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Exercise and Nutrition Science

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## **Protein, how much to eat per meal?:**

# **A systematic review in maximizing the muscle protein synthetic response in resistance-trained athletes.**

By

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*This work is original and has not been submitted previously in support of a degree qualification or other course.*

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This thesis is submitted to the Department of Clinical Sciences of the University of Chester in partial fulfilment of the requirements of the Master of Science degree in Exercise and Nutrition Science.

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## **Abstract**

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**INTRODUCTION:** It is common practice in strength sports to spread out nutrient intake, and more specifically protein, into small amounts during the day with the belief that this is not only optimal but absolutely necessary in order to render in optimal response in terms of protein utilization and growth. Moreover this notion mainly stems from the belief that only a limited amount of protein ( $20 - 30 \text{ g}^{-1}$ ) can be utilized per meal.

**OBJECTIVE:** To evaluate existing evidence into the amount of protein that might optimize the muscle protein synthetic response (MPS) in the post prandial period.

**DESIGN:** Systematic review of randomized controlled trials (RCTs).

**SUBJECTS:** ‘Healthy’ adult subjects (18 – 64 years of age) either after an acute bout of resistance training and/or systematic involvement in resistance training (minimum of 3 days/week).

**RESULTS:** 56 studies were identified as primary research, of which 12 were assessed for eligibility. Of these 12 studies, 3 met the predetermined inclusion criteria. Synthesis of the evidence showed included studies varied considerably in terms of study design, quality and outcomes, yet showed no evidence that only a limited amount of protein can be utilized per meal.

**CONCLUSION:** At this point there is no evidence that only  $20 - 30 \text{ g}^{-1}$  of protein per meal can be utilized per meal by resistance trained athletes while on the other hand there is, at best, very minor evidence that more than these amounts ( $\sim 40 \text{ g}^{-1}$ ) might stimulate MPS to a greater degree. Further studies should focus on comparing various amounts of protein using ‘realistic’ administration of nutrients as well as with the usage of ‘realistic’ training protocols.



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## Section 1: Introduction

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The chapter serves to present the reader with guidelines, to introduce the topic and its background, to propose the problem statement, elaborate on the aims and objectives of this thesis, as well as to serve as an introduction to the various upcoming chapters.

### **1.1 A word for the audience**

This section is meant to serve as reading guidelines audience as well to introduce some basic terminology that will be used throughout this thesis. The intention of this thesis is to reach a broad spectrum of readers, which could be sports nutritionists, academics, students, coaches, athletes or general public interested in the field of sports nutrition. The aim of the thesis is therefore to be as clear and understandable as possible with regards to the, at times difficult, terminology used. It would however be recommended to have some general background knowledge regarding (sports) nutrition and more specifically the role of protein in the athlete's diet to fully understand the thesis.



## 1.2 Terminology

In order to first of all understand basic terminology used in the subsequent sections of this chapter, as well as to understand what is meant by the terminology used in the aforementioned thesis title, the basic terminology consists of the following:

- **Systematic Review:** “Systematic reviews aim to identify, evaluate and summarise the findings of all relevant individual studies, thereby making the available evidence more accessible to decision makers” (Centre for Reviews and Dissemination, 2009, p. v).
- **Resistance trained athletes:** Any athlete looking to increase muscular size and strength as a result of diet and exercise, whereas weightlifting is the principle form of exercise used. This could be bodybuilders, power lifters or other athletes who seek increase muscular size and/or strength such as footballers, rugby-players, sprinters, hockey-players etc. in at least some period off their training (offseason).
- **Muscle Protein Synthesis:** The term skeletal muscle protein synthesis (MPS) is commonly used with regard to alteration of protein expression in skeletal muscle (McDonald, 2007; Norton et al., 2009) and should be regard in such a manner throughout the review. That is additional contractile proteins are incorporated to existing tubular myofibrils (the basic units of muscle) in the process of skeletal muscle protein synthesis (Zatsiorsky & Kraemer, 2006).

### **1.3 Background**

This section describes the key contextual factors related to this topic and why the review was required. Additionally it serves to provide the rationale for underpinning the inclusion criteria, such as the choice of intervention to be considered in the review, as well as the focus of the review question (Centre for Review and Dissemination, 2009).

#### **The discrepancy**

Resistance trained athletes are a group of athletes that tend to have austere dietary patterns (Andersen, Barlett, Morgan, & Brownell, 1995; Brownell & Rodin, 1992; Heyward, Sandoval, & Colville, 1989; Kleiner, Bazzarre, & Litchford, 1990; Patelli et al., 1987) and their strictly disciplined habits of eating have more than once been compared with several eating disorders such as Anorexia Nervosa, Bulimia Nervosa as well as BSM-IV (more commonly known as binge eating disorder) (Goldfield, Blouin, & Woodside, 2006; Mangweth et al., 2001). Mangweth et al. (2001) for instance described the eating patterns of resistance training population such as bodybuilders as “obsessive as that of subject with eating disorders but with a ‘reverse’ focus of gaining muscle as opposed to losing body fat” (p. 38). While this thesis is not out to investigate whether strict diet adherence often seen in resistance trained athletes and specifically bodybuilders is a disorder or not, the above does however illustrate that these athletes tend to be very much disciplined when it comes to dietary intake.

While most of the above mentioned studies tend to look at dietary habits in terms of food selection and total caloric intake (Brownell & Rodin, 1992; Goldfield, et al., 2006; Heyward, et al., 1989; Kleiner, et al., 1990; Mangweth, et al., 2001; Patelli, et al., 1987) one study did subject these dietary habits to a more in depth investigation and the study by Andersen et al. (1995) shows that it is common practice for resistance trained athletes to spread out nutrient

(protein) intake into several meals during the day (Andersen, et al., 1995). A notion that has been suggested in more recent literature as well stating that especially resistance trained athletes practice “eating small amount of protein (and calories) in small amounts throughout the day with the belief that this is necessary for optimal protein utilization or growth” (McDonald, 2007, p. 65) Moreover the idea of eating small amounts of nutrients (protein) 2-4 hours throughout the day with the belief that this is optimal is to one degree or another suggested by several (leading) sports nutrition books as well (Bernadot, 2006; Clark, 2008; Kleiner & Greenwood-Robinson, 2007).

While the relatively low amount/high frequency approach thus seems common practice amongst resistance trained athletes (Andersen, et al., 1995; McDonald, 2007) and certainly seems to be recommended by several (leading) sports nutrition books as well, they do not seem to provide any scientific evidence or to as to why eating small meals throughout the day containing protein, ‘feeds your muscles more efficiently’ (Kleiner & Greenwood-Robinson, 2006, p. 9). A closer look various sources of literature reveal that this persistent idea of high meal frequency is mainly based upon the belief that only a given amount of protein (20-30 g<sup>-1</sup>) can be utilized from a given meal (McDonald, 2007; Norton & Wilson, 2009).

If this premise regarding this limit was to be correct, any excess protein would just be oxidised and excreted by the body (Tarnopolsky et al., 1992). This notion however is hard to reconcile in the light of various other studies in scientific literature that seem to suggested, that much higher amounts of protein can be utilized from per meal.

Soeters et al., (2009) for instance compared two weeks of intermittent fasting involving 20 hour fasting cycles and four hour eating cycles with a more conventional diet of eating three times a day. The researchers found no difference in preservation of lean mass and muscle

proteins, despite the consumption of  $101 \text{ g}^{-1}$  of protein once a day followed by a 20-hour fast (Soeters et al., 2009).

Stote et al., (2007) even found improvements in lean mass and a decrease of body fat when only one meal a day (of which the protein content was approximately  $86 \text{ g}^{-1}$ ) was consumed as opposed three meals a day for the study period of eight weeks. It can be argued for this reason that when only  $20 - 30 \text{ g}^{-1}$  of protein was 'used' the rest would simply have been wasted, as is thus often suggested as the reason for the ingestion relatively moderate amounts of protein per meal throughout the day (Kleiner & Greenwood-Robinson, 2007; McDonald, 2007; Norton & Wilson, 2009) an occurrence increases in lean body mass (LBM) would have seem highly unlikely. The opposite was however the case in this study, as the one meal group increases LBM with  $0.7 \text{ kg}^{-1}$ , as opposed to no changes in terms of LBM in the three meal group (Stote et al., 2007).

This work is however in contrast to the work by Iwao, Mori & Sato (1996) on boxers who were on a hypocaloric diet of  $1200 \text{ kcal}^{-1}/\text{day}$  and were eating either two or six meals per day. While both groups experienced decreases in lean body mass, the six meal group lost less, suggesting that 6 meals might be anti-catabolic in the case of low-calorie diets (Iwao, Mori, & Sato, 1996).

Unequivocally the most relevant research on protein distribution comes from unpublished work by Øyvind, Therese & Truls (2007). In the study 33 man and 15 women with at least one year of previous resistance training experience were placed either on a three- or six- meal per-day-diet. Both groups were instructed to eat the same amount of calories, providing approximately a  $300 \text{ kcal}^{-1}$  per day surplus with a protein intake of  $1.7 \text{ g}^{-1}/\text{kg}^{-1}$ . It was reported that the three meal group gained more total lean body mass and fat mass as opposed to the six meal group. This could have had several reasons; food intake was self-reported and

their accuracy may therefore be questioned (Hill & Davies, 2001; Schoeller, 1990) and it has been suggested that differences in food intake over the length of the study could have impacted the results (McDonald, 2007).

Like wise to results of Soeters et al. (2009) and Stote et al. (2007) yet along a different line of measurement (i.e. nitrogen balance) research by Irwin & Feeley (1967) as well as Finkelstein & Dryer (1971) also found no difference between three meals and six meals assuming equal total protein intake (approximately  $58 \text{ g}^{-1}$  in the case of Irwin & Feeley (1967) and  $110 \text{ g}^{-1}$  in the study of Finkelstein & Dryer (1971)). This is in line with the work by Young et al. (1971) whom fed the subjects  $118 \text{ g}^{-1}$  of protein in either one, three or six meal frequency per day and work by Arnal et al (1999, 2000) whom found no difference in terms of fat-free mass and nitrogen retention, when 80% of total daily protein ( $54 \text{ g}^{-1}$ ) was eating in one meal as opposed to evenly spread out across four meals.

In contrast to these results another study found that six meals versus three meals per day led to slightly greater weight loss and better nitrogen retention (Antoine, Rohr, Gagey, Bleyer, & Debry, 1984). This is in line with work by Garrow et al. (1981), that gave their subjects a rather low amount of protein per day ( $25\text{-}30 \text{ g}^{-1}$  on average), whom found five meals was superior to three meals per day in grams of nitrogen balance, but not in terms of weight- and fat-loss.

It must be noted however that most of these aforementioned studies used nitrogen balance method of measurement, a method that is inherently flawed to assess protein requirements for skeletal muscle protein metabolism for a number of reasons, including the fact that losses are difficult to measure accurately (Fern, Bielinski, & Schutz, 1991; Lemon & Proctor, 1991; Tome & Bos, 2000; Waterlow, 1999), it does not take into account the transient role (stimulatory role despite oxidation) of amino acids (Millward & Rivers, 1988; Millward &

Rivers, 1989), contains a strong adaptive component that may give skewed results (nitrogen balance can be achieved over a numerous amount of protein intakes despite static caloric intakes) (Millward, Bowtell, Pacy, & Rennie, 1994; Rand, Pellett, & Young, 2003; Young, 1986) and more importantly perhaps, nitrogen balance is a measurement of whole body protein flux and can rarely provide relevant information about protein turnover changes in specific tissue (Tessari, 2006).

A more feasible way suggested in recent scientific literature is to use of more direct measures of skeletal muscle protein synthesis (La Bounty et al., 2011) such as the use of stable isotopes to assess muscle protein fractional synthetic rate (FSR)(Garlick, McNurlan, & Caso, 1997; Wolfe, 1992). This method will be described more thoroughly in *section 1.4.4.1 Outcome criteria*.

Coming back to the issue of protein amounts and frequency, there is in contrast to some of the research above (Arnal et al., 2000; Arnal et al., 1999; Finkelstein & Fryer, 1971; Irwin & Feeley, 1967; Soeters, et al., 2009; Stote, et al., 2007) that state that differences in protein distribution throughout the day does not matter in terms of lean body mass and/or nitrogen balance more direct research, that assessed the muscle protein fractional synthetic rate (FSR), that muscle protein synthesis is in fact regulated on a meal to meal basis (Bohé, Low, Wolfe, & Rennie, 2003; Norton et al., 2007). In the study by Bohé et al. (2003), where the investigators intravenously infused human subjects AAs at four different ranges (either 43.5, 87, 162, 261 mg<sup>-1</sup>/kg<sup>-1</sup>/h<sup>-1</sup>) found that after an initial increase, MPS returned to baseline. This however despite the maintenance of AA levels, that were initially high enough to elicit a MPS response, yet were thus not able to maintain MPS (Bohé, et al., 2003). In the work by Norton et al. (2007) using a rodent model, the researchers came to similar conclusions that is ,in the case of hyper aminoacidemia (sustained elevated amino acid levels in the bloodstream) muscle protein synthesis became refractory (the cell was incapable of repeating another bout

of muscle protein synthesis). This has led some to suggest that protein distribution does make a difference in resistance trained populations (McDonald, 2007; Norton & Wilson, 2009).

#### **1.4 Problem statement**

It is clear at this point that scientific literature is in disregard whether protein distribution throughout the day makes a difference and whether there is an optimal amount of protein that can be ingested per meal. On one hand resistance trained athletes their selves (Andersen, et al., 1995) as well as several leading sports nutrition books advocate, to one degree or another, a high meal frequency (6 – 8 intakes per day), spacing protein intake out in these meals to render an optimal protein response. A notion that thus mainly stems from the (unsubstantiated) belief that only 20 to 30 g<sup>-1</sup> of protein can be utilised per meal (McDonald, 2007). On the other hand there is however research that suggests that protein distribution throughout the day does not matter based on several studies assessing nitrogen balance and/or lean body mass (Arnal, et al., 2000; Arnal, et al., 1999; Finkelstein & Fryer, 1971; Irwin & Feeley, 1967; Soeters, et al., 2009; Stote, et al., 2007). That at least based on their findings do not seem to confer with the belief that only 20 to 30 g<sup>-1</sup> can be utilised per meal.

