UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

PhD thesis

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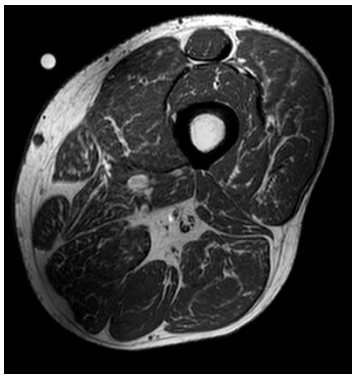
Preservation of muscle mass and function through protein supplementation and exercise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This thesis has been submitted to the Graduate School of Health and Medical Sciences April 30th, 2020.

PhD Thesis

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1. Preface and acknowledgements

This PhD thesis cover the work performed during my PhD scholarship at University of Copenhagen. The CALM intervention study formed the basis for the results in this thesis, and was funded by the University of Copenhagen Excellence Program for Interdisciplinary Research with generous support from Arla Foods Ingredients P/S. The study was conducted at the Institute of Sports Medicine Copenhagen at Bispebjerg Hospital from 2014 to 2018.

I would like to thank my supervisors for their support and guidance during this process. I am especially grateful to Michael Kjær for giving me the opportunity to do this PhD scholarship. You have created an outstanding research and working environment that has been a joy to be a part of so far, and which I look forward to continue being a part of. To Lars Holm, I also owe you a great amount of gratitude. Since I first stepped into your office nearly 7 years ago, you have provided an enormous amount of enthusiastic feedback and support. I would not have made it this far in research without your help – Not just because of all the work you have done to give me these opportunities, but mainly because I never considered achieving a PhD an option for me until you opened my eyes to this possibility.

In relation to the CALM study, I want to thank the many participants who provided their time and interest in the study. This study would also not have been possible without the assistance of the many students and scientific personnel that have been involved in the study throughout the years. With regards to making the CALM study possible, I am especially grateful for the incredible effort done by Rasmus Bechshøft and Søren Reitelseder. Without the amazing work you did in setting up the logistics of the study, this study had been an unmanageable task.

Thank you to everyone who contributed to making it a joy to come to work at the ISMC during the past years, especially those of you who were a part of the legendary CALM office. This office provided interesting scientific discussions (and lengthy monologues by Jacob), as well as friendly competitions (A.K.A Girth´s chances for glory). Although this office might have looked like a dorm room from the outside, everyone in this office contributed to a unique working environment that was essential for my sanity during my efforts to make it through analyzing ~1000 MRI images of thighs.

Lastly, I want to thank my friends and family for their support throughout the years. My girlfriend, Mathilde, has had a pivotal role in the making of this thesis, with her unrelenting cheering and support through the many ups and downs during the last years. Especially during the last months of writing this thesis in Covid-19 lockdown, your support has been invaluable. Thank you.

Kenneth H. Mertz, Copenhagen May 1st.

2. List of Papers

Paper 1:

Kenneth H. Mertz, Søren Reitelseder, Mikkel Jensen, Jonas Lindberg, Morten Hjulmand, Aide Schucany, Søren Binder Andersen, Rasmus L. Bechshoeft, Markus D. Jakobsen, Theresa Bieler, Nina Beyer, Jakob Lindberg Nielsen, Per Aagaard, Lars Holm. Influence of between-limb asymmetry in muscle mass, strength, and power on functional capacity in healthy older adults. *Scand J Med Sci Sports 2019 p. 1-8. https ://doi.org/10.1111/sms.13524*

Paper 2:

Kenneth H. Mertz, Søren Reitelseder, Rasmus Bechshoeft, Jacob Bulow, Grith Højfeldt, Mikkel Jensen, Simon R. Schacht, Mads Vendelbo Lind, Morten A. Rasmussen, Ulla R. Mikkelsen, Inge Tetens, Søren B. Engelsen, Dennis S. Nielsen, Astrid P. Jespersen, Lars Holm. The effect of daily protein supplementation with or without resistance training for 1 year on muscle size, strength and function in healthy older adults. A randomized controlled trial. *(Under review)*

Paper 3:

Kenneth H. Mertz, Søren Reitelseder, Morten A. Rasmussen, Jacob Bülow, Grith Højfeldt, Mikkel Jensen, Morten Hjulmand, Jonas Lindberg, Mathilde U. Kramer, Rasmus Bechshoeft, Lars Holm. Temporal changes in muscle mass, strength and function in older adults during and after a center-based or home-based resistance training intervention. The CALM trial. *(In preparation).*

3. Abbrevations

ADL:	Activities of Daily Living
ASMI:	Appendicular Skeletal Muscle Index
BMI:	Body Mass Index
BMR:	Basal Metabolic Rate
CI:	Confidence Interval
CT:	Computed Tomography
DIAAS:	Digestible Indispensable Amino Acid Score
DXA:	Dual Energy X-Ray Absorptiometry
EI:	Energy Intake
IDAA:	Indispensable Dietary Amino Acid
mITT:	Modified Intention-to-Treat
MVIC:	Maximal Voluntary Isometric Contraction
LEP:	Leg Extensor Power
LEF:	Lower Extremity Function
LTM:	Lean Tissue Mass
MPS:	Muscle Protein Synthesis
MRI:	Magnetic Resonance Imaging
PDCAAS:	Protein Digestibility-Corrected Amino Acid Score
qCSA:	Quadriceps cross-sectional area.
RCT:	Randomized Controlled Trial
RDA:	Recommended Daily Allowance
RFD:	Rate of Force Development

RM: Repetition Maximum

SD: Standard Deviation

SE: Standard Error

3. Abstract

Muscle mass, strength, and power begins to decline around the 5th decade of life, increasing the risk of developing functional limitations with increasing age. These age-related declines are caused by a complex nexus of biological alterations in hormonal milieu, inflammation, neural function, tendon function etc. While this development is likely inevitable, strategies for counteracting these declines are of great interest, in order to maintain physical function of older adults for as long as possible.

The absolute levels of muscle mass, strength, and power has been shown numerous times to be predictive of current as well as future functional capabilities of older adults. However, limited research has looked into the impact of between-limb asymmetry in these parameters on functional capabilities. In paper 1, we investigated the prevalence of between-limb asymmetry in measures of lower extremity muscle mass, strength and power, as well as its association to functional capabilities in a cohort of healthy older adults. We found that the average degree of between-limb asymmetry in measures of muscle strength and power was ~10%, whereas the asymmetry in leg muscle mass was ~3%. However, measures of between-limb asymmetry in muscle mass, strength, and power were not consistently associated with functional capabilities. In contrast, the absolute levels of muscle mass, strength, and power showed moderate to strong association to functional capabilities. Based on this, we concluded that interventions aiming to improve or maintain functional capabilities of healthy older adults should focus on increasing muscle mass, strength and power, whereas the effects of reducing between-limb asymmetry seem of less importance.

In order to best possibly maintain muscle mass, it has been suggested that older adults need more a higher dietary protein intake than what is currently recommended. A potential strategy for counteracting age-related loss of muscle mass, could therefore be protein supplementation. Heavy resistance training is known to be effective in increasing muscle mass, strength, and function in older adults. However, many older adults do not enjoy this training modality, and thus long-term adherence to training might be limited. Research into the effectiveness of alternative training modalities are therefore of interest.

In paper 2, we therefore investigated the effects of protein supplementation alone as well as combined with either heavy resistance training or light intensity, home-based resistance training for 1 year in healthy older adults. We found that protein supplementation alone was not associated with any benefits in relation to preserving muscle mass, strength, or function. As expected, the addition of heavy resistance training increased isometric muscle strength and was associated with a better preservation of quadriceps cross-sectional area compared to protein supplementation alone. Despite a high adherence to training, light-intensity, home-based resistance training did not provide any benefits compared to protein supplementation alone.

In paper 3, we investigated the temporal changes in muscle mass, strength and function during and after the 1-year training intervention. We found that while both heavy resistance training and light intensity, home-based training were capable of increasing muscle strength during the initial 6 months of training, only heavy resistance training were capable of inducing further increases in muscle strength during the last 6 months of training. Furthermore, 6 months after the intervention, the heavy resistance training was associated with higher strength and rate of force development than protein supplementation alone, whereas light intensity, home-based training did not provide this benefit.

In conclusion, the findings do not indicate that protein supplementation alone is a beneficial strategy for counteracting age-related loss of muscle mass, strength, or function in healthy older adults. The addition of light intensity, home-based training was not an effective long-term training strategy, although it provided some increases in muscle strength during the first 6 months of training. Adding heavy resistance training to protein supplementation was the most beneficial long-term strategy, and thus it is suggested that future research and innovation efforts aim to investigate how to increase participation of older adults for this training modality.

4. Dansk Resumé

Muskelmasse, -styrke og -effekt begynder at falde fra omkring det 4. leveårti, hvilket med stigende alder vil udgøre en øget risiko for begrænse fysisk formåen. Disse tab skyldes en kompleks blanding af ændringer i hormonelt miljø, inflammation, senefunktion, neural funktion mv. Selvom disse ændringer er højst sandsynligt uundgåelige, er strategier til at mindske de aldersrelaterede tab meget vigtige for at kunne opretholde funktionsevnen hos ældre så lang tid som muligt.

Adskillige studier har vist at mængden af muskelmasse, styrke- og -effekt er gode prædiktorer for både nuværende samt fremtidigt funktionsniveau. Kun få studier har dog undersøgt hvorvidt graden af asymmetri mellem lemmerne i disse parametre har betydning for funktionsevnen. I artikel 1 undersøgte vi derfor graden af asymmetri i en kohorte af raske ældre, samt betydningen af asymmetrien for funktionsevnen. Vi fandt at den gennemsnitlige asymmetri mellem benenes styrke og effekt var omkring ~10%, mens asymmetrien i benenes muskelmasse var ~3%. Graden af asymmetri mellem lemmerne var dog ikke gennemgående associeret med funktionsevnen, mens det absolutte niveau af muskelmasse, -styrke og -effekt var positivt korreleret med funktionsevne. Baseret på disse fund konkluderede vi at indsatser der har til formål at vedligeholde eller fremme funktionsniveau hos raske ældre bør fokusere på at øge muskelmasse, -styrke og -effekt, mens effekterne af at mindske asymmetri mellem lemmerne formentlig er af mindre betydning.

For at bedst muligt bevare muskelmasse, er det blevet foreslået at ældre bør indtage mere protein end hvad der i øjeblikket anbefales til den generelle befolkning. Proteintilskud kunne derfor udgøre en potentiel strategi til at modvirke det aldersrelaterede tab af muskelmasse. Tung styrketræning kan øge muskelmasse, -styrke og funktion hos ældre. Dog bryder mange ældre sig ikke om denne træningsform, og tilslutningen til denne træningsform kan derfor være begrænset. Effektive alternativer til denne træningsform er derfor af stor interesse.

I artikel 2 undersøgte vi derfor effekten af proteintilskud alene samt kombineret med enten tung styrketræning eller hjemmebaseret, let styrketræning henover 1 år hos raske ældre. Vi fandt at proteintilskud alene ikke gav nogle positive effekter på muskelmasse, -styrke, eller funktionsevne. Som forventet resulterede tung styrketræning i en forbedret isometrisk styrke, samt en bedre vedligeholdelse af quadriceps tværsnitsareal end proteintilskud alene. På trods af en god

tilslutning til træningen, gav den hjemmebaserede, lette styrketræning ingen positive effekter på sammenlignet med proteintilskud alene.

I artikel 3 undersøgte vi tidsopløsning i ændringerne i muskelmasse, styrke og funktionsevne under og efter den 1-årige træningsintervention. Vi fandt at både tung styrketræning og hjemmebaseret, let træning medførte en øget styrke efter de første 6 måneders træning, men at kun den tunge styrketræning var i stand til fortsat at forbedre styrken som følge af de efterfølgende 6 måneders træning. Derudover var tung styrketræning forbundet med en højere styrke og kraftudviklings rate end proteintilskud alene 6 måneder efter træningens ophør, mens dette ikke var tilfældet efter den hjemmebaserede, lette træning.

Fundene i denne afhandling indikerer at proteintilskud uden sideløbende træningsinterventioner ikke er en fordelagtig strategi for at modvirke aldersrelateret tab af muskelmasse, styrke, og funktionsevne. Tilføjelsen af hjemmebaseret, let træning var ikke en effektiv langsigtet træningsstrategi, selvom denne form for træning medførte en styrkeforbedring efter de første 6 måneders træning. Kombinationen af tung styrketræning og proteintilskud var den mest effektive langsigtede strategi og resultaterne i denne afhandling lægger derfor op til at fremtidig forskning og innovation søger mod at klarlægge hvorledes træningstilslutningen blandt ældre kan øges til denne træningsform.

5. Introduction

"*No decline with age is more dramatic or potentially more functionally significant than the decline in lean body mass (…) There may be no single feature of age-related decline that could more dramatically affect ambulation, mobility, calorie intake, and overall nutrient intake and status, independence, breathing, etc"* – Irwin Rosenberg, 1989.

Those were the remarks made in Rosenberg´s 1989 plea for attention towards the phenomenon of age-related loss of muscle mass[1]. A phenomenon he suggested to term sarcopenia, derived from the Greek words *sarco* (meaning flesh) and *penia* (meaning loss). Since this call for attention, increasing focus has been put on the importance of this decline, culminating in the recognition of sarcopenia as a disease state through its official ICD-10 code in 2016[2]. While the current definition of sarcopenia has been undergoing debated revisions[3,4], to now also include losses of physical function[5,6], strategies to counteract age-related losses of skeletal muscle mass remain of utmost importance due to its many important roles functions in relation to locomotion as well as metabolism[7]. Given the varying definitions of the term Sarcopenia, "age-related loss of muscle mass" will be used in this thesis to describe this phenomenon.

The age-related decline in muscle mass has been suggested to begin as early as in the 5th decade of life[8]. The rate of decline is somewhat debated, but is around ~0.5% $\cdot\, year^{-1}$, likely accelerating to ~0.8 % $\cdot\, year^{-1}$ during the 8th decade of life[9]. Importantly, accompanying losses of muscle strength are typically reported to occur at faster rates, of around ~2% $\cdot\, year^{-1}$ [10]. Muscle power (defined as $force \cdot velocity$), seems to decrease at an even faster rate than strength[11], potentially having large implications for overall functional capabilities such as walking or rising from a chair[12–14], as well as risk of falls[15]. The biological mechanisms underlying age-related losses of muscle mass, strength, and function are a complicated matter to comprehend, being a nexus of alterations in hormonal milieu[16], inflammation[17], tendon function[18], neural function[19] etc. The matter is further complicated by the fact that the age-related declines are also negatively affected by behavioral factors such as inactivity and inadequate intake of energy and protein[5,20].

Although the age-related losses of muscle mass, strength, and function are likely inevitable, strategies on how to counteract these losses are of great interest for both society as well as the individual. The present thesis will investigate the effects of two potential strategies: Protein

supplementation and resistance training. Furthermore, as most activities of daily living (ADL) are characterized by bilateral limb movements, this thesis will also investigate if between-limb asymmetry in muscle mass, strength, and power should be a focus in interventions aiming to preserve functional capabilities of older adults.

6. Background

6.1. The relationship between muscle mass and functional capabilities

Maintaining a sufficient muscle mass throughout life seems imperative for maintaining functional capabilities with age. In a classic cross-sectional study from Janssen and colleagues[21], it was observed that older adults with low muscle mass had substantially higher risks of functional impairment compared to age-matched older adults with a normal muscle mass. The link between risk of functional impairments and low muscle mass is likely mainly mediated by the fact that a larger muscle mass is capable of generating higher strength. Strength has been shown to be a good predictor of both current functional capabilities[12,22–24], as well as risk of developing future functional impairments[25] and mortality[26]. Some studies suggest that muscle power is an even better determinant of functional capabilities compared to muscle strength[12,27]. However, for both muscle strength and power the association to functional capabilities are likely curvilinear (illustrated in Figure 1), as losses of strength and power at very high initial levels is unlikely to ADL[24,28]. While the association between strength/power and functional capabilities are relatively well established, studies investigating the association between muscle mass per se and performance in functional tests (such as gait speed, chair stand tests etc.), show mixed results[22,29–34]. However, some of the discrepancy between studies might be explained by a lack adjustment in the statistical models for other important factors such as physical activity and body fat levels, potentially disguising the beneficial effects of a higher muscle mass in the analysis[35]. Furthermore, as with muscle strength, the relationship between muscle mass and functional capabilities is likely not linear, and thus detrimental effects on functional capabilities may not be apparent until the muscle mass decreases below a certain threshold.

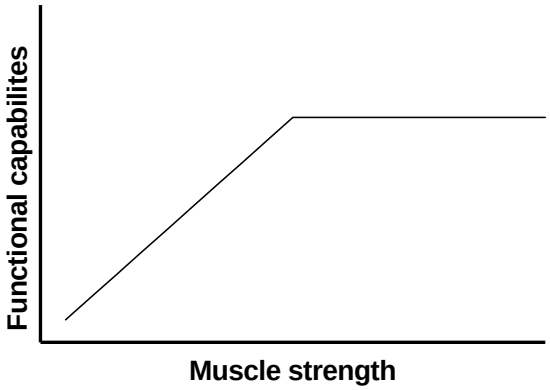


Figure 1. Illustration of the suggested curvilinear relationship between muscle strength and/or power and functional capabilities. The graph is based on the findings of Ferruci and colleagues as well as Cress and Meyer.

A large proportion of ADL are characterized by being dependent on bilateral lower limb function (e.g. walking, stair climbing, etc.). Thus, it is likely that the weaker of the lower limbs, as well as the degree of between-limb asymmetry in muscle strength, could the limiting factor in the abilities of

the older adults to perform these tasks. Previous studies have observed that a large degree of between-limb asymmetry in leg extensor power (LEP) as associated with a decreased postural balance as well as an elevated incidence of falls[14,36]. Studies investigating the association between functional capabilities and between-limb asymmetry in lower extremity strength and power have shown inconclusive results[23,37,38]. Some of the discrepancy in these findings could however potentially be related to differences in testing methods (e.g. whole-leg vs. single-joint strength testing). If between-limb asymmetry in lower extremity strength and power limits functional capabilities in older adults, reestablishing between-limb symmetry should be a focus in exercise programs for older adults, and thus more research is needed on this matter.

Given the vast implications of impaired muscle function on not just the personal autonomy[21,39,40] and mortality risk[26] of the individual, but also the major costs for society considering health care and nursing expenditures, strategies to counteract age-related losses of muscle mass, strength and function, are of great importance for both the individual and for society. Currently, the main suggested non-pharmacological strategies for maintaining muscle mass and function with age include increasing dietary protein and exercise interventions[20,41–46]. The following sections will investigate the evidence regarding the effectiveness of these two intervention strategies in counteracting muscle mass, strength and function in older adults.

6.2. Protein and prevention of muscle loss

Current global dietary guidelines specify a recommended daily allowance (RDA) of 0.8 $g \cdot kgBW^{-1} \cdot day^{1}$ [47] for all adults, irrespective of age. This recommendation is primarily based upon findings from studies using nitrogen balance to asses dietary protein requirements[48]. The nitrogen balance method is based on the concept that protein in the primary source of nitrogen in the body, and thus, any losses or gains in bodily nitrogen should be reflective of a decrease or increase in protein. The amount of protein intake that induces a net zero nitrogen balance, should therefore in theory be sufficient to maintain muscle mass. While some studies using this method has shown that protein requirements for older adults are higher than the current RDA[49,50], this was not observed in the meta-analysis by Rand and colleagues[48]. However, the nitrogen balance method has received critique due to issues in accuracy in measuring intake and excretion of nitrogen, resulting in possible underestimation of the protein needs of older adults[42,51]. Recently, the indicator amino acid oxidation (IAAO) method has emerged as a new method of investigating

protein requirements. This method is based on the assumption that when one indispensable dietary amino acid (IDAA) is deficient for protein synthesis, all other excess IDAA, including the indicator amino acid, will be used as energy substrate and thereby oxidized. At increasing dietary protein intakes, IAAO will thus decrease until reaching a plateau at when the intake of the limiting amino acid is sufficient[52]. Results using this method seem to suggest that the RDA of protein for older adults should be increased to ~1.2 0.8 $g \cdot kgBW^{-1} \cdot day^{1}$ [53–55]. It should be noted however, that while the IAAO method provides considerable advantages over the nitrogen balance method including being less invasive as well as using a more valid endpoint measure[52], the method has also received critique for not providing enough evidence behind the claim that the IAAO reflects overall protein requirements rather than just the requirement for the indicator amino acid[56].

Several epidemiological studies have suggested that protein intakes beyond the current RDA are associated with higher muscle mass[57–62], strength[57,63], and risk of functional limitations[64], although these findings are not unanimous throughout the literature[65,66]. Perhaps the strongest evidence for increased dietary protein recommendations in relation to counteracting age-related loss of muscle mass, is the results from the Health ABC study[60]. In this prospective cohort study including older adults ageing 70-79 years, it was found that losses of lean mass were appr ~40% less for the participants in the highest quintile of protein intake (>1.1 $g \cdot kgBW^{-1} \cdot day^{1}$) compared to the participants in the lowest quintile of protein intake (<0.7 $g \cdot kgBW^{-1} \cdot day^{1}$)[60]. In the same cohort, it was recently found that participants in the upper tertile of protein intake (>1.0 $g \cdot kgBW^{-1} \cdot day^{1}$) had a lower risk of developing mobility limitations during a 6-year follow-up period compared to participants consuming <1.0 $\cdot kgBW^{-1} \cdot day^{1}$ [64]. While these observational findings seem to suggest higher protein intakes as a promising tool to counteracting age-related loss of muscle mass, causal relationships cannot be deducted from observational studies. It is worth noting that on a cross-sectional basis, higher protein intakes are often associated with other factors with large potential impact on muscle mass, such as higher physical activity levels[59,60], alcohol consumption[59], and overall diet quality[67].

These aforementioned findings have formed the basis for arguments by several research groups towards increasing dietary protein recommendations for older adults (65+ years)[42,68,69]. In the Nordic countries, an increased dietary protein recommendation for older adults has even been incorporated into the recent edition of the Nordic Nutrition Recommendations[70]. However, here it

is acknowledged that there is a lack of randomized controlled trials (RCTs) investigating the effects of manipulating the dietary protein content of older adults on muscle mass, strength, and function. Given the relatively small annual loss in muscle mass of around 0.5-0.8%[9], such randomized clinical trials will likely need a large sample size as well as a long intervention period in order to detect any difference related to protein supplementation alone. In a recent attempt to perform such a study, Bhasin and colleagues[71] found that in older functionally limited men (N = 92) whose usual protein consumption was around the RDA, increasing daily protein intake to 1.3 $g \cdot kgBW^{-1} \cdot day^{1}$) for 6 months was not associated with an increase in muscle mass, strength, or function compared to peers continuing to consume the RDA. However, that study only had a duration of 6 months, and used DXA to estimate changes in muscle mass. As DXA has previously been shown to be less sensitive to age-related changes in muscle mass compared to magnetic resonance imaging (MRI)[72], the lack of an effect could be due to inadequate intervention length and method sensitivity. Zhu and colleagues[73] investigated whether 30 g daily whey protein supplementation for 2 years would be beneficial in preserving muscle mass and strength in older women already consuming more protein than the RDA. To my knowledge, that study has used the longest study duration to date when investigating the effectiveness of increase dietary protein intake in healthy older adults. This study even used highly sensitive computed tomography (CT) scans of the calf muscles to investigate changes in muscle size. Although Zhu and colleagues were able to detect significant reductions in muscle size over the 2-year period, they observed no beneficial effect of whey protein supplementation[73]. However, to my knowledge, that is the only study to date investigating the effects of protein supplementation for >6 months on muscle mass and strength. Thus, further studies are still needed in this area.

6.3. Protein distribution

While the total daily protein intake of older adults has been a topic of interest for several years, interest into the distribution of protein intake throughout the day has received increasing interest during the recent years. Muscle mass is constantly regulated through simultaneous synthesis and degradation, with the net protein balance being defined as the difference between protein synthesis and breakdown. Net protein balance is maintained through the ingestion of amino acids, which will result in systemic hyperaminoacidemia, stimulating the synthesis of new proteins[74,75]. The increase in protein synthesis is transient, and returns to fasting levels within few hours despite

continued elevations amino acid availability[76–78]. Thus, long-term protein net positive balance is dependent on multiple stimulations of protein synthesis throughout the day. Hence, it has been hypothesized that distributing the total daily protein intake into servings of the amount approximating the optimal dose for stimulating muscle protein synthesis (MPS), would be the most favorable way of ingesting the daily protein amount. The ability to increase MPS in response the face of hyperaminoacidemia seems to be impaired in older adults[79,80]. Moore and colleagues[79] found that older adults were able to increase postprandial protein synthesis to the same extent as young adults, but required larger doses of protein for maximal stimulation. As older adults in the Western part of the world often consume their dietary protein in a skewed fashion, consuming the most in the evening and little in the morning[81,82], this could pose a risk of suboptimal stimulation of muscle protein synthesis at breakfast and/or lunch. Although observational studies investigating the importance of an even protein distribution in relation to muscle mass have shown mixed results[58,65,83,84], optimal timing and distribution of ingested protein might still be worth a consideration as long as all servings can meet the optimal dose.

6.4. Protein quality

The provision of amino acids with the dietary protein will affect its ability to stimulate and enhance the muscle protein synthesis. Like the approach used in the IAAO method, the maintenance of protein synthesis is dependent on the availability of the amino acids coded for in the synthesized proteins. Thus, dietary proteins for humans should deliver all required amino acids in relative quantities comparable to the human body's tissue and in a readily digestible and accessible manner. Based on such requirements protein quality is defined. The most common method of describing protein quality is through a scoring system, the two most commonly used being the protein digestibility-corrected amino acid score (PDCAAS)[85] and latest the digestible indispensable amino acid score (DIAAS)[86]. However, these scoring systems describe the amount of a given protein that is needed in order to avoid a whole-body protein deficit and are therefore not specifically aimed towards describing the anabolic potential of a given amino acid. As digestion rate[87] as well as essential amino acid content[88] have been shown to be important factors in determining the anabolic potential of a protein, two proteins with equal protein quality scores might elicit different anabolic responses[87,89]. The amino acid leucine has been shown to be the major determinant of increases in MPS[90–92]. As animal-based proteins typically have a higher

leucine content compared to plant-based proteins, this seems to be one possible factor explaining the higher anabolic potential of some animal-bases protein sources[89]. In observational studies, is has been shown that while protein intake from animal-based protein sources are well correlated with muscle mass, this relationship is not as evident for plant-based protein sources[59,93]. It should be noted however, that these findings are not unanimous in the literature[65]. In young adults, supplementation with animal-based protein supplements have been shown to be superior to soy protein supplementation in increasing muscle mass during resistance training interventions[94,95]. However, limited evidence exists regarding the importance of the protein quality of the supplement, when supplied as part of a mixed diet without any exercise intervention in older adults. In a recent study by Oikawa and colleagues[96] it was observed that supplementation with whey proteins elicited greater increases in MPS in older women over a 6-day period compared to supplementation with collagen proteins, both in the resting state as well as after resistance training. The participants in that study consumed on average ~1.8 $g \cdot kgBW^{-1} \cdot day^{1}$ of protein, an thus these findings are somewhat surprising, as previous studies have suggested that the importance of protein quality diminishes at higher intakes of protein[97,98]. However, it should be noted that measures of MPS do not generally correlate well with long-term changes in muscle mass[99], and thus studies employing measures of long-term changes in muscle mass after protein supplementation of varying protein qualities are still needed.

6.5. Potential concerns regarding a high-protein diet

As advancing age is associated with a gradual decline in kidney function, clinicians have for a long time been concerned whether high-protein diets might negatively impact this development. In patients with renal disease, low-protein diets have been shown to counteract the rate of renal function decline[100]. However, in adults with no renal impairments, the protein content of the diet does not seem to affect renal function[101–103]. It should be noted however, that Knight and colleagues[103] found that in women with mild renal insufficiency, higher protein intakes seemed to accelerate the loss of renal function.

Another common concern regarding high-protein diets, is the potential negative effects on bone health. This concern arose from early studies observing an increase urinary calcium excretion related to high-protein diets[104,105], giving rise to a hypothesis that high protein intakes should induce metabolic acidosis, which would be buffered through alkalinizing compounds derived from

bone. However, this hypothesis has been dismissed, as a recent meta-analysis found no negative effects of high protein intakes, and even found trends towards positive effects of high protein intakes on many bone sites[106].

In conclusion, while protein supplementation might have detrimental effects on kidney function in individuals with preexisting renal disease, the majority of evidence indicate that protein supplementation is not associated with any health risks in healthy older adults.

6.6. Exercise and ageing

Sufficient physical activity is an important factor in preserving muscle mass, strength and function with ageing. Prospective cohort studies indicate that older adults with moderate to high physical activity levels have a ~50% lower risk of disability compared to low-active individuals[107]. Conversely, even short periods of low activity levels or disuse has been shown to have dramatic negative impact on muscle mass and strength[108–110]. As physical activity levels seem to be lower in older adults compared to young[111], physical inactivity might play a substantial role in the degree of age-related loss of muscle mass. As proposed by Lazarus and Harridge[112], the performance of master athletes might therefore be the best way to gain insight into the "true" age-related changes in physical function, as these are highly unlikely to be affected by the negative effects of lifestyle factors such as physical activity, diet, smoking etc. Notably, the world record performances in masters events decline with increasing age[112], thus it is evident that regular exercise is not capable of completely preventing age-related losses of muscle function. In master endurance athletes ageing 55-79 years, Pollock and colleagues[113] found no association between age and muscle mass, indicating that the high volume of exercise training was protective against losses of muscle mass. However, although muscle mass was not decreased with age, both strength and cardiovascular fitness showed negative associations with age[113]. In line with these findings, Mikkelsen and colleagues[114] found that quadriceps muscle size was preserved in master endurance athletes compared to young adults whereas quadriceps strength was not. Importantly, Piasecki and colleagues[115] recently observed no significant differences in leg lean mass between life-long competitive master endurance athletes and master endurance athletes who took up intense training and competition after the age of 50. Further, a recent study from our group observed significant increases in muscle size and strength after resistance training of older adults over the age of 83 years[116]. However, when investigating changes at the muscle fiber level,

resistance training had no effect on fiber size or satellite cell content, suggesting limited muscle plasticity in this population[117]. These findings suggest that while life-long exercise is likely the superior way of maintaining optimal health, it is never too late to start exercising in order to reap health benefits.

