DISSERTATION FROM THE
NORWEGIAN SCHOOL OF
SPORT SCIENCES
2017

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Effects of strength training and supplementation with different milk proteins on regulation of muscle mass in young and elderly

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Table of contents

Summary

The ability to maintain a normal muscle masss or to increase muscle mass from a subotimal level, is of great interest from both a sports and health perspective. The health perspective is especially important for our growing elderly population, because the age dependent loss of muscle mass and strength limits participation in daily activites and increases the presence of comorbidities and risk of mortality. In healthy adult individuals changes in muscle mass is mainly regulated by changes in physical activity and diet; especially protein intake and energy balance. The importance of a well balanced diet with a suffcient protein intake is undisputed. Still, whether supplementing the diet with additional protein in the form of supplements offers an extra effect is debated. Furthermore, the role of protein quality of these supplements is unclear. The aim of this thesis was to compare the acute and long-term muscle anabolic effects of supplementation with three differnet milk protein products: 1) native whey, containing high levels of the amino acid leucine, 2) regular whey protein concentrate 80 (WPC-80) and 3) regular milk.

Three acute and two long-term training studies were conducted. In total 77 young and 60 elderly men and women took part in the studies. The primary endpoints of the thesis are changes in muscle protein synthesis (acute studies) and changes in lean muscle mass (training studies). Secondary endpoints are changes in *quadriceps* cross sectional area, *m. vastus lateralis* thickness, muscle fiber cross sectional area, muscle function measured as maximal tests (1RM) and functional test (only in elderly), and phosphorylation of central kinases involved in the regulation of muscle protein synthesis (p70S6K, 4E-BP1 and eEF-2).

In study I we found native whey to to increase blood concentrations of leucine to a greater extent than several forms of whey proteins and milk. Despite greater leucine concentrations in blood with native whey, we observed no differences in phosphorylation of p70S6K, 4E-BP1 and eEF-2, or muscle protein synthesis between native whey and WPC-80 in young (study II) or elderly (study III) individuals. However, native whey induced a greater post resistance exercise phosphorylation of p70S6K and higher rates of muscle protein synthesis than milk in young and elderly. In the training studies (IV and V) native whey was compared to milk. The acute difference for p70S6K phosphorylation between native whey and milk observed in the acute study was not reproduced in these studies. Most participants experienced gains in muscle mass, and all improved their muscle function. Still, no differences were observed between the supplements for any outcome in the training studies.

In summary, no clear benefit on acute anabolic outcomes were evident with post resistance exercise ingestion of native whey compared to WPC-80. The potentially greater acute anabolic effect of post resistance exercise ingestion of native whey compared to milk is unclear, as our results were equivocal. However, for the long-term outcome measures, no differences were observed between native whey and milk.

Sammendrag

Evnen til å opprettholde en normal muskelmasse, eller å øke muskelmassen fra suboptimale nivåer er interessesant både fra både et idretts- og helseperspektiv. Helseperspektivet er spesielt viktig for vår stadig økende eldre populasjon, da aldersrelatert tap av muskelmasse og styrke begrenser evnen til å klare seg selv i hverdagen og øker tilstedeværelsen av risikofaktorer for sykdom og død. I friske voksne individer er endringer i muskelmasse primært regulert av endringer i fysisk aktivitet og ernæring, særlig proteininntak og energibalanse. Viktigheten av et godt og balansert kosthold med et tilstrekkelig proteininntak er hevet over enhver tvil. Men hvorvidt det å øke proteininntaket i kostholdet i form av proteinsupplementering gir en ekstra effekt er debattert. Videre er det uklart hvor stor rolle kvaliteten på proteinet i disse supplementene spiller. Målet med denne doktorgraden var å sammenligne hvordan supplementering med tre de tre ulike melkeproteinproduktene (1) nativ myse, som inneholder høye nivåer av aminosyren leucin, (2) vanlig myseprotein konsentrat (WPC-80) og (3) vanlig melk påvirker anabole prosesser i muskel akutt og over tid.

Tre akutt- og to langtidsstudier ble gjennomført. Totalt deltok 77 unge og 60 eldre menn og kvinner i studiene. De primære endepunktene i doktorgraden er muskelproteinsyntesehastighet (akutte studier) og endringer i fettfri masse (treningsstudier). Sekundære endepunkter er endringer i tverrsnittet av *quardiceps*, tykkelse av *m vastus lateralis*, muskelfibertverrsnitt, muskelfunksjon målt med maksimale styrketester (1RM) og funksjonelle tester (bare for eldre) og fosforylering av sentrale kinaser involvert i reguleringen av muskelproteinsyntese (p70S6K, 4E-BP1 og eEF-2).

I studie I fant vi at nativ myse økte konsentrasjonen av leucin i blod i større grad en flere typer myseprotein og melk. På tross av den høyere konsnetrasjonen in blod så vi ingen forskjeller i fosforylering av p70S6K, 4E-BP1 og eEF-2, eller i muskelproteinsyntesen mellom nativ myse og WPC-80 i yngre (studie II), eller eldre (studie III) etter styrketrening. Nativ myse førte til en økt fosforylering av p70S6K og høyere muskelproteinsyntese sammenlignet med melk, i yngre og eldre etter styrketrening. I treningsstudiene (IV og V) sammenlignet vi nativ myse med melk. De akutte forskjellene i p70S6K fosforylering mellom nativ myse og melk observert i de akutte studiene lot seg ikke reprodusere i disse studiene. De fleste deltakerne økte muskelmassen og alle økte muskelfunksjonen, men vi fant ingen forskjeller mellom supplementene for utfallsmålene i treningsstudiene.

Oppsummert fant vi ingen klare fordeler, ved inntak av nativ myse sammenlignet med WPC-80 etter styrketrening, for akutte anabole utfallsmål. Hvorvidt det er en større akutt anabol effekt av inntak av nativ myse enn melk etter styrketrening er uklart, da resultatene våre er tvetydige. Det var imidlertid ingen forskjell mellom supplementering med nativ myse og melk for langtids-utfallsmålene i disse studiene.

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Oslo, July 2017

Håvard Hamarsland

List of papers

I. Hamarsland H, Laahne JAL, Paulsen G, Cotter M, Borsheim E, Raastad T. Native whey induces higher and faster leucinemia than other whey protein supplements and milk: a randomized controlled trial. BMC Nutrition 2017 3:1. BioMed Central; 2017;3:10.

II. Hamarsland H, Nordengen AL, Aas SN, Kristin Holte, Ina Garthe, Gøran Paulsen, Matthew Cotter, Elisabet Børsheim, Benestad HB, Raastad T. Native whey protein with high levels of leucine results in similar post-exercise muscular anabolic responses as regular whey protein, in young individuals. In review

III. Hamarsland H, Aas SN, Nordengen AL, Kristin Holte, Ina Garthe, Gøran Paulsen, Matthew Cotter, Elisabet Børsheim, Benestad HB, Raastad T. Native whey induces similar post exercise muscle anabolic responses as regular whey, despite greater leucinemia, in elderly individuals. Submitted

IV. Hamarsland H, Handegard V, Kåshagen M, Benestad HB, Raastad T. Native whey with high levels of leucine induces similar adaptation to strength training as milk protein, in young individuals. Manuscript

V. Hamarsland H, Johansen MK, Seeberg F, Brochmann M, Garthe I, Benestad HB, Raastad T. Native whey induces similar adaptation to strength training as milk protein, despite higher levels of leucine, in elderly individuals. Manuscript

Abbreviations

AMP	adenosine mono phosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
AMPK	AMP-activated protein kinase
4E-BP1	4E binding protein 1
BCAA	branched chained amino acids
CV	coefficient of variability
DIAAS	digestible indispensible amino acid score
DXA	dual-energy X-ray absorptiometry
EAA	essential amino acids
eEF-2	eukaryotic elongation factor 2
eIF4E	eukaryotic initiation factor 4E
MPB	muscle protein breakdown
MPS	muscle protein synthesis
mTOR	mechanistic target of rapamycin
mTORC1	mechanistic target of rapamycin complex 1
p70S6K	ribosomal protein S6 kinase beta-1
PKB	protein kinase B
PDCAAS	protein digestibility corrected amino acid score
PA	phosphatidic acid
PLD	phospholipase D
PRAS40	proline-rich Akt substrate, 40kDa
Rheb	ras homolog enriched in brain
TSC2	tuberosclerose complex 2
1RM	1 repetition maximum

1. Introduction

Skeletal muscle is an essential tissue to human motion and physical performance, in both sports and activities of daily life. Furthermore, skeletal muscle is pivotal in metabolic regulation and changes in skeletal muscle mass and function affects the risk of several diseases such as obesity, insulin resistance, diabetes, cardiovascular disease and osteoporosis (Wolfe & Chinkes, 2005; Ruiz *et al.*, 2008). Therefore, knowledge on how to maintain or increase skeletal muscle mass and function is important to both athletes and clinical populations. The most remarkable feature of skeletal muscle is its ability to adapt to the physical demands placed upon it. It does this by integrating and responding to an array of external stimuli from e.g. physical exercise and nutrition. The purpose of this introduction is to give a brief overview of how skeletal muscle is regulated by protein feeding and resistance exercise in young and elderly individuals.

1.1. Protein turnover – regulation by feeding and exercise

Proteins are constantly built and broken down in all tissues of the body. Two continuously on-going processes regulates this turnover of protein, namely protein synthesis[1] (MPS) and protein breakdown[2] (MPB). The balance between these processes controls the quantity of protein in muscle, and is called net protein balance. Whether we maintain, loose or gain body protein is determined by whether protein synthesis or protein breakdown is greatest over a period of time. Only about 20% of proteins for protein synthesis come from the ingestion of amino acids, the remaining 80% are recycled from protein breakdown (Wolfe & Chinkes, 2005). As a result a relatively high protein turnover compared to the amino acid intake and nitrogen excretion is evident. The rate of protein turnover may change without any changes in protein balance, as long as protein synthesis and breakdown are elevated to the same extent. Theoretically, a higher protein turnover will result in an amplification of the control of net protein production and potentially a better quality of proteins in the tissue (Wolfe & Chinkes, 2005). Protein turnover in different tissues varies significantly (Waterlow, 1984), and the turnover in muscle is considered slow at about 1-2% per day (Bouchard, 2015).

[1] Making of protein or peptides from amino acids.
[2] Degrading proteins or peptides to amino acids.

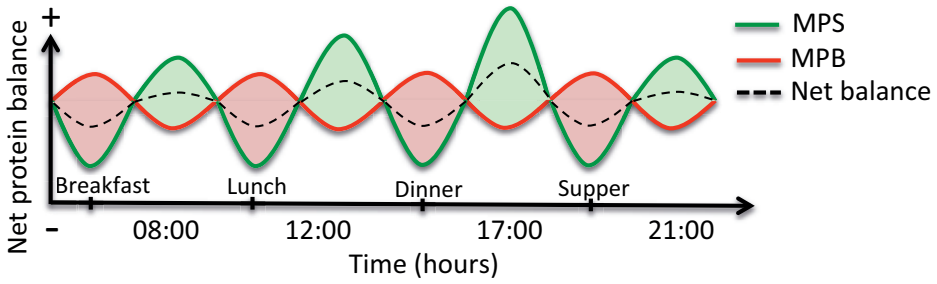

Figure 1.1 *Muscle protein synthesis (MPS) and musslce protein breakdown (MPB) fluctuating during a day. MPS increases and MPB decreases with protein intake, leading to a positive net protein balance after a meal (green areas). In the postabsorptive state MPS decreases, while MPB increases, leading to a negative net protein balance (red area).*

The dynamic regulation of muscle protein turnover in healthy individuals is primarily regulated by essential amino acid availability (Volpi *et al.*, 2003) and exercise (Biolo et al., 1995b). In the post-absorptive state, MPB exceeds MPS, resulting in a net negative muscle protein balance and loss of muscle protein (Rennie *et al.*, 1982; Biolo *et al.*, 1995*b*; Volpi *et al.*, 1999). With protein ingestion, MPS typically doubles (Glynn *et al.*, 2010*a*; Atherton *et al.*, 2010; Dickinson *et al.*, 2011), whereas MPB is decreased resulting in a few hours of positive net muscle protein balance and accretion of muscle protein occurs (Biolo *et al.*, 1995*a*; 1997; Volpi *et al.*, 1999; Glynn *et al.*, 2010*a*). Given a stable activity level and sufficient amounts of protein, a steady state with no gain or loss of muscle mass occurs. This balance can be tipped in favour of muscle protein accretion with regular exercise, especially resistance exercise (Tesch, 1988; Staron *et al.*, 1990; Hather *et al.*, 1991; Biolo *et al.*, 1997). Within one hour after heavy-load resistance exercise in untrained individuals, MPS and MPB increase by approximately 65% (Reidy & Rasmussen, 2016) and 30-50% compared to rest, respectively (Biolo *et al.*, 1995*b*; Phillips *et al.*, 1997; 1999). Accordingly, without post exercise amino acid provision net muscle protein balance remains negative, although less negative than in the non-exercised postprandial state. When post-exercise protein is provided MPB remains relatively stable (Tipton *et al.*, 1999*a*; Rasmussen *et al.*, 2000; Borsheim *et al.*, 2002), while MPS increase (+130% from resting values (Reidy & Rasmussen, 2016)) to a greater extent and for a prolonged period compared to exercise or protein feeding alone (Witard *et al.*, 2009; Moore *et al.*, 2009*a*; Churchward-Venne *et al.*, 2012; Camera *et al.*, 2015). Thus, the combined effects of exercise and protein are additive, with addition of post exercise protein increasing myofibrillar protein synthesis by around 50%, compared to an isocaloric placebo (Borsheim *et al.*, 2004; Koopman *et al.*, 2005; Tang *et al.*, 2007). MPS stays elevated for one to five hours after feeding, depending on the resistance exercise and feeding protocol, before returning to baseline rates (Mitchell *et al.*, 2015*c*; Atherton *et al.*, 2010; West *et al.*, 2011; Churchward-Venne *et al.*,

2012). In the rested state MPS returns to baseline levels in spite of continued administration of high levels of amino acids (Bohé *et al.*, 2001; Atherton *et al.*, 2010). This phenomenon is termed the "muscle full effect" and has yet to be shown after axercise. The exercise-induced increased sensitivity to amino acids in muscle seems to extend to at least 24 hours after resistance exercise (Miller *et al.*, 2005; Burd *et al.*, 2011*b*). Thus, a bout of resistance exercise may potentiate the anabolic effect of all meals within 24 hours after exercise.

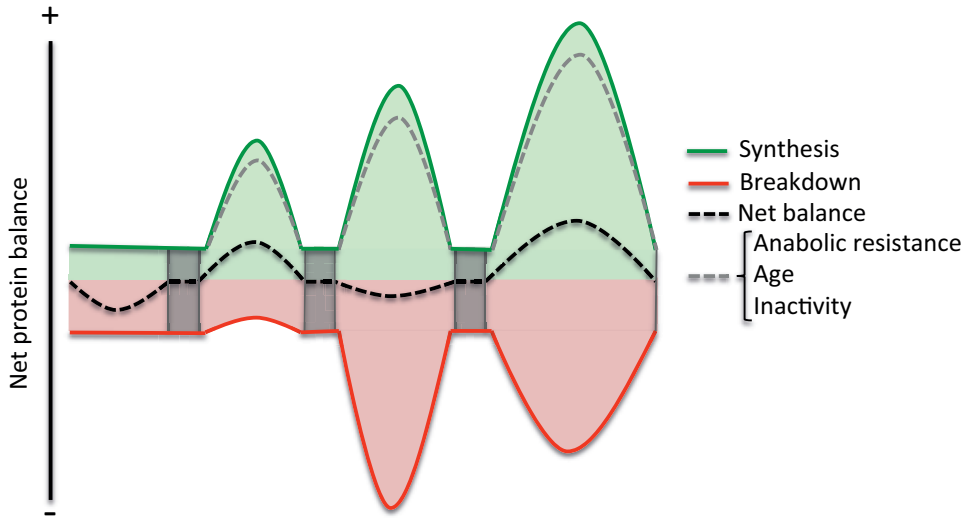

Figure 1.2 *The effect of fasting, protein ingestion, resistance exercise, caloric restriction, age and anabolic resistance on muscle protein synthesis, muscle protein breakdown and net protein balance.*

1.1.1. Muslce protein synthesis rate – regulation by amino acids

The connection between feeding, whole body protein synthesis, and muscle protein synthesis was established over 30 years ago (Rennie et al., 1982). Later amino acids (Bennet *et al.*, 1989; Biolo *et al.*, 1997; Volpi *et al.*, 1999), and subsequently the essential amino acids[3] were shown to be the nutritional factor initiating protein synthesis with feeding (Tipton *et al.*, 1999*b*; 1999*a*; Borsheim *et al.*, 2002; Volpi *et al.*, 2003). EAAs differ from non-essential [4]amino acids in that the body is not

[3] The essential amino acids are leucine, isoleucine, lysine, phenylalanine, valine, threonine, tryptophan, methionine and histidine.
[4] The non-essential amino acids are alanine, aspartic acid, aspargine, glutamic acid and serine. Arginine, cysteine, glycine, glutamine, proline and tyrosine are conditionally essential, meaning that under certain conditions the body needs them to be supplied through the diet.

able to produce them by *de novo*[5] synthesis. As a consequence they must be provided through the diet. Among the EAAs the branched chain amino acids[6] are unique in that they mostly by-pass the first pass splanchnic extraction[7], which makes them readily available to muscle (Wahren *et al.*, 1976; Hagenfeldt *et al.*, 1980; Matthews *et al.*, 1993). Studies in vitro[8] (Buse & Reid, 1975), in animals (Anthony *et al.*, 2000) and in humans (Alvestrand *et al.*, 1990; Smith *et al.*, 1992*a*; Wilkinson *et al.*, 2013; Churchward-Venne *et al.*, 2014) have pointed to a unique role of one of these branched chain amino acids (BCAA), namely leucine, in stimulating MPS. Other EAAs such as valine (Smith *et al.*, 1992*b*), phenylalanine, and threonine (Smith *et al.*, 1998) have also been shown to stimulate MPS at rest in human muscle.

1.1.2. Amino acids and protein synthesis at the molecular level

Protein synthesis is a dynamic and energy demanding process, responding to stimuli, such as amino acids, exercise, growth factors[9], cellular stresses[10] and the energy status of the cell. In order to ensure the appropriate response, MPS is controlled by a set of complex signalling pathways[11]. Central to the control of these pathways is a protein kinase called mechanistic target of rapamycin (mTOR). mTOR can form two structurally and functionally different multi-protein complexes called mTORC1 and mTORC2. mTORC1 is the central complex for control of cellular growth (Laplante & Sabatini, 2012).

Growth factors, mechanical stress (induced by e.g. resistance exercise), amino acids and the energy status of the cell all have the ability to regulate mTORC1 activity, but seem to do so through different signalling mechanisms. Ras homolog enriched in brain (Rheb) is believed to be the most vital activator of mTORC1 (Laplante & Sabatini, 2012). It can be activated by growth factors binding to membrane-bound tyrosine kinase[12] receptors, activating Akt[13] (Engelman *et al.*,

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[5] Latin, meaning from the beginning.
[6] Leucine, isoleucine and valine.
[7] Digested amino acids are transported by the portal vein to splanchnic tissues (gut and liver), which absorbs about 50% (Groen *et al.*, 2015), before the remaining amino acids are released to pheripheral tissues.
[8] Studies performed on cells outside the body.
[9] Group of naturally occuring hormones or proteins capable of stimulating cellular growth and differentiation.
[10] A wide range of changes challenging the cellular homeostasis. In response to exercise these may include heat, free radicals, mechanical strech and damage, hypoxia, changes in pH and changeing lelvels of molecules, such as calcium (Marcotte *et al.*, 2014; Hoppeler, 2016; Sharples, 2017).
[11] Sensing of the microenvironment and communication with other cells are conveyed through cascades (pathways) of proteins coordinating the cells response and actions.
[12] A protein capable of attaching a phosphate group to another protein (phosphorylate), thereby changeing the activity of the phosphate-recieving protein.

2006). Once activated Akt can phosphorylate tuberosclerose complex 2 (TSC2) cancelling its inhibitory effect on Rheb (Inoki *et al.*, 2003*a*; Tee *et al.*, 2003). Further, Akt can phosphorylate the mTORC1 complex bound proline-rich Akt substrate 40kDa (PRAS40), resulting in disassociation and relief of its inhibitory effect (Sancak et al., 2007). During e.g. resistance exercise the anabolic effect of mechanical stress is thought to be conveyed through phospholipase D (PLD) and phosphatidic acid (PA) directly to mTORC1 (O'Neil *et al.*, 2009; Joy & Gundermann, 2014). The anabolic[14] effect of amino acids appears to involve a group of GTPases[15] called Rags (RagA, RagB, RagC, RagD). When activated by amino acids, the Rags bind to Raptor within the mTORC1 complex and subsequently translocate mTORC1 to the lysosomal membrane, where Rheb resides (Sancak *et al.*, 2008; 2010; Bar-Peled *et al.*, 2012). Thus, both the activation of central kinases and the cellular localization of mTORC1 are ways of regulating mTORC1 activity. As protein synthesis is an energy-demanding process it needs to be controlled in times of low cellular energy. A central kinase in sensing the energy levels within the cell is the AMP-activated protein kinase (AMPK), which senses changes in cellular AMP/ATP ratio (Hardie, 2011). When activated AMPK can inhibit mTORC1 activity by activating TSC2 (Inoki *et al.*, 2003*b*), by phosphorylating Raptor within the mTORC1 complex (Gwinn et al., 2008) and by direct phosphorylation of mTOR at Thr^{2446} (Cheng *et al.*, 2004).

The effects of mTORC1 are conveyed on the translational machinery[16] through phosphorylation of its downstream targets ribosomal protein S6 kinase beta-1 (p70S6K) and eIF4E-binding protein 1 (4E-BP1; (Ma & Blenis, 2009). When phosphorylated 4E-BP1 loses its inhibitory action against eukaryotic initiation factor 4E (eIF4E), thus allowing the assembly of the eukaryotic initiation factors (eIF4E, eIF4G and eIF4A), interaction with the ribosomal subunit and mRNA transcription to occur (Ma & Blenis, 2009). mTORC1 phosphorylates 4E-BP1 at several sites[17], including Thr^{37}, Thr^{46}, Ser^{60} and Thr^{70} in a sequential manner (Gingras et al., 2001). $Thr^{37/46}$ can be directly phosphorylated by mTORC1 *in vitro* (Burnett *et al.*, 1998) and act as a primer for subsequent phosphorylation (Gingras et al., 2001).

Another well-characterized target of mTORC1 is p70S6 kinase (p70S6K), which regulates several

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[13] Also called protein kinase B (PKB)
[14] The energy demanding process of building molecules from smaller units.
[15] A molecule that can bind and hydrolyze guanotide triphosphate for enegy demanding processes.
[16] mRNAs, ribosomes, tRNAs and translation factors involved in the process of translating mRNA to proteins.
[17] Phosphates are bound to specific sites on proteins, usually serine, threonine, tyrosine, and histidine residues. The specific sites are often called by what amino acid residue it is and it's number along the amino acid chain.

factors involved in cap-dependent translation initiation[18] and translation efficiency (Ma & Blenis, 2009). The effect on peptide elongation is mediated by phosphorylation of eukaryotic elongation factor 2 kinase (eEF-2K), and relief of its inhibitory effect on eukaryotic elongation factor 2 (eEF-2), leading to enhanced peptide elongation (Redpath *et al.*, 1993). P70S6K have several phosphorylation sites, which needs to be phosphorylated for maximal activity (Pullen *et al.*, 1998; Alessi *et al.*, 1998). Among these the Thr[389] is regarded as the one most representative of activity (Weng et al., 1998).

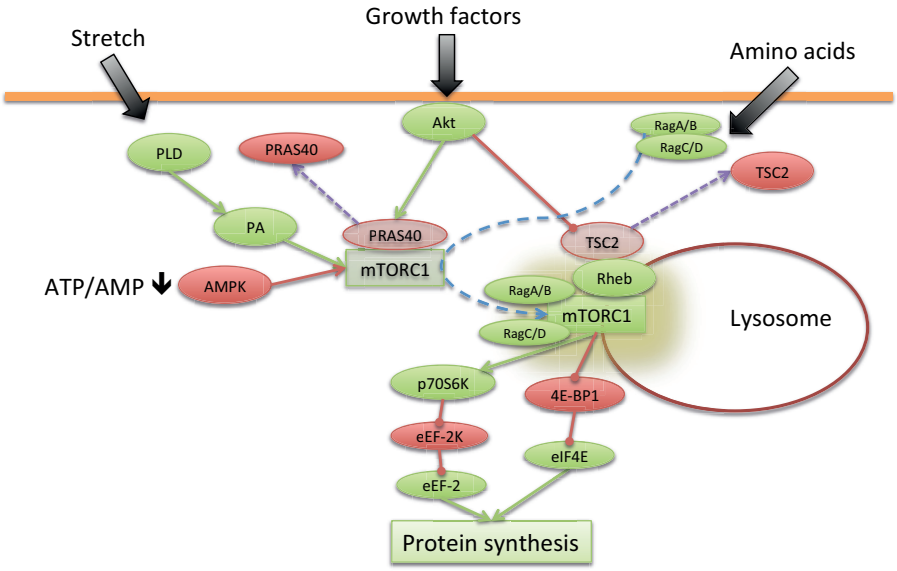

Figure 1.3 *Simplified illustration of mTORC1 regulation by stretch, growth factors and amino acids. Green and red circles indicate positive and negative regulators of MPS, respectively. Green arrows indicate a stimulating effect and red arrows indicate an inhibitory effect. Blue dashed arrows indicate translocation. Purple dashed lines indicate dissacociation.*

1.2. Protein quality and pattern of intake

Protein intake pattern refers to the dose, timing and frequency of intake. Together with the quality of the protein source, these factors determine the impact of ingested protein on MPS.

1.2.1. Protein quality

Protein quality in this thesis refers to the potential of a protein source to stimulate MPS after

[18] The process by which ribosomes makes proteins from a mRNA transcript. The initiation of this process requires the assembly of the small and large ribosomal subunits and is regulated by a set of initiation factors.

ingestion. The Protein Digestibility Corrected Amino Acid Score[19] (PDCAAS) and Digestible Indispensible Amino Acid Score[20] (DIAAS) are the most widely used indexes to describe the nutritional value of a protein source. Both of these indexes give information about where a protein source stands in relation to the minimum nitrogen and amino acids needed to avoid whole body deficiency. However, the "true" skeletal muscle anabolic potential is not predicted by either of these methods. For this we must turn to methods utilizing stable isotope amino acid tracers[21]. By use of these methods several studies have shown that the MPS response depends on both the digestion[22] and absorption[23] kinetics of a dietary protein (Koopman *et al.*, 2009*a*; Pennings *et al.*, 2011*a*), and the amino acid composition (Tipton *et al.*, 1999*b*; 1999*a*; Borsheim *et al.*, 2002; Volpi *et al.*, 2003).

Animal-derived protein sources are generally considered to have a high quality and digestibility (>90%, (Gilani *et al.*, 2012), depending on the processing method[24] (Rutherfurd & Moughan, 2012). Still, studies have found differences in the potential to stimulate MPS after intake of e.g. whey and micellar casein (Tang *et al.*, 2009; Pennings *et al.*, 2011*a*; Yang *et al.*, 2012*b*; Burd *et al.*, 2012). Whey, making up about 20% of the proteins in milk, is considered a "fast" protein, meaning it is rapidly digested and amino acids enter the circulation quickly after ingestion. In contrast, the native form of casein found in milk (micellar casein) is considered "slow", as it clots in the stomach and enters the small intestine and circulation slowly (Boirie *et al.*, 1997*a*; Bos *et al.*, 2003). Changes in blood concentration of essential amino acids, especially leucine, seen after protein intake has been suggested as an important regulator of the MPS-response (Katsanos *et al.*, 2006; Dreyer *et al.*, 2008; Tang *et al.*, 2009; West *et al.*, 2011). According to the leucine-trigger hypothesis, a rapid and large increase in leucine concentrations in blood is needed to maximize the MPS-response to protein intake (Anthony *et al.*, 2001; Phillips, 2014). The importance of

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[19] Method for measurement of protein value of human nutrition and is given as (mg of limiting amino acid in 1 g of test protein • mg of same amino acid in 1 g of reference protein^{-1}) • fecal true digestability (Schaafsma, 2000).

[20] The current recommended method for measurement of protein value of human nutrition and is given as 100 • (mg of digestible indespensible amino acid in 1 g of protein • mg of the same digestible indespensible amino acid in 1 g of the reference protein) (Leser, 2013).

[21] Amino acids labelled with e.g. one or more atoms to make them different from the naturally occuring molecule, and thus detectable by e.g. mass spectrometry.

[22] Breakdown of food to small water-soluble molecules in the, mainly in the gut and small intestine

[23] Transport of digested food molecules from mainly the small intestine to the blood stream, through either diffusion or active transport.

[24] E.g. beef has a slower digestion than minced meat (Pennings *et al.*, 2013), and hydrolysed casein is more readily digestible than intact casein (Boirie *et al.*, 1997*a*), (Calbet & Holst, 2004), (Koopman *et al.*, 2009*a*), further heating, enzymatic treatment (Schmidt *et al.*, 1984) and presence of anti-nutritional agents (van Vliet *et al.*, 2015) may affect the digestive properties and nutritional availability of a protein source.

digestion and absorption as independent regulating factors of postprandial MPS has been challenged by studies finding only temporal differences in postprandial[25] MPS, with MPS being similar when measured over 6 hours (Reitelseder *et al.*, 2011). Due to differing amino acid composition, it is hard to compare the effects on absorption rate of different types of protein. In an attempt to circumvent this challenge, studies have compared a "slow" and "fast" aminoacidemia[26] by manipulating pattern of intake of whey protein (West *et al.*, 2011) or EAAs (Mitchell *et al.*, 2015*d*). The results of these studies are somewhat equivocal and suggest that a rapid amino academia holds a greater anabolic potential than a slow aminoacidemia after resistance exercise (West *et al.*, 2011), and that the total amount of EAAs ingested is the major regulating factor at rest (Mitchell *et al.*, 2015*d*). Based on these and other studies it may seem that a certain peak or increase of leucine concentration in blood is needed to maximally trigger MPS, whereas the other EAAs are required to maintain MPS and the dose of protein will thus affect the duration of the increased MPS (Churchward-Venne et al., 2012).

Thus, consuming a rapidly absorbed protein (West *et al.*, 2011) with high amounts of EAAs and/or leucine may allow for a greater anabolic potential for a given amount of protein (Churchward-Venne et al., 2014), at least for the first hours after exercise. This effect holds true up to a given dose, at which maximal postprandial MPS is reached (Moore *et al.*, 2009*b*; Witard *et al.*, 2014*b*). Consequently, by ingesting a large enough serving of a given protein source it is possible to compensate for a low essential amino acid content (Joy et al., 2013).

1.2.2. Protein dose

In young healthy individuals dose effects studies have shown a "maximal" stimulation of MPS with around 20 g of high quality protein, at rest (Cuthbertson et al., 2005), and after unilateral leg exercise in the fasted state (Moore *et al.*, 2009*b*) and after receiving breakfast (Witard et al., 2014b), with little added effect of 40 g of protein. This dose corresponds to about 8-9 g of EAA and 1.8 g leucine. These findings have been supported by a retrospective analysis of several studies investigating the effect of protein ingestion on MPS, observing a breakpoint for protein stimulation of MPS at around 0.24 g • kg body mass for young men (Moore et al., 2014). Some uncertainty still remains with regards to the optimal protein dose in young, as the use of a whole body workout, as opposed to unilateral leg exercise allowed for a greater stimulation of MPS with 40 g than 20 g of whey protein in young individuals (Macnaughton et al., 2016).

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[25] After a meal.
[26] An excess of amino acids in the blood.

The optimal dose of protein for maximal stimulation of MPS is less established in elderly. A saturation of MPS was observed as ingestion of 20 g of EAA (≈40 g of protein) elicited the same response as 40 g of EAA (≈80 g of protein (Cuthbertson et al., 2005)). However, dose response studies in elderly have not been able to observe a saturation of MPS with doses up to 40 g of whey protein (Pennings *et al.*, 2012; Yang *et al.*, 2012*a*) or soy protein (Yang *et al.*, 2012*b*). The retrospective analysis by Moore and colleagues (Moore et al., 2014) reported an intake of about 0.4 g • kg body mass^{-1} of high quality protein as optimal for stimulation of MPS in elderly men.

It is important to note that the dose of protein needed to maximize MPS is conditional and has been shown to decrease with prior exercise (Pennings *et al.*, 2011*b*), increase with inactivity (Breen et al., 2013) and ageing (Katsanos *et al.*, 2006). Yang and colleagues (Yang *et al.*, 2012*a*) observed a greater anabolic effect of 40 g of whey protein after resistance exercise compared to rest, in elderly individuals. Indicating a greater ability to use large doses of protein for MPS after exercise.

1.2.3. Protein timing

Acute studies on effects of protein intake and resistance exercise in young untrained men show the MPS-response is similar when protein is ingested 1, 2 or 3 hours after exercise (Tipton *et al.*, 2001; Witard *et al.*, 2014*a*). The potentiating effect of resistance exercise on the MPS-response to protein ingestion has been shown to last at least 24 hours after exercise in young individuals (Rasmussen & Phillips, 2003; Burd *et al.*, 2011*b*). Thus, acute studies indicate the anabolic window after resistance exercise to be greater than advocated by some (Lemon *et al.*, 2002; Ivy & Portman, 2007). The results from training studies are harder to interpret. Two studies report an increased lean mass accretion over a training period with protein intakes in close temporal proximity of exercise, compared to protein intake at other time points in young (Cribb *et al.*, 2006) and elderly (Esmarck et al., 2001). Others find no difference (Hoffman et al., 2009) or even an inferior response with protein in close temporal proximity to exercise (Burk *et al.*, 2009) in young. It should be pointed out that some of these studies (Esmarck *et al.*, 2001; Burk *et al.*, 2009) report surprisingly small effects[27] of the training intervention and should therefore be interpreted with caution. A more recent meta-analysis found no evidence for an effect of protein timing on changes in muscle mass or strength (Schoenfeld *et al.*, 2013).

[27] A 1 kg decrease in LBM and neglible increases in muscle strength in the group ingesting protein 2 hours after exercise for 12 weeks, whereas the group ingesting protein immediately after exercise had an increase in LBM of 1 kg. Thus, in light of other training studies in elderly, immediate post exercise protein does not seem to hold any advantage compared to unsupervised nutrition.

1.2.4. Protein intake frequency

The human body does not possess a long-term storage[28] of protein, in the same way as carbohydrates and fat. This necessitates a more frequent intake of protein than other macronutrients in order to maintain a stable muscle net protein balance. With a given total amount of protein to consume during a day, the frequency of ingestion determines the amount of protein in each serving. The frequency and the amount of protein in each serving have been shown to affect the cumulative MPS response during a period of 12 to 24 hours (Areta et al., 2013; Mamerow et al., 2014). Areta and colleagues (Areta et al., 2013) observed a greater aggregated 12-hour MPS response to 4 x 20 g of whey protein compared to 8 x 10 g and 2 x 40 g. Similarly, Mamerow and colleagues (Mamerow et al., 2014) found an even distribution of 90 g of protein to stimulate MPS over a 24-hour period more than an unbalanced pattern (70% of protein at dinner). Supporting the studies reporting diminishing returns in terms of MPS, when consuming 40 g versus 20 g of high quality protein (Moore *et al.*, 2009*b*; Witard et al., 2014b). Despite promising results over 12-24 hours, no effect of protein intake pattern was found for whole body protein synthesis over 4 days in elderly (Kim *et al.*, 2014), for changes in lean body mass over 14 days in young (Arnal et al., 2000) and elderly women (Arnal et al., 1999), or lean body mass and net protein balance over 8 weeks in elderly (Kim *et al.*, 2017). It is, however, hard to make definite conclusions based on these studies, due to methodological challenges such as few participants (Arnal *et al.*, 1999; 2000; Kim *et al.*, 2014; 2017) and a short time frame to observe differences in lean body mass (Arnal et al., 1999; 2000).

1.3. Muscular anabolic resistance

Aging is associated with loss of muscle mass and strength, and if allowed to advance, this condition may proceed to sarcopenia[29], which plays a part in loss of independent living (Baumgartner et al., 1998) and comorbidities (Dominguez & Barbagallo, 2007; Atkins *et al.*, 2014). The loss of muscle mass has been estimated to be 1-2% per year after the age of 65 yr (Frontera

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[28] During starvation or protein deficiency muscle protein is broken down to meet the requirements of energy production and more vital cellular processes, such as respiration enzymes and production of blood cells.

[29] The Scociety of Sarcopenia, Cachexia and Wasting Disorders define sarcopenia as "a person with muscle loss whose walking speed is equal to or less than 1 m/s or who walks less than 400 m during a 6-minute walk, and who has a lean appendicular mass corrected for height squared of 2 standard deviations or more below the mean of healthy persons between 20 and 30 years of age of the same ethnic group" (Morley *et al.*, 2011). However, other definitions exist and a clear consensus has yet to be reached (Anker *et al.*, 2014).

et al., 2000), and results from a negative net muscle protein balance (Puthucheary et al., 2013). Thus, both changes in MPS and MPB could contribute to loss of muscle mass. Basal values for MPS do not seem to differ between young and elderly (Volpi *et al.*, 2001; Paddon-Jones *et al.*, 2004; Kumar *et al.*, 2009; Markofski *et al.*, 2015). Although understudied compared to MPS, the available evidence suggests that fasting values of MPB do not differ between young an elderly (Volpi *et al.*, 2001; Paddon-Jones *et al.*, 2004; Wilkes *et al.*, 2009). The lack of differences between young and old in fasting values turned the focus towards the acute MPS response to protein ingestion and exercise. Several studies have reported a reduced MPS response in elderly compared to young in response to resistance exercise (Kumar *et al.*, 2009; Mayhew *et al.*, 2009; Fry *et al.*, 2011; Kumar *et al.*, 2012), protein intake (Volpi *et al.*, 2000; Guillet *et al.*, 2004; Cuthbertson *et al.*, 2005; Babraj *et al.*, 2005; Katsanos *et al.*, 2006) and the combination of resistance exercise and protein (Drummond et al., 2008). The reduced ability to respond with increased MPS to protein or exercise stimuli has been termed anabolic resistance (Rennie, 2009; Phillips, 2012). The MPS and MPB response to amino acid intake can be blunted by changes in several steps. Elderly may show impairments of protein digestion or absorption (Boirie *et al.*, 1997*b*), increased splanchnic extraction (Boirie *et al.*, 1997*b*; Volpi *et al.*, 1999), decreased postprandial blood flow to muscles (Rasmussen *et al.*, 2006; Timmerman *et al.*, 2010*a*; Mitchell *et al.*, 2013), decreased capacity of amino acid uptake in muscle (Dickinson *et al.*, 2013), and less responsive anabolic signalling (Cuthbertson et al., 2005) leading to a blunted MPS.

However, not all studies report evidence for this anabolic resistance to protein (Volpi *et al.*, 1999; Paddon-Jones *et al.*, 2004; Koopman *et al.*, 2009*b*; Symons *et al.*, 2009; Pennings *et al.*, 2011*b*; Chevalier *et al.*, 2011; Kiskini *et al.*, 2013; Gorissen *et al.*, 2014) or the combination of resistance exercise and protein (Koopman *et al.*, 2006; Symonsi *et al.*, 2010; Pennings *et al.*, 2011*b*; Atherton *et al.*, 2016). The discrepancy between studies is likely a result of many factors including amount, type and method of administration[30] of amino acids, the training load[31], the initial protein intake and physical activity status of the participants, and methodological differences. The anabolic resistance in elderly can in combination with critical incidences leading to periods of inactivity and bedrest, lead to substantial losses of muscle mass over a short time period (Gruther et al., 2008), from which elderly seem to have a hard time recovering from (Hvid et al., 2010). Maintenance of young levels of muscle mass and strength into very old age is unlikely, still the role of the dynamic and likely multifactorial anabolic resistance in the loss of muscle mass and

[30] Amino acids may be administered through ingestion or infusion, as food items, isolated proteins, hydrolysed proteins or amino acids

[31] Resistance (kg) • repetitions • sets for all exercises involving the muscle group of interest.

function remains elusive.

1.4. Strength training and protein supplementation

If maintaining the activity level and receiving sufficient nutrition young healthy individuals will maintain a rather stable muscle mass. Increasing protein intake as well as energy intake may produce small gains in muscle mass along with greater increases in fat mass (Bray et al., 2012). The main regulator for changes in muscle mass in this group is the activity level and type of activity. During periods of reduced activity or immobilization muscle mass is lost (Glover et al., 2008; Krogh-Madsen et al., 2010). Although low-load activities such as running and cycling may induce increases in muscle mass in untrained or older individuals (Kubo *et al.*, 2008; Harber *et al.*, 2010), they are not very effective compared to heavy-load resistance exercise. Abundant evidence shows a robust effect of resistance training on muscle hypertrophy and strength (Rhea *et al.*, 2003; Peterson *et al.*, 2004; Wernbom *et al.*, 2007). In the acute post exercise setting there are clear benefits of protein ingestion compared to placebo (Biolo *et al.*, 1997; Tipton *et al.*, 2004; Reitelseder *et al.*, 2014), with detectable differences between types of protein (Wilkinson *et al.*, 2007; Tang *et al.*, 2009; Reitelseder *et al.*, 2011). Thus, the combination of heavy resistance exercise and protein supplementation over time should hold a great potential to increase muscle mass in young and elderly individuals. However, the long-term effects of protein supplementation in combination with resistance training are not entirely clear. Most studies do not find an effect of protein supplementation on muscle mass or strength in young (Lemon *et al.*, 1992; Antonio *et al.*, 2000; Rozenek *et al.*, 2002; Chromiak *et al.*, 2004; Rankin *et al.*, 2004; Beck *et al.*, 2007; White *et al.*, 2009; Thomas *et al.*, 2011; Weisgarber *et al.*, 2012; Babault *et al.*, 2014; 2015; Mitchell *et al.*, 2015*b*; Reidy *et al.*, 2016), or elderly participants (Verdijk *et al.*, 2009; Kukuljan *et al.*, 2009; Bemben *et al.*, 2010; Chale *et al.*, 2012; Leenders *et al.*, 2013; Arnarson *et al.*, 2013; Mitchell *et al.*, 2015*b*) while some studies do (Young: (Burke *et al.*, 2001; Andersen *et al.*, 2005; Candow & Burke, 2006; Cribb *et al.*, 2006; Hartman *et al.*, 2007; Hulmi *et al.*, 2009*a*; Josse *et al.*, 2010; Volek *et al.*, 2013), elderly: (Holm *et al.*, 2008; Tieland *et al.*, 2012; Dirks *et al.*, 2017)). Discrepancies between studies might result from different study designs, duration, outcome variables, populations, exercise programs, type, amount, frequency of protein and placebo given and the initial protein content of the diet (Morton et al., 2017). Based on the current literature four meta-analyses have concluded in favour of protein supplementation during resistance training in young and elderly (Cermak *et al.*, 2012; Miller *et al.*, 2014; Hidayat *et al.*, 2017; Morton *et al.*, 2017)

Table 1.1 *Summary of human studies comparing different protein supplements in combination with strength training in young individuals.*

Study	Age (yrs)	n	Fitness	Program duration (weeks)	Days /wk	Program	Intensity	Supplementation Protein	Placebo	Outcome	Effect Protein	Placebo	Group diff
Joy et al., 2013	21±2	24	Trained	8	3	WB 3 sets 2-12 reps	RM	Whey protein 48g on training days	Rice protein 48g on training days	Lean mass 1RM leg press 1RM Bench press	+3.2kg (5.4%) + 80kg (38%) +9kg (10%)	+2.5kg (4.3%) +67kg (31%) +10kg (12%)	No No No
Volek et al., 2013	23±3	41	Healthy	36	3	WBR	30-90% 1RM	Whey protein 22g/day	Soy protein 20g/day	Lean mass 1RM squat 1RM bench press	+3.3kg (6.6%) +36kg (43%) +20kg (39%)	+1.8kg (3.7%) +40kg (67%) +16kg (36%)	Yes No No
Wilborn et al., 2013	21±3	16	Trained	8	4	WB 2-split 1-3 sets 12-15 reps	Not decleared	Whey protein 24g before and after workout	Casein protein 24g before and after workout	Lean mass 1RM lean mass 1RM bench press	+1.5kg +89kg +7.5kg	+1.4kg +90kg +4kg	No No No
Denysschen et al., 2009	21-50	18	Over-wheight	12	3	WB 3-split 2-4 sets 8-12 reps	Not decleared	Whey protein 26.6g/day	Soy protein 25.6g/day	Fat free mass 1RM squat 1RM bench press	+1.2kg (1.7%) +39kg (52%) +15kg (21%)	+1.8kg (2.6%) +39kg (51%) +18kg (24%)	No No No
Hartman et al., 2007	18-30	37	Trained	12	5	WB 3-split 2-4 sets 4-12 reps	80% 1RM	500ml milk 17.5g protein/day	Soy protein 17.5g/day	Fat free mass Type I fiber CSA Type II fiber CSA 1RM leg press	+3.9kg (6.2%) ++ ++ +191kg(102%)	+2.8 (4.4%) + + +210kg (98%)	No Yes Yes No
Candow et al. 2006	24±6	27	Un-trained	6	5-6	WB 3-split 4-5 sets 6-12 reps	60-90% 1RM	Whey protein 3 x 0.4g x kg^{-1} x day^{-1}	Soy protein 3 x 0.4g x kg^{-1} x day^{-1}	Lean mass 1RM squat 1RM bench press	+2.5kg (4.7%) +27kg (39%) +8kg (+14%)	+1.7kg (3.1%) +24kg (34%) +8kg (13.4%)	No No No
Brown et al. 2004	19-25	27	Trained	9	?	WB 3 sets 4-6 reps	Not decleared	Whey protein 3 x 11g/day	Soy protein 3 x 11g/day	Lean mass	+2%	+1.8%	No

WB; whole body, 1RM; 1 repetition maximum, CSA; cross sectional area, +; significantly increased from baseline.