To further complicate things there is at least one study done on resistance trained population that stated that a lower meal frequency of three meals might in fact be more beneficial in terms of lean body mass (Øyvind, Therese, & Truls, 2007). Using lean body mass gain in this particular study by Øyvind, et al. (2007) and/or nitrogen balance method of measurement in other studies (Arnal, et al., 2000; Arnal, et al., 1999; Finkelstein & Fryer, 1971; Irwin & Feeley, 1967; Soeters, et al., 2009; Stote, et al., 2007) may however have given skewed results (Kopple, 1987; McDonald, 2007; Waterlow, 1999). It recently has been suggested that a more feasible way of approaching the problem might be by more direct assessment of

muscle protein synthesis, namely the assessment of the muscle protein fractional synthetic rate (FSR) (La Bounty, et al., 2011). Moreover previous reviews regarding protein intake in resistance trained populations mostly tend to base the recommendations of protein intake for resistance trained athletes on nitrogen balance studies (Kreider et al., 2010; Phillips, 2004; Phillips, Moore, & Tang, 2007; Rodriguez, Di Marco, & Langley, 2009; Tipton & Wolfe, 2004) and this review is thus somewhat different from previous reviews to begin with. Recommended intakes from these studies generally range from 1.5 – 2 g<sup>-1</sup>/kg<sup>-1</sup> bodyweight/day for that matter.

Given all the above, this systematic review was set out to evaluate what evidence exists for the notion that only 20 to 30 g<sup>-1</sup> of protein can be utilised per meal, as evidence appears conflicting. The post-prandial period of nutrient intake in resistance trained populations will therefore be assessed using a more direct assessment of muscle protein synthesis, namely FSR. Furthermore systematically reviewing the evidence regarding how often a given intake per meal should be ingested during the day, thus the protein intake frequency was considered as well.

Based on scoping search of the literature a (strong) assessment of this latter notion (frequency) seems unfeasible at this time, simply because no specific literature on resistance trained populations exists assessing at which time point it would be beneficial to ingest another amount of protein. That is not to say there is not at least some literature to suggest that there is a refractory response (the cell is incapable of repeating an action in this case muscle protein synthesis) to protein intake (Anthony et al., 2002; Bohé, et al., 2003; el-Khoury et al., 1995; Norton, et al., 2007; Rennie, Bohe, & Wolfe, 2002), the research that was done on humans (who were also not engaged in resistance training for that matter)



(Bohé, et al., 2003; el-Khoury, et al., 1995; Rennie, et al., 2002) provides no real answer regarding when protein is best ingested again in resistance trained population. Moreover these studies have no real similarity in terms of their measurement, yet merely provide some room for speculation and arguably no appropriateness for systematic reviewing.

For this reason it was decided to investigate the actual amount of protein per meal that might stimulate muscle protein synthesis in resistance trained populations and moreover to provide an answer to whether this notion regarding 20 - 30 g<sup>-1</sup> seems justified, as this thus seems to be the major driver for high meal frequency in resistance trained athletes (McDonald, 2007; Norton & Wilson, 2009).

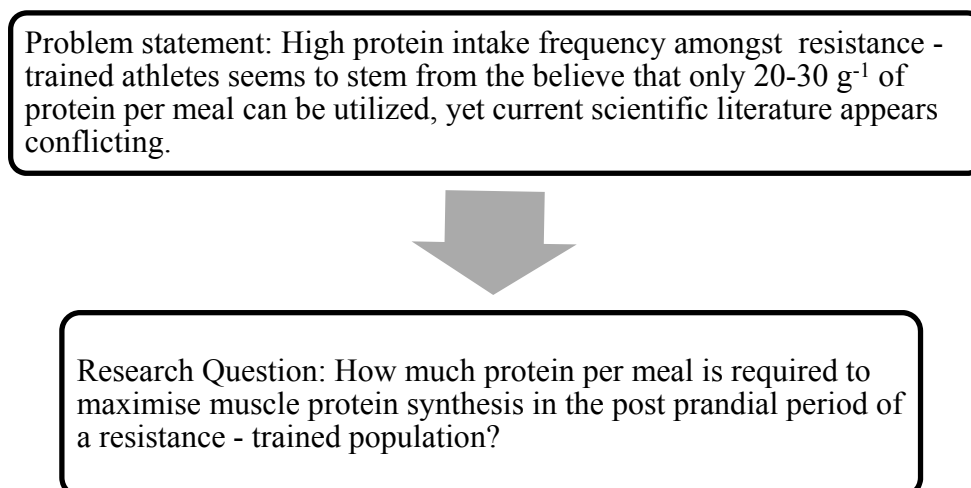
## Section 2: Review Question and Inclusion Criteria

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This chapter describes the review question and the inclusion criteria. It is important that inclusion criteria were set in advance to minimise bias related to the review process (Centre for Review and Dissemination, 2009).

### 2.1 Review question

This thesis is of explorative nature and the primary aim of the systematic review was to review the available evidence regarding of how much protein per meal is needed to maximally stimulate muscle protein synthesis in a resistance trained population. According to the Centre for Review and Dissemination (2009) ‘Systematic reviews should set clear questions’ ... and ... ‘ these should be stated clearly and precisely in the protocol’. In order to fulfil this aim the following problem statement and research question was addressed in hierarchical order (**figure 2.1.1**)



**Figure 2.1.1:** A schematic representation of the research question of the master thesis.

In essence this thesis thus deals with the straightforward question regarding protein intake and its role in maximising muscle protein synthesis in the post prandial period (the period after a meal).

Now that the main problem statement and the review question have been stated the next part addresses the predetermined inclusion criteria of the studies.

## **2.2 PICOS**

The review question and the inclusion criteria were further framed in terms of Population(s), Intervention(s), Comparator(s), Outcome(s) and Study design or in short PICOS (Centre for Review and Dissemination, 2009). Before this thesis goes into the specific inclusion criteria it must be noted that the inclusion criteria assessment was done based on scoping search of the literature and more specifically literature (reviews) related to meal frequency and protein intakes in resistance trained athletes (Kreider, et al., 2010; La Bounty, et al., 2011; Lemon & Proctor, 1991; Norton & Wilson, 2009; Phillips, 2004; Phillips, et al., 2007; Tipton, 2008; Tipton & Wolfe, 2004; Wolfe, 2000). This was done in order to make a realistic and reasonable assessment of how broad or strict the inclusion criteria related to the elements of PICOS were to be set.

### 2.2.1 Population

According to the Centre for Review and Dissemination (2009) the included population should be relevant to the population to which these findings will be applied, which in this thesis would be resistance trained athletes. However the authors also state that “in the absence of individual patient data (IPD) or very detailed reporting of data ” ...“ it is unlikely that inclusion can be restricted to particular types of participants (Centre for Review and Dissemination, 2009, p. 9). The following inclusion criterion regarding population was therefore set:

- Healthy adult subjects (18 – 64 years of age) either after an acute bout of resistance training and/or systematic involvement in resistance training (minimum of 3 days/week).

Given scoping research of existing reviews in the field however, that have been addressed in *section 2.2 PICOS*, there have been studies done using experienced (> 1 year of previous resistance training experience) resistance trained athletes (Kreider, et al., 2010; Phillips, et al., 2007; Tipton & Wolfe, 2004; Wolfe, 2000). The majority however did not assess muscle protein synthesis (see *section 1.4.4. Outcomes* for a full description of relevant outcomes) and setting the inclusion criteria too strict would likely exclude much relevant data.

For these reasons it was decided to keep the population criteria rather broad, which meant that studies regarding participants who were not previously engaged in resistance training, yet were investigated after an acute bout of resistance training were included in the review as well. This was done as literature seems to suggest that even after an acute bout of resistance training (with no previous resistance training experience), MPS is significantly higher (107 % as opposed to 50 %) and thus differs in response to the feeding of protein than

in the absence of resistance training when young healthy subjects were fed the same meal (340 grams of lean beef) (Symons, Sheffield-Moore, Mamerow, Wolfe, & Paddon-Jones, 2011). As similar effect is observed as well as in times of rest (e.g. the resistance trained group has higher MPS rated than the group who did not exercise) (Kim, Staron, & Phillips, 2005; S. M. Phillips et al., 2002).

Subjects not engaged in resistance training for that matter were excluded on the basis that in the absence of resistance training, there is simply no stimuli to maintain a higher net protein balance (synthesis exceeds breakdown) (Dunford & Doyle, 2007; Millward, Price, Pacy, & Halliday, 1991) and moreover as illustrated above will most likely lead to a different response as seen in subjects engaged in resistance training.

### **2.2.2 Interventions**

According to the Centre for Review and Dissemination (2009) the inclusion criteria regarding interventions should specify the precise nature of intervention (e.g the method of administration) as well as whether any co-interventions will affect the eligibility for inclusion. In looking at the actual amount protein intake that is needed to maximise muscle protein synthesis several relevant intervention methods have been accepted:

- Oral ingestion of protein as part of a mixed meal either one- or multiple following times during a day without any further co-interventions other than amino acid supplementation.
- Oral ingestion of protein in isolation one- or multiple following times during a day without any co-interventions other than amino acid supplementation and/or carbohydrate and/or fat supplementation.

- Oral ingestion of a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one- or multiple following times during a day without any further co-interventions other than macro nutritional (protein/ carbohydrate /fat) ones.
- Intravenous infusion of a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one time or multiple following times during a day without any further co-interventions other than macro nutritional (protein/ carbohydrate /fat).

In the case of stimulating muscle protein synthesis, the amino acid content of the meal, and more specifically the *relative* change in extracellular levels (levels in the bloodstream) of the branched chain amino acid (BCAA) leucine plays the primary stimulatory role of muscle protein synthesis (Blomstrand, Eliasson, Karlsson, & Köhnke, 2006; Kimball & Jefferson, 2006; Norton & Layman, 2006; Rennie, Bohe, Smith, Wackerhage, & Greenhaff, 2006), whereas a secondary, yet a far more minor role is played by the hormone insulin (Fukagawa et al., 1985; Grizard et al., 1999; Rennie, et al., 2002).

Although the inclusion of other macronutrients in measuring muscle protein synthesis response as is the case in a mixed meal brings up other factors (insulin) that can influence muscle protein synthesis however the presence of other macronutrients namely carbohydrate and fats does not seem to be a major issue. First of all only small amounts of insulin (approximately  $70\text{-}140\text{ pmol}^{-1}\text{ /l}^{-1}$  plasma) are needed for stimulation of protein synthesis and its role seems to be permissive rather than modulatory (Rennie, et al., 2002), which means that while insulin is necessary, large(r) increases in insulin appear to have no further effect on muscle protein synthesis.

More importantly while insulin is mostly associated with the macronutrient carbohydrate, these amounts of insulin necessary to maximally stimulate muscle protein synthesis

(approximately  $70\text{-}140\text{ pmol}^{-1}\text{ /l}^{-1}$  plasma) would be secreted as a result of oral protein intake and oral administration of free form amino acids (such as leucine) to begin with (Floyd, Fajans, Conn, Knopf, & Rull, 1966). For instance Holt et al. (1997) found that the consumption of a popular protein-rich food such as beef steak ( $158\text{ g}^{-1}$ ) to cause an insulin response that would lead to plasma insulin concentrations of  $7910 \pm 2193\text{ pmol}^{-1}\text{ /l}^{-1}$  (for other protein rich foods and its insulin response is referred to the paper by Holt et al. (1997)) which is thus in far excess of the suggested  $70\text{-}140\text{ pmol}^{-1}\text{ /l}^{-1}$  plasma insulin levels that are suggested to be needed to maximally stimulate muscle protein synthesis from the perspective of this hormone (Rennie, et al., 2002). For this reason the administration of macronutrients other than protein does not seem to affect the muscle protein synthetic response to a degree that this is an issue. Therefore studies containing co-interventions related to altering other nutrients as well, thus ingesting protein amounts as part of a mixed meal, were therefore included.

For the same main reason as was described above, studies feeding its subjects protein in isolation (thus protein only) will lead to the insulin secretion that is necessary to maximally stimulate muscle protein synthesis from insulin's standpoint, were accepted. Moreover it is for this reasoning that studies providing oral AA administration were accepted as well.

Another reason why free from amino acid intake was accepted for inclusion criteria is related to the fact that this even when administered in free form (thus not as a whole protein) they still stimulates muscle protein synthesis (Rennie, et al., 2006; Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999) and can provide relevant information regarding different amount of actual protein and their stimulation of muscle protein synthesis (McDonald, 2007; Norton & Wilson, 2009). Research suggests that for stimulation of muscle protein synthesis to take place with amino acid ingestion, at least all eight essential amino acids need to be present and not for instance just the amino acid leucine (Rennie, et al., 2006). Moreover while all EEAAs

are needed to those stimulate muscle protein synthesis, the other (non-essential and/or conditionally essential) amino acids appear to have no direct influence on protein synthesis (Rennie, et al., 2006). For these reasons only studies that administered an oral amino acid solution containing *at least* all EEAs were set eligible for inclusion and consequently studies that only administered the amino acid leucine or BCAAs were excluded.

Another intervention that, based on scoping search of the literature is sometimes used in studies, is intravenous AA infusion (Bohé, et al., 2003; Rennie, et al., 2002). While its applicability to consumption of meals may be questioned since the digestive processes and hormonal responses that occur with eating obviously do not take place, this is not the focus of this thesis and it appears muscle protein synthesis reacts to intravenous AA infusion in a similar way that it would to the consumption of protein in meal form (Rennie, et al., 2006). Given its potential to provide relevant information this type of intervention was included as well.

