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PhD Thesis

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Long-term effects of prolonged resistance training:

Muscle, brain, and bone trajectories in aging

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PhD thesis

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PREFACE & ACKNOWLEDGEMENTS

This thesis covers the work performed during my time as a PhD student at the Faculty of Health and Medical Sciences at the University of Copenhagen. The studies include data from the LISA study. This research was made possible and supported by the Nordea Foundation with a grant from Center for Healthy Aging, University of Copenhagen, Denmark. During the past 7 years I have had the pleasure of working at Institute of Sports Medicine Copenhagen (ISMC), Department of Orthopedic Surgery M81, Bispebjerg and Frederiksberg Hospital. I greatly appreciate all the wonderful people that I have met and who have helped me along the way.

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Mads Bloch-Ibenfeldt, April 9, 2024

LIST OF PAPERS

Paper 1

Mads Bloch-Ibenfeldt, Anne Theil Gates, Karoline Karlog, Naiara Demnitz, Michael Kjaer, Carl-Johan Boraxbekk

Heavy resistance training at retirement age induces 4-year-lasting beneficial effects in muscle strength: a long-term follow-up of an RCT.

BMJ Open Sport & Exercise Medicine 2024;10. 10.1136/bmjsem-2024-001899.

(Study 1)

Paper 2

Mads Bloch-Ibenfeldt, Naiara Demnitz, Anne Theil Gates, Hartwig R. Siebner, Michael Kjaer, Carl-Johan Boraxbekk

No long-term benefits from resistance training on brain grey matter volumes in aging.

Submitted to BMC Geriatrics, April 2024 (in review).

(Study 2)

Paper 3

Mads Bloch-Ibenfeldt, Anne Theil Gates, Niklas Rye Jørgensen, Allan Linneberg, Mette Aadahl, Michael Kjær, Carl-Johan Boraxbekk

Heavy resistance training provides short-term benefits on bone formation in well-functioning older adults.

Submitted to Bone, March 2024 (in review).

(Study 3)

LIST OF ABBREVIATIONS

AFRIS	Attitudes to Falls-Related Interventions Scale
ANOVA	analysis of variance
BDNF	Brain-derived neurotrophic factor
BMD	bone mineral density
BMI	body mass index
BREQ-2	Behavioral Regulation in Exercise Questionnaire-2
CON	non-exercising control group
CSA	cross-sectional area
CTX	C-terminal telopeptide of type I collagen
dlPFC	dorsolateral prefrontal cortex
dp-ucMGP	dephosphorylated-uncarboxylated Matrix Gla-Protein
DXA	dual-energy X-ray absorptiometry
eGFR	estimated glomerular filtration rate
fMRI	functional magnetic resonance imaging
HbA1c	hemoglobin A1c (glycated hemoglobin)
HDL-C	high-density lipoprotein cholesterol
HRT	heavy resistance training
IGF-1	insulin-like growth factor 1
IL-6	interleukin 6
LDL-C	low-density lipoprotein cholesterol
LISA	LIve active Successful Aging
LLM	lean leg mass
MIT	moderate intensity training
MRI	magnetic resonance imaging
PASE	Physical Activity Scale for the Elderly
PINP	procollagen type I N-propeptide
RCT	randomized controlled trial
RM	repetition maximum
ROI	region of interest
RPM	revolutions per minute
SCL-90	Symptom Checklist-90

SD	standard deviation
SEM	standard error of the mean
SF-36	36-Item Short Form Health Survey
VEGF	Vascular endothelial growth factor
vlPFC	ventrolateral prefrontal cortex

ABSTRACT

Objectives Many different physiological systems decline with age, and for these, exercise is a promising solution. One form of exercise with many health-related benefits is resistance training, and it has been shown that older adults may profit just as much as younger people. Nonetheless, most studies that have examined the effects of resistance training, and even exercise in general, have done so in the short run (months). What is lacking is knowledge from a long-term perspective (years). In this PhD-thesis, I present studies about the long-term effects of engaging in 1 year of resistance training during a critical time period in life, retirement age. The muscular benefits of resistance training are presented in study 1, the relationship with changes in brain structures in study 2, and in study 3 the relation between resistance training and bone measures.

Methods Data for all three studies comes from the LISA (LIve active Successful Aging) study, which originally was designed as an randomized controlled trial (RCT) examining physical activity as intervention for age-related loss of muscle mass and function in older individuals. In total, 451 community-dwelling older women and men at retirement age (at study start: 66 ± 3 years, 61% women) were randomized to a 1-year intervention of either heavy resistance training (HRT), moderate intensity training (MIT), or a non-exercising control situation (CON). Training was performed 3 times per week. When the 1-year intervention was completed, the participants were neither guided to training nor any particular lifestyle, but remained in the study and were invited for follow-ups at years 1, 2 and 4 from study start. Test procedures included a general health assessment (including blood sampling), different measures of muscle function and body composition, and magnetic resonance imaging (MRI) of brain and muscle.

Results At the 4-year follow-up, general participant characteristics had not changed compared with baseline, and there was no difference between intervention groups. The main finding of study 1 was a significant group x time interaction in isometric leg strength where only individuals in HRT preserved strength over the four years, while strength was decreased for individuals in MIT and CON. In study 2, there was a significant decrease in grey matter volume and increase in white matter hyperintensities across all groups. Further, increase in white matter hyperintensity volume was weakly negatively correlated ($r^2 = 0.01$, $p = 0.05$) with the corresponding change in leg strength. In study 3, elevated levels of PINP in HRT at year 1 indicated a training-related increase in bone formation. At year 4, no group differences were observed. Other bone measures including total and regional bone mineral density and bone degradation (CTX) changed similarly across all 3 groups during the 4-year study period.

Conclusion In older adults at retirement age, engaging in regular resistance training with heavy loads for 1 year benefitted the long-term trajectory of leg muscle strength over 4 years. Preserving leg muscle strength from levels at baseline did not translate into changes in grey matter volumes of the brain or total and regional bone mineral density. The results in this thesis, altogether, showed how challenging it is to induce multiple changes across several physiological systems in aging. From a group-level perspective, the results imply that resistance training load may be of great importance for long-term benefits, but the large individual variability in response to training needs to be better understood to provide better recommendations for this age group.

DANSK RESUMÉ

Baggrund For mange af de forskellige fysiologiske systemer, hvis evner mindskes med alderen, er fysisk aktivitet en lovende løsning. Én form for fysisk aktivitet med mange sundhedsgavnlige effekter er styrketræning, og det er blevet vist at ældre mennesker har mulighed for at profitere i samme grad som yngre. De fleste studier har dog kun undersøgt effekterne af styrketræning, og fysisk aktivitet i al almindelighed, på den korte bane (måneder). Hvad der mangler, er viden om det langsigtede perspektiv (år). I denne PhD-afhandling præsenterer jeg studier, der undersøger de langvarige effekter af 1 års styrketræning, på et kritisk tidspunkt i livet, omkring pensionsalderen. De gavnlige muskulære effekter af styrketræning er forelagt i studie 1, sammenhængen med ændringer i hjernens strukturer i studie 2, og i studie 3 sammenhængen mellem styrketræning og knoglemålinger.

Metode Data fra alle tre studier kommer fra LISA (Lev akIvt Sund Aldring) studiet, der oprindeligt var designet som et randomiseret kontrolleret studie (RCT), hvor effekterne af fysisk aktivitet som intervention for aldersrelateret tab af muskelmasse og -funktion i ældre individer blev undersøgt. Samlet blev 451 ældre samfundsborgere (både kvinder og mænd) omkring pensionsalderen (ved studiestart: 66 ± 3 år, 61% kvinder) randomiseret til en 1-årig intervention bestående af enten tung styrketræning (HRT), moderat intensitets træning (MIT) eller en kontrolsituation hvor deltagerne blev bedt om at fortsætte med deres sædvanlige motionsaktiviteter (CON). Begge træningsinterventioner trænede 3 gange om ugen. Da den 1-årige intervention var afsluttet, blev deltagerne hverken guidet til træning eller specifik livsstil, men fortsatte i studiet og blev inviteret til opfølgende undersøgelser 1, 2 og 4 år efter studiestart. Testprocedurerne inkluderede en general helbredsundersøgelse (herunder blodprøvetagning), forskellige målinger af muskelfunktion og kropskomposition, samt magnetisk resonans (MR) skanninger af hjerne og muskel.

Resultater Ved 4-års opfølgningen var de generelle karakteristika for forsøgsdeltagerne ikke forandret i forhold til baseline, og der var ingen forskel mellem interventionsgrupperne. Det primære fund i studie 1 var en signifikant interaktion af gruppe x tid i isometrisk styrke i benet, hvor deltagerne i HRT havde vedligeholdt styrke over de fire år, mens styrken var faldet for deltagerne i MIT og CON. I studie 2 var der et signifikant fald i volumen af grå substans og stigning i hyperintense forandringer i den hvide substans for alle grupper. Derudover var den øgede mængde af hyperintense forandringer i den hvide substans svagt negativt korreleret ($r^2 = 0.01$, $p = 0.05$) med den tilsvarende ændring i styrke i benet. I studie 3 indikerede forhøjede niveauer af PINP i HRT ved år 1 at der var en træningsrelateret stigning i opbygningen af knoglevævet, mens der ved

år 4 ikke blev observeret nogle gruppeforskelle. Andre knoglemålinger, herunder total og regional knoglemineraltæthed samt knoglenedbrydning (CTX) forandredes på samme vis i alle 3 grupper hen over studiets 4-årige periode.

Konklusion For ældre borgere omkring pensionsalderen var 1 års regelmæssig tung styrketræning fordelagtig for det langsigtede forløb over 4 år i benets muskelstyrke. Vedligeholdelse af muskelstyrke i benet fra niveauet ved baseline blev ikke omsat til ændringer i voluminer i hjernen eller ændringer i total eller regional knoglemineraltæthed. Tilsammen viser resultaterne i denne afhandling, hvor udfordrende det i aldring er at inducere mange ændringer på tværs af flere fysiologiske systemer. På gruppeniveau indikerer resultaterne, at styrketræningens belastning kan være af stor betydning for længerevarende gavnlige effekter, men vi har brug for bedre at forstå den store individuelle forskel i træningsrespons for at kunne give bedre anbefalinger til den ældre befolkning.

INTRODUCTION

I have been physically active throughout my life, engaging in many kinds of training, and I plan to keep on training for as long as I can. Training, however, is not a regular part of many people's lives when they age, even though there is a general belief that training is good for overall health. The concept of studying training-related effects is not new. Nonetheless, we are missing some key aspects in the studies presented so far. For example, much more emphasis has been on immediate effects and a rather short time perspective. We are lacking knowledge about the long-term consequences or beneficial effects of engaging in training in relation to aging. This is a problem, since the proportion of older individuals world-wide is rapidly increasing, which is changing the traditional population pyramid to a more pillar shape. In turn, this is going to possess a major challenge to our health and social systems (WHO, 2022). However, as depressing as this may seem, research does suggest that the process of aging may be modifiable (Christensen, Doblhammer, *et al.*, 2009). In this thesis focus will be on the long-term benefits of training.

In general, physical training has been studied substantially, starting with classical studies looking into the biochemical adaptations of exercise (Holloszy, 1967) and the effects of age, physical activity, and training on skeletal muscle (Shephard, 1986; Thompson, 1994; DiPietro, 2001; Paterson, Jones and Rice, 2007). There has been a plethora of trials looking into the effects of physical activity, including different training modalities, on general physical health, and on specific physiological mechanisms (Warburton, Nicol and Bredin, 2006). With much evidence on the association between exercise and health and how endurance training improves health outcomes (Joyner and Green, 2009), endurance training and its effects on cardiovascular fitness has been the centre of attention for many years (Ruegsegger and Booth, 2018). More recently, there has been an increased focus on the beneficial effects of resistance training – in particular with regards to aging (Latham *et al.*, 2004; Fragala *et al.*, 2019). On a national level in Denmark, this increased focus has led to new recommendations for physical activity, where resistance training is now encouraged for older adults. National guidelines have been updated, with a detailed report published by the Danish health authorities (Danish Health Authority - Sundhedsstyrelsen, 2023). This thesis will focus on components that may be influenced by resistance training, hereby examining the potential effects on physical function, muscle, brain, and bone.

Healthy aging

Recent years there has been much research on the prospects of slowing human aging and prolonging longevity (Carmona and Michan, 2016). Aging is a complex field, driven by a variety of components – see **Figure 1**.

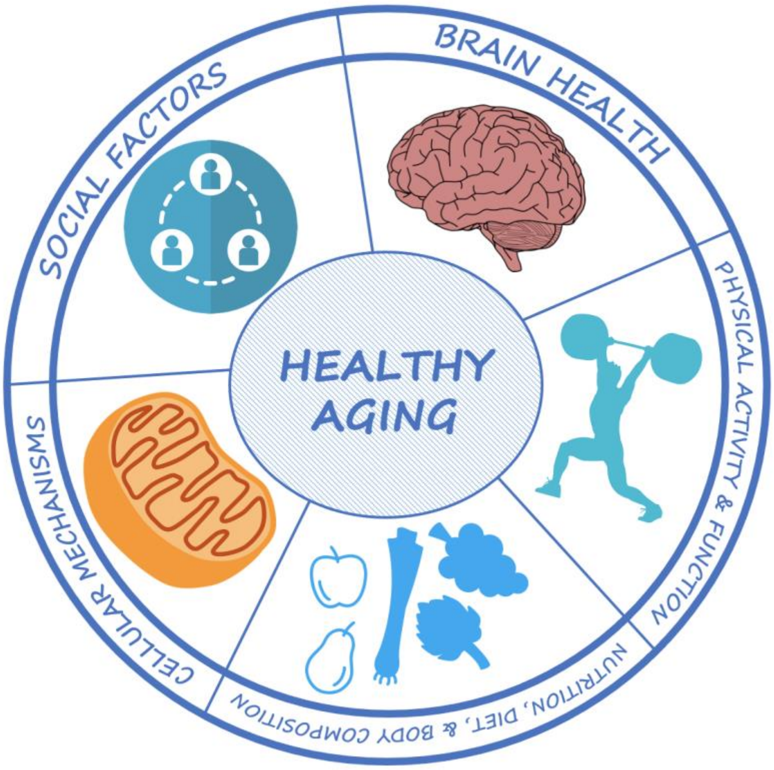

Figure 1. Components of healthy aging, including the fields of physical activity and function, nutrition, diet and body composition, cellular mechanisms, social factors, and brain health.

The components of aging are all somewhat interconnected and are easily categorized into further underlying elements. For example, only for the cellular mechanisms 12 hallmarks of aging have recently been proposed (López-Otín *et al.*, 2023). Other components that influence or are manifested with age include social factors (Takács and Nyakas, 2021), nutrition (Lee *et al.*, 2015), and brain health (Mattson and Arumugam, 2018; Zia *et al.*, 2021). One part of aging is the discrepancy between healthspan and lifespan, leaving a healthspan-lifespan gap (Garmany, Yamada and Terzic, 2021), which may eventually lead to an impacted quality of life and loss of individual autonomy late in life. The health benefits of physical activity are well documented, with physical activity and healthy aging being associated, regardless of definition or measure (Garatachea *et al.*, 2015; Daskalopoulou *et al.*, 2017; Gopinath *et al.*, 2018; Langhammer, Bergland and Rydwik, 2018). In one example of a study where this was outlined, muscle mass was reduced and metabolic health markers negatively influenced following only 14 days of step-reduction in healthy older adults (Breen *et al.*, 2013). Furthermore, it is known that the amount of physical activity decreases with age (Westerterp, 2018).

Age-related physical changes

With age, skeletal muscle function, a major determinant of physical function, is known to decline, accelerating at advanced age (Fielding *et al.*, 2011; Cruz-Jentoft *et al.*, 2019; Suetta *et al.*, 2019). This decline in muscle function is caused by many factors that are related to the nervous, muscular, and skeletal system, such as a loss of muscle mass and muscle strength, due to a reduction in muscle fiber number and size (primarily type II muscle), and impaired neuromuscular function (Carlson *et al.*, 2009; Aagaard *et al.*, 2010; Nilwik *et al.*, 2013; Distefano and Goodpaster, 2018; Tieland, Trouwborst and Clark, 2018; Soendenbroe, Andersen and Mackey, 2021).

The importance of muscle mass per se is for example outlined in studies where muscle mass has been predictive of all-cause mortality (Landi *et al.*, 2013), and post-operative progress and mortality following colon cancer surgery (Xiao *et al.*, 2020). Simultaneously, body composition is altered with age-related increases in both total body fat (Zong *et al.*, 2016) and visceral fat (Huffman *et al.*, 2009). Additionally, bone health, a major component in aging due to particularly osteoporosis and the risk of falls and fractures (Black and Rosen, 2016), declines with age (Colón *et al.*, 2018), with decreased bone mineral density (Berger *et al.*, 2008). In the brain, there is age-related atrophy occurring throughout the brain (Fjell *et al.*, 2009; Nyberg *et al.*, 2023) in addition to increases in white matter lesions, strokes, and dementia, as well as a cognitive decline (Peters, 2006; Murman, 2015).

Resistance training

Resistance training is an effective countermeasure to many of these negative age-associated changes (Fragala *et al.*, 2019). One of the first studies examining the effects of engaging in a resistance training programme, observed beneficial effects on muscle strength, size, and neural activation (Narici *et al.*, 1989). For many years, it has also been known that such adaptations are achievable in the older population, as only some years later it was shown in a small sample of older men and women that 1 year of resistance training led to increases in muscle strength and size (Pyka *et al.*, 1994). Following these findings, the potential benefits of resistance training have been studied extensively. For many years, it has been proposed that health and performance greatly benefit from resistance training (Kraemer, Ratamess and French, 2002), and fundamentals for a successful implementation of resistance training, such as progression and exercise prescription, have been put forward (Kraemer and Ratamess, 2004). When the potential differences in the age-related response to resistance training were studied, older (compared with young) men demonstrated a maintained capacity for increases in muscle strength and power (Newton *et al.*, 2002). Likewise, in studies where neuromuscular adaptations to resistance training were examined in older men and women, neural adaptations seemed to play a great role (Häkkinen and Häkkinen, 1995; Häkkinen *et al.*, 2000). Overall, resistance training positively influences several systems of the human body. The idea of non-responders to exercise, given that some individuals do not improve in line with others during training interventions, has been proposed throughout the years (Booth and Laye, 2010; Pickering and Kiely, 2019). However, it is questionable whether individuals who do not adapt to every possible exercise – irrespective of modality – really exist. Rather, perhaps it is a matter of modifying exercise variables such as intensity, volume, and duration, in turn individualizing prescriptions and not accepting the one-size-fits-all approach.

Skeletal muscle

Upon muscle strength, a beneficial effect of resistance training in older adults is well-established. In a meta-analysis of randomized control trials, in which the effects of resistance training on measures of muscle strength and morphology in older individuals ≥ 65 years were determined, all 25 studies that were included reported a favorable effect on strength (Borde, Hortobágyi and Granacher, 2015). Likewise, other resistance training-interventions have shown improvements in muscle strength in older adults (Leenders *et al.*, 2013; Churchward-Venne *et al.*, 2015; Snijders *et al.*, 2019). Concomitantly, resistance training is known to positively influence size of the aging muscle, with increases in muscle mass, either at whole-body level or at a specific site such as the leg, and muscle cross-sectional area (Peterson, Sen and Gordon, 2011; Leenders *et al.*, 2013; Borde, Hortobágyi and Granacher, 2015; Churchward-Venne *et al.*, 2015; Snijders *et al.*, 2019; Gylling, Eriksen, *et al.*, 2020). Another measure that has seen improvements with resistance training is muscular power, which has been identified as a critical determinant of physical function in older adults (Reid and Fielding, 2012), with power likely more related to daily functional activities than strength (Fielding *et al.*, 2002; Macaluso and De Vito, 2004). However, not all resistance training studies observe an improvement in measures of power in older individuals (Gylling, Eriksen, *et al.*, 2020), and accordingly it has been proposed that power specific training is a more optimal means when the goal is an increase in activities of daily living (Hazell, Kenno and Jakobi, 2007; Katula, Jack and Marsh, 2008; El Hadouchi *et al.*, 2022). Similar to muscular strength and power, some studies have observed additional benefits in relation to physical function, with performance in the chair stand test an excellent example (Leenders *et al.*, 2013; Churchward-Venne *et al.*, 2015). In another study, chair stand performance accordingly improved with training, but this was identical to the non-exercising control group where performance surprisingly also improved after a resistance training intervention (Gylling, Eriksen, *et al.*, 2020).

Brain

For the brain, aerobic training initially showed promise for executive performance in older sedentary adults (Kramer *et al.*, 1999), which was followed up by a meta-analysis showing robust benefits on cognition with aerobic training (Colcombe and Kramer, 2003). Additionally, physical activity in general has been linked to improved cognition (Weuve *et al.*, 2004; Larson *et al.*, 2006). Overall, aerobic exercise, in particular, is suggested to potentially benefit improved brain health and cognition (Colcombe *et al.*, 2006; Kramer, Erickson and Colcombe, 2006; Erickson *et al.*, 2009, 2011; Thomas *et al.*, 2012; Jonasson *et al.*, 2017). Despite being encouraged for brain health for more than a decade (Liu-Ambrose and Donaldson, 2009), resistance training has been less studied, yet initial results have shown great promise. For example, in community-dwelling older women, between 65 and 75 years, resistance training once or twice weekly for 12 months improved executive cognitive functions via improved task performances in both groups, compared with a control group that did balance and toning training (Liu-Ambrose *et al.*, 2010). For the same older women, but only for those who resistance trained twice weekly, functional plasticity in cortex was positively impacted (Liu-Ambrose *et al.*, 2012). Additionally, a subset of the participants had magnetic resonance imaging (MRI) of the brain. At a 2-year follow-up, beneficial long-term effects were observed on cognition in all women who had resistance trained during the first year, and on white matter volume in those who had resistance trained twice weekly (Best *et al.*, 2015). A more recent meta-analysis similarly proposed that resistance training appears to have a positive effect on cognition (Landrigan *et al.*, 2020). In a more short-term setting, in older adults aged 60-80 years, a 12-weeks lower body resistance training programme, performed twice weekly, seemed to better preserve brain metabolism than in a passive control group (Sheoran *et al.*, 2023).

The potential effects of resistance training, and a crosstalk between muscle and brain, are suggested to be mediated by a muscle-brain endocrine loop and the release of myokines during exercise (Pedersen, 2019), with an increase in myokine levels after some resistance training interventions (Wang *et al.*, 2023). One myokine, brain-derived neurotrophic factor (BDNF), is produced by skeletal muscle during contraction (Matthews *et al.*, 2009). BDNF is believed to be a key factor for brain plasticity (Kowiański *et al.*, 2018), and is suggested to be linked to hippocampus (Erickson *et al.*, 2011). Muscle specific myokines could also include interleukin 6 (IL-6), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), irisin, cathepsin B, and lactate (Chen and Nakagawa, 2023). However, not all previous results on resistance training and brain health show that there is a positive influence of training. Prolonged studies on both brain structure and cognition have failed to show an immediate effect on hippocampal atrophy (Gylling, Eriksen, *et al.*, 2020) and cognitive function (Sink *et al.*, 2015), although the latter did not report on any measures of physical function or performance to assess the muscular effects of training.

Body composition

There are multiple ways to assess body composition, including measures of adipose tissue (fat), visceral fat, and bone mineral density (BMD) using state-of-the-art dual-energy X-ray absorptiometry (DXA), as well as various blood-based markers. It has been shown that resistance training is able to induce favorable changes to body composition, in relation to aging. Several studies have observed a decrease in body fat after resistance training interventions, varying from 12 to 56 weeks, in older adults (Campbell *et al.*, 1994; Snijders *et al.*, 2019; Gylling, Eriksen, *et al.*, 2020). For visceral fat, previous research has predominantly focused on the effects of cardiovascular training, however recent research suggests that resistance training is also beneficial for a decreased visceral fat content (Gylling, Eriksen, *et al.*, 2020; Wewege *et al.*, 2022).