While the studies on master endurance athletes indicate that life-long endurance training is capable if rescuing age-related losses of muscle mass[113,114], endurance training does not seem to increase muscle mass or strength in previously sedentary older individuals[118]. In contrast, it is well established that resistance training is capable of inducing marked increases in muscle mass and strength in untrained older adults[116,119–124]. Pearson and colleagues[125] found that in master weightlifters, muscle strength and power declined at the same rate as healthy age-matched controls. However, muscle strength and power were substantially higher for the weightlifters, with the oldest weightlifters being as strong and powerful as untrained individuals ~20 years younger[125]. Resistance training therefore seems as an effective intervention strategy to increase muscle mass and strength, as well as a viable long-term training method to maintain muscle function.

6.7. Resistance training intensity

When training is aimed towards increasing muscle mass and strength, most available evidence indicates that this is best achieved at training loads of >70% of 1 repetition maximum (RM)[126,127]. Although the higher training intensities seem to elicit the greatest physiological responses, training using lighter loads also has been shown to increase muscle mass. In young adults, Holm and colleagues[128] found that resistance training at 15.5% 1 RM induced a ~2.5% increase in quadriceps muscle size, whereas training at 70% 1 RM induced a 7.5% increase. Importantly, the training in that study was volume-matched (load x repetitions), meaning that the number of reps performed at the light training intensity was decided to equate to the same total volume as the heavy training intensity. Thus, training was not performed to failure on either intensity. In a recent meta-analysis, Schoenfeld and colleagues[129] found that when training was performed until muscular failure, heavy-load resistance training (>60% 1 RM) induced greater increases in strength compared to moderate-load resistance training (<60% 1 RM), but both methods were equally effective in promoting muscle hypertrophy. Csapo and Alegre[130] performed a meta-analysis on studies comparing the effect of heavy resistance training (~80% 1 RM) to training using moderate

loads (~45% 1 RM) in older adults. In that meta-analysis it was found that while heavy training loads were the most effective for increasing muscle size and strength, training using moderate loads were also able to increase strength, hypertrophic responses to training using moderate loads were not significant[130]. Notably, the observed differences between training using moderate and heavy loads were smaller when training volumes were matched, and in the case of muscle hypertrophy, not significantly different[130].

Overall, the available evidence indicates that while heavy resistance training seems to be the most effective method of increasing muscle mass and strength, training using lighter loads might also be capable of improving these parameters when a sufficient training volume is performed. This might especially be relevant in conditions where heavy loading is not well tolerized, such as osteoarthritis[131], or in cohorts excluded from participation in due to contraindications for heavy resistance training, such as uncontrolled hypertension or cardiovascular disease[132]. Furthermore, many older adults prefer lighter intensity training programs[133,134], and might therefore not enjoy resistance training using heavy loads. In a study comparing the adherence to walking interventions at moderate or high intensities, it was found that training interventions using higher intensities had lower adherence, resulting in a lower exercise volume[135]. If these findings also apply to resistance training at varying intensities, training at lighter loads could potentially be a more effective long-term strategy for improving muscle mass, strength and function in older adults. It should be mentioned however, that studies so far have not found differences in adherence to resistance training at heavy loads compared to light-moderate loads[136,137].

While the impact of altering training load per on training adherence is somewhat speculative, it should also be considered that training using lighter training loads can more easily be performed at other locations than traditional commercial gyms. Heavy resistance training requires specialized equipment, and thus heavy resistance training requires the participant to have access to facilities with this type of equipment – Typically commercial gyms. These commercial gyms might pose challenges for training participation of older adults due to an intimidation of the environment as well as the economic costs associated with membership fees in these gyms[138,139]. Furthermore, while resistance training interventions in supervised, center-based settings have been shown to be very effective during the intervention period, it has also been shown that exercise continuation after the interventions is low[136,138]. It is important to remember that exercise only induces

physiological adaptations when it is performed, thus making adherence an important aspect when recommending exercise modalities and setting. When targeting a high degree of adherence, the most effective exercise modality might not be an option. Alternative effective settings and methods of resistance training are therefore of great interest in order to increase exercise participation and continuation from older adults.

6.8. What happens when the interventions end?

Given the low exercise continuation after resistance training intervention, it is of interest to uncover whether the benefits of the training persist, or if the improvements in muscle mass, strength and function are lost shortly after training cessation. Previous reports on this subject have found that while improvements in muscle mass are generally lost once the intervention ends[140–143], strength gains have been observed to be maintained above pre-training levels for up to a year after the training intervention[140–144]. However, changes in muscle mass and strength are dependent on the degree of exercise continuation after the intervention. Trappe and colleagues[142] found that improvements in muscle size and strength in older men in response to a 12-week resistance training intervention, could be maintained by one training session per week for 6 months. Somewhat in contrast with these findings, Bickel and colleagues[143] observed that the effects of 16 weeks of resistance training on myofiber cross-sectional area in older adults, were not maintained with exercise continuation once weekly. However, improvements in specific strength (strength per unit of lean mass) were maintained even without exercise continuation, and continued to improve with exercise continuation[142]. Both of the aforementioned studies continued exercise supervision during the exercise continuation. As supervised training interventions seem to be superior to unsupervised interventions[145], this might have a have major effect on the efficiency of the exercise continuation. Snijders and colleagues[141] recently found that irrespective of self-reported exercise continuation, muscle mass was lost 1-year after a 6-months resistance training intervention. However, the participants who continued unsupervised training were still able to maintain muscle size above pre-training levels. Also in that study, strength was maintained irrespective of training continuation[141].

6.9. Effect of training length – Timing of adaptations.

As described in the previous section, once the adaptations to resistance training interventions have been achieved, older adults are typically able to maintain some of these benefits for a

prolonged period of time. It is therefore of interest to know when these adaptations occur, and for how long a training intervention can continue to elicit improvements in muscle mass, strength, and function.

In young adults, significant increases in muscle size and strength are typically observed after ~3-4 weeks of resistance training[146–148]. The initial rapid increases in muscle strength seem to be mainly attributed to neural adaptations, whereas prolonged increases seem more reliant on continued increases in muscle size[149,150]. Brook and colleagues[146] observed that in response to a 6-week resistance training intervention, the vast majority of hypertrophic adaptations had occurred within the first 3 weeks. This was reflected by the measures of long-term protein synthesis, showing that myofibrillar fractional synthesis rate was only elevated during the first 3 weeks of training[146]. However, as the differences in muscle volume between an long-term resistance trained and an untrained is vastly larger than what is typically observed after a typical resistance training intervention study[151], it is evident that training adaptations can occur for extended periods of time, although at a slower rate. In older adults longer resistance training interventions (>6 months) are generally associated with greater improvements than shorter interventions[127]. However, these studies are typically investigating supervised, heavy resistance training. To my knowledge, no studies to date have investigated the temporal changes in muscle mass and strength in response to unsupervised resistance training using lighter loads. It could be speculated that while the progressive increase in training load during a heavy resistance training program might elicit continued increases in muscle size and strength, an unsupervised lighter-load training regimen might result in initial improvements in these parameters, but fail to elicit continued improvements due to insufficient progression in training load.

7. Objectives and hypotheses

The aim of this thesis is to investigate the efficacy of protein supplementation and resistance training interventions in preventing age-related loss of muscle mass, strength, and function. This aim is investigated in three papers with the following objectives:

In **Paper 1** the objective was twofold:

- To quantify the magnitude of between-limb asymmetry in lower limb muscle mass, strength and power in a large cohort of healthy home-dwelling older adults.
- Two investigate to which extent lower extremity function (LEF) could be predicted by measures of muscle mass, strength and power, as well as between-limb asymmetry in these measures.

The hypotheses were that between-limb asymmetry in lower limb muscle mass, strength, and power would be present in the investigated cohort, and that the magnitude of asymmetry, as well as absolute levels of muscle mass, strength and power, would be predictive of LEF.

In **Paper 2** the objective was to investigate the effect of protein supplementation alone or combined with light intensity or heavy load resistance training on muscle size, strength, and function on older adults. This was investigated through a RCT involving 208 healthy older adults (>65 years) who were randomized to one of five 1-year interventions: 1) Carbohydrate supplementation. 2) Collagen protein supplementation. 3) Whey protein supplementation. 4) Light-load home-based resistance training with whey protein supplementation. 5) Center-based heavy resistance training with whey protein supplementation. The hypotheses were twofold:

- Supplementation with high quality protein (whey) would be better at preserving muscle mass, strength and function compared to lower quality protein (collagen).
- Adherence to light-load home-based resistance training would be higher than to center-based heavy resistance training, and thus, when analyzed using a modified intention-to-treat principle (mITT), exert equally effective long-term strategies for increasing/preserving muscle size and strength.

In **Paper 3** the objective was to investigate temporal changes in muscle mass, strength and function during and after 1 year of light-load home-based resistance training compared to center-based heavy resistance training or no training. The hypotheses were twofold:

- When performing per protocol (PP) analysis, heavy resistance training would be the most beneficial during the intervention, with continued increases in these measures throughout the intervention, whereas the light-load training will only improve these parameters during the first half of the intervention.
- Light-load home-based training would be associated with better preservation of muscle mass, strength, and function after the intervention period.

8. Methods

8.1. Study design

The 'Counteracting Age-Related Loss of Skeletal Muscle Mass' (CALM) intervention study has generated the data for the three papers in the present thesis. The CALM intervention study was an interdisciplinary project investigating the effect of protein supplementation and resistance training from a clinical as well as an ethnological perspective.

The CALM intervention study was a 1-year RCT, including a total of 208 healthy older participants (>65 years of age) into one of five groups: 1) Carbohydrate supplementation (CARB). 2) Collagen protein supplementation (COLL). 3) Whey protein supplementation (WHEY). 4) Home-based light intensity training with whey protein supplementation (LITW). 5) Center-based heavy resistance training with whey protein supplementation (HRTW). Stratified randomization was done by an investigator not involved in interventions or not sensitive to blinding, stratifing by sex and number of completed repetitions on the 30-s chair stand test (<16 or ≥16).

The 5 intervention groups in the study composed the two study arms of the project (see figure 2); A nutrition arm, and a training arm. The nutrition arm of the study investigated the effect of supplementation with a high-quality protein (WHEY) compared to a lower quality protein (COLL) and muscle mass, strength, and function. The training arm investigated the effects of adding home-based lighter-load training on top of whey protein supplementation, compared to the addition of heavy resistance training, on muscle mass, strength, and function. The WHEY group was therefore both a part of the nutrition arm as well as the training arm.

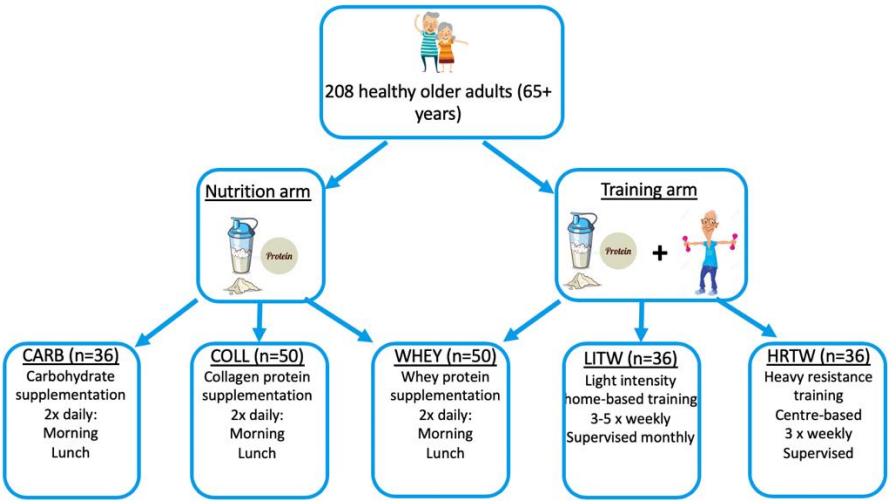


Figure 2. Overview of study arms and intervention groups in the CALM intervention study.

The study trial design included 4 main timepoints of measurements: 1) Baseline (0 months). 2) Midway through the intervention (6 months). 3) End of intervention (12 months). 4) Follow-up 6 months after the end of the intervention (18 months) (Figure 3).

For **paper 1**, measurements obtained at 0 months were used for cross-sectional analysis. Therefore, all participants enrolled in the CALM intervention study (n= 208) were included in analysis for this paper. For **paper 2,** measurements obtained at 0 and 12 months were used from all participants included in the CALM intervention study (n= 208) to assess the effects of the interventions in mITT analysis (described in detail in 9.8.3. *Paper 2*). For **paper 3**, measurements obtained at all timepoints for the participants in the training arm only were used to assess the temporal changes in muscle mass, strength and function in response to light-home based training versus center-based heavy resistance training during and after the intervention. We wanted to investigate temporal changes under conditions where adherence to the interventions were satisfactory, and we therefore only included participants fulfilling the requirements for the per protocol (PP) (Described in detail in 9.8.4. *Paper* 3) analysis in the training arm (n= 64) in that analysis.

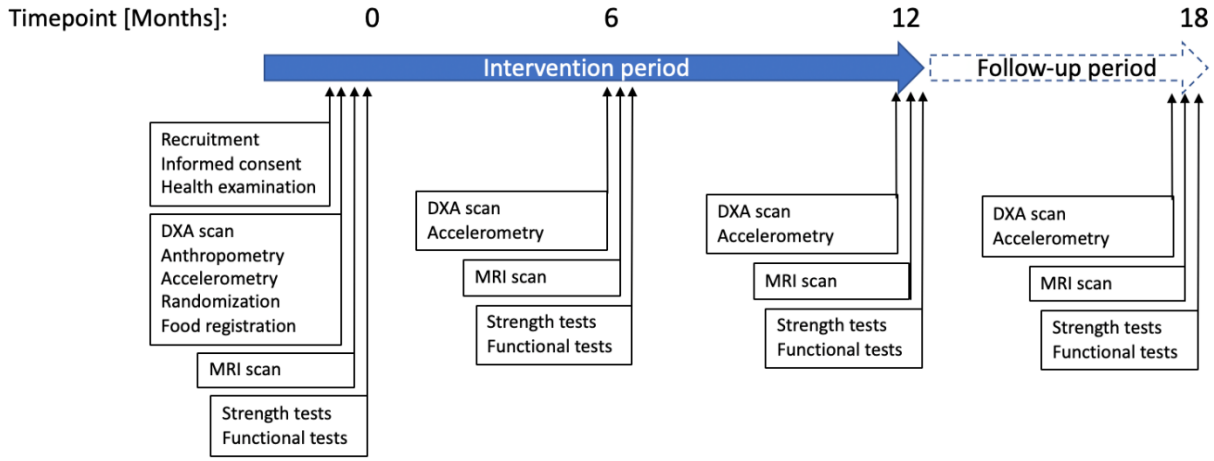


Figure 3. Overview of the trial design

8.2. Participants

Healthy older adults over the age of 65 years were recruited through advertisements in local newspapers, radio programs, social media etc. to be included in the CALM intervention study, participants were not allowed to participate in >1 hour of heavy resistance training per week, but were allowed to perform other types of exercise. Furthermore, participants were excluded if they possessed any disease or other chronic condition potentially hindering them from safely completing the intervention.

As shown in the CONSORT flow diagram (Figure 4). We had 1285 initial contacts by phone or mail, out of which 1163 were screened for the exclusion criteria by phone. 280 were scheduled for a screening visit, and 208 were included in the study and randomized to one of the five intervention groups. The vast majority of excluded participants were excluded from participation due to medical reasons (diseases or medication possibly interfering with their participation in the study). A large number of participants declined to participate, mainly due to the time demanded for participation and associated transportation needs. Of the included participants, a total of 24 participants did not complete the intervention.

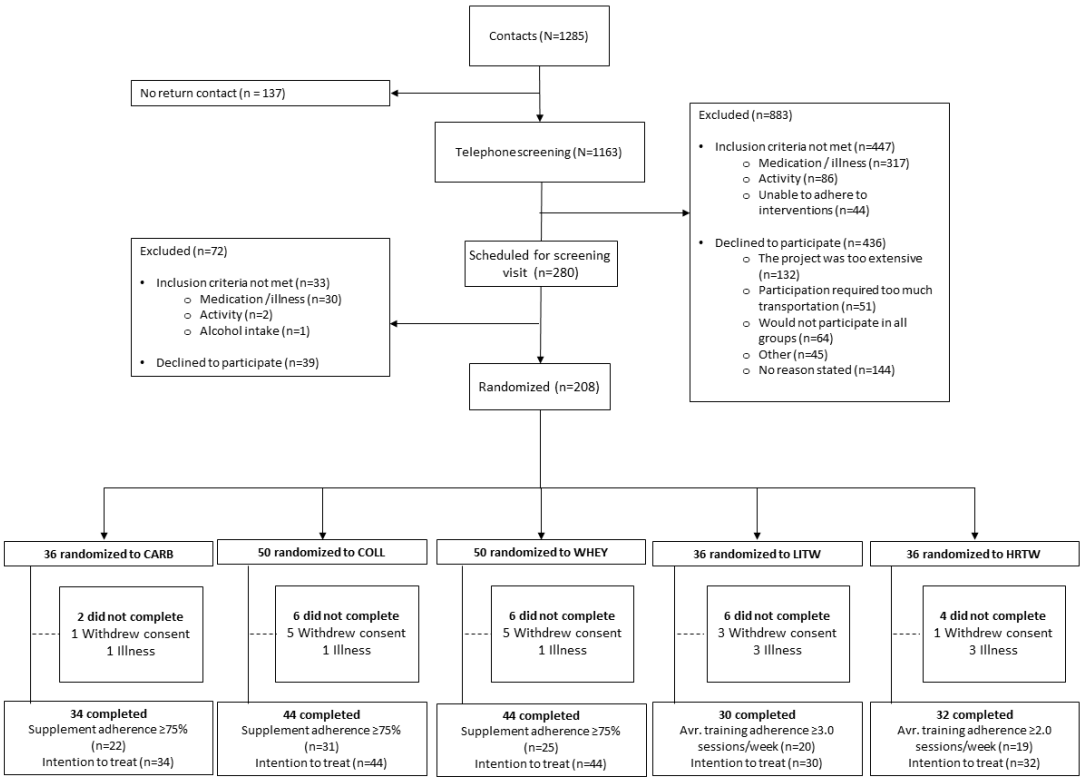


Figure 4. CONSORT flow diagram for the CALM intervention study.

8.3. Interventions

As described in previous sections, the CALM intervention study trial design was composed of a nutrition focused study arm investigating the effects of protein supplementation, and a training focused study arm investigating the effects of adding resistance training on top of protein supplementation.

8.3.1. Nutritional supplements

All intervention groups received a nutritional supplement to ingest twice daily for entire intervention period. Composition of the supplements is shown in table 1. Participants were instructed to ingest the supplements in the morning and at midday, preferably just before breakfast and lunch to increase satiety, thereby minimizing excess caloric intake. On training days, LITW and HRTW were instructed to ingest one of the daily supplements just after completing the

training sessions. All participants noted adherence to the supplements in hard copy diaries and received new supplies of supplements every 6 weeks. All supplements were developed, prepared, and individually packaged by Arla Foods Ingredients Group P/S, Viby, DK. Participants randomized to the groups in the nutrition arm were blinded regarding the content of the supplement. Due to the trial design, participants in the training arm could not be blinded to the content of the supplement.

Group	Supplement content	Protein [g]	EAA [g]	Leucine [g]	Carbohydrate [g]	Energy [kJ]
CARB	Maltodextrin + sucrose	0	0	0	30	510
COLL	Bovine collagen protein hydrolysate + sucrose	20	3.4	0.6	10	510
WHEY	Whey protein hydrolysate + sucrose	20	10.3	2.2	10	510
LITW	Whey protein hydrolysate + sucrose	20	10.3	2.2	10	510
HRTW	Whey protein hydrolysate + sucrose	20	10.3	2.2	10	510

Table 1. Supplement composition in all groups. Composition is shown per supplement.

The protein content of the supplements were chosen based on findings from acute studies showing that muscle protein synthesis is optimally stimulated at ~25-35 g of high quality protein[79,152]. As mentioned previously, older adults in western countries tend to consume their daily protein intake in a skewed fashion, consuming most protein in relation to dinner, less in relation to lunch, and the least in relation to breakfast[81,82]. As illustrated in figure 5, supplementation of 20 g of protein in relation to breakfast and lunch would theoretically cause these meals to surpass the ~30 g of protein needed for optimal stimulation of MPS.

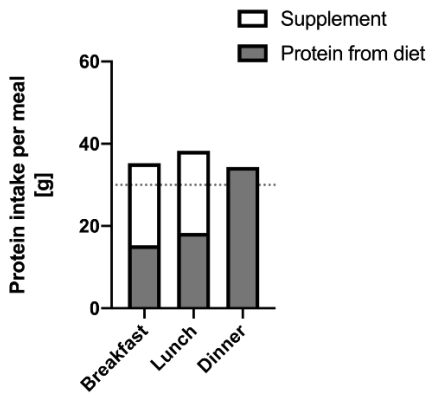


Figure 5. Illustration of the impact of the potential effect of the chosen supplementation strategy on protein intake. Data on protein intake from the diet are adapted from (Berner et al, 2013). The dotted line represents the protein dose needed for optimal stimulation of MPS (~30 g).

8.3.2. Training interventions

The heavy resistance training for HRTW was performed 3 times weekly at Bispebjerg Hospital under supervision of experienced staff. The training program consisted of 5-10 minutes of warm up on stationary bikes followed by 5 resistance training exercise, mainly focused on the lower extremities (Leg press, leg extension, leg curl, shoulder pull-down, shoulder press). Loading was periodized in 3-month cycles starting at 3 sets of 12 reps at 12 RM, and progressing to 5 sets of 6 repetitions at 6 RM. The participants performed a 3 RM test in the beginning of the training intervention and at the end of each training cycle. The 3 RM was then converted to a 1 RM using Brzycki´s formula; *1 RM = w*36/(37-r)*, where *w* is the weight lifted, and *r* is the number of repetitions performed[153]. The initial training load was then set to 70% of the 1 RM, but was adjusted after each session to ensure that the participant could perform the targeted number of repetitions in the final set, increasing the weight if the participant could perform additional repetitions after the final set. Participants were instructed to perform the lifts in a controlled fashion, with ~1 s in both the concentric and eccentric phases of the lifts. Adherence to the training was noted by the staff.

The exercises LITW program were chosen to mimic the muscle activation and range of motion of HRTW using body weight and elastic bands for resistance. As for HRTW, the program consisted of five exercises (Chair stand/squat, leg extension, leg curl, shoulder pull, push ups). For bilateral exercises (chair stand/squat, shoulder pull, push ups) participants performed as many repetitions as possible in a controlled tempo within 1.5 min intervals for 3 sets. Each set was separated by 1.5 min rest. In unilateral exercises (leg extension, leg curl), participants performed as many repetitions as possible within 1 min intervals on each leg. Each leg was trained alternating for a total of 6 sets (3 per leg). Training loads were adjusted by using stiffer elastic bands and adjusting seat height in the chair stands. Training frequency was varied in a cyclic manner, performing 3-4-5-4-3-4-5… etc sessions per week, amounting to an average planned training frequency of 4 sessions per week over the duration of the intervention. Participants mainly performed the training sessions unsupervised, but received supervision once weekly for the first month, followed by supervision once monthly for the remainder of the intervention. Adherence to the training sessions were noted by the participants in hard-copy diaries.

8.4. Measurements of muscle mass

The primary outcome of the CALM intervention study (reported in paper 2 and 3), was quadriceps cross-sectional area (qCSA) assessed by MRI scans. MRI and Computerized tomography (CT) scans are the golden standard methods in measurements of muscle size[154], with MRI providing the substantial advantage of not applying X-rays. All MRI scans in the study was performed at the Department of Radiology, Bispebjerg Hospital. Both thighs were scanned in a Siemens Verio 3 Tesla scanner by blinded radiographers. Our intention was to measure qCSA at 50% femur length. However, due to time constraints at the radiology department, the most feasible solution was to measure qCSA at set distances from the tibia plateau. The scans were composed of 6 axial slices, each 8 mm thick separated by 60 mm, with the first slice being placed at the tibia plateau (see figure 6). We used slice 4 on the dominant thigh for further analysis, as this was the scan that was closest to 50% femur length for all participants. Scans were then analysed in a blinded fashion using OsiriX v. 5.5.2 (OsiriX medical imaging software, Geneva, Switzerland). Each image was analysed twice, with the mean coefficient of variation between measurements of 0.7%. The average of the two measurements were used for further analysis.

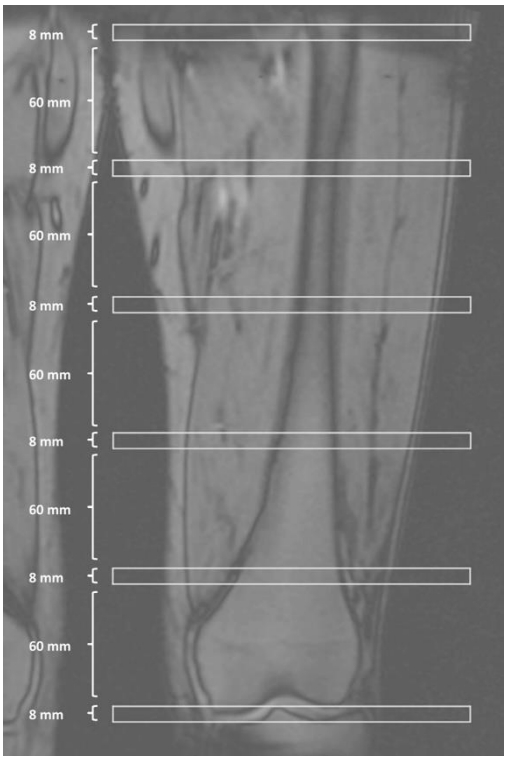


Figure 6. Illustration of the MRI slice placement. Adapted from Bechshøft et al, 2016.

To assess body composition, we used full-body DXA scans (Lunar iDXA, GE Medical Systems, Pewaukee, WI, USA). DXA is considerably cheaper and easier to use than MRI but rely on tissue-specific X-ray absorption to estimate body composition. From the DXA scans, we obtained total lean tissue mass (LTM), leg LTM, arm LTM, total fatmass, and body fat percentage. Using arm and leg LTM, we calculated appendicular lean tissue mass (aLTM) by adding the LTM in the arms and legs. Appendicular skeletal muscle index was calculated by dividing aLTM by height squared[39]. At 0 and 12 months, scans were performed while the participants were in an overnight fasted state. Due to practical issues, it was not possible to perform the scans in the morning at 6 and 18 months, and therefore these scans were not performed in the fasting state. These differences are likely to cause a systematic overestimation of LTM and aLTM at the 6 and 18 month timepoints

due to differences in hydration status, although the overestimation of aLTM is likely smaller than LTM[155]. The differences in hydration status might also have an impact on the measures of fat mass. However, this effect is likely minor[156]. At all timepoints, participants were asked to refrain from strenuous physical activity for 48 hours prior to the scans.

8.5. Measurements of muscle strength and function

A number of tests of muscle strength and power as well as functional capabilities were performed. All tests were performed on the same day at all 4 timepoints, in the order described below. Unilateral tests (Leg extensor power, grip strength, dynamic peak torque, MVIC, and RFD) were measured on both limbs at 0 months to obtain between-limb asymmetry measures for **paper 1**, but were only measured for the dominant limb at 6, 12, and 18 months.

8.5.1. 400 m gait

Participants were instructed to walk 400 m as fast as possible without running on a 20 m track, marked by colored cones. Performance in this test has previously been shown to be a strong predictor of mortality and risk of future mobility limitations[157]. Generally, tests of habitual and maximal gait speeds at both short distances (<10 m) and long distances (> 400m and 6 minute walk) are good predictors of overall functional capabilities[158,159]. However, the shorter distances and habitual speeds might suffer from a ceiling effect in healthy older adults[159]. Therefore, in the CALM intervention study, the 400 m gait test was chosen in order to minimize the risk of a ceiling effect. Results are reported as time to complete 400 m. For the composite measure of lower extremity function (LEF, described in section 8.8.2.), results were converted to average gait speed, by dividing 400 m by the time to complete the 400 m.

8.5.2. Leg extensor power

We measured maximal unilateral leg extensor power in the Nottingham Power Rig (Queens Medical Center, Nottingham University, UK), which measures leg extensor power against a fixed load[160]. Participants were seated with hands folded across the chest and were instructed to press down a pedal as hard and fast as possible by rapidly extending the hip and knee joint of one leg. Based on the acceleration of a flywheel, average power production during the movement was calculated by the software.

8.5.3. 30-s chair stand test

The 30-s chair stand test was performed as another measure of the functional capabilities of the participants. Participants were seated in a chair without armrests and with their hands folded across the chest. From this position, participants completed as many sit-to-stands as possible in 30 seconds, without assisting with their arms. This test has previously been shown to be a valid test of functional lower body strength in older adults[161].

8.5.4. Grip strength

Grip strength was chosen as a marker of upper body strength, and was performed using a hand grip dynamometer (DHD-1 [SH1001]; SAEHAN Corporation, Changwon City, South Korea). Participants were seated with one arm resting at the armrest with a 90° elbow angle. From this position, participants were instructed to squeeze the dynamometer as hard as possible for ~5 seconds with strong verbal encouragement.