All nutritional interventions that have co-interventions of supplements other than AAs (or AAs studies that that have any other co-interventions besides nutritional ones) as well as any form of anabolic steroids or other performance enhancing drugs were to be excluded. For instance a supplement like creatine may have direct effects on muscle protein synthesis (Volek et al., 1999). The same goes for anabolic steroids as they are suggested in multiple literature reviews to directly influence muscle protein synthesis (Kuhn, 2002; Wilson, 1996). Needless to say this makes it more difficult to assess if the muscle protein synthetic response was the result from nutritional intervention or the result from the administration of a particular supplement and/or drug.

Co-interventions of AAs and nutritional interventions, that is both whole foods and free form amino acid supplementation were administered simultaneously, are accepted as well since



they have been suggested to stimulate muscle protein synthesis in the same way. Meaning that when whole food proteins already maximally stimulate muscle protein synthesis extra free form AA supplementation is not likely to stimulate muscle protein synthesis any further and vice versa (Verhoeven et al., 2009).

### **2.2.3 Comparators criteria**

Comparators or comparative studies for that matter in medical science can best be defined as a study where two or more types of treatments or interventions are compared to each other (Centre for Review and Dissemination, 2009). This section is thus largely an extent to the previous section namely *2.2.2 Interventions*, yet describes the inclusion criteria that were set in terms of comparators in these studies.

In terms of amounts the following inclusion criteria regarding comparison were set (and these are the thus largely an extent to the inclusion criteria related to *section 1.4.2 Interventions*):

- Comparison of any different amounts of oral protein administration as part of a mixed meal either one- or multiple following times during a day without any further co-interventions other than amino acid supplementation.
- Comparison of any different amounts of oral protein administration in isolation one - or multiple following times during a day without any co-interventions other than amino acid supplementation.
- Comparison of any different amounts of a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one- or multiple following times during a day without any further co-interventions other than macro nutritional ones.
- Comparison of any different amounts of intravenous infusion a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one- or multiple

following times during a day without any further co-interventions other than macro nutritional ones.

Comparing different amounts of protein and/or free from AAs is the most obvious and moreover the most feasible way to assess effect on muscle protein synthesis based on scoping search (Kreider, et al., 2010; Lemon & Proctor, 1991; McDonald, 2007; Norton & Wilson, 2009; Phillips, 2004; Phillips, et al., 2007; Tipton, 2008; Tipton & Wolfe, 2004) and the comparators described as above were included for this reason.

#### **2.2.4 Outcomes**

This section describes a clearly defined set of relevant outcomes and their justification (Centre for Review and Dissemination, 2009).

The following relevant outcome criterion was accepted for inclusion:

- Assessment of mixed muscle protein fractional synthetic rate (FSR) in the post-prandial period through the use of muscle biopsy samples using the infusion of stable isotopes of the amino acid(s).

Only when the study met the inclusion criterion above, the following surrogate outcomes were also accepted:

- Measurement of activity of one or more of, to the mTORC1 pathway related factors through the use of antibodies (immunoblotting):
  - Eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1)
  - Eukaryotic translation initiation factor 4 gamma (eIF4G)
  - Ribosomal protein S6 kinase (p70<sup>S6K</sup>)
  - Ribosomal protein s6 (rpS6)

## **Muscle protein fractional synthetic rate**

The general procedure for measuring muscle protein synthesis begins first of all the injection of a particular amino acid or several amino acids labelled with the chosen isotope into the bloodstream. To now determine the rate of protein synthesis several measurements have to be taken. First of all a tissue sample using a muscle biopsy is taken from the subject and this sample is tested for the enrichment of the specific labelled amino acid in tissue protein ( $E_p$ ). Second the mean intracellular muscle free enrichment of the amino acid must be determined ( $E_t$ ). Third the tracer incorporation time must be determined ( $t$ ). The rate of protein synthesis (e.g. the percentage change of renewed protein tissue in a given time span) can then be calculated with, amongst others, the following equation (Garlick, et al., 1997):

$$\text{FSR} = (E_p \times 100) / (E_t \times t)$$

or alternatively when two samples are taken

$$\text{FSR} = ([E_{p1} - E_{p2} \text{ or } \Delta E_p] \times 100) / (E_t \times t)$$

and so on.

This method of assessing protein synthesis is considered reliable and valid (Burd et al., 2011; Garlick, et al., 1997; Toffolo et al., 2003) and changes in muscle protein FSR was therefore accepted as the main outcome. FSR for that matter is expressed in % change per hour.

## Measuring mTOR activity

The mTOR (Mammalian Target of Rapamycin) system is a serine/threonine protein kinase that consists of two complexes with distinct involvements. Namely the rapamycin-sensitive complex mTORC1 (that will have focus in this thesis), which is amongst other things, is involved in protein synthesis (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009; Liu, Jahn, Wei, Long, & Barrett, 2002; Wu, 2009), and the rapamycin-insensitive complex mTORC2, which main involvements include cell proliferation, differentiation, migration, and cytoskeletal reorganization (Sarbasov, Guertin, Ali, & Sabatini, 2005; Wu, 2009).

As mentioned in *section 2.2.2 Intervention criteria* extracellular changes in plasma levels of the amino acid leucine is the main stimulator of muscle protein synthesis (Bohé, et al., 2003; Norton & Layman, 2006). It has been suggested that it mostly does so by stimulating mTORC1 (who in its turn activates its two main targets the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) and the phosphorylation of ribosomal protein p70s6 kinase (p70<sup>S6K</sup>) and it turn its target substrate of ribosomal protein s6 (rpS6) (Drummond, et al., 2009; Norton & Layman, 2006; Wu, 2009)) and to some extent independently trough the activation of eukaryotic translation initiation factor 4 gamma (eIF4G) (Bolster, Vary, Kimball, & Jefferson, 2004; Crozier, Kimball, Emmert, Anthony, & Jefferson, 2005). See **figure 2.4.1** on the next page.

The eukaryotic translation initiation factors eIF4G and (eIF4E)-binding protein 1 (4EBP1) for that matter are proteins involved transcribing coding information from DNA to the sites of the ribosomes (Hay & Sonenberg, 2004). The aforementioned ribosomes such as rpS6 in turn create protein from amino acids and DNA coding (Alberts et al., 2002).

**Figure 2.4.1:** Graphical overview of the metabolic effects of leucine and its pathways involved. Leucine concentrations influence protein synthesis, through activation of initiation factors eIF4E and eIF4G and the phosphorylation of ribosomal protein p70s6 kinase (p70<sup>S6K</sup>) and it turn its target substrate of ribosomal protein s6 (rpS6)

Adapted and edited from Norton, L. E., Layman, P. Garlick, D. Brana, T. G. Anthony, Zhao, L., et al. (2007). Translational controls of muscle protein synthesis are delayed and prolonged associated with ingestion of a complete meal *FASEB J*, 21. Retrieved from <http://www.fasebj.org/>

## 2.5 Study design

It has been established that the types of studies included in the review will play a major role in determining the reliability as well as the validity of the estimates (Centre for Review and Dissemination, 2009) and while certain study designs are clearly more robust than others (see **table 2.5** for an hierarchal overview of study types) if based on scoping search of the literature there are likely to only be a limited amount of randomised controlled trials, other study designs may be accepted as well (Centre for Review and Dissemination, 2009; Margaliot & Chung, 2007). The following study designs have been found eligible for inclusion:

- Randomised controlled trials (RCTs) and more specifically:
  - Randomised crossover trials
  - Cluster randomised trials

Based on scoping search of the literature there are enough randomised controlled trials (almost all work done on nutrient provision and muscle protein synthesis are RCTs to begin with, based on the scoping search) to address the issue of the amount of protein to maximise muscle protein synthesis. RCTs in specific have been chosen for systematic reviewing since they are considered to provide the highest levels of evidence (Centre for Review and Dissemination, 2009; Margaliot & Chung, 2007) (see **table 2.5.1** on the next page for a hierarchal overview of study designs) as well as minimise the risk for bias (Centre for Review and Dissemination, 2009; Margaliot & Chung, 2007).

**Table 2.5.1 Evidence-based Medicine’s Level of Evidence: Highest to Lowest**

Level of Evidence	Definition
1A	Systematic reviews of RCTs
1B	Individual RCT
2A	Systematic review of cohort studies
2B	Individual cohort study, low-quality RCT
2C	Ecological study
3A	Systematic review case-control studies
3B	Individual case-control study
4	Case series, poor-quality cohort and case-control studies

Adapted from Patsopoulos, N. A., Analatos, A. A., & Ioannidis, J. P. A. (2005). Relative Citation Impact of Various Study Designs in the Health Sciences. *JAMA: The Journal of the American Medical Association*, 293(19), 2362-2366.

Whereas bias in this case can be referred to as the ‘systematic deviations from the true underlying effect brought about by poor study design or conduct in the collection, analysis, interpretation, publication or review of data’ (Centre for Review and Dissemination, 2009, p.34). To minimise this possibility of not finding the true effect, only RCTs were to be included. The types of RCTs that were to be accepted are randomised crossover trials (where all subjects receive all the interventions) and cluster randomised trials (where clusters of subjects rather than single individuals are randomised to different interventions) (Centre for Review and Dissemination, 2009). For a full description of the two aforementioned types randomised controlled trials that have been accepted in this systematic review, it is referred to the Centre for Review and Dissemination (2009).

## **Section 3: The Systematic Review**

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The purpose of this chapter is to provide the reader with a basic understanding of a systematic review as well as to serve the reader with some of the features that set systematic reviews apart from other type of reviews. In addition several types of biases will be defined as well as their respective steps that have been taken to minimize the possibility of their occurrence. This is then followed by a description of the stepwise process of conducting a systematic review.

### **3.1 Systematic reviews**

Reviews are considered one of the most useful methods to summarize existing literature, attain unambiguous conclusions and develop best practice and policy in maze of different studies that can come to apparently conflicting conclusions (Albanese & Norcini, 2002; Centre for Review and Dissemination, 2009; Jones & Evans, 2000; Tranfield, Denyer, & Smart, 2003). Yet in the absence of standardization and transparency in the review process, reviews of the literature can become subjective and reflect the implicit biases of the reviewer(s) and not necessarily form a reliable representation of the literature in a given field (Albanese & Norcini, 2002; Centre for Review and Dissemination, 2009).

The review protocol is one of the distinct feature that sets a systematic review apart from ‘traditional’(narrative) reviews, where the inclusion or exclusion of studies as well as the appraisal of their quality are thus largely dependent on the author(s) interest or bias and are not considered a validated method of reviewing (Albanese & Norcini, 2002; Deeks, 1998). This led several authors arguing that systematic reviews are advantageous in respect to



traditional reviews (Albanese & Norcini, 2002; Jones & Evans, 2000; Margalot & Chung, 2007; Tranfield, Denyer, & Smart, 2003).

Described below are some of the more distinct features as to why the process of a systematic review in this thesis was favoured and chosen over other more 'traditional' or narrative reviews with

- The actual review process of the systematic review consists of a strict set of steps concerning reviewing that are designed *a priori* to be transparent and of reproducible nature, while the process of a 'traditional review' would be far more subjective and dependent upon the author's opinions or previous beliefs (Albanese & Norcini, 2002; Jones & Evans, 2000; Margalot & Chung, 2007). A type of bias that is more commonly known as reviewer bias (Deeks, 1998)
- The systematic review strives for the inclusion of all relevant data, while the inclusion of relevant data in the case of a traditional review could be dependent on the above mentioned interest and biases of the author (Margalot & Chung, 2007).
- Systematic reviews are more commonly cited than all other types of study designs, including randomized controlled trials and decision analyses, and continue to be cited many years after publication (Patsopoulos, Analatos, & Ioannidis, 2005).
- The systematic review addresses a specific topic or question (in this case MPS in the response to feeding), while traditional reviews often address a broad clinical topic (Jones & Evans, 2000).

- The systematic reviews employs and specify that hand search of some high yield journals was done (see *section 4.2 Resources searched*), thereby recognizing the limitations of computer searches using key word strings (Albanese & Norcini, 2002). Haig (2001) for instance found that the sensitivity of a computer search as opposed to hand search ranged from 6.5 % in a computer search to 61.3 % in a hand search, whereas computer search sensitivity was defined as total number of relevant studies retrieved by computer search and hand search sensitivity was defined as total number of studies retrieved by hand search.
- While traditional reviews may specify inclusion criteria similar to systematic reviews, the systematic review (B.3, appendix B) will also include a bibliography of articles that were excluded from the review and the reasons as to why they were rejected (Albanese & Norcini, 2002).
- Systematic reviews, unlike traditional reviews, require a protocol. This comprehends a summary sheet on which data from each of the included studies is recorded in the systematic review (Appendix C) (Albanese & Norcini, 2002).
- When looking at primary studies (the studies included in systematic review) it is possible to synthesize and combine results from different studies into a meta-analysis (Glass, 1976). Meta-analysis provides the opportunity to statistically combine results from different primary studies that are compatible with regards to their quality level. This may highlight different facts that individual studies do not reveal when looked at in solitary. I.e. the by-the-meta-analyses-synthesized effect is more likely to find meaningful related effects, or on the other end of the spectrum differential effects,

than less systematic approaches such as traditional reviews (Egger & Smith, 1997; Lipsey & Wilson, 2000; Tranfield, et al., 2003). It must be noted however that given the large differences in terms of characteristics, methods of administration, population studies it was anticipated that a meta-analysis was not possible in this systematic review.

The systematic reviews like any other review also possesses several limitations and below are some of the more general limitations as well some that are specifically recognized with respect this systematic review in particular.

- The quality of a systematic review is partly dependent of the quality and the availability of the primary studies available in the literature (Margalioth & Chung, 2007). While the large degree of variation of study design, methods of reporting, external validity etc. amongst different studies can to some extent be overcome with decent study appraisal techniques, there is always the possibility to find variable or no evident results from a systematic review, simply because the studies vary to such a great degree in the field. Given the relative immaturity of the field of nutrition intake and resistance trained athletes (McDonald, 2007) this limitation was recognized when commencing this systematic review. In addition there is the possibility that when a review contains studies that are biased, the final conclusion of the review will be biased as well, although good quality assessment techniques can to some degree overcome this issue (Deeks, 1998). See *section 5.1 Tools for assessing quality* for further explanation of the quality assessment techniques.