For bone health, in addition to known lifestyle factors such as general physical exercise and diet, which have been suggested to be beneficial (Muñoz-Garach, García-Fontana and Muñoz-Torres, 2020; Chen and Avgerinou, 2023), there has been much research on exercise specifics. Currently, which still holds two decades later, weightbearing exercises with impact are recommended (NIH Consensus Statement, 2000). For resistance training, there is no consensus with very mixed results on BMD. In several studies, there has been no clear effect of resistance training (Pruitt *et al.*, 1992; McCartney *et al.*, 1995; Pruitt, Taaffe and Marcus, 1995; Fujimura *et al.*, 1997; Maddalozzo and Snow, 2000). In other studies, beneficial effects on BMD have been observed, even though these have often been small or only at regional bone sites such as the spine or hip (Lohman *et al.*, 1995; Yarasheski, Campbell and Kohrt, 1997; Layne and Nelson, 1999; Rhodes *et al.*, 2000; Vincent and Braith, 2002; Hinton, Nigh and Thyfault, 2015; Shojaa *et al.*, 2020; Herda and Nabavizadeh, 2023). Blood-based markers procollagen type I N-propeptide (PINP) and C-terminal telopeptide of type I collagen (CTX), which are the international reference markers for bone formation and resorption respectively (Vasikaran *et al.*, 2011), can be used to measure bone turnover. In relation to resistance training, the beneficial effects on bone turnover may be attenuated with age (Gombos *et al.*, 2016; Smith *et al.*, 2021; Stunes *et al.*, 2022). Even so, to some degree resistance training has shown the ability to positively influence bone markers. Shorter durations (6-16 weeks) of high-intensity resistance training have increased bone turnover suggesting a favored bone formation in older adults around their 60's, while in elderly women 16 weeks of resistance training increased some, but not all, bone markers (Sartorio *et al.*, 2001; Karabulut *et al.*, 2011; Huovinen *et al.*, 2016). Recently, also dephosphorylated-uncarboxylated Matrix Gla-Protein (dp-ucMGP) has been linked to bone formation, through its reflection of vitamin K status (Cranenburg *et al.*, 2010; Villa *et al.*, 2017; Akbari and Rasouli-Ghahroudi, 2018). Not much literature exists on the relation between resistance training and dp-ucMGP, though one study showed that plasma levels of dp-ucMGP were correlated to sarcopenia parameters such as muscle mass and physical performance in a cohort of adults, who were on average 58 years old (Schweighofer *et al.*, 2022).

Training in the long-term perspective

As per above, it is evident and well-documented that resistance training has many beneficial effects. It is also very well documented that these beneficial effects or hard-fought gains in particularly muscle mass and muscle function are often easily lost following resistance training interventions, either partly or totally, within a particular time frame in a use-it-or-loose-it manner (Trappe, Williamson and Godard, 2002; Fatouros *et al.*, 2005; Kalapotharakos *et al.*, 2007; Karinkanta *et al.*, 2009; Tokmakidis *et al.*, 2009; Bickel, Cross and Bamman, 2011; Correa *et al.*, 2013, 2016; Uusi-Rasi *et al.*, 2017; Sakugawa *et al.*, 2019; Snijders *et al.*, 2019; Blocquiaux *et al.*, 2020). Consequently, considering the question of how much is needed to do to maintain adaptations, it is proposed that a continuation of regular training is necessary to preserve adaptations and change trajectories (Gylling, Bloch-Ibenfeldt, *et al.*, 2020). However, the length of both training and follow-up periods are modest in most of the abovementioned studies. In a recent meta-analysis, where some of these studies were included, the effects of resistance training cessation in older adults were analyzed. Here, again limited to shorter training durations (9-24 weeks) and also small sample sizes, it was concluded that muscle loss might be related to the duration of detraining – translatable to the length of follow-up – as the amount of muscle lost generally was greater in the studies with longer follow-up periods (up to 52 weeeks)(Grgic, 2022). Unfortunately, the literature is very sparse with regards to studies where prolonged resistance training is combined with a lengthy follow-up, to study the true long-term consequences. One study examined the effects of a 1-year training intervention on muscle performance in older adults 7-years post study start (Kennis *et al.*, 2013). Originally, individuals were randomized to a combination of resistance and aerobic training on one hand or whole-body vibration training on the other, compared with a control group that did not participate in training. The results were, however, analyzed with a single combined group of all individuals who had trained in one of the two training groups. After the 1 year of training, participants were encouraged to pursue a physically active lifestyle but were not informed of a follow-up. Notably, some beneficial long-term adaptations were evident. While muscle performance was decreased from baseline in both the training group and controls at follow-up, there was some preservation of training-induced gains, as the degree of decline in measures of static and dynamic strength differed between groups with similar annual decline rates from year 1 to 7 (Kennis *et al.*, 2013). In another study, where there was a long-term training intervention of 5 years, the effects of exercise on mortality and brain health in older community-dwelling adults were examined (Stensvold *et al.*, 2015, 2020). The training focused on cardiovascular fitness and was performed at either high or moderate intensity, with a control group following the Norwegian recommendations for physical activity. Notably, no muscular measure was included, but adding high-intensity training to an already high level of cardiovascular fitness did not influence all-cause mortality, cardiovascular disease, and cancer (Stensvold *et al.*, 2020), or long-term trajectories of brain health (Pani *et al.*, 2021, 2022; Arild *et al.*, 2022; Reitlo *et al.*, 2023).

Training intensity

One parameter of resistance training, and training in general, which can be influenced, is training intensity. For resistance training this can also be expressed as load. Several studies have reported on intensity-dependent outcomes, and one meta-analysis showed that for increases in muscle strength and size, resistance training with heavy loads was more effective than moderate loads in older adults (Csapo and Alegre, 2016). The aspect of intensity is also of interest in combination with the long-term perspective. Combining 24 weeks of resistance training at two different intensities with a prolonged follow-up period of 48 weeks, one study further highlighted the importance of training intensity, showing in older men that the higher intensity training elicited greater gains in both muscle strength and physical function and also that these gains were better preserved during the follow-up period (Fatouros *et al.*, 2005). Similarly, resistance training with heavy loads has proven more beneficial than moderate training on several parameters in older adults, including the preservation of muscular gains over a 1-year period (Gylling, Bloch-Ibenfeldt, *et al.*, 2020; Gylling, Eriksen, *et al.*, 2020). When periods of both resistance training and follow-up were short, differences between high-intensity and moderate training showed the same patterns in muscular measures in older adults (Tokmakidis *et al.*, 2009). Altogether, training intensity seems to impact adaptations to training and trajectories during follow-up, but this has primarily been studied after both training interventions and follow-up periods of shorter durations.

AIMS & HYPOTHESES

There is a need for and importance of longitudinal studies in relation to the effects of training in aging, where individuals are followed over many years beyond the end of a training intervention. Further, as outlined in the introduction, aging is a multifaceted process. Therefore, in order to fully appreciate how training can promote healthy aging, a multi-dimensional approach is necessary. In this thesis I have been interested in the interplay between different biological systems of the body and whether it is possible to pick up interactions between different age-related changes, to see whether there is a commonality between e.g., muscle, brain, and bone. The overall aim of this thesis was therefore, to investigate the long-term effects of engaging in 1 year of resistance training during a critical time period in life, retirement age.

To match this overarching goal, three studies were performed. In study 1, the aim was to investigate whether improvements in muscle mass and muscle function observed both immediately and 2-years post study start would be maintained also at year 4. It was hypothesized that, compared with non-exercising controls, individuals randomized to training would still show positive training-induced effects in muscle strength at the 4-year follow-up.

In study 2, the influence of resistance training on age-related structural brain changes was examined. It was hypothesized that there would be beneficial long-term training-related effects on brain health, with smaller declines in brain structural changes at the 4-year follow-up.

The third study explored how bone mineral density and markers related to bone turnover were influenced by resistance training. It was hypothesized that positive effects of training would translate into attenuated loss of bone mineral density.

METHODS

All three studies in this thesis are based on the LISA (LIve active Successful Aging) cohort, which in 2014 was initiated as a multidisciplinary randomized controlled trial to examine physical activity as intervention for age-related loss of muscle mass and function in older individuals (see Eriksen et al., 2016 for study protocol).

At the start, advertisements in local newspapers and social media were used to recruit older adults around retirement age. From these advertisements, 1026 community-dwelling older adults volunteered to take part in the study. These were further screened by telephone and provided oral and written information about the study. Following screening, the remaining individuals went through a medical exam and finally baseline assessments. Of the original 1026 individuals, 451 persons aged between 62 and 70 (mean age: 66 ± 3 years) met inclusion criteria, passed the medical exam, gave written consent, and were subsequently enrolled in the study.

Excess levels of habitual physical activity, defined as more than 1 hour of regular strenuous physical activity per week, as well as specific diseases and medications that would interfere with the ability to train were the main exclusion criteria (Gylling, Eriksen, *et al.*, 2020). Chronic diseases with no direct effect on exercise ability were not considered a reason for exclusion. Participants were stratified according to sex (man/woman), body mass index (BMI) (≤28 or >28), and performance in the chair-stand test (≤11 or >11 repetitions), and randomized to one of three groups: heavy resistance training (HRT); moderate intensity training (MIT); or a non-exercising control group (CON).

Following baseline and post-intervention assessments, participants were longitudinally followed-up at years 2 and 4, with further follow-ups planned at years 7 and 10. See **Figure 2** for study flow chart, including details about dropouts for each of the three intervention groups respectively.

Intervention

Training was performed 3 times per week for a whole year in both training groups. HRT met at a local commercial gym, for a supervised full-body program with heavy loads. Participants began their training with 6-8 weeks of habituation, starting off with 1 set of 15 repetitions for each exercise. They were instructed that the last repetitions should feel exhaustive. Total training volume was then gradually increased before the periodization regimen commenced in week 7-9.

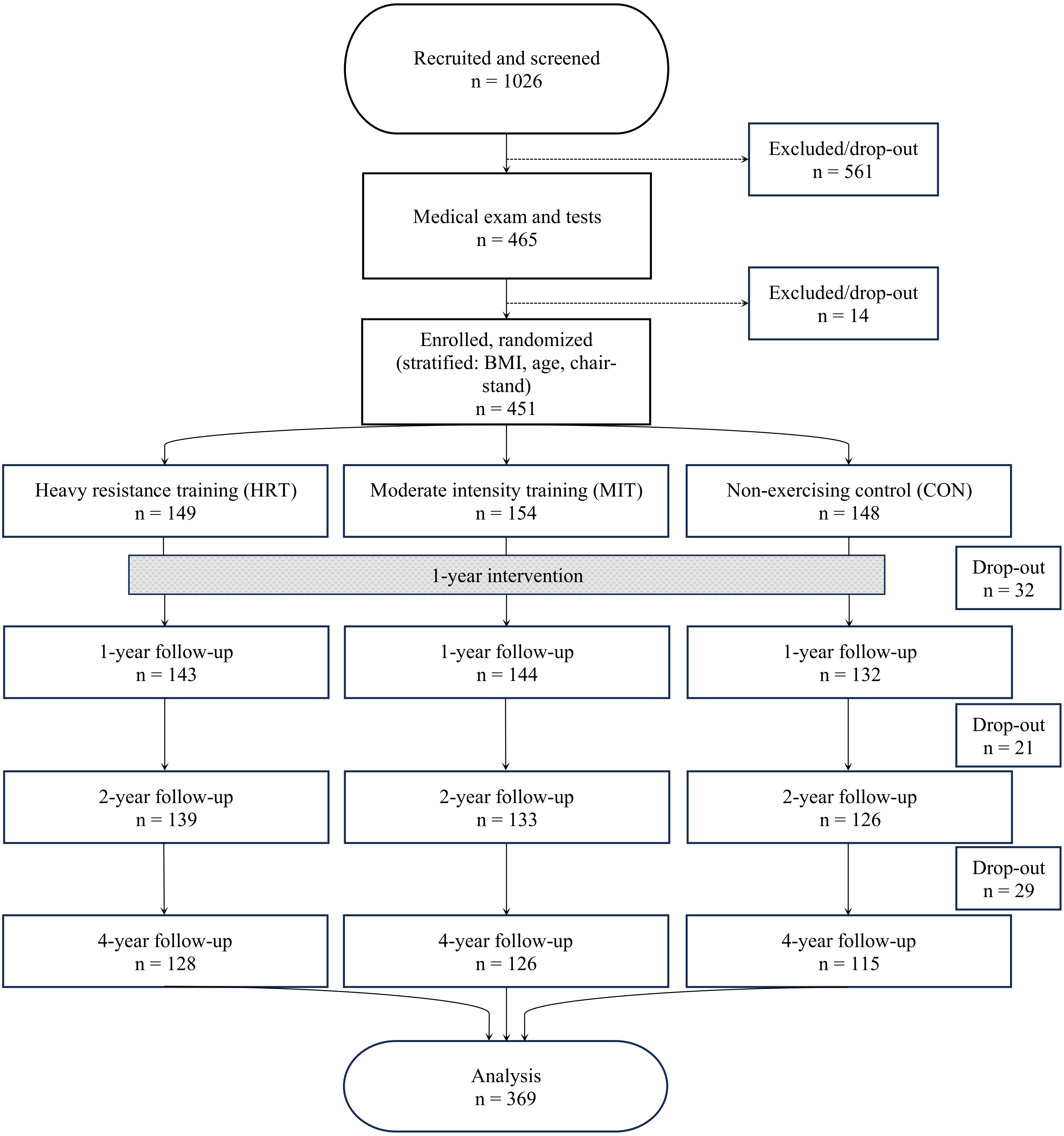

Figure 2. Flow chart, including enrollment, assessments at baseline, group allocation, intervention, and follow-up.

This training regimen was machine based with 6-12 repetitions at ~70-85% of 1 RM (1 RM was estimated according to methods by Brzycki, 1995 (Wood, Maddalozzo and Harter, 1993), with 1-2 minutes of rest between sets, and full restitution every 9th week. MIT engaged in a more moderate intensity training regimen, which also started with 6-8 weeks of habituation and initially was performed with one set of 10 repetitions for each exercise. These participants were also instructed that the last repetitions should feel exhaustive. Training was performed as circuit training with 10-18 repetitions at ~50-60% of 1 RM (estimated similarly to HRT), no rest, and consisted of bodyweight and resistance band exercises that were set to mimic the exercises in HRT. Training was also periodized with a progression in the load of the resistance bands (TheraBand, Akron, Ohio, USA – red to gold bands) and supervised once a week at the hospital, while the other two weekly training sessions were performed individually at home. See **Table 1** for exercises in HRT and MIT.

Heavy resistance training (HRT)	Moderate intensity training (MIT)
Leg press	Squat
Chest press	Pushup
Knee extension	Seated knee extension
Low row	Seated low row
Leg curl	Standing hip abduction
Ankle plantar flexion	Standing hip extension
Hip abduction	Heel raise
Abdominal crunch	Abdominal crunch
Lower back exercises	Lower back exercises

Table 1. Specific exercises in the two training regimes.

In both HRT and MIT, training was supervised by experienced personnel. The non-exercising control group was encouraged to maintain habitual physical activity level and individuals in this group were invited to social-cultural activities at a regular basis (approximately 1 per month), both at the hospital and at other sites primarily in the greater Copenhagen area. These activities included e.g. guided tours at attractions, lectures on specific topics, and bridge sessions.

Test procedures

Assessments were conducted on three separate days – see **Figure 3** for overview.

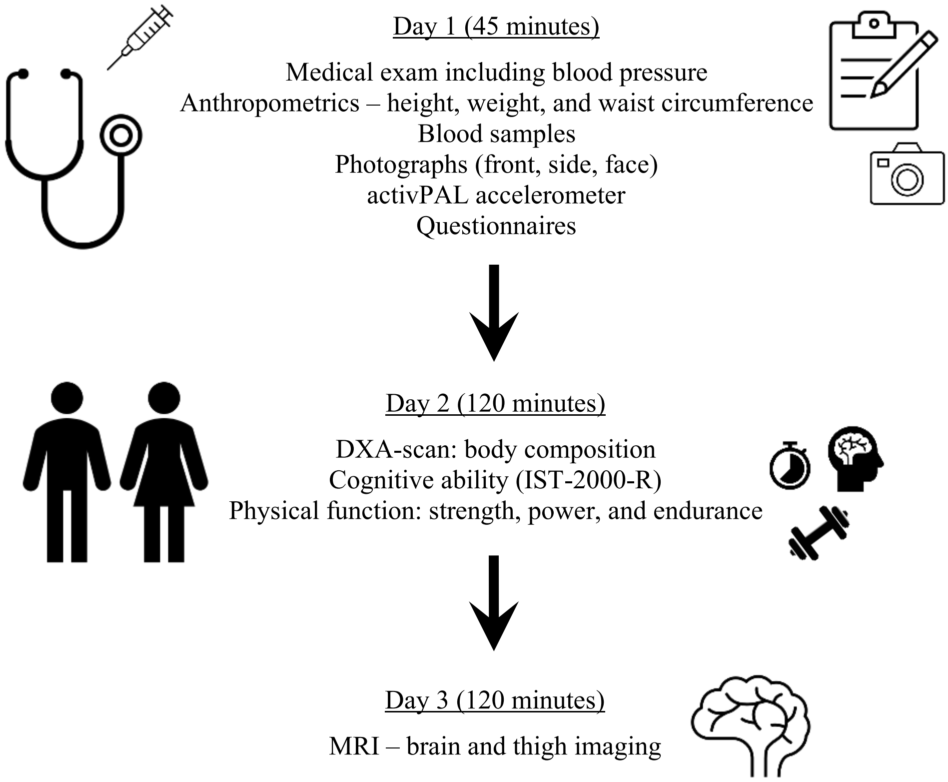

Figure 3. Overview of the three days with assessments and tests.

Day 1

On day 1, participants met before noon at the hospital in a fasted state but allowed to drink water and take their regular medication. A medical exam was performed, with medical history registered. Further, height and bodyweight were measured without shoes, on a digital scale (Seca 285 wireless measuring station), waist circumference measured with a tape measure, and a single measure of blood pressure and heart rate with an electronic sphygmomanometer. Fasting venous blood samples were drawn from the cubital vein in the elbow joint, for analysis at the Hospital biochemistry laboratory. Samples were analyzed for traditional markers of health, such as markers of glucose and lipid metabolism, inflammation, and immune function (including cholesterol (total, HDL-C and LDL-C), inflammation (C reactive protein), blood glucose (hemoglobin A1c), estimated glomerular filtration rate (eGFR), and leucocytes). Further, two 9 ml samples for storage of additional serum and plasma were drawn. The plasma sample was placed on ice for 30 minutes while the serum sample was placed at room temperature. To separate the plasma and serum from cells, samples were centrifuged at 3970 RPM, 4°C, for 10 minutes (Centrifuge 5810 R, Eppendorf, Germany). Immediately after centrifugation samples were pipetted into Eppendorf tubes® and stored at -80°C until further analyzed.

For assessment of perceived age, photographs with participants in their regular clothes were taken from three angles, according to previous methods (Christensen, Thinggaard, *et al.*, 2009). An accelerometer (activPAL™ micro, PAL Technologies Ltd), for measuring daily physical activity through step counts, was placed on the mid-thigh of the dominant leg, and worn for 5 consecutive days, including three weekdays and the weekend. The activPAL is considered a well-established device to collect data on physical activity (Edwardson *et al.*, 2017; Blackwood *et al.*, 2022). Finally, a series of questionnaires were administered, including the exercise self-efficacy questionnaire (Marcus *et al.*, 1992), which was answered at the hospital during the health screening, and questionnaires regarding background and lifestyle (modified from the CAMB cohort (Lund *et al.*, 2016)), personality (NEO-FFI (Mortensen, Flensborg-Madsen, Molbo, Christensen, *et al.*, 2014)), mental distress (a shortened version of the SCL-90 (Olsen, Mortensen and Bech, 2004)), physical activity (PASE (Washburn *et al.*, 1993, 1999; Dinger *et al.*, 2004)), health-related quality of life (SF-36 (Brazier *et al.*, 1992; Bjorner *et al.*, 1998)), and attitude to intervention (AFRIS (Yardley *et al.*, 2007; Mikolaizak *et al.*, 2018)) which were handed out and completed at home.

Day 2

The second day was planned a minimum of 6 days after the medical exam and featured dual-energy X-ray absorptiometry (DXA) scans, cognitive tests, and physical tests. The participants were encouraged to avoid vigorous physical activity in the preceding 72 hours. Upon arrival they emptied their bladder and handed in accelerometer and questionnaires. To evaluate body composition and BMD, DXA scans (Lunar iDXA, GE HealthCare – enCORE software V.16), which is the state-of-the-art measure for BMD, were completed with the participants lying in a supine position. In total participants had 4 scans (**Figure 7**): a full-body scan, for determining bone mineral density, lean body mass, fat mass, and visceral fat content, and 3 scans for evaluation of bone mineral density at specific bone regions: lumbar spine (L1-L4), right femoral neck, and left femoral neck. Some of the participants did not have (all) bone specific scans, due to for example total hip arthroplasty.

Afterwards, a Danish shortened version (IST-2000-R) of the intelligence structure test IST-2000 (Liepmann *et al.*, 2007) was performed to assess general cognitive ability. Three subtests of 6, 7 and 10 minutes each were performed, similarly to previous studies (Mortensen, Flensborg-Madsen, Molbo, Fagerlund, *et al.*, 2014).

The final part of day 2 consisted of physical tests, which were used to determine physical function - particularly strength and endurance. Endurance was assessed with a 400m walking test, which previously has been used to estimate cardiorespiratory fitness in older adults (Simonsick, Fan and Fleg, 2006; Pettee Gabriel *et al.*, 2010) and has linked performance to mortality, cardiovascular disease, mobility limitation, and mobility disability (Newman *et al.*, 2006). The test was performed over a 20-meter course, with the instruction to walk as fast as possible and with verbal encouragement in form of lap progression during the walk. Afterwards, leg extensor power was assessed on each leg in a Nottingham leg extensor power rig (University of Nottingham, Queen's Medical Centre), similarly to previous studies (Bassey and Short, 1990; Blackwell *et al.*, 2009; Aadahl *et al.*, 2011; Suetta *et al.*, 2019). Two submaximal repetitions were used as warm-up, whereafter maximal extensor power (force x velocity, Watt) was determined as the highest value of a minimum of 5 attempts, with rest in between attempts. Participants were instructed to kick as hard and fast as possible. To further evaluate strength and endurance in the lower extremities, on a standardized chair participants performed the 30-second chair-stand test, previously reported as a valid indicator of lower body strength in active, community-dwelling older adults (Jones, Rikli and Beam, 1999). Strength was further assessed by measures of handgrip strength, widely considered a reliable measure of overall muscle strength as well as health and functional status (Bohannon and Schaubert, 2005; Rijk *et al.*, 2016; McGrath *et al.*, 2020). Maximal handgrip strength (kilograms), measured with a SAEHAN DHD-1 Digital Hand dynamometer, was determined as the peak value of a minimum of 3 attempts on each hand. Attempts were performed with verbal encouragement from the test personnel and each measurement lasted 3-5 seconds. Participants were seated in an armchair in a standardized position as previously described (Aadahl *et al.*, 2011). Finally, isometric leg strength was assessed in a Good Strength chair with the complementary device (Bluetooth V.3.14, Metitur Oy). Maximal isometric quadriceps torque (Newton metres), with knees flexed at 70° and a strain-gauge fastened to the ankle by a velcro strap (see **Figure 4** for setup), was measured as the highest value of at least three 5-second contractions on each leg, with 30 seconds of rest between attempts. Like handgrip strength, participants were verbally encouraged during the test. Similar procedures for this specific device have previously been described, in which maximal isometric thigh muscle strength was measured in healthy older adults (Tiainen *et al.*, 2004; Bieler *et al.*, 2014).

Figure 4. Setup for measuring leg muscle strength.

Day 3

The final day took place at Hvidovre Hospital, at the Danish Research Centre for Magnetic Resonance. Participants were again encouraged to avoid vigorous physical activity in the preceding 72 hours. Magnetic resonance imaging (MRI) of the brain and thigh were performed by an experienced radiographer, with participants lying in a supine position in a 3.0 Tesla scanner (TX Phillips Achieva Scanner, Philips Healthcare). Brain MRI consisted of 3D T1-weighted, 3D T2-weighted, 3D Flair, resting-state fMRI, brain perfusion (ASL), and diffusion-weighted imaging. Thigh MRI (see **Figure 5**) was 2D T1-weighted with slices above tibia plateau for delineation of m. vastus lateralis. Region of interest (ROI) was on the mid slice, 20 cm above tibia plateau.
Some individuals did not participate in the MRI due to contraindications, such as pacemakers, metallic components, or claustrophobia.