Table 1.2 *Summary of studies comparing protein supplementation to placebo in combination with strength training in elderly individuals.*

Study	Age (yrs)	n	Fitness	Program duration (weeks)	Days /wk	Program	Intensity	Supplementation Protein	Placebo	Outcome	Effect Protein	Placebo	Group diff
Dirks et al., 2017	77±1	34	Frail	24	2	WB 4 sets 15-8 reps	50-75% 1RM	Milk protein 2x15g per day	Lactose 2x7.1g Per day	Lean mass Lean leg mass 1RM leg press Chair rise	+1.4kg (2.9%) +0.8kg (5%) +51kg (40%) ÷19%	- - +49kg (39%) ÷24%	Yes Yes No No
Maltais et al. 2016	60-75	30	Sarco-penic men	16	3	WB 3 sets 6-8 reps	80% 1RM	12g protein (7g EAA, 3,5g leucine) After workouts	Rice milk, isocaloric	Lean mass 1RM bench press 1RM lat pull-down Chair rise	+2.2kg (4%) -11.7kg (25%) +7.5kg (13%) -	+1.6kg (2,9) -5.7kg (12%) 9.8kg (17%) -	
Mitchell et al., 2015	74.4 ± 5.4	16	Healthy	12	3	WB 3-4 sets	75-85% 1RM	Chocolate milk, 14g protein per day	Carbohydrate, 66g, isocaloric Per day	Type I fiber CSA Type II fiber CSA 1RM leg press 1RM chest press	+ - +10kg (52%) +8kg (52%)	+ - +17kg (36%) +2kg (5%)	No No No No
Arnarson et al., 2013	65-91	161	Healthy	12	3	WB 6-8 sets 6-8 reps	75-80% 1RM	20g whey + 20g carbohydrates After workouts	40g carbohydrates After workouts	Lean mass Appendicular lean mass	+0.7kg (1.5%) +0.6kg (2.5%)	+0.9kg (1.9%) +0.5kg (2.3%)	No No
Leenders et al., 2013	70±1	53	Healthy	24	3	WB 4 sets 8 reps	60-80% 1RM	Milk protein 2x15g per day	Lactose 2x7.1g Per day	Lean mass Lean leg mass Type I fiber CSA Type II fiber CSA	+1.4kg (2.7%) +3% - +44%	1.1kg (1.9%) +3% - +44%	No No No No
Chale et al., 2012	70-85	80	Mobility-limited	24	3	WB 2-3 sets 10-12 reps	80% 1RM	Whey 2x20g + carbohydrate 2x20g per day	Carbohydrate 2x45g Per day	Lean mass 1RM leg press Stair climb Chair rise	+0.6kg (1.3%) +21% ÷7.8% ÷22%	+0.3kg (0.7%) +17% ÷5.9% ÷20%	No No No No
Kim et al., 2012	>75	116	Sarco-penic	12	2	Lower body 8 reps	Moderate, progressive	EAA 2x3g per day	No placebo given	Leg muscle mass Knee extensions Walking speed	+3% +9% +13%	+2.5% +4% +17%	No No No
Tieland et al., 2012	>65	53	Frail	24	2	WB 4 sets 15-8 reps	50-75% 1RM	Milk protein 2x15g Per day	Lactose 2x7.1g Per day	Lean mass Lean app. mass 1RM leg press Chair rise	+1.3kg (2.8%) +0.9kg (4.5%) +45kg (36%) ÷14%	-0.3kg -0.2kg 48kg (41%) ÷24%	Yes Yes No No
Bemben et al., 2010	48-72	21	Healthy	14	3	WB 3 sets 8 reps	80% 1RM	Whey protein 35g + 480ml Gatorade After workouts	480ml Gatorade After workouts	1RM leg press 1RM bench press	+50% +35%	+30% +32%	No No
Kukuljan et al., 2009	50-79	91	Healthy	72	3	WB 1-3 sets 8-20 reps	50-85% -> power training	Milk protein 2x6.6g per day	No placebo given	Lean mass	+1.2kg	+1.0kg	No
Verdijk et al. 2009	70±2	26	Healthy	12	3	Legs 4 sets 8-10 reps	60-80% 1RM	Casein 2x10g per workout	Water	Lean mass Lean leg mass Type I fiber CSA Type II fiber CSA 1RM leg press	+0.7kg (1.3%) +1kg (6%) +13% +29% +24%	+0.6kg (1.3%) +1kg (6%) +5% +28% +27%	No No No No No

Holm et al., 2008	55±1	29	Frail	24	2-3	Legs + pull down 3-5 sets 20-8 reps	80% 1RM	Whey protein 10g + carbohydrate 31g per workout	Carbohydrate 6g Per workout	Lean mass Type I fiber CSA Type II fiber CSA Knee ex strength	+0.8kg - - +14%	 - - +8%	No No No Yes
Godard et al., 2002	>65	17	Healthy	12	3	Knee extensions 2x10 reps + 3 x max	80% 1RM	12g EAA + 72g carbohydrate per day	No placebo given	Thigh CSA Knee ex strength Knee ex MVC	+7% +46 +24%	+6% +53% +19%	No No No

WB; whole body, 1RM; 1 repetition maximum, CSA; cross sectional area, +; significantly increased from baseline, -; no significant change from baseline, ÷ significantly decreased from baseline.

Hypertrophy is assumed to occur as a result of repeated periods with increased MPS, in response to exercise and protein ingestion, accumulating over time (Booth *et al.*, 1998; Coffey & Hawley, 2007). Based on this, acute differences in ability to stimulate MPS observed between different protein types may result in different long-term outcomes. Indeed, this has been reported in some (Candow & Burke, 2006; Cribb et al., 2006; Hartman et al., 2007; Volek et al., 2013), but not all (Brown *et al.*, 2004; Denysschen *et al.*, 2009; Wilborn *et al.*, 2013; Joy *et al.*, 2013) studies comparing supplementation of different protein types during a period of resistance exercise in young (table 1.1). Few studies have compared different protein types in elderly individuals. Gryson and colleagues (Gryson et al., 2014) compared daily supplementation with 10 g of milk protein or native whey during a 16 week resistance training period, and observed no differences between supplements for changes in measures of strength and total or appendicular lean mass. The studies comparing protein and placebo in elderly individuals report conflicting results (table 1.2).

1.5. Native whey

Dairy products are important food constituents in the Norwegian diet, in the form of milk, cheese, yoghurt, butter and sour crème (Helsedirektoratet, 2011). The protein content of cow milk is usually around 3.3%. By reducing the pH to 4.6 at 30°C about 80% of the proteins in milk will clot and separate from the milk. This group of proteins are called caseins. The remaining 20% consists of several protein fractions but are collectively called whey proteins. Whey protein was traditionally seen as a by-product of cheese production, where casein is the main component. But with the increasing awareness of the nutritional value of whey in human nutrition, whey is today used in several products from formula to sports nutrition and protein supplements for elderly. Based on the concentration of whey protein the produced powder is often referred to as whey protein concentrate (often containing 80% protein, called WPC-80) and whey protein isolate (containing ≥90% protein). Whey protein products can be produced in several ways, and the mode of production will affect the amino acid content and other properties of the whey proteins (Schmidt *et al.*, 1984). Advances in production techniques make it possible to separate casein and whey proteins by filtration, thus avoiding heating and chemical treatment, and the product is often called native whey (Brans *et al.*, 2004). Referring to native whey as a whey protein is, however, somewhat misleading as it differs from other whey proteins by not containing

glycomacropeptides[32] (GMP). In our view the most attractive property of this native whey, compared to regular whey, is its high content of leucine. Manufacturers claim native whey produced by ultrafiltration has great benefits compared to WPC-80 (Lactalis, n.d.). Based on the high levels of leucine, the hypothesis of a greater anabolic potential with native whey compared to WPC-80 hold some theoretical merit, and is an interesting question to pursue. Furthermore, we wanted to a compare the supplementation of protein supplements with just increasing the intake of a readlily available food item containing high quality proteins, such as milk.

[32] Glycomacropeptide is a part of kappa-casein, high in proline, glutamine, serine, threonine, isoleucine, valine, low in leucine and depleted in tryptophan, tyrosine, phenylalanine and cysteine.

2. Research aims and hypotheses

The overall aim of this thesis was to investigate potential beneficial effects of native whey supplementation on muscular anabolic responses and adaptations in response to resistance exercise, compared primarily to WPC-80, but also milk protein.

As knowledge on muscular adaptations to protein supplementation and resistance exercise is highly relevant to several groups of society, both in terms of performance and health, studies (except study I) were made both in young and in elderly.

The specific aim of the studies were to:

1. Compare changes in blood concentrations of amino acids after ingesting 20 g of protein in the form of WPC-80, microparticulated whey, hydrolyzed whey, milk or native whey after resistance exercise in young men (study I).
2. Compare intracellular anabolic signaling and muscle protein synthesis responses when ingesting 20 g of protein form milk, WPC-80 or native whey imediatley and two hours after resistance exercise in young (study II) and elderly (study III) men and women.
3. Compare adaptations in muscle mass and strength when supplementing with two doses of 20 g of protein from milk or native whey daily during a 11-12 week resistance training period in young (study IV) and elderly (study V) men and women.

Our hypotheses were similar for young and elderly, and can be divided into 4 different levels:

Amino acid signal

1. Post-exercise blood concentrations of leucine will increase to a greater extent after post-exercise ingestion of 20 g of native whey compared to WPC-80 or milk.

Signal transduction

2. Post-exercise phosphorylation of p70S6K and 4E-BP1 will increase more after post-exercise ingestion of 20 g of native whey than with WPC-80 or milk.
3. Post-exercise phosphorylation of eEF-2 will decrease[33] more after post exercise ingestion of 20 g of native whey compared to WPC-80 or milk.

Muscle protein synthesis

4. MPS will be stimulated to a greater extent after ingestion of 20 g of native whey immediately and two hours after resistance exercise, compared to ingestion of WPC-80 or milk.

[33] Decreased phosphorylation of eEF-2 increases its activity (Ma & Blenis, 2009).

Adaptation

5. Muscle mass will increase more with native whey than milk, when supplementing as two doses of 20 g daily, during a 11-12 week training period.
6. Muscle strength will increase more with native whey than milk, when supplemented as two doses of 20 g daily, during a 11-12 week training period.

In addition we hypothesized that post-exercise ingestion of native whey would lead to a faster recovery of force-generating capacity than WPC-80 and milk.

3. Methods

This thesis presents data from five studies. Study I was carried out in the autumn 2013, study II and III were conducted simultaneously in the autumn 2014 with a second round of elderly participants in the spring 2016. Study V ran during the autumn 2015, with an additional round of elderly participants simultaneously with study IV in the spring 2016 (figure 3.1).

3.1. Subjects

A total of 77 young and 60 elderly participants gave their written, informed consent to participate in the studies (table 3.1). Five elderly and one young participant participated in both study III and V, and II and IV, respectively. All participants in study I and II were strength trained. Participants in study III were healthy active elderly, and half of them reported being engaged in a form of resistance exercise. In study IV and V only untrained participants, with regards to strength training, were included. The studies were approved by the Regional Ethics Committee for Medical and Health Research of South-East Norway and performed in accordance with the *Declaration of Helsinki* (appendix I). Study II, III, IV and V were registered at clinicaltrials.gov as NCT02968888 (I and II) and NCT03033953 (IV and V).

A total of 9 young and 9 elderly withdrew from the studies. In study I three participants withdrew, two due to reasons not related to the study and one due to knee pain during the training sessions. In study II, two participants withdrew, due to busy time schedules. In study III, one participant withdrew due to health issues. In study IV, four participants withdrew one due to headaches after the first training session and three withdrawals were due to reasons not related to the study. In study V, eight participants withdrew, one due to dizziness after the first workout, two due to arthritic pain during workouts, two due to health issues not related to the study and two due to busy time schedules.

Table 3.1 *Subject characteristics*

Study	Group	N (♂/♀)	Age (yrs)	Body mass (kg)	Body fat (%)	Lean mass (kg)
I		10/0	27 ± 7	80.8 ± 6.3		
II						
	Milk	8/4	25 ± 5	72.8 ±12.4	19.1 ± 7.2	57.1 ± 13.5
	Whey	5/5	5 ± 2	70.0 ± 11.6	21.5 ± 6.4	52.9 ± 9.6
III						
	Milk	7/3	75 ± 4	76.3 ± 17.8	27.7 ± 7.2	52.8 ± 11.4
	Whey	6/5	73 ± 3	70.0 ± 11.6	27.4 ± 7.1	50.5 ± 10.5
IV						
	Milk	10/8	29 ± 5	77.9 ± 16.0	29.1 ± 6.1	53.2 ± 10.7
	Native whey	10/8	30 ± 6	77.9 ±11.7	27.3 ± 7.9	54.2 ± 8.0
V						
	Milk	9/6	74 ± 4	74.6 ± 14.0	30.5 ± 5.5	49.8 ± 9.2
	Native whey	9/6	73 ± 2	78.3 ± 16.2	32.0 ± 9.1	49.5 ± 10.9

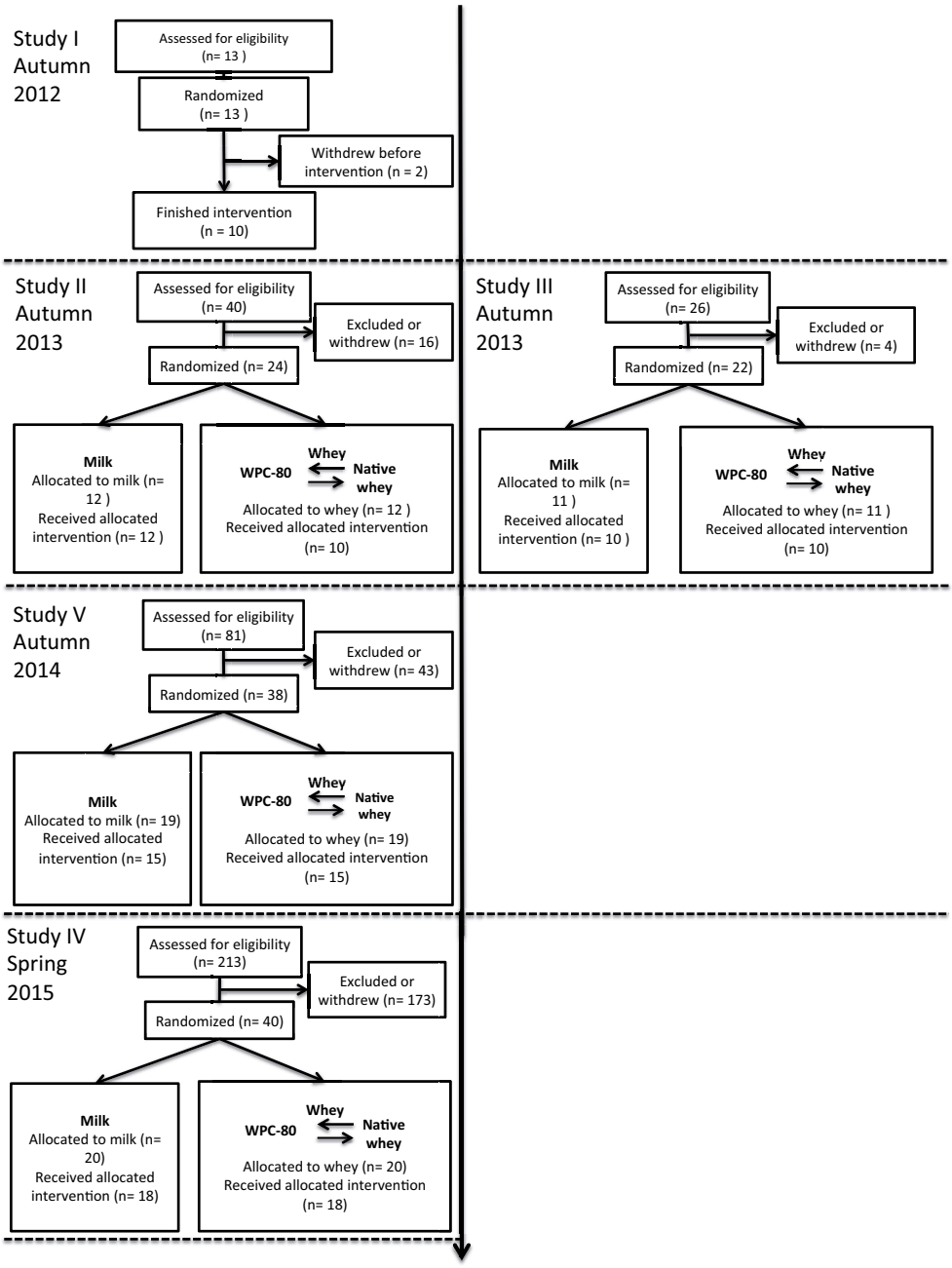

Figure 3.1 *Simple flow charts of the studies included in the thesis.*

3.2. Study design

Study I (figure 3.2) was carried out as a single blinded, randomized, cross-over design to investigate potential differences in plasma amino acid concentrations after four different whey supplements and milk protein when ingested after resistance exercise.

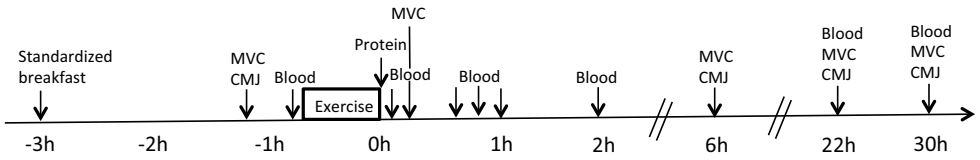

Figure 3.2 *Timeline of study I*

Study II and III (figure 3.3) were designed as double blinded, randomized partial cross-over studies to investigate potential differences in acute signaling and MPS responses to supplementation of milk protein, WPC-80 and native whey after resistance exercise. As the comparison between WPC-80 and native whey was our main comparison and we expected small differences between these supplements, we decided to make this a paired comparison. Therefore, the studies included two groups. The milk group, which did the experiment once, and the whey group, which did the experiment twice, one with WPC-80 and one with native whey, in a randomized counter-balanced manner.

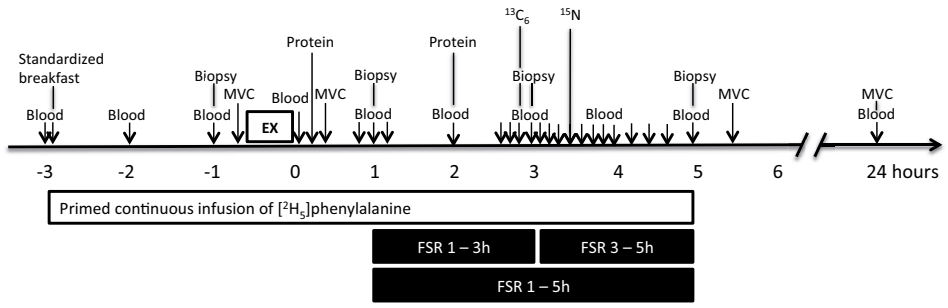

Figure 3.3 *Timeline study II and III*

Study IV and V (figure 3.4) were designed as double blinded, randomized, parallel groups studies to investigate the long-term effect of milk protein and native whey supplementation on adaptations of muscle mass and strength to 12 and 11 weeks of resistance training in young and elderly, respectively. In order to investigate the links between acute and long-term responses to resistance exercise and protein supplementation we also included an acute study at the start and at the end of the training period.

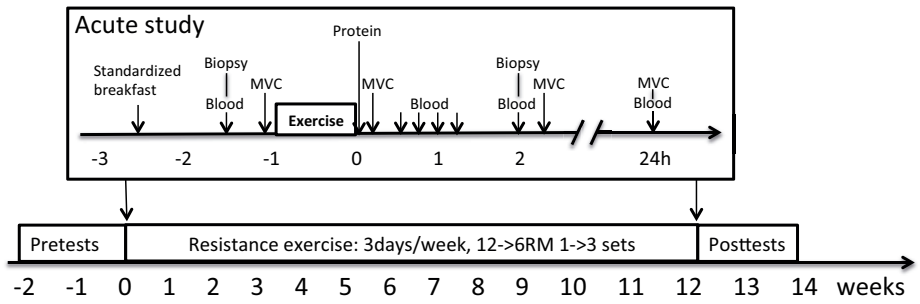

Figure 3.4 *Timeline of study IV and V*

3.3. Supplements

In study I-III, supplements were produced by Tine ASA (Oslo, Norway), whereas in study IV and V the native whey was produced by Lactalis® (Laval, Mayenne, France). In study I, microparticulated whey was produced by microfiltration of WPC-80 at high temperatures and high shear forces, with an end product of micro particles between 1 and 10 μm. Whey hydrolysate was produced using proteolytic enzymes and had a 10% degree of hydrolysis. In order to match all drinks on macronutrients, cream (TINE SA, Oslo, Norway), lactose (VARIOLAC® 992, Arla, Vidby J, Denmark), and water was added to WPC-80 and native whey (table 3.2). Native whey and WPC-80 contained whey protein only, whereas milk contained 20% whey and 80% casein. In study II and III, drinks were enriched with 6% [$^2H_5$] phenylalanine in order to maintain the plasma enrichment after intake.

In study I-III, participants were given bottles containing 636 ml of supplement at the time of ingestion. In study IV and V, supplements were provided to participants weekly as sachets with one serving of dried powder to be dissolved in 400ml of water before ingestion. On days with no training, supplements were consumed in the morning and in the evening. On training days. one serving was consumed immediately after training and one in the morning or evening, depending on whether the participant exercised in the morning or in the evening. Supplements were consumed every day from the first training session to the last test of the study. Compliance to the supplementation was self-reported and noted at each training day by the instructors. All drinks were matched for appearance and flavor.

Table 3.2 *Amino acid and macronutrient content (gram) per serving of milk, WPC-80 and native whey.*

	Study I, II and III			Study IV and V	
	Milk	WPC-80	Native whey	Milk	Native whey
Alanine	0.64	1.02	1.08	0.63	0.95
Arginine	0.70	0.51	0.57	0.61	0.60
Aspartic acid	1.59	2.23	2.54	1.49	2.24
Cysteine	0.19	0.45	0.57	0.15	0.50
Phenylalanine	0.95	0.70	0.83	0.92	0.85
Glutamic acid	4.26	3.56	3.82	4.10	3.88
Glycine	0.38	0.38	0.45	0.37	0.40
Histidine	0.57	0.38	0.45	0.53	0.49
Isoleucine	1.02	1.27	1.21	0.98	1.14
Leucine	1.97	2.16	2.73	1.87	2.46
Lysine	1.72	1.91	2.29	1.57	2.07
Methionine	0.51	0.45	0.51	0.47	0.51
Proline	2.04	1.34	1.14	1.86	1.30
Serine	1.14	1.14	1.02	1.06	1.04
Threonine	0.89	1.46	1.14	0.84	1.05
Tyrosine	0.76	0.45	0.57	0.82	0.72
Valine	1.27	1.21	1.14	1.23	1.20
Tryptophan	0.25	0.32	0.51	0.25	0.41
Protein	20.5	19.7	21.2	19.1	20.0
Fat	6.3	6.7	6.9	6.9	7.5
Carbohydrates	38.2	42.0	40.7	35.6	41.7

3.4. Diet

In study I participants were told not to change their habitual diet during the experimental period. After an overnight fast they received a standardized breakfast consisting of oatmeal and a glass of orange juice (1855 kJ: 9.2 g fat, 69.3 g carbohydrates and 16.6 g protein).

In study II and III participants completed two 24-hour dietary recall interviews prior to the experiment. A trained dietitian conducted the recall interviews and analyzed dietary nutrient content using the software Mat på Data 5.1 (Mattilsynet, Oslo, Norway, 2009). To standardize the diet participants were provided with a diet plan and pre-packaged food for the day before the experiment, and for the rest of the experimental period (2.5 days in total). The diet plan was individualized relative to body mass and provided young participants with 40 kcal/kg and 1.5 g protein/kg per day. The standardized breakfast contained 30 kJ, 0.14 g protein, 0.36 g fat and 0.84 g carbohydrates per kg body mass for young participants, and 25 kJ, 0,11 g protein, 0.30 g fat and 0.70 g carbohydrates per kg body mass for elderly participants.

In study IV and V each participant had two 24-hour dietary recall interviews prior to, midway

and at the end of the training intervention. The standardization of the diet in relation to the acute experiments in study IV and V were identical to study II and III. Oral and written (appendix I) information about the standardized diet was given to all participants. Participants were provided water ad libitum in all experiments.

3.5. Familiarization

In all studies participants did one familiarization session to all 1RM and functional tests (for elderly). In study II and II participants did two (study II) or 6 (study III) familiarization sessions to the workout in order to find the appropriate training load. Four elderly participants did leg press and knee extensions on a weekly basis and did only 2-4 familiarization sessions.

3.6. Exercise protocols

In study I the training protocol consisted of 4 sets of 10RM repetitions of leg press and knee extensions, and 3 sets of 10RM repetitions of bench press and seated rowing. Warm-up sets of 10 repetitions at 50 and 80% of the 10RM weights were carried out in each exercise. A new set started every two minutes, while 3 minutes of rest was given between exercises. On the first study day, participants were allowed to make adjustments to the training load, whereas on the following four study days the training load could not be changed and was identical to the first study day.

In study II and III the training protocol consisted of 4 sets of 8 repetitions to failure (8RM sets) of leg press and knee extension, with a new set starting every 3 min. Warm-up sets of 10 repetitions at 50% and 80% of the 10RM loads were carried out in leg press.

In study IV and V the training protocol consisted of three sets of 10 repetitions in hammer squat, leg press, knee extension, bench press seated row, one set of close grip pull down and two sets of shoulder press. The load was 10RM for all exercises and a new set was started every third minute. Specific warm up was performed with one submaximal set in hammer squat, bench press and seated rowing. The session was standardized, but training load was increased at the end of the training program in order to have the appropriate RM-load.

3.7. Training program

In study IV and V participants followed a traditional whole body strength-training program with three sessions per week (appendix II). Loads ranged from 12 to 6 RM and progressed from higher towards lower RM during the 12 weeks of training. Inter-set rest periods lasted 2-3

minutes. We reduced the number of sets somewhat for the elderly participants, especially for the upper body. This was to prevent potential shoulder issues. Qualified instructors supervised participants during all training sessions. If a participant missed an exercise session another session would be added to their program in order for all participants to reach the target amount of sessions (36 in young and 33 in elderly).

3.8. 1 RM and functional tests

In study IV and V maximal strength in leg press and bench press (chest press for elderly) were assessed by 1 repetition maximum (1RM) before and after the training intervention. After a 10 min treadmill warm up, a specific warm-up was performed with 10, 6, 3 and 1 repetitions at 50, 70, 80 and 90% of expected 1RM, respectively. 2-3 min of rest was given between attempts. Two to five attempts were used to find 1RM. Range of motion was strictly controlled. Knee flexion during leg press was set to 90° and grip width in bench press was standardized. The load could be adjusted with increments as low as 5 kg in leg press and 1 kg in bench press. The coefficient of variation (CV) for these measurements were <5%.

In all studies unilateral maximal knee extension strength was assessed by isometric maximal voluntary contraction (MVC) in a custom-made knee-extension apparatus (Gym2000, Geithus, Norway). Participants were seated in a chair with a four-point belt fixing the chest and hips, with 90° in the hip and knee joints. Three attempts of 5 s with 1 min rest between were given to reach MVC. Force was measured with a force transducer (HMB U2AC2, Darmstadt, Germany). MVC was tested after 5 min warm up on a cycle ergometer, except for when performed immediately after the workout. The CV for these measurements were <5%.

In order to investigate whether changes in strength also affected more functional measures of performance in healthy elderly we included stair climb and chair rise tests in study V. The stair climb was performed in a 2-level staircase (20 steps of length: 30 cm and height: 18 cm). Participants were instructed to walk the stairs as fast as possible without transitioning into running. No arm-swing was allowed. The stair climb was performed with three different loading levels: body weight, wearing a 10kg weight west, and wearing a 10kg weight west while carrying two 5kg weight discs. Participants had two attempts with each load. Trials were timed using photocells (Speedtrap 2, Brower Timing Systems, Utah, USA). The CV of these assessments was 4%.

For the timed chair-rise participants were instructed to stand with their arms crossed in front of a chair (height: 47cm), then to sit and stand five times as fast as possible. When sitting both feet

had to be lifted of the ground and when standing hips needed to be fully extended for the trial to be registered. Trials were timed using a pressure-plate (Speedtrap 2, Brower Timing Systems, Utah, USA) placed on the chair.

3.9. Measures of muscle mass

Body composition was assessed by dual energy X-ray absorptiometry (Lunar iDXA GE Healtcare, Madison, Wisconsin, USA. Using the enCORE Software

Version 14.10.022) before and after the intervention. Participants were scanned from head to toe in a supine position, providing values for lean tissue, fat mass and bone mineral content. The CV for the assessment was <1% for lean mass.

Transverse section images were captured of the thighs (15 images), arms and chest (10 images; GE Signa 1.5 Tesla Echospeed, GE Medical Systems, Madison, WI, USA) before and after the training intervention. Joint-gaps on the right side were used as reference points and all measures were done on the right thigh, arm and chest. The distance between images was individualized based on the length of femur and humerus. The images [Digital Imaging and Communications in Medicine (DICOM)] were analyzed using OsiriX 3.9.3 (Pixmeo, Bernex, Switzerland), giving the cross-sectional area (CSA) of individual muscles. The CV of these assessments were <2%.

3.10. Blood samples

Blood serum samples clotted in room temperature for 30 min before being centrifuged at 1300 g for 10 min at 4°C. Blood plasma samples were collected in lithium heparin tubes and immediately centrifuged at 1300g at 4°C for 10 min. After collection of serum and plasma, samples were stored at -80°C until further analysis. In study I serum glucose and urea were analyzed at Fürst Medical Laboratory (Oslo, Norway). In study II and III plasma glucose and insulin were measured by an enzyme-linked immune sorbent assay (Alpco, Salem, NH, USA), and a Cobas clinical analyzer (Cobas 6000, Roche Diagnostics, Indianapolis, IN, USA), respectively. Serum urea and CK was measured at Fürst Medical Laboratory (Oslo, Norway). In study IV and V serum glucose, insulin, urea and CK were measured at Fürst Medical Laboratory (Oslo, Norway).

3.10.1. Blood amino acid concentration

Amino acid concentration was measured in plasma using the Phenomenex EZ:faast free (physiological) GC-MS analysis kit (Phenomenex®, Torrance, CA, USA). Samples were

concentrated by solid phase extraction, derivatized, and separated by liquid-liquid extraction as directed by the EZ:faast kit. Derivatized samples were then analyzed by gas chromatography-mass spectrometry using a Shimadzu QP-2010 Ultra GCMS (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with the EZ:faast amino acid analysis column (10 m x 0.25 mm x 0.25 μm). The injection was 2 μl at 300°C at a constant helium carrier gas flow of 1.1 ml/min with a split ratio of 1:15. The initial oven temperature was 110°C and was raised to 320°C at 30°C/min. The MS interface was 320°C and the EI source was 240°C. Mass spectra were analyzed from 45-450 m/z at 4 scans/s. Total run time was 7 minutes. Duplicate sample injections were performed to reduce injection variability.

3.11. Biopsies

Muscle biopsies were collected from the mid portion of m. vastus lateralis. The procedure was conducted under local anaesthesia (Xylocaine with adrenaline, 10 mg·ml^{-1} + 5 μg·mg ml^{-1}, AstraZenica London, UK). 200 mg of muscle tissue was obtained by a modified Bergström technique with suction. Blood, fat and connective tissue was quickly removed and the biopsy samples rinsed in physiological saline, before divided in pieces for further analysis. For western blot two specimens of about 60 mg was quickly frozen, one in liquid nitrogen (for analyses on soluble proteins) and one in isopentane cooled on dry ice (for analyses of cellular fractions). Specimens for immunohistochemistry were mounted in Tissue-Tek (Sakura Finetek, Torrance, CA, USA) and rapidly frozen in isopentane cooled on dry ice. All muscle samples were stored at -80°C for later treatment and analysis. For analyses on soluble proteins muscle samples (60 mg) were homogenizes in 1 ml T-PER (Tissue Protein Extraction Reagent, Thermo Scientific, Rockford, IL, USA), 20 μl Halt Protease and Inhibitor Cocktail (Thermo Scientific) and 20 μl EDTA (Thermo Scientific). For analyses on the cytosolic, nuclear and cytoskeletal fraction muscle samples (60 mg) were homogenized using a subcellular extraction kit according to the supplier´s instructions (ProteoExtract Subcellular Proteome Extraction Kit, Calbiochem®, Darmstadt, Germany). Cytosolic, membrane, nuclear and cytoskeletal fractions were obtained by stepwise extraction using different buffers and centrifugation. Protein concentration was measured using a commercial kit (BioRad DC protein micro plate assay, BioRad, Hercules, CA, USA), a filter photometer (Expert 96, ASYS Hitech, Cambridge, UK) and the provided software (Kim, ver. 5.45.0.1, Daniel Kittrich@ok.cz). Protein measurements were run in triplicates (analytical CV was 5%).

3.11.1. Western blots

Depending on the antibody to be used, twenty to sixty micrograms of protein was separated by 4-12% SDS-PAGE gels under denatured conditions and transferred to polyvinylidene difluoride (PVDF) membranes (Immuno-blot, Bio-Rad). Membranes were blocked for 2 h at room temperature in 5% fat-free skimmed milk and 0.05% TBS-t solution [Tris-buffered saline (TBS; Bio-Rad); Tween 20 (VWR International, Radnor, PA, USA); skimmed milk powder (Merck, Darmstadt, Germany) and incubated over- night at 4°C with primary antibodies (table 3.3), followed by incubation with appropriate secondary anti-bodies for 1 h at room temperature. Membranes were washed in 0.05% TBS-t solution between stages. Primary and secondary antibodies (table 3.3) were diluted in a 1% fat-free skimmed milk and 0.05% TBS-t solution. Protein bands were visualized by luminol-based enhanced chemiluminescence (Super Signal West Dura Extended Duration Substrate, Thermo Scientific) and quantified using a Bio-Rad ChemiDoc^{TM} MP System (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were stripped using Restore Western Blot Stripping Buffer (Thermo Scientific). All samples were run as duplicates, samples from each participant was run on the same gel, and intensity comparisons were only done within each blot.

Western blot samples from study II and III were run with NuPAGE Novex bis-tris 4-12% gels on an XCell SureLocke® MiniCell and a XCell II^{TM} Blot Modul (Invitrogen Carlsblad, CA, USA), whereas samples from study IV and V were run on a Bio-Rad system (Mini PROTEAN® Tetra Cell, Bio-Rad Laboratories, Hercules, CA, USA) using stain free gels (Mini PROTEAN® TGX Stain-Free^{TM} Gels, Bio-Rad laboratories).

Table 3.3 *Antibodies used for Western blot*

Antibody	Dilution	Amount of protein loaded on gel (µg)	Cat. no	Manufacturer
P70S6K	1:1000	30	2708	Cell signaling
phospho-P70S6K Thr^{389}	1:1000	30	9234	Cell signaling
eEF-2	1:5000	30	2332	Cell signaling
phospho-eEF-2^{Thr56}	1:5000	30	2331	Cell signaling
4E-BP1	1:1000	60	9452	Cell signaling
phospho-4EBP-1$^{Thr37/46}$	1:1000	60	9455	Cell signaling
Secondary anti-rabbit	1:3000		7074	Cell signaling

3.11.2. Immunohistochemistry

Eight-micrometre-thick cross sections were blocked for 30 min with 1% BSA (bovine serum albumin; A4503, Sigma Life Science, St Louis, MO, USA) in a 0.05% PBS-t solution (Calbiochem®) before incubation for 2 h at room temperature with antibodies against myosin heavy chain II (SC71, hybriodomabank DSHB, IA, USA) and dystrofin (Abcam, Cambridge, UK), disolved in the blocking solution. This was followed by incubation with appropriate

secondary antibodies (A11005 or A11001; Life technologies, Invitrogen) for 30 min at room temperature, before covered with a coverslip and mounted with ProLong Gold Antifade Reagent with DAPI (Invitrogen Molecular Probes, Eugene, OR, USA). Muscle sections were then and left to dry overnight at room temperature. Between stages, the sections were washed 3x 5 min in a 0.05% PBS-t solution. For each section 50 type I fibers and 50 type II fibers were analyzed.

3.12. Mesures of FSR

3.12.1. Infusion protocol

Participants arrived in the lab at 06:45 after an overnight fast. A cannula was inserted into a forearm vein in both arms. A baseline blood sample was drawn before participants received a standardized breakfast to be consumed within 20 minutes. Thirty minutes after the baseline blood sample a primed continuous infusion of [$^{2}H_{5}$]phenylalanine (0.05 μmol·kg^{-1}·min^{-1}; 2 μmol·kg^{-1} prime; Cambridge Isotopes Laboratories, Andover, MA, USA) was started. Biopsies and blood samples were collected according to figure 3.3.

3.12.2. Blood, muscle protein-bound and intracellular free phenylalanine enrichment

Plasma from blood samples for the measurement of phenylalanine enrichment was analyzed as previously described (Wolfe & Chinkes, 2005). Briefly, plasma was deproteinized with 500 μl 15% sulfosalicylic acid, and amino acids were purified using cation exchange chromatography (AG 50W-8X, 100–200 mesh H+ form; Bio-Rad Laboratories, Richmond, CA, USA). Purified amino acids were dried under vacuum (Vacuum Dry Evaporator System, Labconco, Kansas City, MO, USA) and thereafter derivatized with 80 μl (1:1, v/v) acetonitrile: *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA, Sigma-Aldrich) for 45 min at 100°C. Isotopic enrichments of the plasma samples were determined on the tert-butyl dimethylsilyl (TBDMS) derivatives using gas chromatography/mass spectrometry (Shimadzu QP-2010 Ultra GCMS, Shimadzu Scientific Instruments, Columbia, MD, USA) and selected ion monitoring (Wolfe & Chinkes, 2005). Enrichments were expressed as tracer-to-tracee ratios. Appropriate corrections were made for overlapping spectra (Wolfe & Chinkes, 2005).

Plasma from blood samples for the measurement of phenylalanine enrichment was analyzed as previously described (Burd *et al.*, 2011*a*). Briefly, plasma was deproteinized by adding 20% perchloric acid and centrifuged at 1000xg at 4C for 10 minutes. Supernatant was removed, and

the mixed plasma protein pellet was washed three times with 2% PCA, two times with ethanol, and one time with diethyl ether, dried overnight at 50°C and hydrolyzed overnight in 6 N HCl at 110°C. The hydrolyzed mixed plasma protein samples were then processed using the same method as muscle protein bound samples.

Twenty-five to thirty mg of muscle was placed in 800 µl 10% perchloric acid (PCA), homogenized and centrifuged. The supernatant was collected for measurement of intracellular amino acid enrichment. The remaining pellet (bound protein) was washed three times with 2% PCA, two times with ethanol, and one time with diethyl ether, dried overnight at 50°C and hydrolyzed overnight in 6 N HCl at 110°C. Amino acids from the bound and intracellular fractions were then purified by cation exchange chromatography and thereafter derivatized in the same way as for the blood samples. Isotopic enrichment of TBDMS-phenylalanine in the bound protein was determined by gas chromatography-combustion-isotope ratio/mass spectrometry (GC-C-IRMS) set to high temperature conversion (HTC) mode for analysis of deuterium (Delta V Advantage Isotope Ratio Mass Spectrometer with GC Isolink, Thermo Scientific, West Palm Beach, FL, USA). Enrichment is calculated from the relative ratios of hydrogen (mass 3/mass 2) corrected for natural abundance of deuterium, divided by fraction of atoms that could be labeled (5/39).

3.12.3. Calculations

Baseline muscle fractional synthesis rate[34] (FSR) was calculated using the precursor product method (Wolfe & Chinkes, 2005):

$$FSR\ (\%h^{-1}) = E_{p2} - E_{p1} / (E_{pre} \times t) \times 100$$

The product is the difference in enrichment of the bound protein pool (E_{p2}) and the mixed plasma proteins (E_{p1}). The precursor (E_{pre}) is the average plasma free or muscle free D_5 phenylalanine enrichments to estimate the upper (muscle free) and lower (plasma free) limits of the true muscle protein FSR. The tracer incorporation time is denoted by *t*.

Skeletal muscle fractional synthesis rate (FSR) was calculated (as a measure of MPS) according to the precursor product method where the precursor is the mean enrichment of the intracellular pool (E_{IC}) of biopsies being analyzed (Wolfe & Chinkes, 2005). The product is the difference in enrichment of the bound protein (E_{BP}) pools of the two muscle biopsies being analyzed. Skeletal

[34] It represents the fraction of a total pool (in our case muscle bound protein) that is synthesised in a unit of time (Wolfe & Chinkes, 2005).

muscle FSR is expressed as percent per hour: FSR (%/hour) = $((E_{BP t2}-E_{BP t1})/(E_{IC} \cdot (t_2-t_1))) \cdot 100$

Baseline MPS was only calculated during the first experiment for participants in the whey group, and this value was used as a baseline for both supplements in this group.

3.13. Statistics

Non-normally distributed data (D'Agostino and Pearson omnibus normality test) were log-transformed prior to statistical analysis. All data are illustrated in original form and expressed as means ± SD. Statistical analyses were made using Prism Software (Graphpad 6, San Diego, CA, USA). Statistical significance level was set at $p \leq 0.05$.

3.13.1. Study I

A two-way ANOVA with repeated measures (time x group) was used to compare the five experimental trials (milk, MWP, WPH, WPC-80 and native whey). Tukey´s multiple comparisons test was used as a *post hoc* test to specify the significant differences between groups and time points. A one-way ANOVA was used to compare the area under the curve of individual and total BCAA, EAA and total amino acids between protein supplements.