- Systematic reviews preferably require at least two or more reviewers (Albanese & Norcini, 2002). While normally a distinct advantage as opposed to a traditional literature reviews, the thesis only has one author and poses a limitation of this review.
- A problem faced by any type of review is that abstracts can be misleading and in some cases even provide conclusions that are conflicting with those in the main article itself (Albanese & Norcini, 2002). Given the reliance of reviews on the abstract for initial screening this may pose issues with inclusion. While in recent years journals have begun to standardize and regulate formats of abstracts to a greater degree, this in order to provide a more representative overview of the contents of the main article in the abstract, older articles (before the year 2000) must be approached with consideration (Albanese & Norcini, 2002). The systematic review tried to overcome this problem to read to retrieve the entire article and thoroughly read the conclusions as well.
- Despite the transparent and systematic fashion of this review, this in order to minimize bias and error, a problem facing any review is several biases in literature search and inclusion (**table 3.1.1** on the next page) (Centre for Reviews and Dissemination, 2009; Jones & Evans, 2000; Margalio & Chung, 2007)). Since the majority of reviews are dependent on published articles, one of the more common biases is publication bias (Jones & Evans, 2000; Margalio & Chung, 2007; Montori & Guyatt, 2002). This bias has been described by Montori & Guyatt (2002) as when the publication of studies ‘depends on the direction of the study results and whether they are statistically significant’ (p.163). For instance it has been suggested that medical studies, that found certain interventions to be ineffective, have not been published for this reason (Montori & Guyatt, 2002). Moreover it has been suggested

by several authors that the odds that a clinical trial, observational study or laboratory-based experiment with statistically significant results will be published have been found three to eight times greater than studies with statistically insignificant results, despite being found to be of equal quality (Dickersin & Min, 1993; Easterbrook, Gopalan, Berlin, & Matthews, 1991). Montory & Guyatt (2002) propose two solutions to deal with this problem. The first one being a comprehensive search for unpublished studies in conference proceedings, correspondence with experts and search of clinical trial registries, which is in line with recommendations from the Centre for a Review and Dissemination (2009), that also recommends the search of what they describe as 'grey literature'. This includes the aforementioned conference proceedings, clinical trial registries as well as records of on-going research (Centre for a Review and Dissemination, 2009). It must be noted however that the search for unpublished papers is recognised as difficult (Centre for Review and Dissemination, 2009). Another solution proposed by these authors is prospective trial registration in a central database (such as the Cochrane Central registry), where they will remain available irrespective of publication status and direction of outcome (Montori & Guyatt, 2002). To overcome the problems mentioned above grey literature search, as described in A.3, *appendix A*, has been performed.

A type of bias related to the above is citation bias, where studies with statistically significant and/or positive results are cited more frequently than studies that did not found favourable and/or significant results (Jones & Evans, 2000; Margalio & Chung, 2007; Montori & Guyatt, 2002). This poses another potential issue and the solution for this problem is largely in agreement with the solution to publication bias, that was described above (Montori & Guyatt, 2002).

Another type of bias that is threatening to inclusion of all relevant data is English-language bias (Margalio & Chung, 2007). This occurs when reviewers only include studies published in English-language, usually to simplify the search and inclusion process. Moher et al. (1996) argued that this type of bias may exclude 20 to 50 % of relevant clinical trials in foreign language, which are considered of equal quality with respect to their English counterparts. A possible solution for this is to translate search terms into other commonly used languages such as German, Portuguese, Russian and/or Spanish although a professional interpreter of foreign languages is needed and the practicality of this process is debatable.

Another type of bias related to this is Database bias, where primarily North American and West-European journals are searched and consequently several journals that are published in other languages and parts of the world are left unsearched (Margalio & Chung, 2007). The solution described in respect to language bias is likely to deal with this problem as well, yet as mentioned practicality might be an issue.

**Table 3.1** Common types of Biases in Literature Selection

Publication Bias	Selective submission and publication of studies with statistical significance and/or positive results (Dickerson & Min, 1993).
Citation Bias	Studies with statistical significance and/or favourable findings are cited much more often than studies with negative and/or nonsignificant findings (Moher et al., 1996).
English-language bias	The exclusion of papers published in other languages than the English on to simplify search, retrieval process and practicality issues related to inclusion of studies of non-English languages (Moher et al., 1996).
Database bias	The primarily search for relevant data in North-American and West-European Journals while other (relevant) journals elsewhere in the world are left unsearched (Moher et al., 1996).

In order to, to some extent overcome the problem of English Language bias and Database bias, the author translated the search terms into the German and Dutch language, this could be done given the authors knowledge of those languages. The search terms are described in *A.1, appendix A*.

Now that some basic understanding, several distinct features that set systematic reviews apart from other reviews as well as a limitations related to the systematic review have been highlighted, the process of designing and conducting the systematic review will be discussed in the next section.

### **3.2 The review protocol**

The review protocol serves as the foundation of any systematic review (Jones & Evans, 2000; Margalot & Chung, 2007) and a typical systematic review consists of the following key areas (Centre for Review and Dissemination, 2009):.

- Background
- Review question and inclusion criteria
- Identifying research evidence
- Study selection
- Data extraction
- Quality assessment
- Data synthesis

### **3.2.1. Background**

For the description of this section and its related contents the reader is referred to the previously described *section 1.2 Background*.

### **3.2.2 Review question and inclusion criteria**

For the description of this section and its related contents the reader is referred to the previously described *Section 2.1 Review question and inclusion criteria*.

### **3.2.3 Identifying research evidence, study selection, data extraction, quality assessment, data synthesis**

The next sections will in turn describe the identification of research evidence and the processes of study selection, data extraction, quality assessment and data synthesis.



## **Section 4: Identifying Research Evidence and Data Extraction**

This section will elaborate on the preliminary search strategy that was developed to identify the relevant research as well to elaborate on the process of developing data extraction techniques (Centre for Review and Dissemination, 2009; Jones & Evans, 2000 Margalio & Chung, 2007). The next part will specify the search strategy and the search terms that were used as well as the databases of additional sources that have been searched.

### **4.1 Search strategy**

The search strategy has been developed to take into account PICOS. Thus the search terms (*A.1, appendix A*) have been constructed to capture the review question in terms of its accepted population(s), intervention(s), comparator(s), outcome(s) and study design(s) (see *Section 2 Review question and inclusion criteria* for a full description of the inclusion criteria). Based on scoping search of the literature search terms were developed that included a range of text words and their synonyms, commonly found in the abstract and the titles of the relevant study. Furthermore it also included subject indexing terms that were commonly found on the bottom of abstracts. In addition these search terms were combined using the Boolean search strings ‘AND’ and ‘OR’ to make for more complete search strings, this to ensure further relevant studies were identified (Aho & Corasick, 1975; Frants, Shapiro, Taksa, & Voiskunskii, 1999).

While it was recognised in *section 3 The Systematic Review* that English-language bias as well as Database bias (the preliminary search in mostly North American and European (English-based) journals) might exclude relevant studies, practicality related to searching in other languages is an issue (Margalio & Chung, 2007). Yet as mentioned previously, given

the author's knowledge of the German and Dutch language the in the English language specified search terms were also translated and employed in those languages, in order to, to some extent overcome this problem (*see A.1, appendix A* for a full description of the search terms).

#### **4.2 Resources searched**

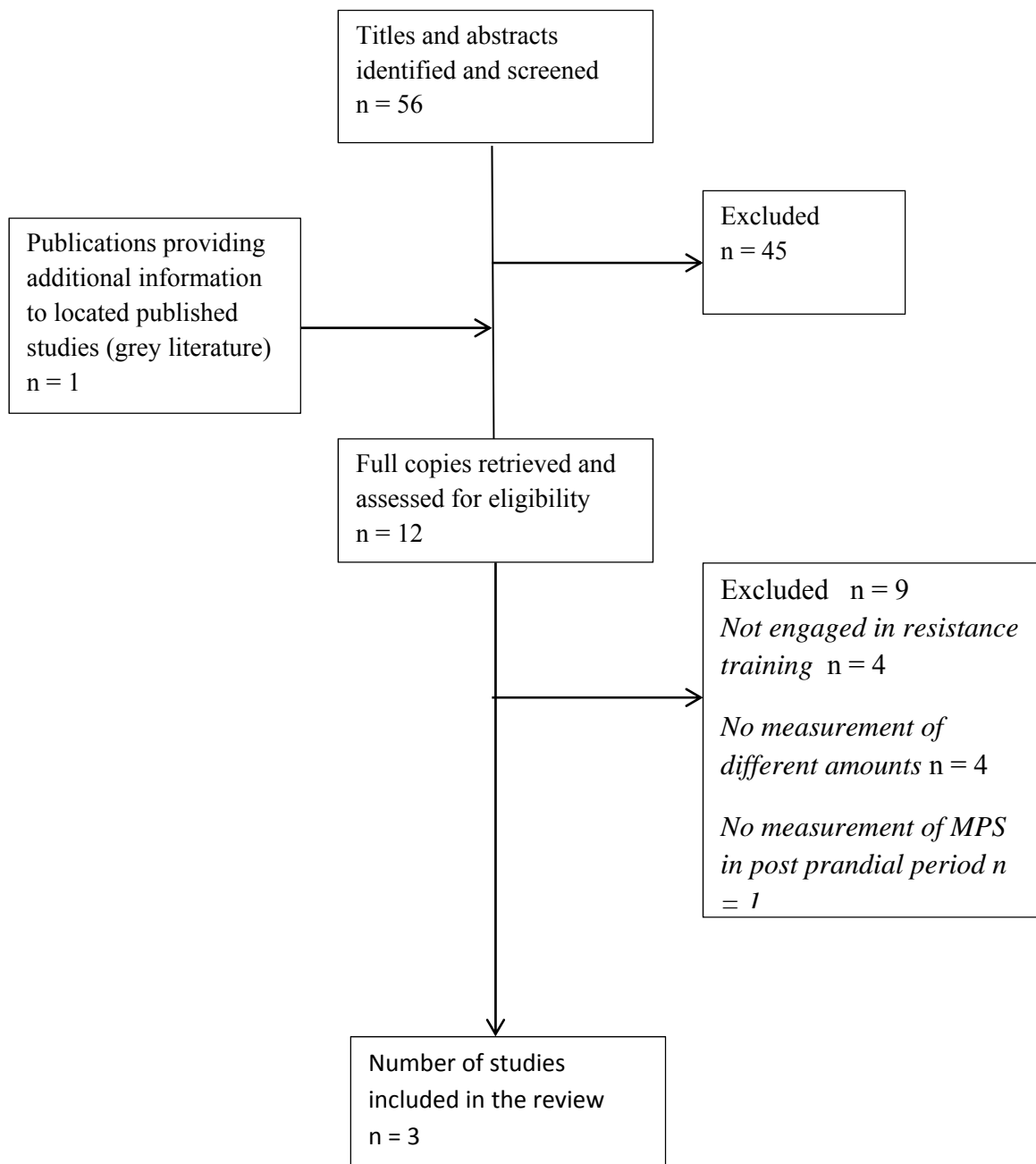
The following databases were search for relevant studies from the period 1980 - present (*A.3.2.1, appendix A*): Google Scholar; PubMed; ScienceDirect provided by Elsevier B.V.; Springer Link; The Cochrane Library. In addition the following journals were hand searched from the period January 2000 - September 2011: American Journal of Physiology-Endocrinology and Metabolism; The American Journal of Clinical Nutrition; The British Journal of Nutrition; The Journal of Nutrition (*A.3.2.1, appendix A*). Given that there is always a risk that relevant primary research is overlooked by electronic database searching (Centre for Review and Dissemination, 2009; Jones & Evans, 2000 Margaliot & Chung, 2007) a range of steps had been taken to minimize the possibility of overlooking possible potential primary research:

The reference lists of included papers were scanned for relevant studies in order to further identify relevant studies could have been missed by the search strings. The following website was searched The 'SuppVersity - Nutrition and Exercise Science for Everyone' website (<http://suppversity.blogspot.com/>) as well as the following conference proceedings (Phillips, 2011; Tipton, 2011). Citation searches in Google Scholar were carried out for papers citing the recent key paper by Norton & Wilson (2009). Finally two recent key book publications were searched for further possible relevant literature (Di Pasquale, 2008; McDonald, 2007) The full strategies are shown in *Appendix A*.

### 4.3 Study selection

The aim of study selection is to identify all relevant primary research and it is important that the selection process minimizes biases, which could occur when the decision to include or exclude studies is affected by pre-formed opinions (Cooper & Ribble, 1989; Oxman & Guyatt, 1993; Slavin, 1995). Given that systematic review was conducted by a single reviewer (thesis author) this is a reasonable threat to the systematic review. However strictly predefined exclusion criteria can to some extent overcome this threat (Centre for Review and Dissemination, 2009).

As was described in *section 4.1 Search strategy* several search methods have been conducted, yet the initial search process commenced with electronic database searching, which were hereafter screened against the inclusion criteria (see *A.4, appendix A*). Initial search of the electronic databases retrieved a total of 56 studies that were selected for further viewing (**figure 4.3.1** on the next page). After careful reading of the titles and abstracts, another 44 studies were rejected based on not meeting the inclusion criteria on first appearance (*A.1 Inclusion criteria*) or (since there was great overlap of studies retrieved) they appeared to be already retrieved in an earlier stadium of the search process (i.e. the different databases were searched on different dates). After full scale reading of the 11 primary studies (see *B.1, Appendix B*), three studies met the inclusion criteria and were decided to be included as primary research. The rationale of excluding the other studies can be found in (*B.3, Appendix B*). Moreover one more study (Fern, et al., 1991) was retrieved by scanning ‘grey literature’ and more specifically the paper was found in a recent key publication by Di Pasquale (2008), yet was rejected after full text retrieval.