8.5.5. Dynamic peak torque, MVIC, and RFD

Dynamic strength of the knee extensors were measured in an isokinetic dynamometer (Kinetic Communicator, model 500-11). The tests were performed at a slow movement velocity (60°/s), in a knee joint range of motion from 90° to 10° knee flexion (where 0° is full extension of the knee).

After completing the dynamic strength measurements, maximal isometric contractions were performed at 70° knee flexion to measure maximal voluntary isometric contraction (MVIC) and rate of force development (RFD). Participants were instructed to push forward as fast and hard as possible, performing contractions of ~5 s duration. RFD was measured as the average rate of force development from onset of force production to 200 ms. The attempt with the highest peak torque at 200 ms after onset of contraction was used for analysis.

8.6. Dietary assessment

To assess the diet composition of the participants, we used 3-day weighed food recordings. Participants were instructed to weigh all their food items for three consecutive days (Wednesday to Friday), and write this information down in hard-copy food logs. Analysis of the food records were done by trained researchers at a collaborating research department, using the electronic dietary assessment tool VITAKOST™ (MADLOG ApS, Kolding, Denmark). Nutrient intakes were

calculated using reference values from the Danish Food Composition Databank (version 7.01; Søborg; Denmark). Potential under-reporters were identified using the ratio between mean daily energy intake (EI) and basal metabolic rate (BMR), excluding participants with a ratio ≤ 1.0, assuming a PAL of 1.5[162]. For each participant, the assessments of diet composition were performed prior to starting the intervention, and after 11 months of intervention.

Participants were instructed to register all food items but exclude supplements in the weighted food records. Total energy and protein intakes from the supplements were therefore estimated by multiplying the supplement adherence of each participant by the protein and energy contents in the supplement. At the 11-month diet assessment, the estimated intakes from the supplements were then added to the registered protein and energy intakes.

We report the average daily energy and protein intake in **paper 2** and **paper 3.** More details on the diet composition of the participants in the CALM cohort can be found elsewhere[163].

8.7. Activity monitoring

To get an objective estimate of daily activity levels, we measured average daily step counts using accelerometer-based activity monitors (activPal 3^{TM}, activPal 3c^{TM}, or activPal micro; PAL technologies, Glasgow, UK). The activPal^{TM} activity monitors have been shown to provide valid estimations of step counts, exhibiting <1% measurement error on step counts irrespective of walking speed[164]. Activity monitoring was done at all timepoints (0, 6, 12, and 18 months).

8.8. Statistics

The following sections will describe the power calculation performed for the CALM intervention study as well as a detailed description of the statistical analysis performed in relation to each paper. Statistical analysis for paper 1 was performed in STATA (v. 15.1, StataCorp), whereas statistical analyses for paper 2 and 3 were performed in R (version 3.5.1), with the function lm() from the stats package (ver 3.5.1), lmer() from the lme4 package (ver. 1.1-20) and glth() from the multcomp package (ver. 1.4-8) installed. For all papers, illustrations of data were made in GraphPad Prism (V. 8.3.0, GraphPad Software, LLC).

8.8.1. Power calculation for the CALM intervention study

The primary outcome in the CALM intervention study was qCSA, and the power calculation was therefore done in relation to this measure, applying a level of significance of 0.05 and a power of 0.80. Based on previous findings from our research group[165], we aimed to be able to detect between-group differences in changes in qCSA from 0-12 months of 2%, corresponding to approximately 0.8 cm^2, expecting a standard deviation (SD) of ~1.4 cm^2. Based on this calculation, 30 participants were needed in each group. Expecting a dropout rate of ~15%, 36 participants were recruited in HRTW, LITW and CARB. 50 participants were recruited in COLL and WHEY partly due to expecting higher dropout rates in these groups, and partly to enable more sensitive pairwise comparisons of the effects of COLL vs WHEY.

8.8.2. Paper 1

The degree of between-limb asymmetry was quantified as the percentage difference between the strongest and weakest limb (or highest/lowest LTM), calculated as: $\%ASYM = \frac{Strongest\ limb - weakest\ limb}{Strongest\ limb} \cdot 100\%$. Differences in between-limb asymmetry between sexes were compared using Wilcoxon rank-sum tests, as data on between-limb asymmetry were assumed to follow a non-Gaussian distribution.

We used the 30-s chair stand test and the 400 m gait test to create a global index of LEF, inspired by other studies[38,166]. Based on each participant´s performance in each of these two tests, a composite Z-score was calculated to provide a single score for the LEF for each participant. To investigate the association between LEF and measures of muscle mass, strength and power, we therefore performed multiple linear regression, with the composite Z-score as the dependent variable, muscle mechanical parameters as independent variables. We included sex, age, steps per day, fat percentage, and BMI as potential covariables in the model, excluding covariables with low weight in the model ($P > 0.1$) through progressive step-wise regression.

8.8.3. Paper 2

This paper included the primary outcomes of the studies, investigating changes in muscle mass, strength and function from 0 to 12 months of the intervention. Changes from 0 to 12 months were investigated in the nutrition arm and training arm separately, using a longitudinal mixed-model with time and intervention group as fixed predictors. If the interaction term was significant, we

performed pairwise contrast analysis between all pairs of groups in the relevant study arm (eg. CARB vs COLL vs WHEY, and WHEY vs LITW vs HRTW). Analysis was performed as a mITT, including all participants who completed the 12-month tests. This is slightly modified to the traditional intention-to-treat (ITT) principle, as this would require all participants who were randomized to be included in the final analysis[167]. As not all participants returned for the 12-month tests, we did not have a complete data set for ITT analysis. Rather than making estimates of missing data, we chose our mITT approach for the present study. We also performed PP analysis, to see the effects of the interventions when performed to with satisfactory adherence. To be included in PP analysis in the nutrition arm, we set a cut-off for supplement adherence at >75% (corresponding to 1.5 daily supplements on average). In the training arm, cut-off points for training adherence were set to 66% for HRTW (corresponding to an average training frequency off 2 sessions per week), and 75% for LITW (An average training frequency of 3 sessions per week). The higher cut-off point for LITW was set because we expected a higher training volume to be necessary for this training intervention to be effective. For both training groups, participants also had to have >75% adherence to the protein supplement in order to be included in PP analysis.

Baseline data are summarized as mean $\pm$ SD unless otherwise stated. Individual treatment effects and between-group differences in treatment effects are reported as mean change and associated 95% confidence intervals (CI).

8.8.4. Paper 3

In paper 3, we only included participants fulfilling the requirements for PP analysis in the training arm. Changes in the measured parameters were analyzed over the entire intervention and subsequent follow-up period (0, 6, 12, and 18 months), using mixed-model analysis on the delta values compared to baseline ((Δ0-6 months, Δ0-12 months, Δ0-18 months). If the time*group interaction term was significant, we performed a 1-way ANOVA at each timepoint followed by subsequent pairwise contrast analysis. Temporal changes of the measured parameters were assessed within each group using contrast analysis between timepoints (0 vs 6 months, 6 vs 12 months, 12 vs 18 months, and 0 vs 18 months), but only if the time*group interaction term was significant.

Baseline data are summarized as mean ± SD unless otherwise stated. Between-group as well as differences between timepoints are reported as mean difference ± standard error (SE).

9. Results and discussion

In this section, results from the three papers will be summarized and discussed separately.

9.1. Paper 1

9.1.1. Participant characteristics

All participants in the CALM intervention cohort was included in analysis for paper 1. Baseline characteristics of these participants can be found in table.

		All			Men			Women			P-value
N =		208			109			99			-
Age	**[y]**	70.2	±	3.9	70	±	3.9	70.4	±	3.9	0.52
Weight	[kg]	75.7	±	12.8	81.4	±	11.2	69.4	±	11.4	<0.0001
Height	[m]	1.72	±	0.08	1.77	±	0.06	1.67	±	0.06	<0.0001
BMI	[kg/m^2]	25.6	±	3.8	26.0	±	3.4	25.1	±	4.1	0.07
ASMI	[kg/m^2]	7.6	±	1.2	8.3	±	0.9	6.7	±	0.8	<0.0001
Fat%	[%]	33.3	±	8.1	29.0	±	6.4	37.9	±	7.2	<0.0001
Visceral fat	[kg]	1.3	±	0.9	1.7	±	0.9	0.9	±	0.7	<0.0001
400 m gait time	[s]	245	±	34	236	±	32	255	±	33	0.0001
30 s chair stands	[reps]	19.7	±	5.0	20.7	±	4.8	18.6	±	5.0	0.001
Daily stepcount	[steps]	10056	±	3958	10040	±	3877	10163	±	4099	0.83

Table 2. Characteristics all participants included in the CALM intervention study and used for analysis in paper 1. All results are reported as mean ± SD. P-values represent the outcomes of unpaired T-tests or Wilcoxon rank-sum test comparisons between sexes. Table is adapted from paper 1.

The CALM cohort consisted of 109 men and 99 women. Sex differences were observed in body weight, height, appendicular skeletal muscle index (ASMI), Fat percentage (Fat%), visceral fat mass, 400 m gait time, and 30-s chair stand performance. However, daily step counts did not differ between sexes. The fact that male participants performed better on both tests of LEF (400 m gait time and 30-s chair stand test), fits well with the literature generally showing a higher risk for women of developing functional limitations and frailty[29,168]. Although none of the participants in the CALM cohort would be considered frail or functionally limited, the 400 m gait speed have been shown to be a good predictor of future risk of mobility limitations[157]. Although the participants in the study by Newman and colleagues were ~4 years older on average compared to the CALM cohort, it is still of interest to note that 90% of the CALM cohort completed the 400 m gait test fast enough to be placed in the best quartile of the cohort in that study[157]. Furthermore, the results on

the 30-s chair stands are comparable to what has been observed previously in a very active age-matched Danish cohort[169]. Combined with the relatively high average daily step counts of >10.000 steps/day[170], these results underline the fact the participants included in the CALM cohort were generally well functioning and physically active. Therefore, the participants in the CALM cohort would not be considered at risk of developing frailty or functional limitations in the near future, and can generally be considered a healthy and physically active.

9.1.2. Unilateral LTM, strength and power

Results on unilateral assessments of leg LTM, muscle strength, and power can be found in Table 3. Male participants had higher levels of LEP, dynamic peak torque, and MVIC, as well as higher leg LTM (all $P < 0.001$), even when normalized to body weight. Due to the higher relative adiposity of the female participants, the differences in these measures might have been less evident if we had instead reported the measures relative to leg LTM. However, strength relative to LTM (typically termed "muscle quality" or "specific tension"[171,172]) also seem to be lower in females compared to men[166,172].

			Strongest limb			Weakest limb			Gender effect
Leg extensor power	[W/kg]	All	2.63	±	0.68	2.32	±	0.63	< 0.001
		Men	3.00	±	0.63	2.65	±	0.60	
		Women	2.23	±	0.48	1.97	±	0.47	
Dynamic peak torque	[Nm/kg]	All,	2.04	±	0.45	1.78	±	0.46	< 0.001
		Men	2.27	±	0.39	2.02	±	0.40	
		Women	1.78	±	0.38	1.51	±	0.39	
MVIC	[Nm/kg]	All,	2.29	±	0.54	2.04	±	0.54	< 0.001
		Men	2.55	±	0.47	2.30	±	0.45	
		Women	2.01	±	0.46	1.76	±	0.49	
LTM legs	[kg]	All,	8.66	±	1.68	8.41	±	1.66	< 0.001
		Men	9.88	±	1.20	9.59	±	1.21	
		Women	7.31	±	0.94	7.09	±	0.94	

Table 3. Unilateral knee extensor strength, leg extensor power, and leg lean tissue mass. LEP, dynamic peak torque, and MVIC are all reported normalized to body weight. Results are reported as mean ± SD. P-values represent the outcome of linear regression analysis. Table is adapted from paper 1.

9.1.3. Between-limb asymmetry

Percentage between-limb asymmetry in LEP, dynamic peak torque, MVIC, leg LTM is shown in Figure 7. Average percentual asymmetry in measures of strength and power ranged between 10% and 13% (LEP: 10.6 ± 7.9%; dynamic peak torque: 13.0 ± 10.8%; MVIC: 11.2 ± 10.3 %), whereas the asymmetry in leg LTM was 3.0 ± 2.3%. Surprisingly, women had significantly higher degree of

between-limb asymmetry in dynamic peak torque compared to men (Men: 11.1 ± 9.5; Women: 15.0 ± 11.8%, P = 0.005), but magnitudes of between-limb asymmetries did not differ between sexes in any other measure. This effect of sex on between-limb asymmetry has, to my knowledge, not been reported elsewhere. However, as the effect of sex was only significant in dynamic peak torque and no other measure, these results should be interpreted with caution.

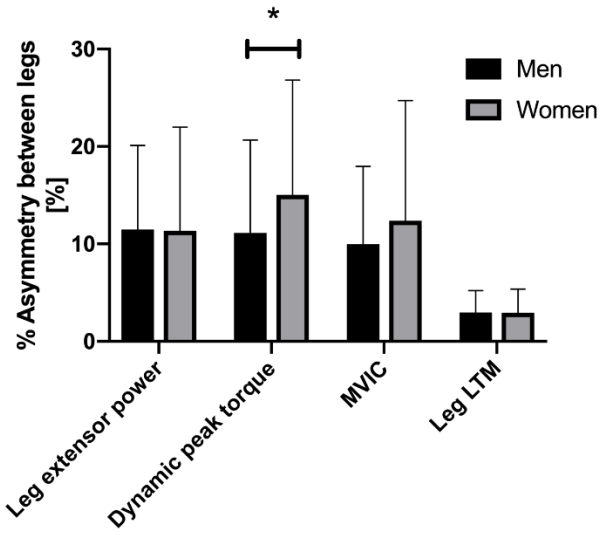


*Figure 7. Percentage between-limb asymmetry in LEP, dynamic peak torque, MVIC, and leg LTM. Results are shown as mean ± SD. * denotes significant difference between sexes P < 0.05 using Wilcoxon rank-sum test.*

It should be emphasized that the reported degrees of between-limb asymmetry were calculated as the differences between the highest value and the lowest values, and not the differences between the self-reported dominant and non-dominant leg. When data are reported as the differences between dominant and non-dominant leg, it is apparent that a large proportion of the participants were actually stronger/had higher LTM on their non-dominant leg (Table 4).

Measure	Dom ≤ Non-dom [%]	Prevalence of degree of asymmetry [% of participants)		
		<10%	10-20%	>20%
LEP	42	50	38	12
Dynamic peak torque	23	46	34	20
MVIC	31	57	27	16
FFM legs	35	100	0	0

Table 4. Prevalence of asymmetry, and number of subjects with higher strength/LTM in their self-reported non-dominant leg.

9.1.4. Association with LEF

LEF was positively correlated with absolute levels of LEP, MVIC, dynamic peak torque and leg LTM (Table 5). The association between strength and LEF have previously been suggested to be curvilinear[24,28], and therefore the moderate-to-strong associations observed were somewhat surprising given the high levels of muscle strength and power in the present cohort. Although similar association have been observed in prior studies of older adults with lower levels of physical function[23,37], the present findings indicate that even in very active, healthy older adults, higher levels of muscle strength and power are still accompanied by a high LEF and vice versa.

In contrast to previous reports[12,13,27,173], we did not find LEP to be a stronger predictor of LEF than MVIC or dynamic peak torque. In the CALM study, we measured LEP using the Nottingham Powerrig, which measures LEP against a fixed load[160]. Another study from our lab also using this apparatus, did also not find LEP to be a consistently better predictor of measures of LEF, compared to isometric knee extensor strength[174]. Other studies have typically tested LEP at against a load relative to max strength (typically 40-80% of 1RM)[12,13,27,173], which might explain why we cannot confirm these findings in the CALM cohort. While the Nottingham Powerrig has several advantages over other methods of assessing lower extremity power development, including being able to easily test unilateral power development of the whole leg instead of only single-joint tests, some considerations should be taken into account when interpreting results from this apparatus. As the tests are performed against a fixed load, the test will be performed at vastly different points of the force-velocity curve, depending on the strength of the participants. In a frail population, between-subject differences in LEP assessed using the Nottingham Powerrig would likely be very dependent on differences in maximal force capacity. Contrary, in very fit populations, such as the CALM cohort, the observed differences in LEP are likely much more dependent on abilities to produce high contraction velocities. The same would be true when comparing results between sexes in the CALM cohort, as the tests will be performed at different points of the force-velocity curve due to the differences in strength been sexes. This does not take away any value of the Nottingham Powerrig, but is an important consideration when interpreting results on lower extremity power tests from different studies, suing varying methods of assessment.

Associations to LEF		Included covariables					P-value	R^2
		Gender	Age	Steps/day	Fat-%	BMI		
Leg extensor power	*Strongest leg*	**	**	*	***	-	<0.001	0.44
	Weakest leg	**	**	**	***	-	<0.001	0.45
	%ASYM	-	-	-	-	-	0.36	0.004
Dynamic peak torque	*Strongest leg*	***	*	**	***	-	<0.001	0.47
	Weakest leg	**	**	**	***	-	<0.001	0.45
	%ASYM	-	-	-	-	-	0.07	0.02
MVIC	*Strongest leg*	**	**	**	***	-	<0.001	0.46
	Weakest leg	**	**	**	***	-	<0.001	0.47
	%ASYM	-	***	*	***	-	0.03	0.40
Leg LTM	*Strongest leg*	-	***	*	***	-	0.02	0.38
	Weakest leg	-	***	*	***	-	0.03	0.38
	%ASYM	-	-	***	-	-	0.05	0.12

*Table 5. Relationships between LEF and lower body LTM, strength and power of the strongest and weakest leg, or the degree of between-limb asymmetry (%ASYM). "P-value" indicates the level of significance for the correlation. Levels of significance for the covariables are shown as; * P < 0.1, ** P < 0.01, *** P < 0.001. "-" P > 0.1. Table is adapted from paper 1.*

The strength of associations for absolute levels of LEP, MVIC, dynamic peak torque, and leg LTM to LEF were comparable for the strongest and weakest leg, indicating that LEF of the present cohort was not limited by the weakest limb. This finding is comparable to earlier findings in a slightly older population at risk of mobility limitations [23]. Somewhat in contrast with these findings, percentage between-limb asymmetry in MVIC and leg LTM were both found to be negatively associated with LEF, although the degree of asymmetry in LEP and dynamic peak torque were not significantly associated to LEF. Given that asymmetry in these measures would be expected to be largely dependent on the same physiological factors, these disparate trends are somewhat surprising. As between-limb asymmetry in MVIC is a matter of between-limb differences in ability to generate maximal force, this parameter might be more affected by an asymmetry in muscle mass than the dynamic measures. As dynamic peak torque and LEP are measured under more dynamic conditions, asymmetry in these measures might be more a matter of between-limb asymmetry in neuromuscular coordination than MVIC. This could potentially explain the disparate trends, although it remains highly speculative.

A finding that might be somewhat controversial, is the positive association between leg LTM and LEF. While a low muscle mass has been shown to be a risk factor for mobility limitations[40,175],

many studies have observed no direct correlation between muscle mass and measures of LEF[22,30–32,34,176]. However, given strong association between strength and LEF, as well as strong association between leg LTM and muscle strength/power, it is somewhat surprising that so many studies find no association between muscle mass and measures of LEF. In relation to the findings in this paper, it is important to note that there was no significant association between leg LTM and LEF in our unadjusted analysis, and that the association only became apparent when we adjusted for age, body fat percentage and physical activity. As physical activity and body adiposity are both important factors in LEF of older individuals[34,166,177] these parameters are crucial to account for when the role of other factors in determining LEF are investigated.

Associations to LTM		Included covariables					P-value	R^2
		Gender	Age	Steps/day	Fat-%	BMI		
Leg extensor power	*Dominant leg*	***	-	-	-	***	0.002	0.72
Dynamic peak torque	*Dominant leg*	***	-	-	-	***	<0.001	0.77
MVIC	*Dominant leg*	***	-	-	-	***	<0.001	0.76
Grip strength	*Dominant arm*	***	-	-	-	***	<0.001	0.84

*Table 6. Associations between relevant extremity LTM (arm LTM for grip strength, leg LTM for lower limb measures) and strength/power. "P-value" indicates the level of significance for the correlation. Levels of significance for the covariables are shown as; * P < 0.1, ** P < 0.01, *** P < 0.001. "-" P > 0.1.*

Based on the results of this paper, between-limb asymmetry might therefore have a minor impact on LEF of healthy older adults, although the associations between the degree of asymmetry and LEF were not consistent for all measures. It should be noted that the associations of absolute levels of lower extremity muscle mass, strength and power to LEF were consistently stronger than the association between degrees of asymmetry and LEF. Therefore, while reducing between-limb asymmetry in muscle mass, strength, and power might have a small beneficial effect on LEF of healthy older adults, the main focus in resistance training for this population should still be to increase absolute levels of muscle mass, strength and power in order to maintain or improve physical function.

9.2. Paper 2

This section will mainly focus on the results from the mITT analysis performed in **paper 2**, as this is the main analysis of this paper.

9.2.1. Participant characteristics by group

Participant characteristics for the separate groups can be found in Table 7. As participant grouping in this study was randomized, any differences between groups at baseline would be expected to be random[178], and thus, no statistical analysis of between group differences were performed at baseline. With that being said, it seemed that the randomization procedure was successful in generating relatively homogenous groups regarding most assessed parameters.

	CARB	COLL	WHEY	LITW	HRTW
Variable	(n = 36)	(n = 50)	(n = 50)	(n = 36)	(n = 36)
Demographics, Mean (SD)					
Age, y	69.6 (3.9)	70.4 (4.1	70.3 (4.3)	70.4 (4.0)	70.3 (3.1)
BMI, kg/m^2	26.0 (3.9)	25.4 (6.0)	25.2 (3.6)	25.7 (3.1)	25.9 (3.5)
Daily activity, Steps/day	10894 (5165)	10590 (3996)	10118 (3590)	10119 (3450)	9777 (3574)
Protein intake, g/kg/day	1.2 (0.3)	1.2 (0.4)	1.1 (0.3)	1.0 (0.3)	1.1 (0.4)
Energy intake, kJ/day	8442 (1804)	8150 (1952)	8529 (2092)	7445 (2220)	8268 (2146)
Body Composition					
Lean tissue mass, kg	48.5 (7.8)	49.2 (8.6)	50.0 (8.5)	48.1 (9.3)	48.8 (9.9)
Fat percentage, %	33.2 (9.3)	32.0 (9.1)	32.7 (7.5)	34.3 (7.5)	34.7 (7.1)
Quadriceps size, cm^2	56.6 (11.3)	56.0 (13.9)	54.5 (11.0)	56.7 (11.4)	55.4 (13.1)
Strength and function					
400 m gait time, s	248 (42)	243 (38)	242 (30)	242 (30)	251 (27)
30 s chair stand, reps	19.9 (5.7)	20.1 (5.3)	19.4 (4.6)	20.1 (4.6)	18.9 (4.9)
Leg extensor power, W	183.1 (56.2)	191.2 (67.2)	189.6 (59.6)	190.8 (61.4)	194.2 (65.8)
MVIC, Nm	158.9 (41.1)	169.0 (53.4)	177.6 (47.0)	171.5 (44.4)	165.0 (50.8)
SF-36					
MCS	59.3 (3.2)	57.3 (4.3)	57.6 (3.6)	57.1 (4.7)	57.5 (4.4)
PCS	55.3 (4.7)	56.0 (4.7)	56.8 (3.1)	56.4 (4.0)	56.5 (4.2)
Laboratory data					
Hba1c, mmol/mol	36.0 (2.2)	35.8 (3.4)	36.2 (3.5)	35.8 (2.9)	35.8 (2.7)
Total cholesterol, mmol/l	5.6 (0.9)	5.7 (1.0)	6.0 (1.2)	5.5 (1.0)	5.8 (0.9)
HDL Cholesterol, mmol/l	1.9 (0.5)	2.0 (0.6)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)
LDL Cholesterol, mmol/l	3.1 (0.8)	3.2 (1.0)	3.4 (0.9)	3.0 (1.0)	3.4 (1.0)
Triglycerides, mmol/l	1.3 (0.6)	1.4 (0.8)	1.7 (0.8)	1.4 (0.6)	1.4 (0.6)
Creatinine, µmol/l	76.8 (14.7)	81.4 (15.9)	80.5 (11.6)	78.8 (14.7)	77.0 (12.7)

Table 7. Participant characteristics by group. Results are presented as mean ± SD.

9.2.2. Adherence to interventions

Results on adherence to interventions are shown in Table 8. Adherence to the supplements did not differ significantly between groups in neither the nutrition arm nor the training arm. Training

adherence was significantly higher for LITW compared to HRTW, both in mITT and PP analyses. Although this confirmed the hypothesis that participants would adhere better to LITW, these results should be interpreted with caution. Self-reported adherence to training has questionable validity, and is likely to overestimate adherence[179]. Therefore, the differences observed might simply be a matter of comparing self-reported adherence to supervised adherence. Nonetheless, self-reported adherence was the only possible way of assessing adherence to the present home-based training setup. Using the same method of assessing training adherence, a previous study in a comparable Danish cohort found very limited adherence to home-based training[180]. It is of therefore of great interest to investigate the factors contributing a high degree of adherence to home-based training, to successfully be able to incorporate this type of training. In the study by Nielsen and colleagues, it was noted that the participants felt uncertain regarding which exercises to perform, and how to correctly perform the exercises[180]. In our study, participants performed the home-based exercise with supervision once weekly for the first month, followed by once-monthly supervision for the remaining 11 months of training. A sufficient degree of initial supervision could therefore be an important aspect in achieving a high degree of adherence to home-based training.

The adherence to the dietary supplements was high but could suffer from the same problems regarding over-reporting due to the adherence being registered by self-reports. This is not as problematic as the training adherence, as adherence reporting methods were similar between supplement groups. Due to less frequent contact with participant in supplement-only groups, there was issues with a high number of non-reporters in the nutrition arm. There was a high number of participants (34 in total – See Table 8) who did not report their adherence to the respective supplement. These participants still came to the research facilities to receive new supplies of supplement as planned, but failed to report their adherence to the supplements, typically due to losing the hard-copy adherence log, or not being willing to fill it out twice daily. For future studies, other methods of assessing adherence to the supplements could be of great interest in order to minimize non-reporters (e.g. participants returning empty supplement packages, more frequently physically meeting the participants, adding tracer to the supplements, etc).

	CARB		COLL		WHEY		LITW		HRTW	
	mITT	**PP**	**mITT**	**PP**	**mITT**	**PP**	**mITT**	**PP**	**mITT**	**PP**
Training adherence (Median [Q1, Q3])	-	-	-	-	-	-	89%* [77%, 96%]	94%* [88%, 97%]	72% [62%, 78%]	78% [75%, 82%]
Supplement adherence (Median [Q1, Q3])	95% [77%, 97%]	96% [89%, 98%]	96% [86%, 99%]	96% [86%, 99%]	88% [82%, 93%]	90% [85%, 96%]	90% [77%, 94%]	93% [85%, 100%]	87% [79%, 97%]	94% [87%, 98%]
Supplement non-reporters (n=)	7	-	11	-	14	-	1	-	1	-
Drop outs (n=)	2	-	6	-	6	-	6	-	4	-
Included subjects (n=)	34	22	44	31	44	25	30	20	32	19

*Table 8. Adherence to interventions by group in modified intention to treat analysis (mITT) and per protocol analysis (PP). Adherence is presented as median adherence and corresponding 25th percentiles (Q1) and 75th percentiles (Q3). Participants were included in per protocol analysis if supplement compliance exceeded 75%, and training compliance exceeded 75% for LITW and 66% for HRTW. *Significant different from HRTW (P<0.05).*

Changes in energy and protein intake from 0 to 11 months are shown in Figure 8. COLL and WHEY increased protein intakes significantly more than CARB, with no differences in changes in energy intake. It seemed that the supplements caused nominal decreases in protein and energy intake from the remainder of the participants diet, causing only minor numeric increases in total energy intake at 11 months.

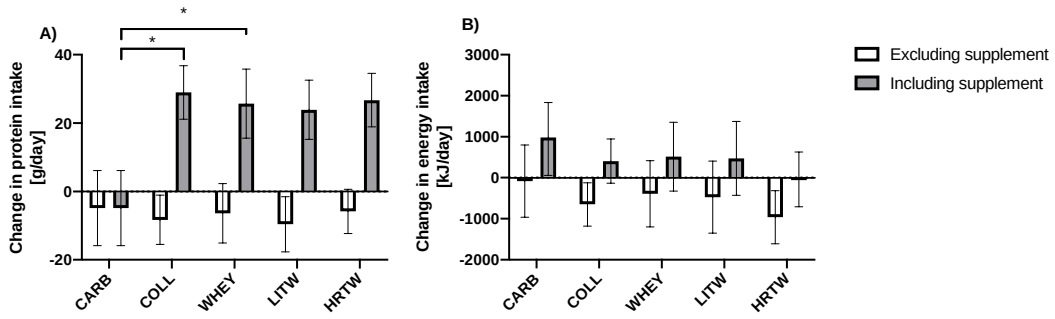


*Figure 8. Changes in energy and protein intake from 0 to 11 months of intervention. Changes are shown both including estimated intake from the supplements, as well as changes without accounting for the supplement. Results are shown as mean changes and corresponding 95% CI. * significant different (P<0.05) from CARB in 1-way ANOVA and subsequent contrast analysis within the nutrition arm and training arm respectively.*

9.2.3. Effects of protein supplementation

This subs-section will focus on the results observed in the nutrition arm of paper 2.

Changes in muscle size and body composition are shown in Figure 9. 1-way ANOVA analysis revealed no between-group differences in any of the performed measures (qCSA: P=0.17; LTM: P = 0.29; Fat percentage: P = 0.95).

