Effect of whey supplementation on blood markers of protein metabolism in young and elderly after resistance exercise

Master thesis by Kristin Holte

Department of Nutrition
Faculty of Medicine

UNIVERSITY OF OSLO

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Kristin Holte

Supervisors:
Truls Raastad
Håvard Hamarsland
Bjørn Steen Skålhegg

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Oslo, November 2014

Kristin Holte

Abstract

Introduction: Ingestion of whey protein has been shown to be superior to casein in the acute stimulation of anabolic responses in muscle. The composition of whey protein may alter how rapidly the amino acids are available after consumption, and thus affect acute anabolic responses in muscle and other tissues.

Aims: To investigate how ingestion of different whey products, influences the acute changes in the blood amino acid and urea concentration following standardized resistance exercise.

Subjects and methods: Twenty two young (20-35 years) and 14 elderly (>70 years), both men and women, were included in this double-blinded placebo controlled (partial) crossover study. The whey group went through the trial twice, one with native whey and one with WPC-80, while the milk group functioned as the control group. The study was designed as an acute exercise trial with a pre workout baseline measurement and post workout measurement for 5 h, and recovery measurement at 24 h. Blood concentrations of amino acids, glucose, insulin and urea was used to investigate the differences in the acute response after ingestion of native whey, WPC-80 and milk.

Results: Ingestion of native whey in the young resulted in a higher concentration of plasma leucine compared to WPC-80 and milk between 45 and 220 min post workout ($p<0.05$), and a greater area under the curve (AUC) for leucine and BCAA compared to WPC-80 ($p<0.05$). The same results were found for the elderly between 60 and 220 min post workout ($p<0.05$) and for AUC for leucine ($p<0.05$). At 45 min post workout, ingestion of native whey and WPC-80 resulted in a higher plasma leucine concentration for young compared to elderly. Ingestion of native whey resulted in a higher percentage increase in urea at 180 and 300 min post workout compared to milk, for the young ($p<0.01$) and the elderly ($p<0.01$).

Conclusion: Ingestion of native whey resulted in a more rapid and greater plasma leucine concentration, compared to WPC-80 and milk, which most likely was attributed to the higher leucine content in native whey, and a faster digestion and absorption kinetics for native whey, compared to milk. Another finding was the significant difference in amino acid uptake between young and elderly. The young experienced a rapid increase in plasma leucine concentration, while the elderly experienced a slower, more sustained response after ingestion of native whey. The higher percentage increase in urea found after ingestion of native whey versus milk, can be attributed to higher BCAA content and the greater aminoacidemia seen after ingestion of native whey.

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Abbreviations

AA	Amino acids
AUC	Area under the curve
BW	Body weight
BCAA	Branched-chain amino acids
DIAAS	Digestible Indispensable Amino Acid Score
DXA	Dual energy X-ray absorptiometry
EAA	Essential amino acids
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
IGF-1	Insulin growth factor 1
IMVC	Isometric maximal voluntary contraction
MG	Milk group
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
mTOR	mammalian target of rapamycin
NNR	Nordic Nutrition Recommendations
PDCAAS	Protein Digestible Corrected Amino Acid Score
RM	Repetition maximum
WG	Whey group
WHO	World Health Organization
WPC-80	Whey protein concentrate

1 Introduction

Every day a part of our muscle protein is broken down, and an equivalent amount is (re)built when we are weight stabile and keep a steady level of activity. In some cases, however, muscle proteins are broken down faster than they are built, and in these phases we lose muscle mass. In cases of immobilization, this occurs very quickly, whilst it is a more gradual process that comes with aging and through an increased level of inactivity (1). For many patient groups, and in general the eldest part of the population, loss of skeletal muscle mass and strength occurs with risk of adverse outcomes such as physical disability, poor quality of life and death (2). There are two main factors that regulate our muscle mass through life; how physically active we are and what we eat. In regards to our diets, the most important issue is to provide our bodies with sufficient energy and nutrients such as essential amino acids (EAA) and lipids combined with sufficient amounts of vitamins and minerals. To maintain muscle mass a sufficient amount of high-quality protein is also required (3, 4). The latter becomes evident when observing that after every meal the speed of building muscle increases simultaneously with the decrease of degradation, given that the meal contains enough energy and EAA (5). Nevertheless, one important observation is that the elderly people do not seem to respond equally well to meals as young people in regards to protein intake (6, 7). It is thus suggested that elderly people may have less sensitivity to meals in the muscle building processes. It means that it is important for elderly to have meals that provide an optimal stimulation of the muscle building process (4, 8). It is equally important for young, active people to have a recovery meal after heavy workouts, in order to quickly initiate crucial processes of protein anabolism and recovery.

1.1 Protein as an important nutrient

Protein is the major structural component of all cells in the body and act as precursor of many coenzymes, hormones, nucleic acids, and a number of other molecules essential for life. Thus an adequate intake of dietary protein is essential to maintain cellular integrity and function, and for general health and reproduction (9). Protein intake, requirements and recommendations have been heavily debated, especially within the field of sports nutrition.

1.1.1 Protein requirements and recommendations

The World Health Organization (WHO) define the protein requirement of an individual as "*the lowest level of dietary protein intake that will balance the losses of nitrogen from the body*" (10). Hence, the protein intake should maintain the body protein mass, in persons at energy balance with modest levels of physical activity. A higher intake is recommended for growing children and in pregnant and lactating women (10). Because of this, the protein requirement for an individual is dynamic and dependent on a complex relation between health, age and physical activity.

WHO`s recommended daily intake of 0.85 g protein/kg bodyweight (BW) per day for adults has been heavily debated (11), and several studies have indicated higher need of dietary protein intake in certain populations (11-15). Based on the Nordic dietary habits and available evidence, Nordic Nutrition Recommendations (NNR) from 2012 recommend a protein intake corresponding to 10-20 energy percent (E%) for adults in Norway (16). For elderly, the recommended protein intake is in the range of 15-20 E%, which corresponds to about 1.1–1.3 protein/kg BW per day (16). This is an increase of 20% compared to the NNR 2004 recommendations (16). The PROT-AGE study group also suggest that optimal protein intake for elderly (>65 years) is higher than the level currently recommended for adults at all ages (10). They recommend an average daily intake in the range of 1.0-1.2 g protein/kg BW per day for elderly (12), while a higher protein intake (>1.2 g/kg BW/d) is advised for those who are physical active (12). Higher dietary protein ingestion is beneficial for good health, to prevent sickness, promote recovery from illness, and to maintain functionality in elderly. Elderly need to make up for age-related changes in protein metabolism, such as a declined anabolic response to ingested protein (12), and they need more protein to combat inflammatory and catabolic conditions associated with chronic and acute diseases that commonly occur with aging (12).

The position statement on 'Nutrition and Athletic Performance' from the American Dietetic Association, Dieticians of Canada, and the American College of Sports Medicine recommend a daily protein intake of 1.2 to 1.7 g/ kg BW in endurance and strength-trained athletes (14). Several other studies have also shown that athletes need more protein than sedentary, with a daily protein requirement in the range of 1.2-1.8 g/kg BW (17-21).

1.2 Amino acid metabolism

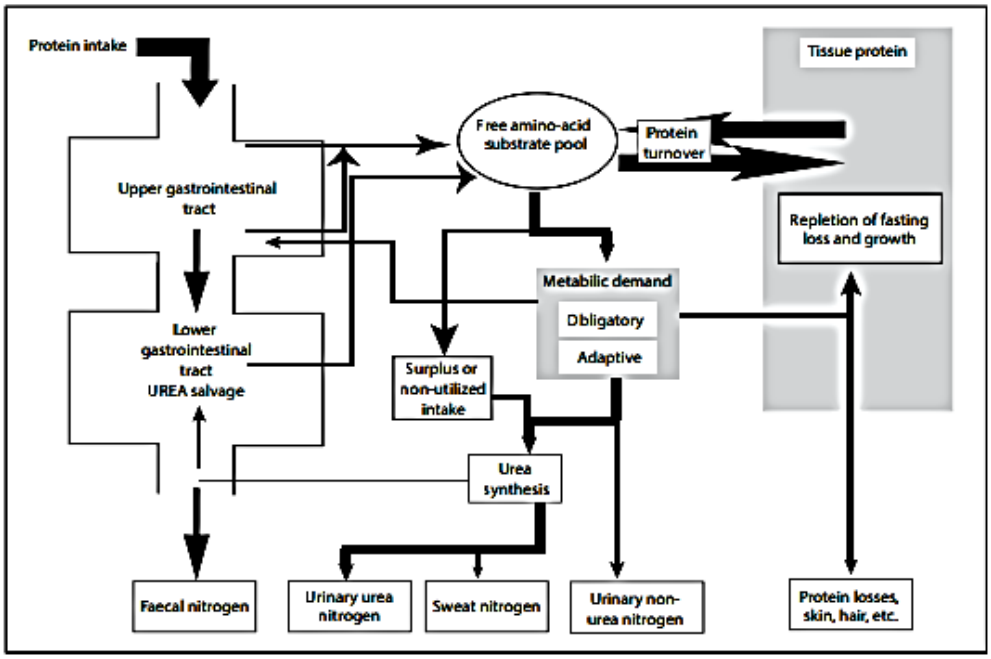

Figure 1.1: Model of protein metabolism in humans from WHO/FAO/UNU 2007 (10).

The most important aspect of protein from a nutritional point of view is its role as source of nitrogen and EAA (9). Amino acids are the building blocks of a protein and they are the defining characteristics of a protein. Amino acids can be categorized as either essential (indespensible) and nonessential (dispensible). With an adequate nitrogen supply, nonessential amino acids can be synthesized within the human body from other amino acids and glucose. The EAA must be provided from the diet as they cannot be synthesized in the body and these include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine (16). In addition to these nine EAA, six others are conditionally essential, as they are synthesized by other amino acids or their synthesis is limited under special pathophysiological conditions (9, 16).

1.2.1 Protein quality

Different sources of protein vary widely in their chemical composition as well as in their nutritional value. The quality of a source of protein is an expression of its ability to provide the nitrogen and amino acid requirements for growth, maintenance, and repair. In practice, protein quality is principally determined by two factors: digestibility and the amino acid composition of the protein (21). There are many ways to determine protein digestibility

efficiency; the most commonly used are the Protein Digestibility Corrected Amino Acid Score (PDCAAS) method (21) or the Digestible Indispensable Amino Acid Score (DIAAS), most recently introduced by the Food and Agriculture organization of the United Nations (FAO) (22). PDCAAS is based on an comparison of the amino acid profile of a food source against a standard amino acid profile (requirement of an 2-5 year old child) (16). PDCAAS calculates the digestibility of a protein by the remaining levels of protein in the fecal matter. DIAAS on the other hand, samples from the end of the ileum, which provide a more accurate measure of the EAA digestibility (16). By using digestibility values, dietary intake can be converted to an estimated uptake into the body of these proteins. Protein from animal sources such as meat, eggs and milk provide all nine essential amino acids in reasonable proportions and get maximal score on the digestible efficiency (16).

1.2.2 Digestion and absorption

After ingestion, proteins are denatured by the acid in the stomach and cleaved into smaller peptides by the enzyme pepsin (9). The proteins and peptides passes into the small intestine, where different digestive enzymes hydrolyze the peptide bonds. The resultant mixture of free amino acids and small peptides is then transported into the mucosal cells by specific carrier systems for amino acids and peptides. The absorbed peptides are hydrolyzed to amino acids in the cell, and are then secreted into the portal blood or further metabolized within the cell. The amino acids in the blood stream pass into the liver, where a portion are taken up and used. The remaining amino acids pass through into the systemic circulation and are utilized by the peripheral tissues (9).

The amino acids leaving the liver into the circulation have a different composition than the ones entering the liver. In particular, they are enriched in the three branched-chain amino acids (BCAA) valine, leucine and isoleucine. These three EAA constitute about 20% of dietary protein, but represent about 70% of the amino acids leaving the liver after a meal (23). The other amino acids are retained in the liver, while BCAA are preferentially removed by peripheral tissues, particularly muscle, after a meal. Skeletal muscle possesses a specific branched-chain 2-oxoacid dehydrogenase, and therefore has the ability to oxidize the BCAAs, providing a source of energy for the muscle (23).

Unlike many amino acids, BCAA are transaminated throughout the body, particular in skeletal muscle. The reaction of transamination involves the transfer of an amino group from

one amino acid to a 2-oxoacid, thus forming a new 2-oxoacid and a new amino acid (23). The main acceptor 2-oxoacid is either pyruvate, forming alanine, or 2-oxoglutarate, forming glutamate. Alanine and glutamine are the predominating amino acids leaving the muscle (9). These further provide links between amino acid and carbohydrate metabolism via the glucose-alanine cycle.

1.2.3 Urea metabolism

Nitrogen from the amino acids leaves the body via the urine in the form of urea, uric acid and creatinine. Small quantities of nitrogen are also lost from feces, sweat, and other secretions and from the skin, hair, and nails (9). Urea is the primarily end product of nitrogen and amino acid metabolism and is produced via the cyclic pathway known as Krebs-Henseleit cycle, or Urea cycle, in the liver (24). Urea is synthesized in larger amounts than is eliminated in the urine. In normal conditions, about 20-30% of the urea synthesized is hydrolyzed by bacterial urease in the gastrointestinal tract, leading to the production of ammonia, which is excreted (24).

It remains controversial whether control of the body nitrogen balance is achieved via changes in urea production in parallel with protein intake or via a regulation of urea hydrolysis. Fouillet et al found that urinary urea was not influenced by the protein source in the meal but was influenced by the protein level in the diet (24). Other studies have also concluded that the rates of urea production and excretion changes in parallel with the level of protein intake (25-27).

1.3 Net protein balance

Whether a person increases muscle mass (hypertrophy), loses muscle mass (atrophy), or remains stable, is dependent on the net protein balance. Net protein balance is defined as muscle protein synthesis (MPS) minus muscle protein breakdown (MPB). MPS and MPB are controlled through intricate intracellular signaling, which is largely regulated by protein intake and exercise (28). Amino acids in blood, and the BCAA leucine in particular seem to be potent stimulators of MPS in both young and elderly (25, 29, 30).

1.3.1 Leucine

Of all nutrients, the single amino acid leucine possesses the most marked anabolic characteristics in acting as a trigger element for the initiation of protein synthesis (11, 31-33). As a BCAA, leucine can be catabolized in the muscle, thus posing the possibility that metabolites of leucine could be involved in mediating the anabolic effects of leucine (33). It should be noted, however, that leucine cannot stimulate a rise in MPS in the absence of a full complement of EAA (11, 31).

1.3.2 Glucose and insulin

After ingestion of carbohydrates, an increase in the concentration of glucose in the blood can be detected within about 15 min, and reach peak at around 30-60 min after intake (23). As the concentration of blood glucose rises, the endocrine pancreas responds with an increased insulin secretion and thereby a rise in the plasma insulin concentrations (34).

Ingestion of certain proteins and amino acids can also stimulate insulin release, particularly when eaten in large quantities. The role of insulin on MPS is somewhat debated, but in most studies it seems like insulin exhibit a greater role in inhibition of MPB, than in stimulating MPS (35-37). As a result of the inhibition of MPB, insulin lowers free amino acid levels in both the plasma and intracellular spaces (38).

1.3.3 Resistance exercise

A key aspect with the acute responses to exercise and the subsequent adaption is the interaction between nutrition and exercise. It is well accepted that resistance exercise is able to stimulate MPS (13, 39), an effect that is found to be enhanced when combined with intake of dietary protein or amino acids (31, 40, 41). It has been suggested that resistance exercise can enhance the sensitivity of the MPS responses to dietary amino acids by sensitizing muscle to the anabolic actions mediated by insulin and amino acids, an effect that appears to peak in the first 3 hours after exercise (29) and may persist up to 48 hours after a bout of exercise (42). Such findings suggest that intake of protein should happen close after exercise to take advantage of its sensitizing effect (12). This is especially important for athletes to optimize the recovery period between training sessions.

1.3.4 Regulation of MPS

The anabolic effects of amino acids and insulin on protein synthesis are enhanced by physical activity and some nutrients, and are impaired by sedentary lifestyle, bed rest and immobilization (**Figure 1.2**).

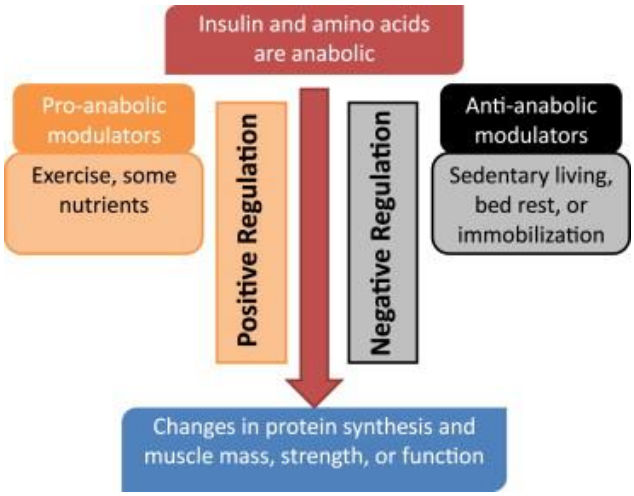

Figure 1.2: Anabolic effects of insulin and amino acids on protein synthesis are enhanced by physical activity and some nutrients and are impaired by sedentary lifestyle, bed rest, or immobilization. Figure from Bauer et al (12).

