bulking and cutting.

On average, both groups behaved as expected, with the bulking group gaining mostly lean mass and the cutting group losing fat mass. However, on individual basis there was a wide variance in results of participants their respective phases. Based on current results, no clear advice can be given on the optimal amount of protein during either a bulking or cutting phase.

In conclusion, this study shows that protein intake had a positive effect on changes in lean mass while bulking. While having no effect on the changes in lean mass while cutting.
6. Recommendation

The study design as an observational study has been a great choice for exploring this relation between protein intake and lean mass change during cutting and bulking phases. As the results of this study show, it is recommend to do further research regarding protein intake and lean mass change during cutting and bulking phases. Unfortunately, during this study there have been too many variables, which interfere with the results of the study. All the participants are following different training regimen, have different macronutrient splits and their caloric surplus or deficit is probably too much of a confounder to observe the effect of protein on muscle mass. It is recommended to design an intervention based on the following changes.

For further research, it is recommended to give scientifically based training programs to the athletes in which the intensity is based on a percentage of the 1RM (1 rep max) for the exercises, specifically for the cutting phase and the bulking phase (25). This will reduce the confounding effects of the diversity in intensity and training volume. This will also show the effects of training experience in diminishing returns on muscle mass gained in the bulking phase. To further minimise the confounding effect of training experience all participants should have the same amount of training experience.

Regarding diet, it is recommended to make multiple groups up to 6 groups in total. This would be a baseline group which is based on current literature and recommendations of 1.8 g/kg of protein and groups with up to 3 g/kg of protein. Considering an increase in protein will also increase the total amount of calories consumed. Therefore, it is advised to maintain a set amount of calories based on kilograms of bodyweight and make changes in either carbohydrate or fat intake.

Basing the dietary intake on 3 days in a single week is not representative for a cutting or bulking phase lasting 10 weeks. Therefore, basing the caloric intake on kcal/kg of body weight will also allow the participants to continue their goal in either gaining or losing weight. Several food tracking apps can be found with the option for the researchers to observe the dietary intake. A commonly used app is Myfitnesspal. This app allows people to befriend each other and view each other’s food diary (26). Based on this, a weekly average of calories can be calculated. Based on the weekly averages, the increments of calories can be tracked and verified with the predetermined protocol.

A final recommendation for further studies is to either test women or men exclusively. The effect of the male hormone testosterone is confounding the effect of protein on muscle mass. It should be noted that exogenous hormones, like steroids, are likely to have a positive effect on food conversion ratios. Meaning the nutrients consumed are more likely to be used for muscle building processes. Unfortunately, the previous studies regarding food conversion ratios are done on fish and other animals. Based on this and the anecdotal remarks from athletes on steroids, it could be an indication that this positive effect on food conversion ratios is also present when humans use anabolic steroids (25).

Finally, a practical recommendation based on the current literature is that it seems likely that in a bulking phase the recommended amount of protein is between 1.8-2.2 g/kg of body weight (20,28,29). Regarding the cutting phase, consuming more than 1.8-2.2 g/kg of body weight of protein could improve muscle retention. This should be studied further and it is
advised to use a maximum protein consumption of 2.8 g/kg of body weight. This prevents consuming a sub-par diet in terms of variety, micronutrient density and adequate nutritional support for training while also positively affecting mood state during dieting (30,31).

Therefore, based on these studies, the following recommendations are made:

Athletes, who are in a bulking phase, should consume up to 2.2 g/kg of body weight of protein, while being in a caloric surplus. The caloric surplus should be based on the amount of weight the athlete needs to gain, while keeping in mind the available time for this phase (20,28,29).

Athletes, who are in a cutting phase, should consume 2.8 g/kg of body weight of protein, while being in a caloric deficit. The caloric deficit should be based on the amount of weight the athlete needs to lose, while keeping in mind the available time for this phase (30,31).
7. References