Contrary to the hypothesis of the nutrition arm, WHEY was not associated with better preservation of muscle mass compared to neither COLL nor CARB. If anything, WHEY was actually associated with the numerically largest loss of qCSA and LTM of the three supplement groups. The results therefore clearly demonstrate that whey protein supplementation did not provide any benefit in preserving muscle mass in this cohort of healthy older adults. Using deuterated water, Oikawa and colleagues[96] recently found that whey protein supplementation increased long-term MPS more than collagen protein supplementation. While whey protein might increase muscle protein turnover more than collagen protein, the present findings underline that this does not translate to any effects on muscle mass per se.

All groups in the nutrition arm seemed to increase body fat percentage. As we did not control the effects of the supplements against normal eating behavior, it is not possible to conclude whether this increase was due to the supplement or an effect of ageing. In a recent study by Bhasin and colleagues[71] it was observed that increasing protein content in the diet of older men resulted in a loss of fat mass. However importantly, this alteration of protein content was done without increasing energy intake. While energy intake in the CALM study, assessed by the weighted food logs, did not seem to increase markedly due to the supplements, it is likely that the minor increases in fat percentage were due to the supplements causing a slightly positive daily energy balance in the participants, causing accumulation of adipose tissue over the course of the 12-month intervention.

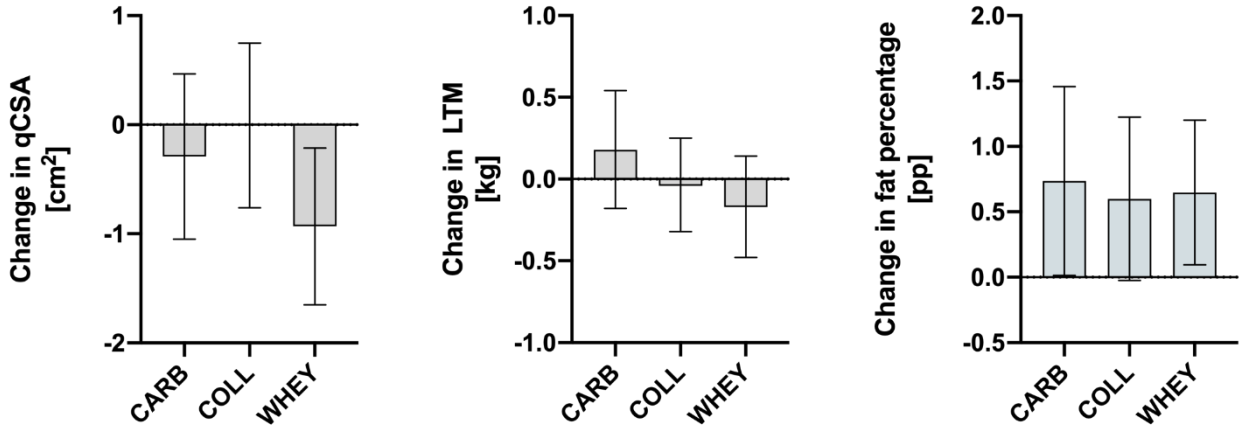


Figure 9. Changes in qCSA, LTM and fat percentage in the nutrition arm of the study. Results are shown as mean change with associated 95% CI.

Changes in measures of strength, power, and functional capabilities are shown in Figure 9. 1-way ANOVA analysis revealed no between-group differences in any of the measured parameters (MVIC: P = 0.13; LEP: P = 0.94; 400 m gait: P = 0.99; 30-s chair stand: P = 0.30). Given that protein supplementation had no effect on muscle mass or body composition, it is hardly surprising that no effects were observed on strength and function, as these effects would be expected to be mediated through the effects on muscle mass.

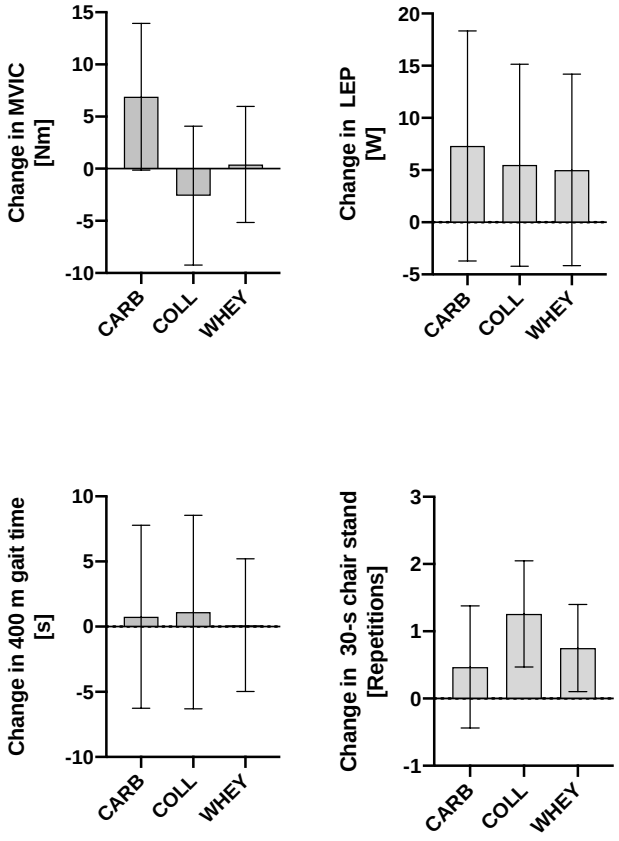


Figure 10. Changes in measures of muscle strength, power and functional capabilities in the nutrition arm of the study. Results are shown as mean changes with associated 95% CI.

Overall, no beneficial effects of protein supplementation were observed in any measured parameter. In a comparable study cohort, Zhu and colleagues[73] performed a 2-year RCT comparing daily protein supplementation to isocaloric placebo in healthy older women (ageing 70-80 years), also not observing any beneficial effects of protein supplementation. Likewise, in a recent meta-analysis, Tieland and colleagues[181] found no beneficial effect of protein supplementation on muscle mass and strength in healthy older adults. The results from these studies as well as the present study therefore underline the lack of beneficial effects of supplementing healthy older

adults with protein without concomitant exercise interventions. Notably, in a recent study in older men with mobility limitations and protein intakes below the current RDA (0.83 protein·kg^{-1}·day^{-1}), Bhasin and colleagues[71] investigated the effects of a high-protein diet (1.3 protein·kg^{-1}·day^{-1}) compared to 0.8 protein·kg^{-1}·day^{-1} over the course of 6 months. Also in this study, the authors observed no beneficial effects of increasing protein intakes. Notably, the authors also had used investigated if the high-protein diet was beneficial when combined with testosterone therapy. Even in this case of increased protein turnover, the high-protein diet still did not provide any benefit compared to the diet lower in protein[71]. However, the intervention duration in that study was 6 months, which could be insufficient in order to detect differences in muscle mass – Especially given that Bhasin and colleagues used LTM assessed via DXA as their marker of muscle mass, which has been shown to be less sensitive to changes compared to MRI[72].

Based on the available evidence, there is therefore no basis for recommending protein supplementation without concurrent training interventions for healthy older adults already reaching protein intakes of >1.0 g·kg^{-1}·day^{-1}.

9.2.4. Effects of adding resistance training to protein supplementation

This sub-section will focus on the results from the training arm of paper 2, where the effects of adding either light intensity, home-based training or heavy resistance training on top of whey protein supplementation were investigated.

Changes in muscle size and body composition are shown in Figure 11. The group*time interaction term was significant for qCSA (P = 0.04). Contrast analysis revealed more positive changes for HRTW compared to WHEY (Between-group difference [mean, 95% CI]: +1.68, +0.41 to +2.95 cm^2, P=0.03) but not for HRTW compared to LITW (+1.29 cm^2, -0.08 to +2.67 cm^2, P=0.16). LITW did not change qCSA compared to WHEY (+0.39, -0.88 to +1.66 cm^2, P=0.82). Surprisingly, no between-group differences were observed for LTM (P = 0.09) or fat percentage (P = 0.10). These the low degree of morphological adaptations to the training interventions are quite surprising. Several studies have observed >5% increases in muscle size of older adults after 3-4 months of heavy resistance training[119,120,124,182]. However, some studies have also observed little to no hypertrophy in older adults after resistance training[148,183,184]. As noted in a previous section, the average protein intakes of the participants in the present study were well above the RDA, and when

including the protein from the supplement, around the protein intakes associated with the largest muscular adaptions in previous reports[185]. Thus, the limited hypertrophy response in the present cohort is of great interest, as the participants should be in a good condition for adapting to the training stimulus. Median adherence to training corresponded to an average training frequency of ~2 sessions/week. This has previously been shown to be sufficient to induce muscle hypertrophy in older adults[186]. Interestingly, other 1-year resistance training interventions have also observed less than expected muscle hypertrophy compared to the shorter-term training studies[187,188]. It has been suggested that most hypertrophy occurs during the initial 4 weeks of training[146–148], possibly explaining why the long-term training interventions do not show higher degrees of muscle hypertrophy compared the shorter interventions. However, it might also be speculated that participants might not exert themselves to the same degree when entering a long-term training intervention compared to a more intensive, short-term intervention. It should also be noted that most participants went on 3-4 weeks of vacation during the 1-year intervention, causing prolonged breaks in training, which could be possibly be limiting the degree of hypertrophy.

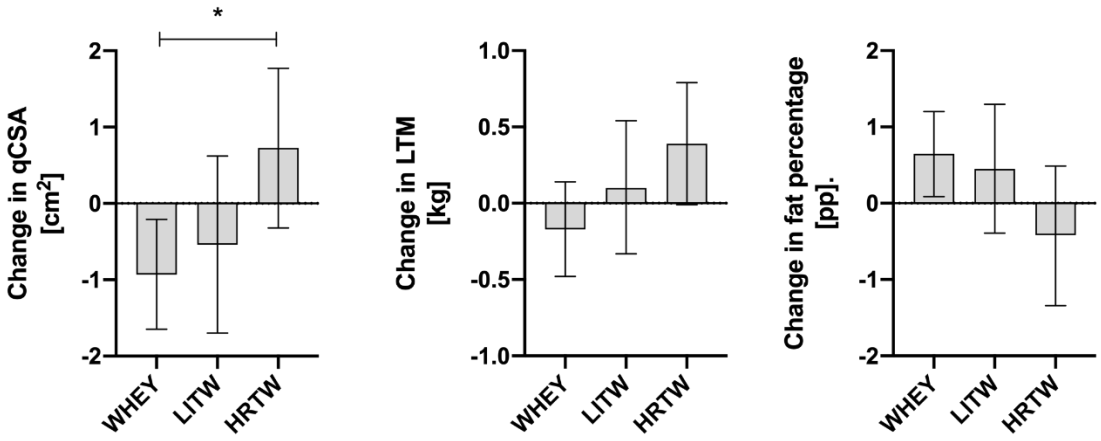


*Figure 11. Changes in qCSA, LTM and fat percentage in the training arm of the study. Results are shown as mean changes with associated 95% CI. *significant between group difference.*

Changes in strength, power and functional capabilities are shown in Figure 12. The group*time interaction term was significant for MVIC ($P < 0.0001$). Contrast analysis revealed that MVIC increased significantly more in HRTW compared to WHEY (+23.9, +14.2 to +33.6 Nm, $P< 10^{-5}$), and LITW (+16.8, +6.1 to +27.4 Nm, $P = 0.01$). However, changes for LITW were not significantly different from WHEY (+7.1 Nm, -2.8 to 17.1 Nm, $P = 0.34$). The group*time interaction term was not significant for LEP ($P = 0.73$), 30-s chair stand ($P = 0.82$), or 400 m gait time ($P = 0.14$).

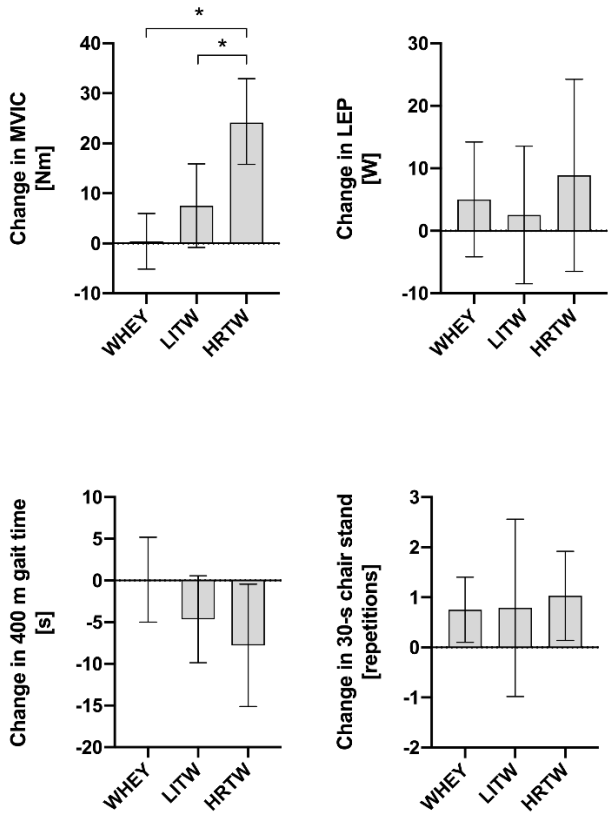


*Figure 12. Changes in measures of muscle strength, power and functional capabilities in the training arm of the study. Results are shown as mean changes with associated 95% CI. *significant between group difference.*

Surprisingly, LITW had little to no effects of muscle strength, power and functional capabilities. Although the results on exercise adherence from this group should be interpreted with caution (see section 9.2.2 *Adherence to intervention*), the results still indicate, that adherence to this training was high. It is therefore unlikely that the lack of an effect from LITW was due to insufficient adherence. In line with the present findings, Gylling and colleagues observed a minor (~5%) increase in MVIC, with no change in muscle mass or function after 1 year of light intensity resistance training in a large cohort of healthy and chronically diseased older adults[187]. The participants in the CALM study were generally quite active, and although they did not perform heavy resistance training prior to enrollment in the study, they still performed a variety of physical activities, such as running, biking, tennis etc. Due to the high general physical activity, the light intensity training likely provided an insufficient additional stimulus in order to cause any physiological adaptations.

Despite the limited muscle hypertrophy, the change in MVIC for HRTW was comparable to the 10-20% increases that have previously been reported after heavy resistance training interventions of 3-12 months duration[120,182,187,189–193]. It was however surprising that we were unable to detect any changes in LEP in response to the heavy resistance training. Previous studies have observed increases lower extremity power in response to resistance training[116,122,194]. The lack of effect of HRTW on LEP might be due to the repetitions in training being performed in training. Participants were instructed to perform all repetitions in training in a controlled fashion, with ~1 s eccentric and ~1 s concentric phases. The studies by Caserotti and colleagues[122] as well as Reid and colleagues[194] both emphasized the power development in the concentric phases of the lifts, which could likely explain the greater degree of transfer to power development. Bechshøft and colleagues[116] observed increases in LEP assessed in the Nottingham Powerrig, using a training protocol similar to what was done in the CALM study, however the participants in that study was older than in the CALM study (average age ~87 years vs ~70 years), and thus had substantially lower (~40% lower) LEP compared to the participants in CALM. As discussed in a previous section (9.1.4 *Associations with LEF*), the Nottingham Power Rig uses a fixed load to assess LEP, causing the test to be performed at different points of the force-velocity relationship. As the participants in the study by Bechshøft and colleagues were substantially weaker than in the CALM study, gains in LEP in that study were likely more dependent on changes in force generating capacity, whereas changes in LEP for the CALM participants would have been more dependent on changes in contraction velocity. Gylling and colleagues[187] also did not observe any improvements in LEP after heavy resistance training in a cohort with comparable initial strength and power levels, using a similar test and training protocol.

No effect of training was observed on the measures of LEF. With the strong positive correlation between strength and LEF in mind from paper 1, it was somewhat surprising that the robust strength gains for HRTW did not translate to improved measures of LEF. Gait times

did improve for HRTW, however the improvements were not significantly different from the change observed in the other groups (P = 0.14). It seems quite likely that the this is a matter of the study being somewhat underpowered in order to detect between-group differences in this parameter. In the 30-s chair stand test, all groups improved (although with a large degree of variation in LITW). Given that WHEY and LITW did not improve any measure of strength

noticeably, it seems unlikely that the small increases in 30-s chair stand performance are representative of improvements in function, but rather some degree of "learning effect", where participants simply improve their performance due to practicing the given test.

In summary, the addition of light intensity, home-based training to whey protein supplementation was not effective in increasing muscle size, strength or function. However, center-based heavy resistance training was effective in increasing muscle strength as well as preserving muscle size over the course of 1-year.

9.3. Paper 3

The sub-section will describe and discuss the results in paper 3, where participants fulfilling the requirements for the PP analysis in the training arm were analyzed further. As this paper focuses on the effects of the addition of resistance training, the intervention group "WHEY" will in this paper be called "no training with whey protein supplementation" (NOTW).

9.3.1. Participant characteristics

Characteristics of participants included in analysis for paper 3 in shown in Table 9. No differences in any of the measured parameters were observed, indicating that the group characteristics were still fairly similar after exclusion of participants not fulfilling the requirements for the PP analysis.

	NOTW			LITW			HRTW			P-value
N (Male/female)	25 (12/13)			20 (10/10)			19 (10/9)			
Age	69.9	±	3.8	70.3	±	3.8	70.2	±	3.4	0.93
Body composition										
Weight (kg)	73.4	±	15.0	72.7	±	9.1	76.5	±	14.4	0.65
Height (m)	1.73	±	0.09	1.70	±	0.07	1.71	±	0.08	0.52
BMI (kg/m^2)	24.5	±	3.8	25.1	±	3.35	26.0	±	3.8	0.42
aLTM (kg)	22.6	±	5.2	21.4	±	4.1	22.9	±	5.6	0.61
Fatmass (kg)	24.8	±	6.6	24.1	±	6.4	22.9	±	8.3	0.67
qCSA (cm^2)	55.1	±	12.7	56.2	±	12.5	57.6	±	14.2	0.83
Strength and function										
MVIC (Nm)	184.0	±	49.9	175.4	±	43.0	170.9	±	55.8	0.68
Dynamic peak torque (Nm)	158.9	±	45.6	149.6	±	39.2	156.8	±	47.5	0.78
RFD (Nm/s)	670.5	±	293.7	634.6	±	200.6	624.0	±	234.9	0.81
LEP (W)	183.6	±	61.0	184.6	±	64.6	206.2	±	67.3	0.45
Grip strength (kg)	36.1	±	11.8	36.1	±	9.5	40.8	±	13.4	0.35
400 m gait time (s)	236.0	±	24.5	237.9	±	34.8	251.4	±	29.4	0.20
Activity										
Steps per day	10774	±	3557	10324	±	3444	9652	±	4241	0.62
Diet										
Protein intake (g/kgBW)	1.1	±	0.3	1.1	±	0.3	1.1	±	0.3	0.68
Energy intake (kJ)	8470	±	1951	7354	±	2017	8252	±	2135	0.21

Table 9. Characteristics of participants included in analysis for paper 3. Results are shown as mean ± SD. P-value indicates the outcome of a 1-way ANOVA between groups.

9.3.2. Adherence to training

Adherence to the training interventions are shown in Figure 13. HRTW had significantly lower adherence to training in the second half of the intervention (6-12 months), compared to the first half (0-6 months). There were no significant differences between adherence to training in first and second half of the intervention for LITW. These findings are interesting, as I am not aware of other studies describing the temporal changes in adherence to long-term resistance training. The slight decrease in adherence to training for HRTW could likely affect the temporal changes in muscle mass and strength and should therefore be of interest in future long-term training studies. Overall adherence to training was higher for HRTW than LITW. However, in this paper that difference is caused by the fact that we used different cut-off criteria for the two intervention groups in this PP analysis (>75% for LITW vs >66% for HRTW).

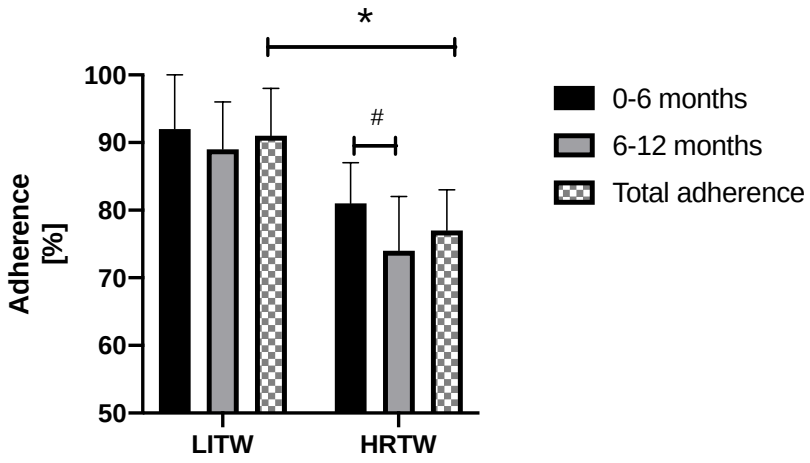
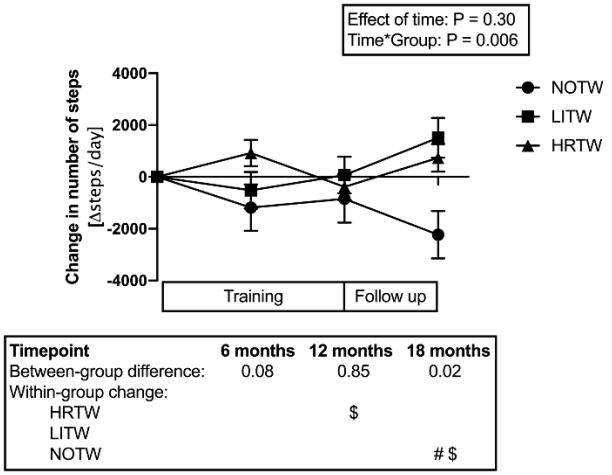


*Figure 13. Adherence to training in the training groups. *: significant between-group difference. #: significant difference between timepoints.*

9.3.3. Changes in activity level

A significant time*group interaction was observed for daily step counts (Figure 14). While we expected that adding an exercise intervention to the daily lives of the older adults would increase overall daily physical activity, this was not the case in the CALM study, as we observed no effect of the of the training interventions on daily step counts during the intervention. In young adults, the addition of an exercise intervention increases overall daily activity level[195]. However, older adults seem to compensate for this increase in exercise related activity, by lowering other types of physical activity[196]. The lowering of habitual physical activity seem to be mainly influenced by fatigue from the exercise session, although a sense of having "earned" the right to be inactive might also be a substantial factor[197]. Unfortunately, we did not investigate whether the training interventions resulted in physical activity compensation. Consequently, the lack of increase in daily step counts could potentially be due to inadequate sensitivity of our measurements of daily activity.

Significant between-group differences were observed at 18 months, where LITW and HRTW had significantly higher daily step counts compared to NOTW. These differences were mainly mediated through significant decreases in step counts for NOTW. It is somewhat surprising that NOTW decreased physical activity during the follow-up period, but this finding indicates that the training modalities motivated the participants to maintain high activity levels after the interventions.



Timepoint	6 months	12 months	18 months
Between-group difference:	0.08	0.85	0.02
Within-group change:			
HRTW		$	
LITW			
NOTW			# $

Figure 14. Changes in daily step counts. #: Significantly different (P<0.05) from baseline. $: Significantly different (P<0.05) from previous timepoint. Results are shown as mean ± SE

9.3.4. Temporal adaptations to training during and after the intervention.

There was no significant effect of training on qCSA, aLTM, or fat mass (Figure 15). This was somewhat expected given the low degree of hypertrophic response observed from 0-12 months in **paper 2**. However, given that the present analysis is based on PP analysis, it is still surprising that we were unable to detect significant hypertrophy in response to training. An important consideration in relation to the present results, is the fact that our mixed-model analysis includes 3 groups and 4 timepoints, which will inevitably cause difficulties in detecting significant time*group interactions and cause an increased risk of type 2 error. However, given that the numerical increase in qCSA during the intervention for HRTW was ~2%, muscle hypertrophy was in any case quite minor. While eyeballing statistics could suggest that minor hypertrophic adaptations seemed to occur in aLTM during the first 6 months, it should be remembered that DXA scans at 6 and 18 months were not performed in a fasting state, while scans at 0 and 12 months were. This could potentially affect the outcomes of the DXA scans, e.g. due to differences in hydration. Although measures of appendicular lean tissue would be expected to be less effected than measures of total lean tissue[155], this could still cause aLTM to be somewhat overestimated at the 6- and 12-month timepoints.

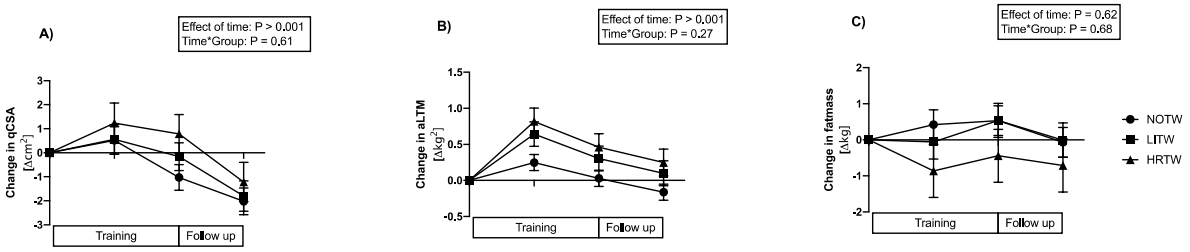


Figure 15. Changes in body composition. A) Quadriceps cross-sectional area (qCSA). B) Appendicular lean tissue mass (aLTM). C) Fat mass. Results are shown as mean ± SE.

The time*group interaction term was significant for MVIC, dynamic peak torque, and RFD (Figure 16). This sub-section will describe the changes in these parameters for each of the training groups.

LITW increased MVIC, dynamic peak torque, and RFD during the first 6 months of the intervention. However, these increases did not differ significantly from the changes observed in NOTW. At the 12-month timepoint, dynamic peak torque and RFD remained above baseline levels, without any further increases, whereas MVIC was not different from baseline. However, of these improvements only dynamic peak torque was significantly improved compared to NOTW at the 12-month timepoint. From 12-18 months, MVIC, dynamic peak torque, and RFD decreased significantly, and did not differ from baseline at the 18-month timepoint.

HRTW increased MVIC, dynamic peak torque, and RFD during the first 6 months of the intervention. However, only the changes in MVIC differed significantly from NOTW, and none of the changes differed compared to LITW. From 6-12 months, only MVIC was further increased. However, at the 12-month timepoint, the changes in MVIC, dynamic peak torque, and RFD were all significantly different from the changes in NOTW, but only MVIC was elevated compared to LITW. From 12-18 months, MVIC and RFD remained elevated above baseline levels, whereas dynamic peak torque decreased to baseline level. However, MVIC, dynamic peak torque, and RFD were all higher for HRTW compared to NOTW at the 18-month timepoint. Furthermore, MVIC was also significantly higher at 18 months compared to LITW, whereas RFD tended to be higher for HRTW as well.

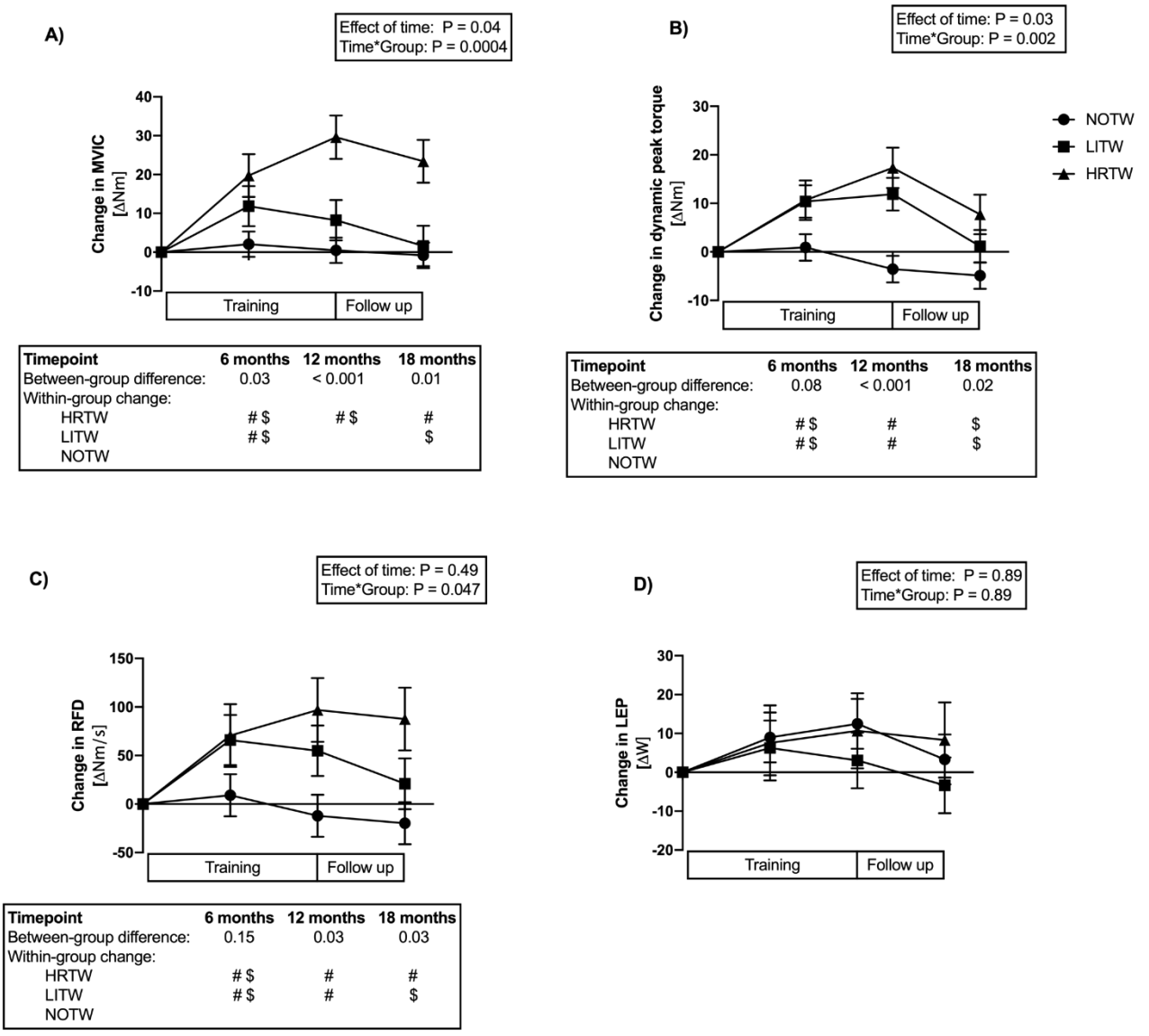


*Figure 16. Changes in lower extremity strength and power. #: Significantly different (P<0.05) from baseline. $: Significantly different (P<0.05) from previous timepoint. Test of between- and within-group differences are only performed if the Time*group interaction is significant (P<0.05). Results are shown as mean ± SE.*

Collectively these results suggest that the LITW had a positive effect on muscle strength, but mainly occurred during the first half of the intervention and were only somewhat maintained during the second half of the intervention. Interestingly, HRTW was not associated with better results than LITW during the first 6 months, but increased MVIC significantly compared to LITW from 6-12 months. While this underlines the superiority of heavy resistance training as a long-term training modality, it also provides highlights the possibilities of LITW for shorter training interventions. Given that the light intensity, home-based seemed to be capable of inducing improvements in muscle strength during the initial 6 months, training modalities such is this could

potentially be used to increase exercise self-efficacy, potentially motivating older adults to participate in more strenuous exercise modalities in the long-term, which could then induce further adaptions.