The mechanisms for the stimulation of protein synthesis by nutrients, and leucine in particularly, is not fully understood, but it is likely that the mammalian target of rapamycin (mTOR) and Rag pathway are involved (39, 43, 44). The mTOR pathway is one of the best understood pathways, which potentially integrates amino acid signaling with other nutrient-related signals as it is activated by insulin and by other anabolic hormones such as insulin growth factor 1 (IGF-1) (34, 44). mTOR complex 1 (mTORC1) is a protein kinase and can target other regulatory proteins (4E-BPs and S6K1) involved in the translation and elongation of mRNA into protein (45). Recent research has revealed that mTORC1 signaling is coordinated primarily at the lysosome membranes (45). This discovery has revealed several different signaling molecules involved in the transducing of the amino acid signal to mTORC1, including the Rag GTPases, MAP4K3, and Vps34/ULK1 (45). The mTOR pathway is also involved in the increased MPS observed after resistance exercise (23). Expression of amino acid transporters in muscle, such as LAT1 and SNAT2, may also be of importance in the regulation of MPS in response to amino acids and exercise (31). Thus, increased availability of EAAs, together with the anabolic signal of insulin, can increase MPS.

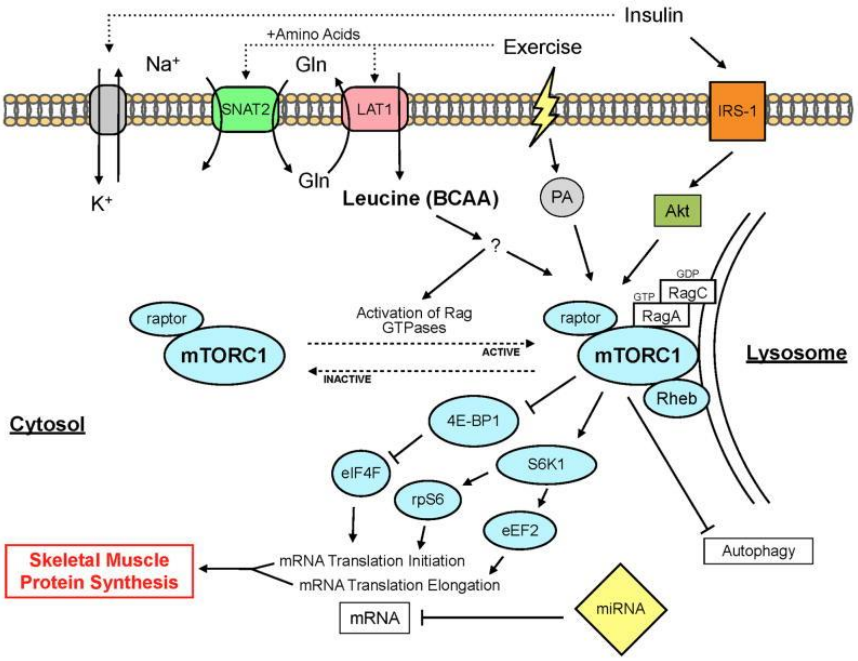

Figure 1.3: Simplified schematic diagram of the proposed cellular mechanisms regulating skeletal MPS in response to amino acids, insulin, and exercise. Figure from Dickinson et al (34).

1.4 Protein dose

The relation between intake of dietary protein/amino acids and MPS is dose-dependent and can be saturated (8, 35, 44). Consumption of 2.5 up to 10 g EAA stimulates myofibrillar and sarcoplasmic MPS in a dose-dependent manner in young, however 20 and 40 g EAA fail to elicit any additional stimulation (8). In agreement, Moore et al showed a dose-dependent increase of mixed MPS with oral ingestion of protein following resistance exercise in young (44). A plateau was reached at 20 g protein (~10 g EAA), from which an increased protein intake elicited an increased protein oxidation instead of an increased MPS. There seems to be a quantitative limitation of the amount of amino acids that can be stored in skeletal muscle during rest, which is termed the "muscle full" concept (46, 47).

Leucine seems to be a key factor in turning on the MPS machinery. It seems that there is a critical trigger threshold of leucine, which has to be reached in the blood before MPS is maximally stimulated. This point is suggested to be around a dose of 2.0 g leucine for young (29).

An intake of 20 g protein, or approximately 10 g EAA, seems to maximally stimulate MPS for a few hours both at rest and in the post exercise recovery period for young. Elderly, on the other hand, may require greater amounts of dietary protein/amino acids to mount a robust increase in MPS in response to feeding (31). Contrary to young, ingesting 40 g of protein increased rates of MPS in the elderly more than 20 g when consumed after resistance exercise (48), suggesting that the elderly may benefit from a higher dose of protein/amino acid to maximize MPS after resistance exercise. It is considered that about 30 g protein, or 15 g of EAA taken as bolus, is required for maximal stimulation of MPS in elderly (12, 49). The anabolic threshold of leucine per-meal seems to be higher in elderly compared to young. The PROT-AGE study group suggests an intake of 2.0-2.5 g leucine in an amino acid mixture as optimal for elderly (12). These results indicate that the quality of protein is very important in the diet of the elderly. The key is to find strategies regarding the amount and type of protein feeding, as well as the timing of the protein intake, as this seems to be important for optimizing the accumulation of muscle mass (11, 12, 50).

1.5 Different effect of various proteins

It seems that the most important factor that determines the size of the acute stimulation on the muscle protein synthesis after a meal is how quickly the blood concentration of leucine increases (29, 51). Thus, in order for a meal to be optimal in regards to the acute response in muscle tissue, it appears that it should contain a sufficient amount of leucine, and it should have an absorption kinetics that allows a quick rise in leucine concentration after intake. Therefore, the whey protein fraction appears to be particularly interesting, as it contains a lot of leucine that is quickly absorbed after intake (52).

1.5.1 Whey, casein and soy protein

Milk (whey and casein) and plant (soy) protein sources contain all of the EAA and therefore provide optimal amino acid composition for maximal stimulation of MPS (21). The difference in the metabolism of milk and soy proteins has been attributed to their digestion kinetics, wherein milk is digested more slowly than soy. Milk contains two protein fractions, 20% whey and 80% casein, which have been characterized based on their rate of digestion as “fast” or “slow” proteins, respectively (37, 53). Whey protein contain β-lactoglobulin which is resistant to denaturation at low pH and thus prevent clothing in the stomach (54). This results

in a rapid digestion and a pronounced aminoacidemia. The effect is transient, and return to resting levels within 2-3 h (21). Casein exists in the form of micelle in milk, which contrary to whey, form a clot in the stomach, and thus provide a slower, but more sustained (6 h) aminoacidemia (21). Soy contains a single homogenous protein fraction, which is digested similar to whey (53).

Table 1.1: Leucine content of different protein sources in % of total amino acid content.

Protein source	**Whey**	**Casein**	**Soy**
Leucine content	11%	8%	8%

Tang et al compared the acute response of mixed MPS of rapidly (whey hydrolysate and soy) to slowly (micellar casein) digested proteins both at rest and after exercise (53). Ingestion of whey protein resulted in a larger increase in blood EAA, BCAA, and leucine concentration than both casein and soy (whey > soy > casein) (53). Wilkinson et al showed that milk ingestion was superior in elevating MPS compared to soy protein when ingested after resistance exercise (55).

Studies have demonstrated greater whole body protein synthesis and leucine oxidation at rest, following ingestion of whey protein versus casein, while a greater role in the inhibition of protein breakdown has been suggested for casein (53, 56). Reitelseder et al found that whey induced a higher, but temporally shorter MPS response compared to casein, which resulted in a more moderate but prolonged MPS response following resistance exercise (37). This resulted in a similar MPS response within the 6 h recovery period. It has been speculated that the difference in the acute MPS response is due to the leucine content of whey and casein (**Table 1.1**) (11, 57). However, in older men, ingestion of 15 g whey protein is better than ingestion of the equivalent amount of EAA (58), thus, whey protein appear to have some anabolic benefit beyond its content of EAA (12).

To optimize the diet to obtain maximal muscle hypertrophy it may be worthwhile increasing the typically low whey protein content of milk, which theoretically would provide both an early (whey) and sustained (casein) stimulation of MPS and an inhibition of MPB.

1.5.2 Different whey products

Whey proteins are fractionated and manufactured in different protein concentrations or are treated to elicit functional changes in the native protein with enzyme hydrolysis methods (54). Such changes to the composition of whey may alter the absorption kinetics and affect how rapidly the BCAA are available after consumption, and thus affect the acute MPS after ingestion (54, 59). Native whey is produced by filtration technology and is a novel whey fraction within sport science. Whey protein concentrate (WPC-80) is the most common commercial whey product which is a byproduct from cheese production (59). The main difference between native whey and WPC-80 is that the whey proteins in WPC-80 are denatured through heating and enzyme interactions. Diverse ways of producing whey protein may cause various biological responses after intake, due to different absorption kinetics and also due to small differences in the amino acid composition. A pilot study by Laahne from 2013 showed that native whey was able to increase leucine concentrations in plasma more rapid and to higher levels than WPC-80, micellar whey protein, whey protein hydrolysate and milk (60). Apart from this study, little has been done to characterize the effect of the various processes of production of whey protein, and hence this will need further investigation.

1.6 Protein through a lifespan

As mentioned, protein intake and efficiency of use appears to decrease with age (12, 61). The reason for this may be due to decreased intake caused by anorexia, appetite loss or gastrointestinal disturbances, reduced ability to utilize protein due to insulin resistance, protein anabolic resistance, high splanchnic extraction or immobility, or a greater need for protein seen in some inflammatory diseases (12).

Studies investigating the differences in MPS and MPB between young and elderly are contradictory and inconclusive for the fasted state. The general consensus is that the rate of MPS and MPB under basal conditions is similar between young and older adults (34). In the fed state, however, most studies show that there is an impaired anabolic response in muscle to dietary protein in elderly (12). The primary factor thought to contribute to decreased muscle mass with aging is an impairment in the ability for skeletal muscle of elderly to respond to anabolic stimuli, commonly referred to as “anabolic resistance” (34).

1.6.1 Anabolic resistance

Figure 1.4 shows a multitude of factors, which may contribute to the anabolic resistance of MPS in elderly. It is speculated that impairment in protein digestion and amino acid absorption may play an important role, along with a reduced muscle perfusion mediated by insulin, reduced amino acid uptake in muscle, or a reduced amount or activity of key signaling proteins (11, 39). However, after ingestion of adequate amounts of protein, these factors may lead to minimal differences in postprandial MPS rates in young and healthy elderly individuals, shown in **Figure 1.5** (39).

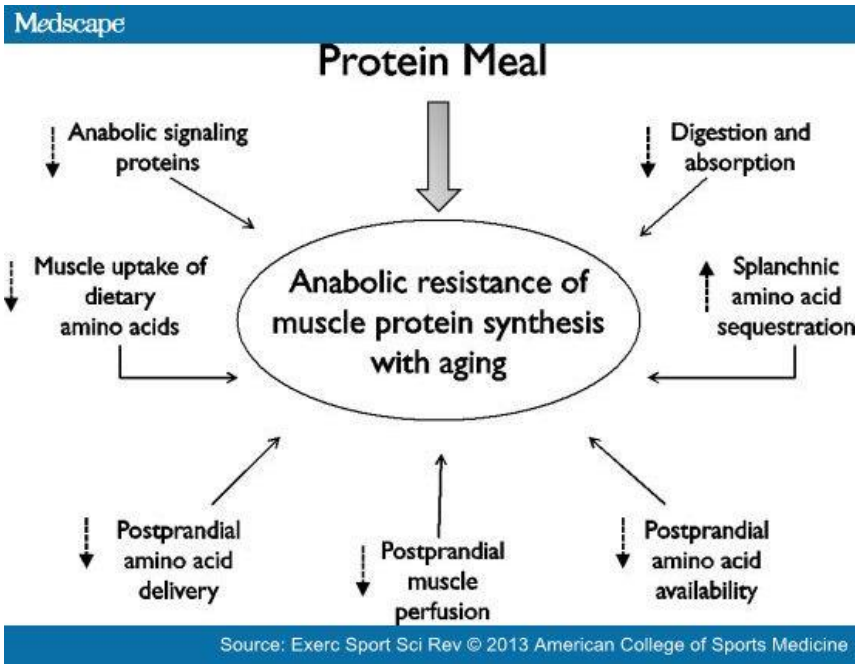

Figure 1.4: Ingestion of protein stimulates MPS. However, several secondary factors may occur between the protein meal and the stimulation of MPS that may lead to anabolic resistance in aging. Figure from Burd et al (39).

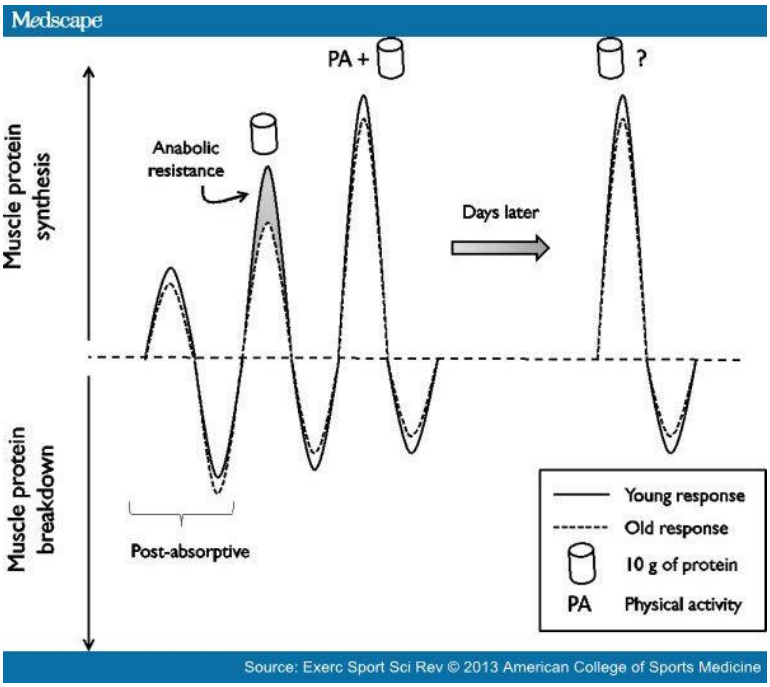

Figure 1.5: Post-absorptive MPS and MPB rates do not differ between the young and healthy elderly. Anabolic resistance of MPS rates may arise after consumption of smaller amounts of dietary protein. These postprandial differences between the young and elderly are diminished after consumption of adequate amounts of dietary protein. Figure from Burd et al (39).

Strategies to improve postprandial anabolic signaling or sensitivity to amino acids and insulin are already been proposed, and include intake of sufficient amounts of protein to maximize the anabolic response and/or use exercise to enhance sensitivity to dietary protein/amino acids and insulin. Other important factors to consider are supplementation of specific amino acids like leucine, the distribution and timing of protein intake during the day and the protein quality (12).

1.6.2 Sarcopenia

Sarcopenia is defined as "*the loss of skeletal muscle mass and strength that occurs with advancing age and with risk of adverse outcomes such as physical disability, poor quality of life and death*" (2). Criteria for diagnosis are based on documentation of low muscle mass plus low muscle strength or low physical performance, and sarcopenia is found to be prevalent in older populations (2). Sarcopenia has multiple contributing factors (**Figure 6**); the aging process with decreased production and reduced sensibility of certain hormones, inadequate diet, bed rest or sedentary lifestyle, chronic diseases and certain drug treatments (2).

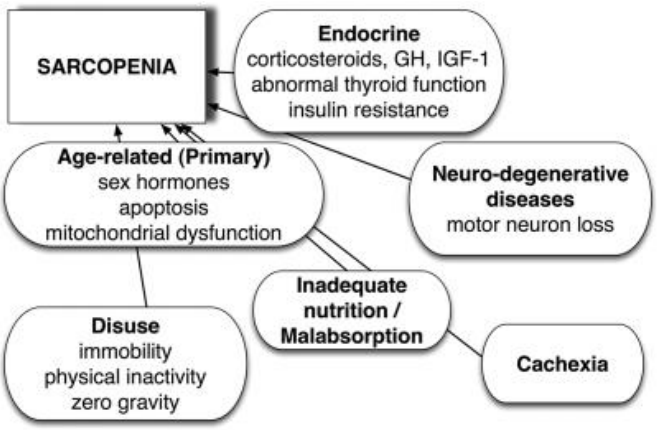

Figure 1.6: Contributing factors of the etiology of sarcopenia in aging. From Cruz-Jentoft et al (2).

Researchers have identified diet and exercise as the two most important factors in the combat against sarcopenia (61). Defining nutritional and exercise interventions that maximally stimulates MPS in elderly are therefore of great interest for the development of strategies to slow down the age-related loss of muscle mass, and prevent sarcopenia (31, 34).

1.7 Summary

Current research has demonstrated that factors such as the dose of dietary protein/EAA ingested, protein food source (whey, micellar casein), and the timing of protein/EAA intake impact the magnitude of MPS in response to feeding and resistance exercise.

Protein ingestion elicits an aminoacidemia that stimulates rates of MPS, an effect that is enhanced when combined with resistance exercise. The stimulation of MPS is driven primarily by EAA, the BCAA leucine in particularly, and occurs in a dose-dependent manner at rest and post exercise. Studies indicate that an intake of about 20 g protein (~10 g EAA) maximally stimulates MPS in young, while elderly, at least in some reports, need 30 g protein (~15 g EAA) to get the same effect. This difference may be due to an impaired ability for skeletal muscle of older adults to respond to anabolic stimuli, called “anabolic resistance”.

The digestion rate of proteins also affects the amplitude of acute increases in MPS. For example, the rapid digested whey protein results in a more rapid and transient aminoacidemia, compared to the slowly digested micellar casein which gives a gradual prolonged aminoacidemia. These stereotypical patterns of aminoacidemia are widely described (50), different whey proteins, on the other hand, have not been studied to that extent. Will different

whey proteins give different responses regarding amino acid kinetics, even though they both are rapidly digested proteins?

