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	Title and Abstract (5%) Title to include: A concise indication of the research question/problem. Abstract to include: A concise summary of the empirical study undertaken.
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The Effect of Whey Protein on Muscle Hypertrophy and Strength: A Case Study

(Dissertation submitted under the discipline of Physiology & Health)

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THE EFFECT OF WHEY PROTEIN ON MUSCLE HYPERTROPHY AND STRENGTH: A CASE STUDY

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Abstract

To increase muscle mass through muscular hypertrophy, individuals must create a positive protein balance between synthesis and breakdown, through adequate protein intake. Whey protein contains high levels of the amino acid leucine, which plays a key role in initiating muscle protein synthesis. The aim of this case study was to examine the effects of a whey protein supplement on the muscular hypertrophy and strength of an individual involved in a resistance training program. The participant completed an eight-week training program; four weeks without a protein supplement, and four weeks with a supplement. The participant's diet was recorded in a food diary during the period without supplementation, and repeated with the addition of the supplement. The supplement increased the participant's daily protein intake to 1.8 g per Kg body mass, and was also consumed post training. The supplement significantly increased both the participant's protein intake, (104 $g \cdot day^{-1}$ ± 19.3 to 148.9 $g \cdot day^{-1}$ ± 6.7), and total energy intake (2280.3 ± 377.8 $kCal \cdot day^{-1}$ to 2459.8 ± 348.5 $kCal \cdot day^{-1}$). Supplementation had little effect on the reduction in total body mass, which decreased 2.7% with the supplement and 2.6% without. However the participant's upper arm and thigh girths increased with the supplement, (+0.6%, +0.8%;) (without: -0.8%, -2.7%) and the skinfold measurements decreased (-12.7%, -19%, -6.2%, -22.6%, -7.9%) (without: -2.7%, -6.4%, +15.4%, -2.4%, +23.5%). The results suggested an increased lean cross sectional area of the upper arm and thigh with the supplement (4.2%, 3%) (without: -0.8% and -6.2%), decreased body fat percentage (-1.2%) (without: +0.7%) and attributed a greater percentage of total body mass reduction to fat mass (With supplement: -2.3% lean mass, -3.9% fat mass; without: -2.8%, -1.9%). The supplement did not affect strength, as squat 1RM increased both with, and without supplementation (3.8%; 1.8%), whilst the bench press demonstrated little change (0%; -2.5% without). This study highlighted that using a whey protein supplement is a simple, effective method of increasing protein intake. The results provide support to the notion that increased protein intakes during an energy deficit can preserve lean mass, but did not have an effect on strength.

Chapter One

Introduction

1.0 Introduction

Nutrition can play a key role in enhancing short term and long term physical performance, such as providing fuel for exercise, enhancing the recovery process and synthesising new tissue (Jeukendreup & Gleeson, 2010; McArdle, Katch & Katch, 2010). Protein, which consists of combinations of amino acids, is one of three macronutrients evident in the diet and is present in the body in the likes of muscle, skin and hair and in the free amino acid pool in the plasma (Jeukendreup & Gleeson, 2010, Tarnapolsky, 2000). It can be found in a variety of foods and can be given a protein rating based on their amino acid content. Highly rated sources that have near to or complete amino acid profiles tend to originate from animal sources, whilst those from vegetable origin tend to rate poorly (McArdle *et al.,* 2010).

Researchers have suggested that those aiming to increase muscle mass through resistance training may require protein intakes of 1 – 1.8 g per Kg of body mass (Hartman *et al.,* 2006; Phillips, Moore & Tang, 2007; Tarnapolsky *et al.,* 1992). This is greater than the recommended intake of 0.8-1.0 g per Kg body mass daily for the general population (McArdle *et al.,* 2010; Tarnapolsky *et al.,* 1992). These values are suggested in order to achieve a positive protein balance, and an increase in body proteins, in this case, increased muscle mass (Jeukendreup & Gleeson, 2010). The lower values recommended represent intakes sufficient to achieve an increase in muscle mass, whilst the higher values are a suggestion of an upper limit. However with some variance evident, studies have also highlighted that individual differences and factors such as; energy requirements, population and training status will affect these requirements, therefore recommendations should take these elements into account (Phillips *et al.,* 2006; Szedlak & Robbins, 2012).

To increase body proteins, such as increasing muscle mass through skeletal muscle hypertrophy, which is the growth and increase in numbers of muscle fibres (McArdle *et al.,* 2010), protein synthesis must exceed protein breakdown, thus creating a positive protein turnover (Jeukendreup & Gleeson, 2010). Resistance training is a method of increasing muscle mass, through stimulating both protein synthesis, and protein breakdown (Kumar, Atherton, Smith & Rennie, 2009; Millward, Garlick, Stewart, Nnanyelugo & Waterloo, 1975; Phillips, Tipton, Aarsland, Wolfe & Wolfe, 1997). Consequently, there must be a sufficient intake of protein from the diet to create a positive protein turnover, in order to achieve skeletal muscle hypertrophy and increase muscle mass (Hartman, Moore & Phillips, 2006; Tarnapolsky *et al.,* 1992).

Resistance training provides the stimulus for muscle protein synthesis to occur, which can be present for up to 48 hours following the training session (Chesley, MacDougall, Tarnapolsky, Atkinson & Smith, 1992; Phillips *et al.,* 1997). Within this time frame, research has identified that the amino acid leucine has a key role in initiating muscle protein synthesis following stimulation by resistance training (Anthony, Anthony, Kimball, Vary & Jefferson, 2005*;* Crozier, Kimball, Emmert, Anthony & Jefferson, 2000; Fujita *et al.,* 2007). Therefore those attempting to increase muscle mass should consider protein sources higher in leucine in order to maximise protein synthesis. When aiming to create a positive protein turnover, muscle protein breakdown must also be considered, as it is stimulated by resistance training and increases following the training session (Biolo, Maggi, Williams, Tipton & Wolfe, 1995; Phillips *et al.,* 1997). If muscle protein breakdown exceeds synthesis, the negative protein balance will result in a reduction of body proteins and a loss of muscle mass, and subsequently must be minimised (Kumar *et al.,* 2009; Millward *et al.,* 1975).

Churchward-Venne, Byrd and Phillips (2012a) have emphasised the importance of consuming adequate protein and leucine content in meals throughout this potential window for synthesis of up to 48 hours post resistance training. However studies have also examined the effects of consuming protein around resistance training, particularly post exercise (Andrews, Maclean & Riechman, 2006; Churchward-Venne *et al.,* 2012b; Esmarck *et al.,* 2001; Hulmi *et al.,* 2008; Moore *et al.,* 2009; Tipton, Ferrando, Phillips, Doyle & Wolfe, 1999). It has been established that consuming protein immediately post exercise can maximally stimulate muscle protein synthesis and reduce protein breakdown (Churchward-Venne *et al.,* 2012; Moore *et al.,* 2009; Tipton *et al.*, 1999). However this has provided inconclusive results in long-term studies, which questions the importance of this notion. In elderly subjects, increased hypertrophy and strength has been demonstrated in those consuming protein post exercise in 12-week resistance training programs (Andrews *et al.*, 2006; Esmarck *et al.,* 2001). However in young, active males, a study by Hulmi *et* *al.,* (2008) demonstrated increases in muscle cross sectional area, but not of a significant level. This may suggest different effect levels in different populations, but could also be due to other factors such as the training programme, level of daily protein intake and specific nutrient timing. However, a recent meta-analysis by Schoenfeld, Aragon & Krieger (2013) concluded that timing protein intake around resistance training did not have a significant effect on hypertrophy and strength, and that daily protein intake was the primary factor in the increase in muscle mass. The authors crucially note that in the majority of these studies, the subjects were untrained, and whilst their conclusions may apply to these subjects, recommendations cannot be made for all populations and training statuses.