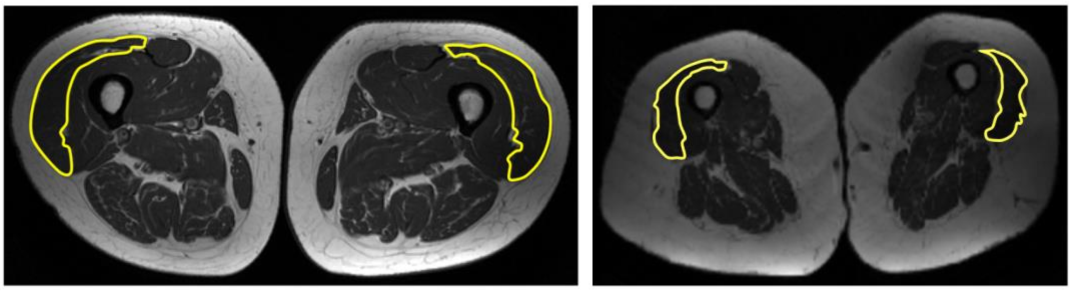

Figure 5. MRI of thighs from two different individuals. ROI, m. vastus lateralis, are highlighted.

Follow-up

After the 1-year intervention, all participants were again tested with identical procedure as the baseline testing apart from the AFRIS questionnaire that was only administered at the beginning of the study since it consisted of questions about the attitude towards the intervention. To be able to identify intervention-induced changes tests-days were planned in good time and as close as possible to intervention-stop (the last training session for individuals in the two training groups). The second test day, including the DXA-scan, was held on average 4.7 ± 0.2 days after intervention-stop, while the corresponding number for the third MRI-day was 12.1 ± 0.6 days after intervention-stop. When the intervention was stopped, all individuals, irrespective of intervention group or adherence to training, were left on their own with no restrictions or official guidance to training or lifestyle changes.

At year 2 and 4 (1 and 3 years after the intervention-stop), the participants were invited for similar assessments as before. Two extra questionnaires regarding continued adherence to the intervention and motivation for exercise (BREQ-2 (Markland and Tobin, 2004)) were added. The former questionnaire was developed singlehandedly for the LISA study, by the original authors of the project, and was used to identify to which extent (frequency and duration) the participants in the two training groups had continued with the intervention-specific training programs, i.e., HRT or MIT, on their own and whether such training was accompanied or replaced by any other form of training (Eriksen *et al.*, 2016). Information from this questionnaire provided an additional opportunity to assess training 'continuation' and whether this had any particular effects.

In addition, after each follow up testing occasion (years 1, 2, and 4), the participants received standardized digital feedback of selected measures to be informed of their own progress.

1- and 2-year results

At year 1, post-intervention, resistance training with heavy loads, compared with the moderate intensity training and the non-exercising control group, improved several muscular, mental, and bodily outcomes (Gylling, Eriksen, *et al.*, 2020). Further, at the 2-years follow-up, muscular leg strength was maintained only in the group that had performed heavy resistance training, and not moderate training, whereas other beneficial effects of the training were not maintained (Gylling, Bloch-Ibenfeldt, *et al.*, 2020). See **Table 2** for a summary of the previous findings.

	1-year (post-intervention)	2-year
Muscle	Leg extensor power ↔	Leg extensor power ↔
	Isometric leg strength – HRT ↑↑ Isometric leg strength – MIT ↑	Isometric leg strength – HRT ↑ Isometric leg strength – MIT ↔
	Handgrip strength ↓	Handgrip strength ↓
	CSA of m. vastus lateralis – HRT ↑ CSA of m. vastus lateralis – CON ↓	CSA of m. vastus lateralis – HRT ↔ CSA of m. vastus lateralis – CON ↓
	Lean body mass – HRT ↑	Lean body mass – HRT ↔
Brain	Hippocampus volume ↓	Hippocampus volume ↓
Bone	-	-
Other	Whole body fat % - HRT ↓	Whole body fat % - HRT ↔
	Visceral fat – HRT ↓	Visceral fat – HRT ↔
	Chair stand test ↑	Chair stand test ↑
	400m walking time ↔	400m walking time ↔
	Mental well-being (SF-36 questionnaire: mental summary score) – HRT ↑	Mental well-being (SF-36 questionnaire: mental summary score) ↓
	Blood pressure ↓	Blood pressure ↓
	HbA1c ↑	HbA1c ↑

Table 2. Results from 1- and 2-years follow-ups. Adapted from Gylling, Eriksen, *et al.*, 2020 and Gylling, Bloch-Ibenfeldt, *et al.*, 2020. Changes are compared with results at baseline.

HRT: heavy resistance training; MIT: moderate intensity training; CON: non-exercising control group.

4-year analysis

This thesis includes the data of the 4-year follow up, which is described in detail below.

MRI

Images were used for quantification of the cross-sectional area (CSA) of m. vastus lateralis (see **Figure 5**), which was estimated by blinded assessors using JIM software (Xinapse systems). Additionally, the brain was segmented into selected ROI using FreeSurfer version 6.0 software for determining volume of regions: brain grey matter, white matter hyperintensities, and hippocampus. The 'caudal-middle-frontal' ROI was used for dorsolateral prefrontal cortex (dlPFC) volume (Nyberg *et al.*, 2023) and the combination of 'pars opercularis', 'pars orbitalis', and 'pars triangularis' ROIs for ventrolateral prefrontal cortex (vlPFC) volume (Jonasson *et al.*, 2017). See **Figure 6A-B** for examples of brain MR images. For all regions, the sum of left and right hemispheres was used.

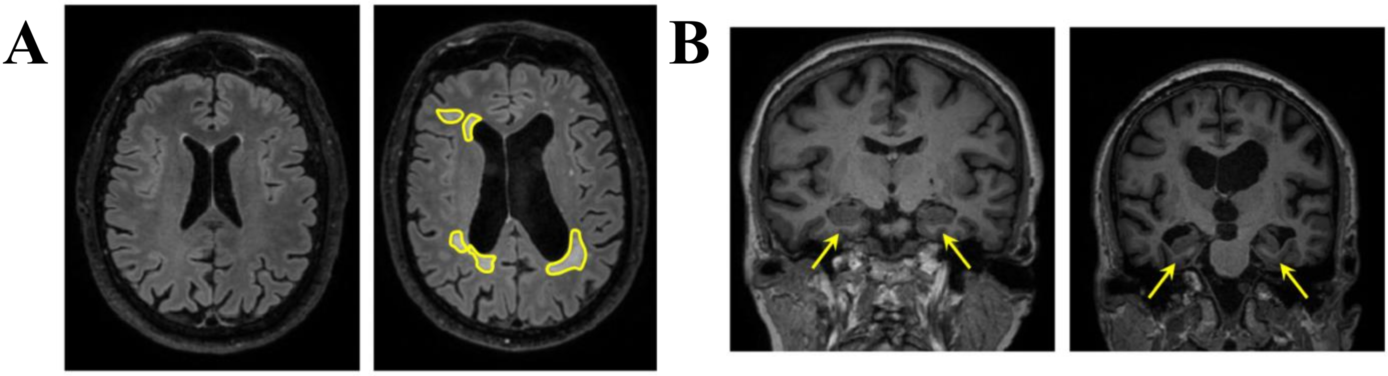

Figure 6. Brain MRI of individuals in the LISA study. **A** Examples of two participants with either small (left) or large (right) white matter hyperintensity volume. Hyperintensities are highlighted. **B** Hippocampus in two participants. Hippocampal atrophy in the image to the right.

DXA

ROI on the DXA images, see **Figure 7**, were automatically determined by scanner software. Additionally, T- and Z-scores were provided, which were used to interpret the results in line with WHO definitions (Blake and Fogelman, 2007). After the last 4-year follow-up had been completed, all scan images were manually re-adjusted for standardization by the same person.

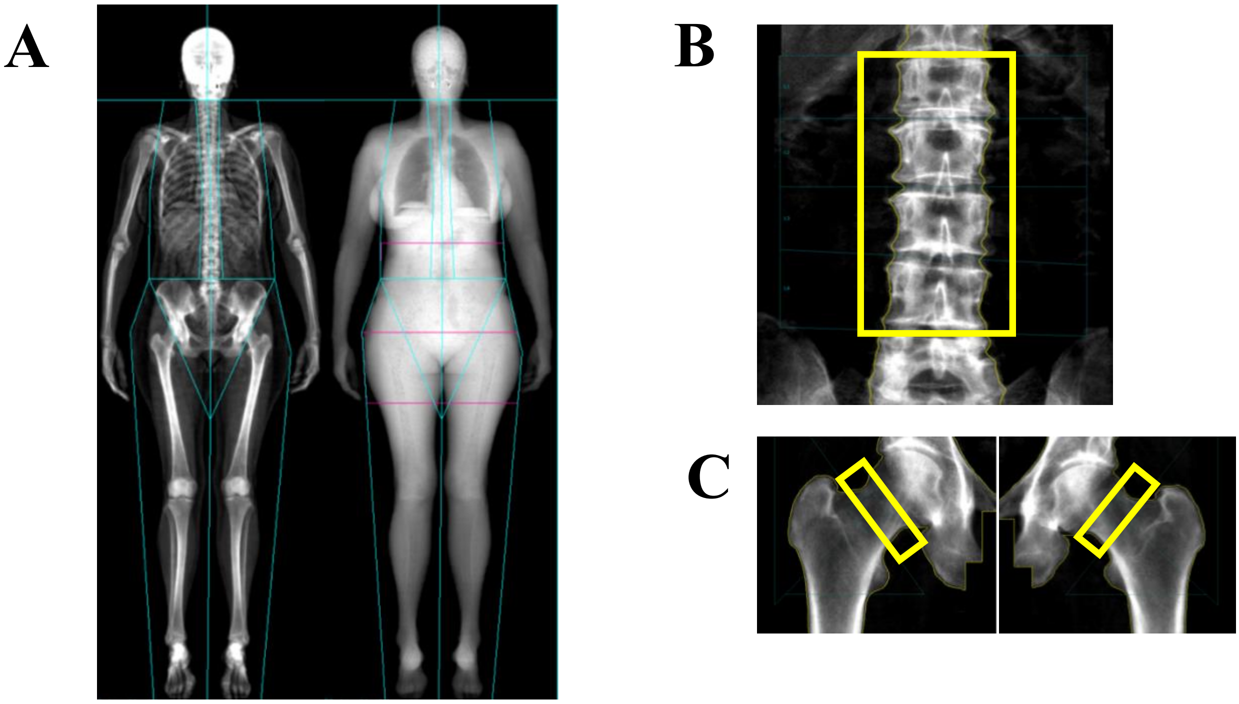

Figure 7. Images from DXA-scans of a study participant. **A** Full-body scan. General regions of interest are delineated. **B** Lumbar spine scan. ROI, lumbar vertebrae L1-L4, is highlighted. **C** Right and left femur. ROI, femoral necks, are highlighted.

Blood samples

Plasma blood samples from all four timepoints were analyzed for CTX, PINP, and dp-ucMGP at the Department of Clinical Biochemistry, Rigshospitalet, Glostrup, Denmark.

The following assays were used to measure plasma CTX and plasma PINP, and biochemically determine vitamin K status: IDS-iSYS CTX (CrossLaps®) assay (Immunodiagnostic Systems, plc, Tyne and Wear, UK), IDS-iSYS intact P1NP assay (Immunodiagnostic Systems), and IDS-iSYS Ina*K*tif MGP assay (Immunodiagnostic Systems) respectively. All three assays were carried out on a dedicated automated analyzer, iSYS (Immunodiagnostic Systems), according to the manufacturer's instructions. Samples had not previously been thawed and were kept frozen at -80°C until analysis.

dp-ucMGP is reportable in the range 300–12.000 pmol/L, with values below 300 pmol/L fixed to 299 pmol/L as previously suggested (Jespersen *et al.*, 2020). CTX is reportable in the range of 33-12.000 ng/L, with values below 33 ng/L fixed to 0 ng/L.

Methodological considerations

The LISA study was conceptualized to the context of a real-life setting where there was no guidance to training or lifestyle after the first year of intervention. This was done to provide a public health perspective, in which it was possible to assess how 1 year of training could translate to a change in an older adults' daily routines and lifestyle. Compared with other resembling studies, where sample sizes have generally been much smaller, the LISA study was a large-scale attempt to induce positive effects using a resistance-exercise based lifestyle change.

The regular project updates and particularly individual feedback on results offered to the participants has been, in my opinion, a key component for a project of this scale and length (up to 10 year), which have kept participants more invested in the study as well as in their own health. In addition, this has likely been a benefit for the study shown by the relatively modest dropout rates during the, so far, four years. However, this could potentially also have influenced participant behavior and results during the follow-up periods.

Initially, the primary brain metric for the analysis of the 1- and 2-year brain structural data was hippocampal volume. Previously it has been shown that exercise has a positive effect on hippocampal volume in older populations (Wilckens *et al.*, 2021), and that hippocampus is important in healthy brain aging (Driscoll *et al.*, 2003; Bussy *et al.*, 2021). In the present thesis, I wanted to acknowledge that aging influences the brain beyond only the hippocampi (see for example Fjell et al., 2009; Nyberg et al., 2023 for studies on whole brain atrophy in relation to aging), and that prefrontal cortex volume (Jonasson *et al.*, 2017) and white matter hyperintensities (Torres *et al.*, 2015) have previously also been linked with fitness. Therefore, in addition to hippocampal volume, I have added these brain structures and variables in the analysis.

For the DXA, scans of left and right femur provided several ROIs (e.g. total hip, Ward's triangle). Here, femoral neck was chosen as the desired ROI. Studies have shown that BMD at the femoral neck is better at predicting hip fractures (Cummings *et al.*, 1993) and is linked to osteoporosis-associated fractures (Warriner *et al.*, 2011). Moreover, scores at the femoral neck are generally used for classification of osteoporosis (Kanis *et al.*, 2008; Sözen, Lale and Başaran, 2017).

Ethics

All participants gave written informed consent before participating in the LISA study. The regional ethics committee approved the study (Capital Region, Copenhagen, Denmark, No. H-3-2014-017), which was also registered on clinicaltrials.gov (NCT02123641).

Statistical analysis

All statistical analyses were performed in R version 4.1.1 and Rstudio 2021.09.0. Figures and tables were created in GraphPad Prism version 10.0.3, Microsoft® Word version 16.83, and Microsoft® PowerPoint version 16.82.

General

Descriptive data are presented as Mean ± SD.

Individuals who completed testing at year 4 were included in the analyses. Sample sizes and number of datapoints in specific measures differed between studies, due to e.g. technical errors, specific medications influencing the results, or contraindicators, with the exact number of participants for each analysis specified in figures, tables, and text.

Potential changes over four years in the general participant characteristics were assessed with student's paired t-tests. When the groups additionally were analyzed separately, two-way mixed-model ANOVAs were used to test group differences. For the assessment of baseline differences between study-participants and dropouts, one-way mixed-model ANOVAs were used. Assessing differences in the response to the intervention, between study-participants and individuals lost-to-follow-up after year 1, two-way mixed-model ANOVAs (group (still in the study vs lost-to-follow-up) x time) were used.

ANOVA analyses were controlled, in the models, for sex and age, and in study 2 additionally for estimated total intercranial volume. All significant interactions were examined with post hoc tests (Tukey). The overall significance level was set at $p < 0.05$ and corrected (Bonferroni) within each study. Effect sizes are reported as η^2 for ANOVAs and r^2 for regression analyses.

Study specifics

To study the longer-lasting effects of supervised resistance training with heavy loads, on measures of strength, body composition, and physical function in study 1, including all 4 timepoints in the analysis, two-way mixed-model ANOVAs were used to test for group x time interactions. One-way mixed-model ANOVAs were used to assess group differences in Δchanges between timepoints. After correcting for multiple comparisons (Bonferroni), significance level was $p < 0.006$ (8 tests).

For brain structural outcomes, in study 2, two-way mixed-model ANOVAs tested group x time interactions on volumes of brain grey matter and white matter hyperintensities, while group differences in Δchanges between timepoints were analyzed with one-way mixed-model ANOVAs. Change-change associations (change from baseline to year 4) were tested with regression analysis. Significance level was corrected to $p < 0.01$ (5 tests).

To study the effect of resistance training on bone health, in study 3, two-way mixed-model ANOVAs were used to test for group x time interactions on measures of BMD, bone turnover, and dp-ucMGP. One-way mixed-model ANOVAs were used to assess group differences in Δchanges between timepoints. Within each sex separately, changes over time in the bone-related outcomes were tested for with one-way mixed-model ANOVAs. Change-change associations (change from baseline to year 4) between measures of bone and muscular strength were tested with regression analysis. Significance level for the bone-related outcomes was corrected to $p < 0.005$ (10 tests).

To test for group x time interactions on performance in functional tests two-way mixed-model ANOVAs were used. For the effects of engaging in regular resistance training beyond the intervention, two-way mixed-model ANOVAs tested group (Continued vs Stopped) x time interactions. 'Continued' was defined as regular (≥2 times/week) resistance training, which included continuing the LISA intervention programme or performing other kinds of resistance training, for the combination of 10-12 months between years 1 and 2 and 19-24 months between years 2 and 4. 'Stopped' included all other participants who had completed the questionnaires.

RESULTS

Participants

In total, 369 older adults participated in the 4-year follow-up. 82 participants had dropped out of the study, primarily due to illness or lack of motivation. Dropout rate between the three intervention groups was roughly the same (individuals participating at year 4: HRT, n = 128; MIT, n = 126; CON, n = 115) – see **Figure 2** for specifics in the flow chart. Individuals that dropped out had higher body weight, BMI, waist circumference, and systolic blood pressure at baseline compared with those that were still part of the study at the 4-year follow-up, while there were no differences in diastolic blood pressure, total cholesterol, hemoglobin A1c or C reactive protein. However, there was no difference in the response to the intervention at year 1 assessments between those who had dropped out at year 4 (individuals lost-to-follow-up post-intervention) and those who stayed in the study, as changes in general characteristics (body weight, BMI, waist circumference, and daily physical activity) and clinical parameters (blood pressure, cholesterol, hemoglobin A1c, C reactive protein), as well as physical function and body composition outcomes did not differ between these subsets of individuals.

At year 4, the participants were 71 ± 3 years on average, 61% women. Compared to baseline there were no changes to bodyweight, BMI, waist circumference, or daily physical activity level, which was still high with almost 10,000 steps/day. See **Table 3** for details. Participant characteristics did not differ between groups at baseline or at the 4-year follow-up.

Clinical outcomes

At study start, ≈ 86% had at least one chronic disease (compared to the ≈ 80% that was reported for individuals included in the analysis of the intervention-response (Gylling, Eriksen, *et al.*, 2020)). At year 4, this number had increased, with ≈ 92% of the participants having at least one chronic disease. Over the four years total cholesterol decreased equally across all groups (Baseline: 5.8 ± 1.0 mmol/L, 4-year: 5.3 ± 1.1 mmol/L, $p < 0.001$). Hemoglobin A1c and C reactive protein did not change. Blood pressure (systolic – Baseline: 143 ± 17 mmHg, 4-year: 140 ± 18 mmHg, $p = 0.004$; diastolic – Baseline: 85 ± 10 mmHg, 4-year: 83 ± 11 mmHg, $p < 0.001$) decreased from baseline to year 4, mostly driven by positive changes in the control group.

	Baseline	4-year follow-up	T-test
Sex (men/women, %)	39/61	39/61	-
Age (years)	66.4 ± 2.5	70.5 ± 2.5	-
Weight (kg)	75.7 ± 13.6	75.3 ± 14.0	p = 0.09
BMI (kg/m^2)	25.8 ± 4.0	25.9 ± 4.3	p = 0.10
Waist circumference (cm) (n = 367)	92.7 ± 11.5	92.5 ± 12.2	p = 0.66
Daily physical activity (steps/day) (n = 349)	9548 ± 3446	9590 ± 3387	p = 0.79

Table 3. Participant characteristics (mean ± SD), n = 369 unless otherwise specified.

Study 1

There was a significant group x time interaction for isometric leg strength ($F_{6,1049} = 8.607$, $p < 0.001$, $\eta^2 = 0.05$; **Figure 8A**). HRT had maintained leg strength over the 4 years (0 yr: 149.7 ± 51.5 Nm, 4 yr: 151.5 ± 51.1 Nm, t(1050) = 1.005, p = 1.00), whereas leg strength was decreased in CON (0 yr: 144.4 ± 43.3 Nm, 4 yr: 134.5 ± 42.2 Nm, t(1050) = -5.261, $p < 0.001$). Leg strength also decreased in MIT, but this was not significant (0 yr: 147.6 ± 54.9 Nm, 4 yr: 142.9 ± 54.6 Nm, t(1050) = -2.594, p = 0.28). The Δchange over four years significant differed between groups, in favor of HRT (HRT > MIT, t(350) = 3.273, p = 0.003; HRT > CON, t(350) = 3.655, $p < 0.001$).

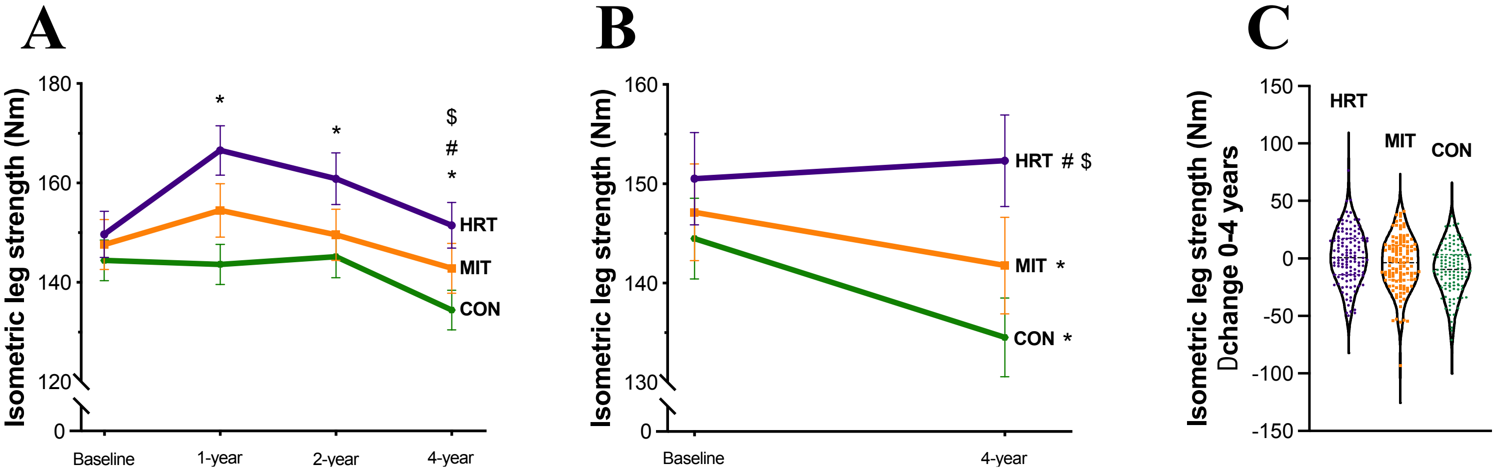

Figure 8. Isometric leg strength (Mean ± SEM) across 4 years for the different groups (heavy resistance training, HRT, moderate intensity training, MIT, and control, CON). **A** (n = 353) Isometric leg strength (Nm) trajectories for all timepoints separated by group. **B** Baseline and 4-year follow-up data (n = 362), each group shown separately. **C** Individual data points showing the distribution of change from baseline to year 4 separated by group.

*, significantly different from baseline (**A**: HRT 1-year, $p < 0.001$; MIT 1-year, p = 0.01; HRT 2-year, $p < 0.001$; CON 4-year, $p < 0.001$) (**B**: MIT 4-year, p = 0.01; CON 4-year, $p < 0.001$).

#, change from baseline significantly different from change in MIT (**A**: HRT 4-year, p = 0.003) (**B**: HRT 4-year, p = 0.03).

$, change from baseline significantly different from change in CON (**A**: HRT 4-year, $p < 0.001$) (**B**: HRT 4-year, $p < 0.001$).