2 Aims and hypothesis

2.1 Aim of the master thesis

Nutrition interventions combined with resistance exercise that enhances MPS may be of great scientific and clinical interest as a strategy to promote positive muscle protein balance. It has been demonstrated that this may be particularly important for athletes to increase muscle mass and for the elderly to reduce the age-related loss of muscle mass and prevent the development of sarcopenia.

Ingestion of whey protein has been shown to be superior to casein in the acute stimulation of MPS. The composition of whey protein may alter how rapidly the amino acids are available after consumption, and thus affect protein synthesis rates.

Based on this the main aim of this thesis was to investigate how ingestion of different whey products, in combination with resistance exercise, influence the acute changes on blood concentration of amino acids, along with other important factors involved in protein metabolism such as glucose, insulin and urea in young and elderly. To accomplish our main aim we tested the products, native whey, WPC-80 and milk, as follows:

- Investigate the effects of intake of native whey after a standardized bout of resistance exercise on blood concentrations of amino acids, glucose, insulin and urea, compared to WPC-80 and milk

- Investigate possible differences in aminoacidemia and the corresponding urea response in the young versus the elderly participants

Based on what has been described in the introduction, our main hypothesis were:

- Native whey will induce a more rapid increase in blood concentration of amino acids, compared to WPC-80 and milk

- Native whey will induce a larger increase in EAA, BCAA and leucine, compared to WPC-80 and milk

- Young participants will experience a larger and more rapid increase in amino acids than elderly participants

2.2 The students task

In this study the master student had the responsibility for carrying out the intervention along with the study group, including planning the diet, recruiting participants to the study, give information to the participant, and implementation of the study at the Norwegian School of Sport Science (NIH). During the study the student had the responsibility to take good care of the participants, preparing of the breakfast and dinner, execution of the resistance exercise and isometric maximal voluntary contraction (IMVC) testing and make sure that the time schedule was followed. After the study was finished, the student registered food intake form 24 hour recalls and from the test period. Finally, the student was responsible for the statistical analysis of the results presented in the master thesis. The student also gave feedback to the participants about their diet.

3 Methods

This master thesis is a part of a larger study project on the effects of different milk protein fractions on muscle protein metabolism in young and elderly. The project is a collaboration between The Norwegian School of Sport Science (NIH), Department of nutrition, University of Oslo (UiO) and Oslo and Akershus University College of Applied Science (HiOA) and it is financed by the Norwegian Research Council (NFR) and TINE SA. This chapter will only include methods that are relevant to the present master thesis.

3.1 Participants

Thirty eight participants, both men and women, were recruited. Of these were 22 between 20 and 35, and 16 above 70 years old. One participant above 70 years withdrew after the first test day and is thus not included in the analysis. Another participant above 70 years did not consume all the protein drinks due to nausea and is therefore not included in the analyzes. The final study population was 36 in total. Participant characteristics are shown in **Table 3.1** and **3.2**.

Table 3.1: Young participants' characterization

	Young (whey)	Young (milk)
Gender	♂ 5 ♀ 5	♂ 8 ♀ 4
Age, year	24,6 (1,5)	25,4 (4,4)
Body weight, kg	70,0 (11,6)	72,8 (12,4)
Lean mass, kg	52,9 (9,6)	57,1 (13,5)
Body fat, %	21,5 (6,4)	19,1 (7,2)

Values are means ± SD.

Table 3.2: Elderly participants' characterization

	Elderly (whey)	Elderly (milk)
Gender	♂ 5 ♀ 1	♂ 5 ♀ 3
Age, year	72,1 (2,4)	76,1 (4,1)
Body weight, kg	73,3 (12,9)	69,0 (8,5)
Lean mass, kg	53,1 (11,1)	48,9 (8,3)
Body fat, %	25,4 (6,8)	26,5 (7,6)
Triglycerides, mmol/L	1,08 (0,27)	1,10 (0,43)
Cholesterol, mmol/L	4,91 (1,11)	6,30 (1,11)
Glucose, mmol/L	5,42 (0,52)	5,16 (0,45)
HDL, mmol/L	1,47 (0,53)	1,66 (0,39)
LDL, mmol/L	2,89 (0,98)	3,87 (1,02)
VLDL, mmol/L	0,47 (0,15)	0,54 (0,17)

Values are mean ± SD.

For the anthropometric data, no significant differences were found between the group receiving whey or milk within the two age categories. There were no differences in lipid profile between the whey group and milk group for the elderly.

The young participants were recruited through locally posted flyers, information via student email, social media and by word of mouth. Two meetings were held to give more information to those who were interested. To be included in the study the young participants had to be regularly engaged in resistance training, with minimum one session a week including leg strength exercises in the past six months. The elderly participants were recruited by locally posted flyers, newspaper advertisement and by information at meetings for elderly. The elderly were screened for blood pressure, cholesterol- and glucose levels and bone mineral density conducted by dual energy X-ray absorptiometry (DXA) before included in the study. The results were checked with Dr. Haakon Benestad[1] before including an elderly in the study. Persons who were allergic to milk or were lactose intolerant were excluded. The participants were not allowed to use any supplements (protein, vitamins, creatine) within two weeks prior to the study start.

Participants were informed of the purpose of the study, the experimental procedures and potential risks. All subjects signed a written consent before enrollment (**Appendix 3 and 4**). This study was approved by the Regional Ethical Committee (REC) and was in compliance with the Declaration of Helsinki.

3.2 Study design

The present study is a double-blinded placebo controlled (partial) crossover study. The study was performed at NIH. Both the young and elderly participants were randomized to a "whey group" (WG, test group) and a "milk group" (MG, control). The WG went through the trial twice, in a randomized order, one with native whey and one with WPC-80. The milk group did the trial only once. This design was chosen to limit the amount of biopsies from each subject, but still keep a strong design for comparison of the whey drinks.

[1] Dr. Haakon Benestad is professor of Medicine (Cell Physiology), now professor emeritus, at University of Oslo (UiO).

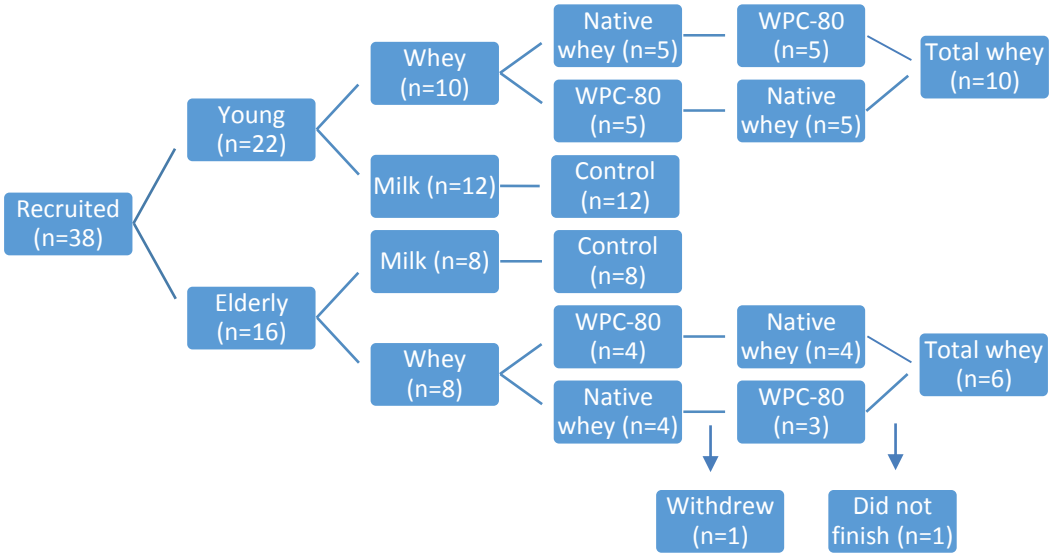

Figure 3.1: Flow diagram of the participants

3.3 Experimental protocol

3.3.1 Pretests

All participants went through pretests before their trail started. Pretests were performed on two separate days for the young and up to six separate days for the elderly. We initially planned six pretests for the elderly, but for some of the participants, who did leg press weekly, the amount of pretest were reduced. Thus, all elderly participants performed at least two pretests, while most of them did six. The aim of the first day was to familiarize the participants to test equipment and protocol, the second day was to establish an eight repetition maximal (8 RM) weight in leg press and knee extension for the participants. Most of the elderly participants were less experienced with heavy weights, and thus went through several pretests to establish their 8 RM weight.

On the first day of pretest, all subjects completed a DXA scan before the familiarization session. In addition, the basal diet of the participants was assessed by two 24 h recalls. All participants received a diet plan for the 2.5 day standardization phase (day before the test day (day-1), on the test day and until lunch the day after the test day) based on their weight and pre-packed food on the last visit before each trial. They were informed and given advice on

how to implement the diet plan during the trial. Participants were asked to refrain from heavy exercise for the 72 h before the study, to be fully rested and recovered for the test day.

3.3.1 Test day

The study was designed as an acute exercise trial with a pre workout baseline measurement and post workout measurement for 5 h, and recovery measurement at 24 h. When participants reported at NIH between 7 AM and 8 AM after an overnight fast (8-10 h), a catheter was placed into veins of the right and left forearms for blood sampling and stable isotope infusion. Blood draw included plasma samples used for amino acid measurements (180 min pre workout to 300 min post workout) and serum samples used for urea measurements (60 and 180 min pre workout and 60, 180, 300 min, plus 24 h post workout). When the venous catheter were in place, participants were served breakfast at 180 min pre workout. Breakfast consisted of microwaved oatmeal made of 55 E% oat, water, 35 E% rapeseed oil and 10 E% sugar. The participants were allowed two protein drinks and water between breakfast and dinner at ~315 min post workout.

On each test day, four biopsies were obtained from the m. vastus lateralis, 2 in each leg. The biopsies were taken at 30 min pre workout, 60, 180 and 300 min post workout. Recovery of the m. quadriceps femoris function was measured by an isometric maximal voluntary contraction (IMVC) test. The IMVC test was conducted 15 min pre workout, 10 and 300 min, plus ~24 h post workout. The test day was conducted as outlined in **Figure 3.2**. It was a minimum of 1 week between the trials for each participant.

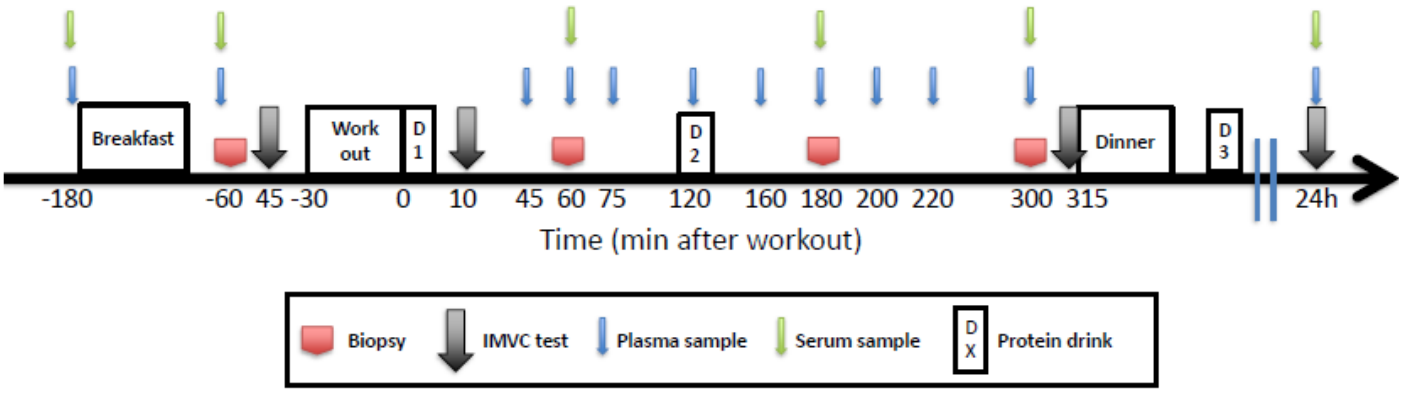

Figure 3.2: Time course for the test day. Plasma samples were used for AA measurements (all times except 24 h) and serum samples were used for urea measurements (60 and 180 min before and 60, 180, 300 and 24 h post workout). Biopsies was obtained from the m. vastus lateralis. Infusion of stabile isotopes is left out. D1, D2 and D3, ingestion of protein drink nr. 1, 2 and 3. IMVC test, isometric maximal voluntary contraction test.

3.3.2 Resistance exercise protocol

The bout of resistance exercise consisted of leg press and knee extension. A weight equivalent to the 8 RM established during the pretests were used. The workout started with leg press and two warm up sets of 10 reps at 70 and 90% of 8 RM weight. Next were four sets of 8 RM leg press with a new set starting every 3rd minute, followed by the same procedure in knee extension.

3.3.3 Standardized diet

Before each trial, participants were provided with an individual diet plan and partly pre-packaged food for the standardized period of 2.5 d (day-1, test day and until lunch the next day) (**Appendix 2**). Participants received cheese, dinner (salmon or meatballs in premade packages (Fjordland, Norway)) and Go`morgen yoghurt (TINE, Norway), in addition to oatmeal on the test day. The rest of the ingredients were basic foods, such as whole grain bread, butter, jam, oil and sugar, which the participants were responsible to get themselves. The diet was designed to meet daily caloric and protein requirement for resistance-trained young individuals and for healthy, active elderly individuals. All food ingested were registered in the diet plan by the participant. Every discrepancy between planned and actual intake were reported in the diet plan.

Body weight (BW) determined the energy intake and the amount of food calculated for each individual. Participants were categorized into weight classes from 50 to 100 kg, with 5 kg intervals.

Recommended energy intake for sedentary adults is 35 kcal/kg BW (62). The requirement for elite athletes is between 45-50 kcal/kg BW (63). As the subjects in this study were in the category between sedentary and elite athletes, 40 kcal/kg BW were set as a standard for all young participants. The amount of protein recommended for athletes is between 1.2-1.8 g/kg BW (14, 17-20) or 10-20 E% (16). We set protein requirement for the young subjects to 1.5 g/kg as this equals 15 E% in a diet that equals 40 kcal/kg BW.

Gaillard et al showed that resting metabolic rate for non-active elderly people (> 70 years) were 20 kcal/kg BW (15). At physical activity levels (PAL) which equals 1.6 on a daily basis, the total energy requirement is approximately 30 kcal/kg BW. Based on this and the fact that energy requirements for elderly (>70 years) compared to sedentary adults is decreased with 10%, which equals 31.5 kcal/kg BW, we set the average energy requirements for the elderly participants in this study to approximately 30 kcal/kg BW. The amount of protein required for elderly above 70 years ranges from 15 to 20 E%, which corresponds to about 1.1–1.3 protein/kg BW per day (16). As all the elderly participants in this study were more active than sedentary older adults, we set the protein requirement for the elderly participants to 1.3 g/kg BW which equals 17.3 E% in a diet of 30 kcal/kg BW.

The constants for energy and protein intake in young and elderly were put into the equation with a constant percentage of energy in each meal (**Table 3.3**), and a diet plan was calculated. Due to individual differences adjustments of the diet plan were allowed if the planned amount of food was too much or too sparse. However, only items on the diet plan were allowed to eat, but the amount could be adjusted if necessary, as long as all discrepancies were reported in the compliance scheme in the diet plan.

Table 3.3: The composition of the diet on day-1. The percentages were the same for young and elderly participants.

Meals	Planned	Actual
Breakfast	20 E%	18-20 E%
Lunch	25 E%	25 E%
Dinner	30 E%	25-35 E%
Supper	20 E%	15-30 E%
Snack	5 E%	0-10 E%

All participants received breakfast equivalent to 20 E%, dinner and at least one yoghurt with the protein drink for supper on the test day. This equalized a minimum of 1000 kcal from food, in addition to approximately 990 kcal from the protein drinks. The total amount of protein ingested was higher on the test day than the day prior, 1.8 g/kg BW for young in both WG and MG and 1.4 g/kg BW and 1.6 g/kg BW for elderly, in the WG and MG respectively. This was due to the high amount of protein in the protein drinks (**Table 3.4**) and the set minimum intake of energy and thereby protein from food. Participants in the WG ingested the exact same amount of energy and protein during each of the two trials.

The nutrient content of the diet were estimated using Mat på Data 5.1 (Mattilsynet, Oslo, Norway 2009). For the food items that were not included in this software, food labels with nutrient content were used for the calculations.

3.3.4 Protein drink

During the test day the participants drank three protein drinks. The amount of macronutrients was supposed to be equal in all supplements, but as **Table 3.4** shows there were some differences between the drinks.

Immediately after completion of the exercise, the participants received a protein drink containing native whey, WPC-80 or milk. All subjects were blinded with regard to what drink they were receiving. The drinks were consumed within 5-10 min.

Table 3.4: Energy and nutrient contents of the protein drinks per serving (636 ml)

	Native whey	**WPC-80**	**Milk**
Energy, kcal	314,5	311,9	292,7
Protein, g	22,4	20,9	20,8
Fat, g	6,9	6,7	6,3
Carbohydrate, g	40,7	42,0	38,2
Leucine, g	2,7	2,2	2,0

The milk and the WPC-80 in this project came from regular production at TINE (Oslo, Norway), while the native whey was produced for this project also by TINE (Oslo, Norway). Cream (TINE, Norway) and lactose (Arla food ingredients, Denmark) were used to adjust the different amount of lactose and fat content of the products. Subsequently, adding water made

each supplement contain a total of 636 ml of liquid. The protein in both native whey and WPC-80 consists of 100% whey, while the milk protein consists of 80% casein and 20% whey.

Table 3.5: Amino acid content in the protein drinks.