With specific regards to strength, studies have demonstrated inconclusive results with varying protein intakes. In a 6-month study with untrained subjects, Vukovick *et al.,* (2004) demonstrated increased upper and lower body strength in those with intakes of 2.2 g per Kg body mass daily, against 1.1 g per Kg. Whilst in a 12-week study of trained subjects, Falvo *et al.,* (2005) only showed increases in lower body strength in those with intakes of 2 g per Kg body mass daily against 1.24 g per Kg. Whilst increases in upper and lower body strength was not consistent across both studies, it must be noted that the subjects had different training status and the studies were of different length. The results of these studies suggest protein intake may only bring significantly greater improvements in strength over longer periods, but there may also be a possibility of a greater effect in untrained subjects. Additionally, factors such as the resistance training program and the slight variations in protein intake may have had an effect. These studies can, however, provide individuals or coaches with an idea of the results that can be expected over certain periods of time, enabling these individuals to make more informed decisions regarding the importance of protein intake.

Sales of dietary supplements have increased progressively in recent times (Grandjean & Ruud, 2003; Gibson-Moore, 2012). There is now a wide range of companies offering a variety of protein supplements such as egg, soy and whey. Churchward-Venne *et al.,* (2012b) demonstrated that whey is an effective protein supplement, with dosages of 25 g, capable of maximising muscle protein synthesis for three to five hours following resistance training. Studies have demonstrated this is due its high leucine content, (Norton, Wilson, Layman, Moulton & Garlick, 2012) which has been shown to initiate muscle protein synthesis (Anthony *et al.*, 2005; Crozier *et al.*, 2000; Fujita *et al.,* 2007).

The aim of this case study is to identify the effect that an eight-week resistance training program has on an individual's skeletal muscle hypertrophy and strength with and without the use of a whey protein supplement. The supplementation will attempt to take advantage of theories previously suggested to promote skeletal muscle hypertrophy and increase strength. These include an increased daily protein intake; in this case to the value of 1.8 g per Kg body mass, as well as the potential benefit of consuming a protein supplement immediately post exercise. A case study will also allow a greater degree of dietary and exercise control due to the lower number of participants, enabling the exact replication of the participant's dietary intake with the addition of a supplement in the second four-week block, which has not always been achieved in previous studies (Esmarck *et al.,* 2001; Hulmi *et al.* 2008). The results will provide valuable feedback to the individual and may provide a coach with information as to the effects a whey protein supplement can have in this period of time, which could be implemented in situations such as off-season training programs. The results of the case study could also be generalised to similar populations and may demonstrate the theories discussed, a benefit of case studies highlighted by Smith (2010).

Chapter Two

Literature Review

2.0 Literature Review

2.1 Nutrition

Nutrition plays a key role in physical performance, providing the fuel for biological work, repairing existing cells and synthesizing new tissues (McArdle *et al.*, 2010). Nutrition can enhance physical performance through means such as providing the correct fuel for exercise or enhancing the recovery process, be it through the use of dietary modifications or nutritional supplements (Jeukendrup & Gleeson, 2010; McArdle *et al*., 2010). A poor diet may prevent an athlete from achieving his/her potential, (Maughan & burke, 2000) which again, highlights the importance of nutrition to sport and exercise performance. Nutrients can be categorised into macronutrients or micronutrients. Macronutrients provide the body with fuel whilst also playing a key role in the structure and function of the human body. These are required in larger amounts than Micronutrients, which are named, as we typically require less than one gram per day, but have roles in the regulation of metabolism in the body (Jeukendreup & Gleeson, 2010). The macronutrients are divided into; Carbohydrates, Fat and Protein, with the latter forming the main focus of this study.

2.2 Protein

Protein is made up of combinations of amino acids and is present in the human body in the form of skeletal muscle and in free form around the body (McArdle *et al.,* 2010). There are 20 different amino acids required by the body that form two groups, essential and non-essential. Essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) must come from the diet, whereas the body is capable of synthesising the remaining 11 non-essential amino acids (Tarnapolsky, 2000).

Protein can be found in a variety of foods, categorised as complete or incomplete proteins based on their amino acid content, and can be given a protein rating based on their biological value. Those that rate highly originate from animal sources such as eggs, dairy, meat, fish and poultry, and those lower on the scale, and are considered incomplete, are typically those from a vegetable origin (McArdle *et al.,* 2010). Protein provides structure to cells within the body, particularly muscle, skin, and hair, which are composed largely of protein. Amino acids also have roles in the metabolism of many organs and tissues, as well as being a precursor for the synthesis of body proteins, such as skeletal muscle tissue (Jeukendreup & Gleeson, 2010).

Following ingestion and digestion, protein now in the form of amino acids, enters the free amino acid pool in the plasma. Amino acids in the plasma can come from dietary intake or protein breakdown. From here, these amino acids can be utilised for protein synthesis, oxidation, or incorporated into many metabolic pathways (Tarnapolsky, 2000). Protein is exchanged between the amino acid pool and the body proteins, this is known as protein metabolism or protein turnover. Protein can be synthesised into the body proteins, or broken down from the body proteins to re-enter the amino acid pool (Jeukendreup & Gleeson, 2010). Protein turnover can be measured by means such as assessing the nitrogen balance, where a positive balance suggests an increase in body protein, and a negative balance suggesting a reduction in body protein (Pellett, 1990). Therefore those looking to increase their body proteins, such as athletes attempting to increase their muscle mass, need to ensure they have a positive protein turnover. This is in addition to a positive energy balance, where intake exceeds expenditure (McArdle *et al.,* 2010). Exercise, and in particular resistance training, stimulates the synthesis and breakdown of protein in skeletal muscle tissue, therefore protein intakes should be adjusted accordingly to create a positive protein turnover in those attempting to gain muscle mass (Chesley *et al.,* 1992; Kumar *et al.,* 2009; Millward *et al.,* 1975; Phillips *et al.,*1997).