3.13.2. Study II and III

As the main goal of the study was the comparison of WPC-80 with native whey, these data were analyzed by one-way repeated measures ANOVA. A Sidak and Dunnett´s test was used as *post hoc* tests to specify the significant differences between trials and time points (within groups), respectively. Comparisons against milk were done with a two-way ANOVA with repeated measures (time x group), with group as non-repeated and time as repeated. Tukey´s multiple comparisons test was used as a *post hoc* test to specify the significant differences between groups. Comparisons between time points within groups were only made against the pre-value, thus a Dunnett´s *post hoc* test was used. Subject characteristics and AUC responses between groups were analyzed with a one-way non-repeated measure ANOVA. A sample size calculation was conducted using a power of 80% based on FSR results from an earlier study comparing whey and casein in young men ((Tang et al., 2009); StatMate, Graphpad Software, San Diego, CA, USA). Based on the power calculation the goal was to include 10 subjects in each group. Statistical analyses were made using Prism Software (Graphpad 6, San Diego, CA, USA).

3.13.3. Study IV and V

A two-way ANOVA with repeated measures (time x group) was applied to test group differences before and after the 12-week training period and relative changes from before to after, and between the acute experiments. Sidak and Tukey´s test was used as post hoc tests to specify significant differences between selected groups or time points and all comparisons, respectively. Dunnet´s test was used as a *post hoc* test for comparisons within groups for blood amino acid concentrations, glucose, insulin, urea and creatine kinase (CK) as comparisons were only made against pre-values. Comparisons of relative changes (%) between groups from before to after the training period were tested with an unpaired Student´s *t* test. Relative changes within each group were assessed with a paired Student´s *t* test. A sample size calculation was conducted with a power of 80% based on lean muscle mass results from an earlier study comparing whey and soy protein supplementation in young men ((Volek et al., 2013); StatMate, Graphpad Software, San Diego, CA, USA). Based on the power calculation, our goal was to include 20 subjects in each group to obtain a 5 % significance for a 1.5 percentage points difference between groups.

4. Results and discussion

In this chapter the main findings from the five studies are discussed, according to the overall aim of the thesis. Detailed results for each specific study are found in the papers and manuscripts, at the end of this thesis. Although very interesting, a comparison between the current studies with regard to the differences between young and elderly will not be discussed in this thesis, but in articles to come.

4.1. Leucine concentrations in blood

In study I our goal was to test the hypothesis that native whey was able to increase blood leucine concentrations to greater levels, not only compared to milk, but also other forms of whey protein products. The hypothesis was confirmed. Through the other studies (II-V) of this thesis, we consistently repeated these findings in both young and elderly participants. Our data (figure 4.1) agrees with findings from previous studies showing markedly different time dependent changes in blood concentrations of essential amino acids and leucine after intake of casein, milk and whey (Tipton *et al.*, 2004; Tang *et al.*, 2009; Reitelseder *et al.*, 2011; Pennings *et al.*, 2011*a*; Dideriksen *et al.*, 2011; Burd *et al.*, 2012; Mitchell *et al.*, 2015*a*).

While some find faster (Grimble et al., 1994) or slower (Farnfield *et al.*, 2009*b*) absorption rates with hydrolyzed whey compared to regular whey, most studies find no effect of hydrolyzing whey protein on absorption rates (Calbet & Holst, 2004; Farup *et al.*, 2016; Mitchell *et al.*, 2017). Based on this we believe the larger increases in blood leucine concentrations after intake of native whey compared to WPC-80 were mainly due to differences in leucine content. Whereas the differences between the whey supplements and milk were likely due to a combination of absorption rate and leucine content (Boirie *et al.*, 1997*a*).

Interestingly, young participants reached higher leucine concentrations in blood at 45, 60 and 75 min in study II, compared to study IV ($P < 0.017$). Moreover, although there was a clear difference between native whey and milk in all studies, these differences were 50-100% greater in study II compared to study IV. These differences were not observed in elderly, but in contrast to study V blood leucine concentrations were maintained at 120 min in study III ($P < 0.007$). Importantly, the native whey protein supplements used in study II and III and in study IV and V were produced by different manufacturers[35]. The native whey in study II and III was "fresh"

[35] Tine SA, Oslo, Norway in study I, II and III and Lactalis®, Laval, Mayenne, France in study IV and V.

from filtration, whereas the native whey in study IV and V was spray dried[36] to powder. As whey proteins are less heat resistant than caseins (Fox *et al.*, 2015), the bioavailability of the amino acids in native whey may be more affected than the milk proteins by this treatment.

Further, the differences in leucine content per serving between milk and native whey were greater in study II and III (0.76 g) than in study IV and V (0.59 g). Why this difference was not detected in leucine concentration in blood of the elderly participants is difficult to answer, but may result from impairments in absorption and transport of amino acids in elderly (Boirie *et al.*, 1997*b*; 1997*a*; Volpi *et al.*, 1999; Rasmussen *et al.*, 2006; Timmerman *et al.*, 2010*a*; 2010*b*; Dickinson *et al.*, 2013; Mitchell *et al.*, 2013). Moreover, the use of a leg based workout in study II and III versus a whole body workout in study IV and V may also have contributed to the differences in blood leucine concentrations, as a larger muscle mass may take up the absorbed amino acids at a faster rate from the blood.

It is important to note that by only measuring the concentrations of amino acids in blood at given time points we cannot conclude on the amino acid absorption rate or uptake from blood into muscle, and whether the fluxes of amino acids differed remains unknown. It is possible that in addition to a faster digestion rate and higher leucine content in the whey proteins, a saturation of leucine transport into muscle or an increased MPB contributes to the higher leucine concentrations in blood with the whey supplements compared to milk. We were unfortunately not able to measure MPB or intramuscular levels of amino acids. Nevertheless, based on previous studies at rest (Bohé *et al.*, 2003; Glynn *et al.*, 2010*b*) and after exercise (Borsheim et al., 2002) we can assume intramuscular levels of EAAs to remain more stable in response to protein intake, and potential intracellular differences between supplements to be less than in blood. Increased amino acid transporter mRNA expression has previously been reported acutely, in response to resistance exercise and protein intake in young (Reidy et al., 2014) and elderly (Dickinson et al., 2014). However, we observed no alterations in the acute changes in blood concentrations of amino acids in response to the training and supplementation period, in young or elderly. Suggesting that no, or minor adaptations in absorption and uptake of amino acids to muscle occurred.

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[36] Method for producing powder from liquid. In short the liquid is sprayed into a chamber with hot air, thus removing the moisture component of the liquid solution (Fox *et al.*, 2015).

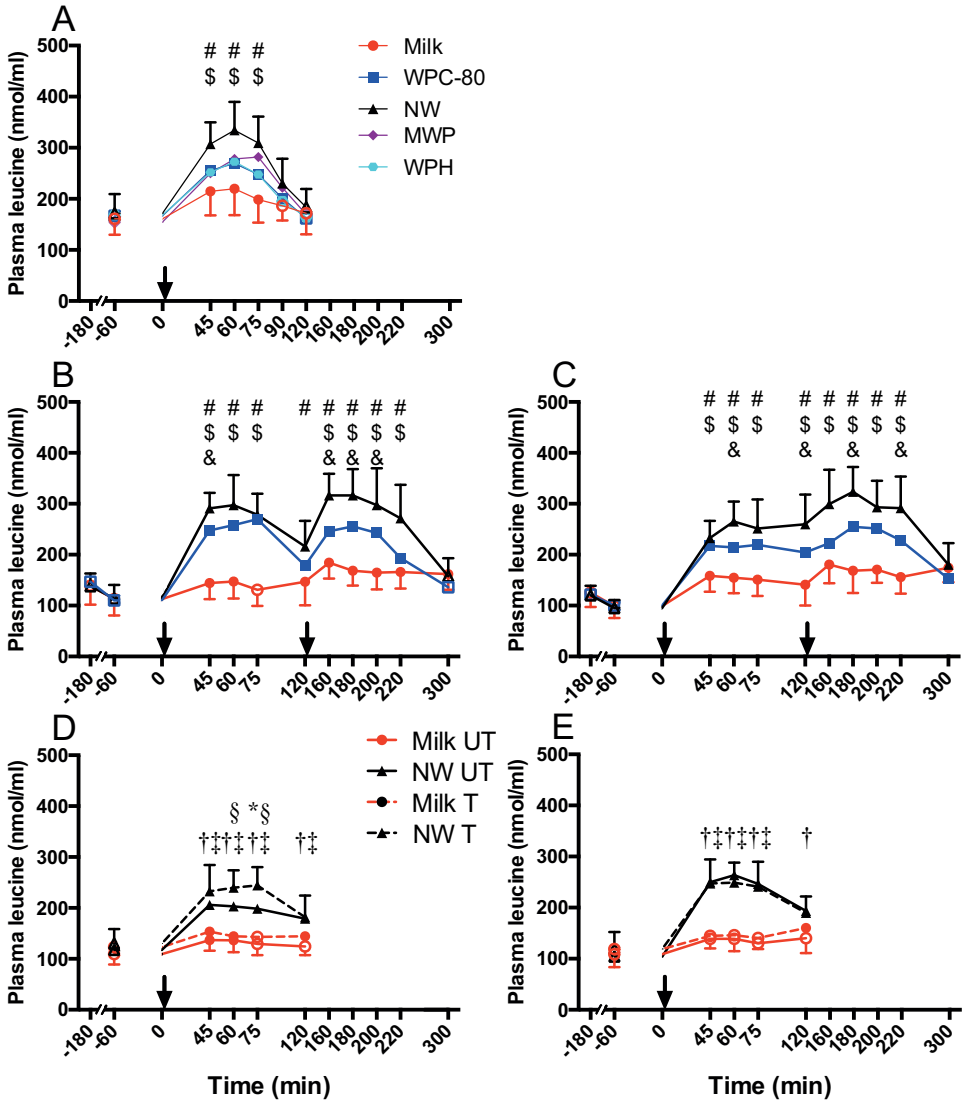

Figure 4.1 *A: blood concentrations of leucine after a post exercise intake of 20 g of milk, microparticulated whey, hydrolyzed whey, WPC-80 or native whey in young resistance trained men. N = 10 for all supplements. B and C: blood concentrations of leucine after a post exercise intake of 2x20 g of milk, WPC-80 or native whey in young and elderly participants, respectively. For young N = 12, 10 and 10 for milk, WPC-80 and native whey, respectively. For elderly N = 10 in all groups. D and E, blood concentrations of leucine after a post exercise intake of 20 g of milk or native whey, before (untrained (UT), whole lines) and after (trained (T), dashed lines) a training period in young and elderly participants, respectively. For young N = 12 and 10 for milk and native whey, respectively. For elderly N = 9 and 11 for milk and native whey, respectively. Only highest and lowest error bars are shown for readability. Arrow indicates the timepoint of supplement ingestion. Filled symbols indicate significantly different from baseline. # native whey different from milk, & WPC-80 different from milk, $ native whey different form WPC-80. * milk different from before to after the training period, § native whey different from before to after the training period, † milk and native whey different before the training period and ‡ milk and native whey different after the training period.*

Based on the apparent superior increase in blood leucine concentrations with native whey compared to the other protein supplements in study I, we wanted to test whether these differences in blood leucine concentrations translated into superior anabolic responses in muscle in study II and III. Specifically, we investigated the effects intake of the different milk proteins after resistance exercise had on acute signaling stimulating the translational process and mixed muscle FSR in young and elderly participants.

4.2. Signaling

The first goal of study II and III was to investigate the hypothesis that native whey would have a greater effect on acute signaling stimulating the translational process in muscle than WPC-80, when ingested acutely and 120 min after resistance exercise. However, no differences were observed in the investigated signaling steps between WPC-80 and native whey. Thus, our hypothesis could not be confirmed. Native whey did, however, increase p70S6K phosphorylation to a greater extent than milk at 180 min postexercise.

4.2.1. p70S6K

We observed a robust increase in the phosphorylation of p70S6K with all protein supplements after resistance exercise, which was greater for native whey than milk, in both young (study II; figure 4.2 A) and elderly (study III; figure 4.2 B). Although, the robust p70S6K-response was reproduced in study IV and V, no differences were observed between native whey and milk (figure 4.2 C and D). There may be several reasons for this. (1) Biopsies were not collected at the same time point relative to exercise and protein supplementation. (2) The differences in p70S6K phosphorylation appeared only after the second serving of supplement in study II and III, and there was no second serving of supplements in study IV and V. (3) The blood leucine concentrations with native whey were higher in study II and III, compared to study IV and V. Unfortunately, we did not measure MPS in study IV and V, and whether the p70S6K response was reflected in MPS therefore remains unknown. Caution is warranted when interpreting the results of p70S6K, as although the phosphorylation of p70S6K increases with anabolic stimuli, it is not considered a good predictor of the MPS response in humans (Greenhaff *et al.*, 2008; Atherton *et al.*, 2010) or animal studies (Crozier *et al.*, 2005).

Some previous studies have reported significant impairments in the p70S6K response to protein and resistance exercise in elderly (Guillet *et al.*, 2004; Cuthbertson *et al.*, 2005; Drummond *et al.*, 2008; Fry *et al.*, 2011; Francaux *et al.*, 2016). However, we observed a robust p70S6K response in

our elderly participants, as have also been reported by others (Kumar *et al.*, 2012; Atherton *et al.*, 2016). In young (study IV) the resting levels of phosphorylation of p70S6K decreased about 10% (only significant with milk). In our elderly participants (study V) resting levels of both total and phosphorylated p70S6K, in elderly, decreased by ≈25% and ≈45%, respectively. The decrease of total and phosphorylated p70S6K in elderly is interesting in light of a recent study by Markofski and colleagues (Markofski et al., 2015), were elderly participants displayed a higher basal phosphorylation of mTORC1 and p70S6K, and higher phosphorylated/total p70S6K ratio, compared to young. In spite of the increased p70S6K phosphorylation at rest, MPS was similar between young and elderly (Markofski et al., 2015). Whether, this mTORC1 and p70S6K hyperphosphorylation is a compensatory mechanism to maintain MPS in ageing muscle, or maybe contributing to anabolic resistance, as proposed by Markofski and colleagues (Markofski et al., 2015), remains to be elucidated. Without knowing the causes or effects, our results suggest that a hyperphosphorylation of p70S6K may be normalized with training in elderly individuals. Furthermore, these data underlines the importance of reporting baseline levels, and not only change from baseline, when comparing changes in different groups such as young and old, or untrained and trained. Comparing the phosphorylated/total ratios of p70S6K in young and elderly in our studies does not reveal any clear difference, but this comparison is not ideal and should be interpreted with caution[37].

As the acute signaling responses were similar between young and elderly participants we combined them in an effort to see whether increasing the number of participants would reveal more significant differences between whey products. Indeed, when combined the results showed a 25% greater phosphorylation of p70S6K with native whey than WPC-80 at 180 min ($P = 0.038$), and a continued elevation from baseline for p70S6K at 300min with native whey ($P = 0.019$). Both whey supplements reached higher values than milk at both 60 ($P < 0.005$) and 180 min ($P < 0.001$). Further, in study IV and V, combining young and elderly did not result in any group differences. Interestingly, the combined data showed a decreased p70S6K response in the trained state, compared to the untrained state. A similar dampend response to resistance exercise and protein intake after a training period has previously been reported in elderly, but not in young (Farnfield *et al.*, 2012).

[37] Samples from young and elderly were run one year appart, by two different students, on two different Western blot systems, with antibodies from different batches and the exposure time was not standardized between the two studies.

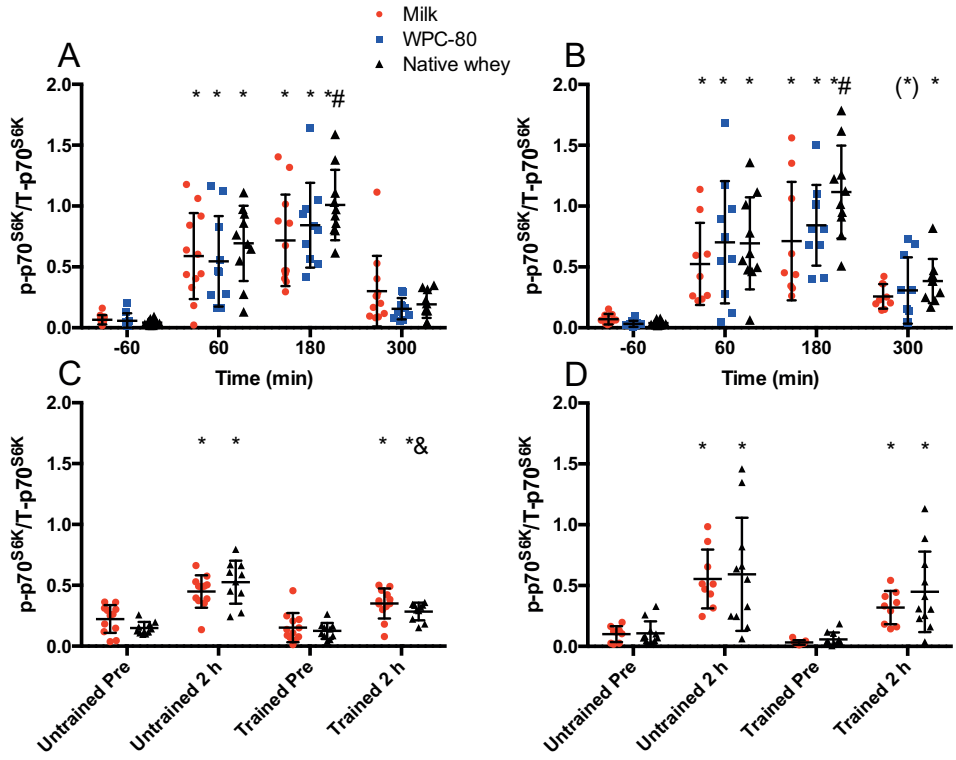

Figure 4.2 *Ratio between phosphorylated and total p70S6K. A and B, following intake of milk, WPC-80 or native whey immediately and two hours after a bout of resistance exercise in young and elderly, respectively. A: n = 12 in the milk group and 10 in the WPC-80 and native whey group. B: n = 10 in all groups. C and D, following intake of milk, WPC-80 or native whey immediately after a bout of resistance exercise in young and elderly, respectively. C, n = 10 in the milk group and 12 in the native whey group. D, n = 9 in the milk group and 11 in the native whey group. Values are mean ± SD. * Different from baseline; # different from milk at the corresponding time point, & different from the untrained state in the same group, p < 0.05.*

Combining young and elderly from study II-V, we observed significant correlations between area under the curve for blood leucine concentration and phosphorylation of p70S6K at 180min ($r = 0.63$, $P < 0.01$). This correlation supports the hypothesis of leucine as an important for the mTORC1 pathway (Anthony *et al.*, 2001; Phillips, 2014).

4.2.2. 4E-BP1

Resting total and phosphorylated levels of 4E-BP1 did not change during the training period. In study II and III phosphorylation of 4E-BP1 decreased before returning to resting levels at 300 min in young with native whey, and remained stable before increasing at 300 min in elderly with all supplements (figure 4.3 A and B). In study IV and V phosphorylation of 4E-BP1 did not change appreciably 120 min after exercise in young or elderly (figure 4.3 C and D). The low

response of 4E-BP1 was somewhat surprising given the fact that 4E-BP1 phosphorylation is expected to increase as a part of the translational signaling cascade related to increased MPS in response to resistance exercise and protein ingestion (Farnfield *et al.*, 2012; Churchward-Venne *et al.*, 2012; Kakigi *et al.*, 2014; Mitchell *et al.*, 2015*c*). However, not all studies observe an acute increase in phosphorylation of 4E-BP1 (Koopman *et al.*, 2007; Reitelseder *et al.*, 2011; West *et al.*, 2011; Reidy *et al.*, 2013; Glynn *et al.*, 2013; Areta *et al.*, 2013; Mitchell *et al.*, 2014).

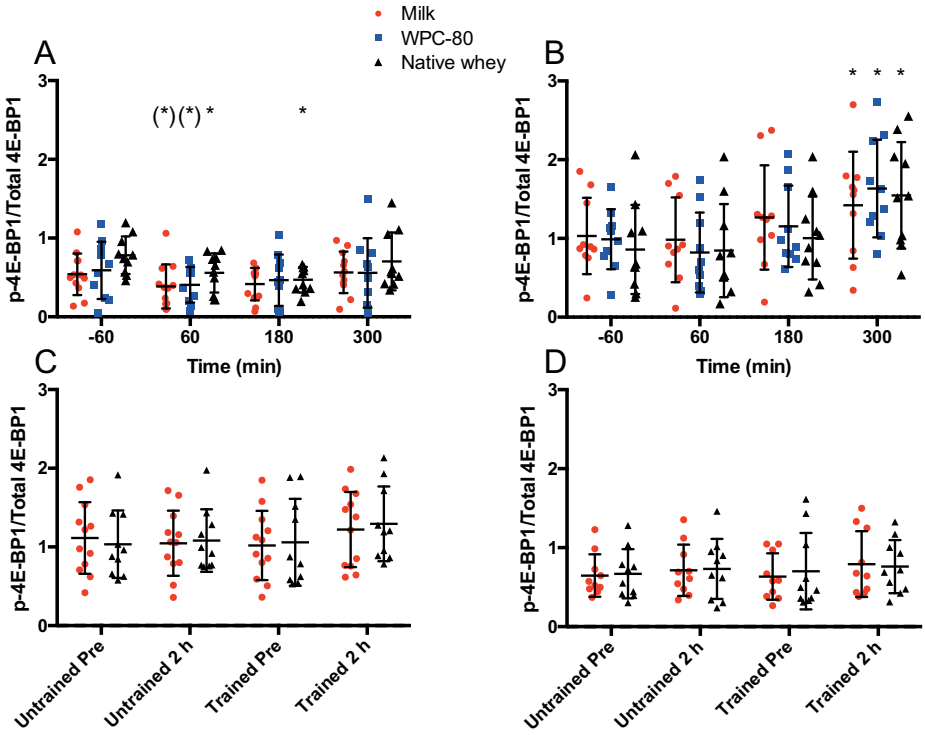

Figure 4.3 *A and B, ratio between phosphorylated and total 4E-BP1 following intake of milk, WPC-80 or native whey immediately and two hours after a bout of resistance exercise in young and elderly, respectively. A, n = 12 in the milk group and 10 in the WPC-80 and native whey group. B, n = 10 in all groups. C and D, ratio between phosphorylated and total 4E-BP1 following intake of milk, WPC-80 or native whey immediately after a bout of resistance exercise in young and elderly, respectively. C, n = 10 in the milk group and 12 in the native whey group. D, n = 9 in the milk group and 11 in the native whey group. Values are mean ± SD. * Different from baseline, p < 0.05.*

As both p70S6K and 4E-BP1 are downstream targets of mTORC1 (Ma & Blenis, 2009), one might expect them to have similar responses to mTORC1 stimulation. This does, however, not seem to be the case as p70S6K Thr^{389} is highly responsive to rapamycin[38] treatment, whereas 4E-

[38] Also called sirolimus and is an mTOR inhibitor used in several medical settings, such as an immunosupressant and to prevent organ rejection.

BP1 Thr$^{37/46}$ phosphorylation is unaffected by rapamycin treatment (Choo *et al.*, 2008; Thoreen *et al.*, 2009). Intriguingly, sensitivity towards rapamycin has been shown to follow the response to nutrients and growth factors (Kang et al., 2013). The consequences of 4E-BP1 phosphorylation are not entirely clear. Some studies report no effect of 4E-BP1 phosphorylation on 4E-BP1 association with eIF4E (Burnett et al., 1998; Gingras et al., 2001), whereas others observe a god agreement between 4E-BP1 phosphorylation and MPS (Mitchell *et al.*, 2015*c*). Based on the findings that MPS in response to amino acids and resistance exercise is inhibited by rapamycin (Drummond *et al.*, 2009*b*; Dickinson *et al.*, 2011; Gundermann *et al.*, 2014), and the p70S6K sensitivity to rapamycin, p70S6K activation is suggested to have a central role in mediating the amino acid response to MPS. This further agrees with the overall patterns of p70S6K and MPS in study II and III. In addition, 4E-BP1 appears to be dephosphorylated during exercise, supposedly to suppress the ATP-demanding process of MPS (Koopman *et al.*, 2007; Dreyer *et al.*, 2008; Apró *et al.*, 2015). Therefore, 4E-BP1 may remain dephosphorylated or at baseline levels for some time after exercise, before an increased phosphorylation is evident (Dreyer *et al.*, 2008; Reitelseder *et al.*, 2011; West *et al.*, 2011; Moberg *et al.*, 2016). The delayed phosphorylation of 4E-BP1 might relate to a greater training volume in these studies compared to studies showing a more immediate increase in phosphorylation of 4E-BP1 (Farnfield *et al.*, 2009*a*; 2012; Kakigi *et al.*, 2014). However, not all studies follow with this trend (Moore *et al.*, 2011; Churchward-Venne *et al.*, 2012).

Lastly, in contrast to most other studies we gave the participants a standardized breakfast. In contrast to the rapamycin treatment studies (Choo et al., 2008; Thoreen et al., 2009), 4E-BP1 have previously been shown to respond to protein intake (Moore et al., 2011), and phosphorylation could already be elevated in our resting samples, thereby reducing the post exercise phosphorylation of 4E-BP1 observed in this study compared to other studies.

Due to differing temporal patterns in 4E-BP1 phosphorylation we did not combine study II and III. Further, combining study IV and V did not change the results from what seen in the individual studies.

4.2.3. eEF-2

Resting levels of total and phosphorylated eEF-2 remained stable during the training period. Interestingly, we did not observe any nutrient or exercise induced acute changes in eEF-2 phosphorylation (figure 4.4 A, B, C and D). Studies investigating eEF-2 in young and elderly show conflicting results, with either no change (Hulmi *et al.*, 2009*b*; Moore *et al.*, 2011; Areta *et al.*, 2013; Churchward-Venne *et al.*, 2014; Mitchell *et al.*, 2015*c*) or a reduced phosphorylation

indicating increased activity (Drummond *et al.*, 2009*a*; Dreyer *et al.*, 2010; Glynn *et al.*, 2010*b*; Camera *et al.*, 2015; Moberg *et al.*, 2016; Agergaard *et al.*, 2016). We do not find a clear pattern to explain the differences between studies. It seems like eEF-2 is more responsive to exercise stimuli than amino acid stimuli, with some exceptions (Glynn *et al.*, 2010*b*). This is in contrast to the upstream, highly nutrient sensitive p70S6K, and do not explain why some studies applying resistance exercise find no effect on eEF-2 phosphorylation (Moore et al., 2011). From these data it is clear that our understanding of how eEF-2 is regulated by resistance exercise and protein supplementation is limited, and needs further investigation. Combining young and elderly did not change the results from what we observed in the individual studies.

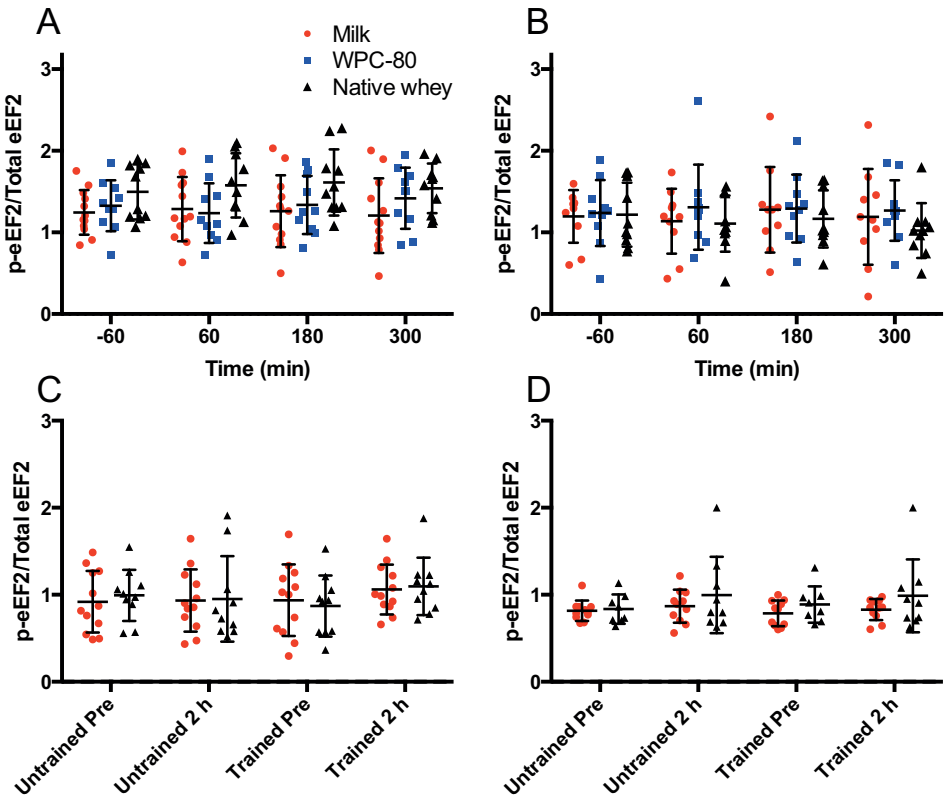

Figure 4.4 *A and B, ratio between phosphorylated and total eEF-2 following intake of milk, WPC-80 or native whey immediately and two hours after a bout of resistance exercise in young and elderly, respectively. A, n = 12 in the milk group and 10 in the WPC-80 and native whey group. B, n = 10 in all groups. C and D, ratio between phosphorylated and total peEF-2 following intake of milk, WPC-80 or native whey immediately after a bout of resistance exercise in young and elderly, respectively. C, n = 10 in the milk group and 12 in the native whey group. D, n = 9 in the milk group and 11 in the native whey group. Values are mean ± SD. * Different from baseline, $p < 0.05$.*

4.3. Muscle protein synthesis

The main goal of study II and III was to investigate the hypothesis that native whey would have a greater effect on mixed muscle FSR than WPC-80, when ingested acutely and 120 min after resistance exercise. We were, however, not able to show any significant differences between native whey and WPC-80 for mixed muscle FSR in the individual studies, or when combining young and elderly (figure 4.5. The hypothesis was consequently not confirmed. Native whey induced higher MPS-rates than milk during the post exercise period, both in young and elderly participants.

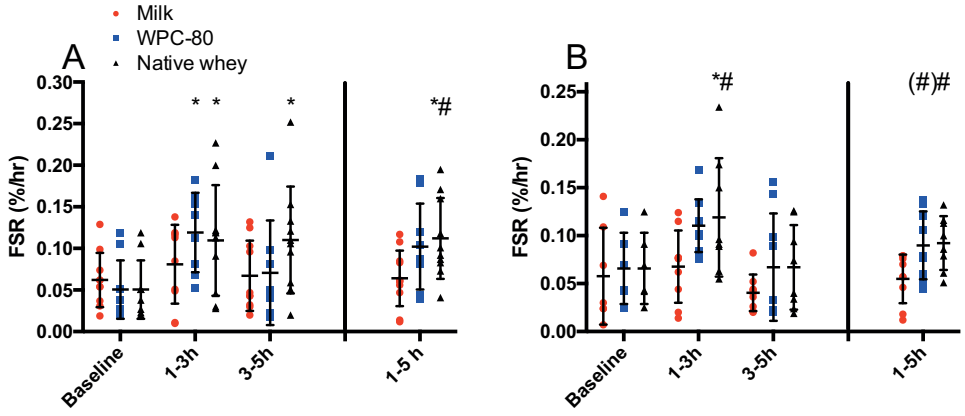

Figure 4.5 *FSR Mixed muscle FSR values following intake of milk, WPC-80 or native whey immediately and 2 hour after a bout of resistance exercise for young (A) and elderly participants (B). A, n = 10 in all groups. B, n = 8 in all groups (at baseline milk was 7 and native whey was 6). Values are mean ± SD. # different from milk over the corresponding time period, p < 0.05. (#) Tendency for a difference from milk (p = 0.05-0.1).*

During the early period (1-3 hours) values of mixed muscle FSR were not significantly different between groups in the young participants. However, the pattern was similar to that of the elderly participants, where MPS values approached and reached significantly higher from milk for WPC-80 and native whey, respectively. In the late period, no significant differences were observed between supplements in young or elderly. For the total period (1-5 hours) native whey showed higher values than milk in young, and both WPC-80 and native whey tended to have higher values than milk in elderly.

As most studies are conducted after an overnight fast and give no more than the supplements of interest during the experimental period (often 4-8 hours), a substantial energy deficit is expected to occur. This experimental setting does not resemble normal conditions and this may affect the results obtained. In an effort to make the setting in our studies closer to a real world practice and to counteract a negative energy balance from the overnight fasting, we provided participants with

a standardized breakfast and two serving of supplements during the experiment. This design makes it difficult to compare our temporal pattern with previous studies giving only one serving of supplement (Reitelseder *et al.*, 2011).

In study II and III native whey contained 0.6g more leucine per serving than WPC-80, totaling a difference in leucine intake of 1.2 g (1.52 g compared to milk) during the 5-hour post exercise period. Previous studies reporting increased stimulation of MPS by adding leucine had a greater difference in leucine content between supplements (3.6-4.2 g). More importantly, in these studies the extra leucine was added to suboptimal doses of protein[39]. The similar MPS-response to WPC-80 and native whey is likely a result of each serving of both supplements exceeding the proposed leucine threshold of 1.8-2.0 g, in order maximally stimulate MPS in young individuals (Reidy & Rasmussen, 2016).

Based on previous studies there seems to be a temporal difference between the effects of whey and casein[40], with whey inducing a faster (Tang et al., 2009) more transient increase in MPS, whereas casein ingestion results in a slower more prolonged increase in MPS after resistance exercise (Reitelseder *et al.*, 2011). However, when measured over a sufficient sampling period, e.g. 4-6 hours only minor differences, if any, are observed in MPS response between ingesting whey and casein after resistance exercise in young (Tipton *et al.*, 2004; Reitelseder *et al.*, 2011). Contrary to these findings, we observed native whey to result in higher values for MPS than milk during the 5-hour post exercise period, in young (tendency in elderly). Both servings of milk protein supplied 1.97 g of leucine (3.94 g in total) during the first two hours after exercise. The proposed leucine threshold is based on whey protein and the slower digestion and absorption of milk proteins may necessitate a greater amount of leucine in the form of milk protein than whey to maximally stimulate MPS. In terms of total EAA and leucine content per serving, WPC-80 was closer to milk. Still, the blood concentrations, effects on p70S6K phosphorylation and stimulation of MPS were more similar to native whey in young and elderly. This points to digestion rate as an additional contributing factor separating milk from the whey proteins, perhaps in addition to other individual anabolic amino acids such as threonine (Smith *et al.*, 1998). These results suggest an advantage of native whey compared to milk in stimulating MPS the first hours after heavy-resistance exercise, when supplemented as in study II and III.

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[39] 6.25 g whey, 10 g milk protein and infusion of 0.4 g of casein * kg body wheight over 5 hours in the studies by Venne and colleagues (Churchward-Venne *et al.*, 2014), Atherton and colleagues (Atherton *et al.*, 2016) and Rieu and colleagues (Rieu *et al.*, 2006), respectively.

[40] As milk is primarily composed of casein (80%) and a smaller part of whey (20%), some information might be gained from studies comparing these purified fractions.

Elderly are generally believed to need up to twice the amount of protein as young in order to maximally stimulate MPS (Pennings *et al.*, 2012; Yang *et al.*, 2012*a*). Katsanos and colleagues (Katsanos et al., 2006) showed that 6.7 g of EAA containing 1.7 g of leucine was sufficient to stimulate MPS in young, with no added effect of increasing the leucine amount to 2.7 g. Their elderly participants, however, did only increase MPS significantly with 6.7 g of EAA containing 2.7 g of leucine. Based on these results we would expect a difference between WPC-80 and native whey to be more likely in elderly, but no difference was observed.

The concept of anabolic resistance in elderly in response to protein and resistance exercise is complex, and whether it is an intrinsic feature of ageing remains contentious (Paddon-Jones *et al.*, 2004; Guillet *et al.*, 2004; Katsanos *et al.*, 2006; Symons *et al.*, 2007; Symonsi *et al.*, 2010). A possible explanation for this discrepancy between studies may be that anabolic resistance in elderly primarily manifests itself when a suboptimal stimulus is applied. Thus, young should respond to lower training volumes (Kumar et al., 2012) and doses of protein than elderly (Moore et al., 2014). On the contrary, if the stimuli are big enough or combined, elderly would reach the same MPS responses as young (Symons *et al.*, 2009; Atherton *et al.*, 2016). In our studies, the combination of a large training volume for the legs and 2 x 20 g of high quality whey proteins may have been sufficient to saturate the MPS-response of our elderly participants, leaving the extra leucine in native whey redundant, when compared to WPC-80. However, it is also possible that the difference between WPC-80 and native whey to be too small to detect with our methods, giving the impression of a "maximal effect" with both supplements. WPC-80 did not increase significantly from baseline in the early period. However, based on the values obtained and previous studies supplementing with whey protein in elderly (Burd *et al.*, 2012; Yang *et al.*, 2012*a*) this seems to be a matter of low statistical power. Milk protein is generally considered a high quality scource of amino acids. Isolated, the lack of an increased MPS in response to milk could be hypothesized to result from anabolic resistance in our elderly participants. However, as we observed the same result in young, we do not believe this to result from anabolic resistance in our elderly participants.

A reduction in activity for a period of two weeks has been shown to induce anabolic resistance in healthy elderly individuals, suggesting a sedentary lifestyle as an important contributor to anabolic resistance (Breen et al., 2013). Participants in study III reported an average of four hours of exercise (including brisk walking) each week. About half the group reported to be engaged in some sort of resistance training weekly. In addition the familiarization sessions for four weeks prior to the study might have further reduced the susceptibility of anabolic resistance in our elderly participants. When we compare the observed responses to exercise and protein intake in

our elderly participants with the response in the young participants, we do not see any evidence of anabolic resistance in terms of signaling or MPS in our active, healthy elderly participants.

As similar responses were observed in young and elderly, we combined the results from study II and III in an effort to increase statistical power and potentially strengthen some of the observed statistical tendencies. Combining young and elderly participants lead to a significantly elevated mixed muscle FSR values with native whey compared to milk (0.114% vs 0.075%, P = 0.042) in the early period. In the late period native whey showed a tendency for higher values than milk (0.091% vs 0.055%, P = 0.056). For the total period both WPC-80 (0.096% vs 0.060%, P = 0.046) and native whey (0.103% vs 0.060%, P = 0.002) had higher MPS values than milk. Thus, although combining young and elderly did reveal a significant effect between all supplements in accordance with the leucine content for phosphorylation of p70S6K, no differences in post exercise MPS between WPC-80 and native whey became more evident. This finding is supported by a previous study in rats, showing that in contrast to the readily saturable MPS-response, anabolic signaling responded to leucine in a dose-dependent manner with no apparent saturation point reached (Crozier et al., 2005).

Although p70S6K phosphorylation and MPS displayed similar patterns on the group level, no correlations were observed between individual changes in p70S6K phosphorylation and MPS. Several factors might explain this: 1) Regulation of MPS is complex and involves several pathways and kinases. Investigating a few of these is likely to give an incomplete picture, 2) The relationship between P70S6K and MPS is not necessarily linear (Crozier et al., 2005), 3) A factual correlation between the "snap-shot" nature of the biopsy for western blotting and the prolonged measurement of MPS may easily be missed (Greenhaff *et al.*, 2008; Atherton *et al.*, 2010).

4.4. Recovery of force-generating capacity

As a measure of muscular stress from the workouts, and to investigate the effects of the protein supplements on recovery of force-generating capacity, we included measurements of maximal force-generating capacity before, 15 min, 2.5 or 5, and 24 hours after the workouts in study II-V. The exercise protocols lead to a 5-40% reduction in force-generating capacity (figure 4.6). These ranges of reductions in muscle function indicate mild to moderate muscular stress, which is supported by small increases in CK across all groups (Paulsen *et al.*, 2012). The clear effect of protein on anabolic signaling and MPS may theoretically accelerate recovery of muscle function, as has been shown in studies applying more damaging eccentric muscle contractions (Cooke *et al.*, 2010; Buckley *et al.*, 2010). In the current studies, a workout considered more "normal" and less

muscle damaging was applied, while at the same time investigating more comparable supplements, making it less likely to find any differences in rate of recovery.

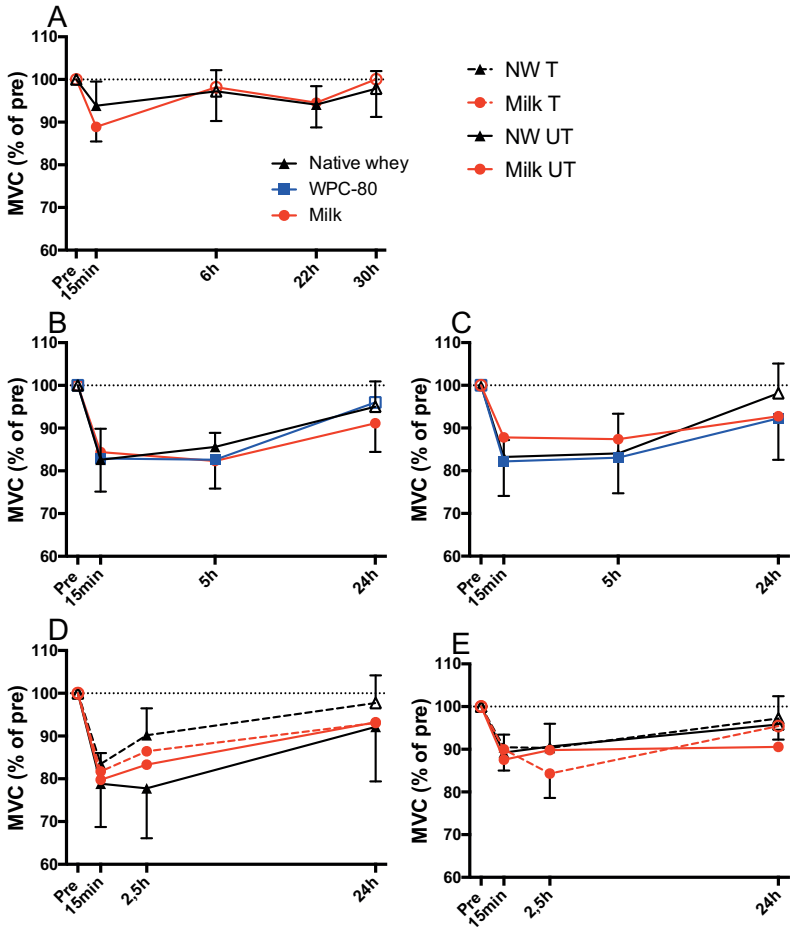

Figure 4.6 *Isometric knee extensor force-generating capacity relative to resting values following resistance exercise and intake of protein supplements in young and elderly participants. A: 20 g of milk or native whey after a bout of resistance exercise in young men, n = 10 in both groups. B and C: 20 g milk protein, WPC-80 or native whey immediately after and two hours after a bout of resistance exercise in young and elderly, respectively. B, n = 12 in the milk group and 10 in the WPC-80 and native whey group. C, n = 10 in all groups. D and E 20 g of milk or native whey after a bout of resistance exercise in young and elderly participants, respectively. D, n = 10 in the milk group and 12 in the native whey group. E, n = 9 in the milk group and 11 in the native whey group. Values are mean ± SD. Only highest and lowest error bars are shown for readability. # milk different from resting values, $ WPC-80 different from resting values; & native whey different from resting values, $p < 0.05$.*

We did not observe any group differences in any of the individual studies. However, when combining the results from young and elderly in study II-V, comparing milk and native whey at 24 hours after exercise, we observed a small but significant difference in recovery of force-

generating capacity of about 3% points (milk: 93%, native whey: 96%, P = 0.008). The relevance of this difference in the groups studied in this thesis is likely trivial. However, it may be of great interest for athletes, and future studies should investigate the potential effects of protein quality on recovery of force-generating capacity.

4.5. Muscle mass and strength

In study IV and V our goal was to test the hypotheses that the previously observed acute differences between native whey and milk supplementation[41] (study I-III) would translate into greater increases in muscle mass and strength during a period of resistance training in young (study IV) and elderly (study V). Both young and elderly participants experienced substantial gains in muscle mass and strength in response to the training and supplementation intervention. However, no group differences were observed for changes in muscle mass or strength (figure 4.7 A and B, figure 4.8 A and B and table 4.1), thus our hypotheses could not be confirmed.

