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Supplementation With Milk Protein Promotes Similar Changes in Strength and Muscle Mass as Isocaloric Supplementation of Native Whey During Strength Training in Elderly Subjects.

An 11 week double blinded randomized controlled trial.

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Summary

Background: Milk protein and especially the whey fraction of milk protein have gained a lot of interest due to its effective stimulation of postprandial muscle protein synthesis. Whey protein can be isolated from pasteurized milk by a multi-filtration technique providing concentrated native whey protein which contains a higher leucine content than the traditional concentrate derived from cheese production. Leucine seems to be a key stimulator of muscle protein synthesis, and is suggested to be even more so in elderly. This is because the optimal protein dosage needed for stimulation of muscle protein synthesis, may be lowered with a higher leucine content. ***Aim:*** In this study we investigated whether daily supplementation of native whey protein could lead to larger muscle hypertrophy during 11 weeks of heavy load strength training than an isocaloric supplementation of milk protein in elderly subjects. ***Methods:*** 26 elderly (age 73.5±2.7 years) men and women received either daily supplementation of 40 g (2x20 g) native whey or milk protein during 11weeks of heavy load strength training (3 sessions per week), following daily undulating linear progression. The experiment was conducted as a double blinded randomized controlled trial. ***Results:*** Similar gains in lean body mass (LBM) (measured by DXA) was achieved in the two groups, with changes of 1.8±0.7 kg (3.8%) (mean ±95% CI) and 2.4±0.7 kg (5.2%) for the native whey and milk groups respectively. Regional changes were also similar, with leg LBM changes of 0.67±0.27 kg (4.2%) and 0.93±0.30 kg (6.0%). Thickness of *m.vastus lateralis* increased by 0.13±0.06 cm (6.1%) and 0.15±0.07 cm (7.2%) for native whey and milk groups respectively. Both supplementations also induced similar changes in muscle strength (1RM tests), with changes of 22% and 20% in chest press, and 38% and 31% in leg press in the native whey group and milk groups respectively. Furthermore, both groups improved performance in functional tests, with improvements of 6.4% and 4.2% in stair climb and by 11.6% and 9.2% in timed sit to stand tests for the native whey and milk groups respectively. Although only the native whey group improved their loaded (+10 kg and +20 kg) stair climb performance (6.4% and 7.5%) there were no differences between groups for any muscular or performance parameters. **Discussion/Conclusion:** Supplementation with native whey and milk protein were equally effective in supporting changes in body composition, strength and functional performance during a period of strength training. This may be due to the high concentration of leucine in the milk protein utilized, resulting in only a small difference between the products. If any difference exists between the two products in eliciting muscular or functional changes following strength training in elderly this could not be detected in the present trial, and the two protein supplementations should therefore be regarded as equally effective.

Keywords: *Protein, Elderly, Native whey, Milk, 1RM , LBM.*

Preface

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Mathias Korterød Johansen

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Contents

Abbreviations

Akt	Protein kinase B (Akt)
BCAA	Branched chain amino acids
CI	Confidence interval
CT	Computed tomography
CV	Coefficient of variation
CSA	Cross-section-area
DXA	Dual x-ray absorptiometry
EEA	Essential amino acids
eIF4E	Eukaryotic translation initiation factor 4E
FSR	Fractional synthetic rate
g/kg/day	Gram per kilo of bodyweight per day
IGF-1	Insulin-like growth factor 1
LBM	Lean body mass
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging
MSc.	Master of Science
mTOR	mammalian target of rapamyocin
MVC	Maximal voluntary contraction
NSSS	Norwegian School of Sport Sciences
NW	Native Whey
P70S6K	70kDa ribosomal protein S6 kinase 1
SPPB	Short Physical Performance Battery
VL	*m.Vastus lateralis*

1. Background

Skeletal muscle is a highly plastic tissue that exhibits a substantial ability to adapt to changes in activity stimulus (Gundersen, 2011). For example, in response to strength training the muscles may adapt by increasing muscle mass and strength (Bassel-Duby & Olson, 2006). Conversely in response to sedentary behavior or immobilization there is usually a net loss of muscle mass and performance (Bassel-Duby & Olson, 2006). In addition, muscle mass also changes due to normal growth throughout the human lifespan (Lexell, Taylor, & Sjostrom, 1988). During childhood and adolescence the muscle mass increases, and during ageing from maturity to senescence, muscle mass declines (Lexell, Henriksson-Larsen, Winblad, & Sjostrom, 1983; Lexell et al., 1988).

Ageing related atrophy of the muscle is reported to begin around 25 years of age and thereafter accelerates (Lexell et al., 1988). Studies have reported that there is a 30-50% decrease in skeletal muscle mass in both men and women, between the ages of 40 and 80 years (Faulkner, Larkin, Claflin, & Brooks, 2007; Lexell et al., 1988). In agreement others have reported that approximately 1% of muscle mass is lost per year after the fourth decade of life (Baumgartner et al., 1998).

The inevitable age-associated decline of muscle mass and physical function is termed sarcopenia (Rosenberg, 1989). Characteristic of this ageing-related atrophy is that the decrease in muscle mass is accompanied by an even greater decrease in strength (Frontera, Hughes, Lutz, & Evans, 1991; Goodpaster et al., 2006) and power (Bassey et al., 1992). In a cohort with 1880 participants (aged 70-79) the annual loss in strength was 3 - 4% faster than the decline in muscle mass in the same individuals (1%) (Goodpaster et al., 2006). This implies that sarcopenia does not only involve loss of muscle mass but also a decline in muscle quality (e.g. changes in basic myofilament structure and function) (Frontera, Zayas, & Rodriguez, 2012).

The loss of muscle mass seen in older adults has significant physiological, functional and health consequences. Often, concurrent with the loss of muscle mass is the decreased ability to perform functional tasks such as climbing stairs, doing household chores and other tasks that require muscular strength (Cawthon et al., 2009). Sarcopenia is also associated with an increased risk for developing chronic metabolic diseases such

as diabetes (Addison, Marcus, LaStayo, & Ryan, 2014; Koopman, 2011). Sarcopenia is therefore considered one of the major causes of frailty and disability in the elderly population and significant impact the quality of life for those affected. The implication of an ageing population worldwide increases the prevalence of sarcopenia and it is assumed that the world's population aged 60 years or above will triple within the next 50 years (Koopman, 2011). For example in Norway there are currently living 1.1 million Norwegians aged ≥ 60 and it is estimated that the share of elderly in the population (aged 67 or more) will increase from its present 15% (2004), to 25% by 2050 and 30% by the year 2100 (Bye, 2004). As a result of this development, sarcopenia has become a major subject of scientific research as well as a public health problem of large dimensions.

In essence, the regulation of muscle mass reflects the balance between muscle protein synthesis and protein breakdown. The relatively slow rate of muscle decline during ageing must mean that there is an overall relatively small negative protein balance. However, it is currently substantial evidence showing that basal fasting protein synthesis and (or) breakdown rates are not different between young and elderly adults (Cuthbertson et al., 2005; Hasten, Pak-Loduca, Obert, & Yarasheski, 2000; Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2005). Therefore, to better understand and to identify interventions that counteract or delay the decline in skeletal muscle mass in the elderly, research have started to focus on the regulation of muscle protein balance in response to anabolic stimuli such as physical activity and food intake. In this regard, both strength training and nutrition interventions have been shown to be promising means to increase muscle mass and strength, and improve physical capabilities in older adults (Frontera, Meredith, O'Reilly, Knuttgen, & Evans, 1988). However, data from more recent studies suggests that the anabolic response to the ingestion of essential amino acids is attenuated in the elderly compared with young controls (Cuthbertson et al., 2005; Katsanos et al., 2005; Volpi, Kobayashi, Sheffield-Moore, Mittendorfer, & Wolfe, 2003). In addition it is shown that the ingestion of glucose together with amino acids does not augment the anabolic response on muscle protein synthesis to the positive effect of amino acids alone in elderly compared to young (Volpi, Mittendorfer, Rasmussen, & Wolfe, 2000). It is therefore postulated that this "anabolic-resistance" is one of the main factors responsible for the age-related decline in muscle mass (Koopman, 2011).

In general there are mainly two factors that govern the effect a given protein intake has on muscle protein synthesis. Firstly, the amino acid composition of the protein intake is a key-determining factor. Essential amino acids exert a significant stimulatory effect on muscle protein synthesis (Volpi et al., 2003), and especially the branched chain amino acid leucine has been shown to be an essential regulator that stimulates protein synthesis the first 1-3 hours after ingestion in young (Glynn et al., 2010) and ageing (Casperson, Sheffield-Moore, Hewlings, & Paddon-Jones, 2012) human skeletal muscle. Notably, in terms of muscle protein synthesis, the addition of nonessential amino acids to an essential amino acid supplement does not stimulate protein synthesis additionally (Volpi et al., 2003). However newer findings suggest that the non-essential amino acids may be needed for continued increased rates of synthesis after the leucine induced initiation in humans (Churchward-Venne et al., 2012). Secondly, the digestibility and/or absorption kinetics of the protein ingested are also shown to affect the rate of muscle protein synthesis. For example, the combination of fast (whey) and slow (casein) proteins found in milk is shown to promote greater increase in muscle protein synthesis than an isonitrogenous and isoenergetic soy-protein beverage after exercise in young individuals (S. B. Wilkinson et al., 2007). When the two fractions of milk protein are investigated against one another, whey protein is found to be superior in stimulating postprandial muscle protein synthesis in the first phase after ingestion (Pennings, Boirie, et al., 2011).

In the context of developing nutritional strategies for preserving muscle mass in elderly it would be desirable to optimize both the amino acid composition and the bioavailability of the protein intake for maximizing muscle protein synthesis. In relation to this, many studies have shown that free-form essential amino acids and whey protein supplements promote muscle synthesis after strength exercise in both young (Burke et al., 2001; Paddon-Jones et al., 2004) and elderly people (Paddon-Jones, Sheffield-Moore, Katsanos, Zhang, & Wolfe, 2006; Paddon-Jones et al., 2004). Previous acute studies in our lab show promising results of native whey protein supplementation. These results show that post-exercise ingestion of native whey results in both higher plasma concentrations of leucine and other essential amino acids (Laahne, 2013; Nyvik Aas, 2014), and increased p70S6K phosphorylation compared to post exercise milk ingestion (Nyvik Aas, 2014).

In this master thesis we aimed to compare the effects of supplementing with these two different protein sources during a long-term strength training intervention in elderly subjects.

Aim:

To compare the effects of daily supplementation with either 40g of milk or native whey protein on muscle mass and muscle function in untrained elderly subjects, during 11 weeks of progressive strength training.

Hypothesis: *The group supplemented with native whey protein will experience larger increases in lean body mass, muscle mass, and strength than the group supplemented with equal amounts of milk protein.*

The rationale for this hypothesis was that native whey protein, due to its higher leucine content was supposed to increase anabolic signaling and the following increase in muscle protein synthesis in the elderly to a larger extent than regular milk protein.

2. Theory

2.1 Regulation of skeletal muscle mass

Skeletal muscle is a plastic tissue that adapts to use and disuse. In response to increased work load (for example heavy strength exercise) the muscle will increase in mass (hypertrophy) and strength. Conversely, in response to disuse the muscle mass will decline (atrophy). Since there is little turnover of skeletal muscle cells, gain or loss of muscle mass mainly reflect the balance between protein synthesis and protein degradation in permanent muscle fibers.

The mammalian target of rapamyocin (mTOR) is considered the main regulator of protein synthesis, which when activated results in an increased translation efficiency (Bodine et al., 2001). The most known activators of mTOR are the Insulin-like growth factor (IGF-1) and its downstream protein kinase B (Akt). When activated, mTOR increase protein translation by phosphorylating the p70S6K which in turn phosphorylates the ribosomal S6 protein (S6K1) (Goodman, Kotecki, Jacobs, & Hornberger, 2012). Phosphorylated S6K1 protein is important for the translation process during protein synthesis (Kimball, 2002). mTOR also phosphorylates the translational repressor 4E binding protein (4EBP1). This relieves the translation factor eIF4E, which then in turn activates the ribosome and protein synthesis (Atherton et al., 2005).

The main regulatory pathway for the process of protein degradation is the myostatin - smad 2/3 pathway (Schiaffino, Dyar, Ciciliot, Blaauw, & Sandri, 2013). The process of protein degradation relies on the ubiquitin-proteasome system and the autophagy-lysosome systems (Schiaffino et al., 2013). The contribution of these two pathways of protein breakdown varies, but they collectively contribute to the turnover of protein and if the rates of protein breakdown exceed those of protein synthesis, muscle mass will be lost (atrophy).

2.2 Anabolic effect of resistance exercise

Many studies report that the mTOR signaling pathway is activated in response to resistance exercise in humans (Eliasson et al., 2006; Terzis et al., 2008; S. B. Wilkinson et al., 2008). The findings that early exercise-induced increase in protein synthesis is blocked by rapamyocin treatment (Drummond et al., 2009), and that the phosphorylation status of S6K1 after exercise is a good indicator for long term increase in muscle mass (Terzis et al., 2008), both indicate that mTOR signaling play a crucial role in mediating the hypertrophic effect of strength training.

The anabolic response following resistance exercise seems to be impacted by both the volume and intensity of the exercise (Burd, West, et al., 2010). High volume-low intensity (30%) exercise performed until failure, elicits a greater anabolic response than low volume–high intensity (90%) performed until failure, or low intensity exercise (30%) work matched to that of high intensity (Burd, West, et al., 2010). These data suggest that performing exercise until failure is important for the synthetic response following exercise; also with a higher volume proving more favorable (Burd, West, et al., 2010). In addition time under tension is shown to be important for the response (Burd, Andrews, et al., 2012). In summation, it seems that several aspects of the resistance exercise (intensity, time under tension, failure and volume) modulate the size of the anabolic response.

2.3 Regulation of skeletal muscle mass and ageing

Muscle protein synthesis is stimulated by exercise in both young and old subjects (Frontera et al., 1988). However, some studies suggest that elderly subjects have a blunted muscle protein synthesis response compared to younger subjects (Kumar et al., 2009). Kumar et al. (2009) reported that mTOR activation (phosphorylation of p70S6K and 4EBP1) was reduced in the elderly compared to young controls after heavy resistance exercise. Aging does however, not affect muscle protein breakdown at rest or following resistance exercise (Fry et al., 2013).

2.4 Anabolic response to food intake

Protein balance in skeletal muscle is affected by nutrient intake (Rennie et al., 1982). Ingestion of essential amino acids is shown to directly activate regulatory proteins in mRNA translation, while non-essential amino acids have no effect on stimulation of muscle protein synthesis. Especially the branched-chain amino acid leucine, is shown to directly increase mTOR activity and its downstream effectors 4EBP1 and S6K1 (Norton & Layman, 2006). Hence, leucine seems to be the main amino acid that stimulates postprandial increase in muscle protein synthesis.

As the positive effect of protein ingestion has been established, the quantities needed for optimal stimulation has been investigated. The amount of protein required to detect an increase in muscle protein synthesis (MPS) is reported to be dose dependent up to 10 g of essential amino acids, or the equivalent amount of whole protein (~20 g), while further increases in dosages fail to elicit further increases in stimulation (at least in young healthy subjects) (Cuthbertson et al., 2005).

Few studies have investigated protein breakdown, possibly due to more complicated and less available measuring methods. When investigated, the plateau in anabolic response seen with synthesis measurements alone is no longer so apparent. In mixed meals it has been suggested that no practical limitation to the anabolic effect of increased dosage sizes exist, as net protein balance continues to rise with higher dosages (Deutz & Wolfe, 2013). However, one important function of protein degradation process is to contribute to the turnover of proteins within the cell. Hence, the complete removal of breakdown would be disadvantageous as protein quality could be impaired.

Sufficient intake of protein has been established to be slightly over 0.8 g/kg/day in elderly individuals (Campbell & Leidy, 2007). The present recommended daily allowance is now 1.2 g/kg/day of protein for the Nordic countries (Nordic Council of Ministers, 2014). When the total protein intake is manipulated there is no difference between a high (1.5 g/kg/day) or very high protein (3.0 g/kg/day) intake on postprandial muscle protein synthesis in both young and elderly subjects (S. Walrand et al., 2008).

This implies that an adequate intake is sufficient. Furthermore, since high protein intake may have adverse effects on kidney functions in the elderly (S. Walrand et al., 2008), it is important to investigate the effect of protein quality for this age group.

2.5 Anabolic response to combined exercise and nutrition

The effect of combining two anabolic stimuli such as resistance exercise and protein supplementation results in synergistic effects, leading to increased muscle protein synthesis, and a positive net protein balance (Biolo, Tipton, Klein, & Wolfe, 1997; Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000; Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999).

When synthetic rates of different muscle fractions are investigated following unilateral exercise and protein consumption, myofibrillar fractional synthetic rate (FSR) is shown to be increased to a further extent in the exercised muscle than in resting muscle (Moore, Tang, et al., 2009). Interestingly the sarcoplasmic FSR is found to be exclusively stimulated by feeding, as no differences are found between exercised and rested muscle (Moore, Tang, et al., 2009).

Investigation of protein intake following resistance exercise reveals a plateau in MPS stimulation with a dosage of 20 g of egg protein. Increasing the dosage up to 40 g only results in a significantly increased oxidation of traced amino acids with no additive effect on the rate of MPS (Moore, Robinson, et al., 2009). Similar results are found when a whey protein supplement is used. Increasing the dosages from 20 g to 40 g of whey protein, does not lead to higher muscle protein synthesis, but instead an increased oxidation and excretion of nitrogen. However, increasing the protein dosage up to 20 g seems to be important because intake of low dosages of protein (10 g of whey) does not seem to be sufficient to increase protein synthesis from baseline (Witard et al., 2014).