2.2.1 Protein Intake

Athletes involved in resistance training programs may require between 1.0 g - 1.8 g per Kg of body mass each day, greater than the recommended daily amount (RDA) for the sedentary population of 0.8 - 1.0 g per Kg body mass (Table 1). These values have been suggested for individuals attempting to achieve a positive protein turnover and an increase in muscle mass through the skeletal muscle hypertrophy associated with resistance training. Studies have demonstrated that intakes of 1.0 - 1.4 g per Kg body mass of protein per day are sufficient to achieve skeletal muscle hypertrophy (Hartman *et al.,* 2006; Phillips *et al.,* 2007; Tarnapolsky *et al.,* 1992). An upper limit to daily protein intake for athletes involved in resistance training, where no further benefit would be seen of 1.6 – 1.8 g per Kg (McArdle *et al.,* 2010; Phillips *et al.,* 2007; Tarnapolsky, 2000; Tarnapolsky *et al.*, 1992). Whilst, the literature tends to agree on a minimum and an upper limit for protein intake, the figures suggested do between authors, which led to Szedlak and Robbins (2012) highlighting that protein requirements may differ with training status. The authors cited the studies of Hartman *et al.,* (2006) who analysed novice lifters and Tarnapolsky *et al.,* (1992) who studied both novice, and experienced strength athletes, and came to different conclusions (Table 1). In light of these findings, factors such as population, training status may cause individual requirements to differ, which should be taken into account when recommending protein intakes for athletes.

Table 1. Recommended protein intakes for athletes involved in resistance training.

Researchers	**Protein Intake (g per Kg Body Mass per day)**
RDA (McArdle *et al.* 2010; Tarnapolsky, 1992)	0.8 - 1.0
Hartman *et al.* (2006)	1.2
McArdle *et al. (*2010)	1.2 - 1.8
Phillips *et al.* (2007)	1.2 - 1.6
Szedlak and Robbins (2012)	1.2 - 1.7
Tarnapolsky *et al.* (1992)	1.4 - 1.8
Tarnapolsky (2000)	1.0 - 1.7

However, Tarnapolsky (2000) showed that average protein consumption within strength athletes was 2 g per Kg body mass daily, but ranged from 1.6 g to 2.8 g, exceeding the upper limits recommended by previous research (Table 1). This suggests that strength athletes may be consuming above the recommended amount of protein in an attempt to promote further increases in strength and hypertrophy, particularly when the upper limit varies in the research. However, athletes must be careful when consuming high amounts of protein, if for example, there is a reduction of carbohydrates in the diet, which may be essential for certain athletes, this may lead to a lack of energy and therefore reduced performance (Phillips *et al.,* 2007). Higher protein intakes also show increased rates of amino acid oxidation (Bowtell *et al.,* 1998; Tarnapolsky *et al.*, 1992), a potential concern for athletes, as less amino acids may be synthesised into muscle tissue. Athletes must therefore consider these notions, as changes to their dietary intake may prove detrimental to performance. Phillips *et al.,* (2007) also note that higher protein intakes do not carry any serious health risks, and that intakes up to 3 g per Kg body mass per day, may in fact benefit individuals in an energy deficit, helping to decrease fat mass and preserve lean mass. This suggestion, as well as the points discussed previously, highlight that protein intake should be decided based on the individual and their goals, particularly when varying circumstances will effect the dietary requirements of that individual.

2.2.2 Protein Synthesis

Protein synthesis begins with a signal such as a nutrient, hormonal or mechanical signal, which induces gene expression. This initiates a process called transcription, where a messenger RNA (mRNA) is formed from the gene template. Once in the cytosol, the mRNA is translated into a protein through the process of translation via ribosomes. Translation requires three distinct steps termed initiation, elongation and termination of the protein to form an amino acid (Tarnopolsky, 2000). The mammalian target of rapamycin (mTOR) is seen as an important regulator of translation within the cell, contributing to the overall control of protein synthesis (Proud, 2007).

Research has identified that the amino acid leucine plays a key role in initiating muscle protein synthesis (Anthony *et al.,* 2005; Crozier *et al.,* 2000; Fujita *et al.,* 2007). Leucine can stimulate protein synthesis through means such as acting upon mTOR (Anthony *et al.,* 2005) and activation of eIF4G phosphorylation, another means of achieving muscle protein synthesis (Bolster, Vary, Kimball & Jefferson, 2004). Exercise however, provides the stimulus for muscle protein synthesis to occur, and although it remains at rest during exercise (Durham *et al.,* 2004), it is elevated following resistance training, and the stimulus can remain for up to 48 hours (Chesley *et al.*, 1992; Phillips *et al.,* 1997). These studies highlight that whilst exercise provides the stimulus, the diet must enforce the adaptation. More specifically, following a bout of resistance training, individuals can take advantage of this stimulus for up to 48 hours through the consumption of leucine rich foods and an adequate protein intake, again highlighting the importance of the content of the diet during this time period.

2.2.3 Protein Breakdown

Protein breakdown or degradation is the process of breaking down proteins to their constituent amino acids. These amino acids contribute to the intra-cellular free amino acid pool, which can be exported into the plasma, oxidised for energy provision or resynthesized into tissue protein (Tarnapolsky, 2000). Research has shown that resistance training does not cause muscle protein breakdown during the exercise bout itself (Durham *et al.,* 2004). But it has been demonstrated that muscle protein breakdown rates increased following resistance training, including more so during eccentric contractions (Biolo *et al.,* 1995; Gibala, MacDougall, Tarnapolsky, Stauber and Elorriaga, 1995; Phillips *et al.,* 1997). Muscle protein breakdown, and muscle protein synthesis can therefore be considered the mechanisms stimulated by resistance training, to induce muscle protein turnover, which is the primary factor in the accretion of body proteins (Phillips *et al.,* 1997).

2.3 Muscle hypertrophy

Muscle hypertrophy refers to the growth or increase in number of the muscle fibres or the sarcoplasm within the muscle (Baechle, Earle and Wathen, 2008; Fisher, Steele & Smith, 2013) which is typically triggered by resistance training, specifically exercises using 60 - 75% of an individual's one repetition max, for repetitions in the range of six to fifteen (Baechle *et al.,* 2008). However, for body proteins to increase, a positive net protein balance between muscle protein breakdown and muscle protein synthesis must be achieved (Kumar *et al.,* 2009; Millward *et al.,* 1975; Phillips *et al.,* 1997). This can be attained through increasing muscle protein synthesis, and reducing muscle protein breakdown. If for example, multiple resistance training sessions occur over a weekly period, the research would suggest that the stimulus for muscle protein synthesis would be present for large periods of time, perhaps even constantly (Chesley *et al.*, 1992; Phillips *et al.,* 1997). Therefore meals containing adequate protein and leucine content, consumed during this period will potentially result in the hypertrophy of the skeletal muscle, through increased protein synthesis and a positive net protein balance. Tarnapolsky (2000) suggested that increasing muscle protein synthesis would prove a more valuable intervention than attempting to reduce muscle protein, a notion supported by other researchers (Kumar *et al.,* 2009, Phillips *et al.,* 1997). This concept places further emphasis on the role the diet has in creating a positive net protein balance through increased synthesis to ensure the body adapts to the stimulus that resistance training provides.

2.4 Nutrient Timing

The total daily intake of protein has been considered vitally important when muscle hypertrophy or muscle building is concerned (Hartman *et al.,* 2006; Tarnapolsky *et al.,* 1992). However, research has also been conducted into the timing of protein intake and the effects on muscle protein synthesis, particularly near the resistance training session.