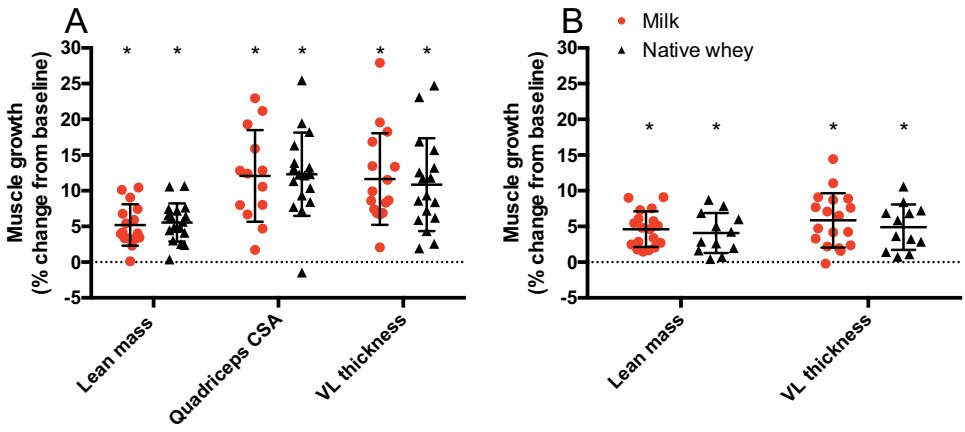

Figure 4.7 *Relative changes in lean mass, quadriceps cross sectional area (CSA) and m. vastus lateralis thickness in young (A) and elderly (B), after a 12-week strength training and protein supplementation with milk or native whey. Young n = 16 and 18 in the milk group and native whey group, respectively. Elderly n = 15 in both groups. Values are mean ± SD. * difference between pre and post within group, p < 0.05.*

The higher MPS-rates with native whey compared to milk protein in study II and III would suggest a greater potential for gains in muscle mass and strength with native whey during a training period. But, no differences were observed for acute, except blood amino acid

[41] Due to the similar acute response to WPC-80 and native whey in study II and III, and a limited capacity to include participants in the training study, we did not include a group receiving WPC-80 in study IV and V.

concentrations, or long-term outcomes between supplements in study IV and V. The results from study IV and V do, however, agree with previous studies measuring the acute signaling and MPS responses to milk and whey after exercise over a 4-6 hour period at rest (Mitchell *et al.*, 2015*a*), and casein and whey after resistance exercise (Tipton *et al.*, 2004; Reitelseder *et al.*, 2011). In study II and III we gave the supplements as two servings immediately and 120min after exercise. In study IV and V we divided the two supplement servings between morning and evening. Consequently, we cannot exclude that ingesting both supplements in closer temporal proximity to exercise could have produced different results in study IV and V.

In study IV and V, the difference between milk protein and native whey should be less than between protein and placebo, and harder to observe. Still, Hidayat and colleagues (Hidayat *et al.*, 2017) observed greater gains in fat free mass in studies supplementing with whey compared to other forms of protein, such as casein and milk concentrate. Based on previous studies in young (table 1.1), it seems like the effects of differing protein quality on changes in muscle mass, are only evident with suboptimal doses of protein (Hartman et al., 2007; Volek et al., 2013), and disappear as protein dose increases (Brown *et al.*, 2004; Denysschen *et al.*, 2009; Wilborn *et al.*, 2013; Joy *et al.*, 2013). However, not all studies follow this trend (Candow & Burke, 2006; Cribb et al., 2006). By supplementing our young participants with 2x20 g of native whey or milk protein daily, both groups likely received sufficient protein to maximally stimulate post exercise MPS. Further, Morton and colleagues (Morton et al., 2017) found no added effect of protein supplementation when individuals ingested beyond 1.6 $g \cdot kg \cdot day^{-1}$, which is what our young participants initially consumed.

As discussed previously, elderly seem to need larger amounts of protein in order to maximally stimulate MPS. Cermak and colleagues (Cermak et al., 2012) observed a smaller effect of combining resistance exercise with protein on changes in lean mass in elderly, compared to young individuals. Simmilarily, Morton and colleagues (Morton et al., 2017) found that elderly, in contrast to young, did not show an additional effect of protein supplementation in combination with resistance exercise. This may relate to a lower number of studies in elderly, supplementing less frequently with smaller doses of protein (table 1.2), compared to studies in young individuals (table 1.1). Combining this with a potential anabolic effect in elderly is likely to give a reduced effect of the intervention. From table 1.2 we see an increase in lean muscle mass of between 0.6 and 1.4 kg over the course of 12 weeks to 18 months with protein supplementation, in elderly. In study V we observed a 2.1 kg increase in lean muscle mass over 11 weeks, which in relative terms is similar to what we observed in young (study IV). Unfortunately, without a placebo group we are not able to determine the separate contributions from resistance training and protein

supplementation. This does, however, point to the need for more "optimal intervention" studies before we can conclude on the effects of protein supplementation in combination with resistance training in elderly.

Based on the baseline characteristics and acute responses to resistance exercise and protein intake, the elderly participants included in this thesis are very similar to the young participants (table 4.1 and 4.2). The only clear sign of ageing in our elderly participants was observed in study V, where the type II fibers were 20 ± 26% smaller than type I fibers before the training period. Whether this reduced type II fiber area is a result of anabolic resistance or some other mechanism related to ageing is not clear. In any case, the type II fiber atrophy was reversed with training and protein supplementation, as type II fiber area increased by 34 ± 45% (table 4.2), and reached similar areas as type I fibers. We observed only a small non-significant increase in type I fiber area of 4 ± 21% in elderly.

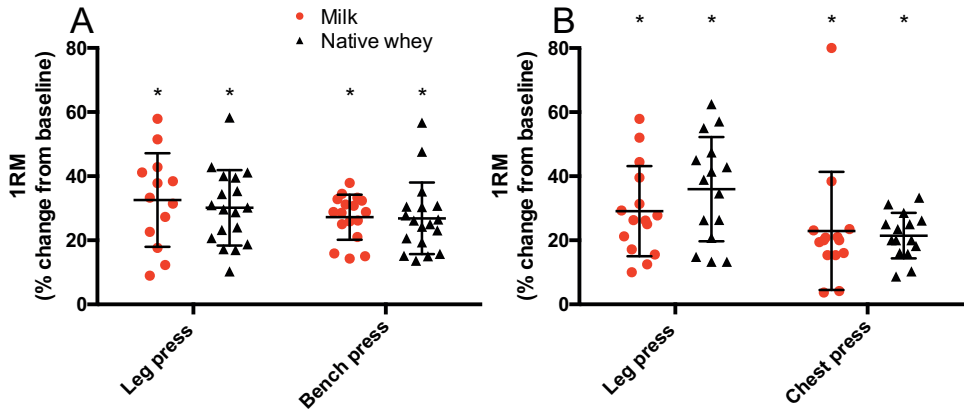

Figure 4.8 *Relative changes in leg press and bench press 1RM in young (C) and elderly (D) after a 12-week strength training and protein supplementation intervention. Young: n = 16 and 18 in the milk group and native whey group, respectively. Elderly: n = 15 in both groups. Values are mean ± SD. * difference between pre and post within group, p < 0.05.*

The observed increases in strength were within the range of previous studies for both young and elderly participants (table 1.1 and table 1.2). In contrast to individual studies (table 1.1 and 1.2) the meta-analyses by Cermak and colleagues (Cermak et al., 2012) and Morton and colleagues (Morton et al., 2017) reported an effect of protein supplementation on gains of strength. This may relate to the small effect that protein supplementation potentially contributes to strength, in addition to the resistance training (Morton et al., 2017) , and the inherent difficulty of measuring performance.

Combining results from young and elderly participants for measures of muscle mass or strength

did not result in any differences between milk and native whey supplementation. In contrast to a previous study (Volek et al., 2013), we did not observe any correlations between fasting levels of blood leucine concentrations during a supplementation period and gains in lean body mass. Furthermore, no meaningful correlations were observed between the rise in acute blood leucine concentrations, signaling responses and changes in lean mass during the training period.

Table 4.1 *Changes in body composition, regional lean mass, fiber spesiffic muscle fiber area and nuclei per fiber after a 12-week strength training and protein supplementation intervention, in young individuals.*

	Milk			Native whey			P values for group difference (% change)
	Pre	Post	% change	Pre	Post	% change	
Body mass (kg)	77.8 ± 16.0	80.0 ± 15.3*	3.1 ± 3.4*	77.9 ± 11.7	81.3 ± 12.1*	4.4 ± 3*	0.21
Fat mass (kg)	22.0 ± 7.1	21.7 ± 6.4	-0.9 ± 6.9	20.9 ± 7.8	21.4 ± 8.3	3.26 ± 9.3	0.15
Lean mass (kg)	53.2 ± 10.7	55.8 ± 11.4*	4.9 ± 3.2*	54.2 ± 8.0	57.2 ± 8.2*	5.6 ± 2.7*	0.48
Leg lean mass (kg)	18.8 ± 4.3	19.9 ± 4.5*	5.5 ± 3.6*	19.0 ± 3.0	20.2 ± 3.1*	6.4 ± 4.5*	0.56
Arm lean mass (kg)	6.31 ± 1.76	6.77 ± 1.98*	7.5 ± 6.18*	6.38 ± 1.48	6.89 ±1.56*	8.23 ±3.78*	0.53
Trunk lean mass (kg)	24.8 ± 4.4	25.9 ±4.6*	4.2 ± 3.7*	25.5 ± 3.6	26.9 ± 3.7*	5.8 ± 3.7*	0.16
VL thickness (mm)	2.46 ± 0.39	2.74 ± 0.39*	11.5 ± 6.2*	2.64 ± 0.46	2.91 ± 0.49*	10.8 ± 6.5*	0.75
MFA type I	4299 ± 799	5071 ± 691	20.6 ± 22.3*	4605 ± 1037	4987 ± 1092	10.9 ±25.4	0.66
MFA type II	4761 ± 1187	6145 ± 1320	31.7 ± 24.6*	4841 ± 1378	6116 ±1684	28.4 ± 27.4*	0.71
Type I nuclei	1.56 ± 0.34	1.80 ± 0.41	20 ± 37*	1.57 ± 0.27	1.76 ± 0.29	15 ± 25	0.63
Type II nuclei	1.69 ± 0.29	2.01 ± 0.36*	22 ± 29*	1.74 ± 0.4	2.03 ± 0.40*	23 ± 42	0.94

Table 4.2 *Changes in body composition, regional lean mass, fiber spesiffic muscle fiber area, and stair climb and chair rise performance after a 11-week strength training and protein supplementation intervention, in elderly individuals.*

	Milk			Native whey			P values for group difference (% change)
	Pre	Post	% change	Pre	Post	% change	
Body mass (kg)	74.6 ± 14.0	77.2 ± 14.0	3.6 ± 2.2*	75.9 ± 16.1	78.3 ± 16.2	3.3 ± 1.6*	0.618
Fat mass (kg)	22.1 ± 7.0	22.4 ± 6.8	1.7 ±5.3	23.9 ± 8.9	24.4 ± 8.9	2.4 ± 4.9	0.721
Leg lean mass (kg)	17.0 ± 3.8	18.0 ± 3.8	6.3 ± 3.6*	17.3 ± 4.7	18.0 ± 4.5	4.6 ± 3.4*	0.199
Arm lean mass (kg)	5.6 ± 1.5	6.0 ± 1.6	6.4 ± 3.6*	5.3 ± 1.5	5.6 ± 1.5	5.2 ± 3.9*	0.377
Trunk lean mass (kg)	24.0 ± 3.9	24.8 ± 3.7*	3.4 ± 3.6*	23.7 ± 4.8	24.4 ± 5.0*	3.0 ± 3.1*	0.791
Stair climb							
BW (s)	7.48 ± 1.0	7.18 ± 0.97	-3.8 ± 5.4*	7.54 ± 0.94	7.03 ± 0.93	-6.56 ± 7.1	0.257
10kg (s)	7.35 ± 1.0	7.21 ± 1.07	-3.3 ± 5.0*	7.62 ± 1.34	7.09 ± 1.07	-6.29 ± 8.1	0.267
20kg (s)	7.91 ± 1.53	7.58 ± 1.50	-3.8 ± 8.4*	8.07 ± 1.73	7.41 ± 1.26	-7.26 ± 8.57	0.293
Chair rise (s)	7.0 ± 2.1	6.29 ± 1.3	-8.1 ± 13.9*	6.70 ± 1.20	5.92 ± 0.97	-11.0 ± 9.2	0.504
MFA type I (μm^2)	4519 ± 1030	4667 ± 1187	4.7 ± 22.0	4897 ± 919	4933 ± 785	2.8 ± 19.7	0.823
MFA type II (μm^2)	3862 ± 1984	4502 ±1397	34.2 ± 56.7*	3740 ± 1608	4692 ± 1441*	33.8 ± 27.4*	0.935

4.6. Acute MPS vs. Hypertrophy

The differences in acute measures of MPS between milk and native whey, in study II and III, does not align with the long-term outcomes in study IV and V. Based on the few existing studies, it appears difficult to relate acute changes in MPS to hypertrophy during a training period on an individual level (Mayhew *et al.*, 2009; Mitchell *et al.*, 2014). Due to methodological challenges most studies do not include a measure of MPB. Thus, the lack of MPB measures in most studies might explain some of the disagreement between MPS and hypertrophy. However, the magnitudes of changes in MPB are considered much smaller than in MPS (Glynn *et al.*, 2010*a*), and seem to be regulated in parallel with MPS (Phillips *et al.*, 1997). Consequently, a measure of MPS is thought to give a relatively good indication of changes in the net protein balance. On a group level, acute interventions have demonstrated similar patterns in stimulation of MPS as long-term hypertrophic outcomes in other studies, with repeated exposure to similar stimuli (Wilkinson *et al.*, 2007; Hartman *et al.*, 2007; Tang *et al.*, 2009; Volek *et al.*, 2013).

As the MPS response in the same individual may change significantly with training status (Kim *et al.*, 2005; Tang *et al.*, 2008; Wilkinson *et al.*, 2014), the measurement of MPS in the untrained state is unlikely to represent changes occurring during a period of training. How fast these changes appear are unclear, but measurable differences in MPS response have been reported in as little as two workouts over a period of 8 days. This rapid adaptation in untrained individuals embarking on a training program has been linked to muscle damage, disappearing within 3 weeks of training, at which measures of protein synthesis agreed well with the hypertrophy outcomes after 10 weeks of training (Damas et al., 2016).

Another important point is the fact that also endurance type exercise stimulates MPS, without any apparent muscle hypertrophy (Carraro *et al.*, 1990; Harber *et al.*, 2010). Thus, not all increases in MPS is related to hypertrophy; it can be related to repair and remodeling[42] of the muscle, without necessitating a change in mass (figure 9).

Several other factors may also contribute to the differing results of acute measures of MPS and long-term outcomes. Measures of MPS have generally been made in the first 2-6 hours after exercise. Although measures over 3 hours post exercise have been found to reflect the 24 hour MPS (Tipton *et al.*, 2003), we know little about MPS in the period 6-24 hours post exercise. Perhaps extending the "acute" measurement to 12 or 24 hours will give a better agreement

[42] Combined, repair and remodelling is often reffered to as reconditioning.

between measures of acute changes in MPS and hypertrophy in a subsequent training period. Further, several challenges exists with the methods of measurement of MPS (Smith *et al.*, 2011), which may preclude the relation between acute changes in MPS and long-term hypertrophy.

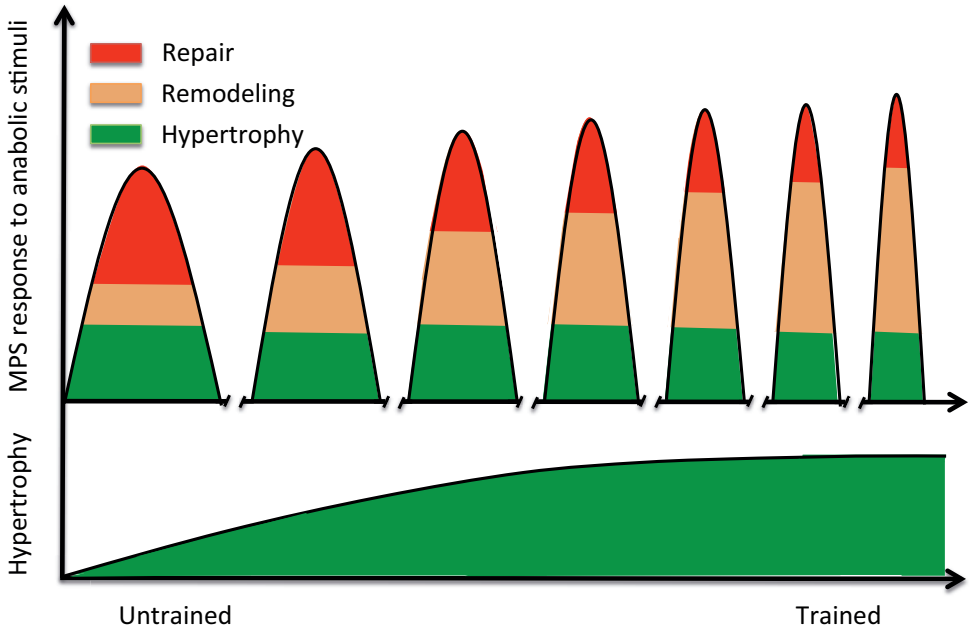

Figure 4.9 *Hypothetical illustration of the acute MPS response and long term training adaptation. The upper part is an illustration of the MPS response to anabolic stimuli during a transition from untrained to trained. The MPS response contributes to repair, conditioning and hypertrophy, possibly explaining the disagreement between acute measures of MPS and long-term hypertrophy. The lower part shows the accumulated muscle mass over time with training. The figure is based on (Phillips* et al.*, 1999; Kim* et al.*, 2005; Tang* et al.*, 2008; Damas* et al.*, 2015).*

Disregarding the reasons behind differences between acute and long-term results, one may still ask the question as to whether the acute or the long-term outcomes are the most correct results. The large variability in individual responses to resistance training, and the array of confounding factors as variability in diet, sleeping patterns, alcohol consumption, daily stress and compliance to the intervention, may all challenge the validity and reliability of a training intervention. Acute measures are more easily controlled and small differences between interventions are detectable. One could make the argument that acute studies are more capable of observing small differences between interventions, which are easily missed in long-term studies. Thus, showing the actual differences between interventions. On the other hand the limited window of measurement, lack of information about MPB, and the contribution of MPS to other processes, such as repair and remodeling, in addition to hypertrophy, argues for a reliance on the long-term studies. In my view, to understand the integrated responses, that over time constitute the long-term effect of a

treatment or intervention, the responses should be assessed by long-term measurement techniques. As a supplement to these methods, acute measures of protein metabolism, in combination with other methods (IHC, Western Blot, RT-PCR and OMICs-methods) can provide valuable mechanistic insight into responses to manipulations of anabolic stimuli.

4.7. Methodological considerations

All studies in this thesis were designed as RCTs, which is considered the gold standard when investigating treatment effects. In order to have a strong comparison between supplements, study I was conducted as a crossover design, where all participants did the experiment with all supplements. In study II and III, we decided to keep the crossover design to compare WPC-80 and native whey, as we expected small differences between these supplements. Due to the number of biopsies, milk was ingested by another group of participants. Thus, making this study a partial crossover. The design without a placebo group is unusual and limits our ability to distinguish effects of protein supplementation and resistance exercise individually. However, the absence of a placebo group apparently concerns doing research *lege artis* (according to the rules of the game) more than the conclusions of the thesis.

Although the numbers of participants included in the studies of this thesis are comparable to other similar studies, they seem to suffer from low statistical power. This becomes apparent when combining the results from young and elderly in study II and III, resulting in more significant differences between groups. From estimates based on previous studies the training interventions include too few participants (Reidy & Rasmussen, 2016). This is further supported by an estimation of the number of participants needed in each group to observe a difference between groups in study IV (about 300). In study IV the difference in lean mass between groups were not regarded as clinically significant and increasing the number of participants is unlikely to have changed the conclusion. In study V differences in lean mass was larger than in study IV, but it was in favor of the milk supplement. Adding participants to this study would have made it easier to make a firm conclusion.

To increase the reliability of the performance tests participants were instructed not to do any physically demanding tasks for two days prior to the tests. In addition warm up and order of tests were standardized. In addition participants performed the test on two occasions with at least three days in between. Such familiarization has been show to improve the test-retest reliability (Levinger *et al.*, 2009; Ritti-Dias *et al.*, 2011). In support of this we observed a 4-5% increase in 1RM in young, an 8-9% increase in 1RM and a 4-7% increase in functional tests for elderly at the

second pre-test.

Elderly individuals make up a heterogeneous group raging from frail and sarcopenic to active and healthy individuals. As our elderly participants showed no clear signs of anabolic resistance we cannot say anything about the effect of the protein supplements on this phenomenon, only conclude that it seems like age, at least up to about 75 years, per se does not necessitates a muscular anabolic resistance. At lest not when 20 or 2 x 20 g of protein was combined with heavy resistance exercise. In order to investigate the nature of anabolic resistance further, future studies should determine the actual existence of the condition in the group of participants prior to interventions. The conclusions from our studies are made on healthy active elderly and may not necessarily apply to frail or sarcopenic elderly. The need for a better understanding of the mechanisms behind e.g. sarcopenia is great. Yet, increasing our understanding of how to extend the healthy period of elderly individuals is also of great importance, as preventing a condition is likely to be more effective than treating it.

The calculations involved in measuring FSR are based on a set of assumptions. One of these is the assumption of steady state of the tracer to tracee ratio (TTR). Thus, in order to minimize the fluctuations in tracer to tracee ratio we enriched the protein supplements with 6% [$^{2}H_{5}$]phenylalanine. Based on the blood samples collected during the study this approached worked well, as no fluctuations in TTR were observed after intake of supplements. There was, however, a short period with an increased TTR at 175 and 180 min in young, and between 175 and 190 min in elderly. This short-lived increase in [$^{2}H_{5}$]phenylalanine TTR was due to a bolus infusion of [$^{13}C_{6}$]phenylalanine not related to the results in the presented studies. This would not affect measures of bound protein by the IRMS, but the GCMS measures of intracellular [$^{2}H_{5}$]phenylalanine could potentially be altered. When analyzed, intracellular enrichment of [$^{2}H_{5}$]phenylalanine did not reflect the brief fluctuations in blood. Mixed muscle FSR calculations should therefore not be affected.

In study II and III we used mixed muscle FSR as a measure of MPS. As we were primarily interested in specific adaptations in the contractile properties of muscle, isolation of the myofibrillar fraction of muscle for FSR measures could have been a stronger approach. Although contractile proteins are most abundant in muscle, the non-contractile proteins generally have a higher turnover and may significantly contribute to the mixed FSR after resistance exercise (Jaleel et al., 2008). Therefore, caution is warranted when interpreting changes in mixed muscle FSR. However, these challenges seem mainly to be a problem when doing unaccustomed exercise (Wilkinson *et al.*, 2008). Thus, the inclusion of resistance trained young participants and extensive familiarization of elderly participants is a strength of study II and III.

We did only measure MPS from 1-5 hours after the workout. A previous study did find this period to be representative of the 24-hour MPS response (Tipton et al., 2003), but we are likely to have missed some of the increased MPS in response to the last serving of supplements, perhaps milk in particular (Reitelseder *et al.*, 2011).

In study II and III we observed values of mixed muscle FSR that were higher than what is usually expected with this method (Reidy & Rasmussen, 2016). However, large variations in reported FSR values are not uncommon (Smith *et al.*, 2011), and our values are within the range of previous studies investigating resistance-trained participants in the postprandial state (Tang *et al.*, 2008; Paulsen *et al.*, 2014).

The lack of a direct measure of MPB is a limitation in this study, as both MPS and MPB are needed to calculate net protein balance in muscle. Previous studies have shown MPS to respond with greater changes than MPB (Phillips *et al.*, 1997; Borsheim *et al.*, 2002) and MPB to be a minor determinant of net muscle protein balance in the acute response to protein intake following resistance exercise (Glynn *et al.*, 2010*a*). Thus, we assume that our MPS measurements to large extent reflect the major part of the net protein balance response.

4.8. Ethical considerations

In study II-V we included muscle biopsies in order to measure protein signaling, MPS and muscle growth at the cellular level. Being an invasive technique, a biopsy is associated with a risk of infection. We applied a modified Bergstrøm biopsy technique with suction. This method has a reduced risk of infection compared to alternative procedures, such as the open biopsy technique (Tarnopolsky *et al.*, 2011). By the use of suction and 6 mm needles, instead of the more common 5 mm needle, the method yields more tissue and necessitates a fewer "clips" (Evans *et al.*, 1982; Melendez *et al.*, 2007; Tarnopolsky *et al.*, 2011), further reducing the risk of infection. In addition we designed the study so that we could collect two biopsies from each incision, where needed. This reduced the amount of incisions and the risk of infection and discomfort. A biopsy can be a stressful situation and involve some pain. Therefore, care was taken to inform the participants about the procedure and make it as manageable as possible. If a participant had second thoughts before the biopsy procedure, it was canceled. In the training studies, participants withdrawing from the biopsy were still allowed to take part in the study without biopsies, if they wished to. The biopsy procedure applied in the studies of this thesis seems appropriate and ethically defensible.

The days of acute measures of MPS and cell signaling were demanding on the participants,

involving several biopsies and a very heavy workout. Still, we did our best to make the day as comfortable as possible. We believe this was achieved as only one participant choose not to take part in the second acute day due to the stressfulness of partaking and the taste of the supplement.

Heavy resistance exercise involves a risk of injury. In the training studies care was taken to avoid injuries by a close supervision of all workouts in elderly and the heavy workouts in young. If pain was experienced during exercise measures, such as modifications of technique, short periods of reduced training volume and in some cases stretching, were taken to allow for continued participation, if it was the wish of the participant.

Ethical concerns, especially elderly, about providing high doses of protein over an extended period of time and the potential negative effects on kidney function have been raised in Norwegian media and by academics (Øimoen, 2016). These claims likely arise from a publication by Brenner and colleagues (Brenner *et al.*, 1982), suggesting protein intake as a possible risk factor for chronic kidney disease. These suggestions were primarily based on animal studies and patients with kidney disease. Later reviews have, concluded that a higher than recommended intake of protein does not pose a risk for developing kidney disease in healthy individuals (Martin *et al.*, 2005; Tipton, 2011), as has the World Health Organization (Joint WHO/FAO/UNU Expert Consultation, 2007). Thus, based on available literature there is no risk of supplementing healthy young and elderly individuals with protein up to the level of intake reached in the studies of this thesis.

5. Conclusions

The overall aim of this thesis was to investigate potential beneficial effects of native whey supplementation on muscular acute anabolic responses and adaptations in response to resistance exercise, compared primarily to WPC-80, but also milk protein. Main findings are summarized below and in figure 5.1).

Amino acid signal

- Post exercise ingestion of native whey increased post exercise blood concentrations of leucine more than WPC-80 and milk protein, both in young and healthy elderly individuals.

Signal transduction

- Post-exercise increases in p70S6K phosphorylation was not significantly different after ingestion of WPC-80 and native whey. However, combining young and elderly revealed a greater p70S6K phosphorylation with native whey than with WPC-80. Native whey induced a greater phosphorylation of p70S6K than milk in study II and III, but not in study IV and V. Thus, no firm conclusion can be made.
- No differences between supplements were observed for 4E-BP1 and eEF-2 in young or elderly healthy individuals.

Muscle protein synthesis

- No difference was observed in MPS between WPC-80 and native whey. Native whey reached higher post exercise levels of MPS than milk in young (1-5 hours) and elderly (1-3 hours) individuals.

Adaptation

- Supplementation with milk and native whey did not differently affect the adaptation of muscle mass or strength to a 12 and 11 week training program in young and elderly, respectively.

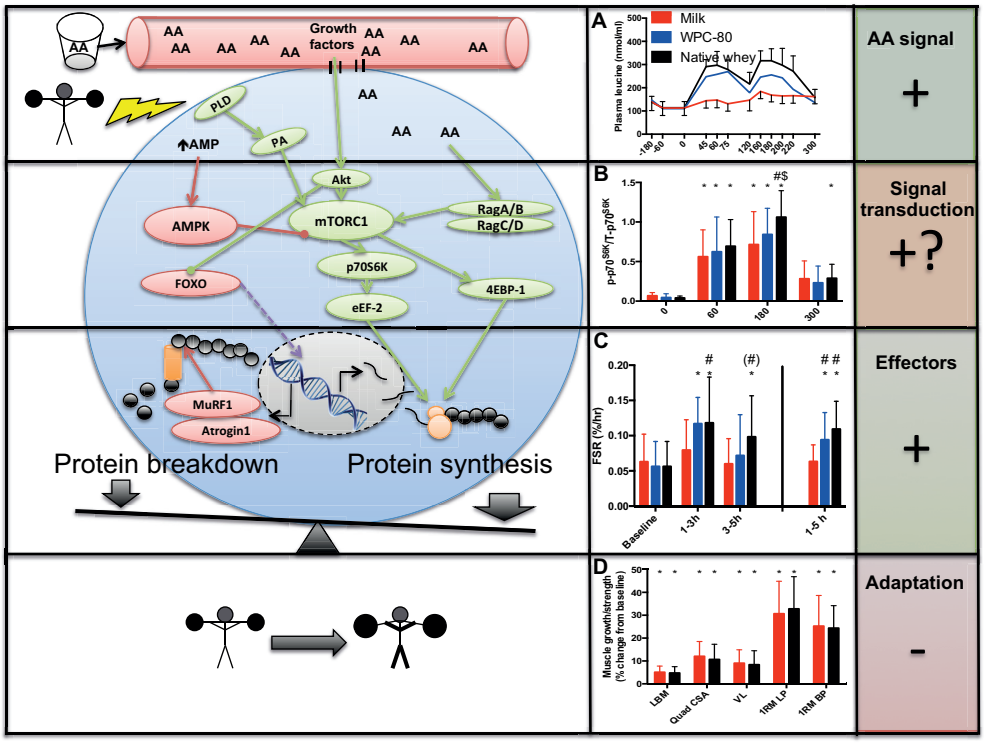

Figure 5.1 *Summary of main findings of the thesis. A, B and C shows combined results from young and elderly participants in study II and III. D shows the combined results of young and elderly participants in study IV and V. Green circles and arrows indicate kinases and actions with a positive effect on MPS, here represented by a simplified mTORC1-signaling pathway. Red circles and arrows indicate kinases, transcriptionfactors and actions with a negative effect on MPS, or a positive effect on MPB, here represented by the ubiquitin-proteasome pathway. Purple scattered arrows indicate translocation. AA; amino acid. * significantly different from baseline, # significantly different from milk, $ significantly different from WPC-80, p < 0,05.*

6. Perspectives

The positive effect of protein ingestion and resistance exercise on MPS is clear. However, the optimal applications and combinations of these anabolic stimuli have yet to be determined, especially with regards to long-term adaptations. To extend our understanding of these adaptations we need to expand our knowledge on protein ingestion as a part of a diet, as opposed to in isolation. The current recommendations for protein intake does not take into account the complexity of protein metabolism. Given what we know about the effects of protein type, dose, timing, frequency of protein intake and co-ingestion with other macronutrients, a recommendation of a single daily amount of protein may not be sufficient. Most studies on the anabolic effect of protein investigate the effects of a single intake of isolated protein in contrast to a complex meal or several meals. There are still uncertainties regarding how other macronutrients affect the protein stimulation of MPS, and how protein type or dose affects optimal timing and frequency of meals in young and elderly. There is also a lack of studies in middle-aged individuals (40-60 year old) and females.

In terms of optimizing strength training for different groups or individuals more research is needed on the so-called non- or low-responders. In order to understand this phenomenon a greater understanding of the mechanisms regulating MPS and MPB is needed. To date few studies have been able to show a clear relation between the known pathways, e.g. the mTORC1 pathway, acute MPS and long-term adaptations in human training studies. The mechanisms responsible for muscular adaptation over time are complex and likely involve factors still undiscovered. A more exploratory and open-minded approach is likely needed in order to extend our knowledge in this area. New methodologies allowing intermediate measures of MPS and MPB, different "omics" approaches, including fluxomics will allow us to make important discoveries in the future.

7. References

Agergaard J, Bülow J, Jensen JK, Reitelseder S, Drummond MJ, Schjerling P, Scheike T, Serena A & Holm L (2016). Light-load resistance exercise increases muscle protein synthesis and hypertrophy signaling in elderly men. *AJP: Endocrinology and Metabolism*ajpendo.00164.2016.

Alessi DR, Kozlowski MT, Weng QP, Morrice N & Avruch J (1998). 3-Phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylates and activates the p70 S6 kinase in vivo and in vitro. *Curr Biol* **8,** 69–81.

Alvestrand A, Hagenfeldt L, Merli M, Oureshi A & Eriksson LS (1990). Influence of leucine infusion on intracellular amino acids in humans. *Eur J Clin Invest* **20,** 293–298.

Andersen LL, Andersen LL, Tufekovic G, Tufekovic G, Zebis MK, Zebis MK, Crameri RM, Crameri RM, Verlaan G, Verlaan G, Kjaer M, Suetta C, Magnusson P, Magnusson P & Aagaard P (2005). The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. *Metab Clin Exp* **54,** 151–156.

Anker SD, Coats AJS, Morley JE, Rosano G, Bernabei R, Haehling von S & Kalantar-Zadeh K (2014). Muscle wasting disease: a proposal for a new disease classification. *J Cachexia Sarcopenia Muscle* **5,** 1–3.

Anthony JC, Anthony TG, Kimball SR & Jefferson LS (2001). Signaling Pathways Involved in Translational Control of Protein Synthesis in Skeletal Muscle by Leucine. *The Journal of nutrition* **131,** 856S–860S.

Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS & Kimball SR (2000). Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *The Journal of nutrition* **130,** 2413–2419.

Antonio J, Sanders MS, Ehler LA, Uelmen J, Raether JB & Stout JR (2000). Effects of exercise training and amino-acid supplementation on body composition and physical performance in untrained women. **16,** 1043–1046.

Apró W, Moberg M, Hamilton DL, Ekblom B, van Hall G, Holmberg H-C & Blomstrand E (2015). Resistance exercise-induced S6K1 kinase activity is not inhibited in human skeletal muscle despite prior activation of AMPK by high-intensity interval cycling. *AJP: Endocrinology and Metabolism* **308,** E470–E481.

Areta JL, Burke LM, Ross ML, Camera DM, West DWD, Broad EM, Broad EM, Jeacocke NA, Moore DR, Stellingwerff T, Phillips SM, Hawley JA & Coffey VG (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *The Journal of Physiology* **591,** 2319–2331.

Arnal MA, Mosoni L, Boirie Y, Houlier ML, Morin L, Verdier E, Ritz P, Antoine JM, Prugnaud J, Beaufrère B & Mirand PP (1999). Protein pulse feeding improves protein retention in elderly women. *Am J Clin Nutr* **69,** 1202–1208.

Arnal MA, Mosoni L, Boirie Y, Houlier ML, Morin L, Verdier E, Ritz P, Antoine JM, Prugnaud J, Beaufrère B & Mirand PP (2000). Protein feeding pattern does not affect protein retention in young women. *The Journal of nutrition* **130,** 1700–1704.

Arnarson A, Geirsdottir OG, Ramel A, Briem K, Jonsson PV & Thorsdottir I (2013). Effects of whey proteins and carbohydrates on the efficacy of resistance training in elderly people: double blind, randomised controlled trial. *Eur J Clin Nutr* **67,** 821–826.

Atherton PJ, Etheridge T, Watt PW, Wilkinson D, Selby A, Rankin D, Smith K & Rennie MJ (2010). Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. *The American Journal of Clinical Nutrition* **92,** 1080–1088.

Atherton PJ, Kumar V, Selby A, Rankin D, Hildebrandt W, Phillips B, Williams J, Hiscock N & Smith K (2016). Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. *Clinical Nutrition*; DOI: 10.1016/j.clnu.2016.04.025.

Atkins JL, Whincup PH, Morris RW, Lennon LT, Papacosta O & Wannamethee SG (2014). Sarcopenic Obesity and Risk of Cardiovascular Disease and Mortality: A Population-Based Cohort Study of Older Men. *Journal of the American Geriatrics Society* **62,** 253–260.

Babault N, Deley G, Le Ruyet P, Morgan F & Allaert FA (2014). Effects of soluble milk protein or casein supplementation on muscle fatigue following resistance training program: a randomized, double-blind, and placebo-controlled study. *J Int Soc Sports Nutr*; DOI: 10.1186/1550-2783-11-36.

Babault N, Païzis C, Deley G, Guérin-Deremaux L, Saniez M-H, Lefranc-Millot C & Allaert FA (2015). Pea proteins oral supplementation promotes muscle thickness gains during resistance training: a double-blind, randomized, Placebo-controlled clinical trial vs. Whey protein. *J Int Soc Sports Nutr* **12,** 3.

Babraj JA, Cuthbertson DJR, Smith K, Langberg H, Miller B, Krogsgaard MR, Kjaer M & Rennie MJ (2005). Collagen synthesis in human musculoskeletal tissues and skin. *Am J Physiol Endocrinol Metab* **289,** E864–E869.

Bar-Peled L, Schweitzer LD, Schweitzer LD, Zoncu R & Sabatini DM (2012). Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell* **150,** 1196–1208.

Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ & Lindeman RD (1998). Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* **147,** 755–763.

Beck TW, Housh TJ, Johnson GO, Coburn JW, Malek MH & Cramer JT (2007). Effects of a drink containing creatine, amino acids, and protein combined with ten weeks of resistance training on body composition, strength, and anaerobic performance. *The Journal of Strength & Conditioning Research* **21,** 100–104.

Bemben MG, Witten MS, Carter JM, Eliot KA, Knehans AW & Bemben DA (2010). The Effects of Supplementation with Creatine and Protein on Muscle Strength Following a Traditional Resistance Training Program in Middle-Aged and Older Men. *J Nutr Health Aging* **14,** 155–159.

Bennet WM, Connacher AA, Scrimgeour CM, Smith K & Rennie MJ (1989). Increase in anterior tibialis muscle protein synthesis in healthy man during mixed amino acid infusion: studies of incorporation of [1-13C]leucine. *Clin Sci* **76,** 447–454.

Biolo G, Fleming RYD & Wolfe RR (1995*a*). Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *Journal of Clinical Investigation* **95,** 811.

Biolo G, Maggi SP, Williams BD, Tipton KD & Wolfe RR (1995*b*). Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol* **268,** E514–E520.

Biolo G, Tipton KD, Klein S & Wolfe RR (1997). An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* **273,** E122–E129.

Bohé J, Low A, Wolfe RR & Rennie MJ (2003). Human Muscle Protein Synthesis is Modulated by Extracellular, Not Intramuscular Amino Acid Availability: A Dose-Response Study. *The Journal of Physiology* **552,** 315–324.

Bohé J, Low JF, Wolfe RR & Rennie MJ (2001). Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol (Lond)* **532,** 575–579.

Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL & Beaufrère B (1997*a*). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci USA* **94,** 14930–14935.

Boirie Y, Gachon P & Beaufrère B (1997*b*). Splanchnic and whole-body leucine kinetics in young and elderly men. *Am J Clin Nutr* **65,** 489–495.

Booth FW, Tseng BS, Flück M & Carson JA (1998). Molecular and cellular adaptation of muscle in response to physical training. *Acta Physiol Scand* **162,** 343–350.

Borsheim E, Aarsland A, RR W & Wolfe RR (2004). Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. *Int J Sport Nutr Exerc Metab* **14,** 255–271.

Borsheim E, Tipton KD, Wolf SE & Wolfe RR (2002). Essential amino acids and muscle protein recovery from resistance exercise. *Am J Physiol Endocrinol Metab* **283,** E648–E657.

Bos C, Metges CC, Gaudichon C, Petze KJ, Pueyo ME, Morens C, Everwand J, Benamouzig R & Tomé D (2003). Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. *The Journal of nutrition* **133,** 1308–1315.

Bouchard C ed. (2015). *Molecular and Cellular Regulation of Adaptation to Exercise*, First edition. Academic Press, Elsevier.

Brans G, Schroën CGPH, van der Sman RGM & Boom RM (2004). Membrane fractionation of milk: state of the art and challenges. *Journal of Membrane Science* **243,** 263–272.

Bray GA, Smith SR, de Jonge L, Xie H, Rood J, Martin CK, Most M, Brock C, Mancuso S & Redman LM (2012). Effect of dietary protein content on weight gain, energy expenditure, and body composition during overeating: a randomized controlled trial. *JAMA* **307,** 47–55.

Breen L, Stokes KA, Churchward-Venne TA, Moore DR, Baker SK, Smith K, Atherton PJ &

Phillips SM (2013). Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. *J Clin Endocrinol Metab* **98,** 2604–2612.

Brenner BM, Meyer TW & Hostetter TH (1982). Dietary Protein Intake and the Progressive Nature of Kidney Disease:. *N Engl J Med* **307,** 652–659.

Brown EC, DiSilvestro RA, Babaknia A & Devor ST (2004). Soy versus whey protein bars: Effects on exercise training impact on lean body mass and antioxidant status. *Nutr J*; DOI: 10.1186/1475-2891-3-22.

Buckley JD, Thomson RL, Coates AM, Howe PRC, DeNichilo MO & Rowney MK (2010). Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *J Sci Med Sport* **13,** 178–181.

Burd NA, Holwerda AM, Selby KC, West DWD, Staples AW, Cain NE, Cashaback JGA, Potvin JR, Baker SK & Phillips SM (2010*a*). Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *The Journal of Physiology* **588,** 3119–3130.

Burd NA, West DW, West DW, Rerecich T, Rerecich T, Prior T, Prior T, Baker SK & Phillips SM (2011*a*). Validation of a single biopsy approach and bolus protein feeding to determine myofibrillar protein synthesis in stable isotope tracer studies in humans. *Nutr Metab (Lond)* **8,** 15.

Burd NA, West DWD, Moore DR, Atherton PJ, Staples AW, Prior T, Tang JE, Rennie MJ, Baker SK & Phillips SM (2011*b*). Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *Journal of Nutrition* **141,** 568–573.

Burd NA, West DWD, Staples AW, Atherton PJ, Baker JM, Moore DR, Holwerda AM, Parise G, Rennie MJ, Baker SK & Phillips SM (2010*b*). Low-Load High Volume Resistance Exercise Stimulates Muscle Protein Synthesis More Than High-Load Low Volume Resistance Exercise in Young Men. *PLoS ONE* **5,** 12033.

Burd NA, Yang Y, Moore DR, Tang JE, Tarnopolsky MA & Phillips SM (2012). Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. *Br J Nutr* **108,** 958–962.

Burk A, Timpmann S, Medijainen L, Vähi M & Oöpik V (2009). Time-divided ingestion pattern of casein-based protein supplement stimulates an increase in fat-free body mass during resistance training in young untrained men. *Nutr Res* **29,** 405–413.

Burke DG, Chilibeck PD, Davison KS, Candow DG, Farthing J & Smith-Palmer T (2001). The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. *Int J Sport Nutr Exerc Metab* **11,** 349–364.

Burnett PE, Barrow RK, Cohen NA, Snyder SH & Sabatini DM (1998). RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proc Natl Acad Sci USA* **95,** 1432–1437.

Buse MG & Reid SS (1975). Leucine. A possible regulator of protein turnover in muscle. *J Clin*

Invest **56,** 1250–1261.

Calbet JAL & Holst JJ (2004). Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *Eur J Nutr* **43,** 127–139.

Camera DM, West DWD, Phillips SM, Rerecich T, Stellingwerff T, Hawley JA & Coffey VG (2015). Protein ingestion increases myofibrillar protein synthesis after concurrent exercise. *Med Sci Sport Exer* **47,** 82–91.

Candow DG & Burke NC (2006). Effect of whey and soy protein supplementation combined with resistance training in young adults. *Int J Sport Nutr Exerc Metab* **16,** 233-244.

Carraro F, Stuart CA, Hartl WH, Rosenblatt J & Wolfe RR (1990). Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am J Physiol Endocrinol Metab* **259,** E470–E476.

Cermak NMN, Res PTP, de Groot LCL, Saris WHW & van Loon LJL (2012). Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *The American Journal of Clinical Nutrition* **96,** 1454–1464.