Contrary to the hypothesis, HRTW was the only training modality associated with a preservation of training adaptations at the 18-month timepoint. The finding that muscle strength was preserved above baseline levels 6 months after the heavy resistance training intervention was in line with what has previously been reported[140–144]. However, LITW was not associated with better strength than NOTW at that timepoint. The was despite the fact that both HRTW and LITW were associated with higher activity levels at the 18-month timepoint compared to NOTW. Van Roie and colleagues[198] observed that while training with heavy loads (80% of 1 RM) was more effective in increasing 1 RM than training with light loads (20% of 1 RM), both training intensities were associated with partly preserved strength gains 6 months after the intervention. However, when assessing isometric and isokinetic strength changes, the authors of did not observe significant within-group preservations of muscle strength after detraining. This underlines the importance of the method of strength assessment, as 1 RM changes are likely to be larger due to being tested in the trained movement.

A novel finding in this paper was the preservation of adaptations in RFD after HRTW. While several studies have observed increases in RFD after resistance training in older adults[122,123], this is to my knowledge the first study to show that RFD is still enhanced 6 months after a resistance training intervention. Lovell and colleagues[123] observed that RFD returned to baseline levels 4 weeks after a 16 week resistance training intervention in older men. It could be speculated that the longer intervention period in the present study consolidated the neuromuscular adaptations, causing the better preservation of RFD in the present study. This is a very important finding as RFD is a strong predictor of functional capacity in older adults[199], as well as a crucial component in the prevention of falls[200].

In summary, we found that while LITW was capable of inducing increases in muscle strength during the first 6 months of training, only HRTW was capable of inducing continued increases in strength from 6 to 12 months. Therefore, while light intensity, home-based training could potentially be beneficial in providing initial increases in muscle strength and potentially motivate

older adults to maintain high physical activity levels, the findings from this paper indicate that the persisting degree of progression in loading associated with heavy resistance training is needed to obtain continued increases in muscle strength and RFD. Furthermore, as only HRTW was associated with a preservation of muscle strength and RFD 6 months after the intervention, heavy resistance training seems to be the most viable long-term training modality.

10. Conclusions

In **paper 1** we found that between-limb asymmetry in lower extremity muscle strength and power is highly prevalent in healthy older adults, with average degrees of asymmetry in these parameters being ~10%. The average degree of between-limb asymmetry in lower extremity muscle mass was much smaller (~3%). However, the degree of asymmetry was not consistently associated with functional capacity, and the weakest leg was not a better predictor of functional capacity compared to the strongest leg. Absolute measures of muscle mass, strength, and power were all positively correlated with functional capacity. From these finding we therefore conclude that training interventions for older adults should focus on increasing muscle mass, strength and power, whereas the effects of decreasing between-limb asymmetry in these parameters might be of less importance.

In **paper 2** we found that protein supplementation without any concurrent exercise intervention did not provide any benefits in relation to maintaining muscle size, strength, or function. Based on these findings, there is no basis for recommending protein supplementation for healthy older adults already reaching daily protein intakes of >1.0 $g \cdot kg^{-1} \cdot day^{-1}$. The addition of heavy resistance training on top of whey protein was associated with a preservation of muscle size, as well as increases in muscle strength. Despite a high compliance to light intensity, home-based training, the addition of this training modality was not effective in inducing adaptations in muscle size, strength, or function.

In **paper 3** we found that while both LITW and HRTW were capable of increasing MVIC and dynamic peak torque during the first 6 months of training, only HRTW was associated with continued improvements in MVIC and RFD from 6-12 months. Furthermore, only HRTW preserved MVIC and RFD above baseline levels 6 months after the intervention had ended. We therefore concluded that while light intensity, home-based training was capable of increasing muscle

strength during the initial 6 months of training, heavy resistance training is needed for continued increases in muscle strength. Furthermore, HRTW provides the additional benefit of preserved adaptions in muscle strength 6 months after a training intervention.

11. Perspectives

The results in the present thesis shows that while light intensity, home-based training was associated with slight improvements in strength during the first half of the intervention, heavy resistance was more effective in providing long-term adaptions in muscle mass and strength. However, this finding still leaves us with one of the same issues described in the initial background sections of this thesis; Many older adults do not enjoy training modalities of higher intensities, and do not feel comfortable in the typical settings associated with heavy resistance training (ie. commercial gyms), and adherence to such training modalities therefore might be limited. The premise of the light intensity, home-based training intervention in the CALM study was to design a training modality that the older adults were likely to adhere to, and then investigate if this intervention had an effect on the parameters of interest. In future studies and innovation efforts it could be of interest to turn this approach around, investigating how to increase adherence to the interventions we know to be the most effective in increasing muscle mass and strength (ie. heavy resistance training).

Protein supplementation without concomitant resistance training did not provide. Any beneficial effects in regard to preserving muscle mass, strength, or function in these participants. The lack of beneficial effects in the present cohort was likely due to the participants having daily protein intakes substantially over the RDA without the supplements. As we observed increases in adiposity in all supplement groups, it is likely that the supplements did not suppress appetite sufficiently to decrease energy intakes from other food sources (although we did not observe increases in daily energy intake). These supplements could therefore potentially be of benefit for underweight or frail older adults, where a lack of appetite might cause inadequate protein and energy intakes[201]. However, Gade and colleagues[202] recently observed a low adherence to protein supplementation in geriatric medical patients after discharge from the hospital. In that study, no effects of protein supplementation were observed when combined with low intensity resistance training. The lack of effect of protein supplementation in the study by Gade and colleagues could potentially be due to the poor adherence to the supplement, which were mainly caused by the supplements being

satiating and causing taste fatigue[202]. This underlines the importance of innovation efforts focusing on developing feasible protein supplements, for this type of intervention to be effective in such populations.

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13. Appendix I – Paper 1

"Influence of between-limb asymmetry in muscle mass, strength, and power on functional capacity in healthy older adults."

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Published in: *Scandinavian Journal of Medicine and Science in Sports,* September 2019.

Received: 12 April 2019 | Revised: 5 July 2019 | Accepted: 24 July 2019

DOI: 10.1111/sms.13524

ORIGINAL ARTICLE

WILEY

Influence of between-limb asymmetry in muscle mass, strength, and power on functional capacity in healthy older adults

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Funding information
University of Copenhagen Excellence Programme for Interdisciplinary Research; Mejeribrugets ForskningsFond; Arla foods ingredients group P/S

Purpose: Numerous daily tasks such as walking and rising from a chair involve bilateral lower limb movements. During such tasks, lower extremity function (LEF) may be compromised among older adults. LEF may be further impaired due to high degrees of between-limb asymmetry. The present study investigated the prevalence of between-limb asymmetry in muscle mass, strength, and power in a cohort of healthy older adults and examined the influence of between-limb asymmetry on LEF.

Methods: Two hundred and eight healthy older adults (mean age 70.2 ± 3.9 years) were tested for LEF (400 m walking and 30-seconds chair stand). Furthermore, maximal isometric and dynamic knee extensor strength, leg extensor power, and lower limb lean tissue mass (LTM) were obtained unilaterally.

Results: Mean between-limb asymmetry in maximal muscle strength and power ranged between 10% and 13%, whereas LTM asymmetry was 3 ± 2.3%. Asymmetry in dynamic knee extensor strength was larger for women compared with men (15.0 ± 11.8% vs 11.1 ± 9.5%; $P = .005$) Leg strength and power were positively correlated with LEF ($r^2 = .43$-$.46$, $P < .001$). The weakest leg was not a stronger predictor of LEF than the strongest leg. Between-limb asymmetry in LTM and isometric strength was negatively associated with LEF (LTM; $r^2 = .12$, $P = .005$, isometric peak torque; $r^2 = 0.40$, $P = .03$.) but dynamic strength and power were not.

Conclusion: The present study supports the notion that in order to improve or maintain LEF, healthy older adults should participate in training interventions that increase muscle strength and power, whereas the effects of reducing between-limb asymmetry in these parameters might be of less importance.

KEYWORDS
asymmetry, lower extremity function, mobility, muscle power, muscle strength

1 | INTRODUCTION

Age-related loss of muscle mass, which has been reported to begin around the 5th decade of life,[1,2] can be responsible for an increased risk of metabolic disorders, functional impairment, and frailty.[1,3] While muscle mass is progressively lost by ~0.5% annually,[4] the accompanying impairments in muscle strength and power are observed to occur at a faster

rate of up to 3%-4% annually.[5-7] Impairment in these factors has been shown to be a strong predictor of current functional capacity[8,9] as well as being associated with an elevated risk of developing future functional limitations.[6,10] However, in well-functioning older individuals, the initial loss of muscle strength and power may not have strong impact on functional capacity, as the relationship between muscle strength/power and functional capability appears to be plateauing (ie, reach a ceiling region) at the upper end of this relationship.[11]

A vast number of physical activities of daily living (ADL) involve bilateral lower limb movements (walking, chair stand, stair climbing, etc), and the ability to perform these activities will therefore be limited by bilateral lower limb muscle function. Thus, another possible determinant of functional capacity could be the degree of lower limb asymmetry in the aforementioned factors. Previous studies have observed that high between-limb asymmetry in leg extensor power is associated with impaired postural balance and an elevated incidence of falls.[12,13] These findings suggest that between-limb differences (asymmetry) in lower limb muscle size, strength, and/or power can negatively ADL in old adults. Thus, the magnitude of between-limb asymmetry in lower limb muscle function may represent a separate and early detectable risk factor for impaired functional capacity even in healthy non-frail older adults. This hypothesis has only been sparsely investigated with inconclusive results.[14-16] The discrepancy between observations could potentially be due to differences in testing methods (testing of whole-leg vs single-joint power), as well as lack of statistical adjustments for physical activity and levels of body fat.[17] Therefore, research using both whole-leg and single-joint testing methods to investigate the potential influence of between-limb asymmetry on functional capacity in older adults is warranted. Furthermore, as the risk of functional impairment seems to be higher in women compared with men,[18-20] investigations of sex-specific differences in lower extremity asymmetry are of key interest.

The aim of this study, therefore, was to quantify the magnitude of between-limb asymmetry in lower limb skeletal muscle mass, strength, and power in a large cohort of healthy home-dwelling Danish older men and women. Secondly, we aimed to investigate to which extent lower extremity function (LEF) would be determined (ie, regressionally predicted) by selected measures of muscle mass, strength, and power, and/or by the degree of between-limb asymmetry in these parameters.

2 | MATERIAL AND METHODS

This study was based on cross-sectional analyses of baseline data obtained in the Copenhagen CALM study.[21] A full description of the CALM protocol, as well as detailed exclusion criteria, has been presented elsewhere.[21] A brief description of the experimental methods is provided below.

2.1 | Participants

A total of 208 home-dwelling older adults with a mean age of 70 ± 4 (SD) years were recruited for the study (Women: 99, Men: 109). All participants gave their written consent in accordance with the declaration of Helsinki II, and the study was approved by the Danish Regional Ethics Committees of the Capital Region (H-4-2013-070). Anthropometric data of the included participants are listed in Table 1. Recruitment was conducted via advertisements in newspapers, magazines, and social media, as well as presentations at senior centers and public events. To be included in the study, participants were not allowed to participate in more than 1 hour of heavy resistance training per week, but were allowed to perform

TABLE 1 Characteristics of the research participants

	All	Men	Women	*P*-value
N	208	109	99	-
Age (y)	70.2 ± 3.9	70 ± 3.9	70.4 ± 3.9	.52
Weight (kg)	75.7 ± 12.8	81.4 ± 11.2	69.4 ± 11.4	<.0001
Height (m)	1.72 ± 0.08	1.77 ± 0.06	1.67 ± 0.06	<.0001
BMI (kg/m^2)	25.6 ± 3.8	26.0 ± 3.4	25.1 ± 4.1	.07
ASMI (kg/m^2)	7.6 ± 1.2	8.3 ± 0.9	6.7 ± 0.8	<.0001
Fat% (%)	33.3 ± 8.1	29.0 ± 6.4	37.9 ± 7.2	<.0001
Visceral fat (kg)	1.3 ± 0.9	1.7 ± 0.9	0.9 ± 0.7	<.0001
400 m gait time (s)	245 ± 34	236 ± 32	255 ± 33	.0001
30-s chair stands (reps)	19.7 ± 5.0	20.7 ± 4.8	18.6 ± 5.0	.001
Daily step-count (steps)	10 056 ± 3958	10 040 ± 3877	10 163 ± 4099	.83

Note: Results are reported as mean ± SD. *P*-values derived using unpaired t testing or Wilcoxon rank-sum comparison between sexes.

Abbreviations: ASMI, Appendicular skeletal muscle index; BMI, Body mass index.

other forms of exercise. Participants were excluded if they possessed any medical condition potentially preventing them from safely completing a 1-year intervention including heavy resistance training and twice daily protein/carbohydrate supplementation. A full description of exclusion criteria can be found elsewhere.[21]

2.2 | Physical performance assessment

All physical performance tests were carried out by an experienced assessor on the same day in the order listed below. Measurement of body composition was done on a separate day. The entire test battery was typically completed within 1 hour, and rest periods between tests were administered as needed. Participants arrived at the Laboratory in clothes and shoes intended for physical activity. Prior to the test day participants had been carefully instructed not to perform any strenuous physical activities 2 days prior to the performance tests. Prior to the tests, the dominant leg of the participants was determined by asking them which leg they felt was the strongest.

2.3 | Lower extremity function

The 400 m walk test and the 30-seconds chair stand test were chosen as objective measures of LEF.[22,23]

The 400 m walk test was performed on a 20-m indoor course track marked by two colored cones. The participants were instructed to walk 400 m as fast as possible without running and without receiving personal assistance or sitting down during the test.[22,24] Data were reported as time to complete 400 m walk. For the later calculation of the composite LEF measure, walk time was converted into average walking speed as this parameter has been shown to be a strong predictor of mobility limitations in older adults.[24]

The 30-seconds chair stand test was performed using a chair without armrest (seat height 44.5 cm). Participants completed as many sit-to-stands as possible in 30 seconds with their hands crossed over the chest. A repetition was defined as the participant rising from a seated position to reach full extension of the knees and hips. This test has previously been shown to be a valid and reproducible test of functional lower body strength in older adults.[23]

The composite sum of the *Z*-scores of each of the two test parameters (average 400 m walk speed and number of stands in the 30-seconds chair test) was calculated to provide a global index for LEF, which was used in the subsequent statistical analyses.[16,25]

2.4 | Maximal leg extensor power

Unilateral leg extensor power (LEP) was measured using the Nottingham power rig (Queens Medical Center, Nottingham University, UK) as described in detail elsewhere.[12,26] In brief, participants were seated with their hands folded over the chest and carefully instructed to press a pedal down as hard and fast as possible by extending the knee and hip joint, thereby accelerating a flywheel. Based on the rotational speed of the flywheel, a computer calculated the average power exerted in each single-leg extension movement. The participants were familiarized to the procedure by performing two submaximal warm-up trials, followed by a minimum of five maximal trials each separated by 30 seconds of rest. The test ended when participants performed two consecutive results that were lower than their current peak average power value. The self-reported dominant leg was tested first, followed by the self-reported non-dominant leg.

2.5 | Maximal knee extensor strength

Maximal concentric knee extensor strength (gravity-corrected peak torque) was measured during slow (60°/s) maximal knee extension using an isokinetic dynamometer (Kinetic Communicator, model 500-11) at a knee joint range of motion from 90° to 10° knee flexion (0° = full knee extension). Following three warm-up trials at submaximal effort, participants performed a minimum of 4 maximal knee extension trials with strong verbal encouragement and visual online display of the exerted torque, separated by 30-45 seconds of rest. Subsequently, trials were repeated until participants were unable to improve knee extensor peak torque any further. The self-reported dominant leg was tested first, followed by the non-dominant leg. For each leg, the trial with the highest gravity-corrected peak torque (calculated by multiplying the gravity-corrected dynamometer force by the length of the dynamometer lever arm) was selected for further analysis.

Finally, participants performed three maximal isometric knee extensor contractions (MVIC) at 70° knee flexion separated by 30-45 seconds rest. Participants were instructed to contract as hard and fast as possible with strong verbal encouragement for approximately 4 seconds. The trial with the highest peak torque was selected for further analysis. Attempts containing an initial countermovement were disqualified, and a new trial was performed.

2.6 | Body composition

Body composition was assessed using dual-energy x-ray absorptiometry (Lunar iDXA, GE Medical Systems). Study participants refrained from strenuous activities for 48 hours prior to the test. They arrived fasting from 21:00 the night before, but were allowed to drink water as needed prior to the scansand. All scans were performed between 08:00 and 10:00. From these scans, lean tissue mass (LTM) was obtained for the left and right lower limbs (Segmented at the femoral neck). Using these measures, appendicular skeletal muscle mass index

(ASMI) was calculated as previously described[27] by dividing the sum of LTM (subtracted by fat and bone mineral content) of arms and legs by height squared. Body fat percentage and visceral fat content were also assessed. Regions of interest (ROIs) for the extremities and visceral body parts were set based on the defaults definitions provided by the scanner software. The same examiner controlled the default positioning of all regions, which were adjusted slightly when appropriate.

2.7 | Activity monitoring

Daily activity levels were measured by mounting an accelerometer-based activity monitor (activPal 3™, activPal 3c™, or activPal micro; PAL Technologies) on the anterior surface of the thigh.[28] The activity monitor was worn for 96 continuous hours covering two weekdays and a full weekend. Data were reported as the average number of steps per day.

2.8 | Statistical analysis

Group characteristics were compared using unpaired *t* tests or Wilcoxon rank-sum tests for Gaussian and non-Gaussian distributed data, respectively. Unilateral strength and LTM for the strongest and weakest leg were analyzed using multiple linear regression with sex, strongest/weakest limb, and age as independent variables. Relationships between dependent variables (Composite *Z*-score) and independent variables (various muscle mechanical parameters) including covariables (sex, age, steps per day, fat percentage, and BMI) were performed using multiple linear regression analysis. Steps per day were used to control for daily activity levels, whereas the assessment of body fat was used to account for potential effects of differences in body composition. These specific covariables were selected as they have previously been shown to affect LEF[17,20] Covariables with low weight in the model ($P > .1$) were excluded using progressive step-wise regression. Robust standard errors were calculated when linear regression models showed heteroscedasticity. Percentage between-limb asymmetry was calculated as (([Strongest – Weakest]/Strongest)*100). Between sex comparisons for limb asymmetry was performed using Wilcoxon rank-sum tests (assuming non-Gaussian distributions). Results are reported as mean ± SD unless otherwise stated, and the level of significance was $P < .05$ (2-tailed testing). All statistical analyses were performed using STATA 15.1 (StataCorp).

3 | RESULTS

3.1 | Characteristics of research participants

Table 1 presents the characteristics of the included participants. Compared with female participants, male participants demonstrated higher ($P < .0001$) ASMI, lower body fat percentage, higher visceral fat content, and tended to have higher BMI ($P = .07$). Furthermore, male participants demonstrated faster 400 m gait speeds ($P = .0001$) and completed more repetitions on the 30-seconds chair stand test ($P = .001$). No sex differences were observed for age or daily activity level.

3.2 | Muscle strength and mass

Data on maximal unilateral muscle strength and power, as well as muscle mass, were grouped into the strongest and weakest limb (Presented in Table 2). Male participants exhibited greater LEP, dynamic knee extensor strength, and MVIC (all normalized to body mass) compared with female participants, along with larger leg LTM (all $P < .001$).

3.3 | Between-limb asymmetry

Data on between-limb asymmetry are presented in Figure 1. The average between-limb asymmetry ranged between 10% and 13% for various strength and power measurements (LEP: 10.6 ± 7.9%; Dynamic peak torque: 13.0 ± 10.8%; MVIC: 11.2 ± 10.3%), whereas asymmetry in leg LTM averaged 3.0 ± 2.3%. Asymmetry was larger in women compared with men for dynamic peak torque (Men 11.1 ± 9.5%; Women: 15.0 ± 11.8%; $P = .005$). For all other measures, asymmetry did not differ between sexes.

TABLE 2 Unilateral knee extensor strength, leg extensor power, and fat-free mass (LTM)

	Strongest limb	Weakest limb	Gender effect
Leg extensor power (W/kg)			
All	2.63 ± 0.68	2.32 ± 0.63	<0.001
Men	3.00 ± 0.63	2.65 ± 0.60	
Women	2.23 ± 0.48	1.97 ± 0.47	
Dynamic peak torque (Nm/kg)			
All	2.04 ± 0.45	1.78 ± 0.46	<0.001
Men	2.27 ± 0.39	2.02 ± 0.40	
Women	1.78 ± 0.38	1.51 ± 0.39	
MVIC (Nm/kg)			
All	2.29 ± 0.54	2.04 ± 0.54	<0.001
Men	2.55 ± 0.47	2.30 ± 0.45	
Women	2.01 ± 0.46	1.76 ± 0.49	
LTM legs (kg)			
All	8.66 ± 1.68	8.41 ± 1.66	<0.001
Men	9.88 ± 1.20	9.59 ± 1.21	
Women	7.31 ± 0.94	7.09 ± 0.94	

Note: Results are reported as mean ± SD. Data on knee extensor dynamic peak torque, isometric peak torque (MVIC), and leg extensor power are reported normalized to body weight. Lean tissue mass (LTM) measures are reported in absolute values. *P*-values represent the outcome of linear regression analyses.

3.4 | Associations between strength, power and asymmetry and lower extremity function (LEF)

LEF was positively correlated with LEP, MVIC, and dynamic peak torque (r^2 = .43-.47, P < .001; Table 3). In addition, leg LTM was positively correlated with LEF (r^2 = .38, P = .02-.03). Leg LTM was not associated with LEF using the non-adjusted regression model. Associations with LEF were comparable when correlating strength or power levels from either the strongest or weakest leg.

Percentage between-limb asymmetry in MVIC was negatively associated with LEF when adjusted for steps per day and body fat percentage (r^2 = .40, P = .025). Likewise, leg LTM asymmetry was negatively correlated with LEF when adjusted for steps per day, although demonstrating a weaker relationship (r^2 = .12, P = .048). These associations disappeared when using non-adjusted regression analysis. Percentage between-limb asymmetry in LEP and dynamic peak torque was not associated with LEF.

4 | DISCUSSION

The present study evaluated the degree of between-limb asymmetry in maximal leg muscle strength, power, and lower limb LTM in order to investigate its potential association with functional capacity among home-dwelling older individuals.

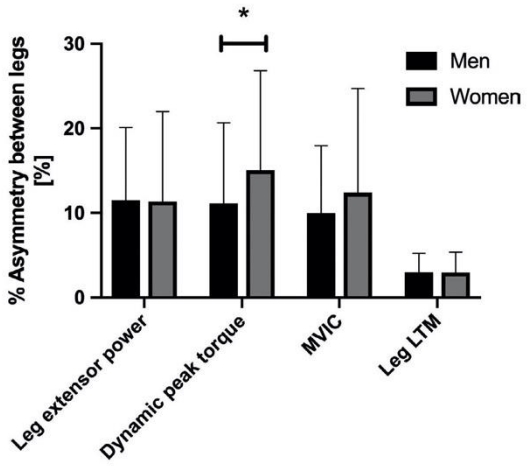

FIGURE 1 Percentage between-limb asymmetry in power, strength, and muscle mass measures. Asymmetry was calculated as (((Strongest – Weakest)/Strongest)*100%). Results are shown as mean ± SD. * denotes significant difference between sexes (P < .05). MVIC; maximal voluntary isometric contraction. Leg LTM, leg lean tissue mass

The data revealed that the mean magnitude of lower limb muscle strength and power asymmetry was in the range of 10%-13%, whereas asymmetry in leg LTM was much lower (3%). At group level, the magnitude of between-limb asymmetry was comparable with values previously reported in healthy older adults of similar age.[13,14,16,29] Notably, however, a significant proportion (11%-20%) of the participants demonstrated much greater (2-3 fold higher) levels of between-limb asymmetry in lower limb strength and power, which might predispose this subpopulation for future mobility limitations. Surprisingly, women demonstrated higher degrees of between-limb asymmetry in dynamic knee extensor peak torque than men. To our best knowledge, this effect of sex on between-limb asymmetry has not been reported previously. This finding could, at least in part, help to explain previous observations of lower LEF and higher risk of developing frailty in older women compared with men.[18,30] However, since sex differences were not apparent for any other outcome measure obtained in the present study, this notion remains purely speculative.

The present study demonstrated moderate-to-strong associations between maximal leg extensor strength/power and LEF (Table 3). Comparable relationships have been observed in previous studies[14,15,31] although these studies generally were performed in elderly with lower functional performance levels than the older adults examined in the present study. For instance, 90% of the participants in the present study completed the 400 m walk in a time that would place them in the fastest quartile reported by Newman and coworkers.[24] Importantly, the present associations suggest that even in healthy independently living and active older individuals, high levels of leg muscle strength and/or power are accompanied by high LEF and vice versa. Some measures of LEF seem to suffer from a ceiling effect when applied in healthy older adults,[32] underlining the importance of choosing sufficiently challenging tests when measuring LEF in this population. In contrast to previous reports,[31,33-35] we did not find LEP to be a stronger predictor of functional performance than isolated muscle strength parameters (dynamic or isometric knee extensor strength). It is possible that this apparent discrepancy arises as a result of the overall high strength and functional performance level of the present group of old adults.

Leg LTM as a measure of lower limb muscle mass appeared to be a moderate predictor of LEF in our cohort when adjusted for age, daily activity level, and body fat percentage. In contrast, leg LTM failed to predict LEF when using a non-adjusted linear regression model. Previous investigations into the relationship between muscle mass and functional performance levels in older adults have shown conflicting results, with some studies reporting positive correlations[1,27,36] while absent in others.[9,37-39] Importantly, leg LTM failed to predict LEF when using a non-adjusted linear regression model. However, a clear positive

TABLE 3 Relationships between lower extremity function (LEF) and lower body strength-/power or fat-free mass (LTM) of the strongest or weakest leg, or between-limb asymmetry (%ASYM)

Associations to LEF	Included covariables						
	Gender	Age	Steps/d	Fat-%	BMI	*P*-value	R^2
Leg extensor power							
Strongest leg	**	**	*	***	-	<.001	.44
Weakest leg	**	**	**	***	-	<.001	.45
%ASYM	-	-	-	-	-	.36	.004
Dynamic peak torque							
Strongest leg	***	*	**	***	-	<.001	.47
Weakest leg	**	**	**	***	-	<.001	.45
%ASYM	-	-	-	-	-	.07	.02
MVIC							
Strongest leg	**	**	**	***	-	<.001	.46
Weakest leg	**	**	**	***	-	<.001	.47
%ASYM	-	***	*	***	-	.03	.40
Leg LTM							
Strongest leg	-	***	*	***	-	.02	.38
Weakest leg	-	***	*	***	-	.03	.38
%ASYM	-	-	***	-	-	.005	.12

Note: "*P*-value" indicates the level of significance for the correlation. Levels of significance for covariables are shown as * $P < .1$, ** $P < .01$, *** $P < .001$. "-" $P > 0.1$.

relationship between leg LTM and LEF emerged when the effects of age, physical activity, and body fat percentage were accounted for. In turn, the observed association between muscle mass (leg LTM) and lower extremity function may have been mainly driven by the positive relationships between lower limb strength and/or power levels and LTM. This can be considered an independent benefit of conserving muscle mass at old age regardless of other potential advantages hereof on metabolic health, systemic inflammatory state, etc[40]

The present study revealed that when using an adjusted regression model, high levels of between-limb asymmetry in MVIC and leg LTM were associated with reduced LEF even when examined in well-functioning community-dwelling healthy older adults. In contrast, the degree of lower limb asymmetry in LEP and dynamic peak torque failed to demonstrate any associations with LEF. These disparate trends are puzzling, as asymmetry in these measures would be expected to depend largely on the same physiological factors, and consequently should be similarly associated with LEF. Although speculative, the disparate trends could possibly be due to asymmetry in MVIC being dependent on differences in maximal force generation capacity of the lower limbs and thus largely rely on skeletal muscle mass (size). In contrast, asymmetry in LEP and dynamic peak torque might to a greater extent depend on between-limb differences in neuromuscular activation and coordination due to the highly dynamic nature of the tests, which involved slow isokinetic to fast non-restricted movement speeds. Further, we intended to examine whether LEF was influenced directly by the strength/power performances of the strongest or weakest leg, respectively. Somewhat unexpectedly, however, neither the prevalence nor the strength of associations to functional performance differed between the strongest or weakest limbs, suggesting that the strength/power capacity of the weakest leg generally does not represent a separate limiting factor for lower extremity function, at least in healthy older individuals. Thus, in terms of lower limb muscle strength and power, the present findings suggest the existence of a substantial physical reserve among healthy older individuals, whereby lower single-limb strength/power levels (and/or potential inter-limb asymmetries herein) may remain beyond any critical threshold below which it would start to negatively affect physical function.[11] Supporting the present observations, LaRoche and colleagues[14] also reported the weakest leg to not be a better predictor of functional performance than the stronger leg in community-dwelling older adults at risk of mobility limitation.