	Native whey	**WPC-80**	**Milk**
Alanine, g	1,08	1,01	0,66
Arginine, g	0,55	0,50	0,68
Aspartic acid, g	2,54	2,21	1,56
Cysteine, g	0,59	0,44	0,16
Glutamic acid, g	3,80	3,56	4,24
Glycine, g	0,43	0,39	0,39
Histidine, g	0,45	0,40	0,55
Isoleucine, g	1,22	1,25	1,02
Leucine, g	2,73	2,15	1,98
Lysine, g	2,29	1,92	1,69
Methionine, g	0,48	0,44	0,51
Phenylalanine, g	0,82	0,68	0,97
Proline, g	1,11	1,34	2,02
Serine, g	1,02	1,13	1,16
Threonine, g	1,12	1,47	0,91
Tryptophan, g	0,48	0,35	0,27
Tyrosine, g	0,55	0,44	0,73
Valine, g	1,15	1,19	1,24
Total AA, g	22,4	20,9	20,8
EAA, g	11,3	10,4	9,8
BCAA, g	5,1	4,6	4,3

AA, amino acids. EAA, essential amino acids. BCAA, branch-chained amino acids.

3.3.5 Dietary assessment

As recommended by European Food Safety Authority (EFSA) we used the 24 hour recall method for dietary assessment (64). Two non-consecutive days were assessed. To make it easy for the participants, the interviews were done the same day as the pretests. It varied from four to 14 days between the interviews. The same person conducted all of the 24 h recalls. The first interview was done by face-to-face interview for all participants, the second was done likewise for all except for two participants, who were interviewed by phone. Food quantities were assessed using household measures. The 24 h recalled day was defined as; from the subject got up the day before until he or she went to bed for the night. The 24 h recall day was conducted using an open structure in the beginning, while time and meals were

added during the interview to help the respondent to remember all foods consumed throughout the day. The interview ended with a checklist of foods, drinks and snacks that might be easily forgotten (**Appendix 1**). The checklist included nutritional supplements.

3.3.6 Body composition measurements

Measurement of body composition was done by a DXA scan at NIH. Participants were not supposed to do strenuous exercise the day before the scan and reported fasting on the day of the DXA scan. Participants had to lie still in the DXA machine for 10 min to complete the test. The DXA machine gives the height and weight of the subject in addition to the body composition of muscles, fat and bones.

3.3.7 Blood samples and preparation

When participants reported to NIH in the morning at the test day, a catheter was placed into veins of the right and left forearms for blood sampling and stable isotope infusion. Blood draw included 11 plasma samples used for amino acid measurements (all time points except 24 h) and six serum samples used for urea measurements (60 and 180 min pre and 60, 180, 300 and 24 h post workout). The collected plasma samples were stored in glass tubes containing lithium heparin and centrifuged at 1,400 rpm for 10 min at 4˚C to separate blood cells from plasma. Plasma was stored at -80˚C until further analysis. The plasma samples were analyzed for insulin, glucose and amino acid concentration at Arkansas Children`s Hospital Research Institute (University of Arkansas, USA). The collected serum samples were stored in room temperature for 20 min before they were centrifuged, and serum was stored at -80˚C until further analysis. The serum samples were analyzed for urea, urat and CK concentration at Fürst Medical Laboratory (Oslo).

3.4 Statistics

The statistical analyses were performed using the Prism ® 6 (GraphPad Software nc., San Diego, CA, USA), Statistical Package for the Social Sciences (SPSS) version 21 for Microsoft (SPSS, Inc., USA) and Microsoft® excel 2011. Normality tests, histograms and Q-Q plots were first used to determine if data followed a normal distribution or not.

Since the aim was to compare the two different whey proteins, a paired design was applied. Participants who were randomized to the whey group were investigated twice. A separate group received milk, and therefore functioned as a control group. Statistical analyzes were carried out as 2-factor (type of protein drink and time) repeated-measures ANOVA. When main effects occurred, Sidak post hoc test were performed to assess specific differences between type and time points. The control group data was analyzed with one-factor (time point) repeated-measures ANOVA. All area under the curve (AUC) data was analyzed with paired t-tests between native whey and WPC-80. When appropriate, unpaired t-tests were conducted between the native whey and the milk group within each age category and between young and elderly within the native whey group.

Missing values were imputed using the linear trend at point method, if they followed the general trend in the group.

The level of statistically significance was set as $p < 0.05$. Data are given as mean and standard deviation ($\pm$ SD) for all parameters as they were normal distributed. Correlation analyzes between variables were performed using Pearson`s correlation.

4 Results

4.1 Diet composition

4.1.1 Baseline measurements

The nutritional baseline measurements were based on the two 24 h recall assessments. When determining baseline intake of nutrients, for both age categories, no differences in energy or protein intake were found between the MG and the WG (**Table 4.1**). For the young participants, the WG had a higher intake of carbohydrate than did the group than ingested milk. For the elderly, the WG had a larger amount of fat in their diet than the MG.

Compared to the elderly, the younger participants had a higher energy intake per kg BW ($p<0.001$), higher E% from protein ($p<0.05$) and higher intake of protein in gram per kg BW ($p<0.001$).

Table 4.1: Baseline measurements of energy content and diet composition for the young and the elderly participants. The results are shown as an average of the two 24 h recall assessments with a p-value for the difference between the groups.

	Young			
	Whey (n=10)	Milk (n=12)	p-value	Recommended intake
Energy (kcal)	2767 (655)	3080 (892)	>0.05	-
Energy (kcal/kg)	40.1 (9.4)	41.7 (6.7)	>0.05	35-50*
Protein (E%)	17.8 (2.1)	19.7 (4.0)	>0.05	10-20
Protein (g/kg BW)	1.8 (0.3)	2.0 (0.5)	>0.05	1.2-1.8*
Fat (E%)	35.2 (5.2)	39.3 (6.2)	>0.05	25-40
Carbohydrate (E%)	43.4 (4.0)	37.5 (6.1)	0.02	45-60

Mean ± SD. E% = energy percentage. Recommended intake from NNR 2012 (16). * For athletes.

	Elderly			
	Whey (n=5)	Milk (n=8)	p-value	Recommended intake
Energy (kcal)	2097 (416)	2024 (593)	>0.05	-
Energy (kcal/kg)	28.6 (6.8)	29.8 (6.8)	>0.05	30
Protein (E%)	15.6 (2.6)	17.4 (3.5)	>0.05	15-20
Protein (g/kg BW)	1.1 (0.2)	1.3 (0.3)	>0.05	1.2-1.5
Fat (E%)	43.5 (3.9)	33.8 (3.7)	<0.01	25-40
Carbohydrate (E%)	35.9 (4.8)	40.0 (4.6)	>0.05	45-60

Mean ± SD. E% = energy percentage. Recommended intake from NNR 2012 (16).

4.1.2 Nutrition during the test period

Characterization of the energy and protein intake during the trial (day-1 and the test day), relative to the usual intake (24 h recall) is shown in **Figure 4.1** and **4.2**. The whey group had the exact same intake of food, and therefore identical energy and protein intake, during both trials.

There were no differences between the groups in protein intake according to the 24 h recall, protein intake at day-1 or test day. Neither the increase in protein intake from 24 h recall to test day or day-1 differed within the two age categories.

The young had a higher protein intake than the elderly, measured by 2 x 24 h recalls (1.9 g/kg BW vs 1.2 g/kg BW, $p<0.001$). The elderly had a greater increase in protein intake from the calculated intake to day-1 (0.3 g vs -0.1 g, $p=0.001$) and the test day (0.1 g vs -0.4 g, $p<0.001$) compared to the young participants.

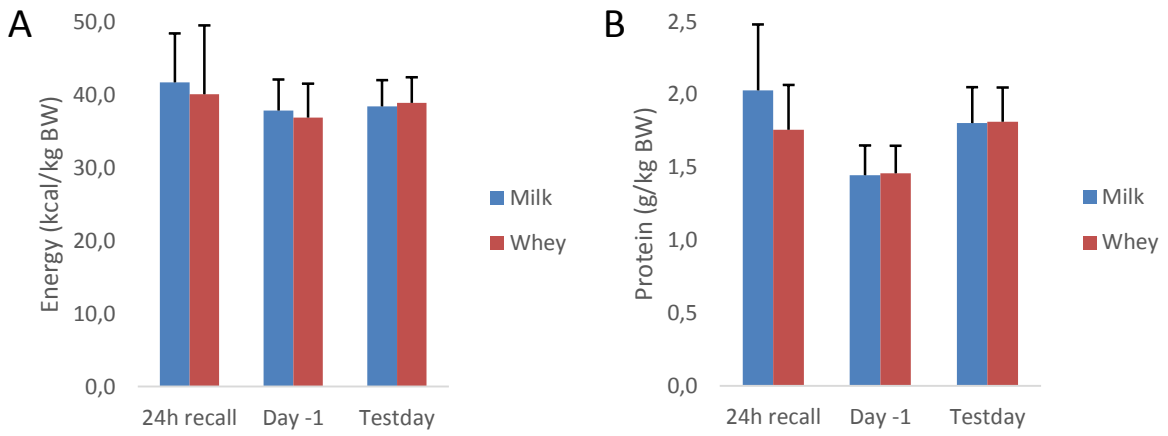

Figure 4.1: Mean + SD energy intake (A) and protein intake (B) for the young participants from 2 x 24 h recalls, on day-1 and on test day for the milk and whey group. The whey group had the exact same food intake during both test periods.

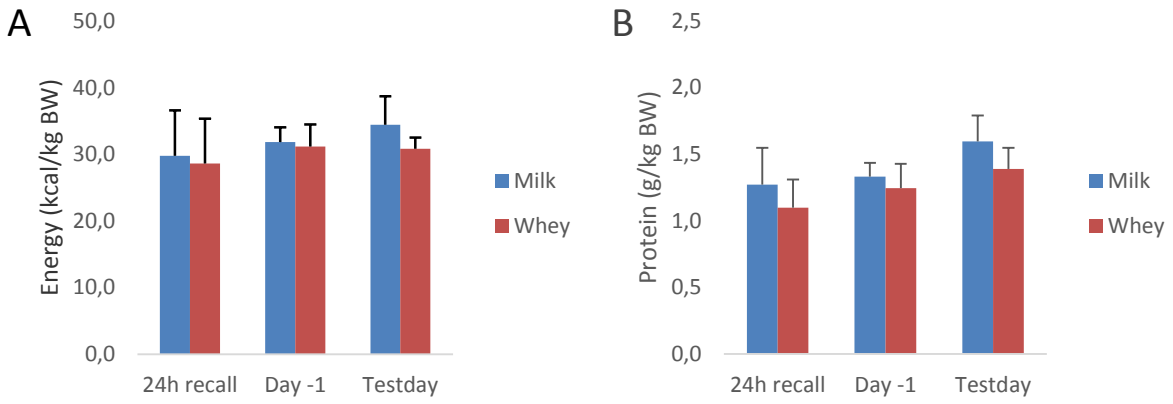

Figure 4.2: Mean + SD energy intake (A) and protein intake (B) for the elderly participants from 2 x 24 h recalls, on day-1 and on the test day for the milk and whey group. The whey group had the exact same food intake during both test periods.

4.2 Blood glucose and insulin

4.2.1 Glucose and insulin young

Ingestion of WPC-80 gave a higher relative blood concentration of glucose, compared to native whey at 45 min and 160 min post workout ($p<0.001$). For WPC-80 and milk we observed a peak at the first measurement after ingestion of protein drink 1 (45 min post workout), while the concentration for the native whey group showed a decrease in blood glucose below baseline. No differences were observed between native whey and WPC-80 for insulin concentrations.

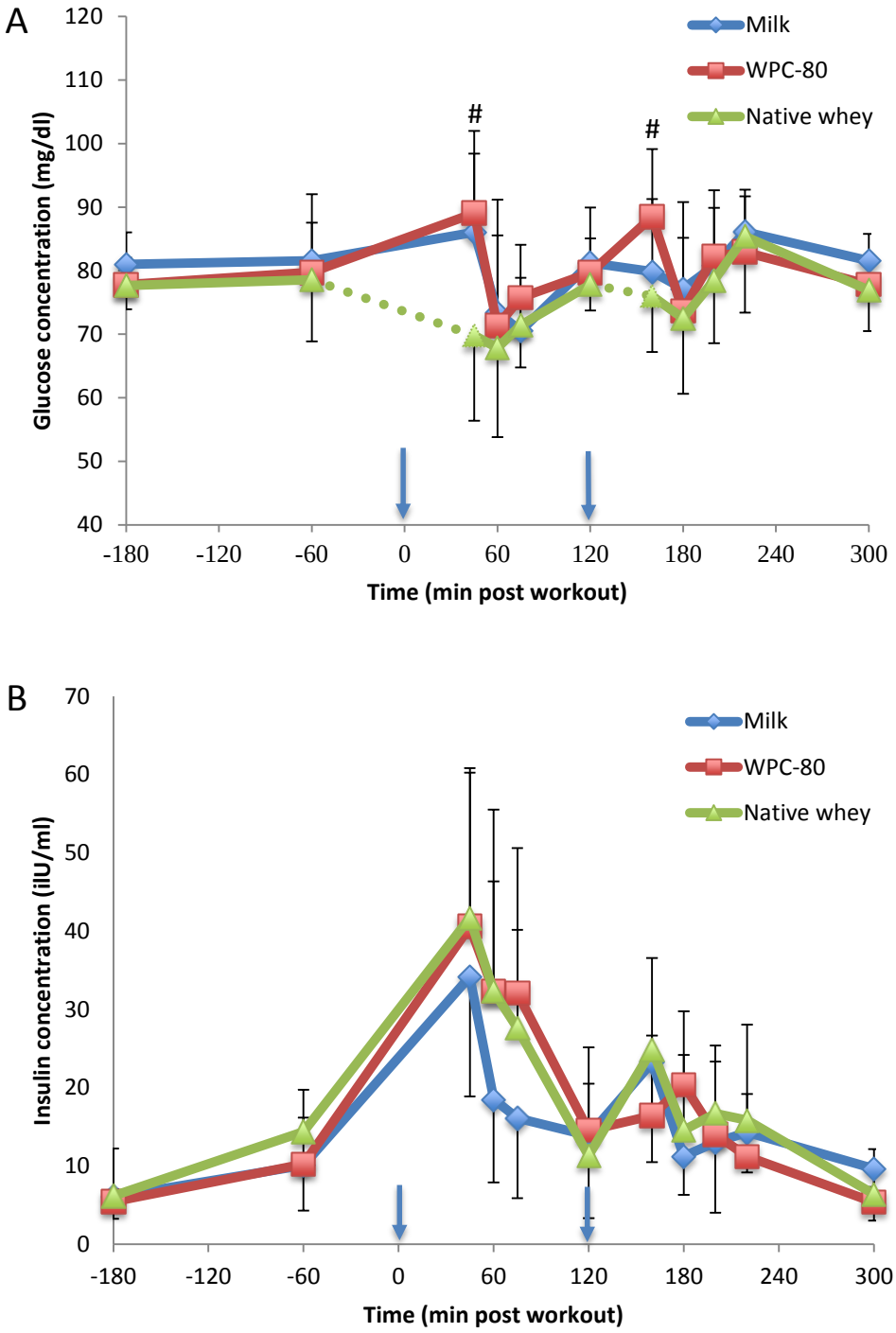

Figure 4.3: Mean ± SD blood concentrations of glucose (A) and insulin (B) for the young participants. # indicates a significant higher concentration of glucose after ingestion of WPC-80, compared to native whey (P<0,05). Arrows show where the proteins drinks were consumed.

4.2.2 Glucose and insulin elderly

No differences in the blood glucose levels were found after intake of native whey and WPC-80 in the elderly group. Ingestion of milk resulted in a greater difference in glucose from baseline (60 min pre workout) to peak compared to native whey ($p<0.05$). Moreover, insulin concentrations peaked at 45 min post workout and were significantly higher after intake of native whey compared to WPC-80 at 60 min post workout ($p<0.05$).