Chale A, Cloutier GJ, Hau C, Phillips EM, Dallal GE & Fielding RA (2012). Efficacy of Whey Protein Supplementation on Resistance Exercise–Induced Changes in Lean Mass, Muscle Strength, and Physical Function in Mobility-Limited Older Adults. *J Gerontol A Biol Sci Med Sci*gls221.

Cheng SWY, Fryer LGD, Carling D & Shepherd PR (2004). Thr2446 is a novel mammalian target of rapamycin (mTOR) phosphorylation site regulated by nutrient status. *J Biol Chem* **279,** 15719–15722.

Chevalier S, Goulet EDB, Burgos SA, Wykes LJ & Morais JA (2011). Protein Anabolic Responses to a Fed Steady State in Healthy Aging. *J Gerontol A Biol Sci Med Sci* **66,** 681–688.

Choo AY, Yoon S-O, Kim SG, Roux PP & Blenis J (2008). Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proc Natl Acad Sci USA* **105,** 17414–17419.

Chromiak JA, Chromiak JA, Smedley B, Smedley B, Carpenter W, Carpenter W, Brown R, Brown R, Koh YS, Koh YS, Lamberth JG, Lamberth JG, Joe LA, Joe LA, Abadie BR, Abadie BR, Altorfer G & Altorfer G (2004). Effect of a 10-week strength training program and recovery drink on body composition, muscular strength and endurance, and anaerobic power and capacity. **20,** 420–427.

Churchward-Venne TA, Breen L, Di Donato DM, Hector AJ, Mitchell CJ, Moore DR, Stellingwerff T, Breuille D, Offord EA, Baker SK & Phillips SM (2014). Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. *The American Journal of Clinical Nutrition* **99,** 276–286.

Churchward-Venne TA, Burd NA, Mitchell CJ, West DWD, Philp A, Marcotte GR, Baker SK, Baar K & Phillips SM (2012). Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *The Journal of Physiology* **590,** 2751–2765.

Coffey VG & Hawley JA (2007). The molecular bases of training adaptation. *Sports Med* **37,** 737–763.

Cooke MB, Rybalka E, Stathis CG, Cribb PJ & Hayes A (2010). Whey protein isolate attenuates strength decline after eccentrically-induced muscle damage in healthy individuals. *J Int Soc Sports Nutr* **7,** 30.

Cribb PJ, Williams AD, Carey MF & Hayes A (2006). The effect of whey isolate and resistance training on strength, body composition, and plasma glutamine. *Int J Sport Nutr Exerc Metab* **16,** 494–509.

Crozier SJ, Kimball SR, Emmert SW, Anthony JC & Jefferson LS (2005). Oral Leucine Administration Stimulates Protein Synthesis in Rat Skeletal Muscle. *JN* **135,** 376–382.

Cuthbertson D, Cuthbertson D, Smith K, Babraj J, Leese G, Leese G, Waddell T, Waddell T, Atherton P, Wackerhage H, Taylor PM & Rennie MJ (2005). Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* **19,** 422–424.

Damas F, Phillips S, Vechin FC & Ugrinowitsch C (2015). A Review of Resistance Training-Induced Changes in Skeletal Muscle Protein Synthesis and Their Contribution to Hypertrophy. *Sports Med* **45,** 1–7.

Damas F, Phillips SM, Libardi CA, Vechin FC, Lixandrão ME, Jannig PR, Costa LAR, Bacurau AV, Snijders T, Parise G, Tricoli V, Roschel H & Ugrinowitsch C (2016). Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage. *The Journal of Physiology* **594,** 5209-5222; DOI: 10.1113/JP272472.

Denysschen CA, Burton HW, Horvath PJ, Leddy JJ & Browne RW (2009). Resistance training with soy vs whey protein supplements in hyperlipidemic males. *J Int Soc Sports Nutr* **6,** 8.

Dickinson JM, Drummond MJ, Coben JR, Volpi E & Rasmussen BB (2013). Aging differentially affects human skeletal muscle amino acid transporter expression when essential amino acids are ingested after exercise. *Clin Nutr* **32,** 273–280.

Dickinson JM, Fry CS, Drummond MJ, Gundermann DM, Walker DK, Glynn EL, Timmerman KL, Dhanani S, Volpi E & Rasmussen BB (2011). Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. *Journal of Nutrition* **141,** 856–862.

Dickinson JM, Gundermann DM, Walker DK, Reidy PT, Borack MS, Drummond MJ, Arora M, Volpi E & Rasmussen BB (2014). Leucine-Enriched Amino Acid Ingestion after Resistance Exercise Prolongs Myofibrillar Protein Synthesis and Amino Acid Transporter Expression in Older Men. *The Journal of nutrition* **144,** 1694–1702.

Dideriksen KJ, Reitelseder S, Petersen SG, Hjort M, Helmark IC, Kjaer M & Holm L (2011). Stimulation of muscle protein synthesis by whey and caseinate ingestion after resistance exercise in elderly individuals. *Scand J Med Sci Sports* **21,** E372–E383.

Dirks ML, Tieland M, Verdijk LB, Losen M, Nilwik R, Mensink M, de Groot LCPGM & van Loon LJC (2017). Protein Supplementation Augments Muscle Fiber Hypertrophy but Does Not Modulate Satellite Cell Content During Prolonged Resistance-Type Exercise Training in

Frail Elderly. *J Am Med Dir Assoc*; DOI: 10.1016/j.jamda.2017.02.006.

Dominguez LJ & Barbagallo M (2007). The cardiometabolic syndrome and sarcopenic obesity in older persons. *J Cardiometab Syndr* **2,** 183–189.

Dreyer HC, Drummond MJ, Pennings B, Fujita S, Glynn EL, Chinkes DL, Dhanani S, Volpi E & Rasmussen BB (2008). Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *Am J Physiol Endocrinol Metab* **294,** E392–E400.

Dreyer HC, Fujita S, Glynn EL, Drummond MJ, Volpi E & Rasmussen BB (2010). Resistance exercise increases leg muscle protein synthesis and mTOR signalling independent of sex. *Acta Physiol (Oxf)* **199,** 71–81.

Drummond MJ, Dreyer HC, Fry CS, Glynn EL & Rasmussen BB (2009*a*). Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *J Appl Physiol* **106,** 1374–1384.

Drummond MJ, Dreyer HC, Pennings B, Fry CS, Dhanani S, Dillon EL, Sheffield-Moore M, Volpi E & Rasmussen BB (2008). Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. *J Appl Physiol* **104,** 1452–1461.

Drummond MJ, Fry CS, Glynn EL, Dreyer HC, Dhanani S, Timmerman KL, Volpi E & Rasmussen BB (2009*b*). Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *The Journal of Physiology* **587,** 1535–1546.

Engelman JA, Luo J & Cantley LC (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* **7,** 606–619.

Esmarck B, Andersen JL, Olsen S, Richter EA, Mizuno M & Kjaer M (2001). Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol (Lond)* **535,** 301–311.

Evans WJ, PHINNEY SD & YOUNG VR (1982). Suction Applied to a Muscle Biopsy Maximizes Sample-Size. *Med Sci Sport Exer* **14,** 101–102.

Farnfield MM, Breen L, Carey KA, Garnham A & Cameron-Smith D (2012). Activation of mTOR signalling in young and old human skeletal muscle in response to combined resistance exercise and whey protein ingestion. *Appl Physiol Nutr Metab* **37,** 21–30.

Farnfield MM, Carey KA, Gran P, Trenerry MK & Cameron-Smith D (2009*a*). Whey protein ingestion activates mTOR-dependent signalling after resistance exercise in young men: a double-blinded randomized controlled trial. *Nutrients* **1,** 263–275.

Farnfield MM, Trenerry C, Carey KA & Cameron-Smith D (2009*b*). Plasma amino acid response after ingestion of different whey protein fractions. *Int J Food Sci Nutr* **60,** 476–486.

Farup J, Rahbek SK, Storm AC, Klitgaard S, Jorgensen H, Bibby BM, Serena A & Vissing K (2016). Effect of degree of hydrolysis of whey protein on in vivo plasma amino acid appearance in humans. *Springerplus* **5,** 532.

Fox PF, Uniacke-Lowe T, McSweeney PLH & O'Mahony JA (2015). *Dairy Chemistry and Biochemistry*. Springer International Publishing, Cham.

Francaux M, Demeulder B, Naslain D, Fortin R, Lutz O, Caty G & Deldicque L (2016). Aging Reduces the Activation of the mTORC1 Pathway after Resistance Exercise and Protein Intake in Human Skeletal Muscle: Potential Role of REDD1 and Impaired Anabolic Sensitivity. *Nutrients* **8,** 47.

Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ & Roubenoff R (2000). Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol* **88,** 1321–1326.

Fry CS, Drummond MJ, Glynn EL, Dickinson JM, Gundermann DM, Timmerman KL, Walker DK, Dhanani S, Volpi E & Rasmussen BB (2011). Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis. *Skelet Muscle* **1,** 11.

Gilani GS, Xiao CW & Cockell KA (2012). Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on Protein Quality. *Br J Nutr* **108,** S315–S332.

Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszynska A, Aebersold R & Sonenberg N (2001). Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes & Development* **15,** 2852–2864.

Glover EI, Phillips SM, Oates BR, Tang JE, Tarnopolsky MA, Selby A, Smith K & Rennie MJ (2008). Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. *The Journal of Physiology* **586,** 6049–6061.

Glynn EL, Fry CS, Drummond MJ, Dreyer HC, Dhanani S, Volpi E & Rasmussen BB (2010*a*). Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *Am J Physiol Regul Integr Comp Physiol* **299,** R533–R540.

Glynn EL, Fry CS, Drummond MJ, Timmerman KL, Dhanani S, Volpi E & Rasmussen BB (2010*b*). Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *Journal of Nutrition* **140,** 1970–1976.

Glynn EL, Fry CS, Timmerman KL, Drummond MJ, Volpi E & Rasmussen BB (2013). Addition of Carbohydrate or Alanine to an Essential Amino Acid Mixture Does Not Enhance Human Skeletal Muscle Protein Anabolism. *Journal of Nutrition* **143,** 307–314.

Gorissen SH, Burd NA, Hamer HM, Gijsen AP, Groen BB & van Loon LJ (2014). Carbohydrate co-ingestion delays dietary protein digestion and absorption but does not modulate postprandial muscle protein accretion. *J Clin Endocrinol Metab*jc.2013–jc.3970.

Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, Wackerhage H, Smith K, Atherton P, Selby A & Rennie MJ (2008). Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* **295,** E595–E604.

Grimble GK, Guilera Sarda M, Sessay HF, Marrett AL, Kapadia SA, Bowling TE & Silk DBA (1994). The influence of whey hydrolysate peptide chain length on nitrogen and carbohydrate absorption in the perfused human jejunum. *Clinical Nutrition* **13,** 46.

Groen BBL, Horstman AM, Hamer HM, de Haan M, Van Kranenburg J, Bierau J, Poeze M, Wodzig WKWH, Rasmussen BB & van Loon LJC (2015). Post-Prandial Protein Handling: You Are What You Just Ate. *PLoS ONE* **10,** e0141582.

Gruther W, Benesch T, Zorn C, Paternostro-Sluga T, Quittan M, Fialka-Moser V, Spiss C, Kainberger F & Crevenna R (2008). Muscle Wasting in Intensive Care Patients: Ultrasound Observation of the M. Quadriceps Femoris Muscle Layer. *Journal of Rehabilitation and Medicine* **40,** 185–189.

Guillet C, Prod'homme M, Balage M, Gachon P, Giraudet C, Morin L, Grizard J & Boirie Y (2004). Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *FASEB J* **18,** 1586–1587.

Gundermann DM, Walker DK, Reidy PT, Borack MS, Dickinson JM, Volpi E & Rasmussen BB (2014). Activation of mTORC1 signaling and protein synthesis in human muscle following blood flow restriction exercise is inhibited by rapamycin. *Am J Physiol Endocrinol Metab* **306,** E1198–E1204.

Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE & Shaw RJ (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* **30,** 214–226.

Hagenfeldt L, Eriksson S & Wahren J (1980). Influence of leucine on arterial concentrations and regional exchange of amino acids in healthy subjects. *Clin Sci* **59,** 173–181.

Harber MP, Konopka AR, Jemiolo B, Trappe SW, Trappe TA & Reidy PT (2010). Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states. *Am J Physiol Regul Integr Comp Physiol* **299,** R1254–R1262.

Hardie DG (2011). Energy sensing by the AMP-activated protein kinase and its effects on muscle metabolism. *Proc Nutr Soc* **70,** 92–99.

Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV & Phillips SM (2007). Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *Am J Clin Nutr* **86,** 373–381.

Hather BM, Tesch PA, Buchanan P & Dudley GA (1991). Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiol Scand* **143,** 177–185.

Helsedirektoratet (2011). *Kostråd for å fremme folkehelsa og forebygge kroniske sykdommer*. Helsedirektoratet.

Hidayat K, Chen GC, Wang Y, Zhang Z, Dai X, Szeto IMY & Qin L-Q (2017). Effects of milk proteins supplementation in older adults undergoing resistance training: A meta-analysis of randomized control trials. *J Nutr Health Aging*1–9.

Hoffman JR, Ratamess NA, Tranchina CP, Rashti SL, Kang J & Faigenbaum AD (2009). Effect of protein-supplement timing on strength, power, and body-composition changes in resistance-trained men. *Int J Sport Nutr Exerc Metab* **19,** 172–185.

Holm L, Olesen JL, Matsumoto K, Doi T, Mizuno M, Alsted TJ, Mackey AL, Schwarz P & Kjaer M (2008). Protein-containing nutrient supplementation following strength training enhances the effect on muscle mass, strength, and bone formation in postmenopausal women. *J Appl Physiol* **105,** 274–281.

Hoppeler H (2016). Molecular networks in skeletal muscle plasticity. *Journal of Experimental Biology*

219, 205–213.

Hulmi JJ, Kovanen V, Selänne H & Kraemer WJ (2009*a*). Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression - Springer. *Amino Acids*.

Hulmi JJ, Tannerstedt J, Selänne H, Kainulainen H, Kovanen V & Mero AA (2009*b*). Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *J Appl Physiol* **106,** 1720–1729.

Hvid L, Aagaard P, Justesen L, Bayer ML, Andersen JL, Ørtenblad N, Kjaer M & Suetta C (2010). Effects of aging on muscle mechanical function and muscle fiber morphology during short-term immobilization and subsequent retraining. *J Appl Physiol* **109,** 1628–1634.

Inoki K, Li Y, Xu T & Guan K-L (2003*a*). Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes & Development* **17,** 1829–1834.

Inoki K, Zhu T & Guan K-L (2003*b*). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115,** 577–590.

Ivy JL & Portman R (2007). *Nutrient Timing: The Future of Sports Nutrition* ed. Carol R. Basic Health Publications Inc.: Laguna Beach, CA, USA.

Jaleel A, Short KR, Asmann YW, Klaus KA, Morse DM, Ford GC & Nair KS (2008). In vivo measurement of synthesis rate of individual skeletal muscle mitochondrial proteins. *Am J Physiol Endocrinol Metab* **295,** E1255–E1268.

Joint WHO/FAO/UNU Expert Consultation (2007). *Protein and amino acid requirements in human nutrition.*

Josse AR, Tang JE, Tarnopolsky MA & Phillips SM (2010). Body composition and strength changes in women with milk and resistance exercise. *Med Sci Sport Exer* **42,** 1122–1130.

Joy JM & Gundermann DM (2014). Nutrition & Metabolism | Abstract | Phosphatidic acid enhances mTOR signaling and resistance exercise induced hypertrophy. *Nutrition & Metabolism* **11,** 29.

Joy JM, Lowery RP, Wilson JM, Purpura M, De Souza EO, Wilson SM, Kalman DS, Dudeck JE & Jäger R (2013). The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr J* **12,** 86.

Kakigi R, Kakigi R, Yoshihara T, Yoshihara T, Ozaki H, Ozaki H, Ogura Y, Ogura Y, Ichinoseki-Sekine N, Ichinoseki-Sekine N, Kobayashi H, Kobayashi H, Naito H & Naito H (2014). Whey protein intake after resistance exercise activates mTOR signaling in a dose-dependent manner in human skeletal muscle. *Eur J Appl Physiol*; DOI: 10.1007/s00421-013-2812-7.

Kang SA, Pacold ME, Cervantes CL, Lim D, Lou HJ, Ottina K, Gray NS, Turk BE, Yaffe MB & Sabatini DM (2013). mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin. *Science* **341,** 1236566–1236566.

Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A & Wolfe RR (2006). A high proportion of leucine is required for optimal stimulation of the rate of muscle protein

synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* **291,** E381–E387.

Kim I-Y, Schutzler S, Schrader A, Spencer H, Kortebein P, Deutz NEP, Wolfe RR & Ferrando AA (2014). Quantity of dietary protein intake, but not pattern of intake, affects net protein balance primarily through differences in protein synthesis in older adults. *AJP: Endocrinology and Metabolism* **308,** ajpendo.00382.2014–ajpendo.00382.2018.

Kim I-Y, Schutzler S, Schrader AM, Spencer HJ, Azhar G, Wolfe RR & Ferrando AA (2017). Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults: A randomized-controlled trial. *Clin Nutr*; DOI: 10.1016/j.clnu.2017.02.020.

Kim PL, Kim PL, Staron RS, Staron RS & Phillips SM (2005). Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *The Journal of Physiology* **568,** 283–290.

Kiskini A, Hamer HM, Wall BT, Groen BBL, Lange A, Bakker JA, Senden JMG, Verdijk LB & Loon LJC (2013). The muscle protein synthetic response to the combined ingestion of protein and carbohydrate is not impaired in healthy older men. *Age (Dordr)* **35,** 2389–2398.

Koopman R, Crombach N, Gijsen AP, Walrand S, Fauquant J, Kies AK, Lemosquet S, Saris WHM, Boirie Y & van Loon LJC (2009*a*). Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *The American Journal of Clinical Nutrition* **90,** 106–115.

Koopman R, Pennings B, Zorenc AHG & van Loon LJC (2007). Protein ingestion further augments S6K1 phosphorylation in skeletal muscle following resistance type exercise in males. *The Journal of nutrition* **137,** 1880–1886.

Koopman R, Verdijk L, Manders RJF, Gijsen AP, Gorselink M, Pijpers E, Wagenmakers AJM & van Loon LJC (2006). Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. *The American Journal of Clinical Nutrition* **84,** 623–632.

Koopman R, Wagenmakers AJM, Manders RJF, Zorenc AHG, Senden JMG, Gorselink M, Keizer HA & van Loon LJC (2005). Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab* **288,** E645–E653.

Koopman R, Walrand S, Beelen M, Gijsen AP, Kies AK, Boirie Y, Saris WHM & van Loon LJC (2009*b*). Dietary protein digestion and absorption rates and the subsequent postprandial muscle protein synthetic response do not differ between young and elderly men. *Journal of Nutrition* **139,** 1707–1713.

Krogh-Madsen R, Thyfault JP, Broholm C, Mortensen OH, Olsen RH, Mounier R, Plomgaard P, van Hall G, Booth FW & Pedersen BK (2010). A 2-wk reduction of ambulatory activity attenuates peripheral insulin sensitivity. *Journal of Applied Physiology* **108,** 1034–1040.

Kubo K, Ishida Y, Suzuki S, Komuro T, Shirasawa H, Ishiguro N, Shukutani Y, Tsunoda N, Kanehisa H & Fukunaga T (2008). Effects of 6 months of walking training on lower limb muscle and tendon in elderly. *Scand J Med Sci Sports* **18,** 31–39.

Kukuljan S, Kukuljan S, Nowson CA, Nowson CA, Sanders K, Sanders K, Daly RM & Daly RM (2009). Effects of resistance exercise and fortified milk on skeletal muscle mass, muscle size, and functional performance in middle-aged and older men: an 18-mo randomized controlled trial. *J Appl Physiol* **107,** 1864–1873.

Kumar V, Atherton PJ, Selby A, Rankin D, Williams J, Smith K, Hiscock N & Rennie MJ (2012). Muscle protein synthetic responses to exercise: effects of age, volume, and intensity. *J Gerontol A Biol Sci Med Sci* **67,** 1170–1177.

Kumar V, Selby A, Rankin D, Patel R, Atherton P, Hildebrandt W, Williams J, Smith K, Seynnes O, Hiscock N & Rennie MJ (2009). Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *The Journal of Physiology* **587,** 211–217.

Lactalis (n.d.). Pronativ.com. *httppronativcomhome-research.* Available at: http://pronativ.com/#home-research [Accessed August 22, 2017].

Laplante M & Sabatini DM (2012). mTOR Signaling in Growth Control and Disease. *Cell* **149,** 274–293.

Leenders M, Verdijk LB, Van der Hoeven L, Van Kranenburg J, Nilwik R & van Loon LJC (2013). Elderly men and women benefit equally from prolonged resistance-type exercise training. *J Gerontol A Biol Sci Med Sci* **68,** 769–779.

Lemon P, Tarnopolsky MA, MacDougall JD & Atkinson SA (1992). Protein-Requirements and Muscle Mass Strength Changes During Intensive Training in Novice Bodybuilders. *J Appl Physiol* **73,** 767–775.

Lemon PWR, Berardi JM & Noreen EE (2002). The role of protein and amino acid supplements in the athlete's diet: does type or timing of ingestion matter? *Current Sports Medicine Reports* **1,** 214–221.

Leser S (2013). The 2013 FAO report on dietary protein quality evaluation in human nutrition: Recommendations and implications. *Nutrition Bulletin* **38,** 421–428.

Levinger I, Goodman C, Hare DL, Jerums G, Toia D & Selig S (2009). The reliability of the 1RM strength test for untrained middle-aged individuals. *J Sci Med Sport* **12,** 310–316.

Ma XM & Blenis J (2009). Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* **10,** 307–318.

Macnaughton LS, Wardle SL, Witard OC, McGlory C, Hamilton DL, Jeromson S, Lawrence CE, Wallis GA & Tipton KD (2016). The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. *Physiol Rep* **4,** e12893.

Mamerow MM, Mettler JA, English KL, Casperson SL, Arentson-Lantz E, Sheffield-Moore M, Layman DK & Paddon-Jones D (2014). Dietary Protein Distribution Positively Influences 24-h Muscle Protein Synthesis in Healthy Adults. *The Journal of nutrition* **144,** 876–880.

Marcotte GR, West DWD & Baar K (2014). The Molecular Basis for Load-Induced Skeletal Muscle Hypertrophy. *Calcif Tissue Int*; DOI: 10.1007/s00223-014-9925-9.

Markofski MM, Dickinson JM, Drummond MJ, Fry CS, Fujita S, Gundermann DM, Glynn EL, Jennings K, Paddon-Jones D, Reidy PT, Sheffield-Moore M, Timmerman KL, Rasmussen BB & Volpi E (2015). Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women. *Experimental Gerontology* **65,** 1–7.

Martin WF, Armstrong LE & Rodriguez NR (2005). Dietary protein intake and renal function. *Nutr Metab (Lond)* **2,** 25.

Matthews DE, Marano MA & Campbell RG (1993). Splanchnic bed utilization of leucine and phenylalanine in humans. *Am J Physiol* **264,** E109–E118.

Mayhew DL, Kim J-S, Cross JM, Cross JM, Ferrando AA & Bamman MM (2009). Translational signaling responses preceding resistance training-mediated myofiber hypertrophy in young and old humans. *J Appl Physiol* **107,** 1655–1662.

Melendez MM, Vosswinkel JA, Shapiro MJ, Gelato MC, Mynarcik D, Gavi S, Xu X & McNurlan M (2007). Wall suction applied to needle muscle biopsy - A novel technique for increasing sample size. *J Surg Res* **142,** 301–303.

Miller BF, Olesen JL, Hansen M, Døssing S, Crameri RM, Welling RJ, Langberg H, Flyvbjerg A, Kjaer M, Babraj JA, Smith K & Rennie MJ (2005). Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J Physiol (Lond)* **567,** 1021–1033.

Miller PE, Alexander DD & Perez V (2014). Effects of whey protein and resistance exercise on body composition: a meta-analysis of randomized controlled trials. *Journal of the American College of Nutrition* **33,** 163–175.

Mitchell CJ, Churchward-Venne TA, Parise G, Bellamy L, Baker SK, Smith K, Atherton PJ & Phillips SM (2014). Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men. ed. Müller M. *PLoS ONE* **9,** e89431.

Mitchell CJ, D'Souza RF, Fanning AC, Poppitt SD & Cameron-Smith D (2017). Short communication: Muscle protein synthetic response to microparticulated whey protein in middle-aged men. *Journal of Dairy Science*; DOI: 10.3168/jds.2016-12287.

Mitchell CJ, McGregor RA, D'Souza RF, Thorstensen EB, Markworth JF, Fanning AC, Poppitt SD & Cameron-Smith D (2015*a*). Consumption of Milk Protein or Whey Protein Results in a Similar Increase in Muscle Protein Synthesis in Middle Aged Men. *Nutrients* **7,** 8685–8699.

Mitchell CJ, Oikawa SY, Ogborn DI, Nates NJ, MacNeil LG, Tarnopolsky M & Phillips SM (2015*b*). Daily chocolate milk consumption does not enhance the effect of resistance training in young and old men: a randomized controlled trial. *Appl Physiol Nutr Metab* **40,** 199–202.

Mitchell WK, Phillips BE, Williams JP, Rankin D, Lund JN, Smith K & Atherton PJ (2015*c*). A Dose- rather than Delivery Profile-Dependent Mechanism Regulates the "Muscle-Full" Effect in Response to Oral Essential Amino Acid Intake in Young Men. *The Journal of nutrition* **145,** 207–214.

Mitchell WK, Phillips BE, Williams JP, Rankin D, Lund JN, Wilkinson DJ, Smith K & Atherton PJ (2015*d*). The impact of delivery profile of essential amino acids upon skeletal muscle

protein synthesis in older men: clinical efficacy of pulse vs. bolus supply. *Am J Physiol Endocrinol Metab* **309,** E450–E457.

Mitchell WK, Phillips BE, Williams JP, Rankin D, Smith K, Lund JN & Atherton PJ (2013). Development of a new Sonovue™ contrast-enhanced ultrasound approach reveals temporal and age-related features of muscle microvascular responses to feeding. *Physiol Rep* **1,** e00119.

Moberg M, Apró W, Ekblom B, van Hall G, Holmberg H-C & Blomstrand E (2016). Activation of mTORC1 by leucine is potentiated by branched chain amino acids and even more so by essential amino acids following resistance exercise. *Am J Physiol, Cell Physiol*ajpcell.00374.2015.

Moore DR, Atherton PJ, Rennie MJ, Tarnopolsky MA & Phillips SM (2011). Resistance exercise enhances mTOR and MAPK signalling in human muscle over that seen at rest after bolus protein ingestion. *Acta Physiol (Oxf)* **201,** 365–372.

Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD & Phillips SM (2014). Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in Healthy Older Versus Younger Men. *J Gerontol A Biol Sci Med Sci* **70,** 57–62.

Moore DR, Moore DR, Tang JE, Tang JE, Burd NA, Burd NA, Rerecich T, Rerecich T, Tarnopolsky MA & Phillips SM (2009*a*). Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *The Journal of Physiology* **587,** 897–904.

Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA & Phillips SM (2009*b*). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *The American Journal of Clinical Nutrition* **89,** 161–168.

Morley JE et al. (2011). Sarcopenia with limited mobility: an international consensus. *J Am Med Dir Assoc* **12,** 403–409.

Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmans M, Helms E, Aragon AA, Devries MC, Banfield L, Krieger JW & Phillips SM (2017). A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med*bjsports–2017–097608.

O'Neil TK, Duffy LR, Frey JW & Hornberger TA (2009). The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. *The Journal of Physiology* **587,** 3691–3701.

Paddon-Jones D, Sheffield-Moore M, Zhang X-J, Volpi E, Wolf SE, Aarsland A, Ferrando AA & Wolfe RR (2004). Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* **286,** E321–E328.

Paulsen G, Hamarsland H, Cumming KT, Johansen RE, Hulmi JJ, Borsheim E, Wiig H, Garthe I & Raastad T (2014). Vitamin C and E supplementation alters protein signalling after a strength training session, but not muscle growth during 10 weeks of training. *The Journal of Physiology* **592,** 5391–5408.

Paulsen G, Mikkelsen UR, Raastad T & Peake JM (2012). Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev* **18,** 42–97.

Pennings B, Boirie Y, Senden JMG, Gijsen AP, Kuipers H & van Loon LJC (2011*a*). Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *The American Journal of Clinical Nutrition* **93,** 997–1005.

Pennings B, Groen B, Groen B, de Lange A, de Lange A, Gijsen AP, Zorenc AH, Zorenc AH, Senden JMG & van Loon LJC (2012). Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *Am J Physiol Endocrinol Metab* **302,** E992–E999.

Pennings B, Groen BBL, van Dijk J-W, de Lange A, Kiskini A, Kuklinski M, Senden JMG & van Loon LJC (2013). Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men. *The American Journal of Clinical Nutrition* **98,** 121–128.

Pennings B, Koopman R, Beelen M, Senden JMG, Saris WHM & van Loon LJC (2011*b*). Exercising before protein intake allows for greater use of dietary protein-derived amino acids for de novo muscle protein synthesis in both young and elderly men. *The American Journal of Clinical Nutrition* **93,** 322–331.

Peterson MD, Rhea MR & Alvar BA (2004). Maximizing Strength Development in Athletes: A Meta-Analysis to Determine the Dose-Response Relationship. *J Strength Cond Res* **18,** 377–382.

Phillips BE, Hill DS, Atherton PJ. (2012). Regulation of muscle protein synthesis in humans. *Clinical Nutrition and Metabolic Care* **15,** 58–63.

Phillips SM (2014). A brief review of critical processes in exercise-induced muscular hypertrophy. *Sports Med* **44 Suppl 1,** 71–77.

Phillips SM, Tipton KD, Aarsland A, Wolf SE & Wolfe RR (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. **273,** E99–E107.

Phillips SM, Tipton KD, Ferrando AA & Wolfe RR (1999). Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *Am J Physiol* **276,** E118–E124.

Pullen N, Dennis PB, Andjelkovic M, Dufner A, Kozma SC, Hemmings BA & Thomas G (1998). Phosphorylation and Activation of p70s6k by PDK1. *Science* **279,** 707–710.

Puthucheary ZA et al. (2013). Acute Skeletal Muscle Wasting in Critical Illness. *JAMA* **310,** 1591–1600.

Rankin JW, Goldman LP, Puglisi MJ, Nickols-Richardson SM, Earthman CP & Gwazdauskas FC (2004). Effect of post-exercise supplement consumption on adaptations to resistance training. *Journal of the American College of Nutrition* **23,** 322–330.

Rasmussen BB & Phillips SM (2003). Contractile and nutritional regulation of human muscle growth. *Exerc Sport Sci Rev* **31,** 127–131.

Rasmussen BB, Fujita S, Wolfe RR, Mittendorfer B, Roy M, Rowe VL, Rowe VL & Volpi E

(2006). Insulin resistance of muscle protein metabolism in aging. *FASEB J* **20,** 768–769.

Rasmussen BB, Tipton KD, Miller SL, Wolf SE & Wolfe RR (2000). An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *Journal of Applied Physiol* **88,** 386-392.

Redpath NT, Price NT, Severinov KV & Proud CG (1993). Regulation of elongation factor-2 by multisite phosphorylation. *FEBS J* **213,** 689–699.

Reidy PT & Rasmussen BB (2016). Role of Ingested Amino Acids and Protein in the Promotion of Resistance Exercise-Induced Muscle Protein Anabolism. *Journal of Nutrition* **146,** 155–183.

Reidy PT, Borack MS, Markofski MM, Dickinson JM, Deer RR, Husaini SH, Walker DK, Igbinigie S, Robertson SM, Cope MB, Mukherjea R, Hall-Porter JM, Jennings K, Volpi E & Rasmussen BB (2016). Protein Supplementation Has Minimal Effects on Muscle Adaptations during Resistance Exercise Training in Young Men: A Double-Blind Randomized Clinical Trial. *Journal of Nutrition* **146,** 1660–1669.

Reidy PT, Walker DK, Dickinson JM, Gundermann DM, Drummond MJ, Timmerman KL, Cope MB, Mukherjea R, Jennings K, Volpi E & Rasmussen BB (2014). Soy-Dairy Protein Blend and Whey Protein Ingestion After Resistance Exercise Increases Amino Acid Transport and Transporter Expression in Human Skeletal Muscle. *J Appl Physiol* **116,** 1353–1364.

Reidy PT, Walker DK, Dickinson JM, Gundermann DM, Drummond MJ, Timmerman KL, Fry CS, Borack MS, Borack MS, Cope MB, Cope MB, Mukherjea R, Mukherjea R, Jennings K, Volpi E & Rasmussen BB (2013). Protein Blend Ingestion Following Resistance Exercise Promotes Human Muscle Protein Synthesis. *The Journal of nutrition*; DOI: 10.3945/jn.112.168021.

Reitelseder S, Agergaard J, Doessing S, Helmark IC, Lund P, Kristensen NB, Frystyk J, Flyvbjerg A, Schjerling P, van Hall G, Kjaer M & Holm L (2011). Whey and casein labeled with L-[1-13C]leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. *AJP: Endocrinology and Metabolism* **300,** E231–E242.

Reitelseder S, Agergaard J, Doessing S, Helmark IC, Schjerling P, van Hall G, Kjaer M & Holm L (2014). Positive muscle protein net balance and differential regulation of atrogene expression after resistance exercise and milk protein supplementation. *Eur J Nutr* **53,** 321–333.

Rennie MJ (2009). Anabolic resistance: the effects of aging, sexual dimorphism, and immobilization on human muscle protein turnover. *Appl Physiol Nutr Metab* **34,** 377–381.

Rennie MJ, Edwards RH, Halliday D, Matthews DE, Wolman SL & Millward DJ (1982). Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clin Sci* **63,** 519–523.

Rhea MR, Alvar BA, Burkett LN & Ball SD (2003). A Meta-analysis to Determine the Dose Response for Strength Development. *Med Sci Sport Exer* **35,** 456.

Rieu I, Balage M, Sornet C, Giraudet C, Pujos E, Grizard J, Mosoni L & Dardevet D (2006). Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *J Physiol (Lond)* **575,** 305–315.

Ritti-Dias RM, Avelar A, Salvador EP & Cyrino ES (2011). Influence of Previous Experience on Resistance Training on Reliability of One-Repetition Maximum Test. *The Journal of Strength & Conditioning Research* **25,** 1418–1422.

Rozenek R, Ward P, Long S & Garhammer J (2002). Effects of high-calorie supplements on body composition and muscular strength following resistance training. *J Sports Med Phys Fitness* **42,** 340–347.

Ruiz JR, Sui X, Lobelo F, Morrow JR, Jackson AW, Sjöström M & Blair SN (2008). Association between muscular strength and mortality in men: prospective cohort study. *BMJ* **337,** a439.

Rutherfurd SM & Moughan PJ (2012). Available versus digestible dietary amino acids. *Br J Nutr* **108,** S298–S305.

Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Markhard AL, Nada S, Nada S & Sabatini DM (2010). Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* **141,** 290–303.

Sancak Y, Peterson TR, Shaul YD, Shaul YD, Lindquist RA, Lindquist RA, Thoreen CC, Thoreen CC, Bar-Peled L & Sabatini DM (2008). The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* **320,** 1496–1501.

Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA & Sabatini DM (2007). PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* **25,** 903–915.

Schaafsma G (2000). The protein digestibility-corrected amino acid score. *The Journal of nutrition* **130,** 1865S–7S.

Schmidt RH, Packard VS & Morris HA (1984). Effect of Processing on Whey Protein Functionality. *Journal of Dairy Science* **67,** 2723–2733.

Schoenfeld BJ, Aragon AA & Krieger JW (2013). The effect of protein timing on muscle strength and hypertrophy: a meta-analysis. *J Int Soc Sports Nutr* **10,** 53.

Sharples AP (2017). Cellular and Molecular Exercise Physiology: A Historical Perspective for the Discovery of Mechanisms Contributing to Skeletal Muscle Adaptation. *Cellular and Molecular Exercise Physiology* **5,** e10.

Smith GI, Patterson BW & Mittendorfer B (2011). Human muscle protein turnover—why is it so variable? *Journal of Applied Physiology*.

Smith K, Barua JM, Watt PW, Scrimgeour CM & Rennie MJ (1992*a*). Flooding with L-[1-13C]leucine stimulates human muscle protein incorporation of continuously infused L-[1-13C]valine. *Am J Physiol* **262,** E372–E376.

Smith K, Essen P, McNurlan MA, Rennie MJ, Garlick PJ & Wernerman J (1992*b*). A multi-tracer investigation of the effect of a flooding dose administered during the constant infusion of tracer amino acid on the rate of tracer incorporation into human muscle protein (Abstract). *Proc Nutr Soc*.

Smith K, Reynolds N, Downie S, Patel A & Rennie MJ (1998). Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. *Am J Physiol* **275,** E73–

E78.

Staron RS, Malicky ES, Leonardi MJ, Falkel JE, Hagerman FC & Dudley GA (1990). Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *Eur J Appl Physiol Occup Physiol* **60,** 71–79.

Symons TB, Schutzler SE, Cocke TL, Chinkes DL, Wolfe RR & Paddon-Jones D (2007). Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr* **86,** 451–456.

Symons TB, Sheffield-Moore M, Wolfe RR & Paddon-Jones D (2009). A Moderate Serving of High-Quality Protein Maximally Stimulates Skeletal Muscle Protein Synthesis in Young and Elderly Subjects. *Journal of the American Dietetic Association* **109,** 1582–1586.

Symonsi TB, Sheffield-Moore M, Mamerow MM, Wolfe RR & Paddon-Jones D (2010). The anabolic response to resistance exercise and a protein-rich meal is not diminished by age. *J Nutr Health Aging* **15,** 376–381.

Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA & Phillips SM (2009). Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol* **107,** 987–992.

Tang JE, Perco JG, Moore DR, Wilkinson SB & Phillips SM (2008). Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am J Physiol Regul Integr Comp Physiol* **294,** R172–R178.

Tang JE, Manolakos JJ, Kujbida GW, Lysecki PJ, Moore DR & Phillips SM (2007). Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men. *Appl. Physiol. Nutr. Metab.* **32,** 1132-1138.

Tarnopolsky MA, Pearce E, Smith K & Lach B (2011). Suction-Modified Bergstrom Muscle Biopsy Technique: Experience with 13,500 Procedures. *Muscle Nerve* **43,** 717–725.

Tee AR, Manning BD, Roux PP, Cantley LC & Blenis J (2003). Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol* **13,** 1259–1268.

Tesch PA (1988). Skeletal muscle adaptations consequent to long-term heavy resistance exercise. *Med Sci Sport Exer* **20,** S132–S134.

Thomas DT, Wideman L & Lovelady CA (2011). Effects of a Dairy Supplement and Resistance Training on Lean Mass and Insulin-Like Growth Factor in Women. *Int J Sport Nutr Exerc Metab* **21,** 181–188.

Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM & Gray NS (2009). An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem* **284,** 8023–8032.

Tieland M, Tieland M, Dirks ML, van der Zwaluw N, van der Zwaluw N, Verdijk LB, van de Rest O, van de Rest O, de Groot LCPGM, de Groot LCPGM & van Loon LJC (2012). Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* **13,** 713–719.

Timmerman KL, Lee JL, Dreyer HC, Dhanani S, Glynn EL, Fry CS, Drummond MJ, Sheffield-Moore M, Rasmussen BB & Volpi E (2010*a*). Insulin stimulates human skeletal muscle protein synthesis via an indirect mechanism involving endothelial-dependent vasodilation and mammalian target of rapamycin complex 1 signaling. *J Clin Endocrinol Metab* **95,** 3848–3857.

Timmerman KL, Lee JL, Fujita S, Dhanani S, Dreyer HC, Fry CS, Drummond MJ, Sheffield-Moore M, Rasmussen BB & Volpi E (2010*b*). Pharmacological vasodilation improves insulin-stimulated muscle protein anabolism but not glucose utilization in older adults. *Diabetes* **59,** 2764–2771.

Tipton KD (2011). Efficacy and consequences of very-high-protein diets for athletes and exercisers. *Proceedings of the Nutrition Society* **70,** 205-214.

Tipton KD, Borsheim E, Wolf SE, Sanford AP & Wolfe RR (2003). Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion. *Am J Physiol Endocrinol Metab* **284,** E76–E89.

Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP & Wolfe RR (2004). Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. *Med Sci Sport Exer* **36,** 2073–2081.

Tipton KD, Ferrando AA, Phillips SM, Doyle D & Wolfe RR (1999*a*). Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* **276,** E628–E634.

Tipton KD, Gurkin BE, Matin S & Wolfe RR (1999*b*). Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J Nutr Biochem* **10,** 89–95.

Tipton KD, Rasmussen BB, Miller SL, Wolf SE, Owens-Stovall SK, Owens-Stovall SK, Petrini BE, Petrini BE & Wolfe RR (2001). Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab*.

van Vliet S, Burd NA & van Loon LJC (2015). The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption. *Journal of Nutrition* **145,** 1981–1991.

Verdijk LB, Jonkers RAM, Gleeson BG, Beelen M, Meijer K, Savelberg HHCM, Wodzig WKWH, Dendale P & van Loon LJC (2009). Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy after resistance training in elderly men. *The American Journal of Clinical Nutrition* **89,** 608–616.

Volek JS et al. (2013). Whey protein supplementation during resistance training augments lean body mass. *Journal of the American College of Nutrition* **32,** 122–135.

Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B & Wolfe RR (2003). Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* **78,** 250–258.

Volpi E, Mittendorfer B, Rasmussen BB & Wolfe RR (2000). The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab* **85,** 4481–4490.

Volpi E, Mittendorfer B, Wolf SE & Wolfe RR (1999). Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol* **277,** E513–E520.

Volpi E, Sheffield-Moore M, Rasmussen BB & Wolfe RR (2001). Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *JAMA* **286,** 1206–1212.

Wahren J, Felig P & Hagenfeldt L (1976). Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. *J Clin Invest* **57,** 987–999.

Waterlow JC (1984). Protein-Turnover with Special Reference to Man. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **69,** 409–438.

Weisgarber KD, Candow DG & Vogt ESM (2012). Whey Protein Before and During Resistance Exercise Has No Effect on Muscle Mass and Strength in Untrained Young Adults. *Int J Sport Nutr Exerc Metab* **22,** 463–469.

Weng QP, Kozlowski M, Belham C, Zhang A, Comb MJ & Avruch J (1998). Regulation of the p70 S6 kinase by phosphorylation in vivo. Analysis using site-specific anti-phosphopeptide antibodies. *J Biol Chem* **273,** 16621–16629.

Wernbom M, Augustsson J, Augustsson J, Thomeé R & Thomeé R (2007). The influence of frequency, intensity, volume and mode of strength training on whole muscle cross-sectional area in humans. *Sports Med* **37,** 225–264.

West DW, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, Moore DR, Stellingwerff T & Phillips SM (2011). Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *The American Journal of Clinical Nutrition* **94,** 795–803.

White KM, Sauer SJ, Hartz KK & Baldridge M (2009). Changes in Body Composition With Yogurt Consumption During Resistance Training in Women. *Int J Sport Nutr Exerc Metab* **19,** 18–33.

Wilborn CD, Taylor LW, Outlaw J, Williams L, Campbell B, Foster CA, Smith-Ryan A, Urbina S & Hayward S (2013). The Effects of Pre- and Post-Exercise Whey vs. Casein Protein Consumption on Body Composition and Performance Measures in Collegiate Female Athletes. *J Sports Sci Med* **12,** 74–79.

Wilkes EA, Selby AL, Atherton PJ, Patel R, Rankin D, Smith K & Rennie MJ (2009). Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *The American Journal of Clinical Nutrition* **90,** 1343–1350.

Wilkinson DJ, Franchi MV, Brook MS, Narici MV, Williams JP, Mitchell WK, Szewczyk NJ, Greenhaff PL, Atherton PJ & Smith K (2014). A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *AJP: Endocrinology and Metabolism* **306,** E571–E579.

Wilkinson DJ, Hossain T, Hill DS, Phillips BE, Crossland H, Williams J, Loughna P, Churchward-Venne TA, Breen L, Phillips SM, Etheridge T, Rathmacher JA, Smith K, Szewczyk NJ & Atherton PJ (2013). Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. *The Journal of Physiology* **591,**

2911–2923.