4.1 | Methodological considerations

Potential limitations may be observed with the present study. A low degree of between-limb asymmetry was observed in the lower limbs LTM (~3%). Given the inherent limitations of DXA scanning to detect subtle differences in lean segment mass,[41] future studies investigating between-limb asymmetry in healthy older adults would benefit from using more sensitive techniques such as magnetic

resonance imaging or CT.[42] Furthermore, it would have been relevant to include measurements of postural balance, since elevated between-limb asymmetry in LEP has previously been observed in fallers compared with non-fallers,[13] although not consistently observed in all studies.[29] Also, given the cross-sectional nature of the present study, no direct causalities could be revealed from the present observations. Longitudinal follow-up on the long-term development in functional capabilities would, therefore, be of strong interest.

In summary, between-limb asymmetry in maximal lower limb muscle strength and power production showed no systematic associations with LEF in a cohort of 208 healthy independently living and active adults aged 65 years and above. Yet, a number of lower limb strength (MVIC) and power (LEP) parameters were moderately-to-strongly associated with LEF.

4.2 | Perspective

The present observations support previous notions that strength training intervention should be introduced in healthy older adults in order to preserve or even better increase maximal muscle strength and power,[43,44] whereas the potential benefits from reducing between-limb asymmetry in selected muscle strength/power or muscle mass parameters seem to remain of lesser importance. Future studies should investigate how specific types of unilateral and bilateral strength/power training will affect lower limb muscle mass, strength, and power of well-functioning older adults, while concurrently assessing to which extent these changes can be translated into improvements in functional capacity.

ACKNOWLEDGEMENTS

Author contributions: SR, RB, NB, TB, PA, and LH designed the study. MJ, JL, MH, AS, and SA collected the data. KM collected the data and drafted the manuscript. JLN and MDJ helped with data analysis. All authors contributed to the revision of the manuscript. This work was supported by the University of Copenhagen Excellence Programme for Interdisciplinary Research, Arla Foods Ingredients Group P/S and the Danish Dairy Research Foundation.

CONFLICT OF INTEREST

None to report.

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How to cite this article: Mertz KH, Reitelseder S, Jensen M, et al. Influence of between-limb asymmetry in muscle mass, strength, and power on functional capacity in healthy older adults. *Scand J Med Sci Sports*. 2019;00:1–8. https://doi.org/10.1111/sms.13524

GRADUATE SCHOOL OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

PHD-THESIS
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1. Declaration by	
Name of PhD student	Kenneth Hudlebusch Mertz
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Name of principal supervisor	Michael Kjær
Title of the PhD thesis	Preservation of muscle mass and function through protein supplementation and exercise

2. The declaration applies to the following article	
Title of article	Influence of between-limb asymmetry in muscle mass, strength, and power on functional capacity in healthy older adults
Article status	
Published ☒ Date: July 24th 2019	Accepted for publication ☐ Date:
Manuscript submitted ☐ Date:	Manuscript not submitted ☐
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3. The PhD student's contribution to the article *(please use the scale A-F as benchmark)* Benchmark scale of the PhD-student's contribution to the article **A.** Has essentially done all the work (> 90 %) **B.** Has done most of the work (60-90 %) **C.** Has contributed considerably (30-60 %) **D.** Has contributed (10-30 %) **E.** No or little contribution (<10 %) **F.** Not relevant	**A, B, C, D, E, F**
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5. Conducting the analysis of data	B
6. Interpretation of the results	A
7. Writing of the first draft of the manuscript	A
8. Finalisation of the manuscript and submission	A
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14. Appendix II – Paper 2

"The effect of daily protein supplementation with or without resistance training for 1 year on muscle size, strength and function in healthy older adults. A randomized controlled trial."

Kenneth H. Mertz, Søren Reitelseder, Rasmus Bechshoeft, Jacob Bulow, Grith Højfeldt, Mikkel Jensen, Simon R. Schacht, Mads Vendelbo Lind, Morten A. Rasmussen, Ulla R. Mikkelsen, Inge Tetens, Søren B. Engelsen, Dennis S. Nielsen, Astrid P. Jespersen, Lars Holm.

Under review

The effect of daily protein supplementation with or without resistance training for 1 year on muscle size, strength and function in healthy older adults.

A randomized controlled trial

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Clinicaltrials.gov identifier: NCT02034760

Registration URL: https://clinicaltrials.gov/ct2/show/NCT020347600

Data described in the article will be made available upon request pending application to the CALM trial.

ABSTRACT

Background: Protein supplementation alone or combined with resistance training have been proposed to be effective strategies to counteract age-related losses of muscle mass and strength.

Objective: To investigate the effect of protein supplementation alone or combined with light intensity or heavy load resistance exercise on muscle size, strength and function in older adults.

Methods: In a 1-year randomized controlled trial (The CALM study), 208 healthy older adults (>65 years) were randomly assigned to one of five interventions: 1) Carbohydrate supplementation (CARB), 2) Collagen protein supplementation (COLL), 3) Whey protein supplementation (WHEY), 4) Home-based light-intensity resistance training with whey protein supplementation (LITW), 5) Center-based heavy-load resistance training with whey protein supplementation (HRTW). All intervention groups received the supplement twice daily. The primary outcome measure was change in m. quadriceps cross-sectional area (qCSA), assessed by magnetic resonance imaging. Secondary outcomes included isometric knee extensor strength (MVIC), 400 m gait speed, 30-s chair stand test, leg extensor power, and body composition.

Results: Protein supplementation did not affect qCSA, strength, body composition, or functional capabilities compared to CARB. Compared to WHEY, HRTW improved qCSA ([Between-group difference, 95% CI]; 1.68, 0.41 to 2.95 cm^2, P = 0.03) and MVIC (23.9, 14.2 to 33.6 Nm, $P< 10^{-5}$). LITW did not improve any measured parameter compared to WHEY.

Conclusions: Protein supplementation alone did not affect muscle size, strength or function. Based on this study, recommending protein supplementation as a stand-alone intervention for older individuals already exceeding daily protein intakes of >1.0 $g \cdot kg^{-1} \cdot day^{-1}$ appears to be ineffective in

improving any of these parameters. Only HRTW was effective in preserving muscle mass and increasing strength. Thus, we recommend that future studies aim to investigate strategies to increase long-term compliance to heavy resistance exercise in healthy older adults. This trial was registered at Clinicaltrials.gov as NCT02034760

Keywords: Protein supplementation, ageing, skeletal muscle, resistance training, randomized controlled trials, exercise

Background

Progressive decline of muscle mass is a hallmark of ageing and is accompanied by decrements in muscle strength[1–3]. The loss of strength leads to a risk of developing functional limitations[4], with potential detrimental effects on health and autonomy of the individual. Thus development of feasible strategies to maintain muscle mass and strength is of great importance[5–7].

The progressive decline in muscle mass and function[8,9] has extensively been suggested to be counteracted by a higher protein intake and usage of muscle through exercise[10,11]. Cross-sectional and prospective cohort studies have shown that protein intake above the current recommended daily allowance (RDA) of 0.83 $g \cdot kg^{-1} \cdot day^{-1}$ [12] is associated with higher muscle mass[13–19], as well as a better preservation of muscle mass in older adults (>65 years)[20–22]. The latter leading to increased recommendations of 1.1-1.3 g protein$\cdot kg^{-1} \cdot day^{-1}$ for older adults in the recent edition of the Nordic Nutrition Recommendations[23]. However, intervention studies investigating the effect of increasing protein intake on muscle mass show mixed results[24–32]. The duration of intervention studies are generally short (≤6 months), and the discrepant findings might therefore be related to inadequate intervention lengths[33]. Furthermore, the importance of protein quality (evaluated by the digestible indispensable amino acid score, DIAAS[34,35]), when supplied as part of a mixed diet, is not known. Oikawa and colleagues[36] recently found that supplementation with a high quality protein supplement (whey) induced greater increases in both acute and 6-days integrated muscle protein synthesis compared to a lower-quality protein supplement (collagen). However, to the present authors´ knowledge, it has not been investigated whether whey protein supplementation results in better preservation of muscle mass compared to collagen during long-term supplementation. Thus, the impact of increasing dietary protein intake on muscle mass and strength in older adults

remains a debated topic, with an urgent need for long-term, well-conducted, human intervention studies[33,37–40].

While heavy resistance training is the most potent exercise modality to increase muscle mass and strength[41–44], some older adults prefer exercise interventions of lower intensity, expensiveness, and situated in more convenient locations like a home-based setting[45,46]. Lower intensity training modalities can be effective in enhancing muscle mass[47–49] and when accounting for adherence, a home-based low intensity exercise program might therefore be an equally (or more) effective long-term exercise intervention as heavy resistance exercise for older adults.

The aim of the present study was to investigate the effect of protein supplementation and resistance training by conducting a 1-year randomized controlled trial, partly single-blinded/partly double-blinded. The hypotheses were:

1) Supplementation with higher quality whey protein will benefit muscle size and strength more than supplementation with lower quality collagen protein in healthy older adults.

2) Adherence to home-based, light intensity resistance exercise is higher than adherence to center-based heavy resistance training, and thus exerts an equally beneficial long-term strategy for gaining/preserving muscle mass and strength.

Methods

The Counteracting Age-Related Loss of Muscle Mass (CALM) trial was conducted at Bispebjerg Hospital, Copenhagen, Denmark between 2014 and 2018. The design of the trial and detailed descriptions of methods and exclusion criteria has been published previously[50]. The regional ethics committee approved the trial protocol (H-4-2013-070), and the subjects gave their written

informed consent to participate. The trial was registered at Clinicaltrials.gov (Identifier: NCT02034760).

Study participants:

208 community-dwelling adults aged 65 years and older were recruited. To be included the participants were not allowed to partake in >1 hour of heavy resistance training per week. Participants were not included if they had any medical condition potentially preventing them from safely completing the 1-year intervention[50].

Participant recruitment:

Recruitment was done through advertisements in newspapers, magazines, and social media, as well as presentations at senior centres and public events. After a brief telephone screening for exclusion criteria, the participants underwent a physical examination including blood samples and measurements of blood pressure to determine if the participants could perform the interventions safely. Subjects also performed a 30-s chair stand test that was used for stratifying randomization.

Randomization:

Following screening and health examination, participants were enrolled in the study and randomized into one of the following five groups using MinimPy 0.3[50,51]: 1) Carbohydrate supplementation (CARB; 20 g maltodextrin + 10 g sucrose), 2) Whey protein supplementation (WHEY; 20 g whey protein hydrolysate + 10 g sucrose), 3) Collagen protein supplementation (COLL; 20 g bovine collagen protein hydrolysate + 10 g sucrose), 4) Heavy resistance training with whey protein supplementation (HRTW), 5) Light-intensity training with whey protein supplementation (LITW). Randomization was done by an investigator not involved in interventions or not sensitive to blinding. We employed a stratified, biased coin minimization with 0.95 base probability, and

used allocation ratios corresponding to the group sizes (see sample size). Randomization was stratified by sex and number of completed repetitions on the 30-s chair stand test (<16 or ≥16).

Interventions:

The five intervention groups comprised the two arms of the study; A supplementation arm and a training arm. The supplementation arm investigated the effect of twice daily protein supplementation, and the impact of protein quality (WHEY, COLL, and CARB intervention groups). Subjects were instructed to ingest the supplements twice daily, at morning and midday, preferably just before or during meals to increase satiety, thereby limiting potential excessive caloric intake. All supplements were developed and packaged by Arla Foods Ingredients Group P/S, Viby J, Denmark. The other arm of the study, the training arm, investigated the effect of resistance training at two different intensities combined with whey protein supplementation against whey protein without training (HRTW, LITW, and WHEY). HRTW performed heavy resistance training 3 times weekly under supervision of trained personnel. Training intensity was periodized into 3-month cycles, increasing the load progressively from 12 repetition maximum (RM) to 6 RM in each cycle. LITW performed light load home-based resistance 3-5 times weekly, using TheraBand® rubber bands (Hygenic Corp., Akron, OH, USA) and bodyweight. To ensure proper execution, study personnel supervised LITW sessions once per week during the first month, and once per month during the remainder of the intervention. Training sessions were mainly focused on the lower extremities, but also included exercises for the shoulders and arms (see Bechshøft et al 2016)[50]. Adherence to HRTW was registered by staff, whereas LITW and supplementation interventions were registered by the participants in hard-copy diaries.

Primary outcome:

The primary outcome was change in midthigh m. quadriceps cross-sectional area (qCSA) of the dominant leg, measured by magnetic resonance imaging (MRI) scans. MRI is considered the gold standard for measuring muscle size, and detecting age-related atrophy[52,53]. MRI scans were performed in a Siemens Verio 3 Tesla scanner by blinded radiographers. Participants were scanned in supine position using a dedicated 32-channel body coil, and a phantom was placed parallel to the femur during the scans. The following protocol was used; 3 plane GRE scout (matrix res. 1.2.0x1.6x6.0 mm, FOV 330mm, TE 3.69ms, TR 7.8ms, scan time 27s); Axial T1 tse from the medial tibia plateau to the pubic symphysis (matrix res. 0.8x0.8x8.0mm, FOV 400mm, TE 8.4ms, TR 500, scan time 3:26). Subjects were instructed to avoid vigorous physical activity for 48 hours prior to the scans. Each scan consisted of six axial slices, with the first slice being placed in the medial tibia plateau. Each slice was 8 mm thick, separated by a 60 mm gap. Slice 4 on the dominant leg was used for assessing quadriceps cross-sectional area (qCSA). All scans were analysed by the same blinded investigator using OsiriX v. 5.5.2 (OsiriX medical imaging software, Geneva, Switzerland). Each scan was analysed twice, showing a mean coefficient of variation between measurements of 0.7%. The mean of the two measurements were used for further analysis.

Secondary outcomes:

To assess lower extremity strength, maximal voluntary isometric contraction (MVIC) of the knee extensors were measured at 70° knee flexion (0° = full extension) in an isokinetic dynamometer (Kinetic Communicator, model 500-11, Chattanooga, TN, USA). Furthermore, leg extensor power was measured in the Nottingham Power Rig (Queens Medical Center, Nottingham University, UK)[54]. The functional capabilities of the participants were assessed using the 400 m walk test[55] and 30-s chair stand test[56]. Assessments of functional capabilities as well as measures of lower extremity strength and power have been described in detail elsewhere[57] Self-perceived quality of

life was measured using the Danish version of the 36-item Short Form Health Survey[58]. We report the physical (PCS) and mental component scores (MCS) for baseline characteristics.

Body composition was assessed using dual-energy X-ray absorptiometry (Lunar iDXA, GE Medical Systems, Pewaukee, WI, USA). Study participants arrived fasting from 21:00 the night before and refrained from strenuous activities for 48 hours prior to the test. All scans were performed between 08:00 and 10:00. From these scans we obtained lean tissue mass (LTM) as well as body fat percentage. Regions of interest (ROIs) for the extremities and visceral body parts were set based on the default definitions provided by the scanner software. The same examiner controlled the default positioning of all regions, which were adjusted slightly when appropriate to take into account inter-individual differences in body placement and body size.

Daily activity levels were measured by mounting an accelerometer-based activity monitor (activPal 3^{TM}, activPal 3c^{TM}, or activPal micro; PAL technologies, Glasgow, UK) mounted on the anterior surface of the thigh[59]. The monitor was worn for 96 continuous hours covering a full weekend. Data are represented as the average number of steps per day.

A detailed description of the dietary assessment can be found elsewhere[60]. Briefly, participants weighed their dietary intake for three consecutive days (Wednesday to Friday), and wrote down the information in food logs. Trained staff then quantified nutrient intake using a dietary assessment tool (VITAKOST^{TM}, MADLOG ApS, Kolding, Denmark). Dietary assessments were performed prior to the intervention, and after 11 months of the intervention. Nutrient intake was assessed for foods only. Protein and Energy intake from the supplement was manually calculated by multiplying the compliance to the supplement with the dietary content of the supplement. For

the participants who failed to report their compliance to the supplement, but who were still receiving the supplement, we used the median compliance rate from the respective groups.

Lastly, HbA1c, blood cholesterol and triglycerides, as well creatinine concentrations were monitored.

Blinding:

Participants in the supplement-only groups (WHEY, COLL, CARB), were blinded to which supplement they received. Training interventions were not blinded to the participants. Staff performing and analysing the MRI images as well as the strength and functional tests were blinded towards the interventions. Unblinded personnel performed DXA scans and blood sampling, but analyses and interpretation of the data output from these were done by blinded researchers.

Sample sizes:

We aimed to detect between-group differences in qCSA changes of 2% over the intervention period, corresponding to approximately 0.8 cm^2. Based on previous data from our lab[61], an SD of ~1.4 cm^2 for qCSA was expected. Thus, applying a level of significance of 0.05 and a power of 0.80, a group size of 30 participants was required. Taking dropout rate into account we included 36 participants in HRTW, LITW and CARB groups and 50 participants in WHEY and COLL groups[50].

Statistical analyses:

Baseline data are summarized by group means ± standard deviations (SD) unless otherwise stated. Effects of the interventions were investigated within each study arm, separately. The individual treatment effects are reported as the mean change and associated 95% confidence intervals (CI)) during the intervention. Between-treatment effects are reported as mean difference in treatment effect and associated 95% CI. The level of significance was set to <0.05. The effects of the interventions were analysed as a modified intention-to-treat, including all participants that

completed at least one test at the 12-month timepoint, irrespective of adherence to the interventions.

Changes from baseline to 12 months were investigated separately in the supplementation arm and in the training arm of the study, using a longitudinal mixed model with time (baseline and 12 month) and intervention group (three levels) as fixed predictors, including their interaction, and person as random term. Treatment inferences were based on significance test of the interaction term, and further investigated by contrasts of intervention group changes from baseline to 12 months between all pairs (CARB vs COLL vs WHEY, and WHEY vs LITW vs HRTW) of group combinations.

R (version 3.5.1) with the function lm() from the stats package (ver 3.5.1), lmer() from the lme4 package (ver. 1.1-20) and glth() from the multcomp package (ver. 1.4-8) were used for data analysis.

Results

In total, we had 1285 contacts from potential participants of which 1148 were screened via telephone. 280 participants were scheduled for an on-site screening visit of which 39 participants declined to participate. 33 were excluded prior to enrollment in the study. Consort diagram is shown in **Figure 1**. 208 participants were randomized and 184 completed the 12-month tests Characteristics of the included subjects are presented in Table 1. 24 participants dropped out during the study; 11 due to illness or injury unrelated to the intervention, 5 due to disliking the supplement, 3 due to the testing being too extensive, and 5 due to personal reasons.

Compliance

Compliance to training was significantly higher in LITW compared to HRTW ([Median [Interquartile range]], LITW: 89% [77%, 96%]; HRTW: 72% [62%, 78%]; $P < 0.01$) (see **supplemental table 1**).

Supplement compliance did not differ significantly between groups (CARB: 95% [77%, 97%]; COLL: 96% [86%, 99%]; WHEY: 88% [82%, 93%], P=0.11), however, a total of 34 participants failed to report their intake of the supplements throughout the intervention (supplemental table 1). These participants all came to the research facilities to receive additional supplements as planned, but they are not included in the compliance values due to their insufficient reporting of supplement intake.

Protein intake increased for COLL ([mean, 95% CI] +29.0, +21.1 to +36.8 g/day), WHEY (+25.7, +15.6 to +35.8 g/day), LITW (+23.9, +15.2 to +32.5 g/day), and HRTW (+26.7, +18.9 to +34.5 g/day) over the intervention period, while energy intake did not change significantly (COLL: +408, -130 to +947 kJ/day; WHEY: +518, -322 to +1358 kJ/day; LITW: +474, -427 to +1375 kJ/day; HRTW: -41, -707 to +625 kJ/day, (see **supplemental table 2**). Energy intake increased for CARB, with no change in protein intake (Energy: +948, +62 to +1835 kJ/day; Protein: -4.9, -15.8 to +6.1 g/day).

Quadriceps size

In the supplementation arm, we observed no between-group differences in changes in qCSA, (P=0.17, **Figure 2A**). In the training arm, HRTW was associated with a more positive change in qCSA compared to WHEY (Between-group difference [mean, 95% CI]: 1.68, 0.41 to 2.95 cm^2, P=0.03), but not compared to LITW (1.29 cm^2, -0.08 to 2.67 cm^2, P=0.16). Changes in qCSA were not significantly different for LITW compared to WHEY (0.39, -0.88 to 1.66 cm^2, P=0.82). Neither HRTW (0-12 month change: +0.73, -0.32 to +1.77 cm^2) nor LITW (-0.54, -1.70 to +0.62 cm^2) exhibited marked changes in qCSA, whereas a decrease was observed for WHEY (-0.93, -1.65 to -0.21 cm^2).

Lower body strength and power

No between-group differences were observed in the supplementation arm for neither MVIC (P = 0.13, **Figure 2B**) or leg extensor power (P = 0.94, **Figure 2C**). In the training arm, changes in MVIC differed between groups, with HRTW inducing greater gains in MVIC compared to LITW (Between-group difference: 16.8, 6.1 to 27.4 Nm, P = 0.01) and WHEY (23.9, 14.2 to 33.6 Nm, $P< 10^{-5}$). However, changes in MVIC for LITW were not significantly different from WHEY (7.1 Nm, -2.8 to 17.1 Nm, P = 0.34). No between-group differences in changes in leg extensor power were observed within the training arm (P = 0.73).

Functional capabilities

In the supplementation arm, between-group differences were observed in changes in 400 m gait time (P = 0.99, **Figure 2D**), or number of repetitions on the 30 s chair stand test (P = 0.30, **Figure 2E**). In the training arm, changes in 400 m gait times were not significantly different between groups (P = 0.14). However, gait times decreased for HRTW (0-12 months change: -7.8, -15.1 to -0.45 s) and decreased nominally for LITW (-4.7, -9.9 to +0.6 s), with no apparent change in WHEY (+0.1, -5.0 to +5.2 s). Changes in number of repetitions on the 30 s chair stand test did not differ between groups in training arm (P = 0.82).

Body composition

In the supplementation arm, changes in fat percentage (P = 0.95, **Figure 2F**) and LTM (P = 0.29, **Figure 2G**) did not differ between groups. However, in all supplementation groups increases fat percentage were observed (CARB: +0.7, +0.1 to +1.5 percentage points (pp); COLL: +0.6, +0.0 to +1.2pp; WHEY: +0.7, +0.1 to +1.2pp), with no marked changes in LTM (CARB: +0.18, -0.18 to +0.54 kg; COLL: -0.04, -0.32 to +0.25 kg; WHEY: -0.17, -0.48 to +0.14 kg). In the training study, between-group differences in changes in LTM did not reach significance (P = 0.09). Nominal increases in LTM were observed in HRTW (+0.39, -0.01 to +0.79 kg), whereas no apparent change was

observed for LITW (+0.10, -0.33 to +0.54 kg). Between-group differences in changes in fat percentage did also not reach significance in the training arm (P = 0.10).

Discussion

This study investigated the effect of two modifiable strategies to counteract age-related loss of muscle mass in older adults; protein supplementation alone and or combined with resistance exercise. Increasing daily protein intake from ~1.1 $g \cdot kg^{-1}$ to ~1.5 $g \cdot kg^{-1}$ by providing daily protein supplements to healthy home-dwelling older individuals had no beneficial effects in any of the performed measures. These results provide strong evidence that an increase in protein intake does not add a benefit in preserving muscle mass or strength in healthy older adults living independently and eating in accordance with current guidelines. Increasing protein content in an iso-caloric diet has been shown to result in loss of fat mass[24], but in the present study supplementation of any kind was associated with an increase in fat percentage. Although this finding was not controlled against normal eating behavior, gaining fat mass indicate that the older adults in the present study did not adjust energy intake and/or expenditure accordingly when supplemented with extra calories, irrespective of the source of supplemented calories (protein/carbohydrate).

Contrary to our hypothesis, WHEY was not associated with more positive changes in qCSA compared to the COLL or CARB. This finding is surprising and contradicts our hypothesis that supplements with high-quality protein should be superior to lower-quality protein supplements in maintaining muscle mass. In a recent study from Oikawa and colleagues[36], it was found that whey protein supplementation induced greater acute and 6-day integrated muscle protein synthesis compared to collagen supplementation in healthy older women. While these findings are contradictory, it should be noted that acute changes in muscle protein synthesis are not well

correlated with long-term changes in muscle mass[62]. Thus, while whey protein supplementation might increase muscle protein turnover to a greater extent than collagen protein supplementation, the present results indicate that this has no functional long-term effect in healthy older adults.

The impact of resistance exercise on top of whey supplementation was also investigated. The effects of LITW were sparse and inferior to those of HRTW, despite the higher compliance to LITW. While HRTW was effective in increasing muscle strength and the increments in MVIC were comparable to what has been previously observed[43,63–65], the lack of change in muscle mass was unexpected. Surprisingly, 1 year of supervised resistance training did not elicit significant increases in qCSA, which have been shown in several studies reporting 5-10% increments in qCSA after 3-4 months of training[66–68]. However, a number of other studies have also struggled to induce muscle hypertrophy in older adults[69–73]. In the present study, median training compliance corresponded to an average of ~2 training sessions per week in HRTW, which has been shown previously to induce hypertrophy in older adults[74]. However, during the present study, most participants went on vacation for 3-4 weeks during the intervention, causing prolonged breaks from training. These breaks from training are likely to attenuate the increases in muscle size, and thus could potentially explain the insignificant hypertrophy observed in the present results. Compared to the very intense 3-4 month training studies previously reported[66–68], we suggest that the present results are more realistic estimates of the effects when recommending older adults to complete resistance training for prolonged periods of time.

While our statistical analysis revealed no between-group differences in changes in functional capabilities, it should be noted that we observed that HRTW improved 400 m gait times. The 400

297 m gait test has previously been shown to be a strong predictor of both functional capabilities and
298 risk of future mobility limitations in healthy older adults[55]. Furthermore, we have previously
299 shown that strength is a good predictor of functional capabilities in our cohort of older adults[57].
300 Albeit speculative in relation to the present results, our findings suggest that heavy resistance
301 exercise is capable of improving functional capacity even in active older adults.

302 *Limitations*

303 We recruited well-functioning home-dwelling healthy older adults with a rather active lifestyle. As
304 a group, they were well-nourished and ingested on average above current RDA of protein in their
305 habitual diet[60]. Hence, the present data cannot be extrapolated to other, more frail elderly people
306 and/or some eating less energy/protein in their normal diet.

307 Our study did not include training groups not receiving protein supplementation. Therefore, the
308 obtained results in the training groups therefore may not be solely attributed to the training per
309 se, and any interaction between protein supplementation and resistance training cannot be
310 derived from the present study. However, while protein supplementation has been shown to be
311 effective in improving adaptations to resistance training in young individuals[44], the additive effects
312 seem to be minor in older adults[44,75].

313 *Conclusion*

314 This 1-year intervention study does not support the hypothesis that protein supplementation
315 benefits preservation of muscle mass and strength in healthy older adults already reaching daily
316 protein intakes of >1.0 g protein·kg^{-1}·day^{-1}. Despite seemingly higher compliance, the addition of
317 light resistance home-based training is not as effective as heavy load resistance training in
318 increasing strength and function. Future research and innovation efforts should focus on

improving long-term compliance to heavy resistance exercise in healthy older adults to obtain greater muscular benefits.

Acknowledgements

Author contributions: Mertz and Rasmussen had full access to all the data in the present study, and take responsibility for the integrity of the data analysis. *Study design:* Reitelseder, Bechshoeft, Højfeldt, Nielsen, Engelsen, Jespersen, Lind, Mikkelsen, and Holm. *Acquisition of data:* Mertz, Reitelseder, Bechshoeft, Bulow, Højfeldt, and Jensen. *Analysis and interpretation of data:* Mertz, Schacht, Tetens, Rasmussen, and Holm. *Drafting of manuscript*: Mertz and Holm. All authors critically revised the manuscript and had the opportunity to comment on the interpretation of results. All have approved the final version. *Statistical analysis:* Rasmussen. Obtained funding: *Holm, Jespersen, Nielsen, Engelsen.*

Funding: This work received funding from the University of Copenhagen Excellence Program for Interdisciplinary Research, the Danish Dairy Research Foundation, P. Carl Petersen´ Fund, Region H research fund, Arla Foods Ingredients P/S, and Innovation Foundation Denmark.

Role of the Sponsor: Arla Foods Ingredients P/S funded part of the study. Mikkelsen, employee at Arla Foods Ingredients was involved in study design, but was not involved in data acquisition or analysis.