Compared with carbohydrate ingestion, protein ingestion post-exercise elicits a greater anabolic response and a higher protein synthesis. Carbohydrate ingestion post-exercise

elicits a small increase in muscle protein synthesis when proteins are not ingested. However, no additive effect on MPS is seen when carbohydrates is co ingested with proteins, compared to an optimal protein intake alone (Koopman et al., 2007). Interestingly, carbohydrate ingestion post-exercise may have a role in reducing muscle protein breakdown rates (Borsheim et al., 2004).

2.6 Training status

Training status seems to affect the FSR response following exercise. There is a stronger immediate response in mixed muscle FSR in trained subjects than untrained subjects (Tang, Perco, Moore, Wilkinson, & Phillips, 2008). However, untrained subject experience a more prolonged and modest effect than trained subjects, which results in a higher total anabolic response (Tang et al., 2008), This shift is initiated during the typical duration of a resistance exercise period (12 weeks), as 8 weeks of training is utilized to differentiate between trained and untrained states within subjects (Tang et al., 2008). A change in postprandial myofibrillar FSR is seen after only a few exercise bouts, suggesting that the shift is almost immediate (D. J. Wilkinson et al., 2014).

Just as resistance exercise increases the anabolic response in muscles following amino acid or protein intake (Biolo et al., 1997), immobilization impairs the mixed muscle protein synthesis response. This was shown during 14 days of full leg-cast induced knee-immobilization which resulted in a 31% reduced MPS in the immobilized leg compared to the used limb (Wall, Snijders, et al., 2013). In contrast, resistance exercise is shown to induce an anabolic window lasting for a prolonged period (up to 48 hours) (Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997) and an anabolic window is even observed with aerobic exercise, showing increased postprandial MPS 15 hours post-session in the elderly (Timmerman et al., 2012).

Collectively physical activity level and training status seems to impact the anabolic response to both exercise and food intake. It seems physical activity increases the anabolic response, while lack of physical activity reduces the response.

2.7 Timing of protein intake

The proximity of protein intake in relation to the exercise bout could also affect the anabolic response. However, in young subjects the synthetic response does not differ between feeding one or three hours after exercise (Rasmussen et al., 2000). This might suggest that the proximity of protein intake after exercise is of little importance for young people. This is also confirmed in meta-analysis showing little effect of protein intake proximity to exercise on hypertrophy in longitudinal training studies (Schoenfeld, Aragon, & Krieger, 2013). This meta-analysis concludes that the dosage of protein intake is more important than timing in determining the anabolic response after exercise.

However, in rats hypertrophy induced by hindlimb suspension was larger and adipose tissue accumulation was less, when rats were fed immediately after performing exercise, compared to delayed feeding (Suzuki et al., 1999). Furthermore, in elderly subjects, data suggest the effect of immediate protein intake following exercise may be more important than in young. In agreement with this, immediate protein supplementation after exercise is shown to increase muscle mass and strength, compared with a delayed intake in elderly humans (Esmarck et al., 2001).

2.8 Muscle protein synthesis in the elderly

Recent studies suggests that elderly individuals need a higher dosage (35 g) of protein to maximize the muscle protein synthesis rate, than younger subjects (20 g) (Pennings et al., 2012), but not all find this difference at rest (Yang, Breen, et al., 2012). Interestingly, resistance exercise seems to increase the sensitivity to amino acids in elderly subjects. After resistance exercise there is a significant increases of myofibrillar FSR in the exercised muscle following the same increase in protein dosage of 20-40 g (Yang, Breen, et al., 2012). A recent report presenting results of acute responses in both young and elderly in studies utilizing similar methods, conclude that a relative dosage of 40 g/kg and 24 g/kg is needed to reach the plateau (maximize) the MPS response in elderly and young men, respectively (Moore et al., 2015).

Furthermore, when essential amino acids are administered to elderly men in similar doses as to young subjects, the plateau in MPS is lower than in younger subjects (Cuthbertson et al., 2005). This could suggest that anabolic resistance occurs with age. One mechanism causing this reduced sensitivity to intake of proteins in the elderly could be the observed lower activation of the mTOR - pathway, which is also found after exercise in elderly subjects (Kumar et al., 2009).

It could be that the differences seen between young and elderly might be caused by the impact of changed kinetics of either digestion or absorption, and that the reduced FSR seen in elderly is due to less availability of amino acids in the circulation. However, when young and elderly consume intrinsically labeled protein at rest and after exercise, identical uptake is seen between both age groups (Pennings, Koopman, et al., 2011), which argues against this notion.

In summation it is established that protein intake and exercise impact the response on muscle protein synthesis, and that age seems to have an effect on the response. The topic of the next segment will focus on how the response is impacted by different protein sources.

2.9 Protein sources

In research affecting protein supplementation, usually three different types of protein are used. These are soy protein and the two milk protein fractions; whey and casein. As they are the most commonly researched protein supplements, the next segment will be limited to these three types.

2.9.1 Milk and soy supplementation

Several studies have investigated the post-exercise muscle protein synthesis response after intake of different protein supplements. Increased muscle protein synthesis rates are reported following milk protein ingestion compared to soy protein ingestion (S. B.

Wilkinson et al., 2007). Furthermore, amino acid uptake across an exercised limb is lower following soy protein ingestion compared with milk supplementation (S. B. Wilkinson et al., 2007). This may be due to different distribution of the proteins in the postprandial state, as soy has been shown to be incorporated into the splanchnic bed to a higher degree than milk proteins (Fouillet, Mariotti, Gaudichon, Bos, & Tome, 2002). The differences in composition may also impact the results in muscle, as milk proteins contain higher concentrations of branched chain amino acids (BCAA). Branched chain amino acids are shown to be transferred to areas outside the splanchnic bed to a higher degree than other amino acids (Biolo et al., 1992). This results in a higher plasma concentration of branched chain amino acids following dairy consumption (Gran et al., 2014). Dairy is also shown to induce a greater activation of the mTOR-pathway (higher phosphorylation of $mTOR^{Ser448}$ and an elevation in ribosomal $S6K^{ser240/244}$), than observed with soy consumption (Gran et al., 2014). However, no difference was seen for Akt or p70S6K (Gran et al., 2014).

When comparing soy ingestion with the different strands of milk protein, soy promotes lower rises in mixed MPS than whey hydrolysate[1] following exercise (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). However at rest the effects are similar (Tang et al., 2009). Compared to micellar[2] casein, the effect of soy is favorable after both exercise and at rest (Tang et al., 2009).

In elderly ingestion of 20 g of soy protein fails to stimulate myofibrillar protein synthesis, both at rest and after exercise. Only after consumption of dosages as high as 40g there is a significant rise in protein synthesis seen in combination with exercise, whereas none is detected at rest (Yang, Churchward-Venne, et al., 2012). This could suggest that elderly are more sensitive to differences between whey and soy, then younger individuals at rest, and that exercise might reduce the threshold for the postprandial MPS response in the elderly.

[1]Hydrolyzed proteins are proteins broken down to its component amino acids.
[2]Micellar casein is the form of casein found in bovine milk.

2.9.2 The Milk proteins

The two main protein fractions in bovine milk are casein and whey, making up 80 % and 20 % of milk protein, respectively. Whey is soluble and the amino acids are absorbed more rapidly than after intake of casein, which is less soluble and clots in the stomach. This result in a slow release of amino acids in the circulation because of a slower gastric emptying and absorption of amino acids (Boirie et al., 1997). The faster absorption rates seen with whey leads to a rapid and strong increase both in plasma concentrations of amino acids and muscle protein synthesis response following feeding with whey, compared to micellar casein in both young (Tang et al., 2009) and elderly subjects (Burd, Yang, et al., 2012; Pennings, Boirie, et al., 2011). These differences are also present post exercise (Burd, Yang, et al., 2012; Tang et al., 2009). When casein is hydrolyzed, it increases the absorption rates, but myofibrillar MPS are still higher following whey supplementation (Pennings, Boirie, et al., 2011).

In the mentioned studies above, biopsies for FSR-calculations after protein intake was taken 2 hours (Burd, Yang, et al., 2012) and 1.5–3 hours post protein intake (Pennings, Boirie, et al., 2011). When measurements are registered over a longer time period (1-6 hours) the initial (1-3.5 hours) FSR is higher following whey ingestion compared to that of casein (Reitelseder et al., 2011). However, in the extended period, 3.5–6 hours, FSR following casein ingestion is still elevated, whereas FSR following whey ingestion is reduced to basal levels. When the net FSR (1–6 hours) is calculated, there is no difference between whey and casein (Reitelseder et al., 2011). Hence, the difference in FSR response may be smaller than what is indicated by the studies taking biopsies over a shorter time period. The casein supplementation used in the Reitelseder (2011) study is calcium caseinate (Reitelseder et al., 2011), which may have impacted the results as this form of casein is more soluble and result in a faster release of amino acids than that of micellar casein. This may induce higher FSR values in the initial period than would have been seen with micellar casein (Reitelseder et al., 2011). Similar results are also found when caseinate intake is compared with whey in elderly subjects (Dideriksen et al., 2011). In addition, casein, but not whey protein, has been found to reduce whole protein breakdown rates (Boirie et al., 1997).

The dissimilar findings in some of these studies may be caused by differences in methodology e.g. different supplementation (micellar casein vs caseinate) and different isotopic tracers, and by the collection time for biopsies. Nevertheless, the results suggest that the effect of whey supplementation is superior to casein for MPS stimulation in the first hours after resistance exercise. However, if another meal is not consumed within six hours, the slow release of amino acids from casein may prove advantageous.

2.9.3 Native whey

Whey protein supplements can be made through several processes. Typically it is made as a secondary step from the whey fractions which is derived from cheese production and preserved as a concentrate or isolate. However, new techniques allow for extraction of whey protein from pasteurized milk with a series of micro- and ultra-filtration techniques, which results in a higher content of essential amino acids, including leucine. It has been shown that ingestion of native whey protein will result in significantly higher blood leucine concentration than both milk and other variations of whey protein (Laahne, 2013; Nyvik Aas, 2014). Production of whey supplements in this way (filtration) do not denaturize protein as is traditionally seen with whey supplements derived from cheese production (Lactalis, 2014). Furthermore being produced in this way, whey protein is more similar to its native form in milk (hence the name). Native whey (30 g) is also shown to induce postprandial whole body protein synthesis significantly more than casein (30 g) following 10 days increased protein intake in elderly subjects (Gryson, Walrand, et al., 2014). However, no difference in postprandial whole body breakdown or synthesis were observed between native whey and casein supplementation for those subjects consuming 15 g of the protein as part of an adequate protein intake over the 10 day period (Gryson, Walrand, et al., 2014). In agreement with this there is also shown a significant increase in myosin FSR following ingestion of 15 g of native whey, comparable to 30 g of native whey or casein, whereas 15 g of casein fails to elicit a response (S. Walrand et al., 2015). Interestingly postprandial mitochondrial FSR increased significantly only in the native whey groups, irrespective of dosage (S. Walrand et al., 2015). An early investigation of native whey suggests that the rate of delivery of amino acids may be too rapid (Lacroix et al., 2006). The

combination with casein may be favorable, as native whey ingestion results in a transient hyperaminoacidemia, compared to milk and casein (Lacroix et al., 2006).

2.10 Leucine trigger

Whey protein is characterized by having a high content of leucine. This is believed to be the main reason why whey protein stimulates protein synthesis stronger than other protein sources in the first phase after ingestion. Leucine has been shown to stimulate muscle protein synthesis through the mTOR-pathway and it is unique among the branched chain amino acids in this regard (Anthony et al., 2000). In rats there is a dose-response relationship between leucine content in meals, mTOR-signaling, and peak muscle protein synthesis. This has been shown both with intake of different protein sources (Norton et al., 2009) and by manipulating the leucine content of a protein source (Norton, Wilson, Layman, Moulton, & Garlick, 2012).

However, leucine is not the sole reason for the effectiveness of whey protein. Through an elegant design, Churchward-Venne et al. (2012) investigated the effects of three different supplement drinks on muscle protein synthesis in humans (Churchward-Venne et al., 2012). After unilateral resistance exercise, subjects consumed either 25 g of whey protein, or 6.5 g of leucine-enriched whey protein with increased leucine content to that of 25 g of whey, or 6.5 g of whey, with all the essential amino acids other than leucine similar to the 25 g dose. They found no difference in the acute post postprandial muscle protein synthesis between drinks. However, only the full 25 g whey protein supplement sustained the post-exercise rates of MPS (Churchward-Venne et al., 2012). This suggests that also other amino acids than leucine has to be available to maximize the protein synthesis response post-exercise.

Even though Churchward-Venne et al. (2012) did not find any difference with their design at rest in young individuals, this may be different in elderly subjects. It has been shown that co-ingestion 2.5 g of leucine and 20 g of casein, increases the rates of postprandial muscle protein synthesis significantly more than casein ingestion alone over a period of 6 hours in elderly subjects (Wall, Hamer, et al., 2013). This suggests that elderly subjects need higher leucine content than young to fully stimulate post-

prandial muscle protein synthesis. In support of this notion it has been shown that the FSR response present in young subjects following ingestion of a low-leucine (1.7 g) EAA mix (~15 g protein) was absent in elderly subjects (Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2006). Only when a higher dosage (2.8 g) of leucine was ingested was an increased response observed in the elderly (Katsanos et al., 2006). Furthermore when elderly have been chronically supplemented with leucine over 14 days, increased postprandial phosphorylation of mTOR, 4E-BP1 and $P70^{s6k1}$, as well as and increased FSR is observed after a mixed meal is consumed (Casperson et al., 2012). Collectively it seems that at least 2 g of leucine has to be ingested for elderly to gain a postprandial response in muscle protein synthesis. This could have attenuated the response in those studies that used low doses of protein and therefore also leucine. It also seems that regular leucine supplementation may help prevent the anabolic resistance that occurs with age (Casperson et al., 2012).

2.11 Longitudinal effects of protein intake with exercise

It is documented in a meta-analysis that supplementation of protein (regardless of type) to a normal diet can provide a positive effect (0.69 kg ± 0.22 CI 95%) on accumulation of muscle mass when resistance exercise is performed over a prolonged period of six or more weeks (Cermak, Res, de Groot, Saris, & van Loon, 2012). However, several of the studies not use a calorie matched placebo, which could make some of the studies hard to interpret (Cermak et al., 2012). This is because an increased energy intake in itself can elicit substantial changes as long as protein requirements are covered (Rozenek, Ward, Long, & Garhammer, 2002).

The effect of consuming protein from different sources during long training interventions have shown a larger effect on lean body mass following milk protein supplementation than isocaloric soy consumption (Hartman et al., 2007; Phillips, Tang, & Moore, 2009; Volek et al., 2013). One study comparing the effect of native whey protein supplementation with that of micellar casein or carbohydrate in conjunction with resistance exercise, did, however, not find any difference in strength or body composition changes between the three groups of young subjects (Babault, Deley, Le Ruyet, Morgan, & Allaert, 2014). However, a better resistance to fatigue was observed

following native whey supplementation. Their dosage sizes may have been suboptimal for both protein groups (10 g) which could explain the lack of effect compared to carbohydrate (Babault et al., 2014).

There are not many interventions combining protein supplementation and strength training on elderly subjects. An overview of interventions using elderly subjects (~70 years old), of considerable duration (12-24 weeks) can be seen in table 2.1 This overview is limited to the interventions using DXA for lean body mass measurements and 1RM measurements, predominantly for leg press, most utilizing a full body resistance exercise programs (as is used in the present study), to allow for comparison of our results with similar interventions.

The changes achieved with prolonged resistance exercise in lean body mass in these studies range from around 0.3-1.5 kg as a result of resistance exercise and protein intervention (table 2.1). As shown in the table, the results are inconsistent with no difference between protein consuming groups in most studies. The table also indicates a trend in most studies favoring protein intake on mean change in lean body mass. When data from multiple studies are compiled, meta-analysis shows a favorable effect for protein supplementation on lean body mass/fat free mass in elderly subjects (Cermak et al., 2012; Finger et al., 2015) as well as young subjects (Cermak et al., 2012). In studies of elderly subjects, most interventions are conducted on sedentary, or individuals inexperienced in resistance exercise. One review supports a link between exposure to resistance exercise and the response to protein supplementation on lean body mass gains in resistance exercise interventions (Pasiakos, McLellan, & Lieberman, 2015). Interestingly, in this review untrained subjects see no benefit of supplementation during the initial weeks of training, while trained subjects gain more lean body mass when protein is consumed. As the duration of training increases, an effect is seen on lean body mass regardless of previous exercise experience (Pasiakos et al., 2015).

In The Tieland study (table 2.1) they investigated frail elderly, making it particularly interesting. They have investigated the effect of protein supplementation with (Tieland, Dirks, et al., 2012) and without exercise (Tieland, van de Rest, et al., 2012) over a 24 week period. The supplements (2x15 g milk protein concentrate) were given in addition to meals, to ensure a higher dosage of protein with each meal than the placebo groups.

Only resistance exercise with added protein induced changes in lean body mass for the frail elderly (Tieland, Dirks, et al., 2012). Furthermore the effect seen by protein supplementation in the frail elderly is not present in elderly subjects who were not frail (Leenders et al., 2013), suggesting that frail elderly see more benefit from supplementation than healthy elderly individuals.