When only baseline and 4-year values were considered (**Figure 8B**), muscle strength was maintained in HRT exclusively (t(124) = 1.98, p = 0.37). In MIT leg strength was decreased by 4% (t(122) = 1.98, p = 0.01), and in CON by 7% (t(113) = 1.98, $p < 0.001$).

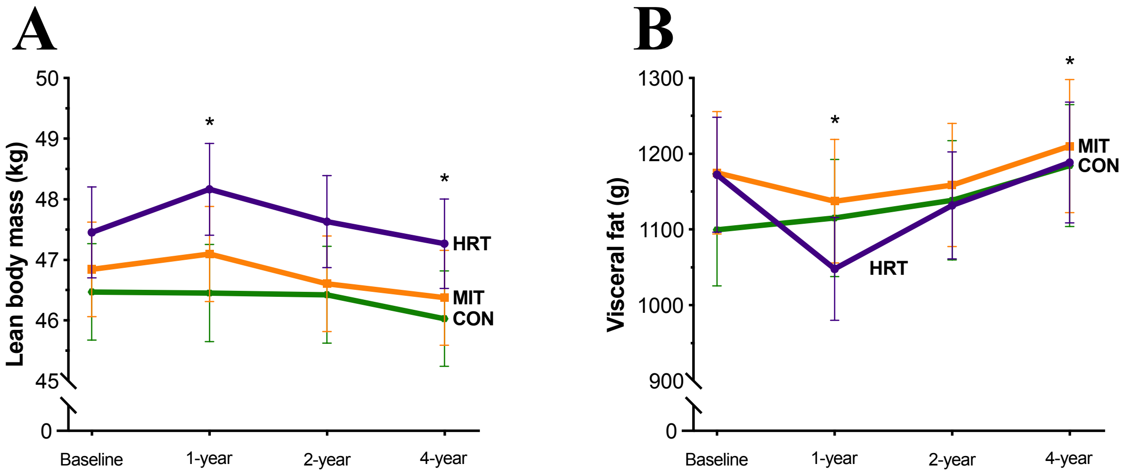

Figure 9. Lean body mass and visceral fat (Mean ± SEM) across 4 years for the different groups (heavy resistance training, HRT, moderate intensity training, MIT, and control, CON). **A** (n = 365) Lean body mass (kg) trajectories for all timepoints separated by group. **B** Visceral fat (g) trajectories (n = 365), for all timepoints separated by group. *, significantly different from baseline (**A**: HRT 1-year, $p < 0.001$; MIT 4-year, $p < 0.001$; CON 4-year, p = 0.003) (**B**: HRT 1-year, p = 0.01; CON 4-year, p = 0.04).

For lean body mass ($F_{6,1085} = 5.353$, $p < 0.001$, $\eta^2 = 0.03$) and visceral fat ($F_{6,1085} = 3.120$, p = 0.005, $\eta^2 = 0.02$) there were additional group x time interactions. Changes in lean body mass were again in favor of HRT (0 yr: 47.5 ± 8.5 kg, 4 yr: 47.3 ± 8.3 kg, t(1086) = -1.813, p = 0.81), compared with MIT (0 yr: 46.8 ± 8.7 kg, 4 yr: 46.4 ± 8.6 kg, t(1086) = -4.506, $p < 0.001$) and CON (0 yr: 46.5 ± 8.5 kg, 4 yr: 46.0 ± 8.5 kg, t(1086) = -4.075, p = 0.003), see **Figure 9A**.

Visceral fat is shown in **Figure 9B**. Fat content was unaltered over the four years in HRT (0 yr: 1172.4 ± 854.6 g, 4 yr: 1188.5 ± 898.3 g, t(1086) = 0.676, p = 1.00) and MIT (0 yr: 1175.0 ± 897.4 g, 4 yr: 1210.1 ± 972.9 g, t(1086) = 1.450, p = 0.95), and increased in CON (0 yr: 1099.6 ± 794.7 g, 4 yr: 1184.4 ± 862.5 g, t(1086) = 3.387, p = 0.04).

The significant group x time interactions for CSA of m. vastus lateralis and the percentage of total body fat (**Table 4**) were due to changes at years 1 and 2, with details reported in Gylling, Eriksen, *et al.*, 2020 and Gylling, Bloch-Ibenfeldt, *et al.*, 2020.

	HRT		MIT		CON		F (group x time)	F (time)
	0yr	4yr	0yr	4yr	0yr	4yr		
Power (W)	195.3 ± 65.1	185.3 ± 60.2	193.2 ± 64.0	179.4 ± 64.1	187.7 ± 61.8	171.2 ± 57.2	$F_{6,1067} = 1.054$ $p = 0.39, \eta^2 = 0.006$	$F_{3,1067} = 43.651$ $p < 0.001, \eta^2 = 0.11$
Handgrip (kg)	35.6 ± 10.5	34.3 ± 9.9	34.3 ± 10.4	32.7 ± 10.2	34.9 ± 10.2	32.8 ± 9.9	$F_{6,1064} = 0.554$ $p = 0.77, \eta^2 = 0.003$	$F_{3,1064} = 27.789$ $p < 0.001, \eta^2 = 0.07$
LLM (kg)	17.0 ± 3.3	16.3 ± 3.1	16.7 ± 3.5	15.9 ± 3.3	16.4 ± 3.4	15.7 ± 3.2	$F_{6,1085} = 1.841$ $p = 0.09, \eta^2 = 0.01$	$F_{3,1085} = 249.742$ $p < 0.001, \eta^2 = 0.41$
CSA (mm^2)	1403.7 ± 339.7	1306.2 ± 328.4	1371.4 ± 358.5	1284.3 ± 352.1	1359.5 ± 330.7	1243.1 ± 293.8	$F_{6,908} = 3.654$ $p = 0.001, \eta^2 = 0.02$	$F_{3,908} = 36.068$ $p < 0.001, \eta^2 = 0.11$
Body fat (%)	34.0 ± 7.9	33.8 ± 8.2	33.5 ± 7.6	33.7 ± 6.4	32.7 ± 8.3	33.1 ± 8.5	$F_{6,1085} = 3.813$ $p < 0.001, \eta^2 = 0.02$	$F_{3,1085} = 16.964$ $p < 0.001, \eta^2 = 0.04$

Table 4. Physical function and body composition variables (Mean ± SD) at baseline and at year 4 separated by group. Note: leg extensor power (Power), handgrip strength (Handgrip), lean leg mass (LLM), CSA of m. vastus lateralis (CSA), and total body fat (Body fat). F-statistics for group x time interaction and effect of time.

For leg extensor power, handgrip strength, and lean leg mass (**Table 4**) there were significant decreases over the 4 years, with no effect of group.

Study 2

For study two, n = 276, with individuals who had MRI at all 4 timepoints: HRT, n = 96; MIT, n = 95; CON, n = 85. Compared with the full sample, body weight significantly decreased over the four years in MIT (0 yr: 74.6 ± 11.9 kg, 4 yr: 73.8 ± 12.3 kg, p = 0.04) and tended to decrease in HRT (0 yr: 76.3 ± 13.0 kg, 4 yr: 75.5 ± 13.4 kg, p = 0.05), while in CON bodyweight did not change (0 yr: 74.4 ± 12.7 kg, 4 yr: 74.8 ± 13.8 kg, p = 0.40).

Figure 10A-E shows the group-by-group changes over 4 years in the specified brain regions. There were no group x time interactions, but significant main effects of time for all 5 regions. Volumes of total grey matter ($F_{3,819} = 231.549$, $p < 0.001$, $\eta^2 = 0.46$), hippocampus ($F_{3,819} = 310.07$, $p < 0.001$, $\eta^2 = 0.53$), vlPFC ($F_{3,818} = 74.380$, $p < 0.001$, $\eta^2 = 0.21$), and dlPFC ($F_{3,818} = 3.640$, $p = 0.01$, $\eta^2 = 0.01$) all decreased, while there was an increase in white matter hyperintensities ($F_{3,819} = 101.876$, $p < 0.001$, $\eta^2 = 0.27$).

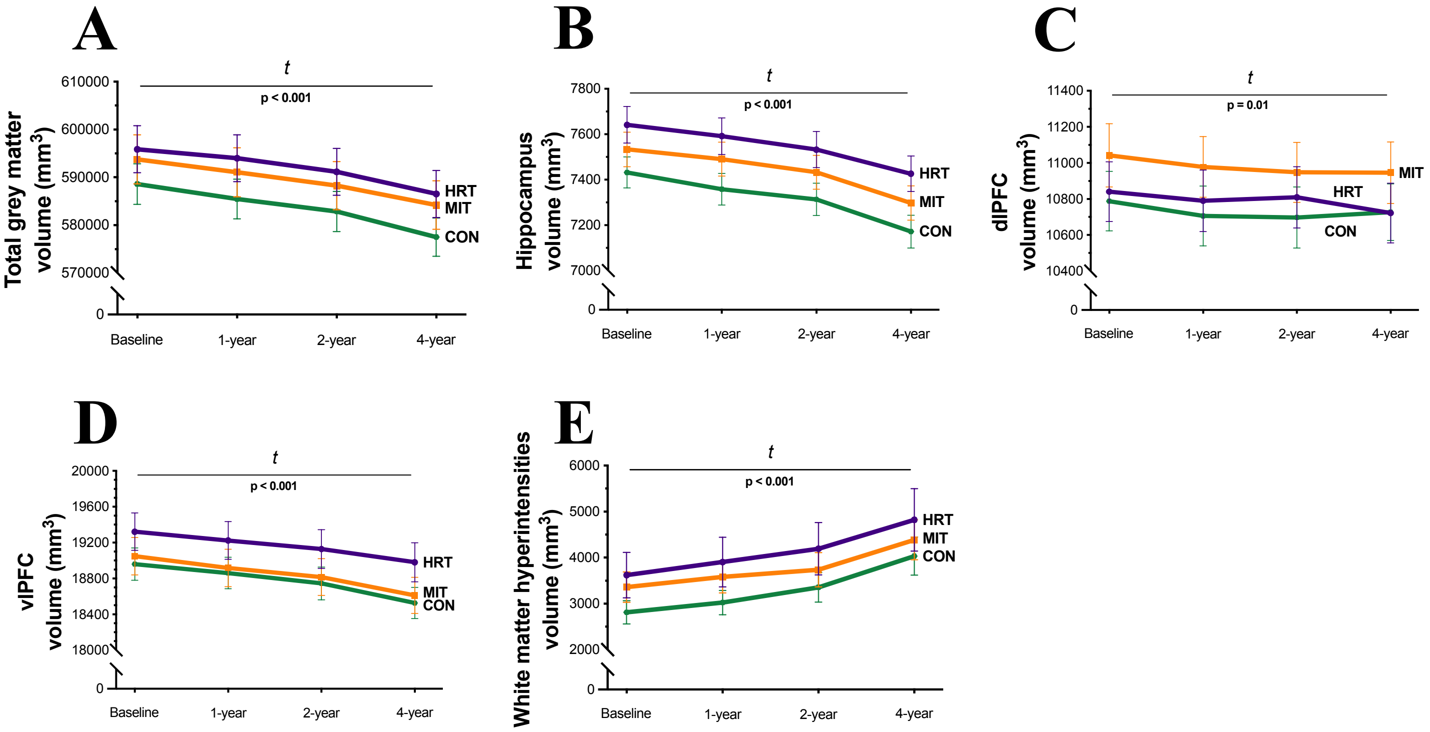

Figure 10. Brain structure volumes (mean ± SEM) for baseline, 1-year, 2-year, and 4-year, across each group. **A** Total grey matter volume (mm^3) **B** Hippocampus volume (mm^3) **C** Dorsolateral prefrontal cortex volume (mm^3) **D** Ventrolateral prefrontal cortex volume (mm^3) **E** White matter hyperintensities volume (mm^3).
t, significant effect of time (**A**, **B**, **D**, **E**: $p < 0.001$; **C**: $p = 0.013$).

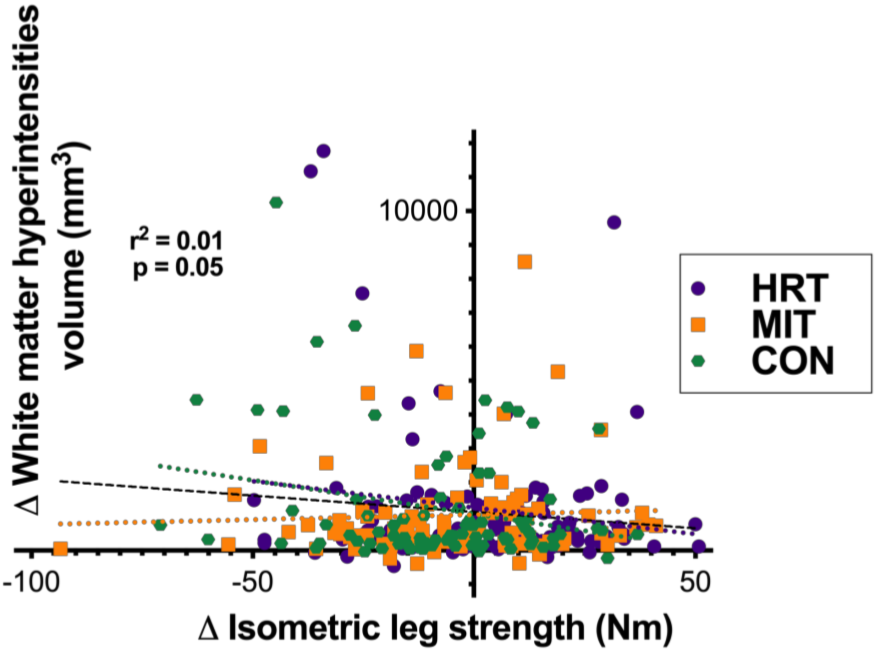

Figure 11. Association between Δ white matter hyperintensity volume (change from baseline to 4-year follow-up) and Δ isometric leg strength (change in leg muscle strength from baseline to 4-year follow-up in the dominant leg). HRT, heavy resistance training; MIT, moderate intensity training; CON, non-exercising control group. For each group, regression slopes are displayed with dotted lines in corresponding colors. The overall regression slope is displayed with a block dotted line.

Changes over the four years in leg muscle strength and white matter hyperintensity volume were negatively correlated ($r^2 = 0.01$, $p = 0.05$), see **Figure 11**. For each group separately, in CON changes were negatively correlated ($r^2 = 0.06$, $p = 0.03$). Changes in HRT ($r^2 = 0.03$, $p = 0.12$) and MIT ($r^2 = 0.002$, $p = 0.65$) were not correlated. Neither of the associations were significant after correcting for multiple comparisons ($p > 0.01$).

For other specified brain regions there were no correlations with leg strength changes.

Study 3

In this study, n = 329 (HRT, n = 116; MIT, n = 109; CON, n = 104). For these individuals, body weight slightly decreased from baseline to year 4 (Baseline: 76.2 ± 12.8 kg; 4-year: 75.8 ± 13.1 kg, $p = 0.03$). Compared with participants in the sample, individuals who subsequently dropped out had a poorer starting condition in some of the bone measures: whole body BMD ($F_{1,429} = 5.504$, $p = 0.02$, $\eta^2 = 0.01$) and femoral neck BMD in the dominant leg ($F_{1,417} = 5.609$, $p = 0.02$, $\eta^2 = 0.01$).

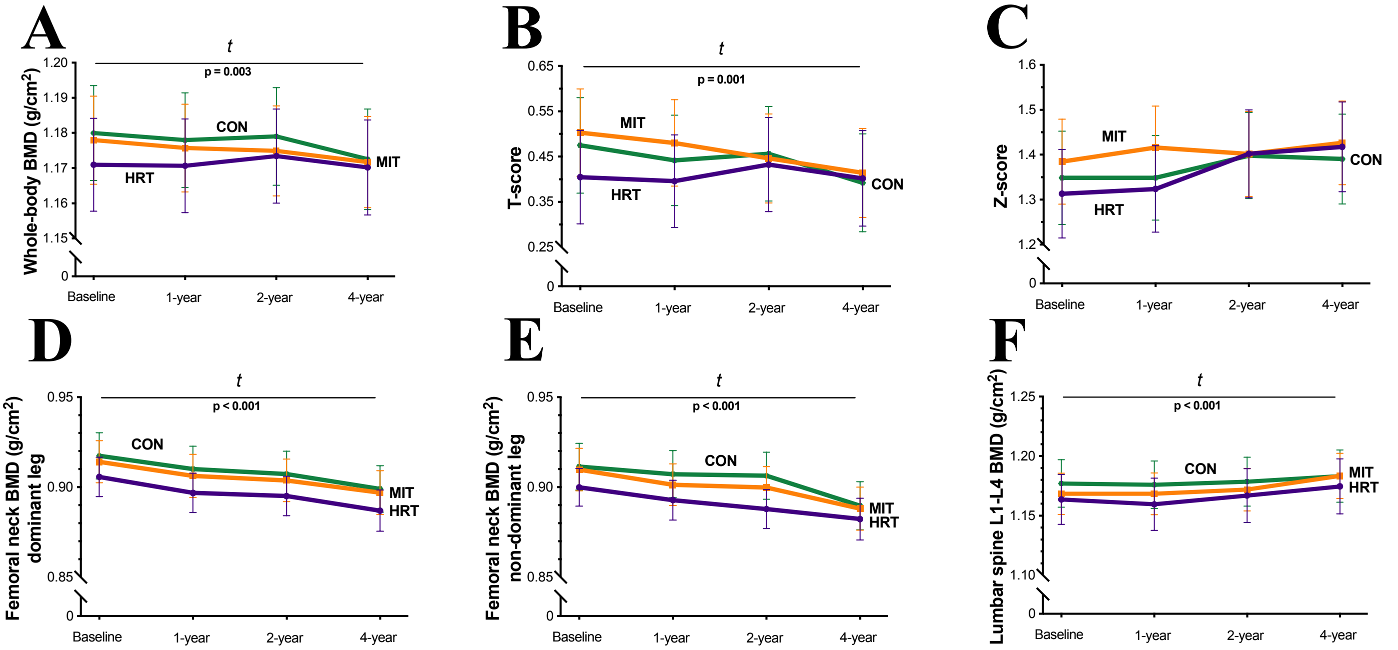

Figure 12. DXA-measures. Mean ± SEM for baseline (black bar - 0yr), 1-year (dark grey - 1yr), 2-year (grey - 2yr) and 4-year (light grey - 4yr), across each group: HRT, heavy resistance training; MIT, moderate intensity training; CON, non-exercising control group. **A** Whole-body bone mineral density (g/cm^2), n = 329. **B** T-score for whole-body bone mineral density, n = 329. **C** Z-score for whole-body bone mineral density, n = 329. **D** Bone mineral density (g/cm^2) in the femoral neck of the dominant leg, n = 301. **E** Bone mineral density (g/cm^2) in the femoral neck of the non-dominant leg, n = 302. **F** Bone mineral density (g/cm^2) in the lumbar spine (L1-L4), n = 251.

t, significant effect of time (**A**, p = 0.003; **B**, p < 0.001; **D**, p < 0.001; **E**, p < 0.001; **F**, p < 0.001) across all groups.

There were no significant group x time interactions for DXA bone measures. As per **Figure 12A-F**, instead there were main effects of time and equal changes across all groups. Whole-body BMD ($F_{3,977}$ = 4.617, p = 0.003, η^2 = 0.01), T-scores ($F_{3,977}$ = 5.499, p = 0.001, η^2 = 0.02), femoral neck BMD in the dominant leg ($F_{3,893}$ = 45.135, p < 0.001, η^2 = 0.13), and in the non-dominant leg ($F_{3,896}$ = 33.821, p < 0.001, η^2 = 0.10) all decreased over the four years. L1-L4 BMD ($F_{3,743}$ = 10.113, p < 0.001, η^2 = 0.04) and Z-scores ($F_{3,977}$ = 4.225, p = 0.006, η^2 = 0.01) increased, but the increase in Z-scores was not significant after correcting for multiple comparisons (p > 0.005).

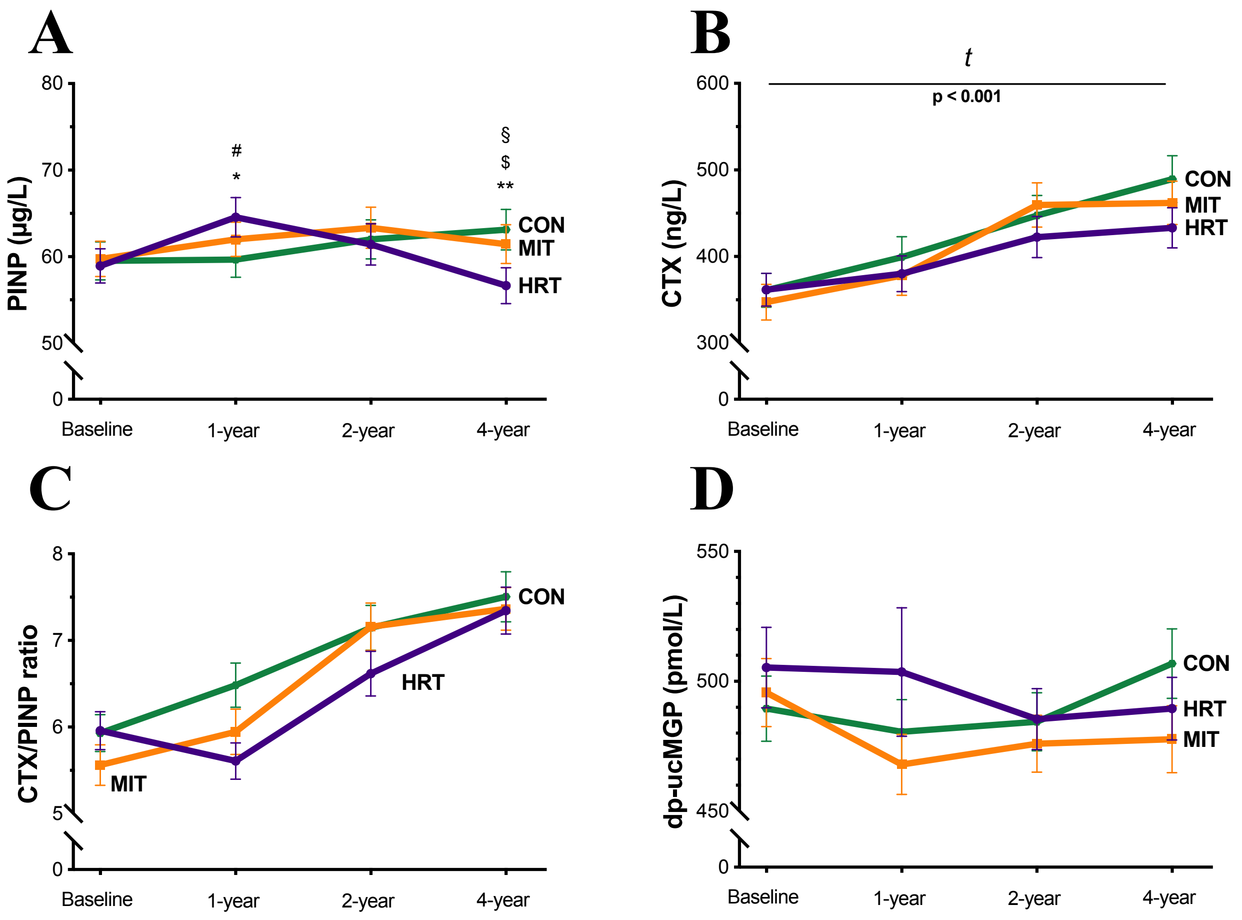

Figure 13. Mean ± SEM for baseline (black bar - 0yr), 1-year (dark grey - 1yr), 2-year (grey - 2yr) and 4-year (light grey - 4yr), for each group: HRT, heavy resistance training; MIT, moderate intensity training; CON, non-exercising control group. **A** Procollagen type I N-propeptide (PINP, µg/L), n = 315. **B** C-terminal telopeptide of type I collagen (CTX, ng/L), n = 314. **C** CTX/PINP ratio, n = 314. **D** dephosphorylated-uncarboxylated Matrix Gla-Protein (dp-ucMGP, pmol/L), n = 313.

t, significant effect of time (p < 0.001) across all groups. *, significantly different from baseline (HRT, p = 0.04). **, significantly different from 1-year (HRT, p < 0.001). #, increase from baseline to year 1 significantly bigger than in

CON (HRT, $p = 0.035$). $, decrease from year 1 to 4 significantly bigger than decrease in MIT (HRT, $p = 0.005$). §. decrease from year 1 to 4 significantly bigger than decrease in CON (HRT, $p < 0.001$).