Wilkinson SB, Phillips SM, Atherton PJ, Patel R, Yarasheski KE, Tarnopolsky MA & Rennie MJ (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *The Journal of Physiology* **586,** 3701–3717.

Wilkinson SB, Tarnopolsky MA, Macdonald MJ, MacDonald JR, Armstrong D & Phillips SM (2007). Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. *Am J Clin Nutr* **85,** 1031–1040.

Witard OC, Cocke TL, Ferrando AA, Wolfe RR & Tipton KD (2014*a*). Increased net muscle protein balance in response to simultaneous and separate ingestion of carbohydrate and essential amino acids following resistance exercise. *Appl Physiol Nutr Metab* **39,** 329–339.

Witard OC, Jackman SR, Breen L, Smith K, Selby A & Tipton KD (2014*b*). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr* **99,** 86–95.

Witard OC, Tieland M, Beelen M, Tipton KD, van Loon LJC & Koopman R (2009). Resistance exercise increases postprandial muscle protein synthesis in humans. *Med Sci Sport Exer* **41,** 144–154.

Wolfe RR & Chinkes DL (2005). *Isotope Tracers in Metabolic Research*. John Wiley & Sons.

Yang Y, Breen L, Burd NA, Hector AJ, Hector AJ, Churchward-Venne TA, Josse AR, Josse AR, Tarnopolsky MA & Phillips SM (2012*a*). Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr* **108,** 1780–1788.

Yang Y, Churchward-Venne TA, Burd NA, Breen L, Tarnopolsky MA & Phillips SM (2012*b*). Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. *Nutr Metab (Lond)* **9,** 57.

Øimoen M (2016). Seks feil og fakta om proteiner. *http//wwwdnno/dn/aktiv/2016/02/18/1000/Kosthold/seks-feil-og-fakta-om-proteiner*. Available at: http://www.dn.no/dnaktiv/2016/02/18/1000/Kosthold/seks-feil-og-fakta-om-proteiner [Accessed May 19, 2017].

Errata

Where in the thesis	Original text/Error	Corrected text
Page 7	Koopman *et al.*, 2009*b*	Deleted
Page 7	Pennings *et al.*, 2011*b*	Pennings *et al.*, 2011*a*
Page 36, figure 4.1	No explanation of UT and T	Explanation inserted
Page 39, figure 4.2	UT and T	Untrained and trained
Page 40, figure 4.3	UT and T	Untrained and trained
Page 42, figure 4.4	UT and T	Untrained and trained
Page 51, table 4.1	Average for MFA type I and II in the milk group were erroneous	Correct numbers inserted
Study 4, page 39, figure 4	Timeline indicated collection of blood at 85 min post exercise	Corrected to 75 min post exercise

Paper I

Native whey induces higher and faster leucinemia than other whey protein supplements and milk: a randomized controlled trial.

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Native whey induces higher and faster leucinemia than other whey protein supplements and milk: a randomized controlled trial

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Abstract

Background: Resistance exercise and protein intake are both strong stimuli for muscle protein synthesis. The potential for a protein to acutely increase muscle protein synthesis seems partly dependent on absorption kinetics and the amino acid composition. The aim of this double-blinded randomized cross-over study was to compare time dependent changes in blood amino acid concentrations after ingesting 20 g of five distinct high quality dairy protein supplements (native whey, whey protein concentrate 80, hydrolysed whey, microparticulated whey, and milk proteins). Furthermore, we investigated whether differences in time dependent changes in blood amino acid concentrations affected acute blood glucose and urea responses, and recovery of muscle function after a bout of strength training.

Methods: Ten young healthy, recreationally active men ingested different milk protein supplements after a whole-body strength training session on five occasions in a randomized manner. Blood concentrations of amino acids, glucose and urea was measured before and 0, 30, 45, 60, 90, 120 min, and 22 and 30 h post-exercise. Maximal voluntary isometric knee extension and counter movement jump were assessed before, immediately after, 6, 22 and 30 h after exercise.

Results: Intake of native whey induced a faster and higher leucinemia than the other protein supplements ($p < 0.001$). All whey protein supplements showed faster time dependent changes in blood amino acid concentrations for total essential and branched chain amino acids compared to milk. There were no major differences between trials in blood concentrations of glucose or urea, or recovery of muscle function after exercise.

Conclusion: Native whey induced the strongest leucinemia and appears to have a greater potential for stimulating muscle protein synthesis than other whey supplements and milk. There were no meaningful differences in blood glucose, urea or recovery of muscle function after the supplements. Future studies should investigate whether the increased leucinemia with native whey translates into greater muscle protein synthesis and muscle mass accretion over time. (NCT02882386, August 16, 2016).

Keywords: Leucinemia, Strength training, Native whey, Hydrolysed whey

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Background

Increasing or maintaining muscle mass is of great importance for populations ranging from athletes to patients and elderly. Resistance exercise and protein ingestion are two of the most important stimuli of muscle protein synthesis (MPS). After ingestion of protein, the increased appearance of circulating essential amino acids stimulates MPS through intracellular kinases [1]. The anabolic response to protein intake is further potentiated by resistance exercise [2–4]. Both the physical characteristics of proteins (e.g., different digestion rates of whey and casein) and the amino acid composition, affect the potential of a certain protein to stimulate MPS [5–7].

Milk contains two protein fractions, the readily digestible and so called "fast" whey fraction, and the slowly digestible and so called "slow" casein fraction. Although both fractions are considered to be of high quality, containing all essential amino acids (EAA), the difference in digestion rate, splanchnic extraction and amino acid profile gives them distinctive properties. Studies on muscle responses show that whey intake induces a large but transient rise in MPS, whereas casein intake produces a moderate more persistent increase in MPS [8]. The leucine threshold hypothesis suggests that a certain intracellular leucine concentration is needed in order to robustly increase MPS [9, 10]. Given its superior ability to rapidly increase blood leucine concentrations to high levels, whey is often considered the most effective protein source to stimulate MPS [11].

Knowledge about how the manufacturing processes of protein containing foods or supplements affects the amino acid profile, absorption kinetics and anabolic potential is important when optimizing diets of e.g. athletes or elderly. Whey protein concentrate (WPC-80) is a by-product of cheese production and thereby subjected to both heat and acidification, denaturing the protein to some extent. Proteins can be further degraded by hydrolysation or microparticulation. Protein hydrolysates are commonly produced by adding proteolytic enzymes, resulting in a complex mixture of peptides with differing lengths, and free amino acids [12]. Hydrolysed casein may be more rapidly digested than intact casein [7, 13, 14]. Grimble and colleges found that hydrolysing whey proteins to mainly di- and tri-peptides improved absorption in the perfused human jejunum [15], however, other studies did not find any effect on absorption rates by hydrolysing whey proteins [13, 16] or a slowed absorption rate [17]. By the use of filtration, heat and shear forces, it is possible to microparticulate proteins to very small particles, giving them distinct properties resembling those of emulsified fat droplets [18]. As a consequence, microparticulated dairy proteins are often used as fat replacements in food. Native whey protein is produced by filtration of unprocessed milk. Because of the direct filtration of raw milk, native whey is a more intact protein compared to WPC-80, and the amino acid profile may also be slightly different (claimed by producers to have higher leucine content than WPC-80 [19]). Consequently, native whey may have characteristics that are positive for stimulation of MPS, but whether these distinct characteristics translate into a more favourable aminoacidemia compared to WPC-80, hydrolysed whey, and microparticulated whey remains to be answered.

The aim of the current study was to compare the time dependent changes in blood amino acid concentrations after ingestion of 20 g of five high quality but distinct dairy proteins; WPC-80, hydrolysed whey (WPH), microparticulated whey (MWP), native whey, and milk. Twenty grams of protein were chosen based on studies showing that 20 g of high quality protein is sufficient to maximally stimulate MPS [4, 20]. We hypothesised that native whey would induce a more rapid and higher leucinemia than WPC-80 due to the higher leucine content (claimed by the producers [19]). Furthermore, because of the rapid absorption rates of amino acids from intact WPC-80, we hypothesised that hydrolysation or microparticulation of WPC-80 would not enhance time dependent changes in blood amino acid concentrations of this product.

Methods

Thirteen healthy male subjects (age: 26.6 ± 7.4 years, height: 180.8 ± 6.3 cm, weight: 80.8 ± 6.3 kg) were recruited to this single blinded, randomized, five-way cross-over, controlled study. Two participants withdrew before the start due to reasons not related to the study. One participant had to withdraw due to knee pain during the training sessions. The results in this article are from the remaining 10 participants. Participants were sport science students recruited through student e-mails and flyers posted around the campus area. Participants were recreationally active and performed strength training 1–3 times per week. All were deemed healthy based on responses to a routine health-screening questionnaire. Resistance trained participants were included to minimize the exercise induced muscle damage, which can occur in untrained individuals, and to avoid learning effects during the study. One week before the start of the study all participants performed a familiarization workout to establish the maximal load they could lift for 10 repetitions (10RM). Performance tests were also practiced during this session. Written informed consent was obtained from all participants before the start of the study. The study was evaluated by the Regional Committee for Medical and Health Research Ethics (REC South East).

Study design

The study protocol is outlined in Fig. 1. All participants completed five study days consisting of a bout of

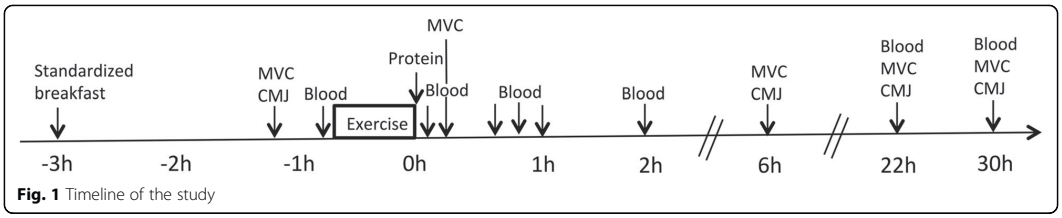

Fig. 1 Timeline of the study

strength exercise and intake of a protein supplement. All measures except maximal voluntary contraction (MVC) and counter movement jump (CMJ), which were only done after native whey and milk, were conducted in the same way with all supplements. Further, the days with native whey and milk had two additional time points of measurements at 22 and 30 h after exercise. Thus, participants and investigators were able to distinguish the days with native whey and milk form other testing days, but not whether they received native whey or milk on these days. The drinks were produced in colour-coded bottles in order to blind the participants and the investigators as much as possible within the design of the study. Training sessions were separated by at least five days, and participants were instructed to refrain from exercise for 72 h before each study day. Participants were instructed to maintain their habitual diet for the whole study period. If consuming any nutritional supplements, participants had to stop using these supplements at least two weeks before the start of the study. Due to short shelf life, all participants consumed the microparticulated whey the first study day. The participants but not the investigators were blinded for this. The other protein supplements were consumed in a randomized order on the following test days.

Participants met in the lab at 07:00 am on each study day after an overnight fast. They received a standardized breakfast consisting of oatmeal and a glass of orange juice (1855 kJ: 9.2 g fat, 69.3 g carbohydrates and 16.6 g protein). Participants were provided water ad libitum. Two hours after breakfast, the participants performed the standardized resistance exercise session in 40 min and consumed one of the five protein supplements within 6 min after the end of the session. Blood serum and plasma samples were collected at 0, 30, 45, 60, 90, and 120 min after ingestion of the protein supplement. Additional blood samples were collected at 22 and 30 h on the study days when milk and native whey were ingested. During the study days with milk and native whey, recovery of muscle function was measured as changes in maximal isometric voluntary contraction knee extensions (MVC), counter movement jump (CMJ) 30 min prior to, and 0, 6, 22 and 30 h after the exercise session. The 0 h time point was immediately after the workout and about 20 min after the last set of leg exercise.

Supplements

Bottles with the supplements were produced and delivered by Tine SA (Oslo, Norway). Each serving consisted of 636 ml and contained 3.3% protein, 1.2% fat, 4.7% lactose and 2% sucrose (Table 1). All supplements were adjusted with cream (TINE SA, Oslo, Norway) and lactose (VARIOLAC® 992, Arla, Vidby J, Denmark) to match milk on macronutrients. The rationale behind this adjustment was to be able to isolate differences to protein composition alone. Proteins in all supplements, except milk, consisted of 100% whey. Milk protein was

Table 1 Amino acid profile and macronutrient content of protein supplements

Amino acids (g/100 g)	Native whey	WPC-80[a]	Milk
Alanine	0.17	0.16	0.10
Arginine	0.09	0.08	0.11
Aspartic acid	0.40	0.35	0.25
Cysteine	0.09	0.07	0.03
Phenylalanine	0.13	0.11	0.15
Glutamic acid	0.60	0.56	0.67
Glycine	0.07	0.06	0.06
Histidine	0.07	0.06	0.09
Isoleucine	0.19	0.20	0.16
Leucine	0.43	0.34	0.31
Lysine	0.36	0.30	0.27
Methionine	0.08	0.07	0.08
Proline	0.18	0.21	0.32
Serine	0.16	0.18	0.18
Threonine	0.18	0.23	0.14
Tyrosine	0.09	0.07	0.12
Valine	0.18	0.19	0.20
Tryptophan	0.08	0.05	0.04
Total protein	3.33	3.10	3.23
Fat	1.08	1.06	0.99
Carbohydrate	6.40	6.60	600

[a]Microparticulated and hydrolysed whey proteins were produced from the WPC-80 and thus had the same composition

80% casein and 20% whey. Sucrose and artificial flavour was added to make supplements similar in taste.

In this study, microparticulated whey was produced by microfiltration of WPC-80 at high temperatures and high shear forces, with an end product of micro particles between 1 and 10 μm. Whey hydrolysate was produced using proteolytic enzymes and had a 10% degree of hydrolysis.

Exercise protocol

Training sessions consisted of 4 sets of 10RM repetitions of leg press and knee extensions, and 3 sets of 10RM repetitions of bench press and seated rowing. Warm-up sets of 10 repetitions at 50 and 80% of the 10RM weights were carried out in each exercise. A new set started every two minutes, while 3 min of rest was given between exercises. On the first study day, participants were allowed to make adjustments to the training load, whereas on the following four study days the training load could not be changed and was identical to the first study day. Each session was initiated by 10 min walk/easy jog on a treadmill.

Tests and measurements

Blood serum samples were clotted at room temperature for 30 min before being centrifuged at 1300 × *g* for 10 min at 4 °C. Blood plasma samples were collected in lithium heparin tubes and immediately centrifuged at 1300 x *g* for 10 min. Blood samples were stored at −80 °C until analyses. Serum samples were analysed for urea (analytic CV: 2.2%), and glucose (CV: 1.7%) at Fürst Medical Laboratory (Oslo, Norway).

Amino acid concentration was measured in plasma using the Phenomenex EZ:faast free (physiological) GC-MS analysis kit (Phenomenex®, Torrance, CA, USA). Samples were concentrated by solid phase extraction, derivatized, and separated by liquid-liquid extraction as directed by the EZ:faast kit. Derivatized samples were then analyzed by gas chromatography–mass spectrometry using a Shimadzu QP-2010 Ultra GCMS (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with the EZ:faast amino acid analysis column (10 m × 0.25 mm x 0.25 μm). The injection was 2 μl at 300 °C at a constant helium carrier gas flow of 1.1 ml/min with a split ratio of 1:15. The initial oven temperature was 110 °C and was raised to 320 °C at 30 °C/min. The MS interface was 320 °C and the EI source was 240 °C. Mass spectra were analyzed from 45–450 m/z at 4 scans/s. Total run time was 7 min. Duplicate sample injections were performed to reduce injection variability. A 3 point standard curve was produced for each measured amino acid (0–200 nmol/ml). The concentration of each amino acid was determined using its standard curve and was then corrected for injection differences using a norvaline internal standard (200 nmol/ml). Total amino acid (Total AA), essential amino acid (EAA), and branched-chain amino acid (BCAA) concentrations reported were the sum of the individual amino acid concentrations in each group.

Unilateral MVC for the knee-extensors was tested using a custom-made knee-extension apparatus (Gym2000, Geithus, Norway). Participants were seated in a chair with a four-point belt fixing the chest and hips, with 90° in hip and knee joints. Three attempts of 5 s with 1 min rest between were given to reach MVC. Force was measured with a force transducer (HMB U2AC2, Darmstadt, Germany). MVC was tested after 5 min warm up on a cycle ergometer, except for immediately after the workout. CMJ was tested using a force plate (HUR Labs Oy, Tampere, Finland). Participants were instructed to keep their hands at the hips and drop down to about 90° in the knee joint, before immediately jumping as high as possible. Three attempts were given, and if the third jump was the highest, participants were given a fourth attempt. A rest period of 30 s was given between each jump. The test-retest CV for jump height in this test is less than 3% in our lab.

Statistics

A two-way ANOVA with repeated measures was used to compare the five experimental trials (milk, MWP, WPH, WPC-80 and native whey) and sampling time points for all dependent variables (variable). A one-way ANOVA was used to compare the area under the curve of individual and total BCAA, EAA and total amino acids between protein supplements. Significant F ratios were further analysed using Tukey´s pairwise comparison. Statistical analyses were made using Prism Software (Graphpad 6, San Diego, CA, USA). All results are expressed as means ± SD. Statistical significance level was set at $p \leq .05$.

Results

There were no reported or observed adverse effects of any of the protein supplements. Baseline values for all dependent variables were not significantly different between studies, except for serum urea, which was higher in the morning of the WPC-80 study.

Blood amino acid profiles

Total amino acid concentration in blood demonstrated a treatment by time effect ($p < 0.0001$; Fig. 2a) revealing an increased amino acid concentration at 30 min after ingesting native whey compared to milk. Blood EAA, branched chain amino acids (BCAA) and leucine showed a similar treatment by time effect ($p < 0.0001$; Fig. 2b-d). Native whey reached the highest values for EAA and BCAA, primarily driven by higher leucine concentration, which was higher than after all other protein supplements during the first hour after intake of protein (Fig. 2d). Blood

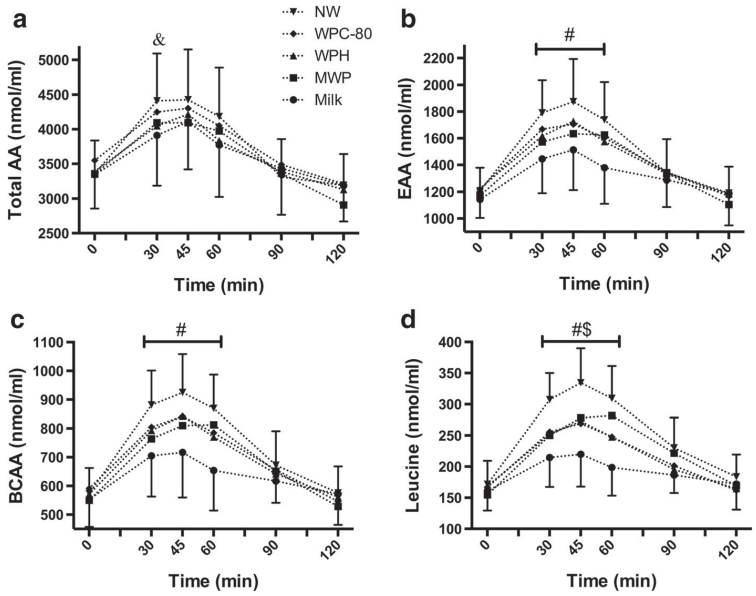

Fig. 2 Blood concentrations of total amino acids (**a**), essential amino acids (**b**), branched chain amino acids (**c**) and leucine (**d**) before and after a bout of strength training and intake of 20 g of protein from milk, microparticulated whey (MWP), whey protein hydrolysate (WPH), whey protein concentrate 80 (WPC-80) or native whey (NW) in young men. Values are means ± SD (only shown for highest and lowest values), n = 10 in each group. Milk and WPH were higher than baseline at 30 and 45 min, MWP, WPC-80 and NW were higher than baseline at time point 30, 45 and 60 min in A, B, C and D (not indicated in the figures; $p < 0.05$). & NW significantly greater than milk at the same time point, $p < 0.05$; # all whey proteins significantly greater than milk at the same time point, $p < 0.05$; $ NW significantly greater than all other whey proteins at the same time point, $p < 0.05$

concentration of isoleucine was higher after intake of WPC-80, MWP, WHP and native whey than milk (see Additional file 1A). For valine, the blood concentrations were only different between native whey and milk from 30 to 45 min, and native whey, WPC-80, MWP compared to milk at 60 min (see Additional file 1B).

The area under the curve did not differ between protein supplements for total AA, EAA or BCAA (see Additional file 2). For leucine, the area under the curve revealed higher values for native whey than for WPC-80, WPH and milk, and higher values for MWP than milk (see Additional file 2 D).

Blood leucine peak was reached at 45 min for most participants when consuming native whey, WPH and WPC-80. After consuming MWP, blood leucine peak was reached after 60 min, whereas leucine peak was reached at 30 min after milk intake for most participants. Regardless of time point, the leucine peak was higher after intake of native whey compared to all other supplements.

Blood glucose and urea

The ANOVA revealed a main effect of time for serum glucose ($p < 0.001$), which for WPC-80, MWP, WPH and native whey, was lower than baseline at 30, 45, 60 and 90 min (Fig. 3a). At time point 30 min, milk was significantly higher than native whey, WPC-80, WPH and MWP ($p = 0.03$).

There was a main effect of time for serum urea, revealing a significant increase in concentration at the 90 and 120 min time points ($p < 0.001$; Fig. 3b). WPC-80 had significantly higher values than MWP, WPH and milk at baseline ($p = 0.04$). This difference was maintained during the trial. At 22 and 30 h, urea levels for milk and native whey were not significantly different from baseline.

Recovery of performance

Both MVC (Milk: −11.1 ± 3.4%, native whey: −6.2 ± 5.7%; Fig. 4a) and CMJ (Milk: −6.4 ± 4.0%, native whey: −4.0 ± 3.6%; Fig. 4b) performance demonstrated a treatment by time effect ($p < 0.001$) indicating muscular fatigue post-exercise at 0 and 22 h after exercise. There were no differences between protein supplements.

Discussion

The main finding of the current study was that 20 g of native whey induced a significantly faster increase and

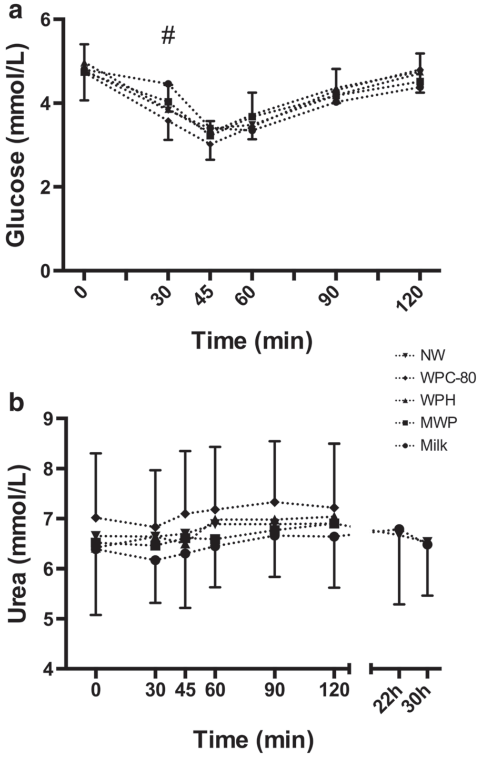

Fig. 3 Glucose (**a**) and urea (**b**) concentrations in serum before and after a bout of strength training and intake of 20 g of protein from milk, microparticulated whey (MWP), whey protein hydrolysate (WPH), whey protein concentrate 80 (WPC-80) or native whey (NW) in young men. Only NW and milk have values at 22 and 30 h. Values are means ± SD (only shown for highest and lowest values), n = 10 in each group. # milk is significantly different from WPH, WPC-80 and native whey

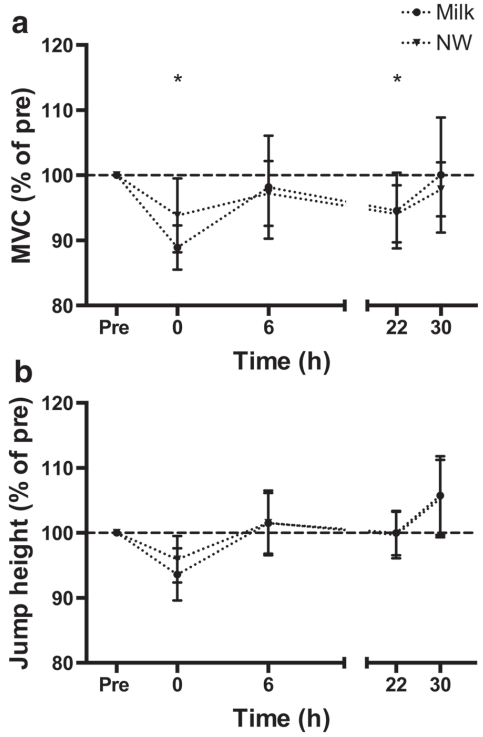

Fig. 4 Muscle function measured as maximal isometric voluntary contraction (**a**) and counter movement jump (**b**) before and after a bout of strength training and intake of 20 g of protein from milk or native whey (NW). Values are means ± SD, n = 10 in each group. *Significantly lower than baseline, $p < 0.05$

higher peak values in blood leucine concentrations than 20 g of MWP, WPH, WPC-80 and milk after a bout of strength training. All whey protein supplements had significantly faster increases and higher peak values of leucine, BCAA and EAA plasma concentrations than milk. Despite these differences in time dependent changes in leucine, BCAA and EAA blood concentrations, we were not able to show any differences in recovery of muscle function after consumption of native whey compared to milk after a bout of heavy load strength training. Finally, hydrolysation or microparticulation of WPC-80 did not increase the rise in blood concentrations of the investigated amino acids (see Additional file 1, 3 and 4).

Increasing extracellular availability of amino acids, especially BCAA and leucine, have been shown to increase protein synthesis both at rest and after exercise [2, 5, 21, 22]. Our data show minimal differences in total amino acid concentration in blood between protein supplements, whereas the whey supplements lead to more favourable time dependent changes in blood concentrations of EAA, BCAA and leucine compared to milk. Our results are in line with other studies showing a more rapid and greater aminoacidemia after whey protein intake compared to casein [5, 8, 16, 23]. Reitelseder and colleagues showed that a rapid aminoacidemia induced by whey was associated with a greater acute (0–3 h post exercise) stimulation of MPS, however when measuring MPS for a longer period of time (6 h), there were no differences between whey and casein [8]. To isolate the effects of aminoacidemia from protein type and amino acid content, West and colleagues provided participants with a bolus (25 g) or a pulse (10 × 2.5 g every 20 min; mimicking casein) of whey protein after resistance exercise [24]. The bolus resulted in a rapid rise in blood concentrations of amino acids and a greater MPS compared to pulse for 5 h post-exercise, indicating that

aminoacidemia affects the anabolic response. However, in the rested state a bolus of 15 g EAA did not provide an anabolic advantage over a pulse of 4×3.75 g of EAA with regards to FSR, leading to the suggestion that the anabolic response is mostly dependent on the total dose [25]. Based on these results one could argue that it seems like a certain peak in blood concentration of EAAs is required to kick-start the protein synthesis, whereas maintenance of increased protein synthesis is relying on the amount of amino acids available over time [6]. Nonetheless, leucinemia has yet to be regarded as an exclusive predictor of MPS. Studies have shown leucine to be an important stimulus for the intracellular kinases regulating MPS, such as mTOR and $p70^{S6K}$ [26, 27]. Based on these studies the high content of leucine and BCAA, and its rapid aminoacidemia suggest a great potential for native whey to increase acute post-exercise intracellular anabolic signalling and MPS. However, whether differences in acute post-exercise MPS translate into long-term effects is more uncertain [28], as the results of Reitelseder and colleagues suggest that the initial differences in MPS between whey and casein might diminish with time [8].

There were no differences between WPC-80, WPH and MWP in time dependent changes in blood amino acid concentrations. Thus, our data are in accordance with others [13, 16, 17] suggesting that, in contrast to casein, there is a limited potential to increase the absorption rate of whey proteins by degradation. We showed that native whey gave the highest leucinemia. As we do not have direct data on absorption kinetics, we are unable to differentiate whether the increased leucinemia after intake of native whey is due to faster absorption, higher leucine content or a combination of these factors. However, when comparing the increases in blood concentrations of amino acids with similar amount in native whey and WPC-80 (e.g., glutamic acid, glycine, methionine and serine), there were no differences between supplements, indicating that differences in amino acid content, and not a faster absorption, was the main cause of differences in blood concentrations.

For total AA, EAA, BCAA and leucine concentrations in blood, some participants reached higher values than others for all drinks. This individual difference in aminoacidemia was not directly related to body mass, which is reasonable to assume correlates with lean mass, as all participants were lean trained young men (see Additional file 5A). Nevertheless, the lighter participants did to some extent reach higher leucine levels with native whey than heavier participants. This observation is in line with a recent study finding no differences in the time dependent changes in blood leucine concentrations after intake of 20 g of whey protein in a group with low lean mass (<65 kg) to a group with high lean mass (>70 kg) [29]. Combined these findings might suggest that a certain amount of leucine is needed on order for leucine kinetics to be affected by body mass. Another interesting observation is the fact that some subjects had no difference in leucine peak or area under the curve after whey proteins compared to milk, whereas other participants increased their values considerably with the whey proteins compared to milk (see Additional file 5A and B). This suggests individual differences in blood leucine concentration response to whey vs. milk proteins.

In contrast to the differences in time dependent changes in blood amino acid concentrations, there were not many differences in blood glucose and urea responses to the various protein supplements. Nevertheless, serum glucose was higher with milk than WPH, WPC-80 and native whey at 30 min. Previous studies have shown a greater stimulation of insulin secretion by certain amino acids compositions and a rapid aminoacidemia compared to a slow and more sustained aminoacidemia [8, 30, 31]. As all drinks in the current study contained the same amount and composition of carbohydrates, the faster and greater aminoacidemia, together with the amino acid composition of the whey proteins could result in higher blood insulin levels and a greater transport of glucose into muscle. Serum urea increased after protein consumption for all groups at 90 and 120 min, indicating that a portion of the ingested amino acids was rapidly oxidized. The differences in absolute urea values between studies were mainly due to a higher baseline value for the WPC-80 trial. The relative change in urea concentration from baseline was higher after intake of WPH than after intake of milk, MWP, WPC-80 at 60 min, and WPC-80 and native whey at 120 min. As the total urea production, aminoacidemia and absolute values were similar between supplements; this relative difference in urea production at two time points probably has no physiological importance.

Our strength training protocol induced a small reduction (0–16%) in maximal force generating capacity, which recovered rapidly (within 22 h), indicating only mild muscular stress during the strength training session [32]. Earlier studies have indicated an attenuated strength decline when consuming whey protein after eccentric exercise compared to carbohydrates or placebo [33, 34]. As the current study involved a less strenuous workout and more comparable supplements a difference in recovery from exercise was less likely than in the previous studies. A more strenuous workout resulting in greater loss of force and increased muscle damage could possibly lead to differences between drinks. However, the workout applied in the current study is considered a normal workout, and thus more representative of typical training, in contrast to a workout of eccentric muscle actions. Moore and colleagues found the protein effect on

MPS in young subjects to be maximized after dosages of approximately 20 g of high quality protein [4, 20]. One can speculate whether MPS reached a ceiling effect in the current study. Consequently, reducing the protein dose to a suboptimal level could be a stronger design to investigate these differences [22, 35]. A greater initial MPS after intake of whey would be considered an advantage for a quick recovery; however, this advantage might be short lived as the MPS after casein intake catches up after the first hours of recovery [8]. Looking at effects over time during an intensive training period can be an alternative approach, as the evidence of beneficial effects of protein on markers of muscle damage seems stronger when protein is consumed over time after daily training sessions [36].

The cross-over design of the current study is considered a strength, as it allows for the participants to be their own control, making the statistical comparison between drinks stronger. Participants and investigators in this study were not completely blinded, which could limit the conclusions. Yet, the authors consider it unlikely that blinding would affect the primary outcome of blood aminoacidemia. Due to short shelf life, all participants consumed MWP during the first training session. In the case of adaptations to the workout during the study this could affect the comparisons between MWP and the other protein supplements. However, by using strength-trained participants, the adaptation to the workout during the study period was minimized. As we did not use stable isotope tracers, we can only assume that the observed changes in blood amino acid concentrations was due to the accelerated appearance of amino acids from the ingested protein source, and not due to an increase in endogenous release from body tissues. Furthermore, it is not possible to know whether the increased amino acid concentration in blood after intake of native whey resulted in a greater anabolic intracellular signalling or increased MPS compared to the other whey supplements or milk, as we did not collect biopsies. The current study included only young trained men. Future studies should include other populations; especially elderly persons might show a greater benefit of increased leucinemia after intake of native whey. Acute studies should include biopsies to measure anabolic signalling and MPS. In order to fully investigate the potential of native whey on muscle mass and strength, long-term supplementation alone or in combination with strength training interventions should be conducted.

Conclusions

Native whey intake induced a greater leucinemia than intake of WPC-80, WPH, MWP or milk the first hour after strength training in young men. All whey supplements led to higher blood peak concentrations for total essential amino acids, BCAA and leucine than milk the first hour after ingestion. In contrast, there were no differences between effects of supplements on glucose, urea or muscle recovery, and time dependent changes in blood amino acid concentrations were not changed by hydrolysation or microparticulation of WPC-80. Future studies should investigate whether the differences in amino acid and leucine kinetics translate into greater anabolic intracellular signalling, MPS and muscle growth.

Additional files

Additional file 1: Blood concentration of total isoleucine (A) and valine (B) before and after a bout of strength training and intake of 20 g of protein from milk, microparticulated whey (MWP), whey protein hydrolysate (WPH), whey protein concentrate 80 (WPC-80) or native whey (NW) in young men. Values are means ± SD (only shown for highest and lowest values), n = 10 in each group. All supplements increased valine concentrations at time points 30, 45 and 60 min and isoleucine at 30, 45, 60 and 90 min, $p < 0.05$. # milk significantly lower then all other drinks at the same time point, $p < 0.05$; & NW significantly higher than milk at the same time point, $p < 0.05$; φ NW, WPC-80 and MWP significantly higher than milk at the same time point, $p < 0.05$; θ NW, WPC-80 and WPH significantly higher than milk and MWP, $p < 0.05$. (TIFF 1117 kb)

Additional file 2: Area under the curve for blood total amino acids (A), EAA (B), BCAA (C) and leucine (D) concentrations after a bout of strength training and intake of 20 g of protein from milk, microparticulated whey (MWP), whey protein hydrolysate (WPH), whey protein concentrate 80 (WPC-80) or native whey (NW) in young men. Individual and mean (± SD) data are shown. * indicate significant difference between groups, $p < 0.05$. (TIFF 1305 kb)

Additional file 3: Blood concentration of total histidine (A), lysine (B), methionine (C), phenylalanine (D), threonine (E) and tryptophan (F) before and after a bout of strength training and intake of 20 g of protein from milk, microparticulated whey (MWP), whey protein hydrolysate (WPH), whey protein concentrate 80 (WPC-80) or native whey (NW) in young men. Values are means ± SD (only shown for highest and lowest values), n = 10 in each group. & NW significantly higher than milk at the same time point, $p < 0.05$. (TIFF 1236 kb)

Additional file 4: Blood concentration of total alanine (A), glutamic acid (B), glutamine (C), glycine (D), proline (E), serine (F), tyrosine (G) and aspargine (H) before and after a bout of strength training and intake of 20 g of protein from milk, microparticulated whey (MWP), whey protein hydrolysate (WPH), whey protein concentrate 80 (WPC-80) or native whey (NW) in young men. Values are means ± SD (only shown for highest and lowest values), n = 10 in each group. (TIFF 1823 kb)

Additional file 5: Individual blood leucine concentration peak values (A) and individual area under the curve values for blood leucine concentrations (B). Each participant is indicated by individual symbols. Filled symbols indicate the five heaviest participants (>80 kg) and open symbols indicate the lightest participants (<80 kg). (TIFF 1088 kb)

Abbreviations

ANOVA: Analysis of variance; BCAA: Branched chain amino acids; CMJ: Counter movement jump; EAA: Essential amino acids; GCMS: Gas chromatography mass spectrometer; MPS: Muscle protein synthesis; MVC: Mean voluntary contraction; MWP: Microparticulated whey protein; NW: Native whey protein; RM: Repetition maximum; SD: Standard deviation; WPC-80: Whey protein concentrate 80; WPH: Whey protein hydrolysate

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Availability of data and materials
The datasets analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
HH contributed with data analysis, drafting and write-up of the manuscript, JALL participated in study protocol development and data collection, TR contributed in protocol development, data collection and write-up of the manuscript. GP contributed in planning and review of the manuscript, MC contributed to in sample analysis and review of the manuscript, EB contributed in planning, sample analysis and review of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Written informed consent was obtained from all participants before the start of the study. The study was evaluated by the Regional Committee for Medical and Health Research Ethics (REC South East), Oslo, Norway. Reference number 2014/834.

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References

1. Fujita S, Dreyer HC, Drummond MJ, Glynn EL, Cadenas JG, Yoshizawa F, et al. Nutrient signalling in the regulation of human muscle protein synthesis. J Physiol. 2007;582:813–23.
2. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol. 1997;273:E122–9.
3. Pennings B, Boirie Y, Senden JMG, Gijsen AP, Kuipers H, van Loon LJC. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. Am J Clin Nutr. 2011;93:997–1005.
4. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. The American Journal of Clinical Nutrition American Society for Nutrition. 2013;99:86–95.
5. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol. 2009;107:987–92.
6. Churchward-Venne TA, Burd NA, Mitchell CJ, West DWD, Philp A, Marcotte GR, et al. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. J Physiol. 2012;590:2751–65.
7. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci U S A. 1997;94:14930–5.
8. Reitelseder S, Agergaard J, Doessing S, Helmark IC, Lund P, Kristensen NB, et al. Whey and casein labeled with L-[1-13C]leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. AJP: Endocrinology and Metabolism American Physiological Society. 2011;300:E231–42.
9. Drummond MJ, Rasmussen BB. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. Curr Opin Clin Nutr Metab Care. 2008;11:222–6.
10. Tang JE, Phillips SM. Maximizing muscle protein anabolism: the role of protein quality. Curr Opin Clin Nutr Metab Care. 2009;12:66–71.
11. Devries MC, Phillips SM. Supplemental Protein in Support of Muscle Mass and Health: Advantage Whey. J Food Sci. 2015;80:A8–A15.
12. Manninen AH. Protein hydrolysates in sports nutrition. Nutr Metab (Lond) BioMed Central. 2009;6:38.
13. Calbet JAL, Holst JJ. Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. Eur J Nutr Steinkopff-Verlag. 2004;43:127–39.
14. Koopman R, Crombach N, Gijsen AP, Walrand S, Fauquant J, Kies AK, et al. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. The American Journal of Clinical Nutrition American Society for Nutrition. 2009;90:106–15.
15. Grimble GK, Guilera Sarda M, Sessay HF, Marrett AL, Kapadia SA, Bowling TE, et al. The influence of whey hydrolysate peptide chain length on nitrogen and carbohydrate absorption in the perfused human jejunum. Clin Nutr. 1994;13:46.
16. Farup J, Rahbek SK, Storm AC, Klitgaard S, Jorgensen H, Bibby BM, et al. Effect of degree of hydrolysis of whey protein on in vivo plasma amino acid appearance in humans. Springerplus. 2016;5:532. Springer International Publishing.
17. Farnfield MM, Trenerry C, Carey KA, Cameron-Smith D. Plasma amino acid response after ingestion of different whey protein fractions. Int J Food Sci Nutr. 2009;60:476–86.
18. Wijayanti HB, Bansal N, Deeth HC. Stability of Whey Proteins during Thermal Processing: A Review. Comprehensive Reviews in Food Science and Food Safety. 2014;13:1235–51.
19. Lactalis. Prolacta: An excellent aminogram. http://ginobio.com/wp-content/uploads/2013/11/%E6%99%AE%E4%B9%90%E5%AE%83%EF%BC%88Prolacta%EF%BC%89.pdf. Accessed 19 May 2016. 2013. p. 1–12.
20. Moore DR, Robinson MJ, Fry JL, Tangx JE, Glover EI, Wilkinson SB, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr. 2009;89(1):161–68.
21. Bohé J, Low A, Wolfe RR, Rennie MJ. Human Muscle Protein Synthesis is Modulated by Extracellular, Not Intramuscular Amino Acid Availability: A Dose-Response Study. J Physiol. 2003;552:315–24. Blackwell Publishing Ltd.
22. Bukhari SS, Phillips BE, Wilkinson DJ, Limb MC, Rankin D, Mitchell WK, et al. Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women, at rest and after exercise. AJP: Endocrinology and Metabolism. 2015;308:E1057–65.
23. Burd NA, Yang Y, Moore DR, Tang JE, Tarnopolsky MA, Phillips SM. Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. Br J Nutr. 2012;108:958–62.
24. West DW, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, et al. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. Am J Clin Nutr. 2011;94:795–803.
25. Mitchell WK, Phillips BE, Williams JP, Rankin D, Lund JN, Smith K, et al. A Dose- rather than Delivery Profile-Dependent Mechanism Regulates the "Muscle-Full" Effect in Response to Oral Essential Amino Acid Intake in Young Men. The Journal of nutrition American Society for Nutrition. 2015;145:207–14.
26. Apró W, Moberg M, Hamilton DL, Ekblom B, Rooyackers O, Holmberg H-C, et al. Leucine does not affect mechanistic target of rapamycin complex 1 assembly but is required for maximal ribosomal protein s6 kinase 1 activity in human skeletal muscle following resistance exercise. FASEB J Federation of American Societies for Experimental Biology. 2015;29:4358–73.
27. Moberg M, Apró W, Ohlsson I, Pontén M, Villanueva A, Ekblom B, et al. Absence of leucine in an essential amino acid supplement reduces activation of mTORC1 signalling following resistance exercise in young females. Appl Physiol Nutr Metab. 2013;39:1–12.

28. Mitchell CJ, Churchward-Venne TA, Cameron-Smith D, Phillips SM. What is the relationship between the acute muscle protein synthetic response and changes in muscle mass? J Appl Physiol. 2014;118:495–7.
29. Macnaughton LS, Wardle SL, Witard OC, McGlory C, Hamilton DL, Jeromson S, et al. The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. Physiol Rep Physiological Reports. 2016;4:e12893.
30. Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP, Wolfe RR. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. Med Sci Sport Exer. 2004;36:2073–81.
31. Floyd JC, Fajans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. J Clin Invest American Society for Clinical Investigation. 1966;45:1487–502.
32. Paulsen G, Mikkelsen UR, Mikkelsen UR, Raastad T, Peake JM, Peake JM. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? Exerc Immunol Rev. 2012;18:42–97.
33. Cooke MB, Rybalka E, Stathis CG, Cribb PJ, Hayes A. Whey protein isolate attenuates strength decline after eccentrically-induced muscle damage in healthy individuals. J Int Soc Sports Nutr BioMed Central. 2010;7:30.
34. Buckley JD, Thomson RL, Coates AM, Howe PRC, DeNichilo MO, Rowney MK. Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. J Sci Med Sport. 2010;13:178–81.
35. Churchward-Venne TA, Breen L, Di Donato DM, Hector AJ, Mitchell CJ, Moore DR, et al. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. Am J Clin Nutr. 2014;99:276–86.
36. Pasiakos SM, Lieberman HR, McLellan TM. Effects of Protein Supplements on Muscle Damage, Soreness and Recovery of Muscle Function and Physical Performance: A Systematic Review. Sports Med. 2014;44:655–70.