Protein supplementation is shown to increase both muscle mass and strength following 24 weeks of resistance exercise in post-menopausal women consuming a nutrient-supplement containing 10 g of whey (Holm et al., 2008). Furthermore, another study conducted on middle-aged and elderly men, found an increased effect of whey protein supplementation on 1RM leg press only, however no difference for lean body mass changes (Eliot et al., 2008). In a recent report, elderly (60 years) men conducting concurrent training (4 months) experienced an increased effect of 10 g of protein (milk or native whey) supplementation on changes in body composition compared to 4 g (milk). However there was no difference between protein sources when given as a 10 g dosage (Gryson, Ratel, et al., 2014).

Solberg et al. (2011) previously conducted a 13 week training intervention on elderly with a mean age of 74.3±4.6 years. The study did not include supplementation, but utilized a similar protocol with essentially identical exercises as in the present study for the strength training group, and was carried out in a similar fashion. The intervention induced a mean change in lean body mass of 1.4 kg (±0.4 kg 95% CI). Changes in 1RM strength were 20% for chest press, and 35% for knee extension (Solberg et al., 2011).

Two possible explanations have been proposed as to why some studies find an effect of protein supplementation while others do not, and they are called the spread and change theory (Bosse & Dixon, 2012). According to spread theory, a sufficient difference in overall protein intake (g/kg/day) between the intervention and control groups must exist (Bosse & Dixon, 2012). In their review, a 66.1% average difference was found in g/kg/day between groups in those studies identifying a positive effect of protein, while the average difference in those studies identifying no effect, was 10.2% greater than controls (Bosse & Dixon, 2012). According to change theory, an increase in protein intake (g/kg/day) from baseline must be present during the training intervention. In their study, the average % increase in those studies finding an additional effect of protein

supplementation was 59.5%, while those studies finding no effect have an average increase of 6.5% from baseline (Bosse & Dixon, 2012). The included studies in this investigation both younger and older individuals. As mentioned previously, simply increasing protein intake may have adverse effects on kidney function in the elderly (S. Walrand et al., 2008), so for this group investigating protein quality with the aim of finding the most effective protein sources remain highly important.

Table 2.1: *Overview of RCT trials, investigating the effects of protein supplementation on prolonged resistance exercise (12-24weeks) in elderly subjects. The overview is limited to studies investigating elderly subjects exclusively, most of them utilizing a whole body resistance exercise-training program. Only studies using dual x-ray absorptiometry as means of measuring lean body mass (LBM) changes are included, as well as changes in 1RM in leg press are presented in the overview (if other means of strength measurement is utilized this is listed).*

Study (Author, year)	Mean Age No. subjects	sex	Training Regimen	Protein / placebo description	Iso caloric	LBM mean change	1RM change	Results favor
Verdijk et al. (2009)	72 years (N=26)	M	3 d/w x 12weeks LBRT 2 exercises: 4 sets x 8-15 reps	Casein hydrolysate 2x10g Flavored water	NO	0.7kg(1.2%) 0.6kg(1.0%)	24 % 24 %	No diff
Chale et al. (2013)	78 years (N=80)	M+W	3 d/w x 24weeks WBRT 5 exercises: 2-3 sets x 10-12 reps	Whey protein 40g maltodextrin	YES	0.6kg (1.3%) 0.3kg (0.6%)	21 % 15 %	No diff
Iglay et al. (2009)	62 years N= 36	M+W	3 d/w x 12 weeks WBRT 8 exercises: 3 sets x 8 reps or fatigue	high protein diet 1.2g/kg/day (egg milk dairy) low protein diet 0.9g/kg/day	YES	~1.2kg ~1.0kg	**32,8 %	No diff
Leenders et al. (2013) (Men)	70 years N=31	M	3 d/w x 12 weeks WBRT 6 exercises: 3-4 sets x 8-15 reps	Milk protein concentrate 15g lactose +calsium drink	NO	1.4kg 1.0kg	26 %	No diff
Leenders et al. (2013)(women)	70 years N=29	W	3 d/w x 12 weeks WBRT 6 exercises: 3-4 sets x 8-15 reps	Milk protein concentrate 15g lactose +calsium drink	NO	1.3kg 1.1kg	31 %	No diff
Gryson, Ratel, et al. (2014)	61years N=48	M	3 d/w x 16 weeks WBRT + aerobic 6 exercises: 3sets x 8-12 reps	Native whey protein 10g milk protein 10g total milk protein 4g	NO	0.6kg 0.8kg -0.9kg	3 % (MVC) 3 % (MVC) no diff	10g protein
Tieland, Dirks, et al. (2012) (frail)	78years N=62	M+W	2 d/w x 24weeks WBRT 6 exercises: 3-4sets x 8-15 reps	Milk protein concentrate 30g (2 x 15g) lactose+ calsium drink	NO	1.3kg -0.3kg	36 % 40 %	Milk protein

WBRT: whole body resistance exercise training, LBRT: lower body resistance exercise training, d/w: days / week, N: Number of participants,1RM: one repetition maximum, No diff: no difference between groups

~ : estimated change based on figure, true mean values not disclosed by author

** Pooled 1RM results of several exercises for all participants.

2.12 Effects on physical performance in the elderly

Several of the studies in table 2.1 have measured effects of protein supplements and exercise on physical performance in the elderly. Typically increases in strength when 1RM is tested seem to be between 20-35%, at least when leg press is tested (table 2.1).

Physical performance tests are conducted in several of the trials in table 2.1, with two of them utilizing the short physical performance battery (SPPB) (Chale et al., 2013; Tieland, Dirks, et al., 2012), which is found to predict future disability (Guralnik, Ferrucci, Simonsick, Salive, & Wallace, 1995). The battery includes a standing balance test, a 2.4 m (8ft) normal-pace walk test, and a 5-times repeated chair rise. For each test the subjects are scored from 0 to 4, higher being better, and their total score represent their performance in the battery, ranging from 0-12 (Guralnik et al., 1995).

Both trials utilizing the whole SPPB report a significant increase following the intervention in SPPB score, as a result of increased physical function (Chale et al., 2013; Tieland, Dirks, et al., 2012), although they do not disclose all individual test values of the battery. The improvement in performance in 5 times chair rise after the first 12 weeks is reported to be 13% (protein) and 5% (placebo) in the Tieland, Dirks, et al. (2012) trial, and 9% (men) and 8% (women) in the Leenders et al. (2013) trial, also utilizing this test. The improvement continues following the next 12 weeks, with total improvements of 14% (protein) and 24% (placebo) in the Tieland, Dirks, et al. (2012) trial, and 18% (men) and 19% (women) in the Leenders et al. (2013) trial following 24 weeks of supplementation and training. The completion times at inclusion in these trials were 15-17 s (frail) (Tieland, Dirks, et al., 2012) and 8 s (healthy) (Leenders et al., 2013). The previously mentioned Solberg et al. (2011) trial at our lab, improved performance to a similar extent, with a mean reduction of 1.0 s (9%) from 11.1 s at inclusion in the same test following the 13 week strength training intervention (Solberg et al., 2011).

Stair climb performance was improved by 8% and 18% for protein (no difference) and carbohydrate groups in the Chale et al. (2013) study. Solberg et al. (2011) improved stair climb performance by 0.6 s (7%) in unloaded and 0.8 s (9%) in loaded (20 kg) in

the same stair climb test utilized in the present study. The completion times of the test at inclusion were 8.4 s and 9.3 s for the unloaded and loaded (20 kg) conditions (Solberg et al., 2011).

It seems the interventions investigating physical performance changes are all able to increase performance following resistance exercise and supplementation, however not different from placebo groups, suggesting resistance exercise is the most important stimuli. Although, a positive effect on both strength and physical performance changes has been identified following protein supplementation alone in the frail elderly (Tieland, van de Rest, et al., 2012), however to a lower extent than in those performing resistance exercise (Tieland, Dirks, et al., 2012).

2.13 Measurements of body composition

There are many means of measuring body composition. The methods used in research include, but are not limited to, underwater weighing, magnetic resonance imaging (MRI), computed tomography (CT) and dual x-ray absorptiometry (DXA) (Heymsfield, Adamek, Gonzalez, Jia, & Thomas, 2014). DXA measurements, which are frequently used in randomized controlled trials are proved to be an practical and accurate approach to measure changes in muscle mass on the whole body level (Heymsfield et al., 2014). Both computed tomography and magnetic resonance imaging have been deemed valid references when compared to cadaver methods for measurement of adipose tissue free skeletal muscle mass (Mitsiopoulos et al., 1998). DXA on the other hand, has been found to have strong correlations with both of these measurement methodologies when investigating body composition (Heymsfield et al., 2014). DXA is reported to have a coefficient of variation of 2.4% for lean tissues, and 1.7% for body fat (Bredella et al., 2010). In controlled physical models, hydration levels increase fat estimation errors, pointing towards the importance of standardized procedures taking feeding and hydration into account (Pietrobelli, Wang, Formica, & Heymsfield, 1998). In our lab the measured coefficient of variation for lean body mass is 1.13% and 4.20% for fat mass, however this is based on repeated measures of only 10 recreationally active young adults (Tofte & Walle, 2013). Ultrasound can be utilized as a means of measuring

muscle characteristics. As ultrasound measurements are hands on, the accuracy is highly dependent on the operator. Furthermore, as hypertrophy does not occur homogenously across a muscle length (Hakkinen et al., 2001), measurements of hypertrophy should probably be paired with whole limb measures.

2.14 Relationship between acute studies and long term changes

Although the effects of protein intake and exercise can be measured both acutely following a single exercise bout or after ingestion alone, and over time, the relationship between these two approaches has recently been called into question (Mitchell, Churchward-Venne, Cameron-Smith, & Phillips, 2015). Hypertrophy is the result of repeated exposure to episodes of positive net protein balance. However, attempts to correlate the measurements of acute muscular protein synthesis rates and subsequent hypertrophy following resistance exercise in the same subject show no correlation (Mayhew, Kim, Cross, Ferrando, & Bamman, 2009; Mitchell et al., 2014). However correlation of hypertrophy with phosphorylation of different signaling proteins has been identified (Mayhew et al., 2009; Mitchell et al., 2014; Terzis et al., 2008). As the measurement of acute responses depend on muscle biopsies, collection time, number of biopsies and analysis of these, might impact the results and make it hard to register subtle differences underpinning the stimuli's effectiveness in promoting changes over time. Factors impacting the ability of these acute measurements in predicting longitudinal changes in muscle mass include the change of subjects training status, as it shifts from untrained to more experienced during the duration of an intervention as described in chapter 2.6.

However as elucidated in the previous segments, the different approaches are in agreement in evaluating different protein sources, with milk proteins promoting favorable effects both in acute responses (S. B. Wilkinson et al., 2007) and in hypertrophy induced over time (Phillips et al., 2009) compared to soy protein. Although acute investigations of myofibrillar FSR may be unable to predict hypertrophy over

time, their investigation remains relevant, as they provide important insight into the mechanistic properties of different exercise and nutrition interventions, as well as their possible potential over time (Mitchell et al., 2015).

2.15 Summary and aim

Sarcopenia, the age associated loss of muscle mass and function, reduce the performance of daily life activities in the elderly. Currently, strength training is the most effective way to counteract the development of sarcopenia as it in some cases can increase muscle mass, or at least prevent the loss of muscle mass. Generally, exercise or/and intake of essential amino acids stimulates muscle protein synthesis in young subjects. However, possibly due to high variation in adaptations between subjects, few studies find an additional effect of protein supplementation to that of exercise with a normal diet in elderly subjects (Table 2.1). When meta-analysis is conducted the documented effect on both fat-free mass and strength in younger subjects seems also to be present in the elderly (Cermak et al., 2012). However, other reviewers find no additional effect of protein supplementation except on lean body mass (Finger et al., 2015). The dairy protein whey is superior to both soy and casein in inducing increased muscle protein synthesis the first hours after exercise. It is believed that its superiority is due to its high leucine content, found to activate the mTOR-pathway exclusively among amino acids. It seems that an amount of at least 2 g of leucine has to be surpassed in order to elicit postprandial changes in MPS in elderly subjects. Furthermore, it seems that a dosage of approximately 20 g of high quality protein may be sufficient in reaching the threshold for a saturation for MPS in young (Moore, Robinson, et al., 2009); although higher dosages may be needed in the elderly (Cuthbertson et al., 2005; Moore et al., 2015; Pennings et al., 2012).

Our aim was to investigate whether a leucine rich native whey protein elicits more extensive longitudinal adaptations in lean body mass, strength and function than a similar dosage of milk protein consumed daily during a prolonged exercise period in elderly subjects.

3. Methods

This master thesis is part of a larger study. In total the study consists of two acute response trials conducted at start and end of an eleven week training intervention (this study) with chronic protein supplementation throughout. The study was approved by the south east regional ethical committee of Norway and carried out in line with the declaration of Helsinki. The study was conducted at the Department of Physical Performance at the Norwegian School of Sport Sciences and funded by the Norwegian Research Council and Tine SA.

3.1 Recruitment and inclusion

30 untrained elderly subjects were recruited through posters on senior-citizen centers, newspaper advertisements, internet articles, and links to the information on the university website that was spread through social media applications like Facebook and Twitter. All recruiting channels led to contact with the project-manager. Upon contact, detailed information sheets were distributed (Appendix II), informing participants of all aspects of the study including potential risks as well as the expected benefits of participation. Various criteria would prevent from participation in the study, among these were: diagnosed diabetes (both types), diagnosed osteoporosis, regular participation in heavy resistance-training, and the use of some pharmaceuticals known to influence relevant cellular signaling. If subjects did not meet any of these immediate exclusion-criteria, they were invited to the Norwegian School of Sport Sciences (NSSS) for further testing and in most cases inclusion in the study.

26 subjects completed the entire study (aged 73.5±2.7 years). Four subjects chose voluntarily to withdraw from the trial due to lack of interest in the study, medical conditions, or other personal reasons.

3.2 Study design

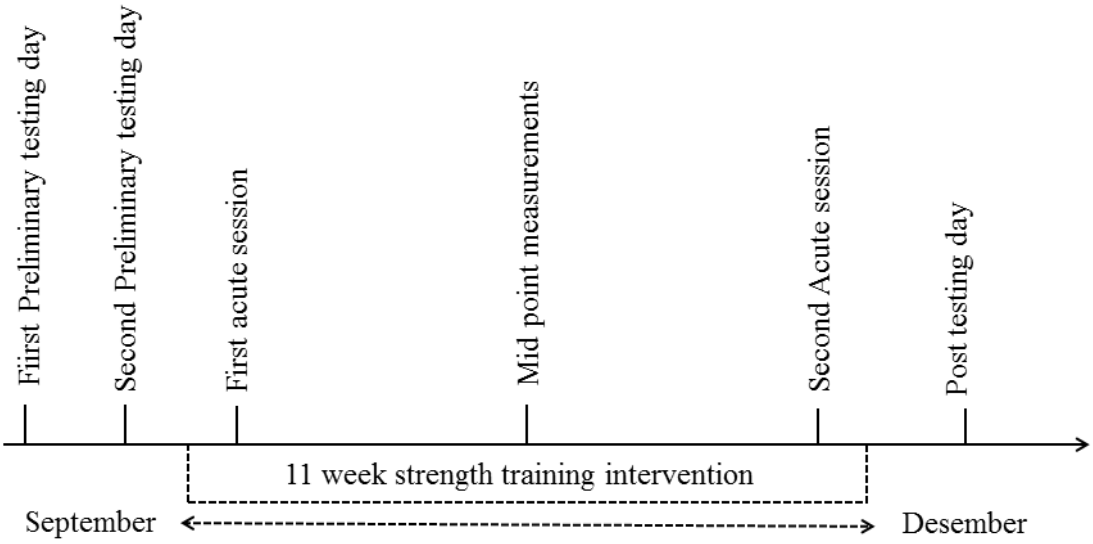


Figure 3.2: *Timeline of the overall study design.*

3.3 The preliminary testing day:

After giving their written consent to participate in the study, subjects arrived at the Department of Physical Performance, at the Norwegian School of Sport Sciences. They arrived in a fasted state (>6hours) between 8 and 10 AM. After meeting with the project manager a Dual X-Ray Absorptiometry (DXA) scan was performed, used for the measurement of body composition. If their bone mineral density was found to be substantially reduced (osteopenic) subjects would be informed, and in severe cases (osteoporotic) excluded from further participation.

The rest of the day involved a series of tests, in short these were: blood samples and blood pressure in a fasted state, a 24-h recall nutrition interview, examination of *m.vastus lateralis*, 1RM tests, functional tests and isometric maximal voluntary contraction in knee- extension.

The entire preliminary testing day (figure 3.3), with the exception of DXA, blood pressure and blood sampling was repeated after a few days of rest. The reason for including two pretests was to minimize the possible learning-effects of especially 1RM and functional tests before the intervention began, and also to estimate the repeatability of the ultrasound measurements on the included subjects, and in order to get a repeated 24-hour recall interview of nutrition.