**Figure 4.3.1:** Flow chart of study selection process

Once the studies, that met the inclusion criteria were identified, data was extracted using a predefined approach (Centre for Review and Dissemination, 2009). This will be discussed in the next section.

#### **4.4 Data extraction**

The process of data extraction has the purpose of obtaining all necessary information regarding study characteristics and findings from the included studies (Centre for Review and Dissemination, 2009). Given that this can be a subjective process and can be prone to errors it is important that this is done in a systematic and reproducible manner, namely through use of a data extraction form (Jones & Evans, 2000; Margalot & Chung, 2007; Tranfield, et al., 2003). It has been suggested that standardised data extraction forms can provide consistency, reduce bias and improve validity and reliability in the process of data extraction (Higgins & Deeks, 2011).

The design of the form is of key value when extracting correct data and it is therefore important that the data extraction form is tailored specifically towards the review question (Centre for Review and Dissemination, 2009). The content of the data extraction form was therefore developed to have a direct link with the pre-determined population-, intervention-, comparators-, outcomes- and study design-criteria in order to obtain the necessary information related to the elements of PICOS (Centre for Review and Dissemination, 2009). The data extraction form furthermore contained general information related to the publication title, author(s), publication date, the search string that retrieved the study as well as the database the study was retrieved from (Centre for Review and Dissemination, 2009).

Another important aspect related to the design of the data extraction form is that the form should be ‘unambiguous and easy to use in order to minimize discrepancies’ (Centre for Review and Dissemination, 2009). As is apparent from the data extraction form (C.1, appendix C) a combination of data extraction methods have been used, namely, numerical methods, fixed test (yes/no), a pick list and free text. The free text fields were mostly used to briefly report on a characteristic or outcome, this in order to simplify the extraction of data as much as possible (Centre for Review and Dissemination, 2009).

The data extraction form thus aided in convenient and straightforward extraction of information, the main outcomes and statistical methods used. Similar extraction of outcome measurement and statistical methods of each of the individual studies moreover adds to the validity of the findings (Centre for Review and Dissemination, 2009). In addition once the relevant information was extracted from the relevant primary research, it could later on be used in the process of data synthesis, this to get a clear and overall view of the amount of protein needed to maximise muscle protein synthesis in resistance trained athletes. Besides the main aim of making the process of being extraction objective, the data extraction form can potentially also serve as a starting point for other professionals seeking similar information as well as aid in the replication process of a systematic review (Centre for Review and Dissemination, 2009).

The actual process of data extraction was done between the 1st and 3th of October 2011. It has been suggested by some that in order to keep the process of data extraction as unbiased and reliable is possible, the person(s) extracting the data should be blinded to the journal and author details (Jadad et al., 1996; Sacks, Berrier, Reitman, Ancona-Berk, & Chalmers, 1987). This, however, is a time-consuming operation which may not alter the results of the review (Berlin, 1997) and its value is therefore questionable. In addition the author of this thesis is a

novice in the field and has no direct affiliation with the several authors nor has any (economic) interest in the results of this thesis.

Once the relevant information and data was extracted from the three studies, the papers were addressed for its quality using a predefined validated approach in the next section.

## **Section 5: Quality Assessment and Data Synthesis**

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As was previously mentioned in section 2.5 *Study design*, there is always the possibility in scientific research of not finding the true effects of the study, i.e. bias. (Centre for Review and Dissemination, 2009; Jones & Evans, 2000; Margalio & Chung, 2007). It is for this reason important that individual quality of the relevant primary studies is assessed (Centre for Review and Dissemination, 2009; Jones & Evans, 2000; Margalio & Chung, 2007). Recording strengths and weaknesses of included studies in a systematic and validated manner provides the researcher with an indication whether flaws in the design or conduct of the study, could possibly have resulted in bias (Jones & Evans, 2000; Margalio & Chung, 2007; Tranfield, et al., 2003). In essence quality assessment of primary studies can be considered an investigation into the extent to which the study results can be ‘believed’ (Centre for Review and Dissemination, 2009).

Quality for that matter is a complex concept and in the context of systematic reviewing refers to the following (Centre for Review and Dissemination, 2009):

- Appropriateness of study design to the research objective
- Risk of bias (e.g. not finding the ‘true’ effect)
- Other issues related to study quality
  - Choice of outcome measure
  - Statistical issues
  - Quality of reporting
  - Quality of intervention
  - Generalizability



It has been suggested that the importance of each aspects of quality depend on the focus and the nature of the review (Centre for Review and Dissemination, 2009). The individual assessment of 'aspect-importance' is moreover considered vital for another reason, namely in considering the tools used for assessing quality (Centre for Review and Dissemination, 2009; Juni, Witschi, Bloch & Egger, 1999). The relative importance of each of these aspects will be addressed with the appropriate study quality assessment technique in the next sections, yet first a rationale will be provided for the quality assessment technique.

### **5.1 Tools for assessing quality**

In general there are two main approaches for assessing quality of studies; one involves the use of checklists (Deeks et al., 2003) and one involves the use of scales, that provide an overall numerical quality score for included studies (Moher et al., 1995).

The use of scales however has been critiqued in scientific literature for the fact that they often have not been developed or tested with the use of standard techniques to establish validity and reliability (Jüni, Witschi, Bloch, & Egger, 1999; Moher, et al., 1995; Olivo et al., 2008). Moreover in at least two systematic reviews, were they were assessed for their potential to produce valid and reliable results in different settings, it became clear that the weighting assigned to several methodological items such the concealment of treatment allocation, blinding of outcome assessment, and handling of withdrawals and dropouts varied considerably (Jüni, et al., 1999; Olivo, et al., 2008). Consequently with some of the scales certain studies were identified as high-quality whereas in other scales they would be identified as low quality (Jüni, et al., 1999) and conclusively only the Jadad-Scale, the Delphi-Scale and the Yates-Scale have been deemed reliable (Olivo, et al., 2008). Their use

in systematic reviews remains debated nevertheless as their influence on the outcome on quality assessment remains unclear (Balk et al., 2002; Herbison, Hay-Smith, & Gillespie, 2006; Jüni, et al., 1999; Verhagen et al., 2000) and more research seems needed whether the use of scales seems justified.

The use of checklists on the other hand has been suggested to be a more feasible and reliable way of ensuring that the studies are assessed of quality in a standardised way (Higgins & Deeks, 2011; Jüni, et al., 1999; Moher, et al., 1995). There are many checklists available for the assessment of study quality; in a previous systematic review nearly 200 checklist were identified yet only six were deemed to be suitable and effective for the use in systematic reviews (Deeks, et al., 2003). The next section will briefly discuss these six checklists and will try to make an assessment of its suitability for this particular systematic review as the implications of the quality assessment for interpreting results should be explicitly considered (Centre for Review and Dissimination, 2008) and moreover should be assessed *a priori* (Olivo, et al., 2008).

## **5.2 The checklists used for quality assessment**

The six potential relevant lists are now discussed with regards to their applicability of this systematic review.

### **Cowley**

This scale was originally developed as a quality assessment tool for a systematic review concerning the use of hip prostheses (Cowley, 1995). This tool provides a list of 13 questions split up into two sections, namely key criteria (seven items) and other criteria (six items). Key

criteria include ‘method of randomisation appropriate and identified’, ‘follow up period, range and mean given’, ‘appropriate statistical tests undertaken’ as well as several to- hip prostheses- specific- criteria (Cowley, 1995). Other criteria includes ‘quantification of outcome criteria’, ‘independence of the investigators’ and several to hip prostheses specific criteria (Cowley, 1995). Albeit very easy and straightforward to apply, given the rather large number of to hip replacement specific criteria and the lack of establishment of validity and reliability of the tool, this checklist was rejected for use in this systematic review.

### **Newcastle – Ottawa**

The Newcastle – Ottawa tool was originally developed using a Delphi process (surveys, questionnaires etc.) to define variable data extraction (Wells et al., 2011). Later on this method was further developed to assess the quality of studies that were to be included in a systematic review (Stang, 2010). However the applicability of this tool specific to this systematic review is questionable as it was specifically developed for epidemiological systematic reviews and moreover contains tools for cohort and case-control studies (Wells, et al., 2011). In addition no information was provided on the reliability or validity of the tool (Deeks, et al., 2003). For these reasons this checklist was rejected as well.

### **Reisch**

Reisch, Tyson & Mize (1989) originally developed their tool to evaluate the design and performance of therapeutic studies. The tool lists a total of 57 items spread out over 12 categories and includes categories such as study design, sample size and randomisation (Reisch, Tyson, & Mize, 1989). The tool was aimed to be very comprehensive in its coverage of internal validity, however the choice of some items may be questionable regarding the connection to internal validity (Deeks, et al., 2003). ‘The statement of purpose’ of the study

for instance is linked to internal validity (Reisch, et al., 1989), however it can be argued that this is more of a reporting issue than a validity issue (Deeks, et al., 2003). Moreover some of the criteria are rather specified towards pharmaceutical studies. For these reasons this checklist was not used in this systematic review.

### **Thomas**

The checklist developed by Thomas (2003) has been developed to specifically address the methodological quality of studies. It can furthermore cover any study design and includes 21 items split up into eight sections namely; selection bias, study design, confounders, blinding, data collection methods, withdrawals and dropouts, intervention integrity and analysis. Upon completion the study can be deemed strong, moderate or weak (Thomas, 2003). While it appeared suitable for this systematic review and was considered given its straightforward assessment of the aspects that can be considered important for its review of study design, blinding, appropriateness of design etc., the absence of reporting on validity and reliability caused it to be rejected as well.

### **Zaza**

The checklists by Zaza et al. (2000) was developed as part of a much larger standardised data extraction form of the 'US Guide to Community Preventive Services: Systematic Reviews and Evidence-based Methods'. The tool is developed to cover any study design and consists of 22 questions grouped into six categories namely; descriptions, sampling, measurement, data analysis, interpretation of results and other (Zaza et al., 2000). A strong point of this checklist is that these questions were generated from a review of methodological literature and moreover that expert opinion was solicited on pilot versions (Deeks, et al., 2003).

While this tool was considered it appears difficult to use in isolation of the entire data extraction form that this checklist is thus part of. In addition given the complexity of the tool it has led Deeks et al. (2003) to suggest that rather good understanding of validity issues is needed and that perhaps expert knowledge is required. Knowledge that is moreover usually not possessed by clinicians or students (Jüni, et al., 1999) and the usage of this tool by these two groups is questionable (Deeks, et al., 2003). For these reasons the use of this checklist was not employed in this systematic review.

### **Downs**

Downs & Black (1998) was developed for assessing the quality of randomised- and non-randomised studies. This checklist consists of 27 questions that are split into four sections, namely ‘external validity’, ‘internal validity – bias’, ‘internal validity – confounding’ and ‘reporting’. In designing this checklist both epidemiological principles as well as methodological literature were used to come to an initial version of this checklist. Hereafter the tool was piloted by several epidemiologists and statisticians and an improved version was produced (Downs & Black, 1998).

The strong point about this checklist is that it has been tested for validity and reliability and scored reasonably high on both. For instance from the assessment of criterion validity it appeared that ‘The Quality Index score correlated highly (0.90) with the score obtained using the instrument of the SRTG (randomised controlled trials only) and with the Global Score (randomised controlled trials + non-randomised studies, 0.89; randomised controlled trials, 0.88; non-randomised studies, 0.86)’ (Dawson & Black, 1998, p. 381). In addition the checklist received a reasonably good inter-rated reliability score of  $r = 0.73$  for RCTs, a test-

retest reliability of  $r = 0.88$  and a rather high internal consistency, with a KR-20 score of 0.89 (Downs & Black, 1998).

Given that this was the only checklist that was assessed for its reliability and validity and scored high on both, this checklist was adopted as the quality assessment method. For the Downs & Black (1998) checklist is referred to Appendix D.

## **5.2 Quality assessment results**

It has been suggested by several authors that some adjustments to a particular checklist might be necessary based on the nature of review and the studies included (Centre for Review and Dissemination, 2009; Deeks et al., 2003; Jui et al, 1999). For this reason the questions in checklist by Downs & Black (1998) were subject to further critical evaluation regarding the applicability of the studies included in these review. Consequently any questions regarding follow-up of subjects (3 in total; Q 9, 17 and 26) were left out, simply because they have no relevance to this review. That is the muscle protein synthetic response is investigated in the post-prandial period in resistance trained populations and follow-ups have no clinical relevance in this.

The checklist would now consists of 24 questions divided into five categories of ‘reporting’, ‘external validity’, ‘internal validity – bias’ and ‘internal validity – confounding’ and ‘power’.

As was previously described in *section 5 Quality assessment and data synthesis* that while it is important to follow systematic and validated principles for quality assessment, and specific

consideration should be given to the relative importance of various aspects related to bias, quality of reporting appropriateness of study design etc. (Centre for Review and Dissemination, 2009; Juni et al, 1999). The next part will therefore interpret the results of the study quality assessment of the primary studies included in this review in terms of the by Downs & Black (1998) suggested sections.

**Table 5.2.1** Downs & Black Checklist Reporting

Study	Q1: Aim clearly described	Q2: Outcomes clearly described	Q3: Patients characteristics clearly described	Q4: Interventions clearly described	Q5: Principal confounders clearly described	Q6: Main findings clearly described	Q7: Random variability for the main outcome provided	Q8: Reporting of adverse effects	Q9: Actual p-value reported
Moore et al. (2008)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Partially (1)	Yes (1)	Yes (1)	No (0)	No (0)
Koopman et al. (2004)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (2)	Yes (1)	Yes (1)	No (0)	No (0)
Tipton et al. (1999)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Partially (1)	Yes (1)	Yes (1)	No (0)	No (0)

It becomes apparent from **Table 5.2.1** that the study by Koopman et al (2008) scores rather high in terms of reporting (8 out of 10 point or 80 %) and can be considered of higher quality the study by Moore et al. (2008) (70 %) and the study by Tipton et al. (1999) (70 %), when it comes to reporting. The main reasons for this can be found in the description of principal confounders and the reporting of possible adverse events during the trial. While Moore et al. (2008) provided description regarding confounding by diet and Tipton et al. (1999) confounding by training, Koopman et al, (2005) provided description regarding possible confounding by both diet and physical exercise.