For changes over time in bone markers, see **Figure 13A-D**. There was a significant group x time interaction for PINP ($F_{6,935} = 3.733$, $p = 0.001$, $\eta^2 = 0.01$), with an increase in HRT after the first year (HRT, Baseline: 58.9 ± 20.9 µg/L; 1-year: 64.6 ± 24.1 µg/L, $t(936) = 3.357$, $p = 0.04$). Additionally, a significant decrease was observed in HRT from year 1 to 4 (HRT, 1-year: 64.6 ± 24.1 µg/L; 4-year: 56.7 ± 21.7 µg/L, $t(936) = 4.723$, $p < 0.001$). For changes between groups, a larger increase was experienced in HRT than in CON during the intervention ($t(312) = 2.494$, $p = 0.04$). From year 1 to 4, the decrease in HRT was larger than in both MIT ($t(312) = -3.154$, $p = 0.005$) and CON ($t(312) = -4.751$, $p < 0.001$). CTX increased equally across groups over the four years ($F_{3,932} = 47.434$, $p < 0.001$, $\eta^2 = 0.13$). For the CTX/PINP ratio there was a group x time interaction ($F_{6,932} = 2.187$, $p = 0.04$, $\eta^2 = 0.01$), driven by changes in HRT at year 1. However, this interaction was not significant after correcting for multiple comparisons ($p > 0.005$).
Levels of dp-ucMGP did not change over time ($F_{3,931} = 1.530$, $p = 0.21$, $\eta^2 = 0.005$) and there was no group interaction ($F_{6,931} = 1.178$, $p = 0.32$, $\eta^2 = 0.008$).

Sex-specific values are displayed in **Table 5**. Men generally had larger values than women, and when sex-differences were analyzed separately, whole-body BMD, T-score, and Z-score all decreased with a main effect of time in women, but not in men. L1-L4 BMD increased with a main effect of time in men, with no change over time in women. For bone turnover markers, women had higher values than men for PINP ($F_{1,310} = 40.679$, $p < 0.001$), CTX ($F_{1,309} = 44.637$, $p < 0.001$), and CTX/PINP ratio ($F_{1,309} = 19.753$, $p < 0.001$). For men, a main effect of time was observed for PINP, with levels increasing over the four years, while in women the levels did not change over time. Levels of dp-ucMGP were not influenced by sex.

	Women					Men				
	0yr	1yr	2yr	4yr	F (time)	0yr	1yr	2yr	4yr	F (time)
Whole-body BMD (g/cm^2)	1.097 ± 0.101	1.094 ± 0.098	1.093 ± 0.102	1.088 ± 0.103	$F_{3,572} = 9.322$ $p < 0.001, \eta^2 = 0.05$	1.287 ± 0.101	1.287 ± 0.101	1.291 ± 0.101	1.289 ± 0.102	$F_{3,407} = 1.666$ $p = 0.17, \eta^2 = 0.01$
T-score	0.156 ± 1.000	0.136 ± 0.976	0.128 ± 1.010	0.073 ± 1.022	$F_{3,572} = 7.877$ $p < 0.001, \eta^2 = 0.04$	0.905 ± 1.038	0.880 ± 1.005	0.911 ± 0.993	0.880 ± 1.010	$F_{3,407} = 0.731$ $p = 0.53, \eta^2 = 0.005$
Z-score	0.094 ± 1.013	0.087 ± 0.979	0.085 ± 1.013	0.087 ± 1.024	$F_{3,572} = 2.749$ $p = 0.04, \eta^2 = 0.01$	1.295 ± 1.101	1.284 ± 1.023	1.339 ± 0.999	1.365 ± 1.014	$F_{3,407} = 2.251$ $p = 0.08, \eta^2 = 0.02$
Femoral neck BMD (g/cm^2) dominant leg	0.876 ± 0.105	0.868 ± 0.103	0.864 ± 0.104	0.854 ± 0.105	$F_{3,518} = 41.418$ $p < 0.001, \eta^2 = 0.19$	0.962 ± 0.118	0.954 ± 0.120	0.953 ± 0.117	0.950 ± 0.120	$F_{3,377} = 9.616$ $p < 0.001, \eta^2 = 0.07$
Femoral neck BMD (g/cm^2) non-dominant leg	0.868 ± 0.107	0.863 ± 0.106	0.862 ± 0.108	0.846 ± 0.105	$F_{3,521} = 41.768$ $p < 0.001, \eta^2 = 0.19$	0.957 ± 0.118	0.953 ± 0.118	0.949 ± 0.116	0.944 ± 0.125	$F_{3,377} = 5.129$ $p = 0.002, \eta^2 = 0.04$
Lumbar spine L1-L4 BMD (g/cm^2)	1.105 ± 0.149	1.099 ± 0.152	1.099 ± 0.156	1.101 ± 0.161	$F_{3,455} = 1.639$ $p = 0.18, \eta^2 = 0.01$	1.271 ± 0.172	1.275 ± 0.171	1.286 ± 0.174	1.305 ± 0.178	$F_{3,290} = 27.910$ $p < 0.001, \eta^2 = 0.22$
PINP (µg/L)	66.5 ± 22.1	67.0 ± 22.8	67.9 ± 24.8	65.6 ± 23.5	$F_{3,539} = 1.051$ $p = 0.37, \eta^2 = 0.006$	49.9 ± 15.3	55.7 ± 18.5	54.8 ± 20.7	53.2 ± 19.9	$F_{3,395} = 5.487$ $p = 0.001, \eta^2 = 0.04$
CTX (ng/L)	417.2 ± 212.8	433.2 ± 237.3	507.9 ± 256.2	536.9 ± 265.8	$F_{3,539} = 34.273$ $p < 0.001, \eta^2 = 0.16$	273.9 ± 153.9	320.1 ± 198.9	354.1 ± 206.9	356.2 ± 201.4	$F_{3,395} = 15.632$ $p < 0.001, \eta^2 = 0.11$
CTX/PINP ratio	6.2 ± 2.4	6.3 ± 2.5	7.4 ± 2.6	8.0 ± 2.6	$F_{3,539} = 43.969$ $p < 0.001, \eta^2 = 0.20$	5.3 ± 2.0	5.6 ± 2.5	6.4 ± 2.7	6.6 ± 2.7	$F_{3,395} = 15.857$ $p < 0.001, \eta^2 = 0.11$
dp-ucMGP (pmol/L)	497 ± 150	480 ± 124	483 ± 120	494 ± 126	$F_{3,536} = 2.342$ $p = 0.07, \eta^2 = 0.01$	496 ± 130	489 ± 242	481 ± 112	486 ± 138	$F_{3,393} = 0.328$ $p = 0.81, \eta^2 = 0.003$

Table 5. Sex-specific values (Mean ± SD) for DXA measures, bone turnover markers and dp-ucMGP. Women and men at baseline (0yr), and years 1 (1yr), 2 (2yr), and 4 (4yr).

Change-change associations (over the four years) for whole-body BMD and CTX/PINP ratio and femoral neck BMD of the dominant leg and isometric leg strength respectively are displayed in **Figure 14A-B**. Overall, both at baseline and as change-change, there was no association between whole-body or regional BMD and bone turnover markers, dp-ucMGP, or isometric leg strength.

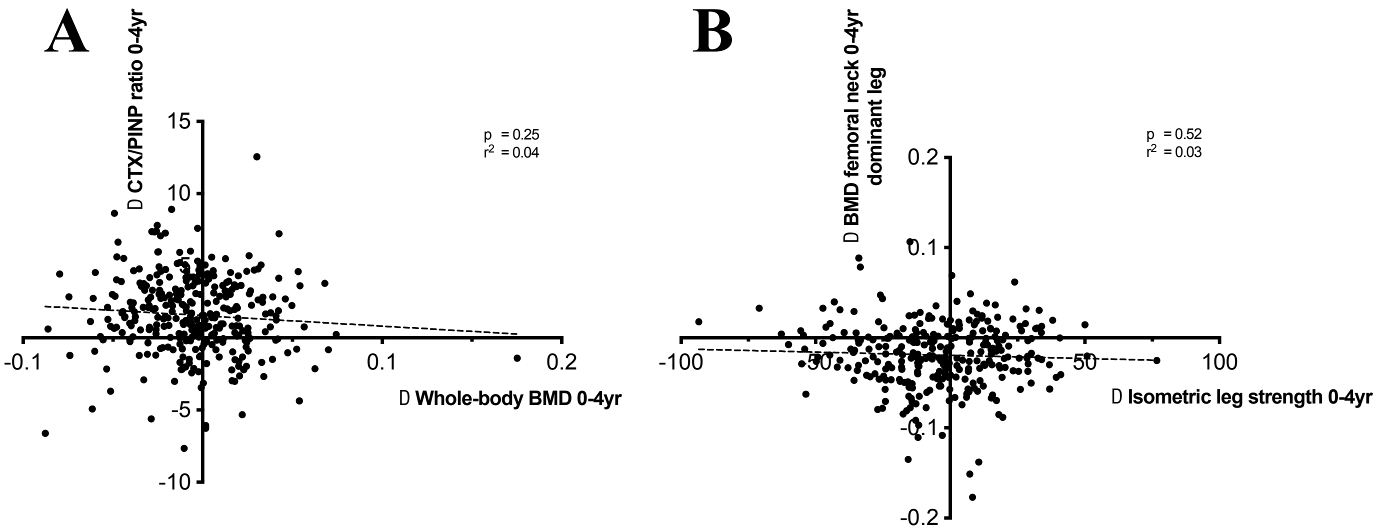

Figure 14. Associations between changes in bone turnover markers and strength from baseline to 4-year follow-up. In **A** Δ Whole-body BMD (g/cm^2) and Δ CTX/PINP ratio, n = 325. In **B** Δ isometric leg strength (Nm) and Δ femoral neck BMD (g/cm^2) in the dominant leg, n = 294. Plots include data from individuals across all groups. Linear regression slopes are displayed with a dotted line.

Functional tests

Performance in the chair-stand test, in which both training groups and the control group simultaneously had improved after the intervention, was still improved at year 4 with a main effect of time (0 yr: 17 ± 4 reps, 4 yr: 19 ± 5 reps, $F_{3,1055}$ = 116.438, $p < 0.001$, $\eta^2 = 0.25$). There was no interaction of group on performance ($F_{6,1055}$ = 1.796, p = 0.10, $\eta^2 = 0.01$).

Similarly for performance in the 400 m walking test, there was a main effect of time ($F_{3,1043}$ = 30.423, $p < 0.001$, $\eta^2 = 0.08$), with a slower time to complete at year 4 (0 yr: 240 ± 30 seconds, 4 yr: 243 ± 36 seconds). Again, there was no interaction of group ($F_{3,1055}$ = 1.085, p = 0.37, η^2 = 0.006).

Training ‘continuation’

Of the individuals in the two training groups, only 3% (n = 7) did not engage in any training (including all forms of training). ≈ 25% (n = 60) regularly participated in resistance training (≥2 times per week) over the 4 years. For the effect of training ‘continuation’ on isometric leg strength, in HRT and MIT respectively, see **Figure 15A-B**. There was an added effect of regular training, with a significant group (Continued vs Stopped) x time interaction for HRT ($F_{1,120} = 5.234$, $p = 0.02$, $\eta^2 = 0.04$), where strength was larger at year 4 for individuals who continued with the training (Continued 4 yr: 167.1 ± 47.2 Nm, Stopped 4 yr: 145.8 ± 51.8 Nm**,** $t(147) = -2.873$, $p = 0.02$). However, for those individuals in HRT who did not regularly participate in resistance training over the 4 years, strength did not drop from baseline to year 4 (Stopped 0 yr: 146.7 ± 53.1 Nm, Stopped 4 yr: 145.8 ± 51.8 Nm, $t(120) = -0.356$, $p = 0.98$). For individuals in MIT, there was a main effect of time with a significant decrease in strength ($F_{1,118} = 6.041$, $p = 0.02$, $\eta^2 = 0.05$), but there was no effect of regular resistance training. Overall, individuals in both HRT ($F_{1,119} = 5.83$, $p = 0.02$) and MIT ($F_{1,118} = 4.546$, $p = 0.04$) who regularly resistance trained were stronger than counterparts who did not regularly train.

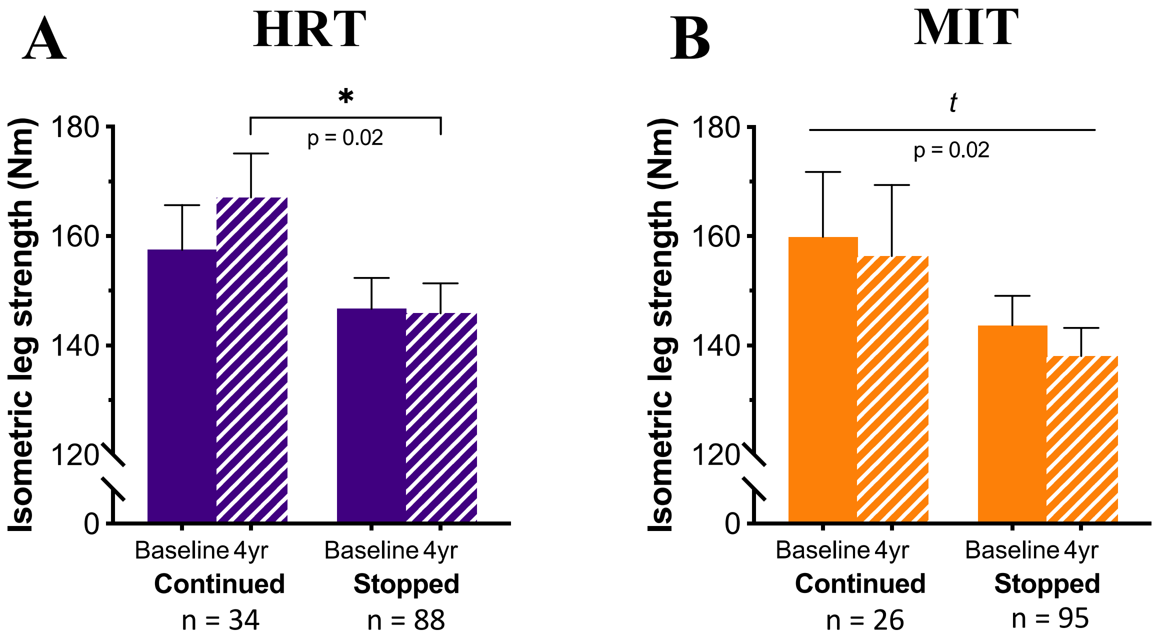

Figure 15. Isometric leg strength (Nm) (Mean ± SEM) at baseline and year 4 (4yr) separated by ‘continuation’ of regular resistance training beyond the intervention. In **A** HRT, heavy resistance training, n = 122. In **B** MIT, moderate intensity training, n = 121.

t, significant effect of time (p = 0.015) across Continued and Stopped. *, significantly different from Continued 4yr (**A**: *Stopped* 4yr, p = 0.02).

DISCUSSION

In this thesis, the long-term effects of engaging in resistance training have been examined. The main beneficial effect observed was a preservation of muscular leg strength from starting condition in the group that initially performed 1 year of resistance training with heavy loads. As opposed to the heavy resistance training, moderate training did not seem potent enough to induce long-lasting effects, which was contrary to the original hypothesis for the LISA trial (Eriksen *et al.*, 2016). The positive influence of HRT on muscular strength was, somewhat surprisingly, not accompanied by many other beneficial effects. In general, the 1 year of training had no longer-lasting effect on other strength and functional outcomes, brain volumes, or different bone measures. Instead, what was observed were expected age-related declines in bone health (Colón *et al.*, 2018) and grey matter volumes (Scahill *et al.*, 2003; Fjell *et al.*, 2009), as well as atrophy rates of hippocampal volume similar to what had been observed during the first two years in this cohort (Gylling, Bloch-Ibenfeldt, *et al.*, 2020; Gylling, Eriksen, *et al.*, 2020). The results, thus, highlights how difficult it indeed is to induce changes at multiple physiological levels in aging. The isolated benefit in strength should not be neglected in any ways, because there is growing evidence showing that independently of age and the length of follow-up period, muscle strength in both the upper and lower extremities is able to predict mortality in otherwise healthy populations (García-Hermoso *et al.*, 2018). The benefit in muscular strength will be a focal point of the discussion in relation to aging, but also in relation to other bodily systems studied, and more intervention-specific factors including when to intervene, what a resistance intervention should look like and whether one-size does fit all.

In the clinical perspective

Participants in this thesis were community-dwelling and by no means a clinical population, however, most individuals did have at least one chronic disease when they entered the study, and several clinically relevant markers (some of these were blood pressure and blood parameters (including markers of inflammation, as well as glucose and lipid metabolism)) have been measured over time. These measures in the LISA study are also interesting from a clinical perspective. There were some changes in cholesterol and blood pressure, but these were negligible and antihypertensives and antihyperlipidemics were generally the most commonly used medications among the participants. Markers for blood glucose, in the form of glycated hemoglobin, and inflammation did not seem to be affected during the four years. Inflammation is an interesting avenue for future studies in the cohort considering the link to healthy aging (Ferrucci and Fabbri, 2018), but a more sensitive measurement would be desirable, for example high-sensitivity C-reactive protein, which is able to detect smaller changes and lower levels of the protein, and as such is considered clinically helpful and an important instrument in cardiovascular risk assessment (Bassuk, Rifai and Ridker, 2004; Seo, 2012).

Body composition

General bodily assessments – bodyweight, BMI, and waist circumference – did not change over the four years, while differences in the total lean body changes were again negligible. Resistance training, both with heavy loads and at moderate intensity, was favorable for the visceral fat, as content was maintained from baseline at year 4. In contrast, an expected age-related increase was observed in the control group (Huffman *et al.*, 2009; Hunter, Gower and Kane, 2010). Although it must be noted that visceral fat content is estimated by DXA, concerns over the precision of estimates have predominately been raised in obese individuals (Shuster *et al.*, 2012). The positive influence on visceral fat is in line with research that has examined the more immediate training-related response, where resistance training, after it was initially thought that benefits were reserved for more endurance-based training, is suggested to benefit, and lower the amount of visceral fat in healthy adults (Wewege *et al.*, 2022).

Muscle strength in the long-term perspective

Whereas resistance training is known to greatly benefit muscular strength in the short run, here the results also showed that in the long-term perspective it was possible to preserve strength. Several muscle markers were measured, and as previously shown total and regional muscle size, as well as leg muscle strength were positively influenced by the intervention (Gylling, Eriksen, *et al.*, 2020), but only leg muscle strength differed between groups at year 4 (Study 1). Supporting this, another study, showed that resistance training can be beneficial on many levels and over many years, which was indicated by results of self-reported time in resistance training in older women who were comparable in age to those studied in this thesis. Assessed over an average of 12 years, in this study self-reported time was associated with decreased all-cause mortality (Kamada *et al.*, 2017). Given the enormous plasticity of skeletal muscle to adapt to a variety of stimuli (Bruton, 2002; Flück and Hoppeler, 2003), it was perhaps not surprising that in the present studies altogether the most prominent benefit of resistance training was observed in muscle strength. The HRT-induced preservation of leg muscle strength was present despite no such benefits in leg lean mass, with the latter decreasing over time in all three intervention groups. It has been known for many years that the response to resistance training, in addition to a subsequent increase in muscle size, is initiated and driven by neural adaptations (Moritani and deVries, 1979; Häkkinen and Häkkinen, 1995; Häkkinen *et al.*, 1998). Moreover, in a more recent review, it was concluded that there is good evidence for beneficial effects of resistance training on neural mechanisms in longitudinal intervention studies on resistance training naïve older adults (Walker, 2021), however, most of the included studies were of shorter duration (months). With changes in leg muscle strength, but not lean leg mass or CSA of m. vastus lateralis, the present results suggest that neural mechanisms may have played a role in the even longer time perspective, and that resistance training may benefit physical function without influencing muscle size. Supporting this, a recent study reported that lifelong recreational exercise was associated with permanently elevated acetylcholine receptors and improved neuromuscular function (Soendenbroe *et al.*, 2022).

Muscle trajectories

The trajectories for muscle strength in aging are thought to be well-known, but there is not a lot of data on actual longitudinal trajectories (Lucas *et al.*, 2020), with much of the knowledge coming from cross-sectional studies investigating age differences (Keller and Engelhardt, 2013; Suetta *et al.*, 2019). Even less is known on what can and needs to be done to change trajectories and what the long-term consequences are of a shorter-term improvement of strength. In one study, muscle strength was measured at a follow-up 48 months after a two-year training-intervention including resistance exercises had ended. Here, studying the combined effect of exercise (a combination of strength, balance, agility, and mobility) and vitamin D-supplementation on fall-induced injuries in older women, some exercise-induced benefits in physical function were preserved at the follow-up (Uusi-Rasi *et al.*, 2017). Of particular interest, along the lines of the benefit in leg muscle strength in study 1, despite only training twice and once per week, respectively, during the first and second year of the intervention, maximal isometric leg extensor strength was increased at a follow-up at 48 months in exercise groups compared with the non-exercising group that had received placebo. In terms of even longer follow-ups, one of the studies with the, to my knowledge, longest follow-up of a training intervention (1 year of training) involving resistance exercises, also showed beneficial effects in muscle performance many years (6 years) after intervention-stop (Kennis *et al.*, 2013).

The results, not surprisingly, indicated that age-related declines can only be attenuated for some years, but interestingly also that some outcomes had similar annual decline rates during the follow-up. In other words, it may not be a matter of attenuating age-related declines, but rather postponing these and changing the trajectories, similar to leg muscle strength in this thesis where trajectories were changed.

Physical activity and behavior beyond a training-intervention may naturally also influence long-term outcomes. In this thesis, leg muscle strength was originally assessed without considering training and behavior beyond the first year. However, one in four participants regularly did resistance training during all four years. Thus, to maintain muscle strength from baseline the HRT intervention was sufficient, but those that continued with resistance training (in HRT) were at an even higher level at year 4.

Strength in relation to other systems of the body

In my thesis, the underlying assumption was that gains in muscle strength would be the key ingredient that would further induce positive effects to other parts of the body, including brain structure in study 2 and bone measures in study 3.

Brain structures

Studies of resistance training on brain structure have in general been limited and yielded mixed findings. Some studies have reported no positive influence of prolonged (up to 24 months) resistance training on brain health in older adults (Sink *et al.*, 2015; Gylling, Eriksen, *et al.*, 2020), whereas other studies (primary and secondary analyses from the same RCT) have supported the influence of resistance training (Liu-Ambrose *et al.*, 2010, 2012; Best *et al.*, 2015). In a systematic review, it was concluded that there was evidence supporting an association between brain structure and muscle structure (Kilgour, Todd and Starr, 2014), but the available literature is limited. This potential link between muscle and brain has further been supported by studies showing a long-term relationship between physical function and brain structure (Demnitz et al., 2017, 2023; Mendez Colmenares et al., 2021). At the molecular level, the muscle-brain crosstalk becomes even more evident with the release of exercise-induced myokines (Matthews *et al.*, 2009; Erickson *et al.*, 2011; Pedersen, 2019). Since myokine expression was not measured in the present cohort, it was not possible to conclude on underlying mechanisms or whether the resistance training had actually benefited myokine release. Nevertheless, levels of BDNF and other myokines are thought to increase with training in general, and some, but not all, resistance training interventions have been able to measure increased myokine levels, although so far this has predominately been in short-term interventions with durations of months and of low to moderate intensity (Wang *et al.*, 2023). In one of the first studies that investigated whether hippocampus would show the same plasticity with aerobic training in older adults as previously reported in younger adults, large individual variabilities in the fitness- and brain-related responses to a 3-month aerobic intervention were observed (Maass *et al.*, 2015). If myokines mechanistically do mediate this interplay between muscle and brain, and considering the individual and interindividual variability that is observed, a better understanding of the chain of events is needed. Whether there is a link at all, may, however, not be what matters the most. Instead, it is more whether resistance training can induce beneficial changes.