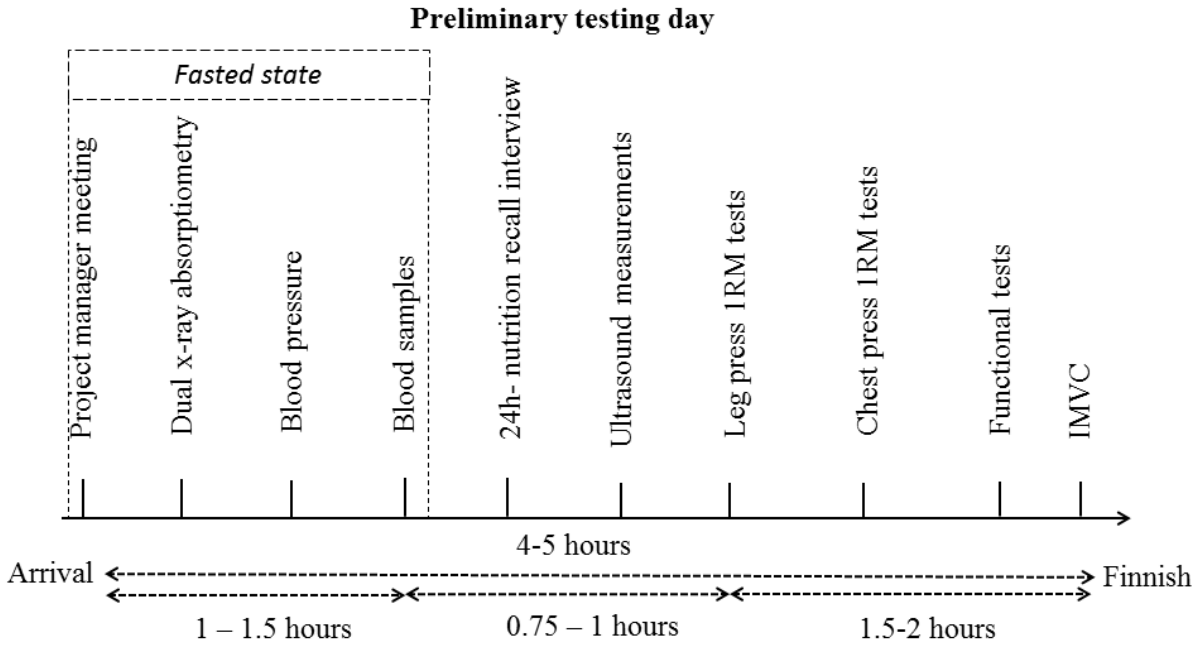


Figure 3.3 *Outline of the preliminary testing day, from arrival to completion of testing. After all blood samples were taken subjects would consume a self-made meal, as they were no longer needed to be in a fasted state.*

3.3.1 Dual x-ray absorptiometry (DXA)

Subjects arrived in a fasted state (>6 hours) before the DXA- measurements. Subjects were positioned according to the manufacturers protocol (Lunar iDXA, GE Healthcare, Madison, USA). For subjects with artificial joints (e.g. knee / hip), the appropriate adjustments were made, for separate limb analysis in these cases. The measured coefficient of variation of repeated DXA measurements in our lab is as mentioned 1.13% and 4.20% for lean body mass and fat mass, measured in young men (Tofte & Walle, 2013). The software version utilized in the current trial was enCORE Software v.14.10.022.

3.3.2 Nutrition interviews

All 24-h recall interviews of the subjects were done by the same MSc. student in nutrition. Subjects that revealed sub-optimal diets with regards to composition of the macronutrient intake were given the necessary instructions to optimize their diet. No subject was allowed to have a protein intake that would result in a total intake less than

1.2 g/kg/day of protein including the sachets. If this was the case they were informed how to make the appropriate adjustments. In all interviews an illustration- sheet of portion sizes for different food types developed by the University of Oslo was used. The collected data were analyzed in the professional application of a Norwegian online nutrition analysis tool (www.diet.no).

3.3.3 Ultrasound measurements

Subjects underwent ultrasound (US) -measurements of the thigh and measurements of the thickness of *m. vastus lateralis* (VL) were done. Several images were taken and the 3 best images were used for calculation of thickness. All measurements were taken at 40% of the measured femur length (distal-proximal). To calculate the 40% distance, measurements spanning from the (VL) insertion (estimated by ultrasound) and trochanter major (estimated by palpation) was used.

When the correct distance was found a pen was used to draw a horizontal line. Assisting lines were drawn when positioning the ultrasound probe along the line to help with the alignment of the probe for retesting (figure 3.4).

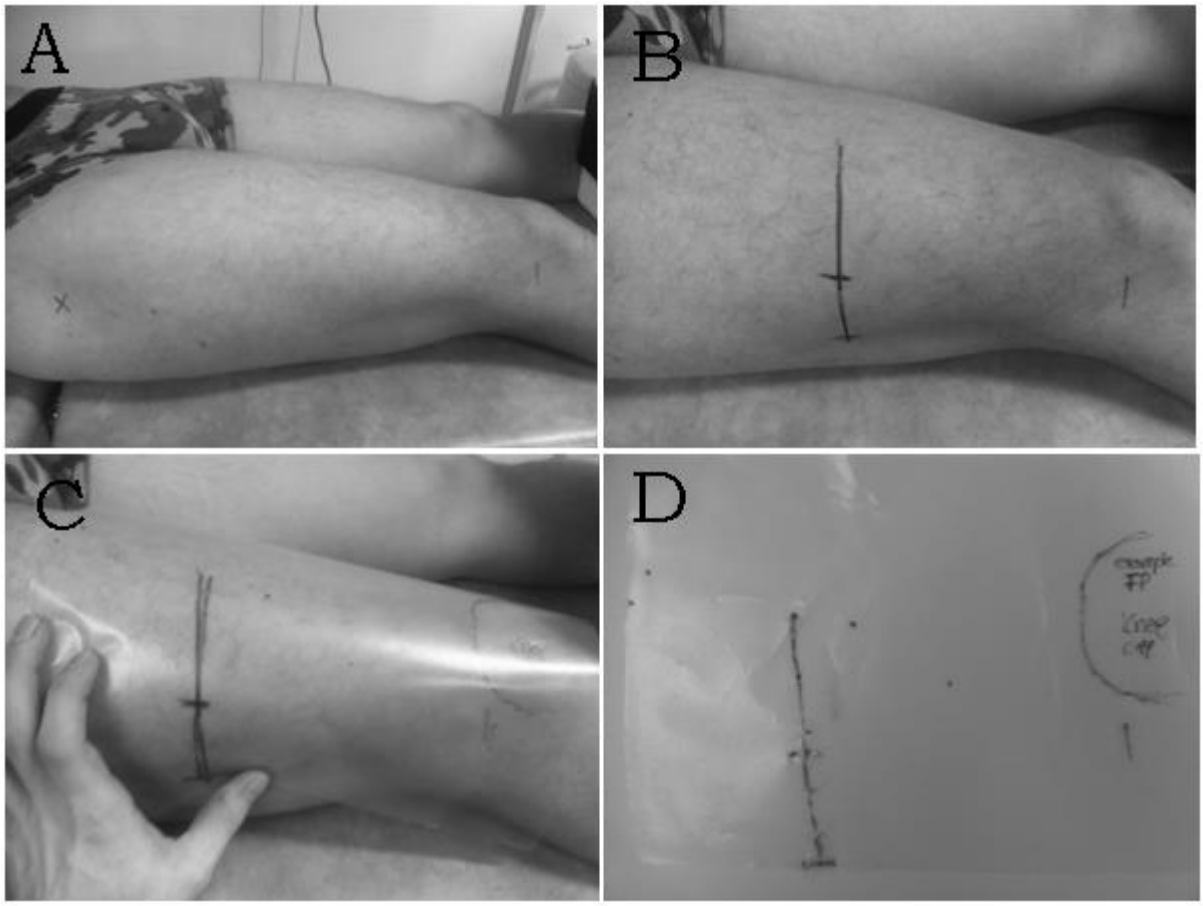


Figure 3.4: *The protocol used, from identification of measurement points (A). Drawing of measurement and assistance lines (B). Transferring the lines to an overlay (C). Overlay with markings and punctured holes to be used on subsequent testing days (D).*

After the lines were drawn, a transparent overlay was placed on the leg. Characteristic features like moles and scars, as well as the kneecap were copied by a permanent marker as well as the measurement line. This was used on subsequent testing days to reposition the line using these various features. The line would be transferred to the skin through punctured holes in the overlay.

On these subsequent days, previously taken pictures were shown on a separate screen, to allow for fine-adjustments of the position in relation to characteristics on the image like lines indicating connective tissue.

All images were analysed three times in OsiriX v 5.5.1 (Pixmeo, Switzerland) and a total of 9 measurement values from three images would together provide the mean value submitted as the PRE, MID or POST value for the specific measurement.

The coefficient of variation (CV) between the preliminary testing days for the subjects that underwent both US-scans included in the study was 2.23%, out of 82 images. Ultrasound was conducted to provide another measurement for changes in muscle mass in addition to regional changes in lean body mass (DXA).

3.3.4 Maximal Strength

Before maximal testing began, subjects performed a 10 min warm up on either an exercise-bike (Technogym, Italy) or a treadmill (Technogym, Italy; Woodway, USA). This preference was noted and repeated on the subsequent testing days. Tests were conducted in machines made by Technogym (Cesena, Italy).

3.3.5 Leg press

Subjects were instructed to position their feet in the center of the platform shoulder width apart, and not allowing their knees to fall in medially. Before any load was added to the sled, subjects would maintain the instructed position while lowering the sled to 90 degree angle of the knee joint. When accomplished, the corresponding wooden pole (18-36 cm) would be selected to ensure all repetitions would meet the desired depth (figure 3.5). This pole was then used as a distance marker for all warm-up sets and 1RM-attempts on all testing days to ensure repeatable testing conditions.

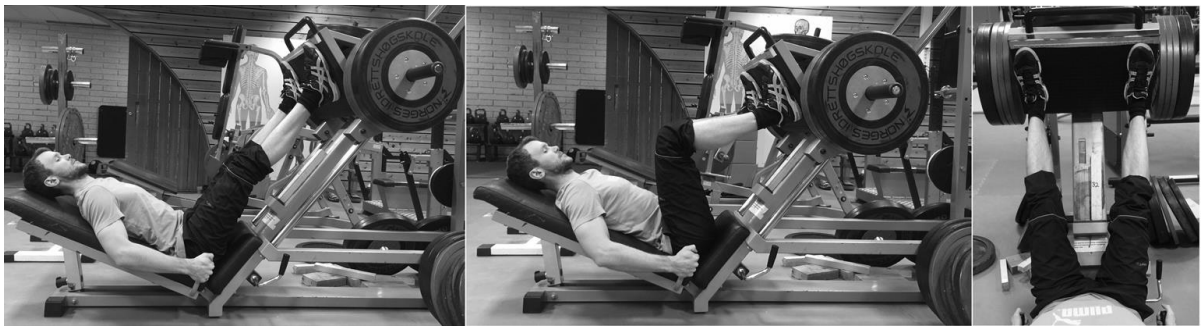


Figure 3.5*: Leg press start and end position (left), middle/bottom position (mid) and foot position (right).*

After the desired conditions were obtained, the subjects would undergo specific warm-up sets before attempting 1RM-lifts. These sets would respectively consist of 10, 6 and 3 repetitions, with progressively increasing loads.

1RM attempts were conducted with maximal effort, starting from the extended position. The subject would then gain complete control of the load, lowering it slowly towards the depth marker, with an aim of barely touching it. Upon contact they would instantly press the sled up towards the starting position. After each successful lift they would attempt a heavier lift after approximately 2 min of rest.

If subjects were unable to lift the load after lowering it to the depth marker, or resting it for a prolonged period (>0.5 s) they would have to repeat at the same weight or the next attempt would be performed at a lower weight. However, if a repetition was completed with sufficient depth but with unsatisfactory technique (most often medial movement of knees) this would count as their 1RM, as the test leader would not allow for increased weights. If subjects completed an attempt with insufficient depth, the test leader would either increase the weight if appropriate or repeat on the same weight if the attempted weight seemed close to the subjects 1RM. If technique was not an issue, the trial would continue until failure was reached. The CV between the measured 1RM values between the two preliminary testing days was 4.43% for leg press (24 subjects).

3.3.6 Chest press

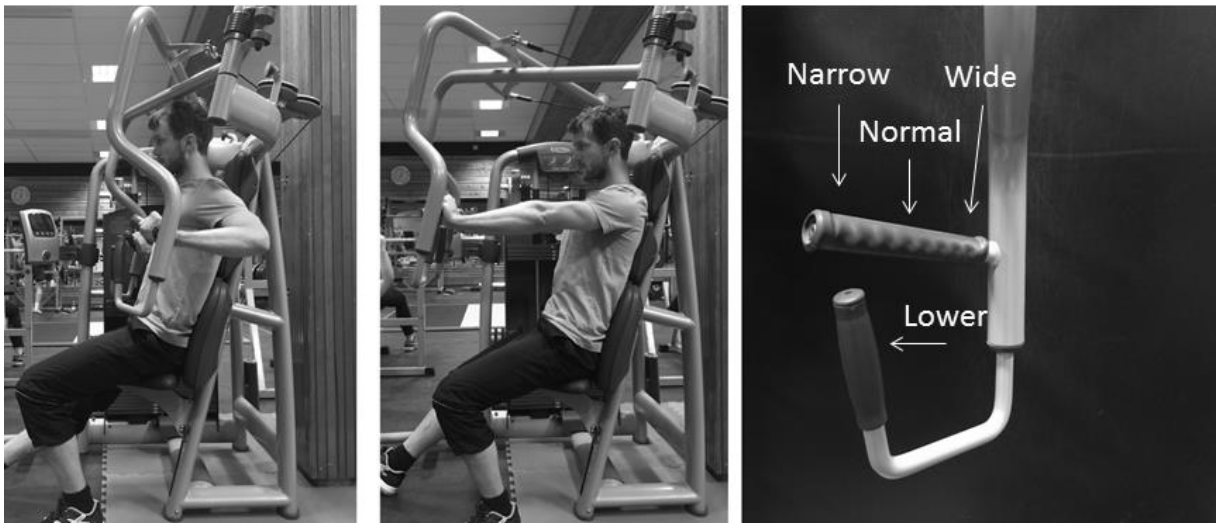


***Figure 3.6**: Chest press start position (left), end position (middle) and grip options narrow/normal/wide and lower (right).*

The subjects were informed that they could not allow their back to leave the back support and that a successful repetition would require straight elbows upon finish. All lifts would begin in the flexed position (elbows bent) and finish with straight arms. They were given the choice of grip width (narrow/normal/wide) and would use the same grip and chair height on subsequent testing days. The assistance lever was kept down in all warm ups and tests, to increase reliability and facilitate for reduced shoulder mobility that was common among our subjects. If subjects were unable to use the normal grips due to shoulder problems/pain, they were allowed to option for the lower grip, and the same option would be used for subsequent testing days. Between the two preliminary testing days, the CV was 4.92% for 1RM in chest press (24 subjects).

3.3.7 Functional tests

Two functional tests were used, these were chosen in accordance with a previous study on a similar population (elderly) performed in our lab (Solberg et al., 2011), and were performed after the testing of 1RM strength.

3.3.8 Timed stair climb

The subjects performed the test 6 times, with 3 different levels of external load. If the subject did not perform the test correctly after instruction in the first trial, they received further instruction at this point, until they were able to meet the test criteria. The stair climb was conducted in a 2-level staircase located in one of the multi-purpose halls within the NSSS and all trials were conducted with the same measurement equipment on these stairs (figure 3.7).

Figure 3.7: *The stair and added weights (10.1 kg west and two 5 kg weight discs) used for the Timed stair climb trials.*

Subjects were instructed to start with their feet in contact with the edge of the first step. Then when ready they would climb the stair as fast as they could without transitioning into running. All trials were closely observed by test leaders, if there was a hint of running within the trial this would be noted, or the trial repeated. After two trials with no external load, they would wear a weight-west (10.1 kg) for two more trials, then finally adding two weight discs (2x5 kg) held in their hands, they would repeat the trial with a total load of 20.1 kg. On this trial they were instructed that arms be kept aside the body at all times, lifting their arms up to their chest or swinging the weights were not allowed, as these weights were simulating daily-life situations like carrying a rucksack and shopping bags.

Trials were timed using Photocells (Speedtrap 2, Brower Timing Systems,Utah, USA), these were placed carefully within the handlebars of the stairs (Top and bottom) in the same position for all trials (figure 3.7). The CV between the preliminary testing days were 4.37%, 4.91% and 4.09% for the bodymass, +10 kg and +20 kg trials respectively (18 subjects).

3.3.9 Timed 5 times Sit-to-stand.

Timed chair raise (sit-to-stand) involved the subjects standing with their arms crossed in front of a chair, then sitting and standing 5 times as rapidly as they were able to within the restrictions of the test. Upon the chair laid a pressure-plate, covered by a wooden board used to increase the surface-area and allow for easier compression of the sensor.

Figure 3.8*: illustration of both the equipment used (left) and satisfactory execution of the protocol for the sit-to stand test.*

Subjects were instructed to start standing (figure 3.8), then when seated they would have to lift their feet from the ground before standing again. Before starting another repetition they would have to demonstrate straight legs, no flexion of the hip or knee joints were allowed. However if all these criteria were met the 5 repetitions would be performed as fast as possible. If not they were repeated until all subjects had repeated 2 trials of satisfactory standards. The measuring device used was a plug-in to the same equipment used for stair-climb (Speedtrap 2, Brower Timing Systems,Utah, USA). The CV between the trials at the preliminary testing days was 5.74% for the sit to stand test (14 subjects).

3.3.10 Maximal Voluntary Isometric Contraction

Maximal Voluntary isometric contractions or MVC was performed last of all tests on all testing days. Prior to the MVC the subjects would cycle on a bike as a general warm up for 5 minutes at their own pace. When finished the chair would be set up for optimal individual settings before testing begun. Great care was given to make sure the axis of rotation would align with the center of the knee-joint for each subject. Also the position of the pressure-point on the shin, chair height and the back-support were noted and repeated on all following tests.