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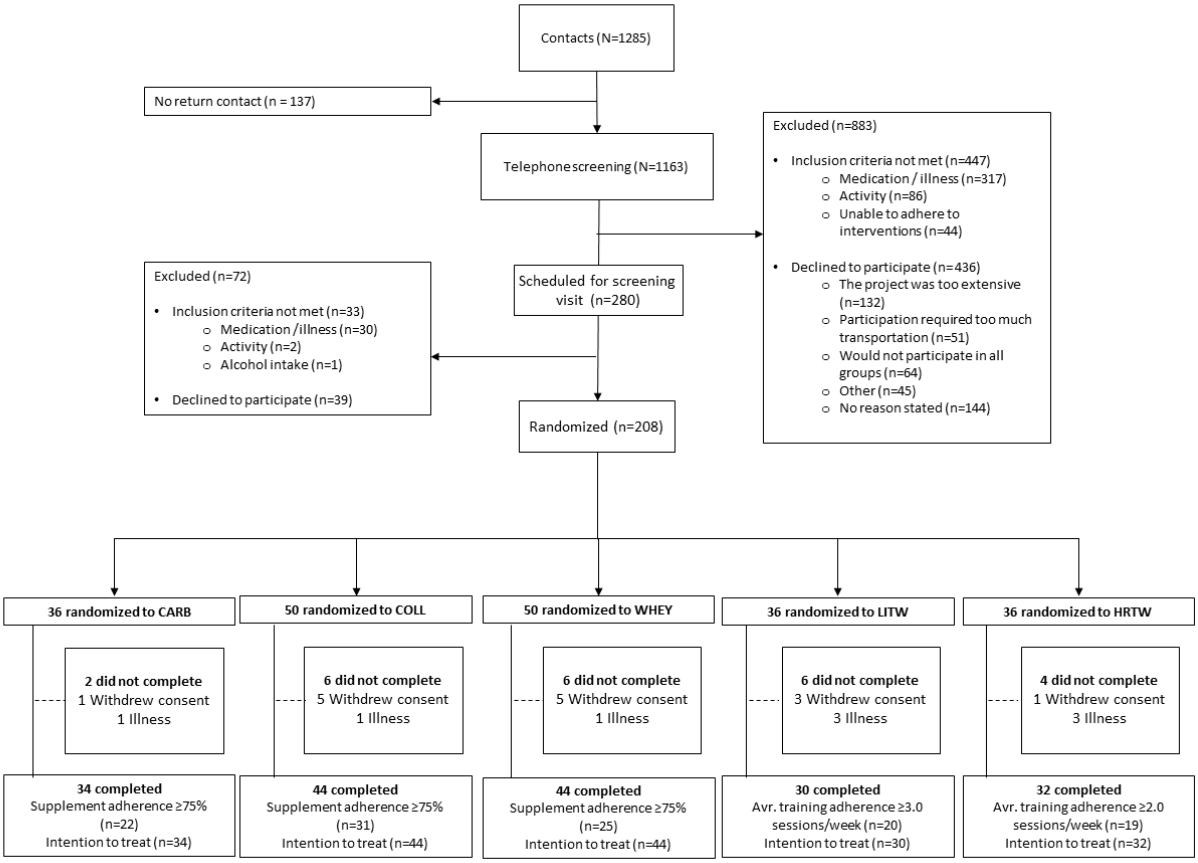
577 FIGURES



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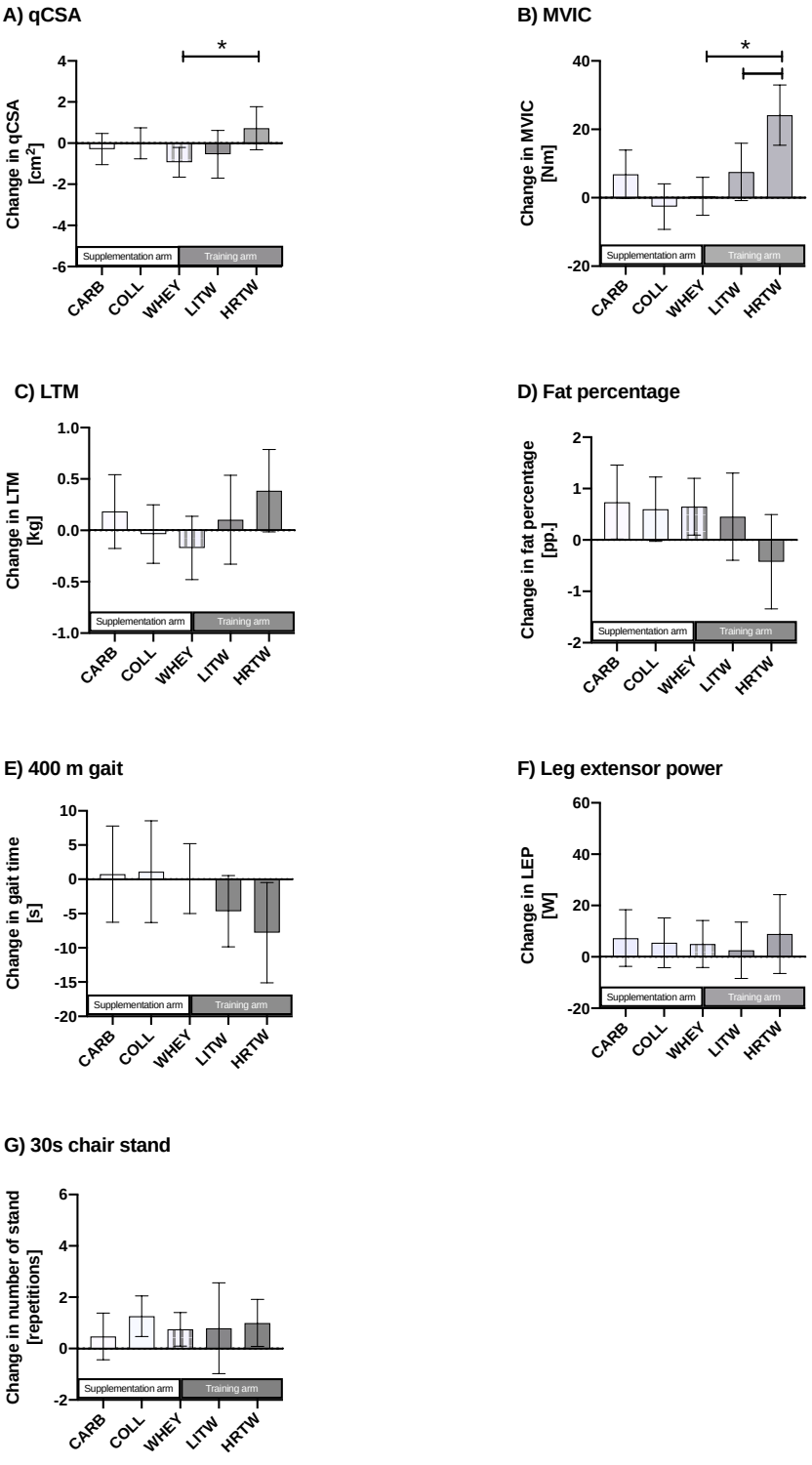
579 Figure 1: **CONSORT diagram showing the flow of participants in the CALM trial.**

580 CARB: Carbohydrate supplementation; COLL: Collagen protein supplementation; WHEY: Whey

581 protein supplementation; LITW: Light-intensity training with whey protein supplementation;

582 HRTW: Heavy resistance training with whey protein supplementation.



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584 Figure 2: **Changes in muscle size, strength and function over the intervention period.**

Changes from baseline to 12 months in A) m. quadriceps cross-sectional area (qCSA). B) Knee extensor maximal voluntary isometric contraction (MVIC) C) Lean tissue mass (LTM). D) Fat percentage. E) 400 m gait time. F) Leg extensor power. G) Reps on the 30-s chair stand test. Results are shown as mean changes [± 95% CI] from baseline to 12 months of intervention. *: Significant between-group difference in changes over the intervention period. CARB: Carbohydrate supplementation; COLL: Collagen protein supplementation; WHEY: Whey protein supplementation; LITW: Light-intensity training with whey protein supplementation; HRTW: Heavy resistance training with whey protein supplementation.

Table 1. Baseline characteristics of the included participants by group.

	CARB	COLL	WHEY	LITW	HRTW
Variable	(n = 36)	(n = 50)	(n = 50)	(n = 36)	(n = 36)
Demographics, Mean (SD)					
Age, y	69.6 (3.9)	70.4 (4.1	70.3 (4.3)	70.4 (4.0)	70.3 (3.1)
BMI, kg/m^2	26.0 (3.9)	25.4 (6.0)	25.2 (3.6)	25.7 (3.1)	25.9 (3.5)
Daily activity, Steps/day	10894 (5165)	10590 (3996)	10118 (3590)	10119 (3450)	9777 (3574)
Protein intake, g/kg/day	1.2 (0.3)	1.2 (0.4)	1.1 (0.3)	1.0 (0.3)	1.1 (0.4)
Energy intake, kJ/day	8442 (1804)	8150 (1952)	8529 (2092)	7445 (2220)	8268 (2146)
Body Composition					
Lean tissue mass, kg	48.5 (7.8)	49.2 (8.6)	50.0 (8.5)	48.1 (9.3)	48.8 (9.9)
Fat percentage, %	33.2 (9.3)	32.0 (9.1)	32.7 (7.5)	34.3 (7.5)	34.7 (7.1)
Quadriceps size, cm^2	56.6 (11.3)	56.0 (13.9)	54.5 (11.0)	56.7 (11.4)	55.4 (13.1)
Strength and function					
400 m gait time, s	248 (42)	243 (38)	242 (30)	242 (30)	251 (27)
30 s chair stand, reps	19.9 (5.7)	20.1 (5.3)	19.4 (4.6)	20.1 (4.6)	18.9 (4.9)
Leg extensor power, W	183.1 (56.2)	191.2 (67.2)	189.6 (59.6)	190.8 (61.4)	194.2 (65.8)
MVIC, Nm	158.9 (41.1)	169.0 (53.4)	177.6 (47.0)	171.5 (44.4)	165.0 (50.8)

SF-36					
MCS	59.3 (3.2)	57.3 (4.3)	57.6 (3.6)	57.1 (4.7)	57.5 (4.4)
PCS	55.3 (4.7)	56.0 (4.7)	56.8 (3.1)	56.4 (4.0)	56.5 (4.2)
Laboratory data					
Hba1c, mmol/mol	36.0 (2.2)	35.8 (3.4)	36.2 (3.5)	35.8 (2.9)	35.8 (2.7)
Total cholesterol, mmol/l	5.6 (0.9)	5.7 (1.0)	6.0 (1.2)	5.5 (1.0)	5.8 (0.9)
HDL Cholesterol, mmol/l	1.9 (0.5)	2.0 (0.6)	1.8 (0.5)	1.8 (0.5)	1.8 (0.5)
LDL Cholesterol, mmol/l	3.1 (0.8)	3.2 (1.0)	3.4 (0.9)	3.0 (1.0)	3.4 (1.0)
Triglycerides, mmol/l	1.3 (0.6)	1.4 (0.8)	1.7 (0.8)*	1.4 (0.6)	1.4 (0.6)
Creatinine, µmol/l	76.8 (14.7)	81.4 (15.9)	80.5 (11.6)	78.8 (14.7)	77.0 (12.7)

Supplemental table 1. Overview of compliance to interventions.

	CARB		COLL		WHEY		LITW		HRTW	
	ITT	**PP**	**ITT**	**PP**	**ITT**	**PP**	**ITT**	**PP**	**ITT**	**PP**
Training compliance (Median [IQR])	-	-	-	-	-	-	89% [77%, 96%]	94% [88%, 97%]	72% [62%, 78%]	78% [75%, 82%]
Supplement compliance (Median [IQR])	95% [77%, 97%]	96% [89%, 98%]	96% [86%, 99%]	96% [86%, 99%]	88% [82%, 93%]	90% [85%, 96%]	90% [77%, 94%]	93% [85%, 100%]	87% [79%, 97%]	94% [87%, 98%]
Supplement non-reporters (n=)	7		11		14		1		1	
Drop outs (n=)	2		6		6		6		4	
Included subjects (n=)	34	22	44	31	44	25	30	20	32	19

Participants were included in per protocol analysis if supplement compliance exceeded 75%, and training compliance exceeded 75% for LITW and 66% for HRTW. ITT: Intention -to-treat analysis. PP: Per protocol analysis. CARB: Carbohydrate supplementation. COLL: Collagen protein supplementation. WHEY: Whey protein supplementation. LITW: Light intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation.

Supplemental table 2. Changes from 0 to 12 months in Intention-to-treat analysis and per protocol analysis

	CARB		COLL		WHEY		LITW		HRTW	
	ITT	PP	ITT	PP	ITT	PP	ITT	PP	ITT	PP
Changes from 0-12m	(n = 34)	(n=22)	(n = 44)	(n=31)	(n = 44)	(n=25)	(n = 36)	(n=20)	(n = 36)	(n=19)
Demographics, Mean (SE)										
Daily activity, Steps/day	-1662 (896)	434 (670)	330 (589)	-132 (716)	-91 (554)	-267 (823)	-322 (582)	113 (536)	-368 (411)	-381 (403)
Protein intake, g/day	-4.9 (5.3)	3.9 (5.9)	29.0 (3.9)*	27.2 (4.5)*	25.7 (5.0)*	31.4 (6.3)*	23.8 (4.2)	26.9 (4.7)	26.7 (3.8)	34.6 (4.0)
Protein intake excluding supplement, g/day	-4.9 (5.3)	3.9 (5.9)	-8.3 (3.6)	-9.8 (4.2)	-6.4 (4.3)	-5.0 (6.1)	-9.6 (3.9)	-9.8 (4.7)	-5.8 (3.2)	-2.3 (4.3)
Energy intake, kJ/day	948 (428)	865.9 (474)	408 (266)	343 (313)	517 (413)	900 (608)	474 (437)	874 (551)	-41 (324)	348 (418)
Energy intake excluding supplement, g/day	-81 (425)	-196 (466)	-649 (260)	-703 (304)	-389 (397)	-130 (603)	-472 (427)	-161 (550)	-961 (315)	-695 (431)
Body Composition										
Fat free mass, kg	0.2 (0.2)	0.4 (0.2)	0.0 (0.1)	-0.1 (0.2)	-0.2 (0.2)	-0.1 (0.2)	0.1 (0.2)	0.2 (0.3)	0.4 (0.2)	0.6 (0.3)
Fat percentage, %	0.7 (0.4)	0.7 (0.3)	0.6 (0.3)	0.6 (0.4)	0.7 (0.3)	0.6 (0.3)	0.5 (0.4)	0.5 (0.5)	-0.4 (0.5)	-0.8 (0.7)
Quadriceps size, cm^2	-0.3 (0.4)	-0.1 (0.5)	0.0 (0.4)	-0.1 (0.4)	-0.9 (0.4)	-1.1 (0.4)	-0.5 (0.6)	-0.2 (0.5)	0.7 (0.5) &	0.8 (0.7)
Strength and function										
400 m gait time, s	0.8 (3.5)	0.5 (2.9)	1.1 (3.7)	5.5 (4.6)	0.11 (2.52)	-4.48 (3.18)	-4.66 (2.55)	-6.79 (3.00)	-7.78 (3.59)	-13.32 (2.94)
30 s chair stand, reps	0.5 (0.5)	0.5 (0.6)	1.3 (0.4)	1.3 (0.5)	0.8 (0.3)	1.0 (0.4)	0.8 (0.9)	1.1 (1.3)	1.0 (0.4)	0.8 (0.3)
Leg extensor power, W	7.3 (5.4)	8.0 (6.9)	5.5 (4.8)	5.7 (6.2)	5.0 (4.6)	12.5 (6.2)	2.6 (5.4)	2.8 (6.3)	8.9 (7.5)	10.7 (10.9)
MVIC, Nm	6.9 (3.5)	10.5 (3.5)	-2.6 (3.3)	1.1 (3.8)	0.4 (2.8)	0.5 (3.3)	7.5 (4.1)	8.7 (4.8)	24.1 (4.3) &, §	29.4 (6.1) &,§
Laboratory data										
Hba1c, mmol/mol	1.06 (0.33)	1.52 (0.42)	0.98 (0.40)	1.03 (0.50)	0.16 (0.33)	0.48 (0.44)	1.27 (0.47)	1.10 (0.51)	0.50 (0.35)	0.42 (0.51)

Total cholesterol, mmol/l	-0.38 (0.12)	-0.41 (0.17)	-0.57 (0.11)	-0.74 (0.12)	-0.54 (0.10)	-0.67 (0.13)	-0.62 (0.09)	-0.69 (0.12)	-0.59 (0.1)	-0.58 (0.14)
HDL Cholesterol, mmol/l	-0.12 (0.05)	-0.17 (0.06)	-0.21 (0.03)	-0.23 (0.04)	-0.14 (0.05)	-0.22 (0.07)	-0.20 (0.05)	-0.20 (0.06)	-0.10 (0.05)	-0.10 (0.06)
LDL Cholesterol, mmol/l	-0.14 (0.10)	-0.12 (0.15)	-0.23 (0.12)	-0.36 (0.13)	-0.16 (0.08)	-0.25 (0.1)	-0.28 (0.08)	-0.34 (0.12)	-0.33 (0.09)	-0.28 (0.15)
Triglycerides, mmol/l	-0.23 (0.07)	-0.24 (0.10)	-0.40 (0.08)	-0.41 (0.10)	-0.52 (0.08) *	-0.43 (0.09)	-0.29 (0.09)	-0.28 (0.12)	-0.39 (0.09)	-0.47 (0.11)
Creatinine, µmol/l	3.71 (1.41)	4.14 (1.88)	3.37 (1.17)	2.19 (1.24)	-0.41 (1.07) $	-0.96 (1.25)	0.87 (1.20)	-0.35 (1.63)	2.50 (1.13)	0.47 (1.09)

*	P < 0.05 vs CARB
$	P < 0.05 vs COLL
&	P < 0.05 vs WHEY
§	P < 0.05 vs LITW

Participants were included in per protocol analysis if supplement compliance exceeded 75%, and training compliance exceeded 75% for LITW and 66% for HRTW. ITT: Intention-to-treat analysis. PP: Per protocol analysis. CARB: Carbohydrate supplementation. COLL: Collagen protein supplementation. WHEY: Whey protein supplementation. LITW: Light intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation.

GRADUATE SCHOOL OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

PHD-THESIS
DECLARATION OF CO-AUTHORSHIP

The declaration is for PhD students and must be completed for each conjointly authored article. Please note that if a manuscript or published paper has ten or less co-authors, all co-authors must sign the declaration of co-authorship. If it has more than ten co-authors, declarations of co-authorship from the corresponding author(s), the senior author and the principal supervisor (if relevant) are a minimum requirement.

1. Declaration by	
Name of PhD student	Kenneth Hudlebusch Mertz
E-mail	khudlemertz@gmail.com
Name of principal supervisor	Michael Kjær
Title of the PhD thesis	Preservation of muscle mass and function through protein supplementation and exercise

2. The declaration applies to the following article	
Title of article	The effect of daily protein supplementation with or without resistance training for 1 year on muscle size, strength and function in healthy older adults. A randomized controlled trial
Article status	
Published ☐ Date:	Accepted for publication ☐ Date:
Manuscript submitted ☒ Date: March 6th 2020	Manuscript not submitted ☐
If the article is published or accepted for publication, please state the name of journal, year, volume, page and DOI (if you have the information).	

3. The PhD student's contribution to the article *(please use the scale A-F as benchmark)* Benchmark scale of the PhD-student's contribution to the article **A.** Has essentially done all the work (> 90 %) **B.** Has done most of the work (60-90 %) **C.** Has contributed considerably (30-60 %) **D.** Has contributed (10-30 %) **E.** No or little contribution (<10 %) **F.** Not relevant	**A, B, C, D, E, F**
1. Formulation/identification of the scientific problem	E
2. Development of the key methods	D
3. Planning of the experiments and methodology design and development	E
4. Conducting the experimental work/clinical studies/data collection/obtaining access to data	B
5. Conducting the analysis of data	B
6. Interpretation of the results	B
7. Writing of the first draft of the manuscript	A
8. Finalisation of the manuscript and submission	A
Provide a short description of the PhD student's specific contribution to the article.[1] Performed most of the data collection in relation to the article, as well as analysis of data. Wrote the first draft for the article and finalised it after receiving comments from co-authors.	

Latest update of the declaration: December 2018

4. Material from another thesis / dissertation[II]	
Does the article contain work which has also formed part of another thesis, e.g. master's thesis, PhD thesis or doctoral dissertation (the PhD student's or another person's)?	Yes: ☒ No: ☐
If yes, please state name of the author and title of thesis / dissertation.	Jacob Bülow, "The ageing skeletal muscle: Effects of training and protein supplementation"
If the article is part of another author's academic degree, please describe the PhD student's and the author's contributions to the article so that the individual contributions are clearly distinguishable from one another.	The PhD student performed most of the experimental work related to the article and wrote the first draft for the article. Jacob Bülow was responsible for daily management of the study, and was involved in data collection as well as contributed to the interpretation of the results.

5. Signatures of the co-authors[III]

	Date	Name	Title	Signature
1.	29/4/20	LARS HOLM	PROF	
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				

6. Signature of the principal supervisor

I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge.
Date: 28/4 2020
Principal supervisor:

7. Signature of the PhD student

I solemnly declare that the information provided in this declaration is accurate to the best of my knowledge.
Date: 27/4-2020
PhD student: Kenneth Hudlebusch Mertz

Please learn more about responsible conduct of research on the Faculty of Health and Medical Sciences' website.

[i] This can be supplemented with an additional letter if needed.
[ii] Please see Ministerial Order on the PhD Programme at the Universities and Certain Higher Artistic Educational Institutions (PhD Order) § 12 (4):
"Any articles included in the thesis may be written in cooperation with others, provided that each of the co-authors submits a written declaration stating the PhD student's or the author's contribution to the work."
[iii] If more signatures are needed please add an extra sheet.

15. Appendix III – Paper 3

"Temporal changes in muscle mass, strength and function in older adults during and after a center-based or home-based resistance training intervention. The CALM trial"

Kenneth H. Mertz, Søren Reitelseder, Morten A. Rasmussen, Jacob Bülow, Grith Højfeldt, Mikkel Jensen, Morten Hjulmand, Jonas Lindberg, Mathilde U. Kramer, Rasmus Bechshoeft, Lars Holm.

(In preparation).

Temporal changes in muscle size and strength in older adults during after a center-based or home-based resistance training intervention.

The CALM randomized controlled intervention trial

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ABSTRACT

Purpose: Heavy resistance training seems to be the most effective method of preventing age-related loss of muscle mass and strength in older adults. However, as such a training regimen is associated with a need for transportation and specialized equipment, long-term adherence to such a training method might be limited in older adults. The present study investigated the temporal changes in muscle mass and strength in during and after a light-load, home-based resistance training intervention, compared to center-based heavy resistance training.

Methods: 64 healthy older adults completed one of three 1-year interventions: 1) Heavy resistance training with whey protein supplementation (HRTW). 2) Home-based light-intensity training with whey protein supplementation (LITW). 3) No training with whey protein supplementation (NOTW). Knee extensor strength isometric strength (MVIC), dynamic peak torque, and rate of force development (RFD) as well as body composition and activity levels were measured after 6 and 12 months of intervention as well as 6 months after the intervention had ended.

Results: Both LITW and HRTW improved strength MVIC and dynamic peak torque during the first 6 months of the intervention, but only HRTW was associated with continued improvements from 6-12 months. Only HRTW was associated with significant improvements in RFD during the intervention. While both LITW and HRTW had higher activity levels 6 months after the intervention compared to NOTW, only HRTW preserved strength and RFD above pre-training levels.

Conclusion: The present study shows that while both LITW and HRTW are capable of increasing muscle strength, adaptations to LITW seem to plateau after 6 months and were not preserved 6 months after the intervention had ended. Therefore, HRTW seems to be the most effective long-term training strategy for older adults.

INTRODUCTION

Muscle mass and strength are lost progressively from around the fifth decade of life[1] and constitutes a risk factor for developing functional limitations, frailty and loss of independence[2,3]. Heavy resistance training seems to be the most effective way of increasing muscle mass and strength, as we (Mertz et al, *Unpublished*), and others have shown[4,5]. In older adults conduction of heavy resistance training for short-term (8-24 weeks) periods results in gains in muscle mass, strength and function[6,7,16,8–15]. However, following a heavy resistance training program is also associated with a demand for transportation as well as economical costs, which might limit prolonged training adherence and especially continuation once guidance, surveillance and supervision are ceased and people are left on their own[17,18]. Adherence to and continuation of training modalities where challenges with transportation and economy are absent, e.g. home-based settings, therefore may be higher. As lighter training modalities have also been shown capable of inducing improvements in muscle mass and strength[14,19,20], such modalities and settings may be a better recommendation for obtaining sustained effects over prolonged periods of time. However, to these author´s knowledge, no studies have described the temporal changes in muscle mass and strength to lighter-load, home-based resistance training in older adults. For heavy resistance training, longer training interventions generally elicit greater improvements in strength than shorter interventions[4,21], with rapid adaptations in muscle mass and strength occurring within the first 3-4 weeks of training[22–25]. It could be speculated that lighter-load, home-based training intervention could elicit short-term increases in muscle mass and strength but fail to elicit long-term improvements due to insufficient progressive loading. However, a more tolerable exercise modality and feasible setting may result in higher degrees of exercise continuation and result in higher overall activity levels, both being important factors in preserving the adaptations from the training intervention[26]

The aim of the present study was therefore to investigate temporal changes in muscle mass and strength during and after a light load home-based training intervention, compared to no training or center-based heavy resistance training. We hypothesized that while heavy resistance training would be the most beneficial during a 1-year intervention period, light-load training would be associated with better preservation of muscle mass, strength and function 6 months after cessation of the intervention.

METHODS

This study reports secondary per protocol analyses on three out of five intervention groups of a large investigation[27], which aimed to study the effect of protein supplementation with or without resistance training on muscle size, strength and function in older adults (Clinicaltrials.gov identifier: NCT02034760).

Participants

122 healthy older adults (65+ years) were recruited for a 1-year randomized controlled trial, involving protein supplementation alone or combined with resistance training. To be included in the study, potential participants were not allowed to do more than 1 hour of weekly resistance training but were allowed to do other types of exercise. Prior to enrollment in the study, all participants were screened, excluding participants with medical conditions potentially preventing them from completing the 1-year intervention safely. A full overview of the exclusion criteria for the main study can be found elsewhere[27]. 64 of the 122 participants fulfilled the requirements for being included in the per protocol (PP) analysis (for details see supplemental flow chart). Briefly, the requirements for the PP analysis were: protein supplementation adherence >75%, and training adherence >75% for LITW (3 out 4 of average weekly sessions) and >66% for HRTW (on average 2 out of 3 weekly sessions). All participants gave written consent in accordance with the declaration of Helsinki II, and the study was approved by the Danish Regional Ethics Committee of the Capital Region (H4-2013-070).

Training intervention and follow up

Participants were randomized to either center-based heavy resistance training with whey protein supplementation (HRTW), home-based light intensity training with whey protein supplementation (LITW), or no training with whey protein supplementation (NOTW).

The interventions are described in detail elsewhere[27]. Briefly, HRTW performed heavy resistance training 3 times weekly under supervision of trained personnel. Training exercises were mainly focused on the lower extremities (leg press, leg extension, leg curl), but also involved upper body exercises (pull-down, push up). Training intensity was progressively increased from 3 sets of 12 repetitions at 12 repetition max (RM) to 5 sets of 6 repetitions at 6 RM over a 3-month training cycle. This cycle was then repeated 4 times over the intervention period. Compliance to the training was monitored by the supervising personnel.

LITW performed home-based training using bodyweight and rubber bands as resistance. The training program consisted of 5 exercises, all chosen to mimic the movements in HRTW. Training was performed 3-5 times weekly (4 times on average) and was supervised monthly by trained personnel to ensure optimal exercise performance, technique and loading. Compliance to training noted by the participants in a hard-copy diary.

All groups received a whey protein supplement (20 g hydrolysed whey and 10 g sucrose, Arla Foods Ingredients P/S, Viby J, Denmark) twice daily. Participants were encouraged to take the supplement in the morning and at midday, preferable just before a meal. On training days, HRTW and LITW were encouraged to ingest their midday-supplement just after a training session. Adherence to the protein supplements was noted by the participants in hard-copy diaries.

After the 12 months of intervention had ended, participants were not allowed to continue using the training facilities on the hospital and did not receive additional rubber bands for continuation of training. However, the participants were encouraged to continue training until the 18-month follow-up measurements.

Physical performance assessment

Detailed descriptions of the strength and power testing protocols can be found elsewhere[28]. Briefly, all physical performance tests were performed on the same day the same experienced researcher, in the order described below. Measurements of muscle size and body composition were performed on separate days. All measurements were performed at baseline, midway through the intervention (6 months), at the end of the intervention (12 months), and 6 months after the end of the intervention (18 months). Prior to all tests, participants refrained from training and strenuous activities for 48 hours. All strength and power tests were performed on the self-reported dominant limb of the participant. Limb dominance was determined by asking the participant which leg and hand they felt was the strongest.

400 m gait test was used as a marker of overall functional capabilities[29]. Participants were instructed to walk 400 m as fast as possible, without running, on a 20 m track. Results are shown as time to complete 400 m.

Leg extensor power (LEP) was assessed using the Nottingham Power Rig (Queens Medical Center, Nottingham University, UK)[30]. Participant received two warm up trials, followed by as many trials as needed to find the maximal LEP. The test ended when the participant had two consecutive trials below the peak value, after a minimum of 5 trials. The highest LEP was used for further analysis.

Grip strength was assessed using a hand grip dynamometer (DHD-1 [SH1001], SAEHAN Corporation, Changwon City, South Korea), and was used as a marker of upper body strength. Participants were seated in a chair with their forearm on the armrest. Participants performed as many trials as needed to obtain the maximal grip strength of the self-reported dominant hand. The test ended when the participant had one trial below the peak value, after a minimum of 3 trials.