Paper II

Native whey protein with high levels of leucine results in similar post-exercise muscular anabolic responses as regular whey protein, in young individuals

Håvard Hamarsland, Anne Lene Nordengen, Sigve Nyvik Aas, Kristin Holte, Ina Garthe, Gøran Paulsen, Matthew Cotter, Elisabet Børsheim, Haakon B. Benestad, Truls Raastad

Paper III

Native whey induces similar post exercise muscle anabolic responses as regular whey, despite greater leucinemia, in elderly individuals

Håvard Hamarsland, Sigve Nyvik Aas, Anne Lene Nordengen, Kristin Holte, Ina Garthe, Gøran Paulsen, Matthew Cotter, Elisabet Børsheim, Haakon B. Benestad, Truls Raastad

Paper IV

Native whey with high levels of leucine induces similar adaptation to strength training as milk protein, in young individuals

Håvard Hamarsland, Vilde Handegard, Mauritz Kåshagen, Haakon B. Benestad, Truls Raastad

Paper V

Native whey induces similar adaptation to strength training as milk protein, despite higher levels of leucine, in elderly individuals

Håvard Hamarsland, Mathias K. Johansen, Fridtjof Seeberg, Marie Brochmann, Ina Garthe, Haakon B. Benestad, Truls Raastad

Native whey induces similar adaptation to strength training as milk protein, despite higher levels of leucine, in elderly individuals

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Abstract

Background: Large amounts of protein (40g), or supplementing suboptimal servings of protein with leucine are able to overcome the anabolic resistance in in elderly muscle. Consequently, with its high leucine content, native whey may hold greater anabolic potential than milk, when supplemented in combination with strength training in elderly. Our aim was to compare the effects of native whey supplementation with milk protein on gains in muscle mass and strength during a period of strength training, in elderly participants.

Methods: In this double-blinded, randomized, controlled study, a total of 30 healthy men and women received two daily servings of 20g of either milk protein or native whey, during an 11-week strength training intervention. Muscle strength, lean mass, *m. vastus lateralis* thickness and muscle fiber cross sectional area were assessed before and after the training period. In addition, the acute phosphorylation of p70S6K, 4E-BP1 and eEF-2 in response to a standardized workout and supplementation was investigated before and after the training period.

Results: Muscle mass and strength increased, by all measures applied (4-7%, $P < 0.001$), with no differences between groups ($P > 0.25$). p70S6K phosphorylation increased (~1000%, $P < 0.045$) 2 hours after exercise in the untrained and trained state, but no differences in signaling were observed between supplements. No correlation between acute measures and changes in long-term outcomes were observed.

Conclusion: Supplementation with milk or native whey during an 11-week strength training period did not differentially increase muscle mass and strength in healthy elderly individuals.

Highlights

- Milk and native whey resulted in similar acute anabolic signaling
- Milk and native whey induced similar gains in muscle mass and strength
- The acute signaling response was similar in the untrained and trained state

Keywords

Protein supplementation, amino acids, protein quality, resistance exercise.

Introduction

Aging is associated with loss of muscle mass and strength and if allowed to advance, this condition may proceed to sarcopenia, which is linked to early loss of independent living (Baumgartner et al., 1998) and several comorbidities (Atkins et al., 2014; Dominguez and Barbagallo, 2007). The most promising intervention to prevent or counteract sarcopenia to date is resistance exercise in combination with appropriate protein consumption (Naseeb and Volpe, 2017). Among the emerging causal mechanisms of sarcopenia, an anabolic resistance in skeletal muscles to the stimuli of resistance exercise and protein intake has received much attention in later years. With a growing aged population, the need to develop effective interventions including exercise and nutrition is increasing. The effect of resistance training and protein on muscle mass is manifested through the summation of transient periods with positive net muscle protein balance (NBAL), leading to accumulation of muscle mass over time (Booth et al., 1998; Coffey and Hawley, 2007). Thus, by optimizing each period of positive NBAL, greater changes in muscle mass can be achieved over time. The anabolic effect of a protein is dependent on the digestion rate and amino acid kinetics (Pennings et al., 2011; Tang et al., 2009; West et al., 2011) and amino acid composition (Atherton et al., 2010; Volpi et al., 2003), in particular levels of leucine (Atherton et al., 2016). Acute studies have shown promising results for high-leucine or leucine enriched proteins in terms of stimulating muscle protein synthesis (Atherton et al., 2016; Bukhari et al., 2015; Wall et al., 2013). Our lab has previously shown native whey to increase signaling related to translation, and MPS to a greater extent than milk in elderly participants after resistance exercise (ref study III). Effects of protein supplementation in long-term training

studies are less clear (Cermak et al., 2012; Chale et al., 2012; Tieland et al., 2012; Verdijk et al., 2009). Native whey is produced by filtration of unprocessed raw milk, leaving proteins intact. Producing whey by this method increases the leucine content of native whey by about 15% and 25% compared to regular whey and milk, respectively (Hamarsland et al., 2017). The aim of the current study was to investigate whether the previously measured short-term differences between native whey and milk (ref study III) translates into differences in long-term adaptations in muscle mass and strength during a 11-week strength training regime in elderly participants. Furthermore, we have limited knowledge about how signaling responses to anabolic stimuli changes with training status. We therefore included an acute study before and after the training intervention to investigate whether the anabolic signaling response changed from the untrained to the trained state.

Methods

Participants and ethical approval

Thirty-eight elderly men and women were included in the study (table 1). Eight participants withdrew from the study after inclusion. One withdrew after the first acute study, after which the participant experienced dizziness. Two withdrew due to arthritic pain. Three withdrawals were due to busy time schedules. Two participants experienced health issues, not related to the study, and were excluded due to low workout attendance. Before entering the study all participants underwent a medical screening. Participants with any injuries to the musculoskeletal system that would impede the execution of strength training were excluded from this study. The use of any dietary supplements was

prohibited during the intervention. If participants were using supplements, usage was halted at least two weeks prior to pre-testing. Participants were untrained and had no prior experience with heavy resistance exercise. The study was approved by the Regional Ethics Committee for Medical and Health Research of South-East Norway and performed in accordance with the *Declaration of Helsinki*. All participants signed a written informed consent form before entering the study. The trial was registered at clinicaltrials.gov as NCT03033953. Figure 1 shows a flowchart of the progress from recruitment through completion of the study.

Study design

This study was conducted as a double-blinded, randomized, controlled trial. The design involved 11-weeks of strength training amounting to a total of 36 workouts. A subgroup of participants (n = 20) took part in an acute study during the first and last workout sessions of the training period. As a result muscle biopsies were collected in four different states: (1) untrained rested, (2) untrained acutely exercised, (3) trained rested, (4) trained acutely exercised. Participants were randomized to one of two groups, receiving either native whey or 1% fat milk.

Supplements

Native whey was bought by Lactalis® (Laval, Mayenne, France) and the 1% milk was produced by Tine ASA (Oslo, Norway). Supplements were matched on macronutrients by adding cream (Tine, Norway) and lactose (Arla food ingredients, Denmark) to the native whey protein. Both supplements were spray

dried into powder form, to be mixed with 400 ml of water before ingestion. The supplements had similar appearance and flavor. Each serving (69 g for milk and 73 g for native whey) were isocaloric and contained 19.3 g and 20.0 g of protein for milk and native whey, respectively (table 2).

On days with no training, supplements were consumed in the morning and in the evening. On training days one serving was consumed immediately after training, replacing the corresponding serving they would have otherwise consumed on non-training days. Supplements were consumed every day from the first training session to the last day of testing. Compliance to the supplementation schedule was self reported and recorded on each training day by the instructors.

Daily food intake

Participants were encouraged to maintain their habitual diet during the intervention. A trained dietitian conducted two 24-hour dietary recall interviews with each participant at the start, midway and at the end of the training intervention. The dietary nutrient content was analyzed using the software Mat på Data 5.1 (Mattilsynet, Oslo, Norway, 2009). Participants with a protein intake lower than 1.0 g $\cdot$ (kg body weight)$^{-1}$ at the first recording were recommended to increase their protein intake and given the necessary guidance.

Training program

Participants followed a traditional whole body strength-training program consisting of three sessions per week. Loads ranged from 12 to 6 RM, for 1-3 sets and progressed from high to low repetition ranges during the 11 weeks of training (supplementary table 1). On Mondays and Fridays, workouts were

conducted with maximal training load and intensity. On Wednesdays workouts were submaximal, aiming at a training load allowing two repetitions in reserve in each set. In addition to the exercises listed under the standardized workout (se later), participants also did weighted back-extensions and abdominal exercises at the end of each workout. Inter-set rest periods lasted 2-3 minutes. Qualified instructors supervised participants during all training sessions, and continuous rotation of instructors between participant groups throughout the period minimized any eventual differences in coaching. If a participant missed an exercise session another session would be added to their program in order for all participants to reach the total 33 sessions.

Dual-energy X-ray absorptiometry

Body composition was assessed by dual energy X-ray absorptiometry (Lunar iDXA GE Healtcare, Madison, Wisconsin, USA. Using the enCORE Software Version 14.10.022) before and after the intervention. Participants were scanned from head to toe in a supine position, providing values for lean tissue, fat mass and bone mineral content. The CV for the assessment was <1% for lean mass.

Maximal strength

Maximal strength in leg press and chest press was assessed by 1 repetition maximum (1RM) tests before and after the training period. Familiarization to the tests was performed one week before testing. After a 10 minute warm up on a treadmill a specific warm-up was performed with 10, 6, 3 and 1 repetitions at 50, 70, 80 and 90% of expected 1RM, respectively. 2-3 min of rest was given between attempts. Two to five attempts were used to find 1RM. Range of motion

was strictly controlled. Knee flexion during leg press was set to 90° and grip width in chest press was standardized. The load could be adjusted with increments as low as 5 kg in leg press and 1 kg in chest press. The CV for these measurements were <5%.

Unilateral maximal knee extension strength was assessed by isometric voluntary maximal contraction (MVC) in a custom-made knee-extension apparatus (Gym2000, Geithus, Norway). Participants were seated in a chair secured by a four-point harness, fixing the chest and hips, with 90° in the hip and knee joints. After three warm up sets at 25, 50 and 75% of perceived maximal effort, three attempts of 5 s with 1 min rest between were performed in order to reach MVC. Force was measured with a force transducer (HMB U2AC2, Darmstadt, Germany). MVC tests were performed after a 5-minute warm up on a cycle ergometer, except when tested immediately after workout sessions for the subgroup taking part in the acute study. The CV for these measurements were <5%.

Functional tests

In order to measure performance changes in daily activities we included a timed stair climb and a timed chair rise. The stair climb was performed in a 2-level staircase (20 steps of length: 30 cm and height: 18 cm). Participants were instructed to walk the stairs as fast as possible without transitioning into running. No arm-swing was allowed. The stair climb was performed with three different levels of load: body weight, wearing a 10 kg weight west, and wearing a 10 kg weight west while carrying two 5 kg weight discs. Participants had two

attempts with each load. Trials were timed using photocells (Speedtrap 2, Brower Timing Systems, Utah, USA). The CV of these assessments was 4%.
For the timed chair-rise participants were instructed to stand with their arms crossed in front of a chair (height: 47cm), then to sit and stand five times as fast as possible. When sitting both feet had to be lifted of the ground to demonstrate full transfer of weight to the chair, and when standing hips needed to be fully extended for the trial to be registered. Trials were timed using a pressure-plate (Speedtrap 2, Brower Timing Systems, Utah, USA) placed on the chair and proper technique ensured by test leaders.

Blood analyses

Serum was analyzed for glucose, insulin, urea and creatine kinase at Fürst Medical Laboratory (Oslo, Norway). Plasma amino acid concentration was measured as described earlier (Hamarsland et al., 2017) with a EZfaast amino acid analysis kit (Phenomenex®, Torrance, CA, USA) and gas chromatography/mass spectrometry (Shimadzu QP-2010 Ultra GCMS, Shimadzu Scientific Instruments, Columbia, MD).

Biopsy collection and pre-analytical processing

Muscle biopsies were collected from the mid portion of *m. vastus lateralis* with a modified Bergström technique with suction. Muscle specimens were used to make a homogenate of soluble proteins and mounted for immunohistochemistry. Pre-analytical processing of muscle tissue was performed as in Paulsen and colleagues (Paulsen et al., 2014).

Western blot

Samples for western blot were treated as previously described (Paulsen et al., 2014), quantified with ChemiDoc MP (BioRad Laboratories CA, USA) and analyzed with Image Lab (v5.1, BioRad Laboratories CA, USA). All samples were run in duplicates, and all comparisons were made within each blot. Primary and secondary antibodies are listed in supplementary table 2.

Immunohistochemistry

Eight-micrometre-thick cross sections were blocked for 30 min with 1% BSA (bovine serum albumin; Sigma Life Science, St Louis, MO, USA) in a 0.05% PBS-t solution (Calbiochem, EMD Biosciences, Darmstadt, Germany) before incubated with antibodies against myosin heavy chain II (SC71, hybriodomabank DSHB, IA, USA), dystrofin (AbCam, Cambridge, UK), in the blocking solution for 2 h at room temperature, followed by incubation with appropriate secondary antibodies (A11005 or A11001; Life technologies, Invitrogen, Carlsblad, CA, USA) for 30 min at room temperature, before covered with a coverslip and mounted with ProLong Gold Antifade Reagent with DAPI (Invitrogen Molecular Probes, Eugene, OR, USA). Muscle sections were then and left to dry overnight at room temperature. Between stages, the sections were washed 3x 5 min in a 0.05% PBS-t solution. Fifty type I fibers and fifty type II fibers were analyzed on each section.

Acute strength training experiment

During the first and the last bout of the strength training intervention 20 participants took part in an additional acute experiment. After an overnight fast

participants received a standardized oatmeal breakfast (25 kJ, 0,11 g protein, 0.30 g fat and 0.70 g carbohydrates · (kg body mass^{-1})). Participants followed an individual diet plan for the length of the acute experiment. The diet plan was based on body weight to meet the daily caloric and protein requirements, providing participants with 30 kcal · (kg body mass)$^{-1}$ and 1.5 g protein · (kg body mass)$^{-1}$ per day. Supplements were to be consumed within 5 min after the exercise session. The session was standardized but training load was increased for the final bout after the training program in order to accommodate their increased strength and ensuring an appropriate RM-load. After a 10 min warm up on a treadmill the session consisted of three sets of 10 repetitions in hammer squat, leg press, knee extension, chest press, seated row, one set of close grip pull down and two sets of shoulder press. The load was 10RM for all exercises and a new set was started every third minute. Specific warm up was done with one submaximal set in hammer squat, bench press and seated rowing. Muscle biopsies and blood samples were collected, and MVC was tested as outlined in figure 2.

Statistics

Non-normally distributed data (D'Agostino and Pearson omnibus normality test) were log-transformed prior to statistical analysis. All data are illustrated in original form. A two-way ANOVA with repeated measures (time x group) was applied to test group differences before and after the 11-week training period and relative changes from before to after, and between the acute experiments. Sidak and Tukey´s test was used as post hoc tests to specify significant differences between selected groups and time points and all comparisons,

respectively. Dunnet´s test was used as a *post hoc* test for comparisons within groups for blood amino acid concentrations, glucose, insulin, urea and creatine kinase (CK) as comparisons were only made against pre values. Comparisons of relative changes (%) between groups from before to after the training period were tested with an unpaired Student´s *t* test. Relative changes within each group were assessed with a paired Student´s *t* test. A sample size calculation was conducted with a power of 80% based on lean muscle mass results from an earlier study comparing whey and soy protein supplementation in young men ((Volek et al., 2013); StatMate, Graphpad Software, San Diego, CA, USA). Based on the power calculation, our goal was to include 20 subjects in each group to obtain a 5 % significance for a 1.5 % points difference between groups. Statistical analyses were made using Prism Software (Graphpad 6, San Diego, CA, USA). All results are expressed as means ± standard deviation (SD). Statistical significance level was set at $p \leq .05$.

Results

Participant characteristics and compliance

We observed no groups differences on baseline characteristics (table 1). Participants attended an average of 33.0 ± 0.9 and 32.5 ± 1.2 sessions in the native whey and milk group, respectively. Total load (repetitions x sets x kg x sessions) lifted during the 11-week intervention was 249,000 ± 65,000 kg and 252,000 ± 60,000 kg in the native whey and milk group, respectively (P = 0.74). The self-reported compliance to the supplementation was 99 ± 1% and 99 ± 1% in the native whey and milk group, respectively (P > 0.99). The total energy intake and protein intake ($g \cdot (kg\ body\ mass)^{-1} \cdot day^{-1}$) increased in both groups

during the supplementation period, with no differences between groups (table 3). The energy percent (E%) from fat decreased in both groups and the E% from carbohydrates increased in the milk group.

Muscle mass and muscle fibre cross sectional area

Significant muscle hypertrophy was evident in lean mass, thickness of the m. *vastus lateralis* and muscle fiber cross sectional area in both groups (table 4, figure 3A). On the cellular level MFA of type II, but not type I, fibres increased in both groups after 11 weeks of training (Milk: $P = 0.035$; native whey: $P = 0.002$; table 4). There were no group differences for any measures of muscle growth.

Muscle strength and performance

After 11 weeks of strength training all participants increased 1RM in leg press and chest press (figure 3B). There were no differences between the groups for strength gains. The time to completion for the stair climb trials and chair rise test improved to a similar extent in both groups (table 4).

Acute experiment

Both in the untrained and in the trained state the acute exercise session resulted in muscular fatigue, demonstrated by a reduced force-generating capacity 15 min after the session in both groups (native whey: -11.8 ± 6.0% vs -9.5 ± 3.0%, milk: -12.4 ± 4.3% vs 10.0 ± 5.0%, in the untrained and trained state, respectively, $P < 0.001$ for all). Force-generating capacity was significantly reduced at 24 hours after exercise in the untrained state with milk (-9.4 ± 6.7%, $P < 0.001$), but not with native whey (-4,2 ± 5.7%, $P = 0.099$). In the trained state

no significant differences from baseline compared to 24 hours after exercise were observed with milk (-4.6%, P = 0.103) or native whey (-2.8%, P = 0.414) There were no group differences for recovery of muscle force-generating capacity.

Blood measures

Fasting serum concentrations of glucose and insulin did not change significantly during the training intervention. Acute changes in serum glucose, insulin, urea and CK are shown in figure 4.

Amino acids concentration in blood

Fasting concentrations of BCAA and some other EAAs (Native whey: lysine, threonine and tryptophan; milk: lysine, methionine, phenylalanine and tryptophan) increased during the intervention period (5-20%, data not shown). The acute increase in blood concentrations of leucine, BCAA and EAA were higher after ingestion of native whey compared to milk after protein ingestion both in the untrained and the trained state (P < 0.001, figure 5). Area under the curve for two hours after protein ingestion was higher in the native whey group for leucine, BCAA and EAA compared to milk in both the untrained and trained state (P < 0.001; data not shown).

Protein signalling

Resting levels of total (milk: -24.4 ± 21.2%, P = 0.009; native whey: -23.2 ± 17.7%, P = 0.003) and phosphorylated (milk: -49.4 ± 27.6%, P < 0.001; native whey: -40.6 ± 31.3%, P = 0.002) p70S6K decreased from before to after the

intervention in both groups (data not shown). Phosphorylation of p70S6K increased with both supplements in the untrained and trained state after resistance exercise and protein supplementation (figure 6A). There were no group differences for p70S6K phosphorylation.

Resting total and phosphorylated levels of 4E-BP1 and eEF-2 remained stable during the training period ($P > 0.51$ for all, figure 6 B and 6C). No acute changes in phosphorylation were observed for 4E-BP1 or eEF-2 after resistance exercise and protein supplementation.

Correlations

Changes in 1RM leg press showed moderate correlations with changes in stair climb performance ($r = -0.5$ to -06, $P < 0.005$).

Discussion

In this study we tested the hypothesis that supplementation with a leucine-rich native whey protein would result in greater increases in muscle mass and strength than milk, during 11 weeks of resistance training in elderly participants. In order to investigate how signaling related to translation responded to protein intake and resistance exercise changes with training status, an acute study was conducted at the beginning and the end of the training intervention. There were 3 primary findings. (1) The increase in muscle mass and strength after 11 weeks of strength training was similar in groups supplemented with native whey or milk (2) We observed no differences between supplements in effects on phosphorylation of p70S6K, 4E-BP1 and eEF-2. (3) Total and resting

phosphorylation levels of p70S6K were reduced during the training and supplementation period.

Amino acid concentrations in blood

Fasting levels of total amino acids, EAA and leucine increased (5-20%) during the intervention. We did not observe the previously reported association between fasting blood levels of leucine and changes in lean mass in young individuals (Volek et al., 2013). As previously reported, the acute changes in blood concentrations of leucine, BCAA and EAA after protein ingestion were greater with native whey compared to milk (ref study III), both in the untrained and trained state. The blood leucine concentrations after exercise and intake of native whey in the current study, are comparable to values previously reported in elderly (ref study III), but lower compared to resistance trained young individuals (ref study II) ingesting 20g of a similar native whey supplement. Impaired digestion and transport of amino acids into muscle have been suggested as possible mechanisms of anabolic resistance in elderly (Burd et al., 2013). We do not have any indications of whether digestion of protein or amino acid uptake into muscle was initially impaired in our participants, but our results indicate no adaptations in these mechanisms in response to 11 weeks of protein supplementation and strength training in elderly. However, we cannot exclude the possibility of a change in amino acid flux, which can occur without changes in blood concentrations.

Intracellular signaling

Milk and native whey supplementation resulted in comparable increases in phosphorylation of p70S6K two hours after resistance exercise, both in the untrained and trained state. These results goes against a previous study by our group, where native whey led to a greater phosphorylation of p70S6K and a concomitant greater stimulation of mixed muscle fractional synthetic rate after exercise, compared to milk (ref study III). This could be due to differences in supplementation regime and timing of biopsies, with 20g of protein immediately after and 2 hours, and biopsies at 1 and 3 hours after resistance exercise. Furthermore, the difference in leucine content per serving between supplements in our previous study was higher than in the current study (0.76g vs. 0.59g; ref study III). This difference in leucine content may have affected the results, but blood concentrations of amino acids, including leucine, over the first 2 hours after ingestion, did not differ between studies.

Farnfield and colleagues (Farnfield et al., 2012) reported a reduced p70S6K response to protein after resistance exercise in elderly after 12 weeks of resistance training. Relating the change in phosphorylation of p70S6K to baseline values, as was done by Farnfield and colleagues (Farnfield et al., 2012), we did not observe a decreased p70S6K response after a bour of resistance exercise and protein intake. Total and phosphorylated p70S6K in the rested state were decreased after the training intervention in both groups. This decrease is interesting in light of a recent study by Markofski and colleagues (Markofski et al., 2015), were elderly displayed a higher basal phosphorylation of mTORC1 and p70S6K, and higher phosphorylated/total p70S6K, compared to young. In spite of the increased p70S6K phosphorylation, MPS was similar between young and

elderly (Markofski et al., 2015). Whether, this mTORC1 and p70S6K hyperphosphorylation is a compensatory mechanism to maintain MPS in ageing muscle, or maybe contributing to anabolic resistance, as proposed by Markofski and colleagues (Markofski et al., 2015) remains to be elucidated.
Without knowing the causes or effects, our results suggest that a hyperphosphorylation of p70S6K may be normalized with training and/or protein intake in elderly individuals. Further, these data underlines the importance of measuring baseline levels, and not only change from baseline, when comparing changes in different groups such as young and old, or untrained and trained.

Recovery of force-generating capacity

Force-generating capacity was reduced by 5-25% after the resistance exercise protocol both in the untrained and trained state. Combined with a small increase in CK this indicates a mild to moderate muscular stress (Paulsen et al., 2012). These data agrees well with a previous study, where we found no difference between milk and native whey on recovery of force-generating capacity after a "normal" bout of resistance exercise in elderly (ref study III).

Effect of protein type on muscle mass and strength

Resistance training in combination with supplementation of milk protein or native whey was effective in terms of increasing muscle mass and strength in our elderly participants. In contrast to our hypothesis, no differences were observed between groups. Previous studies have shown that elderly may need twice the amount of protein as young to maximally stimulate MPS at rest ($0.24 g \cdot kg^{-1}$ vs

0.40g·kg^{-1}; (Moore et al., 2014)) and after moderate volume, heavy resistance exercise (40g vs. 20g; (Yang et al., 2012). Furthermore, adding leucine to a suboptimal dose of protein may rescue the acute MPS in young and elderly (Atherton et al., 2016; Bukhari et al., 2015; Wall et al., 2013). Based on these findings we hypothesized that, due to our supposedly suboptimal dose of protein (20g) and the difference in leucine content between supplements, native whey would lead to superior stimulation of MPS. Which over time would lead to a greater accretion of muscle mass with native whey than milk.

Whey has been shown to acutely stimulate MPS to a greater extent than casein in rested (Pennings et al., 2011) and exercised elderly (Burd et al., 2012), but not all studies report such differences (Dideriksen et al., 2011). A recent review suggests the anabolic resistance in elderly to substantiate mainly when stimuli are suboptimal, and that when combining heavy resistance exercise with "optimal" amounts of protein few studies find evidence for an anabolic resistance in elderly (Shad et al., 2016). Thus, in the present study, the combination of high volume heavy resistance exercise and 20g of high quality protein may have been enough to overcome any potential anabolic resistance, making the higher leucine content in native whey redundant.

Most previous studies investigating the effects of protein supplementation and resistance training in elderly have not found any significant effects of protein supplementation (Arnarson et al., 2013; Bemben et al., 2010; Chale et al., 2012; Kukuljan et al., 2009; Leenders et al., 2013; Verdijk et al., 2009). Despite the lack of significant findings in individual studies, meta-analyses have found greater increases in lean mass and 1RM in leg press, when resistance training was combined with protein supplementation in elderly (Cermak et al., 2012).

However, a more recent meta-analysis by Morton and colleagues (Morton et al., 2017) found no additional effect of protein in elderly, when combined with strength training.

A previous study comparing supplementation with 10 g of milk protein or native whey during a 16 week strength training study in old men reported no differences between these groups for measures of strength, total or appendicular lean mass (Gryson et al., 2014). In contrast Hidayat and colleagues (Hidayat et al., 2017) observed a greater effect in studies supplementing with whey protein, compared to casein or milk protein concentrate, when combining studies with different milk supplements. Several of the studies on protein supplementation in elderly are challenged by a low number of participants, by not exclusively including elderly participants, and by a suboptimal supplementation in terms of frequency and/or amount of protein (Arnarson et al., 2013; Bemben et al., 2010; Kukuljan et al., 2009; Leenders et al., 2013; Verdijk et al., 2009). The few studies reporting additional effects of protein in elderly investigated frail elderly populations, where the potential benefits may be greater than in healthy elderly (Dirks et al., 2017; Holm et al., 2008; Tieland et al., 2012). Without an "optimal" intervention it is hard to conclude on the potential effects of protein supplementation in elderly. Previous studies report between 0.6 and 1.4 kg increases in lean mass (Arnarson et al., 2013; Chale et al., 2012; Dirks et al., 2017; Kukuljan et al., 2009; Leenders et al., 2013; Tieland et al., 2012; Verdijk et al., 2009) with protein supplementation and resistance exercise, whereas we observe an average increase of 2.1 kg increase in our protein groups. Unfortunately, without a placebo group we cannot determine the separate contributions to from resistance exercise and protein supplementation in our

study. Nevertheless, it seems clear that as long as the dietary intake of macro nutrients and protein quality is sufficient, the ability for healthy elderly to respond to strength training with large increases in muscle mass is maintained. In line with changes in muscle mass, we observed no differences in increases in strength or performance in the stair climb or chair rise tests. The 30-35% and 20% increase in 1RM leg press and chest press, respectively, is similar to results from previous resistance training studies supplementing with protein in elderly (Bemben et al., 2010; Chale et al., 2012; Dirks et al., 2017; Tieland et al., 2012; Verdijk et al., 2009). Our moderate correlation between change in 1RM leg press and change in performance indicates a transfer from strength gains to functional tasks in elderly. We did not observe any differences between supplements for any measure of muscle mass or strength. The large variability in individual responses makes it difficult to exclude a type II statistical error in the current study. However, the milk group displayed the greatest increase in measures of muscle mass. Thus, we refute our hypothesis of native whey having a greater anabolic effect on muscle mass and strength than milk protein, when combined with resistance exercise in healthy elderly individuals.

Limitations

The post exercise supplements were consumed immediately after exercise and had a compliance of 100%. All other supplement intakes were self-reported three times per week. Although, participants were highly motivated we suspect an overestimation of adherence to the supplementation scheme.

By recruiting participants through the local newspaper and posters on activity centers, we likely missed the most sedate, less healthy segment of this age group.

As a consequence our results may only apply to healthy, active elderly, and not the groups that may benefit the most.

Conclusion

We observed no differences in muscle mass accretion or gains in strength between milk protein or native whey, when supplemented as 2x20g daily in combination with strength training. In line with the long-term adaptations, protein supplementation after exercise increased phosphorylation of p70S6K equally with milk proteins or native whey. The resting levels of total and phosphorylated p70S6K was reduced during the training period, but the acute anabolic signaling response to resistance exercise and protein supplementation did not change from the untrained to the trained state.

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Disclosures

The authors declare no conflict of interest.

References

Arnarson, A., Geirsdottir, O.G., Ramel, A., Briem, K., Jonsson, P.V., Thorsdottir, I., 2013. Effects of whey proteins and carbohydrates on the efficacy of resistance training in elderly people: double blind, randomised controlled trial. Eur J Clin Nutr 67, 821–826. doi:10.1038/ejcn.2013.40

Atherton, P.J., Kumar, V., Selby, A., Rankin, D., Hildebrandt, W., Phillips, B., Williams, J., Hiscock, N., Smith, K., 2016. Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. Clinical Nutrition In press. doi:10.1016/j.clnu.2016.04.025

Atherton, P.J., Smith, K., Etheridge, T., Rankin, D., Rennie, M.J., 2010. Distinct anabolic signalling responses to amino acids in C2C12 skeletal muscle cells. Amino Acids 38, 1533–1539. doi:10.1007/s00726-009-0377-x

Atkins, J.L., Whincup, P.H., Morris, R.W., Lennon, L.T., Papacosta, O., Wannamethee, S.G., 2014. Sarcopenic Obesity and Risk of Cardiovascular Disease and Mortality: A Population-Based Cohort Study of Older Men. Journal of the American Geriatrics Society 62, 253–260. doi:10.1111/jgs.12652

Baumgartner, R.N., Koehler, K.M., Gallagher, D., Romero, L., Heymsfield, S.B., Ross, R.R., Garry, P.J., Lindeman, R.D., 1998. Epidemiology of sarcopenia among the elderly in New Mexico. Am. J. Epidemiol. 147, 755–763.

Bemben, M.G., Witten, M.S., Carter, J.M., Eliot, K.A., Knehans, A.W., Bemben, D.A., 2010. The Effects of Supplementation with Creatine and Protein on Muscle Strength Following a Traditional Resistance Training Program in Middle-Aged and Older Men. J Nutr Health Aging 14, 155–159.

Booth, F.W., Tseng, B.S., Flück, M., Carson, J.A., 1998. Molecular and cellular adaptation of muscle in response to physical training. Acta Physiol Scand 162, 343–350. doi:10.1046/j.1365-201X.1998.0326e.x

Bukhari, S.S., Phillips, B.E., Wilkinson, D.J., Limb, M.C., Rankin, D., Mitchell, W.K., Koboyashi, H., Greenhaff, P.L., Smith, K., Atherton, P.J., 2015. Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women, at rest and after exercise. AJP: Endocrinology and Metabolism 308, E1057–65. doi:10.1152/ajpendo.00481.2014

Burd, N.A., Gorissen, S.H., Gorissen, S.H., van Loon, L.J.C., 2013. Anabolic resistance of muscle protein synthesis with aging. Exerc Sport Sci Rev. doi:10.1097/JES.0b013e318292f3d5

Burd, N.A., Yang, Y., Moore, D.R., Tang, J.E., Tarnopolsky, M.A., Phillips, S.M., 2012. Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. Br. J. Nutr. 108, 958–962. doi:10.1017/S0007114511006271

Cermak, N.M.N., Res, P.T.P., de Groot, L.C.L., Saris, W.H.W., van Loon, L.J.L., 2012. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. The American Journal of Clinical Nutrition 96, 1454–1464. doi:10.3945/ajcn.112.037556

Chale, A., Cloutier, G.J., Hau, C., Phillips, E.M., Dallal, G.E., Fielding, R.A., 2012. Efficacy of Whey Protein Supplementation on Resistance Exercise–Induced Changes in Lean Mass, Muscle Strength, and Physical Function in Mobility-Limited Older Adults. J. Gerontol. A Biol. Sci. Med. Sci. gls221.

doi:10.1093/gerona/gls221
Coffey, V.G., Hawley, J.A., 2007. The molecular bases of training adaptation. Sports Med 37, 737–763.
Dideriksen, K.J., Reitelseder, S., Petersen, S.G., Hjort, M., Helmark, I.C., Kjaer, M., Holm, L., 2011. Stimulation of muscle protein synthesis by whey and caseinate ingestion after resistance exercise in elderly individuals. Scand J Med Sci Sports 21, E372–E383. doi:10.1111/j.1600-0838.2011.01318.x
Dirks, M.L., Tieland, M., Verdijk, L.B., Losen, M., Nilwik, R., Mensink, M., de Groot, L.C.P.G.M., van Loon, L.J.C., 2017. Protein Supplementation Augments Muscle Fiber Hypertrophy but Does Not Modulate Satellite Cell Content During Prolonged Resistance-Type Exercise Training in Frail Elderly. J Am Med Dir Assoc. doi:10.1016/j.jamda.2017.02.006
Dominguez, L.J., Barbagallo, M., 2007. The cardiometabolic syndrome and sarcopenic obesity in older persons. J Cardiometab Syndr 2, 183–189.
Farnfield, M.M., Breen, L., Carey, K.A., Garnham, A., Cameron-Smith, D., 2012. Activation of mTOR signalling in young and old human skeletal muscle in response to combined resistance exercise and whey protein ingestion. Appl Physiol Nutr Metab 37, 21–30. doi:10.1139/h11-132
Gryson, C., Ratel, S., Rance, M., Penando, S., Bonhomme, C., Le Ruyet, P., Duclos, M., Boirie, Y., Walrand, S., 2014. Four-Month Course of Soluble Milk Proteins Interacts With Exercise to Improve Muscle Strength and Delay Fatigue in Elderly Participants. J Am Med Dir Assoc 15, 958.e1–958.e9. doi:10.1016/j.jamda.2014.09.011
Hamarsland, H., Laahne, J.A.L., Paulsen, G., Cotter, M., Borsheim, E., Raastad, T., 2017. Native whey induces higher and faster leucinemia than other whey protein supplements and milk: a randomized controlled trial. BMC Nutrition 2017 3:1 3, 10. doi:10.1186/s40795-017-0131-9
Hidayat, K., Chen, G.C., Wang, Y., Zhang, Z., Dai, X., Szeto, I.M.Y., Qin, L.-Q., 2017. Effects of milk proteins supplementation in older adults undergoing resistance training: A meta-analysis of randomized control trials. J Nutr Health Aging 1–9. doi:10.1007/s12603-017-0899-y
Holm, L., Olesen, J.L., Matsumoto, K., Doi, T., Mizuno, M., Alsted, T.J., Mackey, A.L., Schwarz, P., Kjaer, M., 2008. Protein-containing nutrient supplementation following strength training enhances the effect on muscle mass, strength, and bone formation in postmenopausal women. J. Appl. Physiol. 105, 274–281. doi:10.1152/japplphysiol.00935.2007
Kukuljan, S., Kukuljan, S., Nowson, C.A., Nowson, C.A., Sanders, K., Sanders, K., Daly, R.M., Daly, R.M., 2009. Effects of resistance exercise and fortified milk on skeletal muscle mass, muscle size, and functional performance in middle-aged and older men: an 18-mo randomized controlled trial. J. Appl. Physiol. 107, 1864–1873. doi:10.1152/japplphysiol.00392.2009
Leenders, M., Verdijk, L.B., Van der Hoeven, L., Van Kranenburg, J., Nilwik, R., Wodzig, W.K.W.H., Senden, J.M.G., Keizer, H.A., van Loon, L.J.C., 2013. Protein supplementation during resistance-type exercise training in the elderly. Med Sci Sport Exer 45, 542–552. doi:10.1249/MSS.0b013e318272fcdb
Markofski, M.M., Dickinson, J.M., Drummond, M.J., Fry, C.S., Fujita, S., Gundermann, D.M., Glynn, E.L., Jennings, K., Paddon-Jones, D., Reidy, P.T., Sheffield-Moore, M., Timmerman, K.L., Rasmussen, B.B., Volpi, E., 2015. Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large

cohort of young and older men and women. Experimental Gerontology 65, 1–7. doi:10.1016/j.exger.2015.02.015
Moore, D.R., Churchward-Venne, T.A., Witard, O., Breen, L., Burd, N.A., Tipton, K.D., Phillips, S.M., 2014. Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in Healthy Older Versus Younger Men. J. Gerontol. A Biol. Sci. Med. Sci. 70, 57–62. doi:10.1093/gerona/glu103
Morton, R.W., Murphy, K.T., McKellar, S.R., Schoenfeld, B.J., Henselmans, M., Helms, E., Aragon, A.A., Devries, M.C., Banfield, L., Krieger, J.W., Phillips, S.M., 2017. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. Br J Sports Med bjsports–2017–097608. doi:10.1136/bjsports-2017-097608
Naseeb, M.A., Volpe, S.L., 2017. Protein and exercise in the prevention of sarcopenia and aging. Nutr Res 40, 1–20. doi:10.1016/j.nutres.2017.01.001
Paulsen, G., Hamarsland, H., Cumming, K.T., Johansen, R.E., Hulmi, J.J., Borsheim, E., Wiig, H., Garthe, I., Raastad, T., 2014. Vitamin C and E supplementation alters protein signalling after a strength training session, but not muscle growth during 10 weeks of training. The Journal of Physiology 592, 5391–5408. doi:10.1113/jphysiol.2014.279950
Paulsen, G., Mikkelsen, U.R., Raastad, T., Peake, J.M., 2012. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? Exerc Immunol Rev 18, 42–97.
Pennings, B., Boirie, Y., Senden, J.M.G., Gijsen, A.P., Kuipers, H., van Loon, L.J.C., 2011. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. The American Journal of Clinical Nutrition 93, 997–1005. doi:10.3945/ajcn.110.008102
Shad, B.J., Thompson, J.L., Breen, L., 2016. Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. AJP: Endocrinology and Metabolism 311, E803–E817. doi:10.1152/ajpendo.00213.2016
Tang, J.E., Moore, D.R., Kujbida, G.W., Tarnopolsky, M.A., Phillips, S.M., 2009. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J. Appl. Physiol. 107, 987–992. doi:10.1152/japplphysiol.00076.2009
Tieland, M., Tieland, M., Dirks, M.L., van der Zwaluw, N., van der Zwaluw, N., Verdijk, L.B., van de Rest, O., van de Rest, O., de Groot, L.C.P.G.M., de Groot, L.C.P.G.M., van Loon, L.J.C., 2012. Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people: a randomized, double-blind, placebo-controlled trial. J Am Med Dir Assoc 13, 713–719. doi:10.1016/j.jamda.2012.05.020
Verdijk, L.B., Jonkers, R.A.M., Gleeson, B.G., Beelen, M., Meijer, K., Savelberg, H.H.C.M., Wodzig, W.K.W.H., Dendale, P., van Loon, L.J.C., 2009. Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy after resistance training in elderly men. The American Journal of Clinical Nutrition 89, 608–616. doi:10.3945/ajcn.2008.26626
Volek, J.S., Volk, B.M., Gómez, A.L., Kunces, L.J., Kupchak, B.R., Freidenreich, D.J., Aristizabal, J.C., Saenz, C., Dunn-Lewis, C., Ballard, K.D., Quann, E.E., Kawiecki, D.L., Flanagan, S.D., Comstock, B.A., Fragala, M.S., Earp, J.E., Fernandez, M.L.,

Bruno, R.S., Ptolemy, A.S., Maresh, C.M., Kraemer, W.J., 2013. Whey protein supplementation during resistance training augments lean body mass. Journal of the American College of Nutrition 32, 122–135. doi:10.1080/07315724.2013.793580

Volpi, E., Kobayashi, H., Sheffield-Moore, M., Mittendorfer, B., Wolfe, R.R., 2003. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am. J. Clin. Nutr. 78, 250–258.

Wall, B.T., Hamer, H.M., de Lange, A., Kiskini, A., Groen, B.B.L., Senden, J.M.G., Gijsen, A.P., Verdijk, L.B., van Loon, L.J.C., 2013. Leucine co-ingestion improves post-prandial muscle protein accretion in elderly men. Clinical Nutrition 32, 412–419. doi:10.1016/j.clnu.2012.09.002

West, D.W., Burd, N.A., Coffey, V.G., Baker, S.K., Burke, L.M., Hawley, J.A., Moore, D.R., Stellingwerff, T., Phillips, S.M., 2011. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. The American Journal of Clinical Nutrition 94, 795–803. doi:10.3945/ajcn.111.013722

Yang, Y., Breen, L., Burd, N.A., Hector, A.J., Hector, A.J., Churchward-Venne, T.A., Josse, A.R., Josse, A.R., Tarnopolsky, M.A., Phillips, S.M., 2012. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. Br. J. Nutr. 108, 1780–1788. doi:10.1017/S0007114511007422

Table 1 Participants characteristics

	Milk	Native whey	*P* values for group differences
N (♂/♀)	15 (9/6)	15 (9/6)	
Age (years)	74 ± 4	73 ± 2	0.18
Body mass (kg)	74.6 ± 14.0	78.3 ± 16.2	0.81
Fat mass (kg)	22.1 ± 7.0	23.9 ± 8.9	0.55
Lean body mass (kg)	49.8 ± 9.2	49.5 ± 10.9	0.95
Body fat (%)	30.5 ± 5.5	32.0 ± 9.1	0.60
VL thickness (cm)	2.1 ± 0.4	2.3 ± 0.5	0.20
Leg press 1 RM (kg)	176 ± 55	158 ± 50	0.36
Bench press 1 RM (kg)	46.8 ± 20	42.8 ± 17	0.56

Table 2 Amino acid and macronutrient content of the milk and native whey supplements.

Amino acids (g per serving)	**Milk**	**Native whey**
Alanine	0.6	1.0
Arginine	0.6	0.6
Aspartic acid	1.5	2.2
Cysteine	0.2	0.5
Phenylalanine	0.9	0.9
Glutamic acid	4.1	3.9
Glycine	0.4	0.4
Histidine	0.5	0.5
Isoleucine	1.0	1.1
Leucine	1.9	2.5
Lysine	1.6	2.1
Methionine	0.5	0.5
Proline	1.9	1.3
Serine	1.1	1.0
Threonine	0.8	1.0
Tyrosine	0.8	0.7
Valine	1.2	1.2
Tryptophan	0.2	0.4
Total protein	**19.1**	**20.0**
Fat	**7.5**	**6.9**
Carbohydrate	**6.9**	**7.5**

Table 3 Daily intakes of energy and macronutrients before and during the training intervention. Values are averaged from two 24-hour recall interviews.

	Milk		Native whey	
	Baseline	Intervention	Baseline	Intervention
Energy (Kj)	7800 ± 2100	9400 ± 1500*	7400 ± 1900	9800 ± 1500*
Protein g·(kg body mass)$^{-1}$	1.0 ± 0.3	1.3 ± 0.2*	1.1 ± 0.3	1.3 ± 0.4*
Protein (E%)	17 ± 4	17 ± 3	19 ± 3	17 ± 2
Carbohydrate (E%)	39 ± 5	46 ± 3*	39 ± 6	43 ± 5*
Fat (E%)	44 ± 8	37 ± 4*	42 ± 7	40 ± 6

Values are means ± SD. * difference between pre and post, p = 0.05.

Table 4 Absolute values and relative changes for anthropometrics, regional muscle mass, muscle cross sectional area (CSA) and muscle fiber area (MFA) in elderly men and women receiving milk or native whey supplementation for 12 weeks combined with strength training.