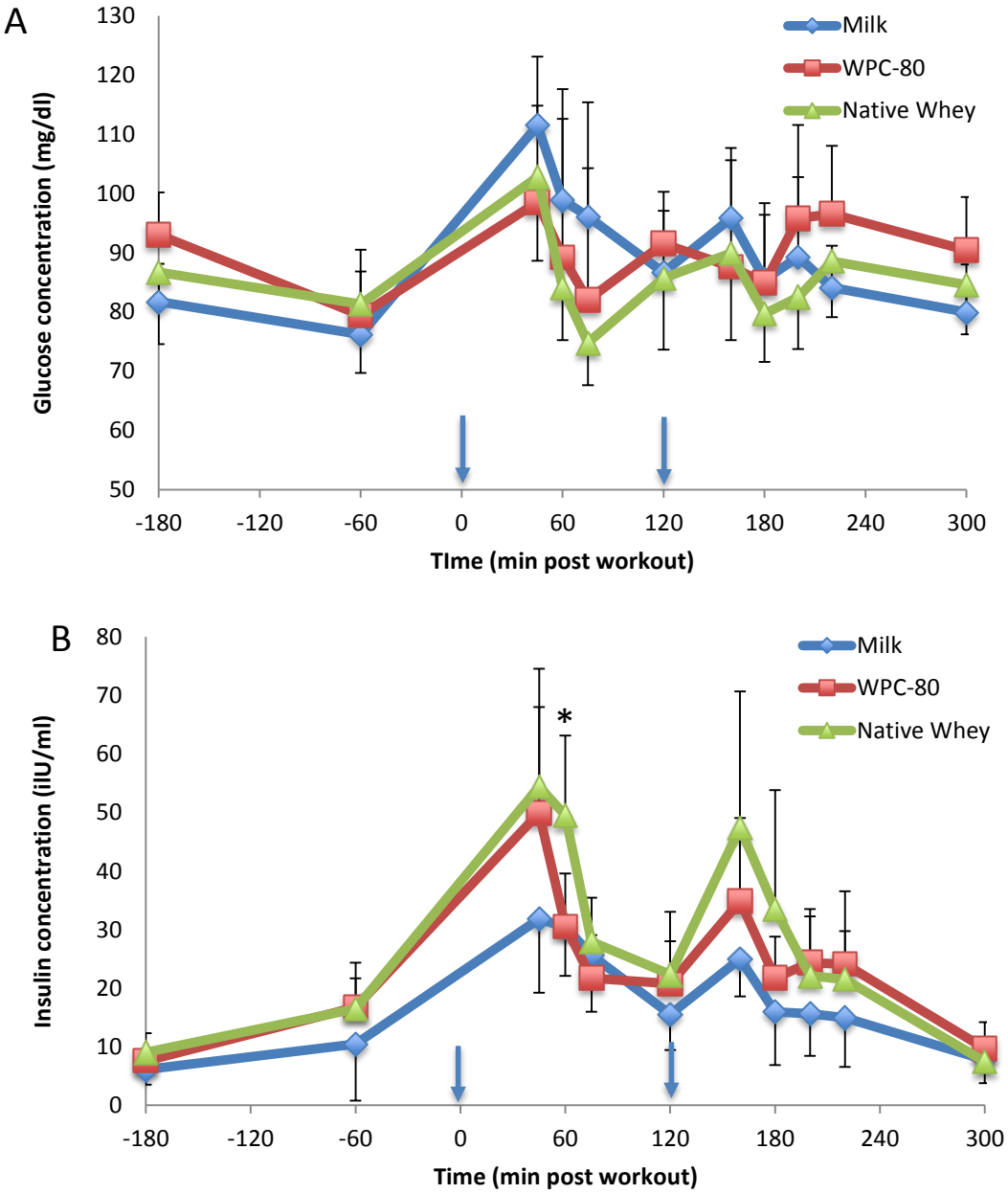

Figure 4.4: Mean ± SD blood concentrations of glucose (A) and insulin (B) for the elderly participants. * indicates a significant higher concentration of insulin after ingestion of native whey, compared to WPC-80 ($P<0,05$). Arrows show where the proteins drinks were consumed.

4.2.3 Young vs elderly

The elderly participants had a higher fasting blood glucose at 180 min pre workout than the young participants (Mean 85.6±9.5 mg/dl vs 79.0±4.6 mg/dl, $p<0.01$). We found no differences at 60 min pre workout.

There were no differences in insulin levels for the young or elderly participants. There was only a tendency of elderly having higher fasting levels of insulin (180 min pre workout) compared to the young group ($p=0.067$).

In **Figure 4.3** and **4.4**, it is depicted that the glucose and insulin levels peak three times for both young and elderly during the time of measurements; after the ingestion of protein drink 1 (45 min post workout), after ingestion of protein drink 2 (160 min post workout) and at 200-220 min post workout. The highest glucose stimulus is after the first drink (45 min post workout) except for the young when ingesting native whey. The milk group had equal or higher glucose stimulus than the whey drinks, even though the milk drink had the lowest concentration of carbohydrates (38.2 g in milk vs 42.0 g in WPC-80 and 40.7 g in native whey).

4.3 Amino acids concentration

4.3.1 Leucine concentration

The plasma leucine concentrations measured were different for all the three drinks for both young and elderly. For the young, native whey and WPC-80 followed the same pattern throughout the test period, while milk revealed a smooth, almost flat curve (**Figure 4.5**) during the time of measurements. Ingestion of native whey gave a significantly higher concentration of plasma leucine compared to WPC-80 at all time-points between 45 and 220 min post workout ($p<0.05$), except at 75 min. Native whey gave a higher concentration of leucine than milk at all times between 45 and 220 min post workout ($p<0.01$).

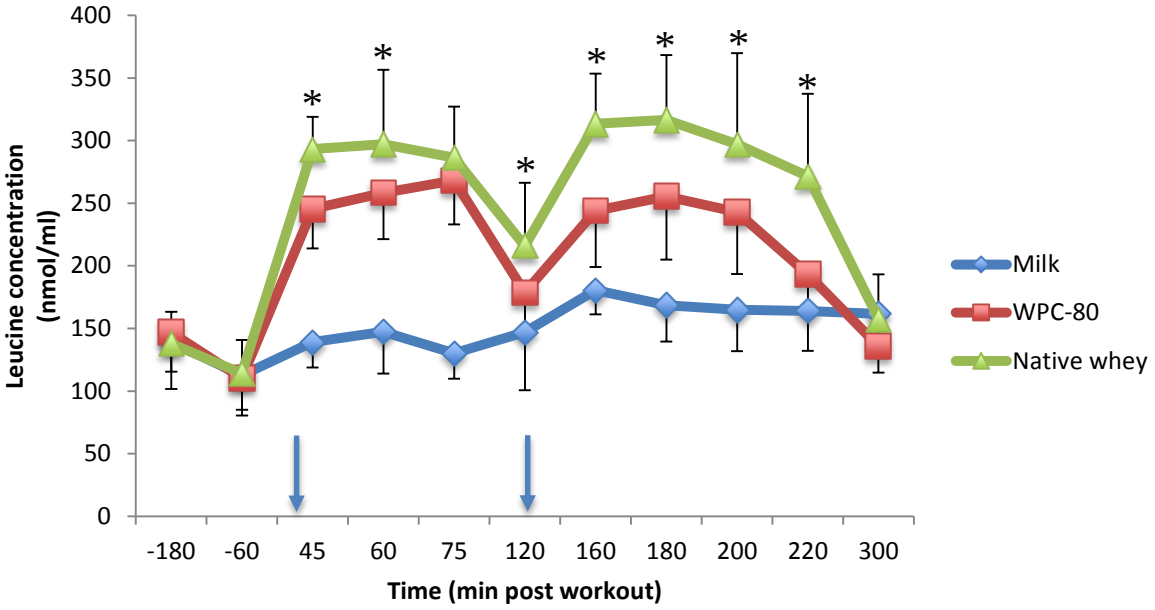

Figure 4.5: Mean ± SD plasma concentrations of leucine for the young participants. * indicates a significant higher concentration of leucine after ingestion of native whey, compared to WPC-80 (P<0,05). Arrows show where the proteins drinks were consumed.

For the elderly the three groups gave a more similar curve for the plasma leucine concentration compared to the young. Intake of native whey resulted in a significantly higher leucine concentration than WPC-80 at 60, 120, 160, 180 and 220 min post workout ($p<0.05$), and we saw a tendency to higher concentration at 75 min post workout ($p<0.10$). Intake of native whey gave a higher leucine concentration in plasma compared to milk at all times between 45 and 220 min post workout ($p<0.05$).

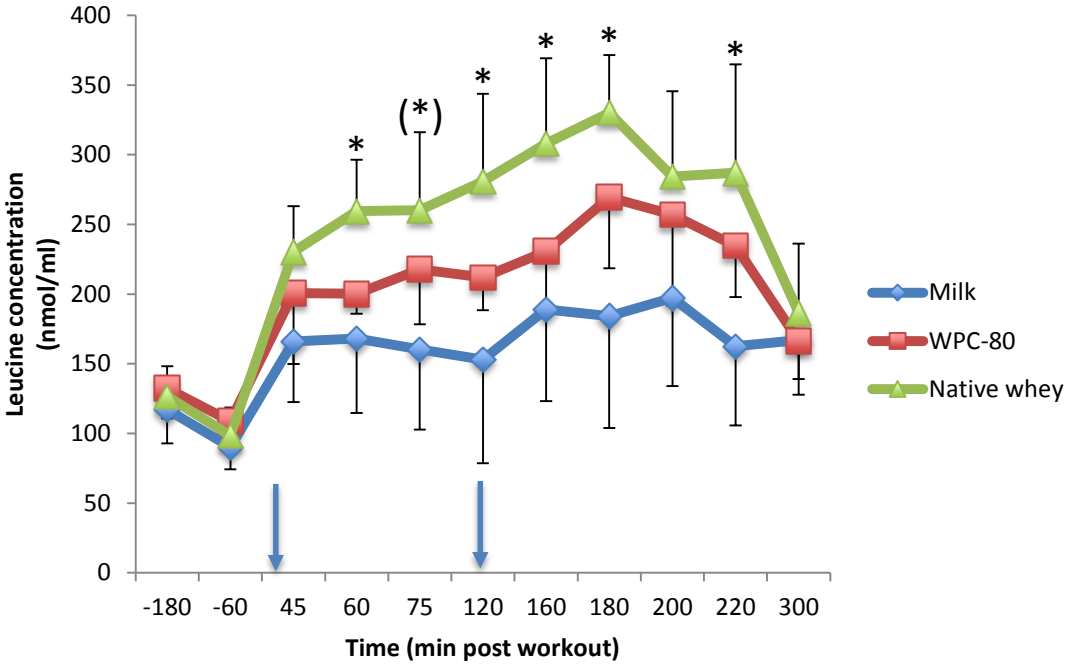

Figure 4.6: Mean ± SD plasma concentrations of leucine for the elderly participants. * indicates a significant higher concentration of leucine after ingestion of native whey, compared to WPC-80 (P<0,05). (*) indicates a tendency to higher concentration of leucine after ingestion of native whey, compared to WPC-80 (p<0.10). Arrows show where the proteins drinks were consumed.

The kinetics of the plasma leucine concentration was different between young and elderly (**Figure 4.5 and 4.6**). Intake of native whey and WPC-80 in the young results in two significant peaks after drink 1 and drink 2, while for the elderly in these two groups, a decrease at 120 min post workout were missing. Instead the relative concentration kept rising until leucine peaked after drink 2. The milk group showed the same shape of the curve for both young and elderly.

At 45 min post workout, ingestion of native whey and WPC-80 resulted in a significant higher plasma leucine concentration for young compared to elderly (p<0.001). After ingestion of native whey, plasma concentrations of leucine reached 293.3 nmol/ml for the young, while for the elderly a level of 230.4 nmol/ml were reached. A tendency to difference in plasma leucine at 45 min post workout in young compared to elderly, were also observed after ingestion of milk (p<0.10). At 60 min post workout, we only observed differences between young and elderly after intake of WPC-80.

After ingestion of native whey, plasma leucine peaked at 60 min post workout after drink 1 and at 180 min post workout (60 min post ingestion) after drink 2 for both the young and the elderly.

For the young participants intake of native whey gave a significantly greater peak after drink 1 (peak 1) and drink 2 (peak 2) and a greater difference from baseline to peak 1 (diff 1) and from baseline at 120 min post workout to peak 2 (diff 2), compared to WPC-80 ($p<0.05$). The same results were found for the area under the curve (AUC) from baseline to 120 min post workout (AUC 1) and the AUC for drink 2 from baseline at 120 min post workout to 300 min post workout (AUC 2) ($p<0.05$).

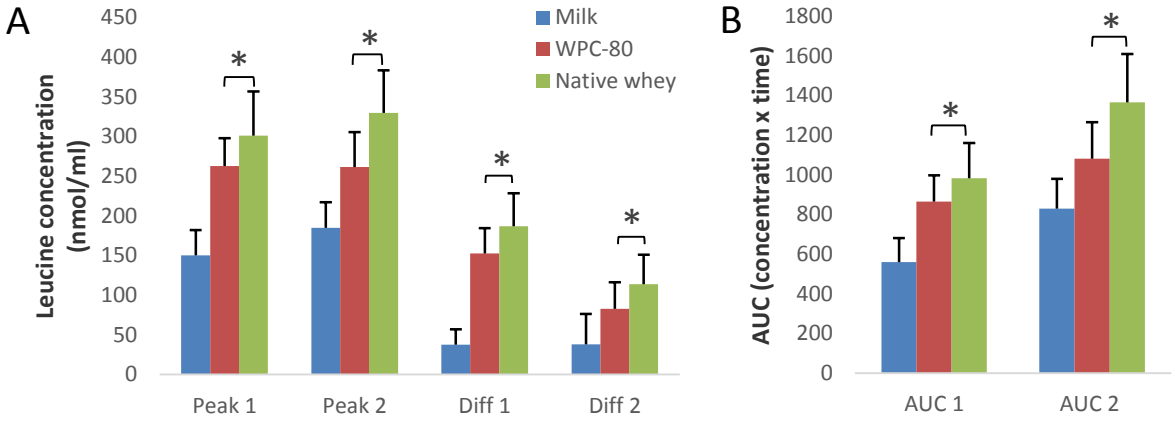

Figure 4.7: Mean + SD leucine kinetics for young participants. A shows peak after protein drink 1 (peak 1) and 2 (peak 2) and difference from baseline to peak for drink 1 (diff 1) and 2 (diff 2). Baseline is 60 min pre workout for drink 1 and 120 min post workout for drink 2. B shows AUC for drink 1 (between 60 min pre workout and 120 min post workout) and drink 2 (between 120 and 300 min post workout). * indicates a significant higher concentration of leucine (peak, diff and AUC) after ingestion of native whey, compared to WPC-80 ($P<0,05$).

For the elderly participants native whey resulted in a higher leucine concentration in peak 2, diff 1 and AUC 1 and 2 compared to WPC-80 ($p<0.05$). We also observed a tendency to higher peak 1 after ingestion of native whey compared to WPC-80 ($p<0.10$).

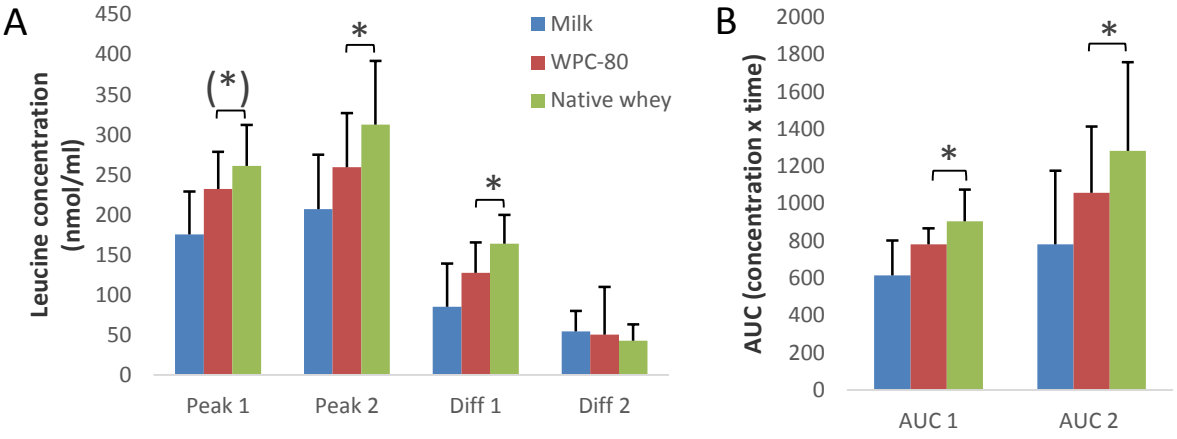

Figure 4.8: Mean + SD leucine kinetics for elderly participants. A shows peak after protein drink 1 (peak 1) and 2 (peak 2) and difference from baseline to peak for drink 1 (diff 1) and 2 (diff 2). Baseline is 60 min pre workout for drink 1 and 120 min post workout for drink 2. B shows AUC for drink 1 (between 60 min pre workout and 120 min post workout) and drink 2 (between 120 and 300 min post workout). * indicates a significant higher concentration of leucine (peak, diff and AUC) after ingestion of native whey, compared to WPC-80 (P<0,05). (*) indicates a tendency to higher peak 1 concentration of leucine after ingestion of native whey, compared to WPC-80 (p<0.10).

4.3.2 BCAA, EAA and total AA

For the young participants intake of native whey resulted in a significantly greater AUC for leucine and BCAA, compared to WPC-80 ($p<0.05$). Intake of native whey resulted in a greater AUC for leucine, isoleucine, BCAA and EAA, compared to milk ($p<0.05$). For the elderly participants intake of native whey resulted in a significantly greater AUC for leucine, compared to WPC-80 ($p<0.05$). Intake of native whey gave a greater AUC for leucine, isoleucine, BCAA and EAA, compared to milk ($p<0.05$). Intake of native whey resulted in a significantly higher peak of BCAA and EAA in young compared to elderly ($p<0.05$, not shown).