Dynamic Peak Torque and Maximal Voluntary Isometric Contraction (MVIC) of the knee extensors were assessed in an isokinetic dynamometer (Kinetic Communicator, Model 500-11). Dynamic peak torque was measured at a contraction velocity of 60°/s at a knee joint range of motion from 90° to 10° flexion (0° equals full extension). Participants received 3 warm up trials at submaximal effort, followed by as many trials as needed to reach peak knee extensor torque. The test ended when the participant had one trial below the peak value after a minimum of 4 trials. MVIC was then measured at a knee angle of 70° flexion. Participants were instructed to contract as hard and fast as possible for 4 s. 3 maximal attempts was then performed, and the highest attained peak torque was used for further analysis. Attempts with any initial countermovement were disqualified, and a new attempt was performed.

Rate of Force Development (RFD) was measured at as the average force development from 0-200 ms after onset of contraction in the MVIC measurements. The attempt with the highest RFD was used for further analysis.

Muscle size and body composition

Quadriceps cross-sectional area (qCSA) was assessed using magnetic resonance imaging as described elsewhere[27]. Briefly, images were obtained 20.4 cm proximal of the tibia plateau and were then analyzed by the same blinded investigator.

Appendicular lean tissue mass (aLTM) and total fatmass was measured using dual energy x-ray absorptiometry (Lundar iDXA, GE Medical Systems). Measurements were performed in the morning, in the overnight fasting state at 0 and 12 months. Due to logistical reasons, scans at 6 and 18 months were performed at noon, and not in the fasting state. aLTM was measured as the sum of LTM in the arms and legs as previously described[28]

Daily step counts were measured using accelerometer-based activity monitors (activPal 3^{TM}, activPal 3c^{TM}, or activPal micro; PAL technologies, Glasgow, UK), and was used as a marker of overall activity levels. The monitor was worn for 96 hours, always covering a full weekend. Average daily number of daily steps was used for further analysis.

Statistical analysis

Baseline data are summarized as mean ± SD for data normally distributed data. Data not normally distributed are summarized as median [Interquartile intervals]. Changes over the intervention period and follow up (6, 12, and 18 months) were investigated using mixed-model analysis on delta values compared to baseline (Δ0-6 months, Δ0-12 months, Δ0-18 months). If the group*time interaction term was significant ($P<0.05$), we performed one-way ANOVA analysis between groups at all timepoints and subsequent

between-groups contrast analysis. Time course and preservation of adaptions were investigated using contrast analysis between timepoints within groups (0 vs 6 months, 6 vs 12 months, 12 vs 18 months, and 0 vs 18 months), but only if the group*time interaction term was significant. Statistical analysis was performed in R (version 3.5.1) with the lme4 package (version 1.1-20) installed.

RESULTS

Baseline characteristics of the included participants are shown in table 1. Note that some results from 0 and 12-month timepoints have been reported previously.

	NOTW			LITW			HRTW			P-value
N (Male/female)	25 (12/13)			20 (10/10)			19 (10/9)			
Age	69.9	±	3.8	70.3	±	3.8	70.2	±	3.4	0.93
Body composition										
Weight (kg)	73.4	±	15.0	72.7	±	9.1	76.5	±	14.4	0.65
Height (m)	1.73	±	0.09	1.70	±	0.07	1.71	±	0.08	0.52
BMI (kg/m^2)	24.5	±	3.8	25.1	±	3.35	26.0	±	3.8	0.42
aLTM (kg)	22.6	±	5.2	21.4	±	4.1	22.9	±	5.6	0.61
Fatmass (kg)	24.8	±	6.6	24.1	±	6.4	22.9	±	8.3	0.67
qCSA (cm^2)	55.1	±	12.7	56.2	±	12.5	57.6	±	14.2	0.83
Strength and function										
MVIC (Nm)	184.0	±	49.9	175.4	±	43.0	170.9	±	55.8	0.68
Dynamic peak torque (Nm)	158.9	±	45.6	149.6	±	39.2	156.8	±	47.5	0.78
RFD (Nm/s)	670.5	±	293.7	634.6	±	200.6	624.0	±	234.9	0.81
LEP (W)	183.6	±	61.0	184.6	±	64.6	206.2	±	67.3	0.45
Grip strength (kg)	36.1	±	11.8	36.1	±	9.5	40.8	±	13.4	0.35
400 m gait time (s)	236.0	±	24.5	237.9	±	34.8	251.4	±	29.4	0.20
Activity										
Steps per day	10774	±	3557	10324	±	3444	9652	±	4241	0.62
Diet										
Protein intake (g/kgBW)	1.1	±	0.3	1.1	±	0.3	1.1	±	0.3	0.68
Energy intake (kJ)	8470	±	1951	7354	±	2017	8252	±	2135	0.21

Table 10. Baseline characteristics of the participants included in analysis. BMI: Body mass index. aLTM: Appendicular lean tissue mass. qCSA: Quadriceps cross-sectional area. MVIC: Maximal voluntary isometric contraction. RFD: Rate of force development. LEP: Leg extensor power. NOTW: No training with whey. LITW: Home-based light-intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation. P-value indicates the results of a 1-way ANOVA.

All participants included in the analysis returned for follow-up measures at 18 months, except one participant in the LITW group. Mean compliance to training differed significantly between training groups (HRTW: 77 ± 6%, LITW: 91 ± 7%, $P < 0.001$, figure 1). Training compliance was significantly lower in the second half of the intervention for HRTW (0-6 months: 81 ± 6 %, 6-12 months: 74 ± 8 %, $P = 0.01$), but not for LITW (0-6 months: 92 ± 8 %, 6-12 months: 89 ± 7%, $P = 0.80$).

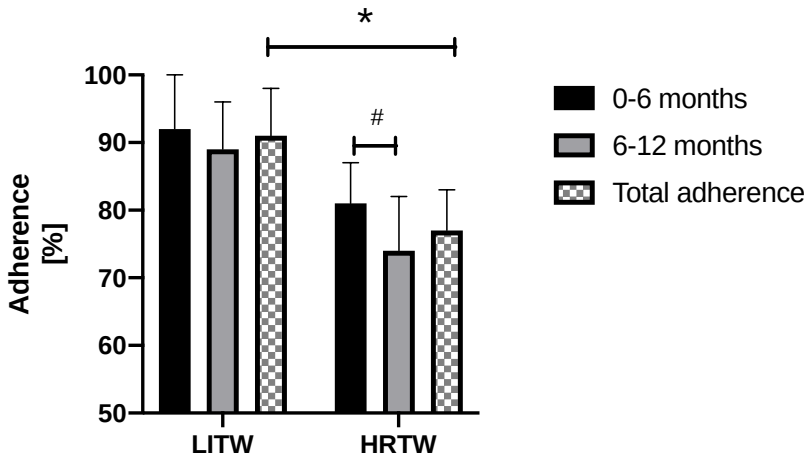


*Figure 17. Adherence to training in the training groups. LITW: Home-based light-intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation. *: significant between-group difference. #: significant difference between timepoints.*

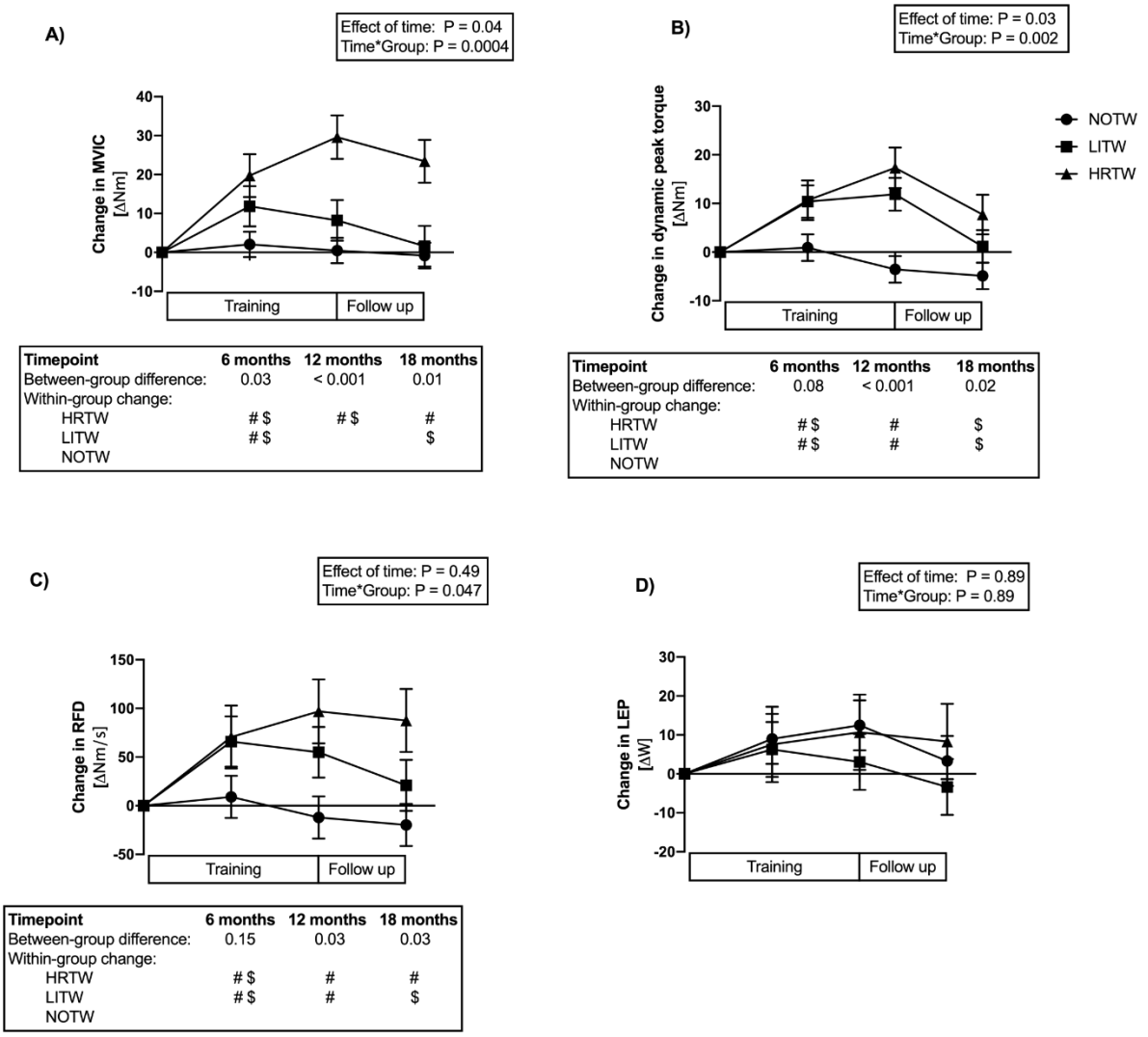
Strength and power:

A significant time*group interaction was observed for MVIC (P = 0.0004, figure 2A), and one-way anova analysis revealed between-group differences at all timepoints (6 months: P = 0.03, 12 months: P <0.001, 18 months: P = 0.01). Contrast analysis revealed that HRTW increased MVIC compared to NOTW at all timepoints (6 months: +17.6 ± 6.3 Nm, P = 0.006; 12 months: +28.9 ± 6.5 Nm, P = $4 \cdot 10^{-5}$, 18 months: +24.2 ± 6.8 Nm, P = 0.0008). LITW increased MVIC less than HRTW at 12 months (-20.7 ± 6.9 Nm, P = 0.004), and 18 months (-21.4 ± 7.2 Nm, P = 0.004), but no difference was observed at 6 months (-7.9 ± 6.5 Nm, P = 0.23). LITW did not increase MVIC significantly at any timepoint compared to NOTW (6 months: + 9.8 ± 6.2 Nm, P = 0.12; 12 months: +8.2 ± 6.4 Nm, P = 0.21; 18 months: + 2.9 ± 6.8 Nm, P = 0.68). Investigating the changes over time within HRTW, MVIC increased during the first 6 months (+19.7 ± 5.5 Nm, P = 0.001), and was further increased from 6-12 months (+ 9.9 ± 4.7 Nm, P = 0.04). At 18 months, MVIC was not significantly different from 12 months (-6.2 ± 4.7 Nm, P = 0.19), and still elevated compared to 0 months (+23.4 ± 5.5 Nm, P = 0.0002). LITW increased MVIC at 6 months (+11.8 ± 5.2, P = 0.03), but did not change from 6-12 months (-3.6 ± 3.1 Nm, P = 0.26). At 18 months, MVIC was not different from 0 months (+1.6 ± 5.2 Nm, P = 0.76).

Also for dynamic peak torque, a significant time*group interaction was observed (P = 0.002, Figure 2B), and one-way anova analysis revealed between-group differences at 12 months (P < 0.001) and 18 months (P = 0.02). HRTW increased dynamic peak torque compared to NOTW at both timepoints (12 months: +20.8 ± 5.0 Nm, P = $9 \cdot 10^{-5}$; 18 months: +12.6 ± 4.3 Nm, P = 0.005). Changes in dynamic peak toque for LITW did not differ significantly from HRTW at any time point (12 months: -5.5 ± 5.2 Nm, P = 0.29; 18 months: -6.7 ±

4.6 Nm, P = 0.15). Dynamic peak torque was increased in LITW compared to NOTW at 12 months (+15.3 ± 4.9 Nm, P = 0.003), but not at 18 months (+5.9 ± 4.3 Nm, P = 0.18). Investigating changes within HRTW, dynamic peak torque increased at 6 months (+10.6 ± 4.1 Nm, P = 0.01), but was not significantly increased further from 6-12 months (+6.7 ± 4.1 Nm, P = 0.12). Dynamic peak torque decreased from 12-18 months (-9.6 ± 4.1 Nm, P = 0.03), but still tended to be increased at 18 months compared to 0 months (+7.7 ± 4.1 Nm, P = 0.07). Investigating changes within LITW, dynamic peak torque increased from 0-6 months (+10.4 ± 3.3 Nm, P = 0.004), but with no further change from 6-12 months (+1.5 ± 2.8 Nm, P = 0.60). From 12-18 months, dynamic peak torque decreased significantly (-10.7 ± 2.8 Nm, P = 0.0005), and was not different at 18 months compared to 6 months (+1.2 ± 3.4 Nm, P = 0.73).

For RFD, a significant time*group interaction was observed (P = 0.047, Figure 2C). One-way ANOVA analysis revealed between-group differences at 12 months (P = 0.03) and 18 months (P = 0.03). Contrast analysis revealed increases in RFD for HRTW compared to NOTW at 12 months (+103.0 ± 38.7 Nm/s, P = 0.01) and 18 months (+107.3 ± 37.9 Nm/s, P = 0.006). LITW did not increase RFD compared to NOTW at any timepoint, although there were trends towards a difference at 12 months (12 months: +66.3 ± 38.1 Nm/s, P = 0.09; 18 months: +39.8 ± 37.9 Nm/s, P = 0.30). Changes in RFD were not different between LITW and HRTW at 12 months (-36.7 ± 40.8 Nm/s, P = 0.37), while LITW tended to have lower increases at 18 months (-67.5 ± 40.1 Nm/s, P = 0.10). Investigating changes within HRTW, RFD was increased from 0-6 months (+70.5 ± 32.4 Nm/s, P = 0.04), and was unchanged from 6-12 months (26.4 ± 27.3 Nm/s, P = 0.34) and from 12-18 months (-9.4 ± 27.3 Nm/s, P = 0.73). At 18 months, RFD was still increased compared to 0 months (+87.6 ±32.4 Nm/s, P = 0.01). For LITW, RFD increased during the first 6 months (+66.0 ± 25.9 Nm/s, P = 0.02), with no further increase from 6-12 months (-11.0 ± 16.4 Nm/s, P = 0.50). At 18 months, RFD decreased compared to 12 months (-34.0 ± 16.4 Nm/s, P = 0.045), and was not different from 0 months (+20.9 ± 26.1 Nm/s, P = 0.43).



Timepoint	6 months	12 months	18 months
Between-group difference:	0.03	< 0.001	0.01
Within-group change:			
HRTW	# $	# $	#
LITW	# $		$
NOTW			

Timepoint	6 months	12 months	18 months
Between-group difference:	0.08	< 0.001	0.02
Within-group change:			
HRTW	# $	#	$
LITW	# $	#	$
NOTW			

Timepoint	6 months	12 months	18 months
Between-group difference:	0.15	0.03	0.03
Within-group change:			
HRTW	# $	#	#
LITW	# $	#	$
NOTW			

*Figure 18. Changes in lower extremity strength and power. A) Maximal voluntary isometric contraction (MVIC). B) Isokinetic peak torque. C) Rate of force development (RFD). D) Leg extensor power (LEP). NOTW: No training with whey protein supplementation. LITW: Home-based light intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation. #: Significantly different (P<0.05) from baseline. $: Significantly different (P<0.05) from previous timepoint. Test of between- and within-group differences are only performed if the Time*group interaction is significant (P<0.05). Results are shown as mean ± SE.*

LEP was unchanged in all groups with no effect of time (P = 0.89, Figure 2D) or time*group interaction (P = 0.89). The same was true for grip strength (Time: P = 0.79, time*group interaction: P = 0.95, figure 3).

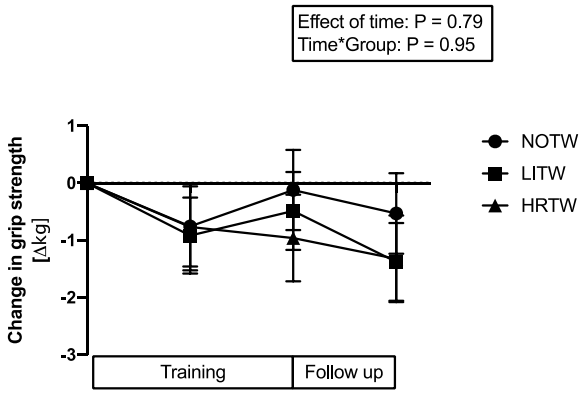


Figure 19. Changes in grip strength. NOTW: No training with whey protein supplementation. LITW: Home-based light intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation. Results are shown as mean ± SE

Body composition and muscle size

A significant effect of time was observed for both qCSA (P = 0.0003, figure 4A) and aLTM (P = 0.0001, figure 4B). However, no time*group interaction was observed (aLTM: P = 0.27; qCSA: P = 0.61). Looking at fat mass, there was not effect of time (P = 0.62, figure 4C) or an time*group interaction (P = 0.68).

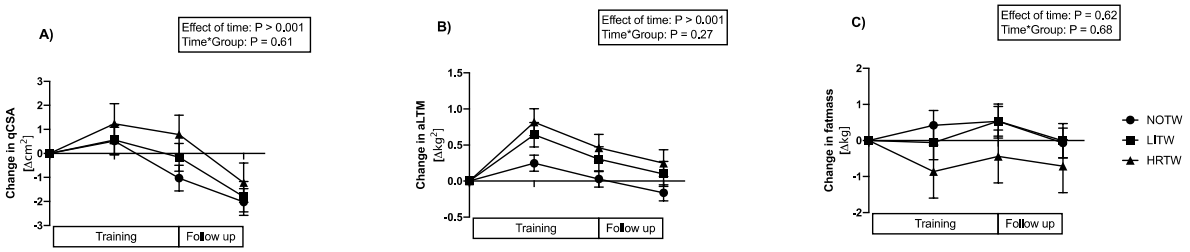


Figure 20. Changes in body composition. A) Quadriceps cross-sectional area (qCSA). B) Appendicular lean tissue mass (aLTM). C) Fatmass. NOTW: No training with whey protein supplementation. LITW: Home-based light intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation. Results are shown as mean ± SE

Gait speed

No effect of time (P = 0.42) or a time*group interaction (P = 0.40) were observed for 400 m gait time (figure 5).

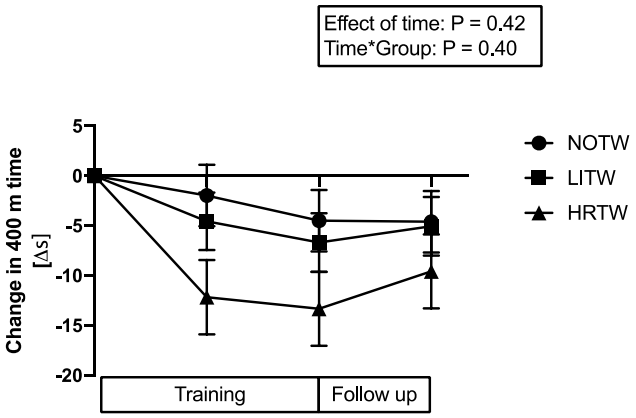
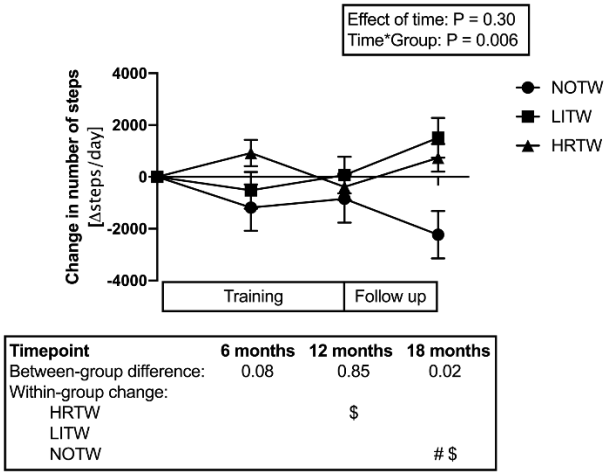


Figure 21. Changes in 400 m gait time. NOTW: No training with whey protein supplementation. LITW: Home-based light intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation. Results are shown as mean ± SE

Activity levels

A time*group interaction was observed for daily steps (P = 0.006, figure 6), and one-way anova analysis revealed between-group differences at 18 months (P = 0.02), but not at 6 months (P = 0.08) or 12 months (P = 0.85). Compared to NOTW, both HRTW and LITW had higher daily step counts at 18 months (HRTW: +2828 ± 1267 steps/day, P = 0.03; LITW: +3691 ± 1313 steps/day, P = 0.01), but LITW did not conduct more daily steps than HRTW (+864 ± 1375 steps/day, P = 0.53). Investigating changes within the groups, HRTW tended to increase daily step count during the first 6 months (+923 ± 508 steps/day, P = 0.08), decreased step counts from 6-12 months (-1303 ± 640 steps/day, P = 0.05), and increased step counts nominally from 12-18 months (+1118 ± 662 steps/day, P = 0.10). LITW did not change step counts during the intervention (6 months: -519 ± 707 steps/day, P = 0.47; 6-12 months: +589 ± 820 steps/day, P = 0.48), but nominally increased steps per day from 12-18 months (+1439 ± 863 steps/day, P = 0.11). NOTW did not change steps per day during the intervention (6 months: -1186 ± 898 steps/day, P = 0.20; 6-12 months: +342 ± 658 steps/day, P = 0.61), but decreased steps per day from 12-18 months (-1385 ± 671 steps/day, P = 0.05).



Timepoint	6 months	12 months	18 months
Between-group difference:	0.08	0.85	0.02
Within-group change:			
HRTW		$	
LITW			
NOTW			# $

*Figure 22. Changes in daily step counts. NOTW: No training with whey protein supplementation. LITW: Home-based light intensity training with whey protein supplementation. HRTW: Heavy resistance training with whey protein supplementation. #: Significantly different (P<0.05) from baseline. $: Significantly different (P<0.05) from previous timepoint. Test of between- and within-group differences are only performed if the Time*group interaction is significant (P<0.05). Results are shown as mean ± SE*

DISCUSSION

To our knowledge, this is the first study to investigate the time course of adaptations to home-based training with lighter loads compared to long-term heavy resistance training compared to a control group also receiving intervention – and thus attention – in the form of supplementation. Several previous studies have found supervised, heavy resistance training to be an effective method over short intervention periods (<24 weeks, often ~12 weeks) in increasing muscle mass and strength in older adults[6–15]. However, the long-term effects of light-load, home-based resistance training are currently not well-described.

In the present study, both LITW and HRTW increased MVIC and dynamic peak torque during the first 6 months. However, only HRTW elicited continued improvements in MVIC from 6-12 months, emphasizing that HRTW is superior to LITW in in inducing long-term improvements in muscle strength. On the other hand, though, LITW was able to maintain the improvements obtained during the first 6 months until 12 months. Interestingly, the relative increase in MVIC and dynamic peak torque for HRTW were ~17% and ~11% respectively, which is comparable to what other studies with similar interventions lengths have reported[31,32], but also to studies with markedly shorter intervention periods[6,8,25,33]. This may suggest that the majority of strength gains to heavy resistance training occur within the first ~12 weeks. However, as MVIC continues to increase from 6-12 months in the present study, the present study supports the notion that longer intervention periods will result in larger improvements in strength. One possible explanation the lack of an additional effect of the long intervention periods compared to shorter interventions, might be a matter of motivation. In the present study, the continued strength increases in response to HRTW occurred despite a lower compliance to training in the second half of the intervention compared to the first

half. Therefore, it could be speculated that shorter interventions might have better adherence and the higher relative load on exercises, simply due to motivation. While exercise adherence was significantly higher for LITW compared to HRTW, it should be noted that the adherence cut-off value for inclusion in the present analysis differed between the groups (>75% for LITW and >66% for HRTW). As exercise adherence was self-reported via dairies in LITW whereas in HRTW training was supervised and adherence followed by research personnel, comparisons of exercise adherence between these two groups should be interpreted with caution. However, the present findings call for future training studies to analyze the temporal changes in adherence to exercise training modalities.

In contrast with previous reports[7,34,35], we did not observe any increases in leg extensor power in response to training in any of the groups. Leg extensor power was measured in the present study using the Nottingham Powerrig, which uses the acceleration of a fixed load to calculate average power development during a maximal leg extension[30]. As the participants in the present study were generally active and healthy, we expect that that the high baseline physical performance of the participants might be the reason behind the lack of increases in leg extensor power. We did however observe an increase in RFD for HRTW, indicating that rapid force production was actually improved in these participants.

Surprisingly, the adaptations to training on strength were not accompanied by significant hypertrophy in response to the training interventions, over 6 months or 12 months. While several studies have detected great degrees of muscle hypertrophy in older adults in response to heavy resistance training[6,8,16,36], other studies have not been able to replicate these increases[37–39]. In the present study, only participants with an average training frequency of ≥2 training sessions per week for HRTW and >3 training sessions per week for LITW were included in analyses. Furthermore, the participants in the present study generally had a high protein intake (<1.0 $g \cdot kg\ BW^{-1} \cdot day^{-1}$ prior to protein supplementation), and good overall nutritional status[40]. Thus, the lack of detectable hypertrophy both at 6 and 12 months in the present study is of great interest, as the participants theoretically should be in good condition for adapting to the training stimulus. The present findings thus seem to indicate limited hypertrophic potential in this population when having engaged in a 1-year intervention period.

6 months after the intervention had ended, HRTW was associated with better preservation of MVIC, dynamic peak torque, and RFD compared to NOTW, whereas LITW did not differ from NOTW in any measure. Both training groups maintained their activity levels, whereas NOTW decreased the activity level. Hence, the lack of strength maintenance for LITW occurred despite a high and unchanged activity level within the group. Previous reports have also found that while muscle mass seems to return to baseline levels within 6 months of detraining, strength gains are somewhat maintained even 12 months after

training [9,41–44]. While the benefits of a heavy resistance training intervention is best maintained if the participants continue (unsupervised) training[9,43,44], the present findings, along with others[9], indicate that older adults participating in a heavy resistance intervention will elicit advantageous long-term adaptations after the intervention has ended – up to 6 months after in this study.

To our knowledge, the present study is the first to demonstrate that heavy resistance training-effects on RFD are maintained at 6 months after cessation of intervention. This is a very important finding as RFD has been shown to be a strong predictor of functional capacity in older adults[45], and is likely to be a crucial component in preventing falls[46].

Some limitations should be considered when interpreting the present results. Firstly, the participants included in the present study were healthy and were generally well nourished[40], and are therefore only transferable to the home-dwelling, rather healthy and active part of the elderly population and should not be extrapolated to e.g. more frail older adults. Furthermore, all groups received twice daily protein supplementation alongside the training regimens. Therefore, the effects observed in the present study cannot solely be attributed to the training regimen. But as the recruited population had protein intakes well above the RDA[47] before inclusion it is unlikely that the protein supplements had a major impact on the training results in the present study.

CONCLUSION & PERSPECTIVES

We conclude that both LITW and HRTW can increase muscle strength in healthy older adults. However, while increases HRTW can induce continuous increases in muscle strength over long-term training interventions, adaptations seem to plateau for LITW after 6 months, though being maintained till 12 months. Both LITW and HRTW explored higher activity levels compared to NOTW during the follow-up period, which dropped activity level from 12-18 months, indicating that exercise training for a long period of time may motivate to remain physically active better than just a nutritional intervention alone. Only HRTW maintained strength and rapid force production above pre-training levels even after 6 months follow-up suggesting this as a superior strategy if training is discontinued. Heavy resistance training seems to be the most efficient long-term resistance training intervention, and hence, future research and innovation efforts should focus on how to engage older adults in such training regimens.

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1. Declaration by	
Name of PhD student	Kenneth Hudlebusch Mertz
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Name of principal supervisor	Michael Kjær
Title of the PhD thesis	Preservation of muscle mass and function through protein supplementation and exercise

2. The declaration applies to the following article	
Title of article	Temporal changes in muscle size and strength in older adults during after a center-based or home-based resistance training intervention. The CALM randomized controlled intervention trial.
Article status	
Published ☐ Date:	Accepted for publication ☐ Date:
Manuscript submitted ☐ Date:	Manuscript not submitted ☒
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5. Conducting the analysis of data	A
6. Interpretation of the results	B
7. Writing of the first draft of the manuscript	A
8. Finalisation of the manuscript and submission	F
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