Churchward-Venne *et al.,* (2012a) highlight the importance of protein and essential amino acid (EAA) provision throughout the window of muscle protein synthesis created by resistance training. The authors state dietary strategy must be carefully considered to maximally stimulate exercised induced rates of muscle protein synthesis in this window of potentially 24 - 48 hours following resistance training. As highlighted previously, for maximal stimulation of muscle protein synthesis, it would seem important to consider protein and leucine content of meals in this window, an area that seems lacking in research as noted by Churchward-Venne *et al.,* (2012a).

The effect of consuming protein immediately post resistance training has also been demonstrated, where muscle protein synthesis is increased immediately and muscle protein breakdown is reduced to maximise the benefit of resistance exercise (Biolo *et al.,* 1997; Phillips *et al.*, 1997). Consuming protein immediately post exercise has been shown to increase muscle protein synthesis (Biolo *et al.*, 1995; Moore *et al.,* 2009; Tipton *et al.,* 1999). Moore *et al.* (2009) concluded that 20g of egg protein, was required to maximally stimulate muscle protein synthesis after resistance exercise in young men. Churchward-Venne *et al.,* (2012b) compared the post exercise supplementation of 25 g whey protein, 6.25 g of whey protein enriched with EAAs and 6.25 g of whey protein enriched with leucine on muscle protein synthesis. The authors discovered that in young, active males, each were effective in maximally stimulating muscle protein synthesis, but whey protein was capable of stimulating muscle protein synthesis for a longer period post exercise, three to five hours in comparison to one to three hours in EAAs and leucine alone. This study in particular highlights that the use of a complete protein such as whey, is superior to supplementing an adequate amount of leucine or EAAs containing leucine that should theoretically maximally stimulate muscle protein synthesis. This should therefore be considered if an individual is considering the use of a post exercise supplement to enhance muscle protein synthesis.

Long-term effects of post training supplementation on muscular hypertrophy and strength have also been demonstrated. Andrews *et al.,* (2006) suggested that consuming a protein supplement post exercise in a 12 week resistance training program, had a greater impact on strength and skeletal muscle hypertrophy than daily protein intake, as elderly subjects achieved increased strength and skeletal muscle hypertrophy despite varying daily protein intakes. Esmarck *et al.* (2001) showed that elderly subjects who took a supplement including 10 g of protein, 7 g of carbohydrate and 3.3 g of fat immediately post resistance training in a 12 week program showed greater increases in muscle cross sectional area (CSA) and strength than when supplementation was delayed by two hours. The study by Andrews *et al.,* (2006) certainly highlights the benefits of protein supplementation post exercise, placing it above daily intake in importance to muscle strength and hypertrophy, whilst the study by Esmarck *et al.,* (2001) shows a potential benefit of consuming protein earlier after resistance training for increased hypertrophy. However, it must be noted that elderly subjects were used in both studies; therefore this notion may not apply to all populations. Hulmi *et al* (2008) demonstrated that young men who supplemented with 15g of whey protein both prior to and post resistance training, increased muscle CSA to a greater degree than those who consumed a placebo over a 21 week resistance training program where macronutrient intake was considered constant. However, the changes in muscle cross sectional area demonstrated in the study were not significant. Whilst these studies do provide support to the supplementation of protein post training, there is conflicting evidence between studies with regards to the significance of the changes and the importance of timing against total protein intake. It would seem that timing intake around the resistance training session may have a significant effect on elderly populations, but only a small, insignificant effect on younger individuals.

However, more recent research, in the form of a meta-analysis (Schoenfeld *et al.*, 2013) has dismissed the importance of the timing of consuming protein immediately following resistance training. They state that the key factor in maximising protein accretion with resistance exercise is adequate protein intake. The authors also highlight a crucial limitation of the literature available, stating that the majority of these studies evaluated subjects who were inexperienced with resistance exercise, and that more experienced individuals may respond differently. This study, whilst dismissing the importance of nutrient timing and emphasising intake as the key factor in untrained individuals, also further supports the notion that different populations and individual factors such as training status may elicit different responses to protein intakes and timing protein consumption around the resistance training session.

2.5 Strength

Strength is closely related to muscle hypertrophy, higher levels of strength have been shown to linearly correlate to higher muscle CSA (McArdle *et al.,* 2010). Whilst greater muscle CSA is evident with strength training, neurological adaptations such as; inter and intra-muscular coordination, increased firing frequency and adaptations in agonist-antagonist activation are also evident in strength based resistance training (Baechle *et al.,* 2008; Folland & Williams, 2007) Strength training uses heavier loads, typically greater than 85% of the one repetition max, for less than 6 repetitions (Baechle, Earle & Wathen, 2008). In relation to protein intake, Vukovich *et al.,'s* (2004) study of a six month strength and conditioning program showed greater increases in strength through using a protein supplement to increase daily protein intake to 2.2 g per Kg of body mass per day than the use of a placebo and an intake of 1.1 g per Kg of body mass per day, when total energy intake was constant. However Falvo *et al.* (2005) only showed strength increases in the squat exercise and none in the bench press exercise when comparing subjects with a daily intake of 2 g per kg of body mass per day against 1.24 g over 12 weeks. The inconclusive results of these studies may suggest that protein intake only affects changes in strength over longer periods of time. However what must also be noted is the training experience of the participants. Whilst the subjects in both studies were young and active, the longer study (Vukovich *et al.,* 2004) used untrained subjects and the shorter study (Falvo *et al., 2005)* used experienced, resistance-trained participants. This may suggest that the untrained subjects had a greater response to the increased intake and the resistance-training programme, but as the studies were of different lengths, this cannot be assumed.

2.6 Protein supplementation

The sales of dietary supplements have increased progressively in recent times (Grandjean & Ruud, 2003; Gibson-Moore, 2012; Maughan, King & Lea, 2004). There are now many supplement companies offering a wide range of nutritional supplements including various sources of protein such as egg, soy and whey. Whey protein has a high biological value, offering a complete amino acid profile and in comparison to other sources of protein contains a higher level of the amino acid leucine (Norton *et al.*, 2012). As discussed previously, when synthesising new muscle tissue is concerned, research has identified that the amino acid leucine plays a key role in initiating muscle protein synthesis, (Anthony *et al., 2005;* Bolster *et al.,* 2004; Crozier *et al., 2000*; Fujita *et al.,* 2007) making whey an ideal source of protein when muscle building is concerned. As discussed previously, Churchward-Venne *et al.,* (2012b) also showed the advantages of whey protein supplementation against EAAs or leucine alone, supporting its use as a post resistance training supplement.

Examples of whey protein supplements are “Maximuscle Promax” and “Maxiraw WPI Intensity” (Maximuscle, 2013; Maxiraw, 2013). The “Maximuscle Promax” is advertised as “The UK’s no.1 whey protein shake” and the recommended usage is two to four servings of 30 g, including 23 g of whey protein with a leucine content of 2.6 g daily, one with breakfast, another after exercise and between meals as required (Maximuscle, 2013). The “Maxiraw WPI Intensity” recommended usage is “Add 25 g to 150 – 200 ml of water or milk, shake and consume. Use 2-3 times daily or as required” (Maxiraw, 2013). It could be argued that the “Maxiraw WPI Intensity” is a purer form of protein, containing 22 g per 25 g serving in comparison to the “Maximuscle Promax” which contains 23 g per 30 g serving. These servings are similar to those used in previous studies (Churchward-Venne *et al*., 2012b; Hulmi *et al.,* 2008), which demonstrated that the supplements were able to maximally stimulate muscle protein synthesis immediately following resistance training, and promote skeletal muscle hypertrophy over a longer period of resistance training. These products would therefore seem to be suitable protein supplements for those attempting to increase muscle mass.