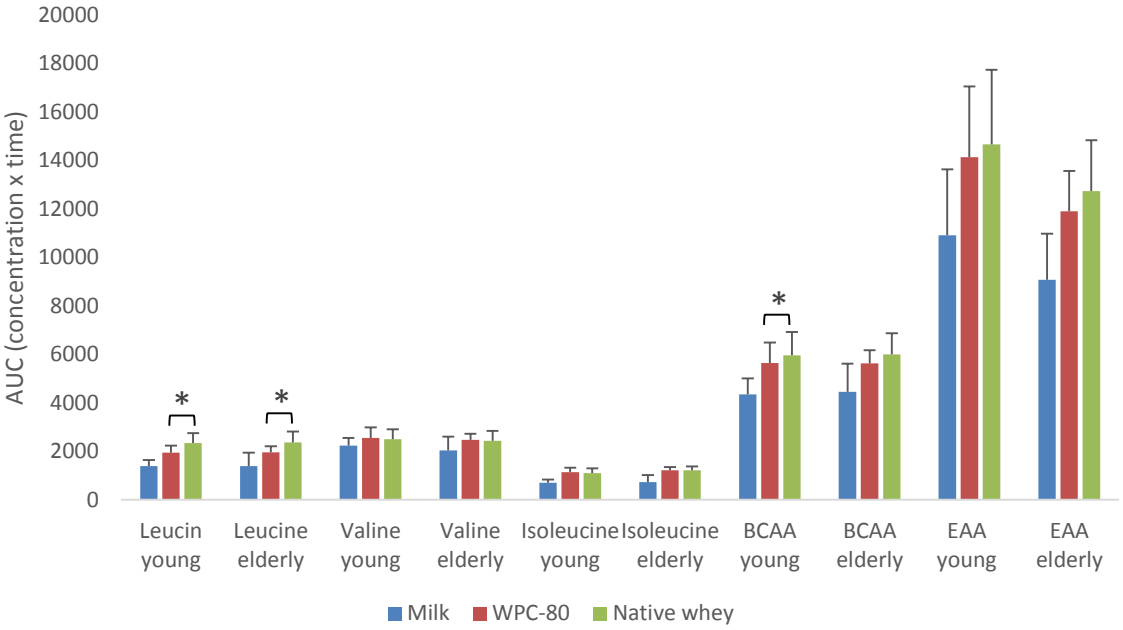

Figure 4.9: Mean + SD AUC for leucine, valine, isoleucine, BCAA and EAA for the young and the elderly participants. * indicates a significant greater AUC after ingestion of native whey, compared to WPC-80 (P<0,05).

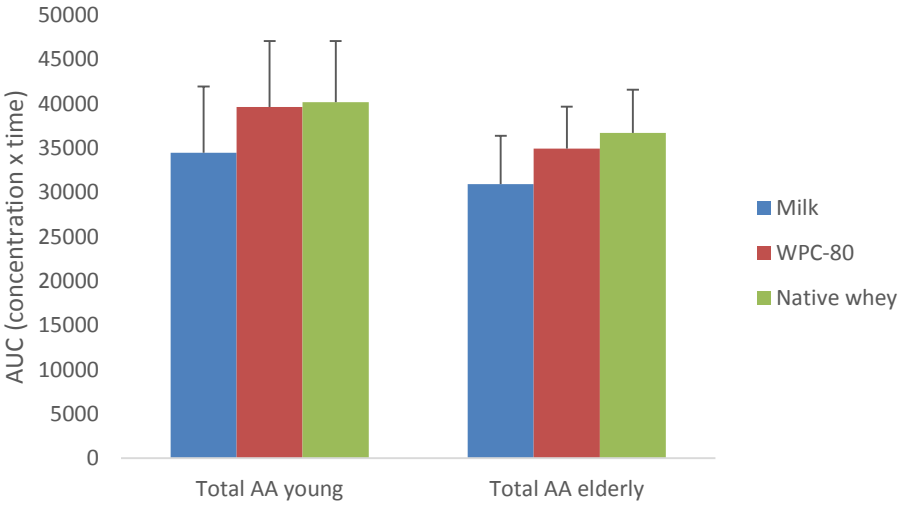

Figure 4.10: Mean + SD AUC for total AA for the young and the elderly participants.

4.4 Urea

4.4.1 Urea young

The changes in urea in percentage of baseline followed the same pattern for native whey and WPC-80 with a slight decrease after baseline and an increase post workout/after ingestion of protein drinks (**Figure 4.11**). Ingestion of milk resulted in a more flat curve and did not give the same change in urea concentration as native whey and WPC-80. No differences were found between native whey and WPC-80 regarding percentage of baseline. Intake of native whey resulted in a higher percentage increase in urea compared to milk at 180 and 300 min post workout ($p<0.01$). No differences were found 24 h post workout.

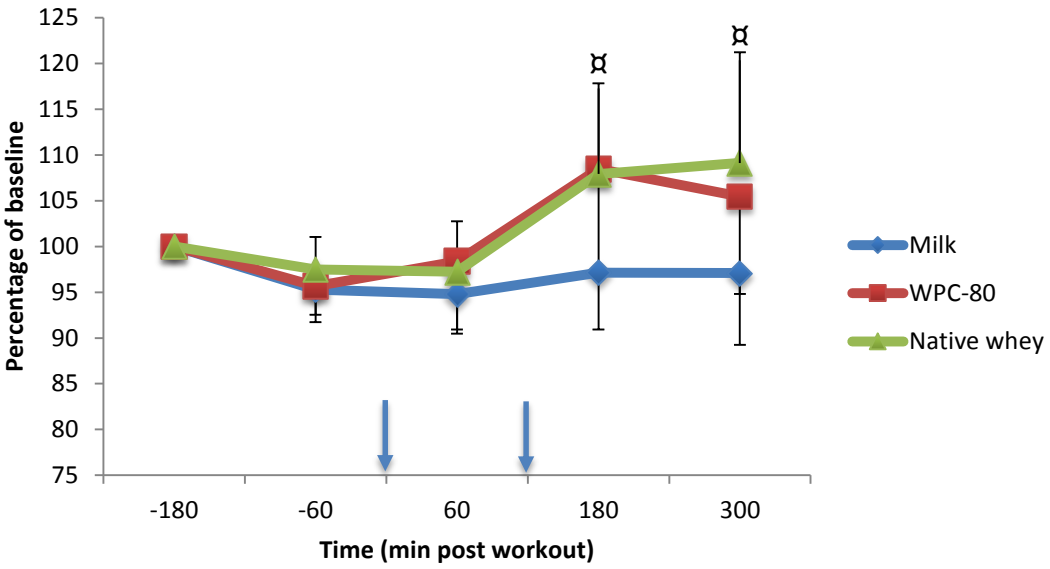

Figure 4.11: Mean ± SD urea in percentage of baseline for the young participants. Baseline at 180 min pre workout were set to 100%. ¤ indicates a higher percentage increase in native whey compared to milk ($p<0.01$). Results for 24 h post workout is not shown.

4.4.2 Urea elderly

The changes in urea in percentage of baseline followed the same pattern for native whey and WPC-80 for the elderly as for the young (**Figure 4.12**). No differences were found between native whey and WPC-80 regarding percentage of baseline. Ingestion of native whey resulted in a higher percentage increase in urea than milk at 180 and 300 min ($p<0.01$). No differences were found 24 h post workout.

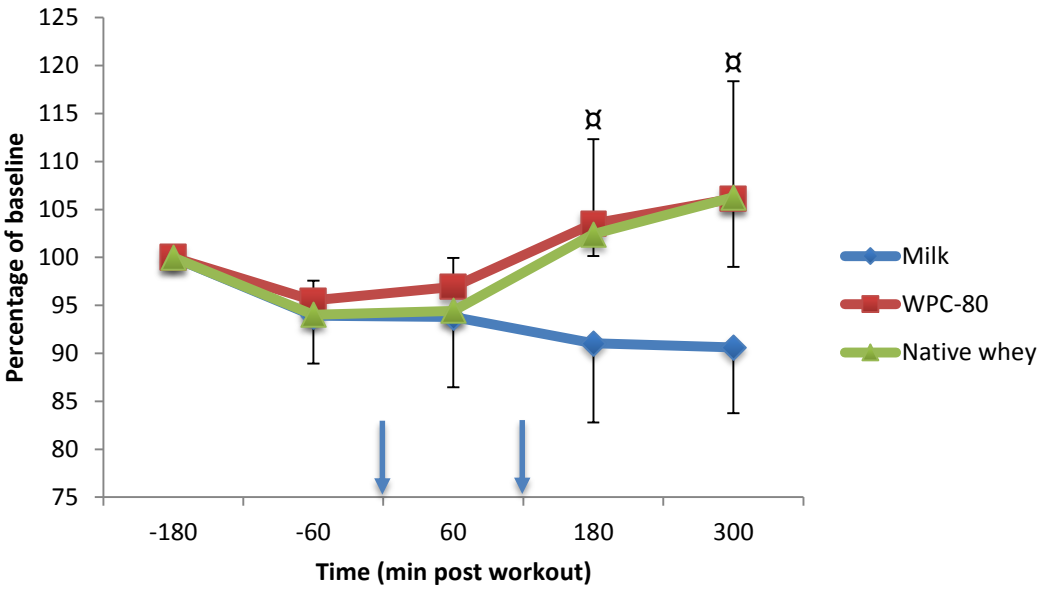

Figure 4.12: Mean ± SD urea in percentage of baseline for the elderly participants. Baseline at 180 min pre workout were set to 100%. ¤ indicates a higher percentage increase in native whey compared to milk ($p<0.01$). Results for 24 h post workout is not shown.

4.4.3 Young vs elderly

Elderly as a group had a higher baseline value of urea compared to the young ($p<0.001$). Intake of native whey resulted in a greater difference between baseline at 60 min pre workout to peak (60-300 min post workout), compared to milk for both young ($p<0.01$) and elderly ($p<0.001$).

Within the native whey group, the elderly participants had a higher peak concentration of urea compared to the young participants (7.4±0.6 mmol/l vs. 6.0±0.8 mmol/l, $p<0.01$). AUC urea was greater for the elderly participants, compared to the young in the same group ($p<0.05$).

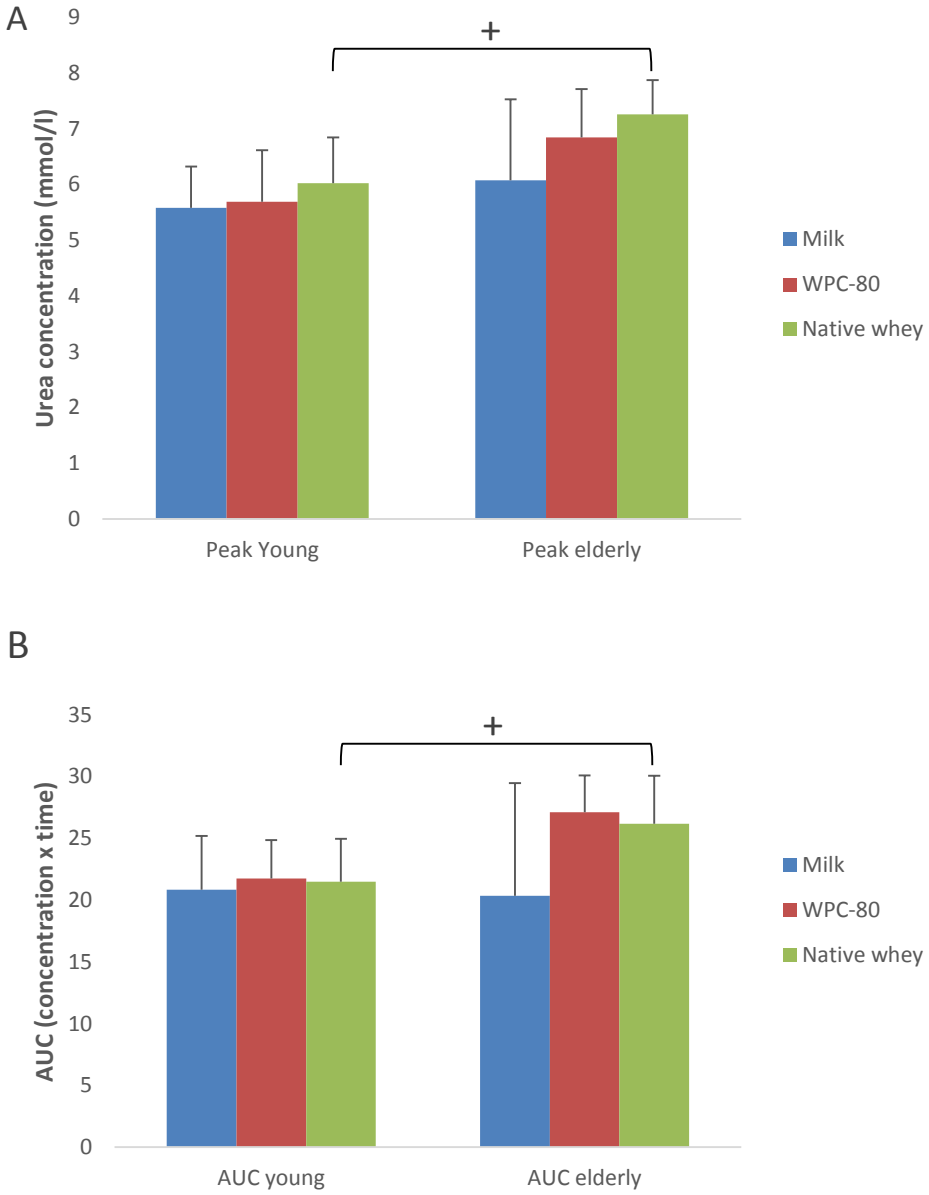

Figure 4.13: Mean + SD urea peak (A) and AUC for urea (B) for young and elderly. + indicates a significant greater peak and AUC for elderly compared to young after ingestion of native whey ($p<0,05$).

4.5 Correlations

4.5.1 Baseline measurements

The relationship between different baseline measurements were investigated using Pearson`s correlation (r).

We found correlations between protein intake in grams from 24 h recall assessment and urea at baseline 180 min pre workout for both young ($r=0.37$, $p<0.05$)) and elderly ($r=0.54$, $p<0.05$), meaning that those with highest protein intakes had the highest urea concentrations in the morning in the fasted state. The same results were shown for protein intake in grams on day -1 and urea at baseline for both young ($r=0.36$, $p<0.05$) and elderly ($r=0.50$, $p<0.05$).

For the young participants ingesting whey, we found correlations between protein intake at the native whey test day and both AUC for BCAA ($r=0.65$, $p<0.05$) and AUC for leucine ($r=0.77$, $p<0.01$). In addition to this we found a correlation between protein intake from 24 h recall and AUC for BCAA ($r=0.72$, $p<0.05$). The same correlations were not seen when the same subjects ingested WPC-80 or in the MG.

4.5.2 Amino acids and Urea

Ingestion of different protein drinks gave different outcomes regarding correlation analyzes. Protein intake from 24 h recalls were correlated with leucine peak 1 for native whey and WPC-80 ($r=0.58$-0.62, $p<0.05$), while the same protein intake was correlated with both leucine diff 1 ($r=0.55$-0.61, $p<0.05$) and AUC for EAA in all three groups ($r=0.59$-0.72, $p<0.01$ for all).

Body mass was correlated with AUC urea for native whey and WPC-80 ($r=0.54$-0.55, $P<0.05$), but not for milk.

For the young as a group we found correlations between urea diff and AUC for leucine, isoleucine and BCAA ($r=0.44$-0.50, $p<0.05$). For the elderly as a group AUC for urea was correlated with AUC for leucine ($r=0.61$, $p<0.05$) and leucine peak ($r=0.56$, $p<0.01$), in addition to AUC for BCAA and EAA ($p<0.01$). The same results were not shown for each protein drink within the two age categories.

5 Discussion

The main findings in this study were that ingestion of native whey resulted in a more rapid and greater plasma leucine concentration, compared to WPC-80 and milk, and a significant difference in the amino acid absorption kinetics between young and elderly. In addition, we found a higher percentage increase in urea after ingestion of native whey compared to milk, for the young and the elderly.

5.1 Discussion of methods

The present study was designed as a double-blinded controlled (partial) crossover study. The study has both advantages and limitations in the methodological design, which are discussed below.

5.1.1 Participants and study design

Inclusion

Based on our power calculations our goal was to include 10 participants in each group for both young and elderly. We fulfilled this with the young participants, but for the elderly we met some challenges. Initially we recruited 20 elderly; however, three of them withdrew before the start of the study due to sickness and injury. As this was at the end of our testing period, there was not enough time to recruit new participants. One elderly withdrew after the first test day, and thus did not finish the study, while another elderly did not ingest all the protein drinks due to nausea. These elderly are thus not included in the analyzes.

We chose to include both men and women in our study. Studies investigating the differences between men and women have come to conflicting results for young and elderly. Whereas several studies have revealed no differences between young men and women in anabolic responses, such as plasma insulin and leucine concentrations (65, 66), Smith et al found that older women, compared with older men, had a blunted anabolic response to both feeding and exercise. Their findings suggest that aging affects muscle protein metabolism differently in men and women (67). We could have made our study objects more homogeny by only including men or only woman. However, this would have given difficulties in recruiting

sufficient number of elderly participants for our study. Due to the limited study population, we did not investigate the sex differences.

We included both young between 20 and 45 years and elderly over 70 years to investigate differences within these two age categories, and if this novel whey fraction can give the same effects in elderly as in well-trained young people.

Randomization and double blinding

The participants in our study were first randomized into two intervention groups, the whey group and the milk group, which also acted as the control group. The participants in the whey group were further randomized into two groups receiving whey at two different days. Randomization by itself may potentially be a confounding factor as it is impossible to obtain identical conditions between the groups (68). To account for this potential error we choose to perform the study double blinded, so neither the participants nor the study personal knew which protein drink the participants received. This was also done to reduce or eliminate biased assessment of the outcome (68).

Study design

A limitation of this study is that we only have a crossover design with native whey and WPC-80, with a different set of participants as a control group. Optimally, we would like to have a crossover with all three drinks. The design was chosen to limit the amount of biopsies from each subject, but still keep a strong design for comparison of the whey drinks. We chose to pair native whey with WPC-80, as we expected smaller differences after intake of these two protein drinks, than either of them compared to milk. We expected to find differences between either of the whey products compared to milk regardless of paired or unpaired groups.

We included an intermediate wash-out period of at least one week, to eliminate the potential effects mediated by the nutrients from the first to the second test drink, so the results would not likely be affected by the previous drink.

5.1.2 Experimental protocol

Standardized diet

Participants were on a standardized diet for 2.5 days in total, which included the day prior to the test day, the test day and until the last test (around 12:00) the day after the test day. This was done in order to control the participants` energy and protein intake the day prior to the test day to reduce the differences among the participants, and to standardize the study as much as possible to reduce the confounding factors.