Contrary to hypothesized, the results did not support an association between muscle adaptation (leg muscle strength) and brain grey matter volumes. However, for white matter hyperintensities, as originally hypothesized, there may be a link to skeletal muscle, considering the negative change-change correlation between progression of hyperintensities and strength. Even though this weak correlation was not significant after correcting for multiple comparisons, this potential link between muscle and brain warrants further investigation, considering the great prevalence of white matter hyperintensities in older adults (Montero-Odasso, 2023) and the association with many negative health outcomes (Debette and Markus, 2010). Further, at least with aerobic exercise, studies have shown that exercise may influence white matter structural integrity in older adults (Sexton *et al.*, 2016; Mendez Colmenares *et al.*, 2021) and in a recent study white matter hyperintensities were proposed as a limiting factor for exercise-induced neuroplasticity (von Cederwald *et al.*, 2023). Importantly, hyperintensities have also been related to impaired gait performance and especially falls in older adults (Zheng *et al.*, 2012; Montero-Odasso, 2023). Highlighting falls, a review of reviews, where barriers and motivators for engaging in strength training were investigated in older adults, established decreasing the risk or fear of falling as a motivation for participating in resistance training (Cavill and Foster, 2018). Falls, including the prevalence of these, is an area of interest, that should be addressed in the present cohort going forward.

Bone mineral density and bone turnover

In view of the inconclusive resistance training-specific literature on bone mineral density, but with exercise (weightbearing exercise with impact) for many years recommended for bone health (NIH Consensus Statement, 2000), it was reasonable to speculate whether positive effects at the muscular level could also somehow translate into effects at the bone level. Such a coupling had even been observed previously in a Korean longitudinal study of community-based older adults ≥ 65 years, where muscle strength in both sexes was the most significant independent factor associated with bone loss at a follow-up 5 years from baseline (Kim *et al.*, 2018). However, the present results did not indicate any association between muscle and actual bone measures. For markers of bone turnover, the results showed greater promise, with the beneficial HRT-related response in bone formation (PINP) – sadly this was not carried on to longer-lasting effects. These findings are somewhat in line with results from other studies, where resistance training (durations of up to 16 weeks) has shown a potential, alongside increasing BMD and muscular measures, to increase at least some markers of bone turnover (Sartorio *et al.*, 2001; Karabulut *et al.*, 2011; Huovinen *et al.*, 2016). Results in this thesis add to the generally sparse literature on longitudinal bone-trajectories, with most data available in different ethnic populations (Ding *et al.*, 2008; Chen *et al.*, 2013; Kim *et al.*, 2018). One Danish study observed an increased BMD over a time period of 2 years in similar aged older men and women and concluded that there is a need for longer follow-up periods (Warming, Hassager and Christiansen, 2002).

For dp-ucMGP values and thereby a reflection of vitamin K status, levels were comparable to those of the Danish general population (Jespersen *et al.*, 2020), but in contrast to bone measures, levels did not change over the four years. Others have otherwise shown that levels of dp-ucMGP are expected to increase with age (Shea *et al.*, 2011). One could speculate that the supposed link between vitamin K status and bone health is expressed at a more advanced age, or that vitamin K status may be predictive of bone health in the even longer run.

When to intervene and what to expect

A central question in lifestyle-related research is at what age one should intervene and how much the intervention can change. Earlier might be better, but we know that this is not always the case, so understanding it is still important to know what age does to an intervention. The participants in this thesis were included around retirement age, as this has been seen as a critical transition in life and also a decade of life where physical parameters such as strength and power, and body composition, including skeletal muscle mass, all decline at an accelerating rate (Skelton *et al.*, 1994; Hughes *et al.*, 2002; Suetta *et al.*, 2019). While this time period in life tends to get much of the attention, more recently several studies have also highlighted the importance of middle age, as a time period prognostic of future health with many risk factors having their roots in mid-life (Brunner *et al.*, 2018; Brown and Covinsky, 2020; Elliott *et al.*, 2021; Sakaniwa *et al.*, 2022). However, at least for the brain, middle age has traditionally been understudied, compared with later stages of life (Dohm-Hansen *et al.*, 2024).

The present results showed, when focusing on the control group, that common annual decline rates of ~ 2% were present in some, but not all, physical parameters, like CSA of m. vastus lateralis, power, leg muscle strength, and visceral fat. The critical transition from work to retirement has been observed in for example cognition, where, irrespective of exercise, at least some domains, such as verbal memory, have shown to decline faster after retirement taking the age-related decline into account (Xue *et al.*, 2018). In an additional study that was not included in this thesis (Bloch-Ibenfeldt and Gates *et al.*, in preparation), using the same sample of participants, predictors for maintained training practice were identified, including the potential influence of retirement status. Briefly, post hoc analyses here suggested that participants on early retirement were the most likely to continue their specific training from the intervention.

With the overwhelming literature on the many beneficial effects of resistance training and given both the short- and long-term benefits in some parameters in this sample of older adults, a strong argument can be made for incorporating resistance training at an even earlier age, backed up by the finding that lifelong exercise positively influenced neuromuscular function in the elderly (Soendenbroe *et al.*, 2022). That some systems of the body did not benefit from the training could be explained by the fact that it was too late to change trajectories, or simply, that having an intervention at this time period may not show so much now, but more in the years to come.

Is adding more always beneficial?

Despite the fact that most participants as mentioned had one or more chronic disease already at study start, general physical activity level assessed as the daily step count, was notably high (~ 10,000 steps/day) throughout all four years. For comparison, in another intervention study, only including women who were also slightly older, they reported ~ 6000 steps/day (Uusi-Rasi *et al.*, 2017). The importance of physical activity and exercise late in life was exemplified in a recent longitudinal study, with trajectories in several measures of physical function showing a marked decrease in older adults that reported themselves as inactive, as compared to an active age-matched cohort (Manning *et al.*, 2023). In the present cohort, even though handgrip strength compared well to peers of similar age (Aadahl *et al.*, 2011; Mayhew *et al.*, 2023) and the number of completed chair-stand repetitions were similar to those of age-matched volunteers who were invited to another Danish study (the Copenhagen Sarcopenia Study) (Suetta *et al.*, 2019), combining physical functioning performances, health evaluations, general physical activity assessments, and preliminary results on physiological well-being (data not published), participants were generally well-functioning, active and probably even healthier than an average age-matched population. This was also reflected in bone measures, where mean Z-scores revealed that BMD did not decrease at the expected magnitude and that it was substantially higher than for averagely age-matched individuals, and when compared to populations of similar nature values were higher than for age-matched persons (Karlsson *et al.*, 1993; Lunt *et al.*, 1997; Rondanelli *et al.*, 2022). Further support is provided by a large older Norwegian population that was followed over several years. In this cohort, the participants in the control group, who followed the national activity guidelines actually showed superior effects to training at two different intensities regarding long-term trajectories of brain structure, development of white matter hyperintensities, all-cause mortality, and other medical conditions (Stensvold *et al.*, 2015, 2020; Pani *et al.*, 2021, 2022; Arild *et al.*, 2022). It should be noted that the Norwegian national activity guidelines are rather demanding with participants encouraged to follow guidelines of at least 30 minutes of moderate intensity exercise almost every day (Stensvold *et al.*, 2015)). In that particular cohort, the Generation 100 study, it had otherwise been hypothesized that high intensity training would be superior to both moderate intensity training and following national activity guidelines for brain and general health. Thus, taken together, is appears to be difficult to expect large effects when adding exercise to an already high level of physical activity. Although speculative, is it possible that the small gains that are not necessarily significant during the 4 years now, play a larger role in the even longer perspective.

Starting condition says it all?

Although it is generally believed that individuals at lower levels to start with have a greater potential to benefit from resistance training, the amount of literature on this matter is not overwhelming, especially when it comes to older adults. In younger people, one meta-analysis did not find an effect of training status (trained vs untrained) on the resistance training-related response in for example muscle strength (Grgic *et al.*, 2022), while results in other studies have suggested that baseline levels do influence adaptations (Mangine *et al.*, 2018; Wetmore *et al.*, 2020). For older adults, one study categorized older women according to their muscular strength at baseline, and after 12 weeks of whole-body resistance training the weaker women had greater improvements in lower-limb strength, but surprisingly not in upper-limb strength or muscle mass (Kassiano *et al.*, 2022).

For bone health in study 3, an analysis of the dropouts indicated that those with a poorer starting condition and thus perhaps the most interesting participants to follow, unfortunately were the ones that did not continue over the years. This has also been shown in other studies. One study showed that brain age was correlated with study attrition (dropping out of a study)(Dunås *et al.*, 2021), whereas some other studies have linked dropping out of a study with poor health outcomes (Goldberg *et al.*, 2006; Beller, Geyer and Epping, 2022). On the other hand, it has also been suggested that a better starting condition might prime the brain for better exercise-induced plasticity (Lloyd *et al.*, 2024).

It could be speculated that this (the significance of a poorer starting condition) would have also been the case considering the characteristics for the whole sample in this thesis, as subsequent dropouts had greater weight, BMI, and waist circumferences at baseline. However, differences in responses to the intervention between participants that completed the 4-year follow-up and those lost-to-follow-up were not apparent, meaning that post-intervention-dropouts seemed to benefit similarly from the intervention. Even so, it is a challenge to maintain the people that we are interested in.

In this thesis, I have focused on results of an interim analysis, treating the cohort like the intervention. A benefit with a large trial like the LISA study, is that it can also be used as a prospective cohort where measures or specific values can be used to predict different trajectories or regressions of parameters, as well as incidents of diseases. For example, in another study where the LISA cohort was used, it was suggested that it is all in the baseline, with the results showing that baseline performance in the chair stand test was associated with cerebellar volume 4 years later in older adults (Demnitz *et al.*, 2023). Thus, characterizing individuals at study start may help to better understand who will benefit, and what to expect, which may be necessary to provide more specific recommendations than the general. Comparably, in a cohort of community-dwelling older adults, vitamin K insufficiency was able to predict the incidence of frailty at a follow-up evaluation 1 year after study start (Azuma et al., 2023).

What should a resistance intervention look like?

There are many factors that may influence resistance training. One such, is loading. The loads or intensity with which resistance training – and training in general – is performed, may influence adaptations. In this thesis, both training groups beneficially maintained visceral fat, suggesting that here intensity of training was not a depending factor. Likewise training intensity did not influence adaptations in the brain and bones. In contrast, as previously mentioned, for immediate muscular adaptations and for the ability to preserve these, training intensity may play a role (Fatouros *et al.*, 2005; Tokmakidis *et al.*, 2009; Gylling, Bloch-Ibenfeldt, *et al.*, 2020; Gylling, Eriksen, *et al.*, 2020). Optimal training intensity or load, as also shown in the Generation 100 study that was mentioned earlier (Stensvold *et al.*, 2020), may differ depending on the system of the body and outcome measured.

In contrast to muscle strength, leg extensor power, which was the primary outcome measure in the original RCT (Eriksen *et al.*, 2016), decreased similarly across groups over the four years. In these individuals, surprisingly this measure had previously also not shown group-differences (Gylling, Bloch-Ibenfeldt, *et al.*, 2020; Gylling, Eriksen, *et al.*, 2020). Other intervention studies have shown improvements in muscle power after 12 weeks of heavy resistance training, and this has been reported in both old and very old individuals (Caserotti *et al.*, 2008; Bechshøft *et al.*, 2017). However, while one study used comparable traditional heavy resistance training, participants may have had a much greater potential for improvement, as they were markedly older and hence both weaker and frailer (Bechshøft *et al.*, 2017). In the other study, training was more explosive and thus favoring training-specific adaptations (Caserotti *et al.*, 2008). For benefits in muscle power to appear, in individuals of this relatively young age, training in the present study was perhaps not optimal and more power specific training is needed (Hazell, Kenno and Jakobi, 2007; El Hadouchi et al., 2022).

Revisiting falls, some have suggested that muscular power, with its great impact on daily and physical function (Fielding *et al.*, 2002; Macaluso and De Vito, 2004; Reid and Fielding, 2012), is more important than strength for falls-prevention in community-dwelling older adults (Simpkins and Yang, 2022). Likewise, power is considered an important component of the chair-stand test

(Alcazar *et al.*, 2021). In this thesis there were no between-group differences in either of these two measures. Other results in older adults have indicated that muscular strength and power are equally important predictors for the time to complete the 400-meter walking test (Marsh *et al.*, 2006).

Training during the intervention in the LISA study was not intended to specifically target for example the brain or bones, where for the latter weightbearing exercises with impact for decades have been recommended for optimizing bone health. For the brain, if, at all, resistance training is beneficial, we need to better understand which components of the training that are essential. While a traditional whole-body programme, whether performed with heavy or moderate loads, might benefit muscle strength, as seen in this thesis, it is perhaps not best suitable for some other adaptations.

Training intensity and 'continuation'

It was originally hypothesized in the RCT that individuals in MIT would be more likely to continue their specified training regimes and that it would be easier to implement routines beyond the intervention (Eriksen *et al.*, 2016). For leg muscle strength, it was interesting that the age-related decline in strength was shifted 4 years only in HRT, despite the fact that most individuals did not do regular resistance training in the follow-up period. Although defining regular resistance training beyond the intervention was quite subjective, one could speculate, along the lines of the hypothesis in the original RCT, that there would be beneficial effects of resistance training 'continuation'. The results showed for HRT that there was an added beneficial effect of regular training in these selected individuals, in line with the hypothesis, but interestingly also that strength on average was maintained from baseline in all other individuals. The results also showed that with moderate intensity training there was no added effect and strength was decreased at year 4, whether or not individuals had regularly resistance trained across all four years. This additive effect for individuals in HRT was somewhat in line with previous reports (Gylling, Bloch-Ibenfeldt, *et al.*, 2020), stressing the importance of training load. Thus, among resistance training naïve older adults, 1 year of heavy resistance training may induce longer-lasting health benefits at a group-level, which from a public health perspective is very exciting and strengthens the reasoning for urging older adults to resistance train with heavy loads.

Even though the additional study of predictors for maintained training practice suggested that individuals in HRT were even likelier to maintain their specific training practice (Bloch-Ibenfeldt and Gates *et al.*, in preparation), contrary to the original hypothesis, surprisingly there was no longer-lasting muscular effect of the moderate training. This training had otherwise been able to improve lean body mass and physical function post-intervention, although not to the same extent as the training with heavier loads. For a deeper understanding of how training was implemented and linked to behavior, and also how the motivation for training was in participants in the non-exercising control group, individual responses to the BREQ-2 questionnaire should be examined.

One-size-fits-all is not always the right fit

On an individual level, it is important to remember that there were many of the study participants who did not respond (that well) to the training intervention. However, these are individuals that might have responded to another type of training – whether that be exercise specifics or another modality. While some of the participants might not have experienced beneficial changes in leg muscle strength, instead these could have improved e.g. physiological well-being. As evident from **Figure 8C** in the results, the individual variability in for example leg muscle strength was rather large. Applying the intention-to-treat principle (McCoy, 2017) is not always optimal. Even though both training adherence and compliance was high (Gylling, Eriksen, *et al.*, 2020), all individuals were included in the analyses irrespective of their behavior during the intervention. Taking the results from a group to individual level is challenging. If we really do believe that resistance training is so potent and beneficial – for many parameters – then why are there not a greater number of individuals (relatively) that markedly benefit from the resistance training? This is something we all need to think about. The individual variability in training response, or just physical activity in general, needs to be better understood and is key for developing personalized interventions for healthy aging.

Returning to the components of aging in **Figure 1**, this thesis has looked at healthy aging via physical activity and function. The present studies revealed that for the brain, training did not influence trajectories of selected grey matter volumes, but there were some indications of a link between muscle strength and white matter hyperintensities. Besides more structural changes, cognition was not assessed, but is included from year 7. Thus, future studies of the LISA cohort will be able to address additional components of brain health. For social factors, even though training was performed (at least partly) in peer groups and individuals in the non-exercising control group were invited for social events, there may have been some social components that were not examined. For example, in one study, whether training was performed individually at home or group-based, seemed to be important for longer-term (64 weeks) training retention, even though this was in even older participants that were 80 years on average (Cyarto, Brown and Marshall, 2006). Similarly, there was no assessment of nutrition and diet, meaning that there was no control of for example protein intake, which by some has been suggested to influence muscle mass and adaptations. However, the Danish population is believed to have a sufficient intake of proteins (Pedersen *et al.*, 2015) and both body weight and daily physical activity remained stable over all four years. Further, training seemed to beneficially preserve visceral fat content, and in the long-term perspective this was irrespective of training load.

Strengths and limitations

There are many strengths of the studies included in this thesis. First, sample size was large. For example, the LISA trial is the largest study to date examining the effects of resistance training on brain structure in humans. Second, it is important to follow the same individuals over several years to better understand long-term trajectories, and to my knowledge the prolonged follow-up period was one of the longest to a resistance training intervention, especially in combination with a prolonged training-intervention. Compared with one of the few studies that had an even longer follow-up to a training-intervention including resistance exercises (Kennis *et al.*, 2013), the present sample sizes were much larger. Third, dropout was notably low, which up-until-now has only accounted to less than 20%, with no difference between women and men. Fourth, there was a great diversity of measures focusing on several biological systems of the body and the interactions between these, where other training studies, also those few that have followed individuals over several years, often have had a narrower approach. Fifth, resistance training was performed at two different intensities/loads, acknowledging that this form of training can be performed in numerous ways. This enabled a deeper investigation of the importance of training intensity/load and whether this factor was paramount for possible adaptations. Sixth, individuals in the non-exercising control group were invited to social and cultural activities during the intervention, attempting to rule out the social component of participating in training groups.

The fact that the older adults were not guided after year 1 and could continue with training and lifestyle as they wished was a limitation of the studies. This approach may have complicated the interpretation of data, as behavior and training practice during the follow-up period may have influenced the results. The rather subjective interpretation of self-reported resistance training (how it was defined as regularly performed), and the fact that it was self-reported, may also have influenced the results of how ‘continuation’ affected the trajectory for leg strength. However, in combination with the broad inclusion criteria, this approach of no guidance beyond the intervention should be seen in the context of a real-life setting, where retention of training practice in older adults is challenging, with a sample which was meant to reflect the average age-matched population.

From the results of in particular bone density, it was, at least partly, evident that there may have been some selection bias, with the worst-of individuals not continuing over time. Whether the participants were comparable to an average age-matched population could be questioned. Further in relation to this, most individuals who agreed to participate in the RCT, were living in Copenhagen or in areas north of the capital. It cannot be ruled out that demographics played a role in the general condition of the older adults. The participants were also aware from study start that they would be invited back for several follow-up measurements, and they were provided with regular newsletters and feedback on their individual results. This may have influenced behavior beyond the training-intervention.

In this thesis, changes in brain structure trajectories were assessed with MRI-derived volumes of the selected brain regions, but other structural changes could be present, as there were no measures of structural connections with diffusion MRI (Mueller *et al.*, 2015). In the LISA study, however, there are other measurements included, such as diffusion and other modalities.

Despite looking at a broad range of adaptations within different biological systems of the body, it is important to stress that this thesis has not covered all aspects of how training can promote healthy aging and that there may be other factors that play a critical role.

CONCLUSION & PERSPECTIVE

In older adults, who were around retirement age at study start, 1 year of resistance training with heavy loads benefitted the long-term trajectory of leg muscle strength, with baseline levels of strength preserved over 4 years. On the contrary, individuals who were randomized to either a moderate intensity resistance training programme or to a non-exercising control situation experienced a similar decline at year 4.

Notably, the benefit of resistance training was not able to translate into influencing age-related changes in brain grey matter volumes. Although a weak long-term association between change in leg muscle strength and white matter hyperintensity volume was observed across all groups, there was no training-related influence on the development of white matter hyperintensities.

For blood-based markers of bone turnover, levels of PINP were transiently increased with heavy resistance training at year 1, indicating that in older adults prolonged resistance training with heavy loads may have an immediate effect on bone formation. This effect was, however, not maintained at the 4-year follow-up. Further, resistance training did not influence changes in both total and regional bone mineral density. Taken together, the results suggested that resistance training had no long-term benefit on selected measures of bone health in these older adults.

In general, engaging in the moderate intensity training did not seem sufficient to induce long-lasting effects, with one exception, visceral fat. Here, irrespective of training load, visceral fat was preserved in both training groups in relation to the control group which experienced an age-related increase.

The three studies in this thesis provide important information on true longitudinal trajectories in aging across multiple physiological systems, and even though little interplay was observed between different biological systems of the body resistance training load should be considered when providing training recommendation for older individuals. The changed trajectories of leg muscle strength may be critical in relation to maintaining independence in the later stages of life.

REFERENCES

Aadahl, M. *et al.* (2011) 'Grip strength and lower limb extension power in 19-72-year-old Danish men and women: The Health2006 study', *BMJ Open*, 1(2). Available at: https://doi.org/10.1136/bmjopen-2011-000192.

Aagaard, P. *et al.* (2010) 'Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure', *Scandinavian Journal of Medicine & Science in Sports*, 20(1), pp. 49–64. Available at: https://doi.org/10.1111/j.1600-0838.2009.01084.x.

Akbari, S. and Rasouli-Ghahroudi, A.A. (2018) 'Vitamin K and Bone Metabolism: A Review of the Latest Evidence in Preclinical Studies', *BioMed Research International*, 2018. Available at: https://doi.org/10.1155/2018/4629383.

Alcazar, J. *et al.* (2021) 'Assessment of functional sit-to-stand muscle power: Cross-sectional trajectories across the lifespan', *Experimental Gerontology*, 152, p. 111448. Available at: https://doi.org/10.1016/j.exger.2021.111448.

Arild, A. *et al.* (2022) 'Five years of exercise intervention at different intensities and development of white matter hyperintensities in community dwelling older adults, a Generation 100 sub-study', *Aging (Albany, NY.)*, 14(2), pp. 596–622. Available at: https://doi.org/10.18632/aging.203843.

Azuma, K. *et al.* (2023) 'Vitamin K insufficiency predicts incidence of frailty in community-dwelling older adults: The Otassha Study', *Journal of Bone and Mineral Metabolism*, 41(6), pp. 817–821. Available at: https://doi.org/10.1007/s00774-023-01457-4.

Bassey, E.J. and Short, A.H. (1990) *A new method for measuring power output in a single leg extension: feasibility, reliability and validity*, *European Applied Journal of Physiology and Occupational Physiology*.

Bassuk, S.S., Rifai, N. and Ridker, P.M. (2004) 'High-sensitivity C-reactive protein: clinical importance.', *Current problems in cardiology*, 29(8), pp. 439–93.

Bechshøft, R.L. *et al.* (2017) 'Improved skeletal muscle mass and strength after heavy strength training in very old individuals', *Experimental Gerontology*, 92, pp. 96–105. Available at: https://doi.org/10.1016/j.exger.2017.03.014.

Beller, J., Geyer, S. and Epping, J. (2022) 'Health and study dropout: health aspects differentially predict attrition', *BMC Medical Research Methodology*, 22(1), p. 31. Available at: https://doi.org/10.1186/s12874-022-01508-w.

Berger, C. *et al.* (2008) ‘Change in bone mineral density as a function of age in women and men and association with the use of antiresorptive agents’, *Canadian Medical Association Journal*, 178(13), pp. 1660–1668. Available at: https://doi.org/10.1503/cmaj.071416.

Best, J.R. *et al.* (2015) ‘Long-Term Effects of Resistance Exercise Training on Cognition and Brain Volume in Older Women: Results from a Randomized Controlled Trial’, *Journal of the International Neuropsychological Society*, 21(10), pp. 745–756. Available at: https://doi.org/10.1017/S1355617715000673.

Bickel, C.S., Cross, J.M. and Bamman, M.M. (2011) ‘Exercise dosing to retain resistance training adaptations in young and older adults’, *Medicine and Science in Sports and Exercise*, pp. 1177–1187. Available at: https://doi.org/10.1249/MSS.0b013e318207c15d.

Bieler, T. *et al.* (2014) ‘Intra-rater reliability and agreement of muscle strength, power and functional performance measures in patients with hip osteoarthritis’, *Journal of Rehabilitation Medicine*, 46(10), pp. 997–1005. Available at: https://doi.org/10.2340/16501977-1864.

Bjorner, J.B. *et al.* (1998) *The Danish SF-36 Health Survey: Translation and Preliminary Validity Studies*, *J Clin Epidemiol*.