When all adjustments were made, subjects would perform 3 voluntary contractions of 25%, 50% and 75% of their perceived maximal effort. After this specific warm-up was completed 3 unilateral MVC trials were then performed, using an elastic strap around the calf to ensure constant contact with the pressure-point before initiation.

Subjects were instructed to press as hard as they could, and as rapid as they could. They received vocal encouragement from the test leader to maintain maximal effort for 3-4 seconds before rest. The leg in question would then rest (~1 min) while the other leg was tested and so on. After 3 trials were completed for both legs, the maximal MVC was noted. For MVC only the best trial was noted between the two testing days (no CV available).

3.4 Resistance Exercise Intervention

The training intervention involved 12-weeks of resistance exercise, with 3 sessions a week following a daily undulating linear progression. For a subgroup (N=15) the period involved 2 acute sessions, one at start (first session) and one in the final week of training. These sessions consisted of the same exercises, however calf raise, pull down, back extension and abdominal crunch were not performed on these two sessions.

Deviations from the program would only happen if a subject could not attend one of the standard training days (Monday, Wednesday and Friday). The different periods is described in more detail in the following segments. Example sheets used for each individual can be seen in appendix IV.

Table 3.4 *Overview of the training periodization for the entire intervention (week 1-12) with details of all periods.*

Week	Exercise	Monday			Wednesday			Friday		
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
1-3	Hammersquat	2	12	RM	2	10	90% of 12RM	1	8	RM
	Leg press	1	12	RM	2	10	90% of 12RM	2	8	RM
	Kne extensions	2	12	RM	2	10	90% of 12RM	2	8	RM
	Calf raise	2	12	RM	2	10	90% of 12RM	2	8	RM
	Chest press	1	12	RM	1	10	90% of 12RM	1	8	RM
	Seated row	1	12	RM	1	10	90% of 12RM	1	8	RM
	Close grip pull-down				1	10	RM			
	Shoulder press	1	12	RM	1	10	90% of 12RM	1	8	RM
	Back extensions	1	max 20		1	max 20		1	max 20	
	Ab crunch	1	max 20		1	max 20		1	max 20	
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
4-6	Hammersquat	2	10	RM	2	10	90% of 10RM	1	6	RM
	Leg press	1	10	RM	2	10	90% of 10RM	2	6	RM
	Kne extensions	2	10	RM	2	10	90% of 10RM	2	6	RM
	Calf raise	2	10	RM	2	10	90% of 10RM	2	6	RM
	Chest press	1	10	RM	1	10	90% of 10RM	1	6	RM
	Seated row	1	10	RM	1	10	90% of 10RM	1	6	RM
	Close grip pull-down				1	10	RM			
	Shoulder press	1	10	RM	1	10	90% of 10RM	1	6	RM
	Back extensions	1	max 15		1	max 15		1	max 15	
	Ab crunch	1	max 15		1	max 15		1	max 15	
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
7-9	Hammersquat	2	10	RM	2	10	90% of 10RM	2	6	RM
	Leg press	2	10	RM	3	10	90% of 10RM	3	6	RM
	Kne extensions	3	10	RM	2	10	90% of 10RM	3	6	RM
	Calf raise	2	10	RM	2	10	90% of 10RM	2	6	RM
	Chest press	2	10	RM	2	10	90% of 10RM	2	6	RM
	Seated row	2	10	RM	2	10	90% of 10RM	2	6	RM
	Close grip pull-down				2	10	RM			
	Shoulder press	2	10	RM	2	10	90% of 10RM	2	6	RM
	Back extensions	1	max 15		1	max 15		1	max 15	
	Ab crunch	1	max 15		1	max 15		1	max 15	
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
10-12	Hammersquat	3	8	RM	3	8	90% of 8RM	2	6	RM
	Leg press	2	8	RM	3	8	90% of 8RM	3	6	RM
	Kne extensions	3	8	RM	2	8	90% of 8RM	3	6	RM
	Calf raise	2	8	RM	2	8	90% of 8RM	2	6	RM
	Chest press	3	8	RM	2	8	90% of 8RM	2	6	RM
	Close grip pull-down				2	10	RM			
	Seated row	2	8	RM	2	8	90% of 8RM	2	6	RM
	Shoulder press	2	8	RM	2	8	90% of 8RM	2	6	RM
	Back extensions	1	max 10		1	max 10		1	max 10	
	Ab crunch	1	max 10		1	max 10		1	max 10	

All sessions were supervised, 3 subjects were followed by one instructor. The instructors would rotate between groups so that all subjects would have conducted approximately the same number of sessions with each instructor. This was to eliminate any differences between the instructors, and the subsequent impact this might have made.

3.4.1 First period weeks 1-3

As can be seen in table 3.4, the total volume lifted during the first weeks of the program is drastically lower (fewer sets) compared to later weeks. The weights systematically increased from session to session the first two weeks, as we were far off the RM weights we aimed to train with these subjects. This easy start was intentionally done to ensure two things, to reduce the risk of injury, and proper technical instruction in all exercises. In week 3 however the load was sufficient enough that the sub-maximal session on Wednesdays was calculated to 90% of the Monday session load. As can be seen in table 3.4 this session involves doing 10 repetitions on a 12RM weight, making it a substantially lighter session.

3.4.2 Second period weeks 4-6

The second period involved continuous progression in both intensity and total volume in the training program. The relative intensity of the sub-maximal sessions also increase as the intensity is now calculated from a weight used with the same amount of repetitions. During the last week of this period the subjects were asked to report to the lab for two midway nutrition-interviews and a ultrasound-scan of the *m. vastus lateralis* thickness. On all sessions during week 6, the subjects would perform leg press with their individual distance- marker used at pretest. This was done to ensure all subjects performed this important exercise with sufficient depth.

3.4.3 Third period weeks 7-9

The third period involved another increase in both intensity and volume to the program, adding another set to various exercises and increasing the intensity on Friday to ensure continued linear progression. As many subjects were now exercising with considerable loads, focus was on keeping both range of motion and technique acceptable. In this period many subjects would frequently reach failure on the last set in various exercises, not limited by their technique.

3.4.4 Final period weeks 10-12

The final period had multiple focus areas. The first was to maintain the linear progression. The intensity was increased while still keeping a high volume in the program, through increasing the intensity from 10 to 8RM on Mondays and Wednesdays (90%) and adding another set on key exercises.

The secondary focus area was making sure that all subjects in the acute-group were able to perform their final acute-session in an identical fashion with regards to previous sessions and the amount of rest days leading up to this session. The same care was taken to make sure subjects would have an identical amount of rest leading up to the final test day (for details see preliminary test day). This test day was done in an identical fashion as the preliminary testing day.

3.5 Supplement intervention

The subjects consumed two sachets of either powdered milk, or native whey protein daily. The Sachets contained 298 (milk) and 299 (Native whey) kilocalories respectively. Being isocaloric the only difference between products should be the composition (table 3.5). The supplements were distributed in a double blinded fashion. All instructors and test personnel were unaware of group affiliation of subjects and batch number contents.

The sachets were consumed twice a day; one in the morning and afternoon or night time on rest days. On training days, one of the sachets would be consumed following exercise sessions. Most sessions were scheduled early in the day, so the post-exercise drink replaced the morning drink for most subjects on exercise days.

New sachets were provided weekly on Mondays, subjects would be asked if they had consumed all sachets since last exercise session after every session. If any sachets had been missed or otherwise this was noted.

Table 3.5: *Composition of supplement sachets, analyzed with the Kjeldahl (total protein) and Eurofins (amino acids) accredited methods.*

Nutrients	**Milk**	**Native whey**	**Difference (% of milk)**
Energy (kcal)	298	299	100
Protein (g)	20.3	19.0	94
BCAA (g)	4.3	4.5	105
EAA (g)	9.3	9.6	105
Carbohydrate (g)	37.8	39.7	105
Fat (g)	7.3	7.2	98
Amino acids (g)	**Milk**	**Native whey**	**Difference (% of milk)**
Alanine	0.7	0.9	136
Arginine	0.6	0.6	88
Asparagine	1.6	2.1	135
Cysteine	0.2	0.5	297
phenylalanine	1.0	0.8	83
Glutamic acid	4.3	3.7	85
Glycine	0.4	0.4	98
Histidine	0.6	0.5	83
Isoleucine	1.0	1.1	105
Leucine	2.0	2.3	118
Lysine	1.7	2.0	118
Methionine	0.5	0.5	99
Proline	2.0	1.2	62
Serine	1.1	1.0	87
Threonine	0.9	1.0	112
Tyrosine	0.9	0.7	78
Valine	1.3	1.1	87
Tryptophan	0.3	0.4	150

% difference values are relative to milk (100%)

3.6 Statistics

All statistics were done in Microsoft Excel 2010 v. 14.0.4760 (Microsoft Corporation, Redmond, USA) and Graphpad Prism 6 (Graphpad Software, Inc., La Jolla, USA). Between group differences were evaluated using two-tailed unpaired student t-tests. Within group differences were evaluated using two-tailed paired student t-tests. The p-value set for statistical difference was $p<0.05$. Absolute values are presented as mean ± standard deviation, with changes presented as mean ± 95% confidence interval.

4. Results

Absolute values are presented in tables and relative changes in figures throughout the results.

4.1 Nutrition

There was no difference in nutritional intake between the groups for either energy intake or any of the macronutrients at baseline (table 4.1). Nutritional values at pre are the mean of calculated values from the two 24-h recall interviews performed during the preliminary testing days. Values for nutritional intake during the intervention are means of the four interviews from mid and post, plus the macronutrient values of the daily supplementation. Significant increases from Pre ($p<0.05$) were found for all values except fat in the milk group. There was no statistical difference in the change in fat between groups, however there was a strong statistical tendency (p-value: 0.06). When examining individual changes in fat intake, several people in the milk group reported lower intake of dietary fat in the recall interviews during the intervention.

The compliance with regards to supplementation resulted in a pooled mean compliance of 99.2%, based on the self-reported intake of sachets.

Table 4.1: *Nutrition data from before (Pre) and during intervention (with supplementation). Values shown as mean (±SD), changes shown as mean changes (± 95% CI).*

		Intake Mean(±SD)		change Mean(± 95% CI)	Between groups
Measurement	**Group**	**Pre**	**During (supplementation)**	**absolute values**	**difference of change (P<0.05)**
Energy intake (Kcal)	Milk	1906 (± 472)	2424*(± 370)	518 (± 275)	NS
	Native Whey	1886 (± 461)	2484*(± 349)	599 (± 208)	
Energy intake / body mass (Kcal/kg)	Milk	27 (± 8)	34*(± 7)	7 (± 4)	NS
	Native Whey	25 (± 6)	33*(± 7)	8 (± 3)	
Protein intake (g)	Milk	75 (± 21)	115*(± 18)	40 (± 10)	NS
	Native Whey	79 (±16)	115*(± 17)	35 (± 9)	
Protein / body mass (g/kg)	Milk	1.03 (± 0.25)	1.58*(± 0.26)	0.55(± 0.15)	NS
	Native Whey	1.07 (± 0.27)	1.56*(± 0.43)	0.49 (± 0.16)	
Carbohydrate intake (g)	Milk	181 (± 49)	255*(± 36)	74 (± 26)	NS
	Native Whey	173 (± 64)	254*(± 52)	81 (± 28)	
Fat intake (g)	Milk	94 (± 38)	94 (± 23)	-1 (± 21)	NS
	Native Whey	83 (± 24)	104*(± 22)	21 (± 13)	

During (supplementation) = Dietary intake + supplementation, SD= Standard deviation, CI= confidence interval, NS = non-significant, stars (*) signifies significant difference from Pre.

4.2 Strength

There were no significant differences between groups in the strength tests performed at baseline (table 4.2).

***Table 4.2**: Performance in the strength tests at pre and post, absolute values presented as mean (± SD), changes as mean (± 95% CI).*

Measurement	Group	Performance Mean(±SD)		Performance change Mean(± 95% CI)	Between groups difference of change(P<0.05)
		Pre	Post	absolute values	
Leg press 1RM (kg)	Milk	171 (± 57,0)	219*(± 59.4)	48 (±9.6)	NS
	NW	162 (± 53.6)	220* (± 64.3)	57 (±14.0)	
Chest press 1RM (kg)	Milk	47 (± 18.5)	56* (± 21.3)	9.0(±2.7)	NS
	NW	43 (±18.1)	52*(± 19.7)	8.5(±1.8)	
MVC (N)	Milk	289 (±82)	321* (±92)	32(±17)	NS
	NW	328 (±115)	357*(± 116)	29 (±6)	

NW= Native whey, MVC= Maximal voluntary contraction, SD= Standard deviation, CI= confidence interval, NS = non-significant, stars (*) signifies significant difference from Pre.

Both the milk group and the native whey group increased 1RM in leg press (31% and 38%, respectively) and in chest press (20% and 22%, respectively), but there were no significant difference between groups (figure 4.2.1).

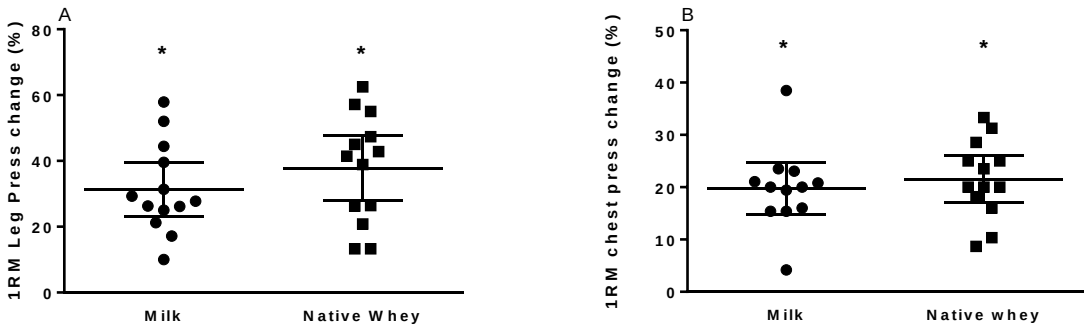


Figure 4.2.1: *Individual and mean 1RM changes (%) in leg press (A) and chest press (B) for milk (p < 0.01) and native whey (p< 0.01) (with 95% CI).*

One individual in the milk group showed an exceptional relative increase of 80% in chest press 1RM. This could be due to shoulder pain at pre-tests, and these values were therefore excluded. There was, however, still no difference of change between groups with this subject included.

A total of two subjects had to have their 1RM at post-test estimated for leg press, as they due to pain could not complete the test until failure. This also applied to one individual for chest press.

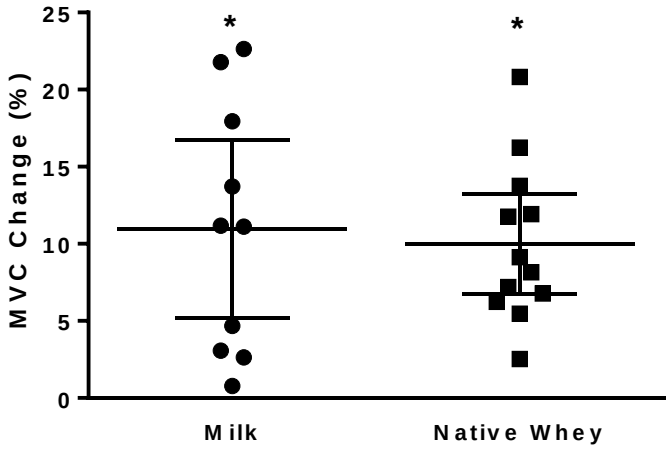


Figure 4.2.2: *Mean MVC changes (%) for milk (p<0.01) and native whey (p<0.01) (with 95% CI).*

Both groups increased maximal force of isometric voluntary contraction in knee-extension (figure 4.2.2) with 11% and 10% for the milk and native whey group, respectively, with no differences between groups. The numbers presented are the mean value of both legs. Seven tests in total of

either one leg or both were not included in this analysis, as due to pain or considerable fatigue, or other circumstances which made these subjects unable to perform the test as intended for both legs.

4.3 Body composition

There were no differences in body composition measurements at baseline between groups (table 4.3). There was no change in total fat mass for either group, however the body fat percentage decreased significantly ($p<0.01$) for the milk group (table 4.3). All individuals except one increased body mass (figure 4.3.1A). This individual was also the individual with the smallest increase of lean body mass in the milk group.