While both supplements recommend increasing daily protein intake, neither takes into account the protein intake of the individual, or advises any protein intakes for increasing muscle mass and promoting hypertrophy. The recommended intakes would result in an increased daily protein intake of 44 - 92 g, depending on the dose chosen. As an example, an 80 Kg male consuming the RDA of protein of 0.8 g per Kg body mass per day, would increase their intake to 1.35 - 1.95 g per Kg, based on these guidelines. As previous research suggested an upper limit of around 1.6 – 1.8 g per Kg body mass daily (Phillips *et al.,* 2007, Tarnapolsky *et al.*, 1992), the larger recommended servings may not provide an additional benefit to the individual at a further financial cost. Additionally, those with higher than the RDA of protein already in the diet would be at more likely to not obtain any further benefit to supplementing with protein. However this would benefit the company, which may explain the recommended servings. Therefore it would seem essential that individuals are aware their daily protein intake prior to considering the use of protein supplementation to increase protein intake, to ensure that they stand to benefit from its use.

2.7 Aim of this study

This study aims to identify whether the increase of dietary protein intake through the use of a whey protein supplement to an individual engaged in a resistance training program results in greater increases in muscle hypertrophy and strength than without protein supplementation. The study will take place over eight weeks, including a four-week block with and a four-week block without an increased intake from protein supplementation. As previous studies have tended to use longer periods (12 - 26 weeks) (Esmarck *et al.,* 2001; Hartman *et al.,* 2006; Hulmi *et al.,* 2008; Vukovich *et al.,* 2004), the present study will provide an indicator of the shorter term effects, which may be of benefit for an individual or a coach intending to implement shorter term interventions to increase muscle mass and strength. The protein intake will be increased to 1.8 g per Kg of body mass daily as per the research discussed previously (Table 1), and will also aim to take advantage of the proposed benefit of consuming a protein supplement immediately post exercise (Andrews *et al.,* 2006; Esmarch *et al.,* 2001; Hulmi *et al.,* 2008).

The study will take the form of a case study of one individual. As afore-mentioned, protein intake should be based on the individual, considering factors such as energy requirements for other sporting activities, whether they wish to increase or decrease overall mass

(Phillips *et al,* 2006) and training status (Szedlak & Robbins, 2012). This study will therefore provide an example of an intervention that could be applied to an individual, and provide specific feedback for the individual and data that may be valuable to a coach. Additionally, previous studies using more participants have failed to control the dietary intakes of the groups throughout their respective studies, only recording each subjects diet at certain times during the studies (Esmarck *et al.,* 2001, Hulmi *et al.,* 2008). A case study of one participant will allow a greater degree of dietary control, enabling the dietary intake to be recorded in the period without supplementation and to be replicated accurately with the addition of the supplement. However researchers have previously highlighted the errors associated with dietary recording, notably the accuracy decreasing over time as participants fatigue (Thompson & Byers, 1994), therefore a case study of one participant aims to decrease this possibility. Smith (2010) outlines that case studies are also helpful in demonstrating theories and models, as well as obtaining results that could be generalised for similar populations.

Chapter Three

Methodology

3.0 Methodology

3.1 Participant

The case study involved one trained, male participant, aged 21, height 178 cm and body mass 84.2 Kg, of Cardiff Metropolitan University. The participant had more than two years' experience of a wide range of resistance training exercises and was not using any nutritional supplements prior to this study.

3.2 Procedure

3.2.1 Experimental Summary

The study consisted of an eight-week resistance training program, divided into two blocks of four weeks. The first four-week block involved the participant recording their dietary intake for all macronutrients. This was done through the use of electronic scales (HoMedics Group, Salter Evo Electronic Scale 1241 BKDR) and macronutrient information on the food's nutritional labelling. The data was collected using a food diary created in Excel (Microsoft, USA) (Appendix A). The second four-week block consisted of the continued use of the resistance training program, the participant replicating the recorded diet, but with the addition of a whey protein supplement. The girths of the upper arm and thigh, as well as skinfolds of the biceps, triceps, subscapular, iliac crest and the mid-thigh were measured in order to calculate skeletal muscle hypertrophy. This was done through calculating the lean cross sectional areas of the upper arm and thigh, as well as enabling the total body fat percentage of the individual to be measured. Strength was also measured through the one repetition max testing of the Bench Press and Squat exercises.

3.2.2 Dietary Assessment and Protein Supplementation

The participant's diet was recorded using a food diary, created using Excel, (Appendix A, Figure 3) and completed by weighing individual foods using an electronic scale, (HoMedics Group, Salter Evo Electronic Scale 1241 BKDR) at the time of consumption, to reduce the error associated with self-reporting dietary intake, as recommended by Thompson and Byers (1992). The macronutrient data was recorded by the participant, from the individual food nutritional labels, in an attempt to increase individual food accuracy. The supplement use followed the guidelines of the research discussed previously, namely the use of a whey protein supplement immediately post resistance training, and to increase the total daily protein intake to 1.8g/Kg, which was a figure consistent with the recommended intakes shown in Table 1. The supplement used was the “Maxiraw WPI Intensity” due to the higher content of protein in comparison to the “Maximuscle Promax” (22g in a 25g serving in comparison to 23g in a 30g serving, respectively). The amount of supplement required each day was calculated from the dietary intake data collected in a food dairy during the initial four-week block and the value measured for total body mass at the halfway stage.

3.2.3 Resistance Training Program

The resistance-training program involved three training sessions per week incorporating a range of exercises designed to incorporate the muscles of the whole body. Previous studies have shown success when using a similar method for skeletal muscle hypertrophy and increases in strength (McCall, Byrnes, Dickinson, Pattany & Fleck, 1996 & DeFreitas, Beck, Stock, Dillon & Kasishke, 2011). The exercises included the barbell squat to incorporate the muscles of the upper leg, the barbell bench press to incorporate the muscles of the chest, shoulder and the triceps, the dumbbell shoulder press incorporated the muscles of the shoulder and the triceps, the bent over barbell row was used to incorporate the muscles of the back and the bicep curl to isolate the bicep muscles (Baechle and Earle, 2008; Graham, 2003; Saeterbakken & Fimland, 2013).

The exercises were performed at 75% of the participant’s one repetition max, involving four sets of six repetitions with 90 seconds of rest. Baechle *et al.* (2008) state strength is best improved using greater loads and lower repetitions, typically below six repetitions at loads of 80% or greater with rest periods of 2 – 5 minutes to recover sufficiently. Whereas hypertrophy; the enlargement and increase in number of muscles fibres, is best achieved using repetition ranges typically between six and twelve repetitions with a load between 60 and 75% and a shorter rest period of 45 - 90 seconds. The load, repetitions and rest period in the resistance-training program were chosen based on these guidelines, in an attempt to deliver both hypertrophy and increases in strength. Table 2 below shows the resistance training program including the exercises, sets, repetitions, rest periods and the muscles involved in each exercise.