	Milk			Native whey			P values for group difference (% change)
	Pre	Post	% change	Pre	Post	% change	
Body mass (kg)	74.6 ± 14.0	77.2 ± 14.0	3.6 ± 2.2*	75.9 ± 16.1	78.3 ± 16.2	3.3 ± 1.6*	0.618
Fat mass (kg)	22.1 ± 7.0	22.4 ± 6.8	1.7 ±5.3	23.9 ± 8.9	24.4 ± 8.9	2.4 ± 4.9	0.721
Leg lean mass (kg)	17.0 ± 3.8	18.0 ± 3.8	6.3 ± 3.6*	17.3 ± 4.7	18.0 ± 4.5	4.6 ± 3.4*	0.199
Arm lean mass (kg)	5.6 ± 1.5	6.0 ± 1.6	6.4 ± 3.6*	5.3 ± 1.5	5.6 ± 1.5	5.2 ± 3.9*	0.377
Trunk lean mass (kg)	24.0 ± 3.9	24.8 ± 3.7*	3.4 ± 3.6*	23.7 ± 4.8	24.4 ± 5.0*	3.0 ± 3.1*	0.791
Stair climb							
BW (s)	7.48 ± 1.0	7.18 ± 0.97	-3.8 ± 5.4*	7.54 ± 0.94	7.03 ± 0.93	-6.56 ± 7.1	0.257
10kg (s)	7.35 ± 1.0	7.21 ± 1.07	-3.3 ± 5.0*	7.62 ± 1.34	7.09 ± 1.07	-6.29 ± 8.1	0.267
20kg (s)	7.91 ± 1.53	7.58 ± 1.50	-3.8 ± 8.4*	8.07 ± 1.73	7.41 ± 1.26	-7.26 ± 8.57	0.293
Sit to stand (s)	7.0 ± 2.1	6.29 ± 1.3	-8.1 ± 13.9*	6.70 ± 1.20	5.92 ± 0.97	-11.0 ± 9.2	0.504
MFA type I (μm^2)	4519 ± 1030	4667 ± 1187	4.7 ± 22.0	4897 ± 919	4933 ± 785	2.8 ± 19.7	0.823
MFA type II (μm^2)	3862 ± 1984	4502 ±1397	34.2 ± 56.7*	3740 ± 1608	4692 ± 1441*	33.8 ± 27.4*	0.935

Values are means ± SD. * difference between pre and post, p = 0.05.

Supplementary table 1 Training program

Week	Exercise	Monday			Wednesday			Friday		
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
1-3	**Hammersquat**	2	12	RM	2	10	90% of 12RM	1	8	RM
	Leg press	1	12	RM	2	10	90% of 12RM	2	8	RM
	Kne extensions	2	12	RM	2	10	90% of 12RM	2	8	RM
	Calf raise	2	12	RM	2	10	90% of 12RM	2	8	RM
	Chest press	1	12	RM	2	10	90% of 12RM	1	8	RM
	Seated row	1	12	RM	2	10	90% of 12RM	1	8	RM
	Close grip pull-down	1	12		1	10	RM	1	8	RM
	Shoulder press	1	12	RM	2	10	90% of 12RM	1	8	RM
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
4-6	**Hammersquat**	2	10	RM	2	10	90% of 10RM	1	6	RM
	Leg press	1	10	RM	2	10	90% of 10RM	2	6	RM
	Kne extensions	2	10	RM	2	10	90% of 10RM	2	6	RM
	Calf raise	2	10	RM	2	10	90% of 10RM	2	6	RM
	Chest press	2	10	RM	3	10	90% of 10RM	2	6	RM
	Seated row	2	10	RM	3	10	90% of 10RM	2	6	RM
	Close grip pull-down	1	10		2	10	RM	1	6	
	Shoulder press	1	10	RM	2	10	90% of 10RM	1	6	RM
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
7-9	**Hammersquat**	2	10	RM	3	10	90% of 10RM	2	6	RM
	Leg press	2	10	RM	3	10	90% of 10RM	3	6	RM
	Kne extensions	3	10	RM	2	10	90% of 10RM	3	6	RM
	Calf raise	2	10	RM	2	10	90% of 10RM	2	6	RM
	Chest press	3	10	RM	3	10	90% of 10RM	3	6	RM
	Seated row	3	10	RM	3	10	90% of 10RM	3	6	RM
	Close grip pull-down	1	10		2	10	RM	1	6	
	Shoulder press	2	10	RM	2	10	90% of 10RM	2	6	RM
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
10-11	**Hammersquat**	3	8	RM	3	8	90% of 8RM	2	6	RM
	Leg press	2	8	RM	3	8	90% of 8RM	3	6	RM
	Kne extensions	3	8	RM	2	8	90% of 8RM	3	6	RM
	Calf raise	2	8	RM	2	8	90% of 8RM	2	6	RM
	Chest press	3	8	RM	3	8	90% of 8RM	3	6	RM
	Close grip pull-down	2	8		3	10	RM	3	6	
	Seated row	2	8	RM	2	8	90% of 8RM	2	6	RM
	Shoulder press	2	8	RM	2	8	90% of 8RM	2	6	RM

Supplementary table 2 Antibodies

Antibody	Dilution	Amount of protein loaded on gel (µg)	Cat. no	Manufacturer
P70S6K	1:1000	30	2708	Cell signaling
phospho-P70S6K Thr^{389}	1:1000	30	9234	Cell signaling
eEF-2	1:5000	30	2332	Cell signaling
phospho-eEF-2^{Thr56}	1:5000	30	2331	Cell signaling
4E-BP1	1:1000	60	9452	Cell signaling
phospho-4EBP-$1^{Thr37/46}$	1:1000	60	9455	Cell signaling
Secondary anti-rabbit	1:3000		7074	Cell signaling

Figure 1. Flow chart diagram of study recruitment, enrollment, randomization follow-up, and analysis.

Figure 2. Timeline of the study.

Figure 3. Relative changes in lean mass and *m. vastus lateralis* thickness (A) and leg press and bench press 1RM (B) after a 12-week strength training and protein supplementation period in elderly. Values are mean ± SD. n = 15 and 15 in the milk group and native whey group, respectivel Data were analyzed with a two-way repeated measures ANOVA (time x supplement). Multiple comparisons tests were used as *post hoc* tests to specify the significant differences between groups (Sidak) and within groups (Tukey). Relative changes were analyzed with a Student´s *t* test for differences between pre and 2 hours within groups (paired) and between groups (un-paired). * difference between before and after the training period within group, $p < 0.05$.

Figure 4. Changes in serum glucose (A), insulin (B), urea (C) and creatine kinase (D) following intake of milk, or native whey immediately after a bout of resistance exercise in elderly. Arrow indicates time point of protein supplement ingestion. Values are mean ± SD (only shown for highest and lowest values). n = 9 and 11 in the milk and native whey group, respectively. Data were analyzed with a two-way repeated measures ANOVA (time x supplement). Multiple comparisons tests were used as *post hoc* tests to specify the significant differences between groups (Tukey), and within groups and training states (Dunett). Black data points indicate difference form resting values, gray data

points indicate no significant difference from resting levels. * milk difference between pre and post, § native whey difference between pre and post, † milk and native whey different at pre, ‡ milk and native whey different at post, $p < 0.05$.

Figure 5. Blood concentrations of essential amino acids (A), branched chain amino acids (B) and leucine (C) following intake of milk, or native whey immediately after a bout of resistance exercise in elderly. Arrow indicates time point of protein supplement ingestion. Values are mean ± SD (only shown for highest and lowest values). n = 9 and 11 in the milk group and native whey group, respectively. Data were analyzed with a two-way repeated measures ANOVA (time x supplement). Multiple comparisons tests were used as *post hoc* tests to specify the significant differences between groups (Tukey) and time points (Dunnet). Black data points indicate difference form resting values, gray data points indicate no significant difference from resting levels. * milk difference between pre and post, § native whey difference between pre and post, † milk and native whey different at pre, ‡ milk and native whey different at post $p < 0.05$.

Figure 6. Phospho/total ratio of P70S6K (A), 4E-BP1 (B) and eEF-2 (C) following intake of milk and native whey immediately after a bout of resistance exercise in elderly. Values are mean ± SD. n = 9 and 11 in the milk group and native whey group, respectively. Data were analyzed with a two-way repeated measures ANOVA (time x supplement). Multiple comparisons tests were used as *post hoc* tests to specify the significant differences between groups (Sidak) and time points (Tukey). Relative changes were analyzed with a Student´s *t* test for

differences between pre and 2 hours within groups (paired) and between groups (un-paired). * different from pre within group, § relative difference from pre, $p < 0.05$.

Supplementary figure 1 Recovery of isometric knee extensor force-generating capacity relative to resting values following intake of milk and native whey immediately after a bout of resistance exercise in elderly. Values are mean ± SD. n = 9 and 11 in the milk group and native whey group, respectively. Data were analyzed with a two-way repeated measures ANOVA (time x supplement). Multiple comparisons tests were used as *post hoc* tests to specify the significant differences between groups (Tukey) and within groups (Dunnett). Black data points indicate difference form resting values, gray data points indicate no significant difference from resting levels.

Supplementary figure 2 Representative blots from Western Blot analysis of phosphorylated and total p70S6K, 4E-BP1 and eEF-2. Bands are shown for each individual group at baseline and 2 hours after exercise. Samples were run in duplicates.

Figure 1

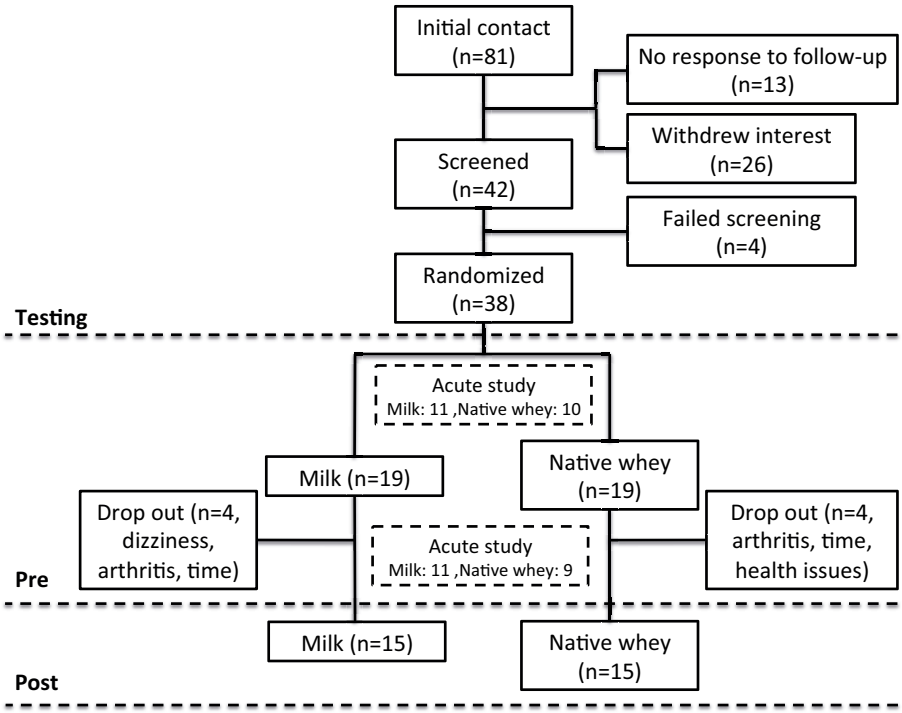

Initial contact (n=81)
No response to follow-up (n=13)
Withdrew interest (n=26)
Screened (n=42)
Failed screening (n=4)
Randomized (n=38)
Testing
Acute study
Milk: 11 ,Native whey: 10
Milk (n=19)
Native whey (n=19)
Drop out (n=4, dizziness, arthritis, time)
Acute study
Milk: 11 ,Native whey: 9
Drop out (n=4, arthritis, time, health issues)
Pre
Milk (n=15)
Native whey (n=15)
Post

Figure 2

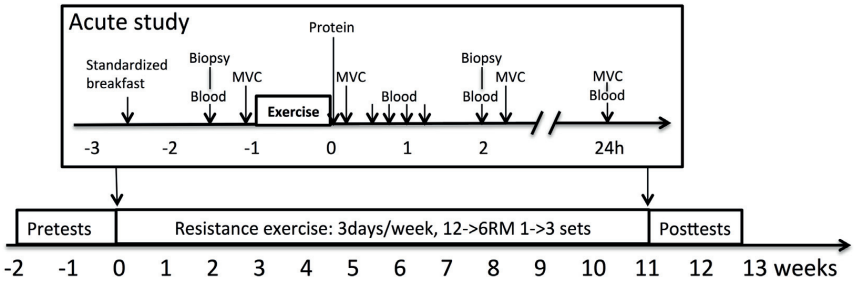

Acute study
Protein
Standardized breakfast
Biopsy
Blood
MVC
Exercise
MVC
Blood
Biopsy
Blood
MVC
MVC
Blood
-3
-2
-1
0
1
2
24h
Pretests
Resistance exercise: 3days/week, 12->6RM 1->3 sets
Posttests
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 weeks

Figure 3

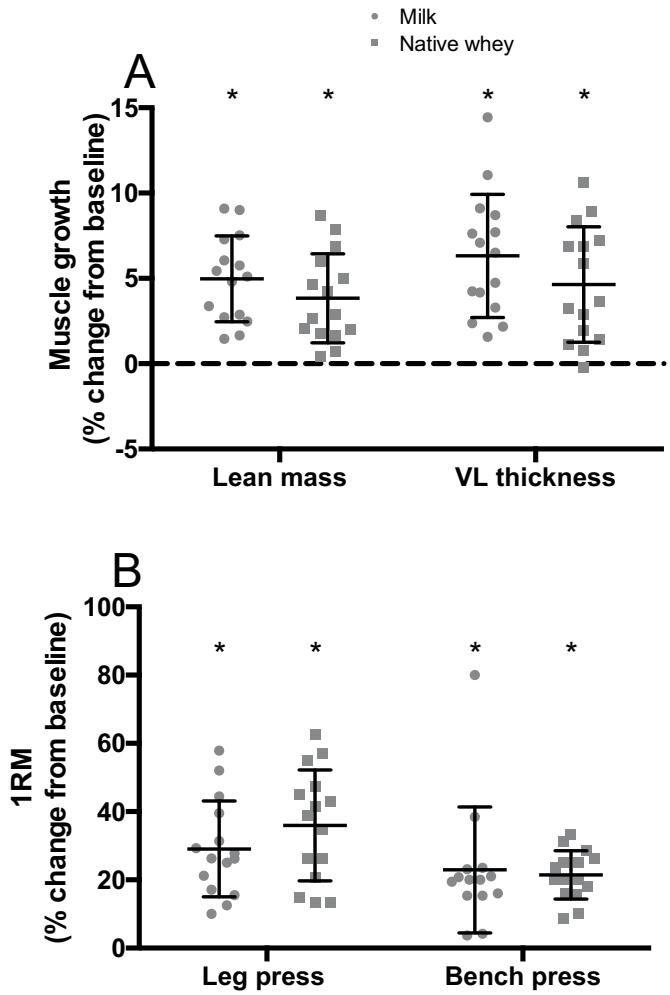

Milk
Native whey
A
Muscle growth
(% change from baseline)
15
10
5
0
-5
*
*
*
*
Lean mass
VL thickness
B
1RM
(% change from baseline)
100
80
60
40
20
0
*
*
*
*
Leg press
Bench press

Figure 4

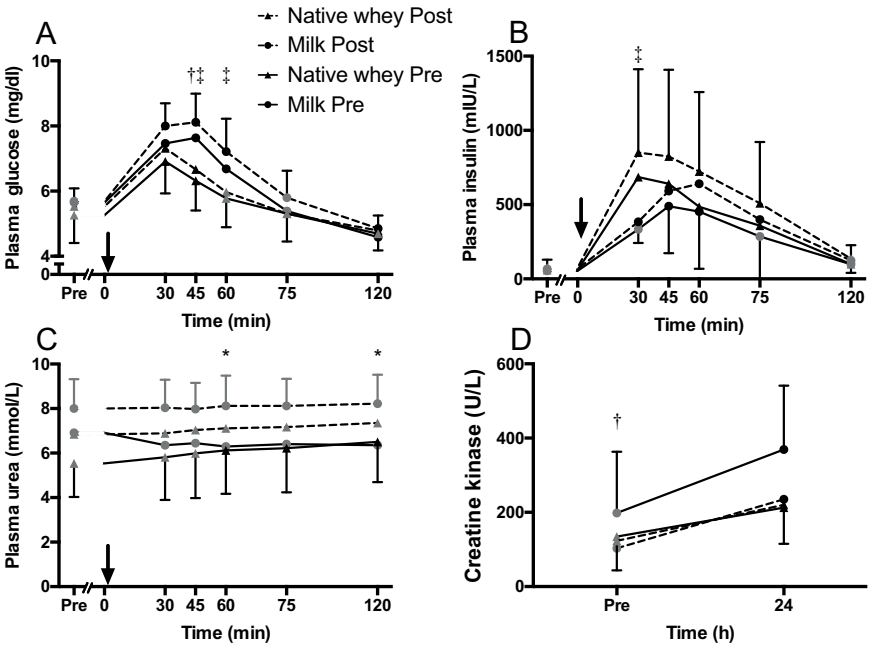

A
Native whey Post
Milk Post
Native whey Pre
Milk Pre
Plasma glucose (mg/dl)
Time (min)
B
Plasma insulin (mIU/L)
Time (min)
C
Plasma urea (mmol/L)
Time (min)
D
Creatine kinase (U/L)
Time (h)

Figure 5

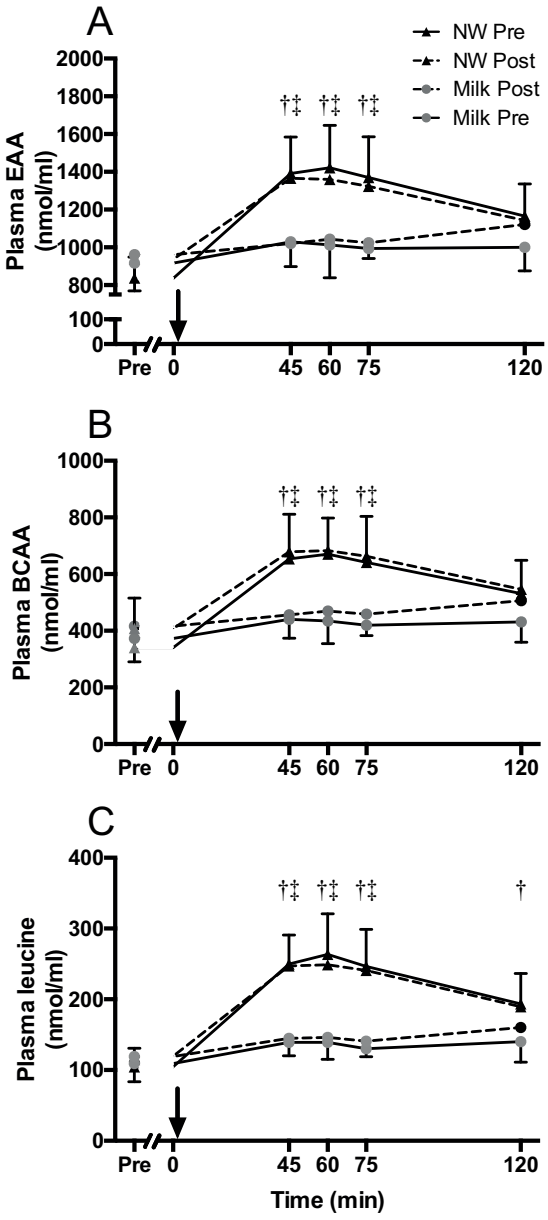
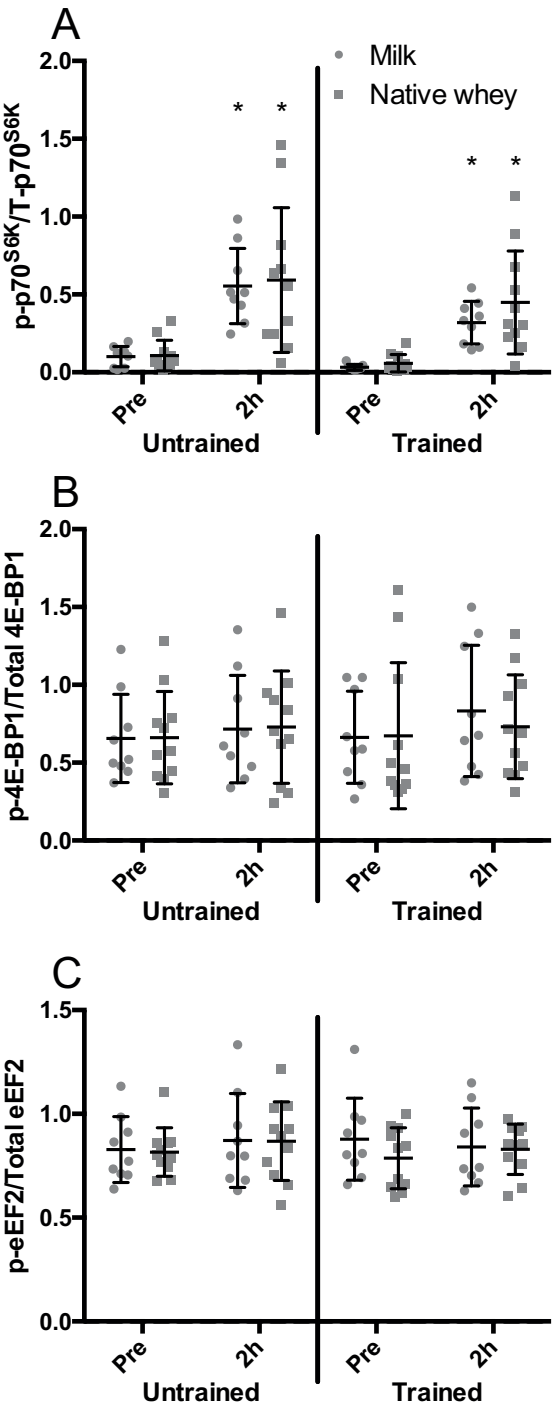
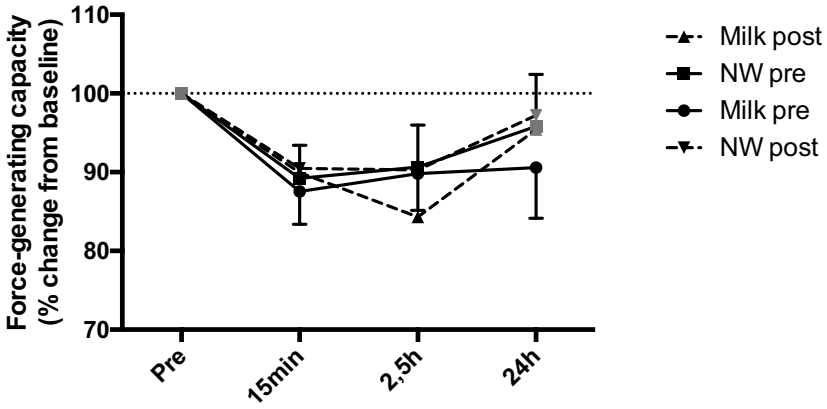

A
NW Pre
NW Post
Milk Post
Milk Pre
Plasma EAA (nmol/ml)
2000
1800
1600
1400
1200
1000
800
100
0
†‡ †‡ †‡
Pre 0 45 60 75 120
B
Plasma BCAA (nmol/ml)
1000
800
600
400
200
0
†‡ †‡ †‡
Pre 0 45 60 75 120
C
Plasma leucine (nmol/ml)
400
300
200
100
0
†‡ †‡ †‡ †
Pre 0 45 60 75 120
Time (min)

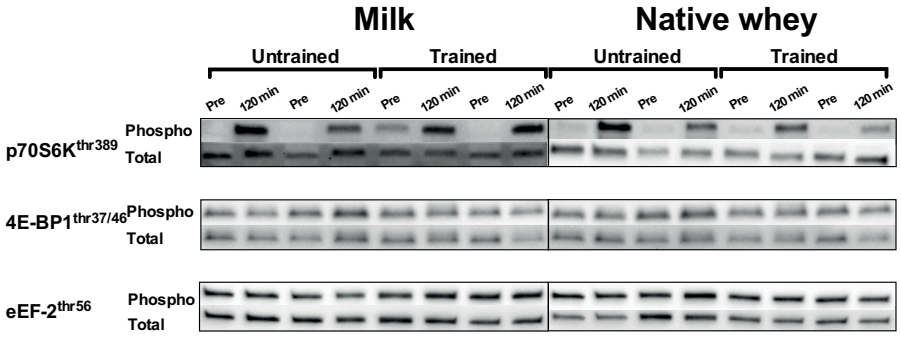
Figure 6


A
Milk
Native whey
p-p70S6K/T-p70S6K
2.0
1.5
1.0
0.5
0.0
*
*
*
*
Pre
2h
Pre
2h
Untrained
Trained
B
p-4E-BP1/Total 4E-BP1
2.0
1.5
1.0
0.5
0.0
Pre
2h
Pre
2h
Untrained
Trained
C
p-eEF2/Total eEF2
1.5
1.0
0.5
0.0
Pre
2h
Pre
2h
Untrained
Trained

Supplementary figure 1



Supplementary figure 2



APPENDIX I

Approval from the Regional Committee for Medical and Health Research Ethics

Participant information and consent form

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Tor Even Svanes	22845521	02.07.2014	2014/834/REK sør-øst C
			Deres dato:	**Deres referanse:**
			13.05.2014	

Vår referanse må oppgis ved alle henvendelser

Truls Raastad
Norges idrettshøgskole

2014/834 Hvordan påvirker inntak av melkeproteiner kombinert med styrketrening muskelmasse og funksjon?

Forskningsansvarlig: Norges idrettshøgskole
Prosjektleder: Truls Raastad

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst) i møtet 10.06.2014. Vurderingen er gjort med hjemmel i helseforskningsloven (hfl.) § 10, jf. forskningsetikklovens § 4.

Prosjektomtale
Sarkopeni (for liten muskelmasse) rammer mange eldre. En svak muskulatur er derfor en viktig årsak til dårlig funksjon og institusjonalisering blant eldre. Tiltak som kan bidra til å bevare muskelmasse gjennom livet vil derfor kunne gi en stor helsegevinst. En kostholdskomponent som er vist å ha positiv effekt på muskulatur, er proteinkvalitet. Det er vist at myse fra melk gir en større stimulering av muskelproteinsyntesen enn andre proteinkilder. Forklaringen er knyttet til at myse absorberes raskt og har et stort innehold av leucin. Man har nå vist at myseprotein fremstilt via filterasjonsteknikk absorberes raskere, gir en større økning av leucin og stimulerer signalveier i større grad enn andre kommersielt tilgjengelige myseprodukter og melk. Nå vil man studere om disse forskjellene man ser akutt etter inntak kan overføres til en større akkumulering av muskelmasse over en periode der inntaket kombineres med styrketrening hos en gruppe eldre (> 70 år) og yngre (18-45 år) voksne. Antall deltakere i Norge: 70 Samtykkebasert

Vurdering
Som en del av intervensjonen i dette prosjektet vil det tas muskelbiopsier, og særlig for de deltakerne som trekkes til akuttforsøket, er det snakk om en relativt betydelig ulempe (totalt seks biopsier). Komiteen mener biopsitakingen er tilfredsstillende beskrevet i selve informasjonsskrivet, men mener at det også påhviler prosjektgruppen et ansvar for at disse opplysningene formidles tydelig og nøkternt som en del av den muntlige informasjonen som gis.

Intervensjonen medfører også DXA-analyser og måling av HbA1c; tester hvor man kan komme over utilsiktede funn som tilsier behandlingsbehov. Komiteen forutsetter derfor også at det er etablert en beredskap i prosjektet som sikrer videre henvisning, for de tilfellene hvor man kommer over slike funn.

Prosjektet er søkt med en sluttdato i 2015, men man ønsker likevel å oppbevare data frem til 2030, uten at dette er videre begrunnet. Komiteen vil her påpeke at femten års oppbevaringstid for opplysninger er lang tid, og viser i den forbindelse til helseforskningslovens § 38: *Opplysninger skal ikke oppbevares lenger enn*

det som er nødvendig for å gjennomføre prosjektet.

Komiteen setter derfor en tidsavgrensning for oppbevaring av prosjektopplysninger på fem år, frem til og med 31.12.2020. Tidsavgrensningen vil også gjelde for forskningsbiobanken som skal opprettes.

Forskningsbiobank
Det søkes om å opprette en spesifikk forskningsbiobank med navn *Nativ myse og effekt på muskelmasse* i prosjektet.

Ansvarshavende for forskningsbiobanken er Truls Raastad. Forskningsansvarlig er Norges Idrettshøyskole.

Forskningsbiobanken vil bestå av muskelbiopsier og blodprøver

Komiteen setter en tidsavgrensning for forskningsbiobanken i tråd med oppbevaringstiden for øvrige data i prosjektet, til og med 2020. Deretter skal materialet behandles i henhold til helseforskningslovens § 30.

Biologisk materiale vil utføres til utlandet i henhold til helseforskningslovens § 29. Deltakerne er orientert om dette i informasjonsskriv.

Informasjonsskriv og samtykkeerklæring
Informasjonsskrivet må revideres med tanke på komiteens tidsavgrensning for prosjektet, slik at deltakerne mottar riktig dato for prosjektslutt og anonymisering/sletting.

Ut fra dette setter komiteen følgende vilkår for prosjektet:

1. Det skal etableres en beredskap for viderehenvisning ved utilsiktede funn i prosjektet.
2. Data og humant biologisk materiale kan ikke oppbevares lenger enn til 2020.
3. Informasjonsskriv skal revideres i tråd med det ovennevnte.

Vedtak
Prosjektet godkjennes under forutsetning av at ovennevnte vilkår oppfylles, jf. helseforskningslovens §§ 9 og 33.

Komiteen godkjenner opprettelse av forskningsbiobanken *Nativ myse og effekt på muskelmasse*, i tråd med det som er angitt i prosjektsøknaden. Biobankregisteret vil bli underrettet ved kopi av dette brev.

I tillegg til vilkår som fremgår av dette vedtaket, er tillatelsen gitt under forutsetning av at prosjektet gjennomføres slik det er beskrevet i søknaden og protokollen, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Tillatelsen gjelder til 31.12.2015. Av dokumentasjons- og oppfølgingshensyn skal prosjektopplysningene likevel bevares inntil 31.12.2020. Opplysningene skal lagres avidentifisert, dvs. atskilt i en nøkkel- og en opplysningsfil. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato.

Komiteens avgjørelse var enstemmig.

Komiteens vedtak kan påklages, jf. helseforskningsloven § 10, 3 ledd og forvaltningsloven § 28. En eventuell klage sendes til REK sør-øst C. Klagefristen er tre uker fra mottak av dette brevet, jf. forvaltningsloven § 29.Dersom vedtaket opprettholdes av REK sør-øst, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Sluttmelding og søknad om prosjektendring
Prosjektleder skal sende sluttmelding til REK sør-øst på eget skjema senest 30.06.2016, jf. hfl.

12. Prosjektleder skal sende søknad om prosjektendring til REK sør-øst dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. hfl. § 11.

Med vennlig hilsen

Britt-Ingjerd Nesheim
prof.dr.med
leder REK sør-øst C

Tor Even Svanes
seniorrådgiver

Kopi til:

Turid Sjøstedt**:** turid.sjostedt@nih.no

Norges idrettshøgskole ved øverste administrative ledelse: postmottak@nih.no

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)

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)

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, være normalt aktiv, og ellers være frisk og uten skader i muskelskjelettapparatet. Du kan ikke bruke noen form for medikamenter eller kosttilskudd (proteinpulver, vitaminer, kreatin eller lignende). Hvis du bruker kosttilskudd kan du likevel delta som forsøksperson ved at du slutter med tilskuddet senest én uke før prosjektstart. Du kan ikke delta om du er laktoseintolerant, har melkeallergi eller er allergisk mot lokalbedøvelse (tilsvarende det man får hos tannlegen).

Bakgrunn og hensikt med forsøket

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 om et nyutviklet myseprotein produsert

av Tine® kan bedre effekten av styrketrening (føre til større økning i muskelmasse og styrke). Dette nye myseproteinet vil sammenliknes med effekten av vanlig lett melk.

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

Gjennomføringen av forsøket

Forsøket går kort fortalt ut på å gjennomføre en teningsperiode på 12 uker med styrketrening tre ganger i uken. Gjennom denne perioden inntas det to enheter på 0,5 l daglig med enten melk eller nativ myse. Du vil bli tilfeldig trukket (randomiseres) til én av gruppene. Før og etter treningsperioden vil det gjennomføres en rekke tester (se under) for å undersøke effekten av de forskjellige drikkene.

Før treningsperioden

Du skal møte på Norges idrettshøgskole 3 ganger for tilvenning til tester og treningsøvelser, styrketester, måling av kroppssammensetning (DXA) og muskelbiopsier i ukene før forsøket. I tillegg må du møte for en MR-analyse hos Curato røntgen. Tidspunkter for de ulike oppmøtene avtales individuelt. Under følger et eksempel på tidsplan for tester:
Dag 1: Underskrevet samtykke og helseerklæring. Fastende DXA-scan, medbrakt frokost, ultralyd av låret, tilvennig til styrketester (ca. 2-3 timer).
Minimum 2 dager hvile.
Dag 2: Gjennomføring av styrketester (ca 1 time).
Minimum 2 dager hvile.
Dag 3: MR hos Curato røntgen (ca. 40 minutter).
Dag 4: Akuttforsøk (ca. 5 timer) eller prebiopsi (ca 45 minutter).

Før trenignsstudien må du også gjennomføre en firedagers kostregistrering, en tilsvarende kostregistrering vil gjentas mot slutten av treningsperioden. **I de to siste dagene før tester og biopsi(er) må du avstå fra all krevende fysisk aktivitet (trening)**. Fra dagen før biopsiene til dagen etter biopsiene skal du

følge en standardisert diett laget av en ernæringsfysiolog.

Akuttforsøk

Tolv deltakere fra hver gruppe trekkes tilfeldig ut til å gjennomføre et akuttforsøk før og etter treningsperioden, dette innebærer 2 biopsier før treningsperioden og 2 biopsier etter treningsperioden (totalt 4 biopsier). De resterende deltakerne i hver gruppe deltar ikke i akuttstudien og tar bare en biopsi før og en etter treningsperioden. Hensikten med akuttforsøket er å måle hvordan proteinsyntesen og proteinnedbrytningen forandres etter treningsperioden. Oppstart denne dagen vil være mellom kl. 0800 og 1000, og forsøket er ferdig mellom kl. 1330 og 1530. Dagen starter med en biopsi og en styrketest i et kneekstensjonsapparat. Så gjennomføres en styrkeøkt som vil være identisk med en av øktene som gjøres senere i treningsperioden. Etter treningsøkten vil du innta en av de to drikkene, og det vil bli tatt biopsier to timer etter økten. Det vil også bli tatt blodprøver gjennom dagen og gjennomført styrketester rett etter økten, 2 timer etter økten og 24 timer etter økten, for å måle restitusjon. Dermed vil du måtte sette av en hel dag til testdagen (fra 0800-1000 frem til ca. 1330-1530) og 30 min til styrketesting dagen etter.

Treningsperioden

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

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

Tester

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

MR: for å måle muskelvekst i lår og overarms-muskulaturen benyttes en MR-analyse. Denne testen innebærer at du må ligge i ro ca. 30 minutter.

Ultralyd: for å måle muskelvekst i m. vastus lateralis (en muskel i låret) gjennomføres en ultralyd av låret.

1RM tester: for å måle styrke vil det testes hvor mye du kan løfte maksimalt en gang i styrkeøvelser for overkropp og bein.

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

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

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

Biopsiene tas ut på følgende måte:

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

Eventuelle ulemper ved å delta

Deltakelse i prosjektet vil kreve en del tid og oppmerksomhet.

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

Venekateter medfører en liten infeksjonsfare og det kan oppleves ubehagelig.

Vevsprøvetakninger (biopsier) medfører en liten infeksjonsfare, og ubehag/smerter kan oppleves under inngrepet. Du kan også oppleve lette til

moderate smerter i 1-2 døgn etter inngrepet.
Du vil få et lite arr etter snittet i huden; arret vil sakte bli mindre tydelig. Enkelte personer vil kunne få en fortykning av huden i arrområdet.

Personvern

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

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

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

Biobank

Biopsiene og blodprøvene vil bli oppbevart i en forskningsbiobank uten kommersielle interesser (vurdert av Regional Etisk Komite). Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Prøvene vil bli lagret til år 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)

Forespørsel om deltakelse som forsøksperson

Hvordan påvirker inntak av forskjellige melkeproteiner muskelproteinbalanse hos eldre?

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

Bakgrunn og hensikt med forsøket

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

styrketrening, som vil være av stor interesse for eldre med tanke på livskvalitet og funksjon i hverdagen.

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

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

Gjennomføringen av forsøket

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

Før treningsperioden

Du skal møte på Norges idrettshøgskole 4 ganger for tilvenning til tester og treningsøvelser, styrketester, måling av kroppssammensetning (DXA), en legesjekk og muskelbiopsier i ukene før forsøket. I tillegg må du møte for en MR-analyse hos Curato røntgen. Tidspunkter for de ulike oppmøtene avtales individuelt. Under følger et eksempel på tidsplan for tester:
Dag 1: Underskrevet samtykke og helseerklæring. Fastende blodprøve, DXA-scan, medbrakt frokost, tilvennig til styrketester og funksjonelle tester (ca. 2 timer).
Minimum 2 dager hvile.
Dag 2: Gjennomføring av styrketester og funksjonelle tester (ca 1 time).
Minimum 2 dager hvile.
Dag 3: MR hos Curato røntgen (ca. 30 minutter).
Dag 4: Akuttforsøk (ca. 8timer) eller prebiopsi (ca 45 minutter).

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

Akuttforsøk

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

Treningsperioden

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

Tester

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

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

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

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

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

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

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

Biopsiene tas ut på følgende måte:

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

Eventuelle ulemper ved å delta

Deltakelse i prosjektet vil kreve en del tid og oppmerksomhet.

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

Venekateter medfører en liten infeksjonsfare og det kan oppleves ubehagelig.

Vevsprøvetakninger (biopsier) medfører en liten infeksjonsfare, og ubehag/smerter kan oppleves under inngrepet. Du kan også oppleve lette til

moderate smerter i 1-2 døgn etter inngrepet.
Du vil få et lite arr etter snittet i huden; arret vil sakte bli mindre tydelig. Enkelte personer vil kunne få en fortykning av huden i arrområdet.

Personvern

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

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

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

Biobank

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

Innsynsrett og oppbevaring av materiale

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

Informasjon om utfallet av studien

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

Forsikring

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

Finansiering

Prosjektet er fullfinansiert av Tine® og Norges forskningsråd.

Publisering

Resultatene fra studien vil offentliggjøres i internasjonale, fagfellevurderte, tidsskrift. Du vil få tilsendt artiklene hvis du ønsker det.

Samtykke

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

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

Dersom du ønsker flere opplysninger kan du ta kontakt med Håvard Hamarsland på tlf: 93 445 916 eller e-post: haavardh@nih.no, Gøran Paulsen på tlf: 93429420, eller Truls Raastad på tlf: 23 26 23 28 el. 913 68 896

Vennlig hilsen
Håvard Hamarsland (Stipendiat)
Gøran Paulsen (forsker)
Truls Raastad (Professor)

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)

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

APPENDIX II

Training program for young (study IV) and elderly (study V) participants

Training program young (study IV)

Week	Exercise	Monday			Wednesday			Friday		
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
1-3	**Squat**	2	12	RM	2	10	90% of 12RM	1	8	RM
	Leg press	2	12	RM	2	10	90% of 12RM	2	8	RM
	Kne extensions	1	12	RM	2	10	90% of 12RM	1	8	RM
	Bench press	1	12	RM	2	10	90% of 12RM	2	8	RM
	Seated row	2	12	RM	2	10	90% of 12RM	1	8	RM
	Close grip pull-down	1	12	RM	2	10	90% of 12RM	2	8	RM
	Shoulder press	1	12	RM	2	10	90% of 12RM	1	8	RM
4-6	**Squat**	2	10	RM	2	10	90% of 10RM	2	8	RM
	Leg press	2	10	RM	3	10	90% of 10RM	2	8	RM
	Kne extensions	2	10	RM	2	10	90% of 10RM	2	8	RM
	Bench press	2	10	RM	3	10	90% of 10RM	2	8	RM
	Seated row	2	10	RM	3	10	90% of 10RM	2	8	RM
	Close grip pull-down	2	10	RM	2	10	90% of 10RM	2	8	RM
	Shoulder press	2	10	RM	2	10	90% of 10RM	1	8	RM
7-9	**Squat**	3	10	RM	3	10	90% of 10RM	3	6	RM
	Leg press	3	10	RM	3	10	90% of 10RM	3	6	RM
	Kne extensions	2	10	RM	3	10	90% of 10RM	2	6	RM
	Bench press	3	10	RM	3	10	90% of 10RM	3	6	RM
	Seated row	3	10	RM	3	10	90% of 10RM	2	6	RM
	Close grip pull-down	2	10	RM	3	10	90% of 10RM	3	6	RM
	Shoulder press	2	10	RM	2	10	90% of 10RM	2	6	RM
10-12	**Squat**	3	8	RM	3	8	90% of 10RM	3	6	RM
	Leg press	3	8	RM	3	8	90% of 10RM	3	6	RM
	Kne extensions	3	8	RM	3	8	90% of 10RM	3	6	RM
	Bench press	3	8	RM	3	8	90% of 10RM	3	6	RM
	Seated row	3	8	RM	3	8	90% of 10RM	3	6	RM
	Close grip pull-down	2	8	RM	3	8	90% of 10RM	3	6	RM
	Shoulder press	2	8	RM	3	8	90% of 10RM	2	6	RM

Training program elderly (study V)

Week	Exercise	Monday			Wednesday			Friday		
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
1-3	**Hammersquat**	2	12	RM	2	10	90% of 12RM	1	8	RM
	Leg press	1	12	RM	2	10	90% of 12RM	2	8	RM
	Kne extensions	2	12	RM	2	10	90% of 12RM	2	8	RM
	Calf raise	2	12	RM	2	10	90% of 12RM	2	8	RM
	Chest press	1	12	RM	2	10	90% of 12RM	1	8	RM
	Seated row	1	12	RM	2	10	90% of 12RM	1	8	RM
	Close grip pull-down	1	12		1	10	RM	1	8	RM
	Shoulder press	1	12	RM	2	10	90% of 12RM	1	8	RM
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
4-6	**Hammersquat**	2	10	RM	2	10	90% of 10RM	1	6	RM
	Leg press	1	10	RM	2	10	90% of 10RM	2	6	RM
	Kne extensions	2	10	RM	2	10	90% of 10RM	2	6	RM
	Calf raise	2	10	RM	2	10	90% of 10RM	2	6	RM
	Chest press	2	10	RM	3	10	90% of 10RM	2	6	RM
	Seated row	2	10	RM	3	10	90% of 10RM	2	6	RM
	Close grip pull-down	1	10		2	10	RM	1	6	
	Shoulder press	1	10	RM	2	10	90% of 10RM	1	6	RM
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
7-9	**Hammersquat**	2	10	RM	3	10	90% of 10RM	2	6	RM
	Leg press	2	10	RM	3	10	90% of 10RM	3	6	RM
	Kne extensions	3	10	RM	2	10	90% of 10RM	3	6	RM
	Calf raise	2	10	RM	2	10	90% of 10RM	2	6	RM
	Chest press	3	10	RM	3	10	90% of 10RM	3	6	RM
	Seated row	3	10	RM	3	10	90% of 10RM	3	6	RM
	Close grip pull-down	1	10		2	10	RM	1	6	
	Shoulder press	2	10	RM	2	10	90% of 10RM	2	6	RM
		Sets	Reps	Load	Sets	Reps	Load	Sets	Reps	Load
10-11	**Hammersquat**	3	8	RM	3	8	90% of 8RM	2	6	RM
	Leg press	2	8	RM	3	8	90% of 8RM	3	6	RM
	Kne extensions	3	8	RM	2	8	90% of 8RM	3	6	RM
	Calf raise	2	8	RM	2	8	90% of 8RM	2	6	RM
	Chest press	3	8	RM	3	8	90% of 8RM	3	6	RM
	Close grip pull-down	2	8		3	10	RM	3	6	
	Seated row	2	8	RM	2	8	90% of 8RM	2	6	RM
	Shoulder press	2	8	RM	2	8	90% of 8RM	2	6	RM