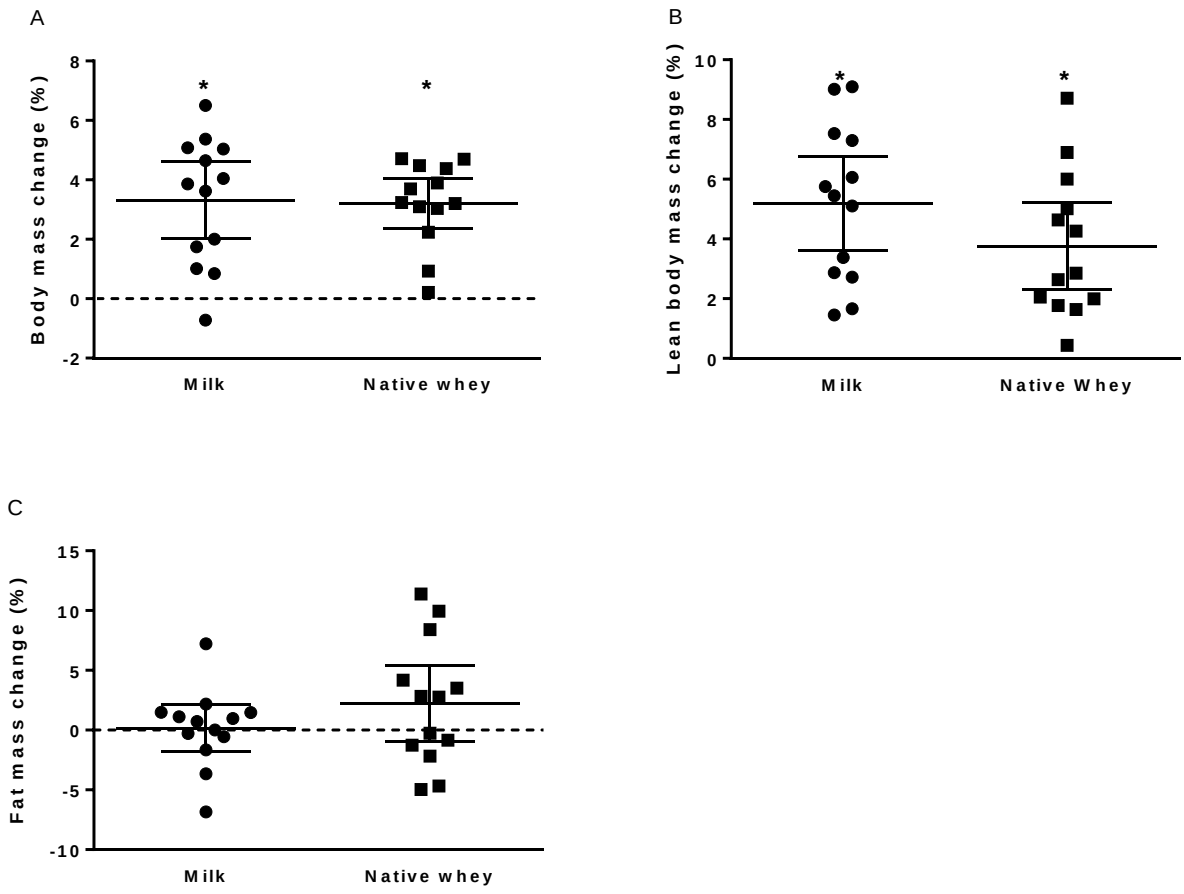


Figure 4.3.1: *Relative changes (%) in body mass (A), Lean body mass (B) and Fat mass change (C). All values shown as Mean with 95% CI.*

The mean changes for lean body mass were 2.4 kg (5.2%) and 1.8 kg (3.8%) for the milk and native whey group respectively (table 4.3; figure 4.3.1B). There were however no significant difference between groups.

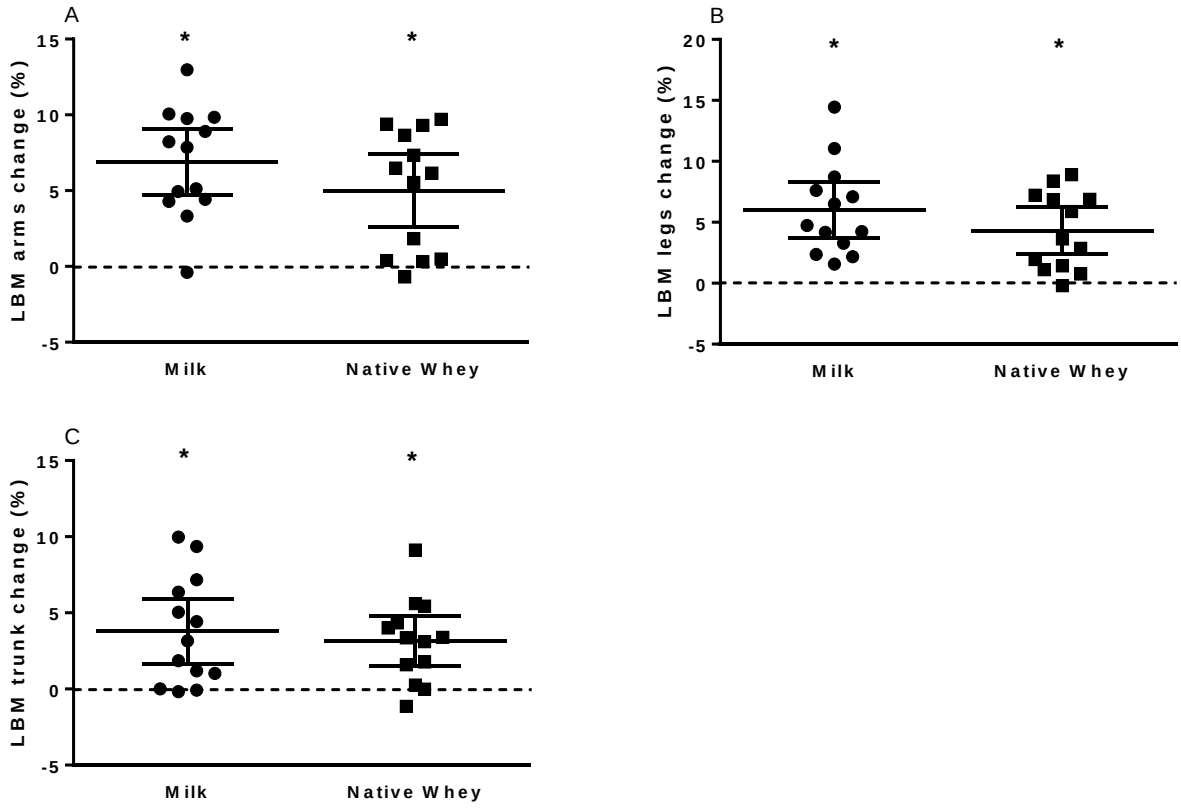


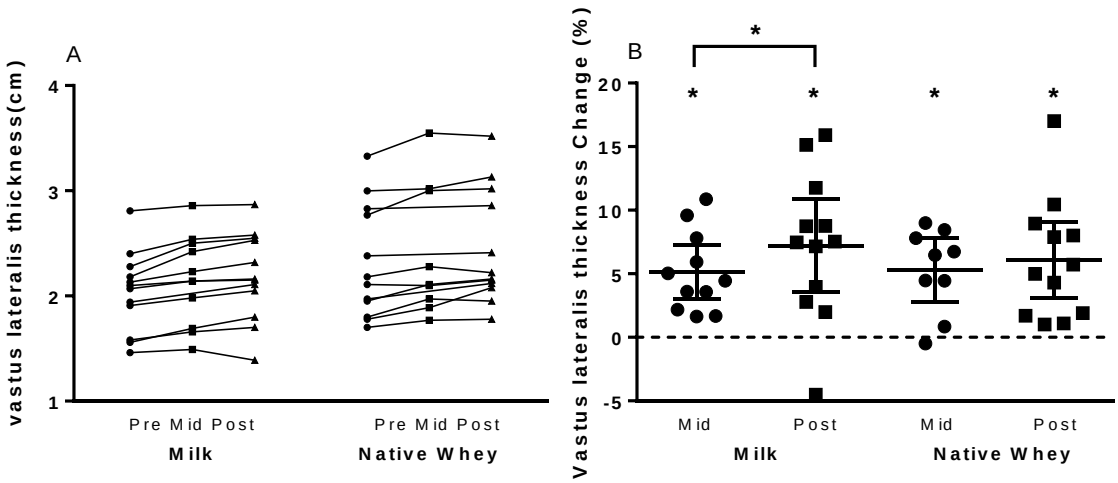
Figure 4.3.2: *Regional changes in lean body mass (LBM) showing relative changes (%) for arms (A), legs (B) and trunk (C). changes are shown as mean (with 95% CI).*

Both groups significantly increased lean body mass for all regions ($P<0.01$) when the DXA measurements were divided into arms, legs and trunk, with no differences between groups. Mean changes were 6.9% vs. 5.0% for arms (figure 4.3.2A), 6.0% vs. 4.2% for legs (Figure 4.3.2B) and 3.8% vs. 3.1% for trunk (figure 4.3.2C) for the milk and native whey groups respectively.

Table 4.3: *Absolute values for measurements of body composition, all measurements presented as absolute values mean (± SD), with changes presented as mean (with 95% CI).*

		Performance Mean(±SD)		Performance change Mean(± 95% CI)	Between groups
Measurement	**Group**	**Pre**	**Post**	**absolute values**	**difference of change(P<0.05)**
Body mass (kg)	Milk	74.1 (± 14.9)	76.4*(± 14.8)	2.3 (± 0.8)	NS
	Native Whey	76.8 (± 16.9)	79.2*(± 16.8)	2.4 (± 0.6)	
BMI (kg/m^2)	Milk	25.2 (± 3.8)	26.1*(± 3.6)	0.82 (± 0.31)	NS
	Native Whey	25.2 (± 4.6)	26.0*(± 4.6)	0.79 (± 0.24)	
Fat mass (kg)	Milk	22.6 (± 7.4)	22.6 (± 7.3)	-0.02 (± 0.35)	NS
	Native Whey	24.0 (± 8.7)	24.4 (± 8.6)	0.41 (± 0.60)	
Body fat composition (%)	Milk	31.43 (± 5.31)	30.37*(± 5.27)	-1.06 (± 0.50)	NS
	Native Whey	31.88 (± 8.58)	31.54 (± 8.56)	-0.35 (± 0.76)	
Lean body mass (kg)	Milk	48.7 (± 9.1)	51.1*(± 9.2)	2.45 (± 0.70)	NS
	Native Whey	50.3 (± 11.5)	52.1*(± 11.5)	1.82 (± 0.67)	
LBM trunk (kg)	Milk	23.6 (± 3.8)	24.4*(± 3.8)	0.86 (± 0.49)	NS
	Native Whey	24.0 (± 5.0)	24.8*(± 5.3)	0.78 (± 0.41)	
LBM arms (kg)	Milk	*5.4 (± 1.4)*	5.8*(± 1.5)	0.36 (± 0.10)	NS
	Native Whey	5.5 (± 1.6)	5.7*(± 1.6)	0.26 (± 0.13)	
LBM legs (kg)	Milk	16.7 (± 3.9)	17.6*(± 3.8)	0.93 (± 0.30)	NS
	Native Whey	17.6 (± 4.9)	18.3*(± 4.7)	0.67 (± 0.27)	
m. vastus lateralis thickness(cm)	Milk	2.04 (±0.38)	2.18*(± 0.42)	0.15 (± 0.07)	NS
	Native Whey	2.32 (± 0.54)	2.45*(± 0.54)	0.13 (± 0.06)	

BMI= body mass index, LBM=lean body mass, SD= Standard deviation, CI= confidence interval, NS = non-significant, stars (*) signifies significant difference from Pre.



Figur 4.3.3: *Individual measurements of m. vastus lateralis thickness (cm) (A) before (Pre), during (Mid) and after (Post) the intervention, and percentage changes from baseline (with 95% CI) with pre values set to zero (B).*

Both groups increased thickness of *m. vastus lateralis* with mean changes of 7.2% for milk and 6.1% for native whey (figure 4.3.3B) with individual changes shown in figure 4.3.3A. Both groups experienced significant ($P<0.01$) changes from the initial measurements however, the milk group also experienced significant ($P<0.05$) changes between measurements taken during (mid) and at the end of intervention (post). At no point were the changes statistically different between the groups.

4.4 Functional tests

The native whey group achieved significant ($P<0.05$) reductions in completion time for the stair climb with body mass (-6.4%) and with added mass of both 10 kg (-6.4%) and 20 kg (-7.5%) (Figure 4.4.1B). The stair climb completion time was also significantly ($P<0.05$) reduced for the Milk group when completing the test with body mass (-4.2%), but with added

mass the milk group did not achieve statistically different reductions (p-values of 0.09 and 0.054 for 10 kg (-3.1%) and 20 kg (-4.5%) trials respectively). At no point were changes in stair climb performance statistically different between groups. Individual changes in completion time can be seen in figure (4.4.1A).

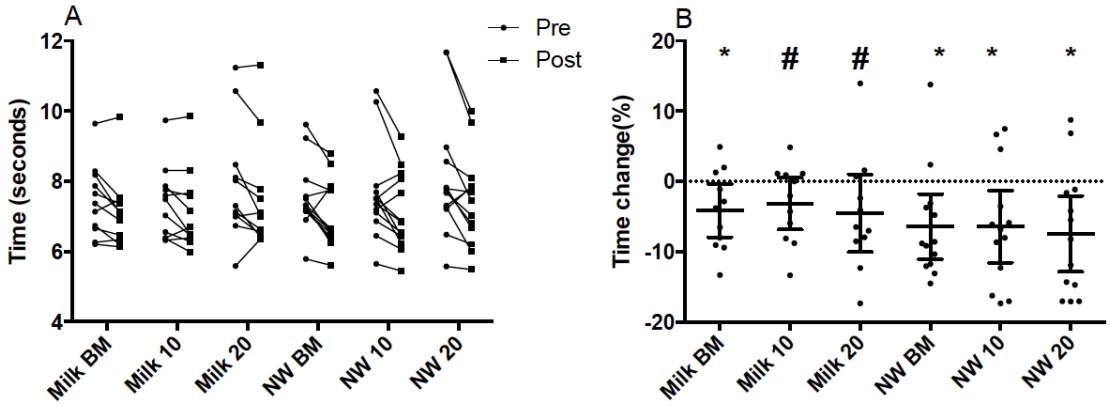


Figure 4.4.1: *Individual changes in seconds (left) and relative (%) changes for timed stair climb with mean changes (right) (with 95% CI) for body mass, 10 kg and 20 kg added load. NW= native whey group, Symbols (*, #) indicate significant(p<0.05) and tendency(p= 0.05-0.10).*

For the timed sit to stand test, both groups experienced significant reductions (P<0.05) in completion time (figure 4.4.2). The native whey group reduced completion time by 11.6%, and the milk group by 9.2%, with no difference in between the two groups.

Absolute values for completion times at baseline and posttest can be seen in table 4.4. There were no between group difference for either pre, post or mean values of change.

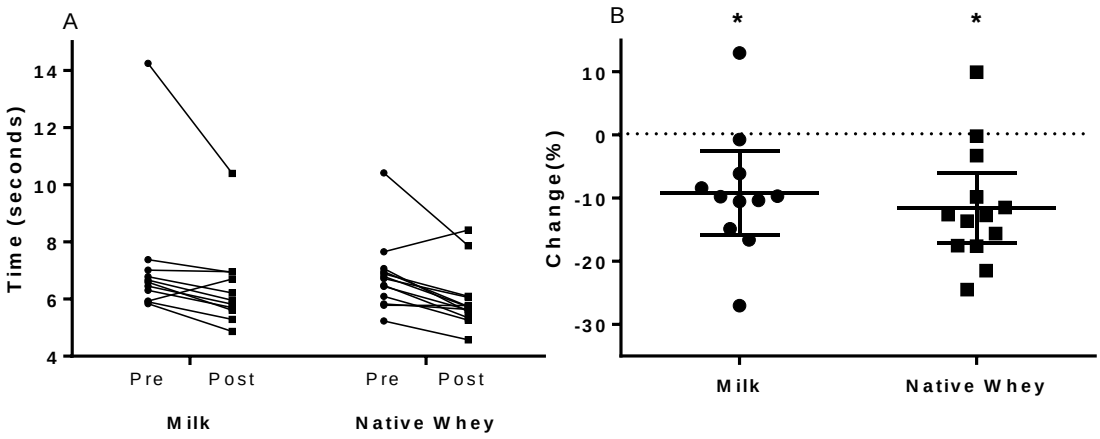


Figure 4.4.2 *(A) Individual values in completion time (seconds) and (B) relative changes (%) in the 5 times sit to stand test showing mean (with 95% CI)*

Table 4.4: *absolute values of completion times (seconds) of functional tests. Group mean values are given as mean (± SD), changes as mean change (± 95% CI).*

Measurement	Group	Performance Mean(±SD)		Performance change Mean(± 95% CI)	Between groups
		Pre	**Post**	**absolute values**	**difference of change(P<0.05)**
Bodyweight stair climb (sec)	Milk	7.5 (±1.0)	7.1* (± 1.0)	-0.32 (± 0.30)	NS
	Native Whey	7.5 (±1.0)	7.0* (±1.0)	-0.50 (± 0.34)	
10kg stair climb (sec)	Milk	7.4 (± 1.0)	7.2 (± 1.1)	-0.23 (± 0.27)	NS
	Native Whey	7.7 (±1.4)	7.1* (±1.1)	-0.53 (± 0.44)	
20kg stair climb (sec)	Milk	8.0 (± 1.6)	7.5 (± 1.6)	-0.40 (± 0.41)	NS
	Native Whey	8.1 (± 1.8)	7.4* (± 1.3)	-0.69 (± 0.49)	
Timed 5x sit to stand (sec)	Milk	7.19(±2.39)	6.40*(±1.48)	-0.79 (±0.76)	NS
	Native Whey	6.79(±1.26)	5.97*(±1.04)	-0.82 (± 0.47)	

SD= Standard deviation, CI= confidence interval, NS = non-significant, stars (*) signifies significant difference from Pre.

5. Discussion

The main findings in this study were: 1) There was no enhanced effect of native whey supplementation compared to milk in eliciting changes in lean body mass, strength or functional performance during an 11 week strength training period. 2) Our intervention seems to have yielded better results than found previously in subjects of this age group.

This chapter will discuss our findings in relation with those by other groups presented in the theory chapter. First a discussion of our main findings will be presented, followed by methodological considerations and the thesis conclusion.