Table 2. Resistance Training program

Exercise	Sets	Repetitions	Rest Period (s)	Muscles involved
Barbell Squat	4	6	90	Gluteus maximus, Semimembranosus, Semitendinosus, Biceps femoris, Vastus lateralis, Vastus intermedius, Vastus medialis , Rectus femoris.
Barbell Bench Press	4	6	90	Pectoralis major and minor, Anterior Deltoid, Serratur anterior, triceps brachii.
Standing Dumbell shoulder press	4	6	90	Anterior and medial deltoids, Triceps.
Bent over Barbell Row	4	6	90	Latissimus dorsi, Teres major, Middle trapezius, Rhomboids.
Machine Bicep Curl	4	6	90	Biceps Brachii, Brachialis, Brachioradialis.

3.2.4 Test Protocols

Testing took place prior to the first four-week block, at the midway point prior to starting the second four-week block, and following the completion of the second block. The participant's height, body mass, skinfold measurements, upper arm and thigh girths were measured in order to calculate body fat percentage, lean cross-sectional areas of the upper arm and thigh. Strength was also measured at these stages. These were used to calculate muscular hypertrophy and changes in strength during both the supplement free program, and the supplemented program.

Height was measured using a fixed stadiometer (Holtain, Fixed Stadiometer, Crymych, UK) and body mass using digital scales (SECA, 770, Hamburg, Germany). Skin fold callipers (Baty International, Harpenden Skin Fold Calliper, Burgess Hill, UK) were used to obtain skinfold measurements of five different sites; the iliac crest, triceps, subscapular, mid-thigh and the biceps as per the recommendations of Eston, Hawes, Martin and Reilly (2009). Girths of the thigh and the upper arm were measured using a tape measure (SECA, 201, Hamburg, Germany). Throughout these tests, three trials were used to obtain a more reliable mean value. The strength of the upper and lower body was measured using the bench press and back squat exercises, respectively (Baechle and Earle, 2008). Strength was measured using the one repetition max test (Harman and Garhammer, 2008), which was performed in a Power Rack, using a 7ft Barbell with Rubber weight plates and an adjustable Bench (Life Fitness, Hammer Strength Athletic Series, Ely, UK). The participant began performing single repetitions at an estimated 50% of their one repetition max and increased the load by approximately 10% following each repetition until reaching approximately 90%, where the increase was decreased to the minimal increase allowed by the weight plates, 2.5Kg.

The skinfolds of the iliac crest, triceps, subscapular and mid-thigh were used with the body fat percentage equation devised by Peterson, Rhea and Alvar (2003). This equation was chosen as previous research has shown including skinfolds from the lower limbs increased the accuracy of body fat percentage predictions (Eston, 2003; Eston, Rowlands, Charlesworth, Davies & Hoppitt, 2005). The lean cross sectional areas (LCSA) of both the thigh and upper arm were then calculated using these girth measurements and the skinfolds of the thigh, the biceps and triceps. Firstly the diameter of each body segment was calculated taking girth as the circumference in the equation Circumference = $\Pi \cdot d$ where d is the diameter. The value for the diameter was then halved, and following removal of the subcutaneous fat from half the relevant skinfolds, the value for lean radius was used in the equation Area = $\Pi \cdot r^2$ to calculate the lean cross sectional area of each body segment. This value was used to determine muscle hypertrophy.

3.3 Data Analysis

Statistical analysis took place within Excel, where a paired T-Test compared dietary macronutrient intake without a protein supplement against the addition of a supplement

Chapter Four

Results

4.0 Results

Figure 1 shows the daily macronutrient content and total calorie intake of the participant's recorded diet over the eight-week program. The supplementation of whey protein during the second four-week block resulted in a significantly greater (P Value < 0.01) protein intake than in the first four-week block. The mean protein intake over the first four-week block was 104 g·day^{-1} (± 19.3) whilst the mean protein intake with supplementation during the second block was 148.9 g·day^{-1} (± 6.7). As the diet recorded during block one was replicated during block two, carbohydrate (CHO) and Fat intakes were not significantly different between blocks one and two. The mean intake for CHO was 231.7 g·day^{-1} (± 47.4) and 230.9 g·day^{-1} (± 49.6) and the mean Fat intake was 98.1 g·day^{-1} (± 25.8) and 98.4 g·day^{-1} (± 26.0) for blocks one and two, respectively. However, mean total calorie intake in block two was significantly greater (P Value < 0.01) than in block one due to the supplementation of protein (2459.8 ± 348.5 and 2280.3 ± 377.8 kCal·day^{-1}). No alcohol was consumed during the duration of the study.