With regards to the actual probability values reported, all studies just reported whether the value was below  $p = 0.05$  ( $< 0.05$ ), yet provided no disclosure regarding the actual p - value

(Koopman et al., 2005; Moore et al., 2009; Tipton, et al., 1999). In addition no study provided any possible adverse effects that could have influenced the results, although it can be argued whether adverse effects were realistic in the absence of any safety issues regarding protein intake (Lowery & Devia, 2009) or controlled training in healthy adults (McArtney, 1999).

**Table 5.2.2** Downs & Black Checklist External Validity

<b>Study</b>	<b>Q10: Sample asked to participate representative of the population</b>	<b>Q11: Sample agreed to participate representative of the population</b>	<b>Q12: Places, facilities representative of the subject's environment</b>
Moore et al. (2008)	Yes (1)	Yes (1)	Yes (1)
Koopman et al. (2004)	UTD (0)	UTD (0)	Yes (1)
Tipton et al. (1999)	UTD (0)	UTD (0)	Yes (1)

In interpreting Q9 and Q10 of the checklist (**table 5.2.2**) it was specifically looked as to whether the sample asked and agreed to participate, would represent the population of resistance trained athletes (i.e. transferability). Only the study by Moore et al. (2008) actually used subjects with previous clearly described weightlifting experience (ranging from four months to eight years). The other two studies however did not use subjects that were previously engaged in resistance exercise (Koopman, et al., 2005; Tipton, et al., 1999). The places and facilities where subjects were studying were representative of the subject's normal environment, namely an environment with resistance training equipment. It becomes apparent from **table 5.2.2** that Moore et al. (2008) achieved a 100 % score on the issue of external validity while the study by Koopman et al. (2004) and the study by Tipton et al. (199) only achieved a 33 % score and were thus considered of lower quality in the aspect of external validity.



**Table 5.2.3** Downs & Black Checklist Internal Validity – Bias

<b>Study</b>	<b>Q13: Attempt to blind participants</b>	<b>Q14: Attempt to blind assessors</b>	<b>Q15: Data dredging results stated clearly</b>	<b>Q16: Appropriate statistics</b>	<b>Q17: Reliable compliance</b>	<b>Q18: Accurate outcome measures</b>
Moore et al. (2008)	Yes (1)	UTD (0)	Yes (1)	Yes (1)	Yes (1)	Yes (1)
Koopman et al. (2004)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)
Tipton et al. (1999)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)	Yes (1)

With regards to the internal validity all three studies score reasonably high (5/6 points or 83 % in the case of Moore et al (2008) and 100 % in the case of Koopman et al. (2004) and Tipton et al. (1999). In the study by Moore et al. (2008) there was no reporting of any attempt to blind the assessors (the researchers themselves) and consideration seems wanted when interpreting the results, as it has been suggested that this may increase the risk of exaggerating the benefits of an intervention (Poolman et al., 2007; Schulz & Grimes, 2002).

**Table 5.2.4** Downs & Black Checklist Internal Validity – Confounding

<b>Study</b>	<b>Q19: Same population</b>	<b>Q20: Participants recruited at the same time</b>	<b>Q21: Randomised</b>	<b>Q22: Adequate allocation concealment</b>	<b>Q23: Adequate adjustment for confounders</b>
Moore et al. (2008)	Yes (1)	UTD (0)	Yes (1)	UTD (0)	Yes (1)
Koopman et al. (2004)	Yes (1)	UTD (0)	Yes (1)	Yes (1)	Yes (1)
Tipton et al. (1999)	Yes (1)	UTD (0)	Yes (1)	Yes (1)	UTD (0)

When looking at **table 5.2.4** it becomes apparent that the study by Koopman et al. (2004) and Tipton scored the highest (80 %) as opposed to 60 % in the case of Moore et al. (2008) and Tipton et al. (1999) for various reasons. With regards to when the participants were recruited this was not stated in either of the studies, which to some extent lowers the quality of the studies.

As was mentioned previously no allocation concealment for the researchers was reported in the study by Moore et al. (2008), therefore at least some caution seems warranted when interpreting the results. The study by Moore et al. (2008) and Koopman et al. (2004) did attempt to provide adequate adjustment for potential confounders, such as diet by supplying their subjects with pre-packaged meals in advance of the trials and give them exercise guidelines in advance of the trial. the case Koopman et al. (2004) attempted to provide adequate adjustment for training, while the study by Tipton et al. (1999) does not report for potential confounding of training and diet and its internal validity may therefore be at least to some degree questioned.

**Table 5.2.5** Downs & Black Checklist Power

Study	Q24: Power calculation
Moore et al. (2008)	N5 - N6 (3)
Koopman et al. (2004)	N8+ (5)
Tipton et al. (1999)	N3 - N4 (2)

As becomes evident from **table 5.2.5** Koopman et al. (2004) score superior in terms of power (100 %) as opposed to the study by Moore et al. (2008) and Tipton et al. (1999) (both 60 %). A lower power for that matter can lead to not finding significant differences between trials, while in fact they may be there (i.e a type II error) (Arnold, Hogan, Colford, & Hubbard, 2011; Dupont & Plummer Jr, 1990). Therefore caution was given when interpreting the result of the latter two studies.

Taking into account all the aspects mentioned in the sections above, the study by Moore et al (2008) achieves a total score of 79 % (23/29), the study by Koopman et al. (2004) achieves a total score of 83 % (24/29) while the study of Tipton et al. (1999) achieves a total score of 69 % (20/29) and can thus be considered the lowest quality study include. The study by Koopman et al. (2004) and Moore et al. (2009) are considered of the highest quality in this review, yet as becomes evident from the section above they do so for different reasons. The study by Moore et al. (2009) for instance scores very high on ‘external validity’ while the study by Koopman et al. (2004) scores much higher on the aspect of ‘power’ and ‘internal validity and confounding’.

Now that the quality of each of the studies has been assessed the next section will consist of data synthesis and conclusions, thereby taking into account the quality scores of the studies on each of the aspects summarized above.

### 5.3 General study characteristics description

**Table 5.3.1** Description of main study characteristics and outcomes

Study	Design	Intervention - N	Previous Res. Train. Exp.	Age (Y)	Height (Cm)	Weight (Kg)	Max Stim of MPS (FSR)
Moore et al. (2009)	C (5x)	0 (0) / 5 (0.4) / 10 (0.8) / 20 (1.6) / 40 (3.2) g <sup>-1</sup> whole egg PRO immediately post workout N = 6	4 months - 8 years	22 ± 2	182 ± 1	86 ± 8	40 g <sup>-1</sup> whole EGG PRO (FSR:104 %)*
Koopman et al. (2004)	C (3x)	~ 11 g <sup>-1</sup> CHO / ~ 11 g <sup>-1</sup> CHO + ~ 7.4 g (0.74 g <sup>-1</sup> ) PRO Hydrolysate / ~ 11 g <sup>-1</sup> CHO + ~ 7.4 g <sup>-1</sup> (0.74 g <sup>-1</sup> ) PRO Hydrolysate + 7.4 g <sup>-1</sup> LEU every 30 min. N = 8	None	22 ± 1	181 ± 2	74 ± 4	22 g <sup>-1</sup> CHO + ~ 15 g <sup>-1</sup> (3.3 g <sup>-1</sup> ) PRO Hydrolysate + 7.5 g <sup>-1</sup> LEU (FSR:0.095%)
Tipton et al. (1999)	C (3x)	PLB (0) / 4 g <sup>-1</sup> MAA (0.44 g <sup>-1</sup> ) / 4 g <sup>-1</sup> EAA (0.88 g <sup>-1</sup> ) every 20 min <sup>-1</sup> N = 6	None	22 ± 2	170 ± 5	66 ± 3	4 g <sup>-1</sup> MAA (FSR:70 %)*

C = Randomized cross over trial; / = OR; CHO = carbohydrates; PRO = Protein; Leu = Leucine; MAA = Mixed Essential Amino Acids; EAA = Essential Amino Acids; MPS; Muscle Protein Synthesis; FSR = Muscle Protein Fractional Synthetic Rate; \* = Relative value of FSR as opposed to O g<sup>-1</sup> intake.

It becomes evident from **table 5.3.1** that the age of the subjects in all three groups was similar (22 ± 2). While the body weights (74 ± 4 kg<sup>-1</sup> respectively 66 ± 3 kg<sup>-1</sup>) in the studies by Koopman et al. (2005) and Tipton et al. (1999) are what you would expect from healthy subjects at their respective heights of 181 ± 2 cm<sup>-1</sup> and 170 ± 5 cm<sup>-1</sup>. The subjects in the study by Moore et al. (2009) although of similar height as the subjects in the study by Koopman et al. (2005), were considerably heavier in terms of body weight (86 ± 8 kg<sup>-1</sup> versus 74 ± 4 kg<sup>-1</sup>) adding to the notion that they at least have been successful in adding muscle mass in their previous period of resistance training, although it must be noted that in neither of the studies lean body mass was given (Koopman, et al., 2005; Moore, et al., 2009; Tipton, et al., 1999). That being said only the subjects in the study by Moore et al. (2009) had

considerable experience with resistance training (ranging from four months to eight years), while the subjects in the other two studies had no previous experience with resistance training (Koopman, et al., 2005; Tipton, et al., 1999).

With regards to design and intervention all studies were randomised crossover trials, meaning that all subjects received all trials adding, given the low number of subjects in two of the studies six ( $n = 6$ ) in the case of Moore et al. (2009) and Tipton et al. (1999), to the strength of the results (Centre for Review and Dissemination, 2009). That is given all subjects received the treatment they act as their own control eliminating possible between - patient - variability. In the study by Moore et al. (2009) all subjects received either 0, 5, 10, 20 or 40 g of whole egg protein immediately after an acute bout of resistance training (12 sets of 8 – 10 to failure) separated by at least a week. In the study by Koopman et al. (2005) subjects received either  $\sim 22 \text{ g}^{-1}$  carbohydrates,  $\sim 22 \text{ g}^{-1}$  carbohydrates +  $\sim 15 \text{ g}$  (3.3) protein hydrolysate or  $\sim 22 \text{ g}^{-1}$  carbohydrates +  $\sim 15 \text{ g}^{-1}$  (3.3  $\text{g}^{-1}$ ) protein hydrolysate +  $7.5 \text{ g}^{-1}$  leucine every 30 min for a period of 330 min after a bout of resistance training (8 set of 8 repetitions at 80 % of the subject's 1-repetition max) separated by at least a week. In the study by Tipton et al. (1999) for that matter, subjects received either a placebo (nothing),  $4 \text{ g}^{-1}$  of mixed essential amino acids (Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine, Arginine, Alanine, Aspartate, Glutamine, Glycine, Proline, Serine, Tyrosine) with a leucine content of  $0.44 \text{ g}^{-1}$  or  $4 \text{ g}^{-1}$  of essential amino acids with a leucine content of  $0.88 \text{ g}^{-1}$  every 20 min over a period of 220 min after an acute bout of resistance training (21 sets of 8-10 reps at 75 % of the subject's 1-repetition max) separated by at least two weeks.

As is evident from the various tables regarding quality assessment and, general study characteristics and outcomes given their large variations in these it is considered

inappropriate to collate and combined the studies in a meta-analysis (Centre for Review and Dissemination, 2009). Therefore a narrative synthesis of the outcomes and evidence is more feasible (Centre for Review and Dissemination, 2009; Popay et al. 2005). The next section will therefore summarise the outcomes and findings of the individual studies taking into account the quality of each of the studies assessing the strength of the evidence.

### **5.5 Narrative synthesis**

With regards to the main outcomes of the studies, the study by Moore et al. (2009) reported that at a consumption level of 5, 10 and 20 g<sup>-1</sup> of protein, FSR was 37 %, respectively 56 % and 93 % higher than fasted conditions (0 g<sup>-1</sup>) ( $p < 0.01$ ) (absolute values were not reported). Furthermore the study reported that with an intake of 40 g<sup>-1</sup> there was no difference in FSR compared to an intake of 20 g<sup>-1</sup> of whole egg protein ( $p = 0.29$ ) and concluded that 20 g<sup>-1</sup> (1.8 g<sup>-1</sup> LEU) per meal is sufficient to maximally stimulate MPS (Moore et al, 2009). However from the plotted figure regarding FSR, the author noticed FSR was in fact higher in the 40 g<sup>-1</sup> (3.2 g<sup>-1</sup> LEU) intakes (figure shown in *Appendix E*). Upon this observation the corresponding author of the paper was contacted, and it appeared that in the case of an intake 40 g<sup>-1</sup> FSR was in fact an additional 11 % higher as opposed to the 20 g<sup>-1</sup>, yet this was not significant. As was mentioned in *section 5.2 quality assessment* on the quality flaws of this study was the amount of subjects and therefore the possibility of a Type II error was reasonable (i.e. not finding significant differences, while in fact there were). It could be argued that this was in fact the case in this study, where the difference was deemed statistically not important. It could however be argued that when looking at optimising muscle protein synthesis (which is the focus of this review) an additional 11% increase in MPS is well worth it for many athletes, as the slightest difference may have a substantial impact on performance. More suggestions to a possible Type II error can be found in the assessment of mTORC1 activity and more specifically in the phosphorylation of ribosomal

protein S6K and it turn its target substrate of ribosomal protein s6 (rpS6). While this study by Moore et al. (2009) reported no significant differences in either of protein intakes ( $p > 0.05$ ) (that is there was no significant differences in phosphorylation in  $0 \text{ g}^{-1}$  opposed to the other extremity of  $40 \text{ g}^{-1}$ ), the plotted figure regarding the phosphorylation S6K and rpS6 does show a higher phosphorylation in at least the S6K measurement and to a lower degree in the rpS6 measurement, although the difference is not as obvious (*See E2, appendix E for figure*). Based on the above there are at least some suggesting that the relative low quality score of power (60 %) has led to a Type II error (not finding significant difference, while in fact they may have been there)

With regards to the quality of the study, the study by Moore et al. (2009) can be considered one of the stronger studies, given the fact that it used relatively experienced resistance trained athletes - and therefore has a rather good transferability -, attempted adequate adjustment for confounding from diet and exercise and provided thorough reporting (Downs & Black, 1998). The drawback of this study can be found in the lack of concealment, in that there is no reporting that the researchers were blinded for the trials, as well the lack of power. Therefore some caution regarding the results seems warranted. Overall however this study is considered of good quality (79 % score).