The diet was designed to meet daily energy and protein requirement for resistance-trained young individuals and for healthy, active elderly individuals. Participants consumed partly prepackaged food during the testing. We chose the products due to convenience, practical reasons, and the fact that we got these products sponsored by the manufactures. The rest of the ingredients were basic foods, which the subjects were responsible to get themselves.

All participants received detailed information about the test protocol and advice on how to implement the diet plan. Regardless of this, one elderly ate breakfast before reporting on NIH on the test day, which most probably affected his baseline measures. As most people that performs physical activity or exercise do so after the intake of breakfast or other meals, we decided to give the participants a light and standardized breakfast that provided enough energy to sustain the bout of resistance exercise.

We chose to give all participants a minimum of 1000 kcal from food, in addition to approximately 990 kcal from the protein drinks, on the test day. This gave a total energy intake of more than 30 kcal/kg for some of the elderly woman. For the well-being of the participants, we had to do it in this manner. The total amount of protein ingested was higher on the test day than the day prior, which was due to the high amount of protein in the protein drinks (~21 g per protein drink) and the additional food intake that had to meet energy requirements.

We allowed for small adjustments of the diet plan if the planned amount of food was too much or too sparse. Some of the participants could not eat all of the food in the diet plan,

while others got hungry and needed to add energy. This was as expected, as energy requirement is dependent on more than just bodyweight, and could be very different in each individual. This led to small differences between planned and actual amount of energy and protein intake. All discrepancies were recorded so calculations and analyzes could be done out of the actual intake.

Protein drink

Each serving of native whey, WPC-80 and milk contained 22.3, 20.9 and 20.8 g protein and 2.73, 2.15 and 1.96 g leucine, respectively. These difference in protein and leucine content between the protein drinks could have led to different effects on the protein metabolism, discussed later.

Dietary assessment

The dietary assessments were done as two 24 h recalls for each subject. At least two independent days are needed to apply statistical modelling to estimate habitual intake (69). According to EFSA days are considered as non-consecutive if there is at least two weeks interval between them (64). Due to limited time-frame and the reason that the recalls had to be done in relation to the pretests, it was not possible to get two weeks in between the recalls for all participants in our study. It varied from 4 to 14 days between the interviews.

Before the first 24 h recall the participants did not know how the diet assessment was conducted, so there was minimal bias due to change in there habitual food pattern. But for the second 24 h recall this could have been an issue. When the recall was announced, the participants could reduce or change their consumption and produce a bias. Moderate underreporting have been reported in this method, particularly in some subgroups (70).

The first 24 h recall was done by face-to-face interview for all participants, whereas the second one was done likewise for all except for two participants, who were interviewed by phone. To reduce the bias of the assessment method, all recalls were conducted by the same person, The disadvantages of the face-to-face interviews was that the subject may have been more vulnerable to exaggerate consumption of foods they perceive to be good and underreport foods perceived to be unhealthy (64).

Food quantities were assessed using household measures. One of the main errors that may occur during the measurement of food consumption in dietary epidemiological surveys is the assessment of accurate portion sizes (71). Use of household measures can result in inaccuracy of amounts eaten, if not conducted with accuracy. It is inevitable that inaccuracies in portion size assessment will remain. These errors will lead to misclassification of subjects according to the amount of food consumed. We did not have any tools in the assessment, but with the same person both collecting and registering the data, the source of error decreased.

The interview ended with a checklist of foods, drinks and snacks that might be easily forgotten (**Appendix 1**), in addition to nutritional supplements. This was useful in the matter that many of the participants realized that they had forgotten items.

The validity of the 24 h recall method mainly depends on the respondents` short-term memory and the accuracy in the reporting of food consumption. The accuracy can be affected my knowledge, memory, and the interview situation. There are conflicting findings of participant characteristics on reporting ability. A number of studies found no significant gender-related differences in ability to estimate the food intake (71-73), while others have reported an effect of gender on the portion-size estimation (74, 75). Some researchers have suggested that the short-term memory required for the dietary assessment may be more difficult for the elderly compared to the young (76).

The registration of food intake in Mat på Data 5.1 (Mattilsynet, Oslo, Norway 2009) was challenging. The program were not complete regarding food items, so food labels had to be used to determine the nutrient content of certain items. Wicked food dishes were difficult to assess correctly, which could have led to small errors in the measurements of energy- and/or protein intake.

Body composition measurements

Among the methods available for direct measurement of lean mass, DXA is reliable, accurate, reproducible and minimally invasive (77). Hydration status can affect the outcome of a DXA scan, but it seems that this is not a major source of variation in DXA body composition estimates in the normal healthy population (77).

Blood samples and preparation

When handling blood samples there are many factors that had to be standardized and taken care of with caution. These factors include procedure for the blood sampling, time of the day the blood sampling was performed, choice of anticoagulant, contamination of the tubes, time before storage and numbers of freeze-thaw cycles (78).

Following standardized protocols and guidelines for blood sampling, preparation and storage can minimize the impact of errors. For plasma samples, this implies that whole blood should be collected in sterile tubes with anticoagulants, placed on ice immediately, and spun down within 30 minutes. This ensures that the inter-assay variations are kept to a minimum (78).

Twenty-two blood draws were taken for every participant at each test day. We had some problems with coagulation of blood samples, which led to a few missing values for some times points in both MG and WG in young and elderly. The missing values had to be imputed to perform statistical analysis. We had to exclude one elderly participant from the analysis due to too many missing values and because the existing values did not follow the pattern of the mean in the rest of the group.

Statistics

Our study population consisted of twenty-two young and fifteen elderly participants. For the elderly only seven participants completed the intervention. This leads to a very narrow set of data and relative few test results to perform statistics calculation on. Because of this it may have been difficult to detect significant results, and potential significant results may have disappeared. However, statistically significant results found in this study suggest strong associations.

5.2 Discussion of results

We have investigated if there is a difference in amino acid uptake and urea turnover after intake of native whey, WPC-80 and milk in young and elderly participants after resistance exercise. In this section the principal results are discussed and put into context of the findings in other scientific studies.

5.2.1 Native whey vs WPC-80

The advantages of whey proteins in terms of muscle anabolism seems to rely on their fast digestion and absorption, along with the high leucine content and the amino acid composition (79).

Glucose and insulin

Increased insulin concentration is a potent anabolic stimulus for MPS, and a number of studies have reported that hyperinsulinemia can increase MPS, particularly when amino acid availability is increased (80).

We showed marked elevations in plasma insulin concentrations following exercise and intake of different whey proteins and milk, which is in agreement with other findings (37, 80). Insulin concentrations peaked at 45 min post workout and were significantly higher after ingestion of native whey compared to WPC-80 at 60 min post workout in elderly. This implies that intake of native whey leads to a prolonged hyperinsulinemia compared to WPC-80, which in turn may affect MPS and MPB in elderly. No differences were found between native whey and WPC-80 for insulin concentrations in the young participants.

Studies indicate that insulin can inhibit muscle proteolysis, and that insulin and amino acids have additive effects on MPS within physiological concentrations (37). Therefore, the high insulin peak seen immediately after the whey intake may have added effects to the high amino acid concentrations regarding anabolic response and MPS.

We encountered some problems with the measurements for native whey in young participants. For glucose, there were missing values in 5 out of 10 participants at the 45 and 160 min post workout samples. These points were therefore extrapolated to complete the curves. We do not know any reasons that ingestion of native whey lead to significantly lower concentrations of glucose compared to WPC-80 at 45 min post workout. The rapid aminoacidemia after ingestion of native whey could have led to a faster insulin response than expected. As our first measurement was at 45 min post workout, we could have missed the top, but we find this less likely. We assume that all three protein drinks would follow the same pattern for glucose in this study therefore we chose to stipulate the curve for the young who ingested native whey.

The glucose results were consistent with what we expected, disregarded the two measurements for native whey in the young. Glucose concentrations peaked after the first protein drink (45 min post workout), decreased to basal levels at 75 min post workout, and increased again after ingestion of the second protein drink. We also found a third peak around 200-220 min post workout. This could be a result of the decreased insulin concentration after its second peak, and thereby higher blood glucose concentration. There were no differences in glucose levels after ingestion of the three protein drinks for young or elderly.

Amino acids

The rate of protein digestion and amino acid absorption determines the extent and rate of amino acid delivery to target tissues, which in turn can affect the overall metabolic response (54).

Smith et al found no differences in amino acid concentrations after ingestion of a mix of carbohydrate and protein drinks containing different types of whey protein (81). Contrary to this, we found that ingestion of native whey resulted in a significantly greater leucine concentration, measured as AUC and peak, compared to WPC-80 in both young and elderly. The young also had a greater AUC for BCAA after ingestion of native whey compared to WPC-80. These results indicate that ingestion of native whey is superior to WPC-80 and milk with regards to increasing the plasma leucine concentration, and thereby may be superior in stimulating the anabolic mechanisms involved in the MPS downstream of leucine. However, several studies have shown that leucine intake in excess of the amount found in adequate whole protein saturate the anabolic pathway and will increase leucine oxidation instead of increasing MPS (43, 44, 82, 83). Thus, when ingesting adequate amounts of high-quality protein, native whey and WPC-80 may impose the same anabolic effect on MPS.

Because of the different protein content in the three protein drinks, we cannot ignore the fact that the differences we found between native whey and WPC-80 and native whey and milk could be a result of the differences in protein content instead of an actual difference between the protein drinks. When we calculated the plasma concentration of leucine per gram of protein, the significant differences between native whey and WPC-80 were diminished, but remained the same between native whey and milk. By adjusting for the difference in amino acid content in native whey and WPC-80, we found that the plasma concentrations of all single amino acids were reflected by their content in the protein drink. These results imply

that it is the higher amount of leucine in native whey that leads to the higher plasma concentrations of leucine, compared to WPC-80. As both native whey and WPC-80 resulted in a more rapid and greater plasma concentration of leucine compared to milk, after adjusting for the difference in protein content, the whey protein are superior to milk in the acute stimulation of protein metabolism, as shown in other studies (53).

Urea

The kinetics of urea production, excretion, and hydrolysis has been widely investigated in humans, as has their modulation by different physiological and dietary factors, including physical activity, recovery and protein intake (24).

Serum urea concentrations decreased slightly after baseline and were steady until 180 min post workout (60 min after ingestion of the second protein drink). Ingestion of native whey and WPC-80 resulted in similar effects on the urea concentration, while ingestion of milk did not cause any changes in the concentration of urea. We found higher percentage increase in urea at 180 and 300 min post ingestion of native whey versus milk for both the young and the elderly. This could be attributed to the higher BCAA content of native whey and the greater aminoacidemia seen after intake of native whey. Increased BCAA availability may lead to increased transamination of the amino acids in muscle and thus higher oxidation rates. This may further result in increased transport of nitrogen to the liver, with a subsequent increased urea production. However, serum urea concentration is affected by both the urea production rate and the excretion via urine (24). Thus, the urea concentration observed in serum is more a reflection of the urea production, and not a direct measure. We found no differences between the protein drinks at 24 h post workout for either the young or the elderly.

Finding from a study by Fouillet et al showed that urinary urea excretion was not influenced by the protein source in the meal but by the protein level in the diet (24). They concluded that urea hydrolysis is an acute nitrogen-sparing mechanism that can counterbalance a postprandial higher urea production, and the efficiency of this recycling is higher when the usual protein intake is lower (24). We found a positive correlation between regular protein intake in grams from the 24 h recalls and urea at baseline (180 min pre workout) for both young and elderly. The same results were shown for protein intake in grams on day-1 and urea at baseline for both young and elderly. Our results suggest that higher protein intake

results in greater serum concentrations of urea, which may be a result of increased urea production due to an elevated protein intake or an increased muscle protein breakdown.

5.2.2 Young vs elderly

Some researchers suggests that a gradual decline in post-absorptive MPS may be the reason for age-related loss of muscle mass (39), while others suggests that there is an impairment in the muscle protein synthetic response to the main anabolic stimuli, food intake and physical activity, in the older population (39). Recent work seems to suggest that the elderly show a blunted response to amino acids, insulin and resistance exercise when compared to young (8, 11, 30, 84).

Glucose and insulin

Blood glucose concentrations were higher in the fasting state for elderly compared to young, and we found a tendency to higher fasting levels of insulin in elderly, but there were no differences between young and elderly after ingestion of the three protein drinks. This is contrary with a previous study in which increased levels of insulin were observed after oral ingestion of 15 g EAA in young, but not elderly (85), while in another study by Pennings et al higher plasma insulin concentrations were observed in the elderly compared to young after ingestion of dietary protein (86).

Several studies suggests that aging is associated with a resistance to the anabolic action of insulin (11, 80, 87). The existence of insulin resistance with aging could represent a key contributor to the reduced muscle anabolic response to feeding and may play a role in the development of sarcopenia (87). Rasmussen et al showed that hyperinsulinemia increased MPS in skeletal muscle of young humans, but not in the elderly. Hence they concluded that healthy aging induces a selective insulin resistance of muscle proteins regardless of glucose tolerance, and is probably due to an impaired response of blood vessels to the dilatory effects of insulin (87). The same results were found by Timmerman and Volpi (88). Further studies are needed to identify the cellular mechanisms for the insulin resistance of MPS in aging.

Amino acids

Several studies have found no differences in post-absorptive amino acid kinetics in the young and elderly (12, 30, 85). Contrary to these, we found that the post-absorptive plasma leucine kinetics was different between young and elderly. The young participants reach peak at 45 min post workout after ingestion of native whey, while the elderly reach peak at 60 min post workout. The young had significant higher leucine concentration at 45 min post workout compared to the elderly after ingestion of both native whey and WPC-80, and we found a tendency to the same result after ingestion of milk. After ingestion of the first serving of native whey the young had a greater peak for EAA and BCAA compared to the elderly. Ingestion of WPC-80 resulted in a tendency to higher peak of EAA, while no differences were found after ingestion of milk. These results together may imply that the young are digesting proteins faster and absorption into the blood are faster after ingestion of about 20 g protein following a bout of resistance exercise compared to elderly, and that the difference between young and elderly are greatest after ingestion of native whey. However, we did not find any differences between young and elderly regarding AUC for leucine, BCAA or EAA, which indicates that the elderly have the same blood amino acid response as the young over the 5 h time-course.

Similar results have been observed in other studies; Fujita and Volpi found that elderly displayed a slower but more prolonged elevation of amino acid in the blood compared to young (80). Similarly, Paddon-Jones et al showed that elderly responded more slowly to MPS after the EAA stimulus but remained in positive net balance for a longer period compared to young, so when the AUC was calculated, no differences between young and elderly subjects were found in the anabolic effect of the amino acid intake (85).

Impairment in protein digestion and amino acid absorption, which limit the appearance of dietary protein-derived amino acids into the circulation, have been postulated as a mechanism underpinning a reduced postprandial muscle protein synthetic response to food intake in the elderly (39). There are several well-documented physiological changes in gastrointestinal structure and function accompanying the aging process, but aging per se appears to have a minor direct effect on most gastrointestinal functions (89, 90). Gastric emptying of liquids is generally slower in older individuals, which may contribute to the different concentration responses in young and elderly participants (85, 89, 90). However, Pennings et al found that

dietary protein digestion and absorption kinetics was not impaired in elderly compared to young (86). The same result were found by Dideriksen et al (11).

Furthermore, it is also possible that the ability to utilize dietary protein/amino acids for muscle anabolism is reduced with aging so that the older population requires a larger amount of amino acids to maintain muscle protein balance and muscle mass (80). Studies have shown that despite the anabolic resistance in elderly, the maximal MPS response that can be obtained by feeding seems to be similar in young and elderly (80). For young people, an intake of 20 g protein, or approximately 10 g EAA, seems to maximally stimulate MPS (44). Elderly, on the other hand, seems to require about 30 g protein, or 15 g of EAA taken as bolus (12, 49), to exhibit the same effect on MPS as the young. This raises the question whether the elderly participants in our study would have shown similar results as the young regarding blood concentrations of EAA, BCAA and leucine, after ingestion of 30 g of protein in each serving instead of 20 g.

Availability of amino acids has shown to be an important factor in promoting net protein synthesis. Hyperaminoacidemia can stimulate MPS acutely by increasing the amino acid transport into the muscle cells (80). The regulation of amino acid transporters and uptake of amino acids into the muscle is different between the young and the elderly (91). This may be another potential mechanism of an impaired response to food intake in the elderly. Dickinson et al found that resistance exercise increased muscle amino acid transporter expression in young and elderly, however, EAA ingestion post exercise enhanced the expression of amino acid transporters only in young, indicating that aging may influence the expression of specific amino acid transporters (91).

We observed a different kinetics of the plasma leucine concentration in elderly compared to young. Ingestion of native whey did not elicit a peak with a subsequent drop after the first serving in elderly, as it did for the young. Instead, the relative concentration kept on rising until leucine peaked about 60 min after the second serving. This may be an effect of the age-related decline in the ability to utilize dietary amino acids. Possible mechanisms include lower muscle mass in elderly and thereby a reduced capacity for amino acid uptake, a reduced blood flow to the muscle and/or an impaired amino acid transport into the muscle for protein synthesis, which all result in decreased availability of amino acids to the muscle (80).

Cuthbertson et al have demonstrated that protein concentration of mTORC1, and its downstream target p70S6K, differ between healthy young and older adults (8). Differences in the availability of such key regulatory proteins may contribute to the reduced capacity of the muscle protein synthetic machinery to "sense" a nutrient signal in older muscle (8). It is beyond the scope of this thesis to discuss this further.