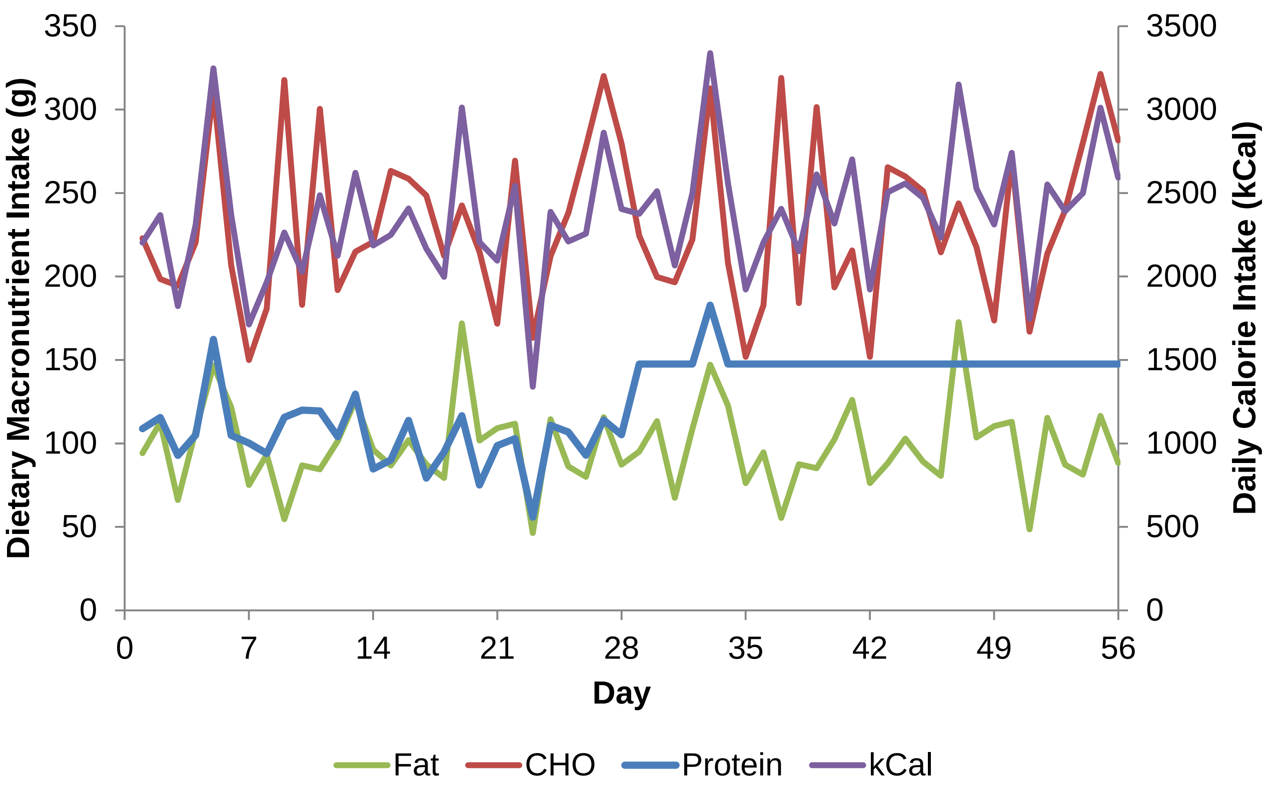

Figure 1. Daily macronutrient and total calorie intake

Table 3 shows the directly measured variables. The resistance training programme with the participant's normal dietary intake brought a decrease in total body mass as well as both upper arm and thigh girth. The change in skinfolds varied, decreasing at the biceps and the subscapular, but increasing at the triceps, iliac crest and mid thigh. Strength increased in the squat exercise, but remained constant in the bench press. In the four week period where the subject consumed an increased protein intake and a protein supplement post exercise, total body mass decreased again, but the girths of the upper arm and thigh increased and the skinfold measurements all decreased. Strength increased in the squat exercise again, but there was a reduction in strength in the bench press exercise.

Table 3. Directly measured variables

		Pre Block 1	Post Block 1 Pre Block 2	Post Block 2	% Change Without Supplement	% Change With Supplement
	Mass (Kg)	84.2	82.0	79.8	-2.6	-2.7
Girth (cm)	Upper Arm	36.4	36.1	36.3	-0.8	0.6
	Thigh	62.3	60.6	61.1	-2.7	0.8
Skinfold (mm)	Bicep	6.7	6.3	5.5	-6.4	-12.7
	Triceps	10.2	11.8	9.5	15.4	-19.0
	Subscapular	12.6	12.3	11.5	-2.4	-6.2
	Iliac Crest	9.1	11.2	8.7	23.5	-22.6
	Mid Thigh	15.5	16.0	14.8	3.2	-7.9
Strength (Kg)	Bench Press	100	100	97.5	0.0	-2.5
	Squat	132.5	137.5	140	3.8	1.8

Table 4 displays the variables calculated from the discrete variables in Table 3. Without the use of a protein supplement, body fat percentage increased, whilst lean mass and fat mass calculated from this percentage both decreased. The lean CSA of both the upper arm and the thigh also decreased during this period. The subject's strength to mass ratio increased in both the bench press and squat exercises without supplementation. With the use of a protein supplement, the participant's body fat percentage decreased over four weeks, whilst more of the total body mass reduction was attributed to a loss in fat mass rather than lean mass in comparison to without supplementation. Unlike without supplementation, lean CSA of both the upper arm and thigh increased with the use of a protein supplement. The strength to mass ratio in the bench press exercise did not change with supplementation, as both strength and mass decreased. However the strength to mass ratio of the squat increased but not to the extent at which it did prior to supplementation.

Table 4. Calculated Variable

	Pre Block 1	Post Block 1 Pre Block 2	Post Block 2	% Change Without Supplement	% Change With Supplement
% Body Fat	23.3	23.5	23.2	0.7	-1.2
Lean Mass (Kg)	64.6	62.8	61.3	-2.8	-2.3
Fat Mass (Kg)	19.6	19.2	18.5	-1.9	-3.9
Upper Arm Lean CSA (cm)	88.9	88.2	91.9	-0.8	4.2
Thigh Lean CSA (cm)	262.4	246.1	253.3	-6.2	3.0
Bench Press Strength to mass ratio	1.19	1.22	1.22	2.5	0
Squat Strength to mass ratio	1.57	1.68	1.75	7.0	4.2

Chapter Five

Discussion

5.0 Discussion

5.1 Summary of Findings

The aim of this study was to demonstrate the effect that a whey protein supplement had on muscle hypertrophy and strength. The supplement did not have an effect on the total body mass reduction evident throughout the study, although there was a significant increase in both daily calorie and protein intake. In contrast to the period without supplementation, there was a consistent and greater decrease in the participant's skinfold measurements with the protein supplement, and an increase in the girths of the upper arm and thigh, which suggested an increased lean CSA of both segments, and reduced subcutaneous fat. These results also suggest that a greater percentage of the decrease in total body mass was attributed to fat mass, and less to lean mass, as percentage body fat decreased only in the supplemented four week period. Strength, and strength to mass ratio increased in the squat exercise but less so with the supplement. Whilst in the bench press, no change was demonstrated prior to supplementation, leading to an increase in the participant's strength to mass ratio, but decreased with the supplement, resulting in no change in the strength to mass ratio.

5.2 Nutritional Profile

The dietary assessment of the participant highlighted that using a protein supplement brought a significant increase to their protein intake. Prior to supplementation, the participant was consuming 104 $g \cdot day^{-1}$ (± 19.3), which equated to 1.2 g (± 0.2) per Kg body mass, before increasing intake to 1.8 g per Kg with the supplement. Based on the values recommended by previous research (Table 1), this demonstrated that whilst the participant may have been consuming enough protein to produce some muscular hypertrophy, the individual may not have been consuming an optimal amount for inducing muscular hypertrophy. Secondly, figure 1 shows that protein intake prior to supplementation varied from day to day, which is also demonstrated by the standard deviation of daily protein intake (± 19.3 g). These points demonstrate that the participant's dietary intake may not have produced the greatest amount of skeletal muscle hypertrophy. Therefore athletes may benefit from carrying out dietary assessments, as well as having an increased awareness of their dietary intake. This will allow an athlete to take advantage of the potential training stimulus, to optimise changes in both body composition and performance. Additionally, this study provides support to the use of a whey protein supplement to increase the dietary intake of protein, as the participant was able to do so with relative ease, without making radical changes to their original diet.

A notable flaw in the participant's diet whilst attempting to increase muscle mass through muscular hypertrophy was a lack of energy provision, which resulted in the reduction in total body mass evident throughout the study. In order to increase muscle mass, the participant would have needed to be in a state of energy surplus (McArdle *et al.,* 2010). Moreover, the increased calorie intake from the supplementation of protein did not result in a reduction of the body mass lost, but caused a slightly greater decrease in total body mass, although the results were very similar during both periods. In this case, the protein supplement alone was not sufficient to achieve a positive energy balance, therefore a further increase in daily calorie intake would be required to increase muscle mass. This again, emphasises the benefit of a dietary assessment and the importance of athletes' awareness of dietary intake.

Whilst the study attempted to improve the accuracy of the nutritional data collected by taking nutritional information directly from the food labels, previous research has recommended food be analysed using a nutritional database or nutritional software (Thompson & Byers, 1994). This could be considered a limitation of this study, as errors may have occurred from the participant recording the data.