When looking at the study of Koopman et al. (2005), FSR was the highest in the CHO + PRO + LEU (total of  $29.6 \text{ g}^{-1} \text{ LEU over } 300 \text{ min}^{-1}$ ), namely  $0.095 \pm 0.005 \%$ . In the CHO + PRO (total of  $7.4 \text{ g}^{-1} \text{ LEU over } 300 \text{ min}^{-1}$ ) and the CHO group FSR was  $0.082 \pm 0.0104 \%$  respectively  $0.061 \pm 0.009 \%$  when free intracellular l-[ring- $^{13}\text{C}_6$ ]phenylalanine was used as the precursor ( $p < 0.05$ ). Note that these are absolute values, as opposed to the study by Moore et al. (2009) that reported relative values. The study by Koopman et al. (2005) is the study with the highest quality in this review. Overall reporting is strong, so is its adjustment

for confounding and bias. The only real drawback of this study is the transferability as subjects were not experienced resistance trained athletes such as in the study by Moore et al. (2008). Moreover the strong power (100 %) combined with the rather large difference in leucine contents ( $29.6 \text{ g}^{-1}$  as opposed to  $7.4 \text{ g}^{-1}$ ) likely contributed to finding significant differences. It must be noted however that the study is a somewhat 'unrealistic' in terms of its administration of the supplements namely  $2.96 \text{ g}^{-1}$  in the CHO + PRO + LEU group as opposed to  $0.74 \text{ g}^{-1}$  in the CHO + PRO every 30 min<sup>-1</sup> and this likely influenced the findings as well.

The study by Tipton et al. (1999) found that FSR was highest in the MAA group ( $4.4 \text{ g}^{-1}$  LEU) as opposed to the EAA ( $8.8 \text{ g}^{-1}$ ) with respective relative values of 70 % and 50 % (FSR) greater than for the placebo trial, where no nutrients were ingested (p - value not reported). In contrast to the studies by Koopman et al. (2005) and Moore et al. (2005) this study thus found a decreased mark in FSR when higher amounts of leucine were ingested, which could provide evidence for the fact that at  $4.4 \text{ g}^{-1}$  of leucine (this would translate to approx.  $55 \text{ g}^{-1}$  of whole protein in the case of an 8 % leucine content, see *figure E.3, appendix E*) MPS is already maximally stimulated. It must be however noted that the ability of finding statistical difference in this study, with a power of 40 % is even weaker, than the study by Moore et al. (2009) and it may also be that, likewise to the study by Moore et al. (2009), there is a type II error (not finding significant difference despite its existence). Following further investigation no strong indications for a type II error were however found in the study by Tipton et al. (1999). Overall this was the lowest quality study (69 %) included in the systematic review, mainly due to low power and weak transferability, that is only six subjects, with no previous resistance training experience participated in this study (Tipton, et al., 1999) and therefore its results must be interpreted with reservation.



## **6. Robustness of synthesis, evidence and conclusions**

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This section will describe the robustness of the synthesis and evidence, thereby coming to conclusions. Robustness for that matter relates to the methodological quality of the studies (such as risk of bias and transferability) as well as the quantity of studies that the evidence base is built on (Centre for Review and Dissemination, 2009). In addition this section will attempt to form conclusions regarding the evidence and provide possible practical recommendations as well as provide some directions for future research.

It is clear at this point no strong and clear evidence exists as to the amount of protein that might maximally stimulate MPS in the post-prandial period. While the study by Koopman et al. (2005) was the study of highest quality in this review and did find significant differences between the trials, the large difference in leucine content of the trials (29.6 as opposed to 7.4 g<sup>-1</sup>) as well as the apparent somewhat ‘unrealistic’ type of administration 2.96 g<sup>-1</sup> in the CHO + PRO + LEU group and 0.74g<sup>-1</sup> in the CHO + PRO every 30 min<sup>-1</sup> are likely to be responsible for this. Consequently translation of the findings from this study to any strong conclusions as to what amount might maximally stimulate MPS seems unfeasible. With regards to the study by Tipton et al. (1999) there is at least some evidence that 4.4 g<sup>-1</sup> of leucine might maximally stimulate MPS as opposed to 8.8 g<sup>-1</sup> when administered over approx. 180 – 200 min<sup>-1</sup>. Moreover this study provides at least a bit more ‘realistic’ way of administering these supplements. In addition this study was considered of the lowest quality study included in this review and more specifically the power was considered low (40 %). Yet no indication of a type II error was found and the quality was with 69 % still not

considered extremely low. However drawing conclusions from this study is difficult as well, as it is unsure whether other (lower) amounts of leucine would have also lead to maximizing the MPS response.

Albeit the strongest and most transferable evidence comes from the study by Moore et al. (2009), who reported that 20 g<sup>-1</sup> (1.6 of g<sup>-1</sup> of total leucine) whole protein would elicit a maximal MPS response and that no further increases were seen when 40 g<sup>-1</sup> (3.2 of g<sup>-1</sup> of total leucine) whole protein was ingested. Yet this conclusion may have been the result of low statistical power (60 %) and notion that moreover was strengthened by the indications of a type II error (that is sample size was not able to pick up significant differences, while they were in fact present) as correspondence with the author revealed that with the 40 g<sup>-1</sup> intake FSR (thus the indicator of MPS) was in fact 11 % higher. In the case of optimization of the performance of athletes it could be argued that this is a worthwhile increase (note that an 11 % increase in MPS would not translate to a 11 % increase in performance of LBM for that matter, yet may give small but significant increases in performance or physique over time), yet strong reservation must also be given to these conclusions.

Conclusively there is at this point no evidence that only 20 – 30 g<sup>-1</sup> of protein per meal can be utilized per meal by resistance trained athletes while on the other hand there is at best very minor evidence that more than these amounts, namely 40 g<sup>-1</sup> in the case of Moore et al. (2009) might stimulate MPS to a greater and more optimal degree.

Although the study by Moore et al. (2009) has its flaws mainly regarding the low amount of statistical power, the study can be considered a step in the right direction and it is felt in this review that more papers should address post prandial MPS (by assessing difference in FSR) in response to different amounts of protein in resistance trained populations yet thus using a

larger sample size. Moreover the usage of more 'realistic' administration of nutrients as well as 'realistic' training protocols seems wanted.

In addition the aspect of refractoriness of MPS (that the incapability to sustain and/or repeat a bout of MPS) warrants further investigation. This in order to make a reasonable assessment regarding frequency, thus investigating as to when given amounts of protein can be ingested again. It is clear that at this point the relative immaturity yet seemingly great (research) opportunities with regards to protein intake and resistance trained athletes should excite everyone interested in the field of sports nutrition.

## **Appendix A:** Search strategy

This appendix provides detailed description the search process employed in the systematic review.

### **A.1. Search Terms**

The following search terms were used to identify relevant research:

1. Muscle protein synthesis
2. Muscle protein fractional synthetic rate
3. Muscle anabolic signalling
4. Muscle anabolism
5. Protein intake
6. Essential Amino Acid intake (EAA)
7. Leucine intake
8. 1 AND 5
9. 1 AND 6
10. 1 AND 7
11. 2 AND 5
12. 2 AND 6
13. 2 AND 7
14. 3 AND 5
15. 3 AND 6
16. 3 AND 7
17. 4 AND 5
18. 4 AND 6
19. 4 AND 7

Note the exact same search terms specified as 1 – 10 have been performed in the German and Dutch language as well.

German search terms:

1. Muskel Proteinsynthese
2. Muskel Proteinsynthese fraktionell synthetische verhältnis
3. Muskel Anabolismus zeichengeben
4. Muskel Anabolismus
5. Protein aufnahme
6. Essentielle Aminosäure aufnahme (EAA)

## 7. Leucin aufnahme

Search strings 8 - 19 were done in a similar fashion as the English search terms.

Dutch search terms:

1. Spiereiwit synthese
2. Spiereiwit fraktioneel synthese verhouding
3. Spier anabolisme signalering
4. Spier anabolisme
5. Eiwitiname
6. Essentiele Amino-zuren inname (EAA)
7. Leucine inname

Search strings 8 - 19 were done in a similar fashion as the English search terms.

## **A.2. Database resources**

### **A.2.1. Electronic database searches**

The following online-sources were searched from 1980 - present using the search strings described in *section A.3.1.* to identify potential relevant primary research evidence.

- Google Scholar (searched on 13/09/11)
- PubMed (searched on 13/09/11)
- ScienceDirect provided by Elsevier B.V. (searched on 14/09/11)
- Springer Link (searched on 27/09/11)
- The Cochrane Library (searched on 07/09/11)
- ‘Grey Literature’ (see *appendix A.3.3.*)

### **A.2.2. Journal hand searches**

The following journals were hand searched for potential relevant research evidence:

- American Journal of Physiology-Endocrinology and Metabolism  
Searched from 2000 (Volume 278(1)) – present (Volume 301(3))

- The American Journal of Clinical Nutrition:  
Searched from 2000 (Volume 71(1)) – present (Volume 94(3))
- The British Journal of Nutrition  
Searched from 2000 (Volume 83) – present (Volume 106(9))
- The Journal of Nutrition  
Searched from 2000 (Issue 130(1)) – present (Issue 141(9))

### **A.2.3 Grey literature search**

The following ‘non-traditional’ searches have been carried out to further identify possible primary research evidence.

#### **A.2.3.1 Search of conference proceedings**

The following conference proceedings were searched:

- Tipton, K. D. (2011). Efficacy and consequences of very-high-protein diets for athletes and exercisers. *Proceedings of the Nutrition Society*, 70(02), 205-214.  
doi:10.1017/S0029665111000024
- Phillips, S. M. (2011). The science of muscle hypertrophy: making dietary protein count. *Proceedings of the Nutrition Society*, 70(01), 100-103.  
doi:10.1017/S002966511000399X

### **A.2.3.2 Search of relevant Internet sources (non-journals)**

The 'SuppVersity - Nutrition and Exercise Science for Everyone' website (<http://suppversity.blogspot.com/>) was searched on 03/10/11 using the on-site search engine with single search terms: 'Muscle protein synthesis', 'Muscle Anabolism', 'Protein intake', 'Essential Amino Acid (EAA) intake', 'Leucine Intake'. The section of the website labelled 'Amino Acids: BCAA, EAA & Co' was scanned in detail.

The search engine of Google was used with the same single search terms as described above in order to further identify possible relevant (unpublished) study results.

### **A.2.3.3 Other searches of relevant literature**

The reference list of studies selected for inclusion (appendix...) were scanned for relevant studies.

Citation searches in Google Scholar (06/09/11) were carried out for papers citing the recent key paper: Norton, L. E., & Wilson, G. J. (2009). Optimal protein intake to maximize muscle protein synthesis: Examinations of optimal meal protein intake and frequency for athletes.

*AgroFood Industry Hi-Tech*, 20(2). Retrieved from

[http://agro-food-industry.teknoscienze.com/pdf/norton\\_AF2\\_09.PDF](http://agro-food-industry.teknoscienze.com/pdf/norton_AF2_09.PDF)

Recent key book publications have been scanned for possible relevant studies:

- Di Pasquale, M. G. (2008). *Amino Acids and Proteins for the Athlete: The Anabolic Edge* (2nd ed.). Boca Raton, FL: CRC Press.
- McDonald, L. (2007). *The Protein Book* (1st ed.). Salt Lake City, UT: Lyle McDonald Publishing.

#### A.4 Inclusion criteria

##### **Inclusion Criteria**

###### *Population:*

- 18 – 64 years old
- Engaged in resistance training: either acute bout or systematic involvement ( $\geq 3$  d/week)

###### *Intervention:*

- Oral ingestion of protein as part of a mixed meal either one time or multiple following times during a day without any further co-interventions other than amino acid supplementation.
- Oral ingestion of protein in isolation one time or multiple following times during a day without any co-interventions other than amino acid supplementation and/or carbohydrate and/or fat supplementation.
- Oral ingestion of a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one time or multiple following times during a day without any further co-interventions other than macro nutritional (protein/ carbohydrate /fat) ones.
- Intravenous infusion a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one time or multiple following times during a day without any further co-interventions other than macro nutritional (protein/ carbohydrate /fat).

###### *Comparators:*

- Comparison of any different amounts of oral protein administration as part of a mixed



meal either one time or multiple following times during a day without any further co-interventions other than amino acid supplementation.

- Comparison of any different amounts of oral protein administration in isolation one time or multiple following times during a day without any co-interventions other than amino acid supplementation.
- Comparison of any different amounts of a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one time or multiple following times during a day without any further co-interventions other than macro nutritional ones.
- Comparison of any different amounts of intravenous infusion a mixture of free form amino acids containing *at least* all essential amino acids (EAAs) one time or multiple following times during a day without any further co-interventions other than macro nutritional ones.

*Outcomes:*

- Assessment of mixed muscle protein fractional synthetic rate (FSR) in the post-

prandial period through the use of muscle biopsy samples using the infusion of stable isotopes of the amino acid(s).