The participants` regular protein intake can affect the way they respond to the protein drinks. Increase in protein intake per se seems to be more important than the actual level of protein intake regarding the efficiency of protein utilization (11). In our study, the young participants had a higher basal protein intake compared to elderly, while the elderly had a greater increase in protein intake from the regular intake to day-1 and to the test day. We found that the level on the regular protein intake from 24 h recalls were positively correlated with peak leucine concentration after the first protein drink for both whey drinks, while the same basal protein intake was positively correlated with both leucine diff 1 and AUC for EAA after ingestion of all three protein drinks. This may imply that higher basal protein intake can prime the absorption of amino acids from the intestine to be more efficient.

Urea

The elderly in our study had higher baseline values of urea compared to the young. A rise in urea concentrations suggests that the capacity to make protein is saturated (25). Factors contributing to increased urea concentration are increased protein intake, increased breakdown of proteins in the intestine or degradation of body proteins due to trauma or sickness (92). The elderly had a greater increase in protein intake from basal intake to both day-1 and test day. The greater increase in protein intake from regular intake to the standardized diet of 1.3 g protein/kg BW, could have led to the higher baseline values for elderly compared to the young. Thus, it seems like it was the increase in protein intake, and not the amount of protein, that determined the serum concentration of urea in our study.

The elderly participants showed higher peak concentrations of urea and AUC for urea compared to the young after ingestion of native whey. As the urea diff did not differ between the young and the elderly, these observed effects was most likely due to higher baseline values for the elderly and not an actual difference in the urea kinetics after ingestion of the protein drinks.

6 Conclusion

The main findings in this study were that ingestion of native whey resulted in a more rapid and greater plasma leucine concentration, compared to WPC-80 and milk, and that we found significant differences in the rate of amino acid uptake between young and elderly. The young participants experienced a rapid increase in plasma leucine concentration, while the elderly experienced a slower, more sustained increase in plasma leucine concentration after ingestion of native whey. However, it is not clear how these differences in amino acid kinetics affect MPS in young and elderly.

We found a higher percentage increase in urea after ingestion of whey protein compared to milk for the young and the elderly, which could be attributed to the higher BCAA content and the greater aminoacidemia seen after intake of native whey.

As we hypothesized, native whey induced a more rapid and greater increase in blood concentrations of leucine, compared to WPC-80 and milk. In line with previous findings ingestion of whey protein resulted in a more rapid transient aminoacidemia, compared to milk which contains a high amount of the slowly digested casein fraction. This effect is attributed to a combination of faster digestion and absorption kinetics of whey, in addition to the higher leucine content compared to milk. The difference between the two whey fractions, native whey and WPC-80, is more likely to be attributed to the higher leucine content in native whey, than the absorption kinetics.

The young participants experienced a more rapid increase in plasma leucine concentrations than elderly, but we did not find a larger peak amino acid concentration in the young. However, it seems that the elderly show a blunted response to the acute stimulations of amino acids and resistance exercise, when compared to young. The mechanisms for the difference in amino acid kinetics in young and elderly populations are not known, but studies indicate that there are multiple factors contributing to the anabolic resistance associated with aging.

Possible mechanisms are:

- Impairment in protein digestion and amino acid absorption due to slower gastric emptying. Seen as lower plasma leucine concentration 45 min post workout in elderly compared to young.

- Reduced ability to utilize dietary protein/amino acids for muscle anabolism in elderly. Seen as a prolonged increase in plasma leucine concentration post whey ingestion and a missing drop at 120 min post workout in elderly. This may be due to reduced blood flow to the muscle, because of age-related insulin resistance, or impaired amino acid transport into the muscle.

- A higher need of amino acids to maximally stimulate anabolic responses in elderly. An increased protein and/or leucine dose in the protein drink for the elderly may have given similar results in the elderly and the young.

All of these factors are among the possible mechanisms for the anabolic resistance seen with aging, and may have an impact on the development of sarcopenia in elderly.

7 Future Perspectives

The present study has generated new questions and hypothesis. Further investigation should include finding the mechanism and potential factors contributing to the differences in amino acid uptake in young and elderly, the cellular mechanisms behind the insulin resistance of MPS in aging and investigate the differences in expression and activity of amino acid transporters in both the gastrointestinal tract and the skeletal muscle in young and elderly.

Skeletal muscle amino acid transporter expression is increased in young and older adults following resistance exercise. Amino acid transporters have a key role in the regulation of muscle protein metabolism because of their ability to activate the mTORC1 signaling pathway. It would be interesting to see if their ability and activity is changed with age and if different whey proteins would elicit different effects.

The present study was an acute study, so the next step is to do a training study over a longer period to investigate if the same results are shown for native whey in young and elderly over a longer time course. Long-term studies are needed to investigate if intake of native whey can reduce the incidence and risk of sarcopenia in elderly, and if intake of supplements with native whey result in a larger increase in muscle mass in athletes, compared to supplements containing WPC-80 or milk.

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Appendices

Appendix 1: Checklist of food, drinks and snack for the 24 h recall

Sjekkliste

Potetgull, peanøtter

Vin, brennevin

Gulrot, kålrot

Kaker, kjeks

Saft, brus

Sjokolade

Pølse

Drops

Frukt

Brød

Øl

Is

Appendix 2: Diet plan

FP:_____

Info til kostplan – "Myseprosjektet"

Drikk:_____

Kjønn: ☐ Mann ☐ Kvinne

Vekt:_____

Mat og drikke

- Du får en individuell kostplan som du skal følge i 2 ½ dag
- Prøv å spis opp alt som står på planen
- Er det noe du ikke orker å spise i ett og samme måltid, så spis resten litt senere
- Hvis du allikevel ikke klarer å spise opp alt, så skriv opp det du ikke har spist på kostplanen
- Du kan drikke ubegrenset med vann
- **Unngå andre mat og drikkevarer enn det som står på planen!!**
- **Unngå alle former for rusmidler (snus, røyk, alkohol)**
- Handleliste:
    - Havregryn, lettkokte
    - Nøytral olje (eks. rapsolje)
    - Sukker
    - Syltetøy
    - Grovt brød (> 50 % grovt)
    - Frukt: banan, eple, pære eller appelsin

Havregrøt i mikrobølgeovn

1. Ha havregryn i en dyp tallerken
2. Hell over ca dobbelt så mye vann som gryn
3. Tilsett olje og salt
4. Sett i mikro i ca 3-5 min, rør om av og til
5. Ha på sukkermengde som beskrevet i planen og kanel etter ønske

Dag -1 (dagen før testdag)

- Alle måltider spises hjemme
- Anbefalt inntak av frukt står på planen, men dette trenger du kun å spise hvis du blir litt småsulten i løpet av dagen
- Du kan drikke vann, saft/brus uten kalorier og kaffe/te uten melk

Testdag

- Frokost serveres når du ankommer NIH
- Du får proteindrikk rett etter trening og 2 timer etter økten
- Middag spises på NIH
- Kveldsmaten spiser du hjemme sammen med en proteindrikk
- Du kan drikke ubegrenset med vann. Eller ingen andre typer drikke denne dagen

Dag 2 (dagen etter testdag)

- Frokost og lunsj spises hjemme
- Du kan drikke ubegrenset med vann og maks 1 kopp kaffe eller te uten melk

Har du spørsmål:

Kontakt Kristin Holte på tlf 99619381 eller kristin.holte@gmail.com

FP:_____

Vekt:_____

Kostplan

Dag -1 (dagen før testdag)

Måltid	Type og mengde mat	Er planen fulgt?
Frokost	Havregryn: Vann: Sukker: Olje: Salt og kanel etter ønske	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor: Hva er ikke spist:
Lunsj	Brød: Hvitost: Syltetøy: Margarin:	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: Hva er ikke spist:
Middag	Fjordland laks à 440 g: Fjordland kjøttkaker à 592 g:	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: Hva er ikke spist:
Kveldsmat (eller i løpet av dagen)	Go`morgen yoghurt Type: Antall:	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: Hvor mye er ikke spist:
Frukt (kan spises når som helst)	Banan, eple, pære, appelsin Antall:	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke:
Drikke	Vann Kaffe/te uten melk Sukkerfri saft/brus	
Annet:		

Testdag

Måltid	Type og mengde mat	Er planen fulgt?
Frokost	Havregryn: Vann: Sukker: Olje: Salt og kanel etter ønske	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor: _____ _____ Hva er ikke spist: _____
Lunsj	**Proteindrikk x 2**	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: _____
Middag	Fjordland laks à 440 g: Fjordland kjøttkaker à 592 g:	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: _____ _____ Hva er ikke spist: _____
Kveldsmat	Go`morgen yoghurt Type: Antall: **Proteindrikk**	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: _____ _____ Hva er ikke spist: _____
Drikke	Vann	
Annet:	_____ _____	

Dag 2 (dagen etter testdag)

Måltid	Type og mengde mat	Er planen fulgt?
Frokost	Havregryn: Vann: Sukker: Olje: Salt og kanel etter ønske	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor: _____ _____ Hva er ikke spist: _____
Lunsj	Go`morgen yoghurt Type: Antall:	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: _____ Hva er ikke spist: _____
Frukt	Banan, eple, pære, appelsin Antall:	Hvis JA, kryss av her ☐ Hvis NEI, hvorfor ikke: _____
Drikke	Vann Maks 1 kopp kaffe/te u/melk	
Annet:	_____ _____	

Appendix 3: Written consent – young participants

Forespørsel om deltakelse som forsøksperson

Hvordan påvirker forskjellige melkeproteinfraksjoner muskelproteinbalanse hos yngre?

Dette skrivet er til alle potensielle forsøkspersoner. Vi ber om din deltakelse i prosjektet, så fremt du oppfyller kriteriene: Du må være i alderen 18-45 år, du skal ha drevet regelmessig styrketrening på hele kroppen under de siste 6 mnd (minst 1 gang per uke), og ellers være frisk og uten skader i muskelskjelettapparatet. Du kan ikke bruke noen form for medikamenter eller ha laktoseintoleranse eller melkeallergi. Du kan heller ikke bruke noen form for kosttilskudd (proteinpulver, vitaminer, kreatin eller lignende); hvis du gjør det kan du likevel delta som forsøksperson ved at du slutter med tilskuddet senest en uke før prosjektstart. Du kan ikke delta om du er allergisk mot lokalbedøvelse (tilsvarende det man får hos tannlegen).

Bakgrunn og hensikt med forsøket

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 både mosjonister, idrettsutøvere og eldre med tanke på prestasjon i idrett og funksjon i hverdagen.

I denne studien ønsker vi å undersøke den umiddelbare effekten på proteinsyntesen og –nedbrytningen av et nyutviklet myseprotein produsert av Tine®. Dette nye myseproteinet vil sammenliknes med vanlig lett melk og WPC-80; myseproteinet som oftest brukes i vanlig proteinpulver.

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 at du gjennomfører én styrketreningsøkt og inntar deretter en drikk på 0,7 liter med myseprotein eller melk. Ulike tester og målinger vil gjennomføres før og etter treningsøkten. Du vil bli tilfeldig trukket (randomiseres) til én av to grupper som inntar enten melk eller myseproteinfraksjoner. Gruppen som inntar myseproteinfraksjoner må gjennomføre forsøket to ganger, en gang med hver mysefraksjon.

Før forsøket

Du skal møte på Norges idrettshøgskole 4 ganger for tilvenning til tester og treningsøvelser, måling av kroppssammensetning (DXA), og en legesjekk i ukene før forsøket. Hver seanse varer i ca. 2 timer. Tidspunkter avtales individuelt. I de tre siste dagene før forsøket må du avstå fra all krevende fysisk aktivitet (trening). Fra dagen før forsøket til forsøket er over (midt på dagen etter hoved-testdagen) skal du følge en standardisert diett laget av en ernæringsfysiolog.

Forsøket

Oppstart på forsøksdagen vil variere fra kl 0700 til 0800. Du vil få en standardisert frokost før forsøket begynner. Måling av proteinsyntesen og –nedbrytingen gjøres ved veneinfusjon av aminosyrer (med stabile isotoper). Det er ingen kjent risiko med stabile isotoper; de forekommer naturlig i maten vi spiser og er ikke radioaktive. Det er en infeksjonsfare, men preparatet klargjøres under sterile forhold og infuseres gjennom et filter som ikke slipper mikrober igjennom. Infusjonen vil innebære at vi setter inn et venekateter i hver arm. Før vi gjennomfører treningsøkten vil vi ta en biopsi og

gjennomføre en styrketest i et kneekstensjonsapparat. Treningsøkten vil bestå av 4 sett av 8 repetisjoner så tungt du klarer, et nytt sett starter hvert 3 minutt. Etter treningsøkten vil du innta en av de tre drikkene, og det vil bli tatt biopsier rett etter økten og etter 2,5 og 5 timer. Det vil også bli tatt blodprøver gjennom dagen og gjennomført styrketester rett etter økten, 5,5 og 24 timer etter treningsøkten, for å måle restitusjon. Dermed vil du måtte sette av en hel dag til testdagen (fra 0700 frem til ca. 1700) og 30 min til styrketesting dagen etter. Deltakere som tilfeldig velges til gruppen med myseproteinene må gå gjennom denne testrunden 2 ganger.

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.

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

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 gruppen som inntar melk blir det til sammen 4 biopsier, mens det for gruppen som inntar mysefraksjonene vil det bli 4 biopsier første runde og 5 biopsier i andre runde, altså 9 biopsier til sammen. Den ekstra biopsien i runde to må tas for å justere for de stabile isotopene som fortsatt kan være igjen i muskulaturen. Flere biopsier kan tas fra samme snitt i huden så det totale antall snitt blir bare 2 for gruppen som inntar melk og 4 for gruppen som inntar mysefraksjoner. 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 muskulaturentas 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. Du må møte ved NIH på totalt 6-8 dager.

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 2028. Ansvarlig for biobanken er Dr. 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, 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)

Samtykke til deltakelse i studien

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 4: Written consent – elderly participants

Forespørsel om deltakelse som forsøksperson

Hvordan påvirker forskjellige melkeproteinfraksjoner 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 på beina. Du kan ikke ha laktoseintoleranse eller melkeallergi. Du kan heller ikke bruke noen form for kosttilskudd (proteinpulver, vitaminer, kreatin eller lignende); hvis du gjør det kan du likevel delta som forsøksperson ved at du slutter med tilskuddet senest en uke før prosjektstart. Du kan ikke delta om du 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 protener har vist seg å kunne motvirke muskelvinnet. 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 den umiddelbare effekten på proteinsyntesen og –nedbrytningen av et nyutviklet myseprotein produsert av Tine®. Dette nye myseproteinet vil sammenliknes med vanlig lett melk og WPC-80; myseproteinet som oftest brukes i vanlig proteinpulver.

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 at du gjennomfører én styrketreningsøkt og inntar deretter en drikk på 0,7 liter med myseprotein eller melk. Ulike tester og målinger vil gjennomføres før og etter treningsøkten. Du vil bli tilfeldig trukket (randomiseres) til én av to grupper som inntar enten melk eller myseproteinfraksjoner. Gruppen som inntar myseproteinfraksjoner må gjennomføre forsøket to ganger, en gang med hver mysefraksjon.

Før forsøket

Du skal møte på Norges idrettshøgskole 6 ganger for tilvenning til tester og treningsøvelser, måling av kroppssammensetning (DXA), og en legesjekk i ukene før forsøket. Hver seanse varer i ca. 2 timer. Tidspunkter avtales individuelt. I de tre siste dagene før forsøket må du avstå fra all krevende fysisk aktivitet (trening). Fra dagen før forsøket til forsøket er over (midt på dagen etter hoved-testdagen) skal du følge en standardisert diett laget av en ernæringsfysiolog.

Forsøket

Oppstart på forsøksdagen vil variere fra kl 0700 til 0800. Du vil få en standardisert frokost før forsøket begynner. Måling av proteinsyntesen og –nedbrytingen gjøres ved veneinfusjon av aminosyrer (med stabile isotoper). Det er ingen kjent risiko med stabile isotoper; de forekommer naturlig i maten vi spiser og er ikke radioaktive. Det er en infeksjonsfare, men preparatet klargjøres under sterile forhold og infuseres gjennom et

filter som ikke slipper mikrober igjennom. Infusjonen vil innebære at vi setter inn et venekateter i hver arm. Før vi gjennomfører treningsøkten vil vi ta en biopsi og gjennomføre en styrketest i et kneekstensjonsapparat. Treningsøkten vil bestå av 4 sett av 8 repetisjoner så tungt du klarer, et nytt sett starter hvert 3 minutt. Etter treningsøkten vil du innta en av de tre drikkene, og det vil bli tatt biopsier rett etter økten og etter 2,5 og 5 timer. Det vil også bli tatt blodprøver gjennom dagen og gjennomført styrketester rett etter økten, 5,5 og 24 timer etter treningsøkten, for å måle restitusjon. Dermed vil du måtte sette av en hel dag til testdagen (fra 0700 frem til ca. 1700) og 30 min til styrketesting dagen etter. Deltakere som tilfeldig velges til gruppen med myseproteinene må gå gjennom denne testrunden 2 ganger.

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.

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

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 gruppen som inntar melk blir det til sammen 4 biopsier, mens det for gruppen som inntar mysefraksjonene vil det bli 4 biopsier første runde og 5 biopsier i andre runde, altså 9 biopsier til sammen. Den ekstra biopsien i runde to må tas for å justere for de stabile isotopene som fortsatt kan være igjen i muskulaturen. Flere biopsier kan tas fra samme snitt i huden så det totale antall snitt blir bare 2 for gruppen som inntar melk og 4 for gruppen som inntar mysefraksjoner. 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 muskulaturentas 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. Du må møte ved NIH på totalt 8-10 dager.

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 2028. Ansvarlig for biobanken er Dr. 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, 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)

Samtykke til deltakelse i studien

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)