Black, D.M. and Rosen, C.J. (2016) ‘Clinical Practice. Postmenopausal Osteoporosis.’, *The New England journal of medicine*, 374(3), pp. 254–62. Available at: https://doi.org/10.1056/NEJMcp1513724.

Blackwell, T. *et al.* (2009) ‘Consistency of leg extension power assessments in older men: The osteoporotic fractures in men (MrOS) study’, *American Journal of Physical Medicine and Rehabilitation*, 88(11), pp. 934–940. Available at: https://doi.org/10.1097/PHM.0b013e3181bbddfb.

Blackwood, J. *et al.* (2022) ‘Use of activPAL to Measure Physical Activity in Community-Dwelling Older Adults: A Systematic Review’, *Archives of Rehabilitation Research and Clinical Translation*, 4(2), p. 100190. Available at: https://doi.org/10.1016/j.arrct.2022.100190.

Blake, G.M. and Fogelman, I. (2007) ‘The role of DXA bone density scans in the diagnosis and treatment of osteoporosis’, *Postgraduate Medical Journal*, pp. 509–517. Available at: https://doi.org/10.1136/pgmj.2007.057505.

Blocquiaux, S. *et al.* (2020) ‘The effect of resistance training, detraining and retraining on muscle strength and power, myofibre size, satellite cells and myonuclei in older men’, *Experimental Gerontology*, 133. Available at: https://doi.org/10.1016/j.exger.2020.110860.

Bohannon, R.W. and Schaubert, K.L. (2005) 'Test-retest reliability of grip-strength measures obtained over a 12-week interval from community-dwelling elders', *Journal of Hand Therapy*, 18(4), pp. 426–428. Available at: https://doi.org/10.1197/j.jht.2005.07.003.

Booth, F.W. and Laye, M.J. (2010) 'The future: genes, physical activity and health', *Acta Physiologica*, 199(4), pp. 549–556. Available at: https://doi.org/10.1111/j.1748-1716.2010.02117.x.

Borde, R., Hortobágyi, T. and Granacher, U. (2015) 'Dose–Response Relationships of Resistance Training in Healthy Old Adults: A Systematic Review and Meta-Analysis', *Sports medicine (Auckland)*, 45(12), pp. 1693–1720. Available at: https://doi.org/10.1007/s40279-015-0385-9.

Brazier, J.E. *et al.* (1992) *GENERAL PRACTICE Validating the SF-36 health survey questionnaire: new outcome measure for primary care*. Available at: https://doi.org/10.1136/bmj.305.6846.160.

Breen, L. *et al.* (2013) 'Two Weeks of Reduced Activity Decreases Leg Lean Mass and Induces "Anabolic Resistance" of Myofibrillar Protein Synthesis in Healthy Elderly', *The Journal of Clinical Endocrinology and Metabolism*, 98(6), pp. 2604–2612. Available at: https://doi.org/10.1210/jc.2013-1502.

Brown, R.T. and Covinsky, K.E. (2020) 'Moving prevention of functional impairment upstream: is middle age an ideal time for intervention?', *Women's Midlife Health*, 6(1), p. 4. Available at: https://doi.org/10.1186/s40695-020-00054-z.

Brunner, E.J. *et al.* (2018) 'Midlife contributors to socioeconomic differences in frailty during later life: a prospective cohort study', *The Lancet Public Health*, 3(7), pp. e313–e322. Available at: https://doi.org/10.1016/S2468-2667(18)30079-3.

Bruton, A. (2002) 'Muscle Plasticity', *Physiotherapy*, 88(7), pp. 398–408. Available at: https://doi.org/10.1016/S0031-9406(05)61265-5.

Brzycki, M. (1995) 'Strength training -- A Practical Approach to Strength Training (3rd edition) by Matt Brzycki', *Scholastic Coach and Athletic Director*, 65(4), p. 86.

Bussy, A. *et al.* (2021) 'Hippocampal shape across the healthy lifespan and its relationship with cognition', *Neurobiology of Aging*, 106, pp. 153–168. Available at: https://doi.org/10.1016/j.neurobiolaging.2021.03.018.

Campbell, W. *et al.* (1994) 'Increased energy requirements and changes in body composition with resistance training in older adults', *The American journal of clinical nutrition*, 60(2), pp. 167–175. Available at: https://doi.org/10.1093/ajcn/60.2.167.

Carlson, M.E. *et al.* (2009) 'Molecular aging and rejuvenation of human muscle stem cells', *EMBO Molecular Medicine*, 1(8–9), pp. 381–391. Available at: https://doi.org/10.1002/emmm.200900045.

Carmona, J.J. and Michan, S. (2016) ‘BIOLOGY OF HEALTHY AGING AND LONGEVITY’, *Revista de investigacion clinica*, 68(1), pp. 7–16.

Caserotti, P. *et al.* (2008) ‘Explosive heavy-resistance training in old and very old adults: changes in rapid muscle force, strength and power’, *Scandinavian Journal of Medicine & Science in Sports*, 18(6), pp. 773–782. Available at: https://doi.org/10.1111/j.1600-0838.2007.00732.x.

Cavill, N.A. and Foster, C.E.M. (2018) ‘Enablers and barriers to older people’s participation in strength and balance activities: A review of reviews’, *Journal of Frailty, Sarcopenia and Falls*, 3(2), pp. 105–113. Available at: https://doi.org/10.22540/JFSF-03-105.

von Cederwald, B.F. *et al.* (2023) ‘White matter lesion load determines exercise-induced dopaminergic plasticity and working memory gains in aging’, *Translational Psychiatry*, 13(1), p. 28. Available at: https://doi.org/10.1038/s41398-022-02270-9.

Chen, C. and Nakagawa, S. (2023) ‘Physical activity for cognitive health promotion: An overview of the underlying neurobiological mechanisms’, *Ageing Research Reviews*. Elsevier Ireland Ltd. Available at: https://doi.org/10.1016/j.arr.2023.101868.

Chen, C.H. *et al.* (2013) ‘Longitudinal changes in bone mineral density of healthy elderly women in southern Taiwan’, *Taiwanese Journal of Obstetrics and Gynecology*, 52(1), pp. 61–65. Available at: https://doi.org/10.1016/j.tjog.2012.08.001.

Chen, H. and Avgerinou, C. (2023) ‘Association of Alternative Dietary Patterns with Osteoporosis and Fracture Risk in Older People: A Scoping Review’, *Nutrients*. Multidisciplinary Digital Publishing Institute (MDPI). Available at: https://doi.org/10.3390/nu15194255.

Christensen, K., Doblhammer, G., *et al.* (2009) ‘Ageing populations: the challenges ahead’, *The Lancet*, 374(9696), pp. 1196–1208. Available at: https://doi.org/10.1016/S0140-6736(09)61460-4.

Christensen, K., Thinggaard, M., *et al.* (2009) ‘Perceived age as clinically useful biomarker of ageing: Cohort study’, *BMJ (Online)*, 339(7735), p. 1433. Available at: https://doi.org/10.1136/bmj.b5262.

Churchward-Venne, T.A. *et al.* (2015) ‘There Are No Nonresponders to Resistance-Type Exercise Training in Older Men and Women’, *Journal of the American Medical Directors Association*, 16(5), pp. 400–411. Available at: https://doi.org/10.1016/j.jamda.2015.01.071.

Colcombe, S. and Kramer, A.F. (2003) ‘Fitness Effects on the Cognitive Function of Older Adults’, *Psychological Science*, 14(2), pp. 125–130. Available at: https://doi.org/10.1111/1467-9280.t01-1-01430.

Colcombe, S.J. *et al.* (2006) *Exercise: An Active Route to Healthy Aging Aerobic Exercise Training Increases Brain Volume in Aging Humans*. Available at: https://academic.oup.com/biomedgerontology/article/61/11/1166/630432.

Colón, C.J.P. *et al.* (2018) 'Muscle and Bone Mass Loss in the Elderly Population: Advances in diagnosis and treatment', *Journal of Biomedicine*, 3, pp. 40–49. Available at: https://doi.org/10.7150/jbm.23390.

Correa, C.S. *et al.* (2013) 'Effects of strength training and detraining on knee extensor strength, muscle volume and muscle quality in elderly women', *Age*, 35(5), pp. 1899–1904. Available at: https://doi.org/10.1007/s11357-012-9478-7.

Correa, C.S. *et al.* (2016) 'Effects of strength training, detraining and retraining in muscle strength, hypertrophy and functional tasks in older female adults', *Clinical Physiology and Functional Imaging*, 36(4), pp. 306–310. Available at: https://doi.org/10.1111/cpf.12230.

Cranenburg, E.C.M. *et al.* (2010) 'Characterisation and potential diagnostic value of circulating matrix Gla protein (MGP) species', *Thrombosis and haemostasis*, 104(4), pp. 811–822. Available at: https://doi.org/10.1160/TH09-11-0786.

Cruz-Jentoft, A.J. *et al.* (2019) 'Sarcopenia: Revised European consensus on definition and diagnosis', *Age and Ageing*. Oxford University Press, pp. 16–31. Available at: https://doi.org/10.1093/ageing/afy169.

Csapo, R. and Alegre, L.M. (2016) 'Effects of resistance training with moderate vs heavy loads on muscle mass and strength in the elderly: A meta-analysis', *Scandinavian journal of medicine & science in sports*, 26(9), pp. 995–1006. Available at: https://doi.org/10.1111/sms.12536.

Cummings, S.R. *et al.* (1993) 'Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group.', *Lancet.*, 341(8837), pp. 72–75. Available at: https://doi.org/10.1016/0140-6736(93)92555-8.

Cyarto, E. V., Brown, W.J. and Marshall, A.L. (2006) 'Retention, adherence and compliance: Important considerations for home- and group-based resistance training programs for older adults', *Journal of Science and Medicine in Sport*, 9(5), pp. 402–412. Available at: https://doi.org/10.1016/j.jsams.2006.06.020.

Danish Health Authority - Sundhedsstyrelsen (2023) *Tab af muskelfunktion og muskelmasse - sarkopeni og effekten af fysisk træning*. Sundhedsstyrelsen.

Daskalopoulou, C. *et al.* (2017) ‘Physical activity and healthy ageing: A systematic review and meta-analysis of longitudinal cohort studies’, *Ageing Research Reviews*. Elsevier Ireland Ltd, pp. 6–17. Available at: https://doi.org/10.1016/j.arr.2017.06.003.

Debette, S. and Markus, H.S. (2010) ‘The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis’, *BMJ*, 341(jul26 1), pp. c3666–c3666. Available at: https://doi.org/10.1136/bmj.c3666.

Demnitz, N. *et al.* (2017) ‘Associations between mobility, cognition, and brain structure in healthy older adults’, *Frontiers in Aging Neuroscience*, 9(MAY). Available at: https://doi.org/10.3389/fnagi.2017.00155.

Demnitz, N. *et al.* (2023) ‘Is it all in the baseline? Trajectories of chair stand performance over 4 years and their association with grey matter structure in older adults’, *Human Brain Mapping*, 44(11), pp. 4299–4309. Available at: https://doi.org/10.1002/hbm.26346.

Ding, C. *et al.* (2008) ‘Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: A longitudinal study’, *Journal of Clinical Endocrinology and Metabolism*, 93(5), pp. 1952–1958. Available at: https://doi.org/10.1210/jc.2007-2325.

Dinger, M.K. *et al.* (2004) ‘Stability and convergent validity of the Physical Activity Scale for the Elderly (PASE)’, *Journal of sports medicine and physical fitness*, 44(2), pp. 186–192.

DiPietro, L. (2001) ‘Physical Activity in Aging: Changes in Patterns and Their Relationship to Health and Function’, *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 56(Supplement 2), pp. 13–22. Available at: https://doi.org/10.1093/gerona/56.suppl_2.13.

Distefano, G. and Goodpaster, B.H. (2018) ‘Effects of Exercise and Aging on Skeletal Muscle’, *Cold Spring Harbor Perspectives in Medicine*, 8(3), p. a029785. Available at: https://doi.org/10.1101/cshperspect.a029785.

Dohm-Hansen, S. *et al.* (2024) ‘The “middle-aging” brain’, *Trends in Neurosciences* [Preprint]. Available at: https://doi.org/https://doi.org/10.1016/j.tins.2024.02.001.

Driscoll, I. *et al.* (2003) ‘The Aging Hippocampus: Cognitive, Biochemical and Structural Findings’, *Cerebral Cortex*, 13(12), pp. 1344–1351. Available at: https://doi.org/10.1093/cercor/bhg081.

Dunås, T. *et al.* (2021) ‘Multimodal Image Analysis of Apparent Brain Age Identifies Physical Fitness as Predictor of Brain Maintenance’, *Cerebral Cortex*, 31(7), pp. 3393–3407. Available at: https://doi.org/10.1093/cercor/bhab019.

Edwardson, C.L. *et al.* (2017) ‘Considerations when using the activPAL monitor in field-based research with adult populations’, *Journal of Sport and Health Science*. Elsevier B.V., pp. 162–178. Available at: https://doi.org/10.1016/j.jshs.2016.02.002.

Elliott, M.L. *et al.* (2021) ‘Disparities in the pace of biological aging among midlife adults of the same chronological age have implications for future frailty risk and policy’, *Nature Aging*, 1(3), pp. 295–308. Available at: https://doi.org/10.1038/s43587-021-00044-4.

Erickson, K.I. *et al.* (2009) ‘Aerobic fitness is associated with hippocampal volume in elderly humans’, *Hippocampus*, 19(10), pp. 1030–1039. Available at: https://doi.org/10.1002/hipo.20547.

Erickson, K.I. *et al.* (2011) ‘Exercise training increases size of hippocampus and improves memory’, *Proceedings of the National Academy of Sciences of the United States of America*, 108(7), pp. 3017–3022. Available at: https://doi.org/10.1073/pnas.1015950108.

Eriksen, C.S. *et al.* (2016) ‘Physical activity as intervention for age-related loss of muscle mass and function: protocol for a randomised controlled trial (the LISA study)’, *BMJ Open*, 6(12). Available at: https://doi.org/10.1136/bmjopen-2016-012951.

Fatouros, I.G. *et al.* (2005) ‘Strength training and detraining effects on muscular strength, anaerobic power, and mobility of inactive older men are intensity dependent’, *British Journal of Sports Medicine*, 39(10), pp. 776–780. Available at: https://doi.org/10.1136/bjsm.2005.019117.

Ferrucci, L. and Fabbri, E. (2018) ‘Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty’, *Nature Reviews Cardiology*, 15(9), pp. 505–522. Available at: https://doi.org/10.1038/s41569-018-0064-2.

Fielding, R.A. *et al.* (2002) ‘High-Velocity Resistance Training Increases Skeletal Muscle Peak Power in Older Women’, *Journal of the American Geriatrics Society (JAGS)*, 50(4), pp. 655–662. Available at: https://doi.org/10.1046/j.1532-5415.2002.50159.x.

Fielding, R.A. *et al.* (2011) ‘Sarcopenia: An Undiagnosed Condition in Older Adults. Current Consensus Definition: Prevalence, Etiology, and Consequences. International Working Group on Sarcopenia’, *Journal of the American Medical Directors Association*, 12(4), pp. 249–256. Available at: https://doi.org/10.1016/j.jamda.2011.01.003.

Fjell, A.M. *et al.* (2009) ‘One-year brain atrophy evident in healthy aging’, *Journal of Neuroscience*, 29(48), pp. 15223–15231. Available at: https://doi.org/10.1523/JNEUROSCI.3252-09.2009.

Flück, M. and Hoppeler, H. (2003) ‘Molecular basis of skeletal muscle plasticity-from gene to form and function’, in *Reviews of Physiology, Biochemistry and Pharmacology*, pp. 159–216. Available at: https://doi.org/10.1007/s10254-002-0004-7.

Fragala, M.S. *et al.* (2019) ‘Resistance Training for Older Adults: Position Statement From the National Strength and Conditioning Association’, *Journal of Strength and Conditioning Research* [Preprint]. Available at: www.nsca.com.

Fujimura, R. *et al.* (1997) ‘Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism’, *Journal of Bone and Mineral Research*, 12(4), pp. 656–662. Available at: https://doi.org/10.1359/jbmr.1997.12.4.656.

Garatachea, N. *et al.* (2015) ‘Exercise Attenuates the Major Hallmarks of Aging’, *Rejuvenation Research*, 18(1), pp. 57–89. Available at: https://doi.org/10.1089/rej.2014.1623.

García-Hermoso, A. *et al.* (2018) ‘Muscular Strength as a Predictor of All-Cause Mortality in an Apparently Healthy Population: A Systematic Review and Meta-Analysis of Data From Approximately 2 Million Men and Women’, *Archives of Physical Medicine and Rehabilitation*, 99(10), pp. 2100-2113.e5. Available at: https://doi.org/10.1016/j.apmr.2018.01.008.

Garmany, A., Yamada, S. and Terzic, A. (2021) ‘Longevity leap: mind the healthspan gap’, *npj Regenerative Medicine*. Nature Research. Available at: https://doi.org/10.1038/s41536-021-00169-5.

Goldberg, M. *et al.* (2006) ‘Health problems were the strongest predictors of attrition during follow-up of the GAZEL cohort’, *Journal of Clinical Epidemiology*, 59(11), pp. 1213–1221. Available at: https://doi.org/10.1016/j.jclinepi.2006.02.020.

Gombos, G.C. *et al.* (2016) ‘Direct effects of physical training on markers of bone metabolism and serum sclerostin concentrations in older adults with low bone mass’, *BMC Musculoskeletal Disorders*, 17(1). Available at: https://doi.org/10.1186/s12891-016-1109-5.

Gopinath, B. *et al.* (2018) ‘Physical Activity as a Determinant of Successful Aging over Ten Years’, *Scientific reports*, 8(1), pp. 10522–5. Available at: https://doi.org/10.1038/s41598-018-28526-3.

Grgic, J. *et al.* (2022) ‘Effects of resistance training performed to repetition failure or non-failure on muscular strength and hypertrophy: A systematic review and meta-analysis’, *Journal of Sport and Health Science*, 11(2), pp. 202–211. Available at: https://doi.org/10.1016/j.jshs.2021.01.007.

Grgic, J. (2022) ‘Use It or Lose It? A Meta-Analysis on the Effects of Resistance Training Cessation (Detraining) on Muscle Size in Older Adults’, *International Journal of Environmental Research and Public Health*. MDPI. Available at: https://doi.org/10.3390/ijerph192114048.

Gylling, A.T., Bloch-Ibenfeldt, M., *et al.* (2020) ‘Maintenance of muscle strength following a one-year resistance training program in older adults’, *Experimental Gerontology*, 139. Available at: https://doi.org/10.1016/j.exger.2020.111049.

Gylling, A.T., Eriksen, C.S., *et al.* (2020) ‘The influence of prolonged strength training upon muscle and fat in healthy and chronically diseased older adults’, *Experimental Gerontology*, 136. Available at: https://doi.org/10.1016/j.exger.2020.110939.

El Hadouchi, M. *et al.* (2022) ‘Effectiveness of power training compared to strength training in older adults: a systematic review and meta-analysis’, *European Review of Aging and Physical Activity*, 19, p. 18. Available at: https://doi.org/10.1186/s11556-022-00297-x.

Häkkinen, K. *et al.* (1998) ‘Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people’, *Journal of Applied Physiology*, 84(4), pp. 1341–1349. Available at: https://doi.org/https://doi.org/10.1152/jappl.1998.84.4.1341.

Häkkinen, K. *et al.* (2000) ‘Neuromuscular adaptation during prolonged strength training, detraining and re-strength-training in middle-aged and elderly people’, *European Journal of Applied Physiology*, 83(1), pp. 51–62. Available at: https://doi.org/10.1007/s004210000248.

Häkkinen, K. and Häkkinen, A. (1995) ‘Neuromuscular adaptations during intensive strength training in middle-aged and elderly males and females.’, *Electromyography and clinical neurophysiology*, 35(3), pp. 137–47.

Hazell, T., Kenno, K. and Jakobi, J. (2007) ‘Functional benefit of power training for older adults’, *Journal of aging and physical activity*, 15(3), pp. 349–359. Available at: https://doi.org/10.1123/japa.15.3.349.

Herda, A.A. and Nabavizadeh, O. (2023) ‘Effect of six weeks of resistance training on bone preservation in older adults: a randomized control trial.’, *Aging clinical and experimental research.* [Preprint]. Available at: https://doi.org/10.1007/s40520-023-02575-9.

Hinton, P.S., Nigh, P. and Thyfault, J. (2015) ‘Effectiveness of resistance training or jumping-exercise to increase bone mineral density in men with low bone mass: A 12-month randomized, clinical trial’, *Bone*, 79, pp. 203–212. Available at: https://doi.org/10.1016/j.bone.2015.06.008.

Holloszy, J.O. (1967) ‘Biochemical Adaptations in Muscle - EFFECTS OF EXERCISE ON MITOCHONDRIAL OXYGEN UPTAKE AND RESPIRATORY ENZYME ACTIVITY IN SKELETAL MUSCLE’, *The Journal of biological chemistry*, 242(9), pp. 2278–2282.

Huffman, D.M. *et al.* (2009) ‘Role of Visceral Adipose Tissue in Aging NIH Public Access Author Manuscript’, *Biochim Biophys Acta*, (10), pp. 1117–1123. Available at: https://doi.org/10.1016/j.bbagen.2009.01.008.

Hughes, V.A. *et al.* (2002) ‘Longitudinal changes in body composition in older men and women: role of body weight change and physical activity’, *The American Journal of Clinical Nutrition*, 76(2), pp. 473–481. Available at: https://doi.org/10.1093/ajcn/76.2.473.

Hunter, G.R., Gower, B.A. and Kane, B.L. (2010) ‘Age Related Shift in Visceral Fat.’, *International journal of body composition research*, 8(3), pp. 103–108.

Huovinen, V. *et al.* (2016) ‘Bone mineral density is increased after a 16-week resistance training intervention in elderly women with decreased muscle strength’, *European Journal of Endocrinology*, 175(6), pp. 571–582. Available at: https://doi.org/10.1530/EJE-16-0521.

Jespersen, T. *et al.* (2020) ‘Uncarboxylated matrix Gla-protein: A biomarker of vitamin K status and cardiovascular risk’, *Clinical Biochemistry*, 83, pp. 49–56. Available at: https://doi.org/10.1016/j.clinbiochem.2020.05.005.

Jonasson, L.S. *et al.* (2017) ‘Aerobic exercise intervention, cognitive performance, and brain structure: Results from the Physical Influences on Brain in Aging (PHIBRA) Study’, *Frontiers in Aging Neuroscience*, 8(JAN). Available at: https://doi.org/10.3389/fnagi.2016.00336.

Jones, C.J., Rikli, R.E. and Beam, W.C. (1999) ‘A 30-s chair-stand test as a measure of lower body strength in community-residing older adults’, *Research Quarterly for Exercise and Sport*, 70(2), pp. 113–119. Available at: https://doi.org/10.1080/02701367.1999.10608028.

Joyner, M.J. and Green, D.J. (2009) ‘Exercise protects the cardiovascular system: effects beyond traditional risk factors’, *The Journal of Physiology*, 587(23), pp. 5551–5558. Available at: https://doi.org/10.1113/jphysiol.2009.179432.

Kalapotharakos, V. *et al.* (2007) ‘The effect of moderate resistance strength training and detraining on muscle strength and power in older men’, *Journal of geriatric physical therapy (2001)*, 30(3), pp. 109–113. Available at: https://doi.org/10.1519/00139143-200712000-00005.

Kamada, M. *et al.* (2017) ‘Strength Training and All-Cause, Cardiovascular Disease, and Cancer Mortality in Older Women: A Cohort Study’, *Journal of the American Heart Association*, 6(11). Available at: https://doi.org/10.1161/JAHA.117.007677.

Kanis, J.A. *et al.* (2008) ‘European guidance for the diagnosis and management of osteoporosis in postmenopausal women’, *Osteoporosis International*, 19, pp. 399–428. Available at: https://doi.org/10.1007/s00198-008-0560-z.

Karabulut, M. *et al.* (2011) ‘Effects of high-intensity resistance training and low-intensity resistance training with vascular restriction on bone markers in older men’, *European Journal of Applied Physiology*, 111(8), pp. 1659–1667. Available at: https://doi.org/10.1007/s00421-010-1796-9.