Only when the study met the inclusion criterion above, the following surrogate outcomes were also accepted:

- Measurement of activity of one or more of, to the mTORC1 pathway related factors through the use of antibodies (immunoblotting):
  - Eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1)
  - Eukaryotic translation initiation factor 4 gamma (eIF4G)
  - Ribosomal protein S6 kinase (p70<sup>S6K</sup>)
  - Ribosomal protein s6 (rpS6)

*Study Design:*

- Randomised crossover trials
- Cluster randomised trials

## Appendix B: Search results and primary studies included

### B.1. Search results

**Table B.1 Search Results**

Database	Studies Retrieved	Studies Selected	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Google Scholar	868620	26	444100	17200	17200	18000	20000	18200	103000	47300	17400	41000	20400	17400	12800	16700	18300	5200	17500	10500	6420
PudMed	538888	12	146910	128	49	319714	57133	3965	928	2370	293	10	10	24	6	3121	374	178	3122	374	179
Science Direct	416484	14	164026	2273	4092	2360	156951	27784	9025	28898	1062	1698	1263	702	844	9836	631	3874	374	423	368
SpringerLink	328623	4	48020	1007	2021	1277	44577	110408	358	892	431	110408	5324	1080	721	292	1241	75	134	185	172
Cochrane Library	547	0	204	22	1	6	282	1	2	4	3	1	1	0	5	3	2	1	2	4	3
Total	2153162	56																			

The numbers 1 – 19 represent the outputs from the search terms as displayed in A.1, appendix A.

### B.2. Primary studies included

(1) Moore, D., Robinson, M., Fry, J., Tang, J., Glover, E., Wilkinson, S., et al. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr*, 89(1), 161 - 168. doi:10.3945/ajcn.2008.26401

(2) Koopman, R., Wagenmakers, A. J. M., Manders, R. J. F., Zorenc, A. H. G., Senden, J. M. G., Gorselink, M., et al. (2005). Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *American Journal of Physiology - Endocrinology And Metabolism*, 288(4), E645-E653.

(3) Tipton, K. D., Ferrando, A. A., Phillips, S. M., Doyle, D., Jr., & Wolfe, R. R. (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol*, 276(4 Pt 1), E628-634. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10198297>

### **B.3 Primary studies excluded**

(1) Bohé, J., Low, A., Wolfe, R. R., & Rennie, M. J. (2003). Human Muscle Protein Synthesis is Modulated by Extracellular, Not Intramuscular Amino Acid Availability: A Dose-Response Study. *The Journal of Physiology*, 552(1), 315-324. doi:10.1113/jphysiol.2003.050674

Reason for exclusion: after full scale reading of the study, the subject appeared not to be engaged in any form of resistance training.

(2) Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P., et al. (2004). Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *The FASEB Journal*. doi:10.1096/fj.04-2640fje

Reason for exclusion: after full scale reading of the study, the subjects were not reported to be engaged in any form of resistance training.

(3) Symons, T. B., Sheffield-Moore, M., Wolfe, R. R., & Paddon-Jones, D. (2009). A Moderate Serving of High-Quality Protein Maximally Stimulates Skeletal Muscle Protein Synthesis in Young and Elderly Subjects. *Journal of the American Dietetic Association*, 109(9), 1582-1586. doi:10.1016/j.jada.2009.06.369

Reason for exclusion: after full scale reading of the study, the subjects were not reported to be engaged in any form of resistance training.

(4) Symons, T., Sheffield-Moore, M., Mamerow, M., Wolfe, R., & Paddon-Jones, D. (2011). The anabolic response to resistance exercise and a protein-rich meal is not diminished by age. *The journal of nutrition, health & aging, 15*(5), 376-381. doi:10.1007/s12603-010-0319-z

Reason for exclusion: after full scale reading of the study, there appeared to be no measurement of different amounts of essential amino acids/protein.

(5) Miller, S. L., Tipton, K. D., Chinkes, D. L., Wolf, S. E., & Wolfe, R. R. (2003). Independent and combined effects of amino acids and glucose after resistance exercise. *Med Sci Sports Exerc, 35*(3), 449-455. doi:10.1249/01.MSS.0000053910.63105.45

Reason for exclusion: after full scale reading of the study, there appeared to be no measurement of different amounts of essential amino acids/protein.

(6) Børsheim, E., Tipton, K. D., Wolf, S. E., & Wolfe, R. R. (2002). Essential amino acids and muscle protein recovery from resistance exercise. *American Journal of Physiology - Endocrinology And Metabolism, 283*(4), E648-E657. doi:10.1152/ajpendo.00466.2001

Reason for exclusion: after full scale reading of the study, there appeared to be no measurement of different amounts of essential amino acids/protein.

(7) Børsheim, E., Aarstrand, A., & Wolfe, R. R. (2004). Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. *Int J Sport Nutr Exerc Metab*, 14(3), 255-271. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/>

Reason for exclusion: after full scale reading of the study, there appeared to be no measurement of different amounts of essential amino acids.

(8) Glynn, E. L., Fry, C. S., Drummond, M. J., Timmerman, K. L., Dhanani, S., Volpi, E., et al. (2010). Excess Leucine Intake Enhances Muscle Anabolic Signaling but Not Net Protein Anabolism in Young Men and Women. *The Journal of Nutrition*. doi:10.3945/jn.110.127647

Reason for exclusion: after full scale reading of the study, the subject appeared not to be engaged in any form of resistance training.

(9) Fern, E. B., Bielinski, R. N., & Schutz, Y. (1991). Effects of exaggerated amino acid and protein supply in man. *Experientia*, 47(2), 168-172. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/>

Reason for exclusion: after full scale reading of the study, muscle protein synthesis did not appear to be measured in the post prandial period yet after four weeks.

## Appendix C: Data extraction form

### 1. General information

- 1.1 Date of data extraction:
- 1.2 Author(s):
- 1.3 Article title:
- 1.4 Citation:
- 1.5 Type of publication:
- 1.6 Country of origin:
- 1.7 Source of funding:

### 2. Study characteristics

- 2.1 Aim/objectives of the study:
- 2.2 Study design:
- 2.3 Study inclusion and exclusion criteria:
- 2.4 Recruitment procedures used:
- 2.5 Unit of allocation:

### 3. Participant characteristics (at the beginning of the study)

- 3.1 Age:
- 3.2 Gender: M/F
- 3.3 Ethnicity:
- 3.4 Previous involvement in resistance training:  
(Y/N) If Y, how long?

### 4. Intervention and setting

- 4.1 Setting of intervention:
- 4.2 Description of intervention(s)
  - 4.2.1 Number of groups:
  - 4.2.2 Number of participants in each groups:
  - 4.2.3 Doses of intervention(s):
  - 4.2.4 Duration of intervention(s):

### 5. Outcomes/results

- 5.1 Statistical technique used:
- 5.2 Reporting of outcomes:
  - 5.2.1 Muscle protein fractional synthetic rate (FSR)
    - Reported (Y/N)
    - Definition used in study:
    - Type of measurement:
    - Amount of intake to maximize outcome:

### 5.2.2 Eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1)

- Reported (Y/N)
- Definition used in study:
- Type of measurement:
- Amount of intake to maximize outcome:

### 5.2.3 Eukaryotic translation initiation factor 4 gamma (eIF4G)

- Reported (Y/N)
- Definition used in study:
- Type of measurement:
- Amount of intake to maximize outcome:

### 5.2.4 Ribosomal protein S6 kinase (p70<sup>S6K</sup>)

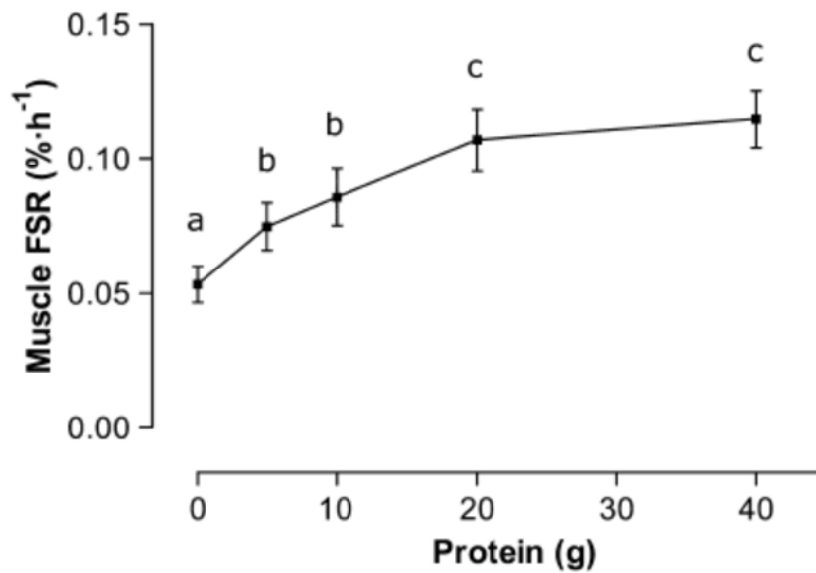
- Reported (Y/N)
- Definition used in study:
- Type of measurement:
- Amount of intake to maximize outcome:

### 5.2.5 Ribosomal protein s6 (rpS6)

- Reported (Y/N)
- Definition used in study:
- Type of measurement:
- Amount of intake to maximize outcome:

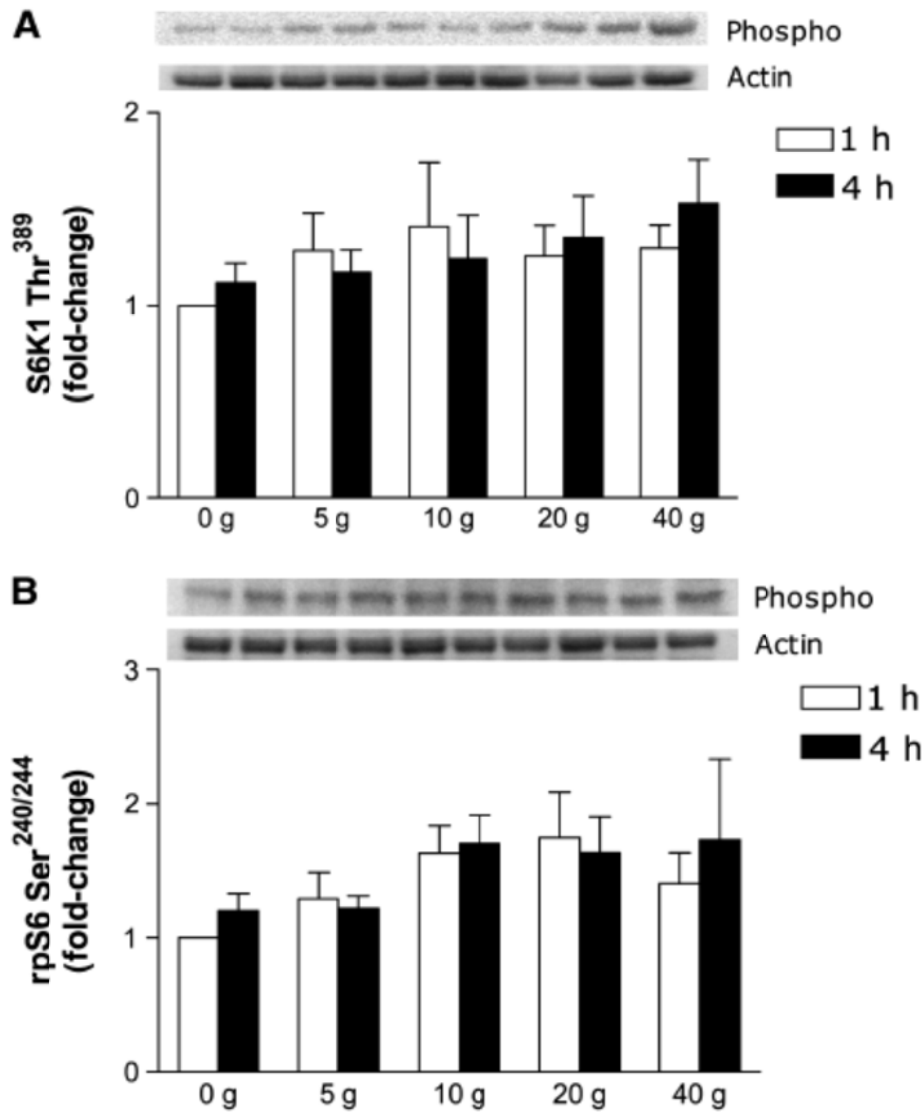


## Appendix D: Relevant figures



**Figure D.1:** Mean ( $\pm$  SEM Mixed muscle FSR) after resistance exercise in response to increasing amounts of dietary protein.

Adapted from Moore, D., Robinson, M., Fry, J., Tang, J., Glover, E., Wilkinson, S., et al. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr*, 89(1), 161 - 168. doi:10.3945/ajcn.2008.26401



**Figure D.2:** Mean ( $\pm$  SEM) phosphorolytion of S6K1 at Thr<sup>368</sup> (A), rpS6 at Ser<sup>240/244</sup> (B) 1 and 4 h<sup>-1</sup> after resistance exercise in response to increasing amounts of dietary protein.

Adapted from Moore, D., Robinson, M., Fry, J., Tang, J., Glover, E., Wilkinson, S., et al. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr*, 89(1), 161 - 168. doi:10.3945/ajcn.2008.26401

**Table D.3: Leucine content of popular protein sources**

<b>Protein Source</b>	<b>Leucine % of total protein</b>	<b>Amount of protein to reach 4.4 g<sup>-1</sup></b>	<b>Amount of food source required</b>
Beef	8 %	55 g <sup>-1</sup>	184 g <sup>-1</sup>
Chicken	7.5 %	59 g <sup>-1</sup>	193 g <sup>-1</sup>
Pork	8 %	55 g <sup>-1</sup>	194 g <sup>-1</sup>
Eggs	8.6 %	51 g <sup>-1</sup>	194 g <sup>-1</sup> or 7 large eggs
Fish	8.1 %	55 g <sup>-1</sup>	236 g <sup>-1</sup>
Whey	12 %	38 g <sup>-1</sup>	Variable depending upon powder type
Milk	9.8 %	45 g <sup>-1</sup>	1294 g <sup>-1</sup>

Data adapted from Layman, D. K., & Baum, J. I. (2004). Dietary protein impact on glycemic control during weight loss. *J Nutr*, 134(4), 968S-973S. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/15051856>

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