5.1 Nutritional changes

The nutritional intake during the intervention was similar in the two groups. The supplements increased the protein intake by 53% and 46% in the milk and native whey groups respectively. It is suggested that an increase in protein intake from baseline elicit an additive effect in addition to that of strength training alone (Bosse & Dixon, 2012). The average increase in protein intake (g/kg/day) from baseline was 59.5% in interventions identifying an additive effect from protein intake (Bosse & Dixon, 2012). This suggests that the supplementation may have induced an appropriate change in protein intake in both groups, as there were no differences between them. However as we had no placebo group we can only compare effects between our two supplementations.

Due to less fat intake among some subjects during the intervention, the milk group did not increase their overall fat intake. However, there were no differences between groups. As there were no differences in nutritional changes, the main difference in food intake between the groups should be their appointed protein supplement. Therefore the resulting physical and performance changes will be discussed mainly based on the different supplementations.

5.2 Body composition changes

There were no differences between the groups in changes in body mass, lean body mass, or fat mass. There were no differences between the changes in appendicular lean body mass, or thickness changes of the *m. vastus lateralis*. However, the muscle thickness increased from mid to post only in the milk group, but no difference was seen from the native whey group. We hypothesized that the group supplemented with native whey would receive the greatest increase in lean body mass, due to its higher leucine content. Although the uptake of branched chain amino acids of native whey has been shown to result in higher plasma concentrations of leucine than milk (Laahne, 2013; Nyvik Aas, 2014), this had no additive effect on lean body mass gain during the period of investigation in the present study. This could be due to us using another product (PROLACTA®) than in the Laahne (2013) and Nyvik Aas (2014) studies, which used native whey produced by Tine SA (Laahne, 2013; Nyvik Aas, 2014).

As mentioned ingestion of less than 2 g of leucine has failed to elicit an optimal protein synthesis response in the elderly (Katsanos et al., 2006) and the leucine concentrations are shown to differ between milk protein fractions (Phillips et al., 2009). The leucine content is 7.7 g per 100 g of protein in skimmed milk powder, which would result in a total of 1.54 g for 20 g of protein (Phillips et al., 2009). Analysis of the milk used in the present study however, showed leucine content of 2.0 g in the provided 20.3 g dose. Furthermore, due to our persistence in keeping the supplement isocaloric, there may have been more protein in the milk sachets, as the analyzed sachets of native whey protein showed a protein content of 19 g. As a result of this, both protein sources have 2 g or above in leucine content, with 18% more in the native whey sachets (2.3 g). This seems a plausible explanation as to why the response is similar in both groups, as it seems that both groups were given an appropriate amount of leucine. However, others do not find an increased effect in either whole body or appendicular muscle mass when investigating the effect of supplementing with 10 g of the same native whey compared with milk protein in the elderly (Gryson, Ratel, et al., 2014). If native whey is indeed a better protein source in eliciting muscle hypertrophy, this is probably due to its ability to stimulate protein synthesis in lower dosages (15 g) than casein (30 g) in the elderly (S. Walrand et al., 2015). However, it has to be taken into account that some authors of these articles, as well as the mentioned Gryson, Walrand, et al. (2014) study, are

employed by Lactalis, the producer of the used whey protein supplement (Gryson, Walrand, et al., 2014).

Whey protein is as mentioned superior in inducing muscle protein synthesis in both young (Tang et al., 2009) and elderly (Pennings, Boirie, et al., 2011) in the immediate hours following intake, compared to soy and casein. Although these acute findings favor whey protein, this is not supported by our longitudinal data, although we investigated milk, it consists mainly of casein proteins (80%). Even though not statistically significant (p=0.16), the mean change in lean body mass was if anything favoring the milk group (2.4 vs. 1.8 kg), suggesting that perhaps milk incorporates important qualities not restricted to the whey proteins.

It has been suggested that the absorption rates following native whey supplementation may be too fast to sustain a postprandial anabolic response due to its transient hyperaminoacidemia (Lacroix et al., 2006). As the dosage in the milk sachets of leucine may be sufficient (2 g), the milk group may have increased their net protein balance also due to casein's effect on whole-body protein breakdown, and slower release of amino acids (Boirie et al., 1997). The possibility of increased effects by combining whey and casein as in milk or as a 50/50 blend has been suggested (Reitelseder et al., 2011). The transient but substantial FSR response following whey ingestion is more moderate but extended following casein ingestion (Reitelseder et al., 2011) and they propose a combination of the two may prove advantageous. It could be argued that the trend in our data support this. However this is merely a trend, with no statistical difference.

The overall protein intake of both groups was above the recommended daily allowance (1.2 g/kg/day) for elderly in the Nordic countries (Nordic Council of Ministers, 2014). No subject consumed less than 1.1 g/kg/day, with only 3 subjects reporting an intake below 1.2 g/kg/day. As elucidated by Katsanos et al. (2006) and Walrand et al. (2015) differences between protein sources seem most apparent when given in smaller boluses. Although the overall intake does not directly reflect the dosage size of protein with each meal, many subjects reported ingesting their protein supplement with breakfast/lunch and supper on non-training days, as they were instructed to ingest one sachet in the morning and in the afternoon/evening. This suggests that our subjects could be ingesting

dosage sizes of proteins at mealtimes nullifying the differences between them (Katsanos et al., 2006).

Even though no differences were seen between groups, a substantial effect was seen from baseline following the intervention. Our intervention induced increases in lean body mass higher then reported in comparable interventions in elderly individuals. The increase in lean body mass following resistance exercise with protein supplementation in elderly during 12-24 weeks of training seem to range from 0.5 kg to 1.5 kg (table 2.1). Our intervention-induced changes were 2.45±0.70 kg (5.2%) for the milk group, and 1.82±0.67 kg (3.8%) in the native whey group, considerably more than the presented gains reported on similarly aged subjects (table 2.1). The next segments will focus on possible contributing factors as to why this difference exists, in addition to the appropriate change in protein intake described already.

Our subjects performed exercise with heavy loads with sets performed to failure, as we increased the load lifted by our subjects progressively with adjustments session to session. We challenged our subjects to lift heavier loads until they reached failure within the prescribed range of repetitions (which happened frequently), or were unable to perform the exercise with the correct technique. As a result, our subjects consistently trained at the prescribed relative intensity, close to failure, proven to be advantageous in the literature (Burd, West, et al., 2010). Some studies describe similar methods as ours for adjusting the intensity. Iglay et al. (2009) adjusted intensity by two methods with the first two sets in an exercise being performed at 80% of 1RM (tested every 4 weeks), while the last set was performed until voluntary failure. If 12 repetitions was exceeded the load was adjusted by 5% for the next session (Iglay et al., 2009). Number of exercises performed by others range from 5 (Chale et al., 2013) to 8 (Iglay et al., 2009) suggesting that the total volume of the exercise conducted by our subjects may be higher, as the amount of reps and sets for the studies in table 2.1 are comparable, 3-4 sets 8-15 reps with an intensity of ~80% of 1RM for each exercise was used in those studies conducting whole body resistance training presented in table 2.1.

The exercise program used in the mentioned study by Solberg et al. (2011) is probably the trial most comparable to the present study, although the duration was extended. They reported an increase in lean body mass of 1.4±0.4 kg with an exercise intervention

identical to ours. Even though their exercise intervention was similar, our subjects experienced a larger increase in lean body mass. This could possibly be due to difference in execution of the program, or an additive effect of the protein supplements, however, direct comparison is not possible.

Another contributing factor could have been physical activity level of the subjects. One requirement for inclusion was as mentioned no prior experience with strength training. However, we did not set any requirements for recreational activity. We believe several of our subjects were quite active for their age, as they reported their fondness for hiking, bicycling and cross-country skiing. Moderate aerobic activity has previously been shown to improve postprandial MPS response (Timmerman et al., 2012). Furthermore as mentioned, disuse reduces the anabolic response to protein intake (Wall, Snijders, et al., 2013). It could be argued that our subjects "lack of disuse" and participation in recreational physical activity (mainly aerobic in nature) could have improved their anabolic sensitivity, resulting in larger adaptations than other studies. That is if their subjects were more sedentary at inclusion and on non-training days (weekends), where the documented post-session anabolic window might be reduced (Phillips et al., 1997).

In addition to the possible contributing factors already mentioned, the high content of energy in our supplementations might have further augmented changes, as an increased energy intake regardless of macronutrient source promote lean mass accretion as long as protein requirements are met through diet (Rozenek et al., 2002). Some trials presents only supplied supplementation post exercise (Verdijk et al., 2009), and supplied protein only in their supplement, with no further caloric content (Verdijk et al., 2009). The caloric content in the protein supplementations of the presented interventions range from under 100 kcal (Leenders et al., 2013; Verdijk et al., 2009) to 378 kcal in two dosages (Chale et al., 2013). The overall daily caloric intake during supplementation in all studies range from 1700 kcal (22 kcal/kg) (Chale et al., 2013) to 2400 kcal (30 kcal/kg) for the male group (33 kcal/kg for females) in the Leenders et al. (2013) trial. Both our groups received close to an extra 600 kcal per day in supplements during the intervention, resulting in an overall intake of over 2400 kcal (33-34 kcal/kg) per day for both groups. This suggests the overall caloric intake in our intervention was close to optimal, as body composition analysis revealed no change or decrease in fat mass, whereas others have observed reductions (Verdijk et al., 2009). This suggests their

intake might have been suboptimal for promoting muscle hypertrophy. The relative mean protein intake is also higher in the present study compared with others, with a reported mean intake of 1.1-1.3 g/kg during supplementation in several studies (Chale et al., 2013; Leenders et al., 2013; Tieland, Dirks, et al., 2012; Verdijk et al., 2009).

5.2.1 Regional changes

The increase in lean body mass was present in all regions (table 4.3). In addition to this, thickness of *m.vastus lateralis* increased similarly in both groups, indicating increases in lean body mass were not related to increases in connective tissue and inner organs, but primarily caused by chances in muscle mass. Since there were no placebo group in this study, we cannot answer whether the change in muscle mass is impacted by the protein intake itself. The meta-analysis by Finger et al. (2015) concluded that protein supplementation only elicits further increases in lean body mass, with no further effect on skeletal muscle mass gains than those induced by resistance exercise alone in the elderly. Of the other interventions in the elderly, several measure appendicular adaptations and a mean increase in leg lean mass of 3% was found across groups by Leenders et al. (2013) while (Verdijk et al., 2009) induced a change of 6% in lean leg mass. Both results are relatively comparable to our results showing an increase of 6.0% and 4.2% in lean leg mass for milk and native whey respectively. Tieland, Dirks, et al. (2012) showed an increase of 5% in appendicular lean mass following resistance exercise and protein intake (Tieland, Dirks, et al., 2012) comparable effects to what we found in our study. However, their study is as mentioned conducted on frail elderly, over a longer intervention period, with a lower training frequency (table 2.1).

In studies measuring CSA changes for quadriceps, induced changes vary from 3% (Chale et al., 2013) to 9% (Leenders et al., 2013; Verdijk et al., 2009). However, as elucidated by Hakkinen et al. (2001), hypertrophy following exercise is not homogenous across the length of all muscles, making these results difficult to compare as small differences in point of measurements could impact the results found by different groups. Although we were not able to utilize MRI or CT measurements in the present study, measurements of muscle thickness were conducted. Both lean leg mass and ultrasound measurement of thickness increased significantly, with no difference between groups. Although not directly comparable with other interventions, the observation that both lean mass for legs, and muscle thickness increased suggest that

skeletal muscle mass in the lower limbs increased significantly in both groups in the current intervention.

5.3 Changes in strength and physical performance

The intervention induced significant changes in strength for both groups. Significant increases in strength were induced in chest press, leg press, and isometric maximal voluntary contraction in leg extension, irrespective of supplementation type. No significant differences were found between the changes in strength.

The increase seen in leg press with mean changes of 31% (milk) and 38 % (native whey) were not different between groups. Furthermore, it is comparable to the relative increase induced over 12-24 weeks in the presented similar interventions. It seems that an increase in 1RM of 20-40% is to be expected in the elderly, which is in line with the expected 1% increase per exercise session (Kraemer et al., 2002) for untrained individuals. The increases in 1RM in chest press were not as substantial; an increase in 20% and 22% in 1RM were seen in the milk and native whey groups respectively. It was expected that the increase in strength was lower in this test since the training program only contained one exercise for the chest muscles. Whereas the subjects performed four lower-limb exercises, all of which could contribute to an increase in leg press strength.

As mentioned in the introduction, one of the defining characteristics of sarcopenia is the impaired ability to perform habitual activities, due to loss of muscle mass and strength. Although none of our subjects had problems performing daily activities, they all increased their muscle strength. This suggests the intervention has been effective in increasing their functional ability, which could reduce their risk of dependability in the future. In frail elderly it has been found that both exercise and protein intake can individually improve physical function (Tieland, Dirks, et al., 2012; Tieland, van de Rest, et al., 2012). Even though our subjects were not frail, the next segment will discuss the effect of the intervention on physical function.

The strength changes also elicited significant improvements in the functional physical performance tests. Both groups reduced their completion time of the stair climb significantly with bodyweight, but only the native whey group reached statistical

significance in both the +10 kg and +20 kg trials. These improvements was, however, not significantly different from the milk group. In the same test, the subjects in the Solberg et al. (2011) study, were ~1 second slower than in the current study for both the bodyweight and 20 kg loaded trials. However, their improvements were similar to those seen in our intervention (Solberg et al., 2011). Their subjects were however considerably slower at both inclusion and end in the sit to stand test, suggesting our subjects may have had a higher level of function at inclusion (Solberg et al., 2011), although some differences exist in the execution of the test which might have affected the absolute time. Their subjects were asked to touch the back support while sitting, while our subjects were required to lift their feet from the ground. Other differences were our utilization of a pressure plate timing system, while they used a stopwatch which may further have impacted the comparability of the test (Solberg et al., 2011). Several others also find an effect in chair-rise tests (Chale et al., 2013; Leenders et al., 2013). An improvement by approximately 10% after 11-12 weeks is seen in this study and others (Leenders et al., 2013). The two subjects with the highest completion times at start also reduced their completion time the most, with improvements of 27% and 24.5% for these two individuals, suggesting they received the most benefit from the intervention.

The changes in functional performance were not uniform among subjects, and some subjects did not improve their performance. This could be due to fatigue from the previous tests conducted, or the change in strength did not have an impact on their physical performance, or that some subjects simply cared less about their performance in the physical tests. This last notion is based on several statements from subjects reporting that they had personal goals for their 1RM performance in leg press and chest press, but not for increasing their performance in the functional tests. Furthermore, there was a considerable learning effect in these tests, resulting in a high CV between the two preliminary testing days (see methods), as subjects generally improved their completion time on the second day (best value noted as PRE). Changes between their best trials at pre and post-tests, suggests that an improvement took place. This notion is supported by the fact that the first testing day was conducted with a focus for learning the execution of the test, while a good performance was the focus for the second preliminary testing day and at posttest.

In summation our high number of exercises may have contributed to the increased effect on changes in body composition of our intervention compared to others, as the volume, intensity, and that exercises are performed until failure, have been shown to impact the response (Burd, Holwerda, et al., 2010). The increases in strength also resulted in an improved physical performance in the functional tests, confirming that strength training with protein supplementation is an effective way of reducing the effects of sarcopenia. Furthermore, as mentioned after the age of forty the average decline in muscle mass is 1% annually (Baumgartner et al., 1998). The current intervention increased lean body mass and lean leg mass substantially, underpinning the potential for counteracting the age related loss with strength training and proper nutrition. However no statistical differences exist that suggest a better effect from either protein supplement.

5.4 Methodological considerations

There was an ambition to perform MRI-scans to estimate regional changes of both thigh and arm muscles in this study. However, coordination issues between our department and the MRI-facility meant the scans would have to be performed after the exercise period had begun, during week 1 or 2 of training and most likely with a different (non - individual) protocol than requested. As this would be less than optimal these funds were redistributed towards the upcoming intervention on younger subjects. The protocol made by the author of this thesis will still prove valuable for this purpose. However, although regional changes obtained by DXA and US (*m. vastus lateralis*) were measured in this study, originally we would have liked to have MRI-scans for these subjects as well to have more sensitive measurements of any possible regional changes.

The resistance exercise was, in our opinion, conducted in an optimal way. This is due several aspects of how the strength training was organized. Rotation of instructors ensured that if different instructors played a role, this would be minimized as subjects were constantly subjected to all instructors. Subjects completed a similar number of sessions, however the time course varied. As subjects missed sessions (e.g. sickness), their training period was extended to allow for completion of the prescribed 33 sessions. All subjects were treated the same, however some consideration were taken in the case of pain. This was most common in the execution of shoulder press, as not all subjects were able to complete the exercise with abducted arms out to the side due to pain or

reduced mobility. For these subjects the exercise was conducted with a neutral grip, allowing the elbows to be kept in front of the trunk during the execution.

The reported compliance (99.2%) to supplementation could have possible dark figures, since no external control of the intake was conducted. We trusted our subjects to consume sachets twice a day and report their intake to us with honesty. We could have implemented a weekly delivery of empty sachets could have improved this aspect of the intervention, as we then would have two measures of compliance to supplementation.

In hindsight the functional tests could have been expanded to include the entire SPPB battery (Guralnik et al., 1995), allowing us to compare our subjects physical function at baseline more accurately with those interventions utilizing this battery. However, our tests are similar to the Solberg et al. (2011) intervention previously conducted in our lab (Solberg et al., 2011).

6. Conclusion and future research

The current study suggests that native whey protein is not more effective in increasing lean body mass, muscle thickness, strength or physical performance compared to that of milk protein in elderly subjects during a prolonged training intervention. Both supplements elicited similar effects when given in a dosage of 2x20 g as co-ingested with carbohydrates and fat. If any differences exist between the two products effectiveness, it could not be found during our 11 week trial, with our measurements. However, more subjects are currently completing the intervention to allow for higher statistical power.