5.3 Body Composition

The increased upper arm and thigh girths, in addition to the consistent reduction in the skinfolds measured at all sites in the period using the protein supplement, suggest that the estimated lean CSA of both segments increased. Whilst the results may lack the accuracy of studies such as Hulmi *et al.,* (2008), which used magnetic resonance imaging (MRI), and suggested this may be the most accurate method for measuring lean CSA, there is evidence to suggest that some hypertrophy of the muscles of these segments occurred. This is in contrast to the decreased girths, and decreased lean CSAs evident without the supplement. These results therefore support the proposals made by previous researchers that higher protein intakes, in this case 1.8 g over 1.2 g per Kg body mass daily (Phillips *et al.,* 2007; Tarnapolsky, 1992; Tarnapolsky, 2000) and protein consumption post resistance exercise (Andrews *et al.,* 2006; Churchward-Venne *et al.,* 2012b; Esmarck *et al.,* 2001; Hulmi *et al,* 2008) will result in greater muscular hypertrophy.

In contrast to the period without the supplement, the skinfold measurements decreased consistently at all sites suggesting a consistent reduction in subcutaneous fat with the supplement. The body fat percentage calculated shows that in the supplemented period, body fat was reduced, in comparison to an increase without supplementation. Values for lean and fat mass derived from this body fat percentage highlight that the use of a supplement caused a greater reduction in fat mass, and a lesser reduction in lean mass. However these results must be treated with caution, as there is a measurement error associated with this method of calculating body fat (Peterson *et al.,* 2003). Nonetheless, this data does support a notion mentioned by Phillips *et al.,* (2007), that increased protein intakes during a period of caloric restriction will result in the preservation of lean mass, which has also been supported in recent reviews by Churchward-Venne, Murphy, Longland and Phillips (2013) and Helms, Zinn, Rowlands & Brown (2013). Whilst these reviews recommended varying daily protein intakes of 1.2 – 2.3 g (Churchward-Venne *et al.,* 2013) and 2.3 – 3.1 g (Helms *et al.,* 2013) per Kg body mass, the results of this study suggest that the higher the protein intake during energy restriction, the more lean mass is preserved.

However these results also provide conflicting notions. Whilst the results suggest that the hypertrophy of the skeletal muscle has occurred, suggesting an increase in muscle mass, it is also evident that there is a reduction in lean mass. Possible explanations of this are whilst the muscles of the segments measured may have hypertrophied, other muscles may not have, and may have atrophied. Muscular atrophy would be expected in the muscles that the resistance training did not include, such as the abdominal muscles or the muscles of the lower leg. As these muscle were not used, muscle protein synthesis would not have occurred, and an excess in breakdown would have resulted in a negative protein balance (Appell, 1990; MacDougall, Elder, Sale, Moroz & Sutton, 1980). This can be considered a notable limitation of the resistance training program. Other suggestions for the loss of lean mass include the depletion of glycogen stores both in the liver and the muscles as well as the water associated with glycogen storage (McArdle *et al.,* 2010).

5.4 Strength

Strength and the participant's strength to mass ratio did not increase a greater amount with the use of a protein supplement over four weeks. The squat exercise showed a smaller increase in both strength, and strength to mass ratio with supplementation, whilst there was a reduction in bench press one rep max but the participant's strength to mass ratio remained the same. A possible explanation for this may be the law of diminishing returns in strength training, where the adaptations to a training programme decrease over time (Peterson, Rhea and Alvar, 2005). This may explain how the resistance training programme caused less of an adaptation during the second four week period, which immediately followed the first, which has been demonstrated in previous studies (Staron *et al.,* 1994). This data does provide some support the notion that protein intake may only affect strength gains in the longer term, as studies have shown greater increases in lower body strength only over twelve weeks, (Falvo *et al.,* 2005) but larger increases in both upper and lower body strength over six months (Vukovich *et al.,* 2004).

Although the resistance training program intended to induce both muscular hypertrophy and increases in strength, it could be argued that the load, repetition and rest period scheme used favoured strength based adaptations, such as neural adaptations, rather than hypertrophic adaptations (Baechle *et al.,* 2008). Whilst adaptations may be possible in both strength and hypertrophy, it could be argued that for more prominent adaptations in either strength or hypertrophy, the training programme should be designed to favour those adaptations, as suggested by Baechle *et al.,* (2008).

5.5 Limitations, Future Research and Practical Applications

In addition to the afore-mentioned limitations of the study, other limitations were evident, which also provide suggestions for future studies and research in this area. This study did not distinguish the importance of protein intake over timing. Although it has been previously demonstrated that timing may have a greater effect on muscular hypertrophy in elderly subjects (Andrews *et al.,* 2006), a recent meta-analysis (Schoenfeld *et al.,* 2013) highlighted that this may not be the case for all populations, and that intake was the main factor in the accretion of muscle mass, but also brought attention to the fact that most of the studies used untrained subjects. Therefore, a suggestion for future research is identifying effects of intakes and timing on trained subjects, a notion also highlighted by Schoenfeld *et al.,* (2013).

However this study did provide an insight into what can be achieved over a four-week period, when using a whey protein supplement and utilising the theories previously suggested to maximise muscle hypertrophy (daily protein intake and timing protein consumption post resistance training) are implemented on a trained subject. Whilst two periods of four weeks may not be of sufficient time to demonstrate larger, long term changes, the study may be of value to individuals and coaches implementing interventions to manipulate muscle mass in individuals, particularly in the shorter term, such as in off season or pre season stages of training. The study highlighted the benefits of carrying out dietary assessments, and having an increased awareness of dietary intake, so that optimal changes in body composition can be achieved. The whey protein supplement also made for an effective, and easily implemented method for increasing an individual's protein intake, which aided in the preservation of lean mass, and may have induced some muscular hypertrophy, whilst in an energy deficit.

5.6 Conclusion

To summarise, it seems that a protein supplement makes for an effective and relatively simple method of increasing an individual's protein intake, as well as providing a convenient source for post exercise nutrition. This study also highlighted the benefits of performing dietary assessments, and that an individual may stand to benefit from a more consistent dietary intake tailored to their goals. In this case study, the protein supplement was able reduce the loss of muscle mass, and may have induced skeletal muscle hypertrophy, on an individual in a four-week resistance training program and in a negative energy balance. This study supports previous studies in the suggestion that this is primarily due to an increased protein intake (Churchward-Venne *et al.,* 2013; Helms *et al.,* 2013; Schoenfeld *et al.,* 2013) but timing the intake of a protein supplement immediately post resistance training may also have a beneficial effect (Hulmi *et al.,* 2008). However over the short period that this study took place, the use of a protein supplement did not promote greater increases in strength.

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Appendices

Appendix A

	Quantity	kCal	Pro	CHO	Fat	Fibre
Wednesday						
Breakfast						
Lunch						
Dinner						
Snacks						
Total		0	0	0	0	0

Figure 2. Food Diary used to record the participant's diet.

Appendix B

Ethical Status

Date: 17th March 2014

To: Sion Evans

Project reference number: 13/10/27U

Your project was recommended for approval by myself as supervisor and formally approved at the Cardiff School of Sport Research Ethics Committee meeting of [include the one that applies 29th May 2013, 26th June 2013, 24th July 2013, 16th October 2013, 27th November 2013].

Yours sincerely

Supervisor