Future research into the possible effects of different protein sources on muscle mass and muscle function in combination with strength training in the elderly should strive to utilize isocaloric and isonitrogenic supplements. Furthermore, in doing this it is important that habitual protein intake is monitored. Differences in protein intake should be limited to the investigated supplementations, as changes in overall protein intake could influence the results. Currently few studies have been conducted in the frail elderly; however they show a significant effect of protein supplementation, both without and with exercise (Tieland, Dirks, et al., 2012; Tieland, van de Rest, et al., 2012). Future research into different protein sources should strive to investigate this group.

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II: Information letter sent to prospective participants.

III: Written consent form.

IV: Session sheet examples (Monday – Wednesday – Friday) used for each participant during the strength training period.

Forsøkspersoner søkes!

«Styrketrening og melkeprotein»

Prosjektet ”**Hvordan påvirker forskjellige melkeproteiner muskelproteinbalanse hos eldre?**” har til hensikt å undersøke effekten av forskjellige melkeproteiner på muskelvekst gjennom en 12 ukers treningsperiode hos yngre og eldre.

Vi søker etter kvinner og menn over 70 år som ikke trener regelmessig, men ønsker å komme i bedre fysisk form

Studien vil foregå i perioden september-desember 2014 og innebærer en rekke tester før og etter treningsperioden. Det vil bli ca. 4 oppmøter på 2-3 timer i forkant og etterkant av treningsperioden for testing.

Treningen vil foregå 3 ganger per uke i grupper på 3 med personlig trener på alle økter ved Norges idrettshøgskole.

Før og etter treningsperioden vil det bli tatt blodprøver og muskelbiopsier

Er du interessert, ta kontakt med :

Håvard Hamarsland: 93445916; haavardh@nih.no

Appendix I: Information poster regarding the study

NORGES IDRETTSHØGSKOLE

Forespørsel om deltakelse som forsøksperson

Hvordan påvirker inntak av forskjellige melkeproteiner muskelproteinbalanse hos eldre?

Dette skrivet er til alle potensielle forsøkspersoner. Vi ber om din deltakelse i prosjektet, så fremt du oppfyller kriteriene: Du må være 70 år eller eldre, være normalt aktiv, og ellers kunne gjennomføre styrketrening. Du kan ikke bruke spesifiserte medikamenter eller kosttilskudd (proteinpulver, kreatin eller lignende). Hvis du bruker slike kosttilskudd kan du likevel delta som forsøksperson ved at du slutter med tilskuddet senest én uke før prosjektstart. Du kan ikke delta om du er laktoseintolerant, har melkeallergi eller er allergisk mot lokalbedøvelse (tilsvarende det man får hos tannlegen).

Bakgrunn og hensikt med forsøket

Sarkopeni (aldersrelatert muskelsvinn) har de siste årene fått mye oppmerksomhet da det i tillegg til å redusere funksjon og livskvalitet i hverdagen også disponerer for flere livsstilssykdommer (bla. type II diabetes og osteoporose). Styrketrening og et økt inntak av proteiner har vist seg å kunne motvirke muskelsvinnet. Inntak av proteiner har i seg selv en umiddelbar muskeloppbyggende effekt ved at proteinsyntesen øker; og kombinerer vi proteininntak med styrketrening får vi en vesentlig kraftigere effekt. Økningen i proteinsyntesen bestemmes i stor grad av mengden og kvaliteten på proteinet, samt hvor raskt proteinet tas opp i blodet. I tillegg til proteinsyntesen vil også proteinnedbrytningen til enhver tid spille inn på proteinomsetningen i muskulaturen. Sammenliknet med proteinsyntesen vet vi lite om hvordan proteinnedbrytningen påvirkes av proteininntak etter styrketrening. Ny kunnskap om dette kan gi oss bedre forutsetninger for å maksimere utbyttet av styrketrening, som vil være av stor interesse for eldre med tanke på livskvalitet og funksjon i hverdagen.

I denne studien ønsker vi å undersøke om et nyutviklet myseprotein produsert av Tine® kan bedre effekten av styrketrening (føre til større økning i muskelmasse og styrke). Dette nye myseproteinet vil sammenliknes med effekten av vanlig lettmelk.

Dette er et dobbelt blindet, randomisert, kontrollert studie, som betyr at verken du eller forskerne du kommer i kontakt med vet hvilken drikk du inntar.

Gjennomføringen av forsøket

Forsøket går kort fortalt ut på å gjennomføre en treningsperiode på 12 uker med styrketrening tre ganger i uken. Gjennom denne perioden inntas det to enheter på 0,6 l daglig med enten melk eller nativ myse. Du vil bli tilfeldig trukket (randomiseres) til én av gruppene. Før og etter treningsperioden vil det gjennomføres en rekke tester (se under) for å se på effekten av de forskjellige drikkene.

Før treningsperioden

Du skal møte på Norges idrettshøgskole 4 ganger for tilvenning til tester og treningsøvelser, styrketester, måling av kroppssammensetning (DXA), en legesjekk og muskelbiopsier i ukene før forsøket. I tillegg må du møte for en MR-analyse hos Curato røntgen. Tidspunkter for de ulike oppmøtene avtales individuelt. Under følger et eksempel på tidsplan for tester:

Dag 1: Underskrevet samtykke og helseerklæring. Fastende blodprøve, DXA-scan, medbrakt frokost, tilvennig til styrketester og funksjonelle tester (ca. 2 timer).

Minimum 2 dager hvile.

Dag 2: Gjennomføring av styrketester og funksjonelle tester (ca 1 time).

Minimum 2 dager hvile.

Dag 3: MR hos Curato røntgen (ca. 30 minutter).

Dag 4: Akuttforsøk (ca. 8timer) eller prebiopsi (ca 45 minutter).

Før treningsstudien må du også gjennomføre to kostintervju, en tilsvarende kostregistrering vil gjentas mot slutten av treningsperioden. **I de to siste dagene før tester og biopsi(er) må du avstå fra all krevende fysisk aktivitet (trening)**.

Akuttforsøk

Ti deltakere fra hver gruppe trekkes tilfeldig ut til å gjennomføre et akuttforsøk før og etter treningsperioden, dette innebærer 2 biopsier før treningsperioden og 2 biopsier etter treningsperioden (totalt 4 biopsier). De resterende deltakerne i hver gruppe deltar ikke i akuttstudien og tar bare én biopsi før og én etter treningsperioden. Hensikten med akuttforsøket er å måle hvordan muskulaturens respons til trening forandres over treningsperioden og hvordan inntak av de to drikkene påvirker dette. Oppstart denne dagen vil være mellom kl. 0800 og 0900, og forsøket er ferdig mellom kl. 1530 og 1630. Før vi gjennomfører treningsøkten vil vi ta en biopsi og gjennomføre en styrketest i et kneekstensjonsapparat. Treningsøkten vil være identisk med noen av øktene som gjennomføres senere i treningsperioden. Etter treningsøkten vil du innta en av de to drikkene, og det vil bli tatt en biopsi tre timer etter økten. Det vil også bli tatt blodprøver (gjennom venekateter) flere ganger i løpet av dagen, og gjennomført styrketester rett etter økten, 3 timer etter økten og 24 timer etter økten, for å måle restitusjon. Dermed vil du måtte sette av en hel dag til testdagen (fra 0700-0800 frem til ca. 1530-1630) og 30 min til styrketesting og blodprøve dagen etter.

Treningsperioden

Treningsperioden starter når man har gjennomført alle testene, og den varer i 12 uker. I disse 12 ukene skal det trenes styrke tre ganger i uken (mandag, onsdag og fredag) i grupper på tre deltakere med oppfølging av en personlig trener på alle økter. Drikkene inntas to ganger om dagen; etter trening og på kvelden på treningsdager, og morgen og kveld på treningsfrie dager.

Etter treningsperioden gjennomføres alle testene på nytt for å måle endringer.

Tester

DXA: ved et av oppmøtene før testingen gjøres en DXA-analyse for å måle kroppssammensetningen som vil danne grunnlaget for de standardiserte måltidene ved testgjennomføringen. Denne testen innebærer at deltakerne ligger stille i ca. 10 minutter.

MR: for å måle muskelvekst i lår- og overarmsmuskulaturen benyttes en MR-analyse. Denne testen innebærer at du må ligge i ro ca. 15 minutter.

1RM tester: for å måle styrke vil det testes hvor mye du kan løfte maksimalt en gang i to øvelser som heter beinpress og brystpress.

Muskelfunksjonstest: testingen av muskelfunksjonen gjøres i et kneekstensjonsapparat som er låst ved 90° i kneleddet.

Funksjonelle tester: en test av hvor raskt du kan reise seg fra en stol fem ganger på rad, samt en test av hvor raskt du kan gå opp en trapp vil bli brukt til å si noe om funksjon i hverdagen og mobilitet.

Blodprøver: blodprøvene vil tas i sammenheng med biopsiene og vil gjøres gjennom venekatetrene slik at det ikke blir noen ekstra stikk for blodprøver.

Biopsier: For de som tilfeldig velges til å være med på akuttforsøket blir det to biopsier før og to biopsier etter treningsperioden. For de som ikke skal være med på akuttforsøket blir det én biopsi før og én etter treningsperioden.

Biopsiene tas ut på følgende måte:

- Huden og bindevevet lokalbedøves der vevsprøven skal taes.
- Et snitt på ca. 1-2 cm gjøres gjennom hud og muskelfascien.
- En nål med diameter på 6 mm føres inn (2-3 cm) og 1-3 små biter av muskulaturen tas ut (total 2-300 mg).
- Snittet lukkes med tape (strips).

Eventuelle ulemper ved å delta

Deltakelse i prosjektet vil kreve en del tid og oppmerksomhet.

Trening skal gjennomføres med stor belastning, og vil medføre en viss risiko for skade og følelse av sårhet/stølhet i muskulaturen.

Venekateter medfører en liten infeksjonsfare og det kan oppleves ubehagelig.

Vevsprøvetakninger (biopsier) medfører en liten infeksjonsfare, og ubehag/smerter kan oppleves under inngrepet. Du kan også oppleve lette til moderate smerter i 1-2 døgn etter inngrepet.

Du vil få et lite arr etter snittet i huden; arret vil sakte bli mindre tydelig. Enkelte personer vil kunne få en fortykning av huden i arrområdet.

Personvern

Vi vil kun lagre informasjon om deg under ditt forsøkspersonnummer. Undervis i forsøket vil vi oppbevare en kodeliste med navn og forsøkspersonnummer. Denne kodelisten vil fysisk være låst inne, slik at det er kun forskerne tilknyttet studien som har adgang til den. Alle som får innsyn i informasjon om deg har taushetsplikt. Innsamlet data vil bli anonymisert etter 15 år (kodelisten destrueres).

Alle prøver vil analyseres ”blindet”, det vil si at forskerne som utfører den enkelte analysen ikke vet hvilken forsøksperson prøven kommer fra (verken forsøkspersonnummer eller gruppe). Prøver vil bli analysert ved NIH (biopsier), Universitet i Oslo (ernæringsinstituttet; biopsier og blod) og Universitetet i Arkansas, USA (biopsier og blod).

Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Biobank

Biopsiene og blodprøvene vil bli oppbevart i en forskningsbiobank uten kommersielle interesser (vurdert av Regional Etisk Komite). Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Prøvene vil bli lagret til år 2020. Ansvarlig for biobanken er Professor Truls Raastad ved Seksjon for fysisk prestasjonsevne ved NIH. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK). Hvis du sier ja til å delta i studien, gir du også ditt samtykke til

at prøver og avidentifiserte opplysninger utleveres til ernæringsinstituttet ved universitetet i Oslo og universitetet i Arkansas.

Innsynsrett og oppbevaring av materiale

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Informasjon om utfallet av studien

Etter at data er innsamlet og analysert vil vi avholde et møte for alle forsøkspersonene der vi presenterer resultatene fra studien.

Forsikring

Deltakere i prosjektet er forsikret dersom det skulle oppstå skade eller kompikasjoner som følge av deltakelse i forskningsprosjektet. NIH er en statlig institusjon og er således selvassurandør. Dette innebærer at det er NIH som dekker en eventuell erstatning og ikke et forsikringsselskap.

Finansiering

Prosjektet er fullfinansiert av Tine® og Norges forskningsråd.

Publisering

Resultatene fra studien vil offentliggjøres i internasjonale, fagfellevurderte, tidsskrift.

Du vil få tilsendt artiklene hvis du ønsker det.

Samtykke

Hvis du har lest informasjonsskrivet og ønsker å være med som forsøksperson i prosjektet, ber vi deg undertegne "Samtykke om deltakelse" og returnere dette til en av personene oppgitt nedenfor. Du bekrefter samtidig at du har fått kopi av og lest denne informasjonen.

Det er frivillig å delta og du kan når som helst trekke deg fra prosjektet uten videre begrunnelse. Alle data vil, som nevnt ovenfor, bli avidentifisert før de blir lagt inn i en database, og senere anonymisert.

Dersom du ønsker flere opplysninger kan du ta kontakt med Håvard Hamarsland

på tlf: 93 445 916 eller e-post: haavardh@nih.no, Gøran Paulsen på tlf: 93429420, eller Truls Raastad på tlf: 23 26 23 28 el. 913 68 896

Vennlig hilsen

Håvard Hamarsland (Stipendiat)

Gøran Paulsen (forsker)

Truls Raastad (Professor)

Appendix II: Information letter sent to prospective participants.

Samtykke til deltakelse i studien Hvordan påvirker inntak av forskjellige melkeproteiner muskelproteinbalanse hos eldre?

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix III: Written consent form.

Uke 1-3	Mandag	Dato:_____	Tatt alle drikker?:			
FP:_____		Instruktør:_____				Tid:
Øvelse 1:	Hammersquat			Reps	Kg	Borg
		Oppv.		12		
		Serie 1		12		
		Serie 2		12		
Øvelse 2:	Beinpress			Reps	Kg	Borg
Kloss:		Serie 1		12		
Øvelse 3:	Knee extensions			Reps	Kg	Borg
Sete:		Serie 1		12		
Fot:		Serie 2		12		
Øvelse 4:	Tåhev			Reps	Kg	Borg
		Serie 1		12		
		Serie 2		12		
Øvelse 5: Chest press				Reps	Kg	Borg
Sete:		Oppv.		12		
		Serie 1		12		
Øvelse 6: Sittende roing				Reps	Kg	Borg
		Oppv.		12		
		Serie 1		12		
Øvelse 7: Nedtrekk				Reps	Kg	Borg
Øvelse 8: Skulderpress				Reps	Kg	Borg
		Oppv.		12		
		Serie 1		12		
Øvelse 9:	Back extension			Max 15	Kg	Borg
		Serie 1				
Øvelse 10: Ab crunch				Max 15	Kg	Borg
		Serie 1				

Uke 1-3	Onsdag	Dato:_____	Tatt alle drikker?:		

FP:_____ Instruktør:_____ Tid:

Alle øvelser gjennomføres ved 90% av 10 RM. *** = 10 RM**

Øvelse 1:	Hammersquat		Reps	Kg	Borg
		Oppv.	10		
		Serie 1	10		
		Serie 2	10		

Øvelse 2:	Beinpress		Reps	Kg	Borg
Kloss:		Serie 1	10		
		Serie 2	10		

Øvelse 3:	Knee extensions		Reps	Kg	Borg
Sete:		Serie 1	10		
Fot:		Serie 2	10		

Øvelse 4:	Tåhev		Reps	Kg	Borg
		Serie 1	10		
		Serie 2	10		

Øvelse 5: Chest press			Reps	Kg	Borg
Sete:		Oppv.	10		
		Serie 1	10		

Øvelse 6: Sittende roing			Reps	Kg	Borg
		Oppv.	10		
		Serie 1	10		

Øvelse 7: Nedtrekk*			Reps	Kg	Borg
		Serie 1	10		

Øvelse 8: Skulderpress			Reps	Kg	Borg
		Oppv.	10		
		Serie 1	10		

Øvelse 9:	Back extension*		Max 15	Kg	Borg
		Serie 1			

Øvelse 10: Ab crunch*			Max 15	Kg	Borg
		Serie 1			

Uke 1-3	Fredag	Dato:_____	Tatt alle drikker?:		
FP:_____		Instruktør:_____			Tid:
Øvelse 1:	Hammersquat		Reps	Kg	Borg
		Oppv.	8		
		Serie 1	8		
Øvelse 2:	Beinpress		Reps	Kg	Borg
Kloss:		Serie 1	8		
		Serie 2	8		
Øvelse 3:	Knee extensions		Reps	Kg	Borg
Sete:		Serie 1	8		
Fot:		Serie 2	8		
Øvelse 4:	Tåhev		Reps	Kg	Borg
		Serie 1	8		
		Serie 2	8		
Øvelse 5: Chest press			Reps	Kg	Borg
Sete:		Oppv.	8		
		Serie 1	8		
Øvelse 6: Sittende roing			Reps	Kg	Borg
		Oppv.	8		
		Serie 1	8		
Øvelse 7: Nedtrekk			Reps	Kg	Borg
Øvelse 8: Skulderpress			Reps	Kg	Borg
		Serie 1	8		
Øvelse 9:	Back extension		Max 15	Kg	Borg
		Serie 1			
Øvelse 10: Ab crunch			Max 15	Kg	Borg
		Serie 1			

IV: Session sheet examples (Monday – Wednesday – Friday) used for each participant during the strength training period.