Karinkanta, S. *et al.* (2009) ‘Maintenance of exercise-induced benefits in physical functioning and bone among elderly women’, *Osteoporosis International*, 20(4), pp. 665–674. Available at: https://doi.org/10.1007/s00198-008-0703-2.

Karlsson, M.K. *et al.* (1993) ‘Bone mineral normative data in Malmo, Sweden Comparison with reference data and hip fracture incidence in other ethnic groups’, *Acta Orthopaedica Scandinavica*, 64(2), pp. 168–169.

Kassiano, W. *et al.* (2022) ‘Weaker older women gain more lower body strength than their stronger counterparts, but not muscle mass, following 12 weeks of resistance training’, *Journal of Sports Sciences*, 40(24), pp. 2714–2721. Available at: https://doi.org/10.1080/02640414.2023.2189789.

Katula, J.A., Jack, W.J. and Marsh, A.P. (2008) ‘Enhancing quality of life in older adults: A comparison of muscular strength and power training’, *Health and Quality of Life Outcomes*, 6. Available at: https://doi.org/10.1186/1477-7525-6-45.

Keller, K. and Engelhardt, M. (2013) ‘Strength and muscle mass loss with aging process. Age and strength loss.’, *Muscles, ligaments and tendons journal*, 3(4), pp. 346–50.

Kennis, E. *et al.* (2013) ‘Long-term impact of strength training on muscle strength characteristics in older adults’, *Archives of Physical Medicine and Rehabilitation*, 94(11), pp. 2054–2060. Available at: https://doi.org/10.1016/j.apmr.2013.06.018.

Kilgour, A.H., Todd, O.M. and Starr, J.M. (2014) ‘A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse’, *BMC geriatrics*, 14(1), pp. 85–85. Available at: https://doi.org/10.1186/1471-2318-14-85.

Kim, K.M. *et al.* (2018) ‘Longitudinal changes in muscle mass and strength, and bone mass in older adults: Gender-specific associations between muscle and bone losses’, *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, 73(8), pp. 1062–1069. Available at: https://doi.org/10.1093/gerona/glx188.

Kowiański, P. *et al.* (2018) 'BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity', *Cellular and Molecular Neurobiology*. Springer New York LLC, pp. 579–593. Available at: https://doi.org/10.1007/s10571-017-0510-4.

Kraemer, W.J. and Ratamess, N.A. (2004) 'Fundamentals of Resistance Training: Progression and Exercise Prescription', *Medicine & Science in Sports & Exercise*, 36(4), pp. 674–688. Available at: https://doi.org/10.1249/01.MSS.0000121945.36635.61.

Kraemer, W.J., Ratamess, N.A. and French, D.N. (2002) 'Resistance Training for Health and Performance', *Current Sports Medicine Reports*, 1(3), pp. 165–171. Available at: https://doi.org/10.1249/00149619-200206000-00007.

Kramer, A.F. *et al.* (1999) 'Ageing, fitness and neurocognitive function', *Nature*, 400(6743), pp. 418–419. Available at: https://doi.org/10.1038/22682.

Kramer, A.F., Erickson, K.I. and Colcombe, S.J. (2006) 'Exercise, cognition, and the aging brain', *Journal of Applied Physiology*, 101(4), pp. 1237–1242. Available at: https://doi.org/10.1152/japplphysiol.00500.2006.

Landi, F. *et al.* (2013) 'Sarcopenia and mortality risk in frail olderpersons aged 80 years and older: Results from iLSIRENTE study', *Age and Ageing*, 42(2), pp. 203–209. Available at: https://doi.org/10.1093/ageing/afs194.

Landrigan, J.F. *et al.* (2020) 'Lifting cognition: a meta-analysis of effects of resistance exercise on cognition', *Psychological Research*. Springer, pp. 1167–1183. Available at: https://doi.org/10.1007/s00426-019-01145-x.

Langhammer, B., Bergland, A. and Rydwik, E. (2018) 'The Importance of Physical Activity Exercise among Older People', *BioMed Research International*. Hindawi Limited. Available at: https://doi.org/10.1155/2018/7856823.

Larson, E.B. *et al.* (2006) *Exercise Is Associated with Reduced Risk for Incident Dementia among Persons 65 Years of Age and Older*. Available at: https://annals.org.

Latham, N.K. *et al.* (2004) 'Systematic Review of Progressive Resistance Strength Training in Older Adults', *The Journals of Gerontology: Series A* [Preprint]. Available at: https://academic.oup.com/biomedgerontology/article/59/1/M48/533579.

Layne, J.E. and Nelson, M.E. (1999) 'The effects of progressive resistance training on bone density: a review.', *Medicine and science in sports and exercise.*, 31(1), pp. 25–30. Available at: https://doi.org/10.1097/00005768-199901000-00006.

Lee, D. *et al.* (2015) ‘Effects of nutritional components on aging’, *Aging Cell*, pp. 8–16. Available at: https://doi.org/10.1111/acel.12277.

Leenders, M. *et al.* (2013) ‘Elderly Men and Women Benefit Equally From Prolonged Resistance-Type Exercise Training’, *The journals of gerontology. Series A, Biological sciences and medical sciences*, 68(7), pp. 769–779. Available at: https://doi.org/10.1093/gerona/gls241.

Liepmann, D. *et al.* (2007) *Intelligenz-Struktur-Test 2000 R*. 2nd edn. Hogrefe.

Liu-Ambrose, T. *et al.* (2010) ‘Resistance training and executive functions: A 12-month randomized controlled trial’, *Archives of Internal Medicine*, 170(2), pp. 170–178. Available at: https://doi.org/10.1001/archinternmed.2009.494.

Liu-Ambrose, T. *et al.* (2012) ‘Resistance training and functional plasticity of the aging brain: A 12-month randomized controlled trial’, *Neurobiology of Aging*, 33(8), pp. 1690–1698. Available at: https://doi.org/10.1016/j.neurobiolaging.2011.05.010.

Liu-Ambrose, T. and Donaldson, M.G. (2009) ‘Exercise and cognition in older adults: Is there a role for resistance training programmes?’, *British Journal of Sports Medicine*, pp. 25–27. Available at: https://doi.org/10.1136/bjsm.2008.055616.

Lloyd, K.M. *et al.* (2024) ‘Data-driven MRI analysis reveals fitness-related functional change in default mode network and cognition following an exercise intervention’, *Psychophysiology*, 61(4). Available at: https://doi.org/10.1111/psyp.14469.

Lohman, T. *et al.* (1995) ‘Effects of resistance training on regional and total bone mineral density in premenopausal women: a randomized prospective study.’, *Journal of bone and mineral research : JBMR.*, 10(7), pp. 1015–1024. Available at: https://doi.org/10.1002/jbmr.5650100705.

López-Otín, C. *et al.* (2023) ‘Hallmarks of aging: An expanding universe’, *Cell*, 186(2), pp. 243–278. Available at: https://doi.org/10.1016/J.CELL.2022.11.001.

Lucas, A.R. *et al.* (2020) ‘Trajectories in muscular strength and physical function among men with and without prostate cancer in the health aging and body composition study’, *PLOS ONE*, 15(2), p. e0228773. Available at: https://doi.org/10.1371/journal.pone.0228773.

Lund, R. *et al.* (2016) ‘Cohort profile: The Copenhagen aging and midlife Biobank (CamB)’, *International Journal of Epidemiology*, 45(4), pp. 1044–1053. Available at: https://doi.org/10.1093/ije/dyv149.

Lunt, M. *et al.* (1997) ‘Population-Based Geographic Variations in DXA Bone Density in Europe: The EVOS Study’, *Osteoporosis International*, 7, pp. 175–189.

Maass, A. *et al.* (2015) ‘Vascular hippocampal plasticity after aerobic exercise in older adults’, *Molecular Psychiatry*, 20(5), pp. 585–593. Available at: https://doi.org/10.1038/mp.2014.114.

Macaluso, A. and De Vito, G. (2004) ‘Muscle strength, power and adaptations to resistance training in older people’, *European journal of applied physiology*, 91(4), pp. 450–472. Available at: https://doi.org/10.1007/s00421-003-0991-3.

Maddalozzo, G.F. and Snow, C.M. (2000) ‘Clinical Investigations High Intensity Resistance Training: Effects on Bone in Older Men and Women’, *Calcified Tissue International*, 66, pp. 399–404.

Mangine, G.T. *et al.* (2018) ‘Influence of Baseline Muscle Strength and Size Measures on Training Adaptations in Resistance-trained Men.’, *International journal of exercise science*, 11(4), pp. 198–213.

Manning, K.M. *et al.* (2023) ‘Longitudinal analysis of physical function in older adults: The effects of physical inactivity and exercise training’, *Aging Cell* [Preprint]. Available at: https://doi.org/10.1111/acel.13987.

Marcus, B.H. *et al.* (1992) ‘Self-Efficacy and the Stages of Exercise Behavior Change’, *Research Quarterly for Exercise and Sport*, 63(1), pp. 60–66. Available at: https://doi.org/10.1080/02701367.1992.10607557.

Markland, D. and Tobin, V. (2004) ‘A Modification to the Behavioural Regulation in Exercise Questionnaire to Include an Assessment of Amotivation’, *Journal of Sport and Exercise Psychology*, 26(2), pp. 191–196. Available at: https://doi.org/10.1123/jsep.26.2.191.

Marsh, A.P. *et al.* (2006) ‘Lower Extremity Strength and Power Are Associated With 400-Meter Walk Time in Older Adults: The InCHIANTI Study’, *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 61(11), pp. 1186–1193. Available at: https://doi.org/10.1093/gerona/61.11.1186.

Matthews, V.B. *et al.* (2009) ‘Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase’, *Diabetologia*, 52(7), pp. 1409–1418. Available at: https://doi.org/10.1007/s00125-009-1364-1.

Mattson, M.P. and Arumugam, T. V. (2018) ‘Hallmarks of Brain Aging: Adaptive and Pathological Modification by Metabolic States’, *Cell Metabolism*. Cell Press, pp. 1176–1199. Available at: https://doi.org/10.1016/j.cmet.2018.05.011.

Mayhew, A.J. *et al.* (2023) ‘Normative values for grip strength, gait speed, timed up and go, single leg balance, and chair rise derived from the Canadian longitudinal study on ageing’, *Age and Ageing*, 52(4). Available at: https://doi.org/10.1093/ageing/afad054.

McCartney, N. *et al.* (1995) ‘Long-term resistance training in the elderly: effects on dynamic strength, exercise capacity, muscle, and bone.’, *The journals of gerontology.*, 50(2), pp. B97-104. Available at: https://doi.org/10.1093/gerona/50a.2.b97.

McCoy, E. (2017) ‘Understanding the Intention-to-treat Principle in Randomized Controlled Trials’, *Western Journal of Emergency Medicine*, 18(6), pp. 1075–1078. Available at: https://doi.org/10.5811/westjem.2017.8.35985.

McGrath, R. *et al.* (2020) ‘What are the association patterns between handgrip strength and adverse health conditions? A topical review’, *SAGE Open Medicine*. SAGE Publications Ltd. Available at: https://doi.org/10.1177/2050312120910358.

Mendez Colmenares, A. *et al.* (2021) ‘White matter plasticity in healthy older adults: The effects of aerobic exercise’, *NeuroImage*, 239. Available at: https://doi.org/10.1016/j.neuroimage.2021.118305.

Mikolaizak, A.S. *et al.* (2018) ‘Adherence to a multifactorial fall prevention program following paramedic care: Predictors and impact on falls and health service use. Results from an RCT a priori subgroup analysis’, *Australasian Journal on Ageing*, 37(1), pp. 54–61. Available at: https://doi.org/10.1111/ajag.12465.

Montero-Odasso, M. (2023) ‘Are falls a manifestation of brain failure? Revisited 40 years later’, *Age and Ageing*. Oxford University Press. Available at: https://doi.org/10.1093/ageing/afac321.

Moritani, T. and deVries, H.A. (1979) ‘Neural factors versus hypertrophy in the time course of muscle strength gain.’, *American journal of physical medicine*, 58(3), pp. 115–30.

Mortensen, E.L., Flensborg-Madsen, T., Molbo, D., Christensen, U., *et al.* (2014) ‘Personality in late midlife: Associations with demographic factors and cognitive ability’, *Journal of Aging and Health*, 26(1), pp. 21–36. Available at: https://doi.org/10.1177/0898264313519317.

Mortensen, E.L., Flensborg-Madsen, T., Molbo, D., Fagerlund, B., *et al.* (2014) ‘The relationship between cognitive ability and demographic factors in late midlife’, *Journal of Aging and Health*, 26(1), pp. 37–53. Available at: https://doi.org/10.1177/0898264313508780.

Mueller, B.A. *et al.* (2015) ‘Diffusion MRI and its Role in Neuropsychology’, *Neuropsychology Review*, 25(3), pp. 250–271. Available at: https://doi.org/10.1007/s11065-015-9291-z.

Muñoz-Garach, A., García-Fontana, B. and Muñoz-Torres, M. (2020) ‘Nutrients and dietary patterns related to osteoporosis’, *Nutrients*. MDPI AG, pp. 1–15. Available at: https://doi.org/10.3390/nu12071986.

Murman, D.L. (2015) ‘The Impact of Age on Cognition’, *Seminars in hearing*, 36(3), pp. 111–121. Available at: https://doi.org/10.1055/s-0035-1555115.

Narici, M. V. *et al.* (1989) ‘Changes in force, cross-sectional area and neural activation during strength training and detraining of the human quadriceps’, *European Journal of Applied Physiology and Occupational Physiology*, 59(4), pp. 310–319. Available at: https://doi.org/10.1007/BF02388334.

Newman, A.B. *et al.* (2006) ‘Association of Long-Distance Corridor Walk Performance With Mortality, Cardiovascular Disease, Mobility Limitation, and Disability’, *JAMA*, 295(17). Available at: https://jamanetwork.com/.

Newton, R.U. *et al.* (2002) ‘Mixed-methods resistance training increases power and strength of young and older men’, *Medicine & Science in Sports & Exercise*, 34(8), pp. 1367–1375. Available at: https://doi.org/10.1097/00005768-200208000-00020.

NIH Consensus Statement (2000) *CONSENSUS CONFERENCE From the National Institutes of Health*. Available at: http://consensus.nih.gov.

Nilwik, R. *et al.* (2013) ‘The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size’, *Experimental Gerontology*, 48(5), pp. 492–498. Available at: https://doi.org/10.1016/j.exger.2013.02.012.

Nyberg, L. *et al.* (2023) ‘Individual differences in brain aging: heterogeneity in cortico-hippocampal but not caudate atrophy rates’, *Cerebral Cortex*, 33(9), pp. 5075–5081. Available at: https://doi.org/10.1093/cercor/bhac400.

Olsen, L.R., Mortensen, E.L. and Bech, P. (2004) ‘The SCL-90 and SCL-90R versions validated by item response models in a Danish community sample’, *Acta Psychiatrica Scandinavica*, 110(3), pp. 225–229. Available at: https://doi.org/10.1111/j.1600-0447.2004.00399.x.

Pani, J. *et al.* (2021) ‘Effect of 5 years of exercise intervention at different intensities on brain structure in older adults from the general population: A generation 100 substudy’, *Clinical Interventions in Aging*, 16, pp. 1485–1501. Available at: https://doi.org/10.2147/CIA.S318679.

Pani, J. *et al.* (2022) ‘Longitudinal study of the effect of a 5-year exercise intervention on structural brain complexity in older adults. A Generation 100 substudy’, *NeuroImage*, 256. Available at: https://doi.org/10.1016/j.neuroimage.2022.119226.

Paterson, D.H., Jones, G.R. and Rice, C.L. (2007) 'Ageing and physical activity: evidence to develop exercise recommendations for older adults', *Applied physiology, nutrition, and metabolism*, 98, pp. S69–S108. Available at: https://doi.org/10.1139/H07-111.

Pedersen, A.N. *et al.* (2015) *Danskernes kostvaner 2011-2013.* 1. udgave. DTU Fødevareinstituttet. Available at: https://backend.orbit.dtu.dk/ws/portalfiles/portal/110628064/Rapport_Danskernes_Kostvaner_2011_2013.pdf (Accessed: 25 March 2024).

Pedersen, B.K. (2019) 'Physical activity and muscle–brain crosstalk', *Nature Reviews Endocrinology*. Nature Publishing Group, pp. 383–392. Available at: https://doi.org/10.1038/s41574-019-0174-x.

Peters, R. (2006) 'Ageing and the brain', *Postgraduate Medical Journal*, 82(964), pp. 84–88. Available at: https://doi.org/10.1136/pgmj.2005.036665.

Peterson, M.D., Sen, A. and Gordon, P.M. (2011) 'Influence of resistance exercise on lean body mass in aging adults: A meta-analysis', *Medicine and Science in Sports and Exercise*, 43(2), pp. 249–258. Available at: https://doi.org/10.1249/MSS.0b013e3181eb6265.

Pettee Gabriel, K.K. *et al.* (2010) *Test-Retest Reliability and Validity of the 400-Meter Walk Test in Healthy, Middle-Aged Women*, *Journal of Physical Activity and Health*.

Pickering, C. and Kiely, J. (2019) 'Do Non-Responders to Exercise Exist-and If So, What Should We Do About Them?', *Sports Medicine*, 49, pp. 1–7. Available at: https://doi.org/10.1007/s40279-018-01041-1.

Pruitt, L.A. *et al.* (1992) 'Weight-training effects on bone mineral density in early postmenopausal women.', *Journal of bone and mineral research : JBMR.*, 7(2), pp. 179–185. Available at: https://doi.org/10.1002/jbmr.5650070209.

Pruitt, L.A., Taaffe, D.R. and Marcus, R. (1995) 'Effects of a one-year high-intensity versus low-intensity resistance training program on bone mineral density in older women.', *Journal of bone and mineral research : JBMR.*, 10(11), pp. 1788–1795. Available at: https://doi.org/10.1002/jbmr.5650101123.

Pyka, G. *et al.* (1994) 'Muscle Strength and Fiber Adaptations to a Year-long Resistance Training Program in Elderly Men and Women', *Journal of Gerontology*, 49(1), pp. M22–M27. Available at: https://doi.org/10.1093/geronj/49.1.M22.

Reid, K.F. and Fielding, R.A. (2012) 'Skeletal Muscle Power', *Exercise and Sport Sciences Reviews*, 40(1), pp. 4–12. Available at: https://doi.org/10.1097/JES.0b013e31823b5f13.

Reitlo, L.S. *et al.* (2023) ‘Hippocampal neurochemicals are associated with exercise group and intensity, psychological health, and general cognition in older adults’, *GeroScience*, 45(3), pp. 1667–1685. Available at: https://doi.org/10.1007/s11357-022-00719-9.

Rhodes, E.C. *et al.* (2000) *Original articles EVects of one year of resistance training on the relation between muscular strength and bone density in elderly women*, *Br J Sports Med*.

Rijk, J.M. *et al.* (2016) ‘Prognostic value of handgrip strength in people aged 60 years and older: A systematic review and meta-analysis’, *Geriatrics and Gerontology International*, 16(1), pp. 5–20. Available at: https://doi.org/10.1111/ggi.12508.

Rondanelli, M. *et al.* (2022) ‘Bone Mineral Density Reference Values in 18- to 95-Year-Old Population in Lombardy Region, Italy’, *American Journal of Men’s Health*, 16(5). Available at: https://doi.org/10.1177/15579883221119363.

Ruegsegger, G.N. and Booth, F.W. (2018) ‘Health benefits of exercise’, *Cold Spring Harbor Perspectives in Medicine*, 8(7). Available at: https://doi.org/10.1101/cshperspect.a029694.

Sakaniwa, R. *et al.* (2022) ‘Impact of modifiable healthy lifestyle adoption on lifetime gain from middle to older age’, *Age and Ageing*, 51(5). Available at: https://doi.org/10.1093/ageing/afac080.

Sakugawa, R.L. *et al.* (2019) ‘Effects of resistance training, detraining, and retraining on strength and functional capacity in elderly’, *Aging Clinical and Experimental Research*, 31(1), pp. 31–39. Available at: https://doi.org/10.1007/s40520-018-0970-5.

Sartorio, A. *et al.* (2001) ‘Effects of a 16-week progressive high-intensity strength training (HIST) on indexes of bone turnover in men over 65 years: A randomized controlled study’, *Journal of endocrinological investigation*, 24, pp. 882–886. Available at: https://doi.org/10.1007/BF03343946.

Scahill, R.I. *et al.* (2003) ‘A Longitudinal Study of Brain Volume Changes in Normal Aging Using Serial Registered Magnetic Resonance Imaging’, *Archives of Neurology*, 60(7), p. 989. Available at: https://doi.org/10.1001/archneur.60.7.989.

Schweighofer, N. *et al.* (2022) ‘Dp-ucMGP as a Biomarker in Sarcopenia’, *Nutrients*, 14(24). Available at: https://doi.org/10.3390/nu14245400.

Seo, H.S. (2012) ‘The Role and Clinical Significance of High-Sensitivity C-Reactive Protein in Cardiovascular Disease’, *Korean Circulation Journal*, 42(3), p. 151. Available at: https://doi.org/10.4070/kcj.2012.42.3.151.

Sexton, C.E. *et al.* (2016) ‘A systematic review of MRI studies examining the relationship between physical fitness and activity and the white matter of the ageing brain’, *NeuroImage*, 131, pp. 81–90. Available at: https://doi.org/10.1016/j.neuroimage.2015.09.071.

Shea, M.K. *et al.* (2011) 'Circulating uncarboxylated Matrix Gla protein is associated with vitamin K nutritional status, but not coronary artery calcium, in older adults', *Journal of Nutrition*, 141(8), pp. 1529–1534. Available at: https://doi.org/10.3945/jn.111.139634.

Sheoran, S. *et al.* (2023) 'Strength gains after 12 weeks of resistance training correlate with neurochemical markers of brain health in older adults: a randomized control 1H-MRS study', *GeroScience*, 45(3), pp. 1837–1855. Available at: https://doi.org/10.1007/s11357-023-00732-6.

Shephard, R.J. (1986) 'Physical Training for the Elderly', *Clinics in Sports Medicine*, 5(3), pp. 515–533. Available at: https://doi.org/10.1016/S0278-5919(20)31108-X.

Shojaa, M. *et al.* (2020) 'Effect of exercise training on bone mineral density in post-menopausal women: A systematic review and meta-analysis of intervention studies', *Frontiers in Physiology*, 11. Available at: https://doi.org/10.3389/fphys.2020.00652.

Shuster, A. *et al.* (2012) 'The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis', *The British Journal of Radiology*, 85(1009), pp. 1–10. Available at: https://doi.org/10.1259/bjr/38447238.

Simonsick, E.M., Fan, E. and Fleg, J.L. (2006) 'Estimating cardiorespiratory fitness in well-functioning older adults: Treadmill validation of the long distance corridor walk', *Journal of the American Geriatrics Society*, 54(1), pp. 127–132. Available at: https://doi.org/10.1111/j.1532-5415.2005.00530.x.

Simpkins, C. and Yang, F. (2022) 'Muscle power is more important than strength in preventing falls in community-dwelling older adults', *Journal of Biomechanics*, 134, p. 111018. Available at: https://doi.org/10.1016/j.jbiomech.2022.111018.

Sink, K.M. *et al.* (2015) 'Effect of a 24-month physical activity intervention vs health education on cognitive outcomes in sedentary older adults: The LIFE randomized trial', *JAMA - Journal of the American Medical Association*, 314(8), pp. 781–790. Available at: https://doi.org/10.1001/jama.2015.9617.

Skelton, D.A. *et al.* (1994) 'Strength, Power and Related Functional Ability of Healthy People Aged 65–89 Years', *Age and Ageing*, 23(5), pp. 371–377. Available at: https://doi.org/10.1093/ageing/23.5.371.

Smith, C. *et al.* (2021) 'The effects of acute exercise on bone turnover markers in middle-aged and older adults: A systematic review', *Bone*. Elsevier Inc. Available at: https://doi.org/10.1016/j.bone.2020.115766.

Snijders, T. *et al.* (2019) 'Muscle mass and strength gains following 6 months of resistance type exercise training are only partly preserved within one year with autonomous exercise continuation in older adults', *Experimental Gerontology*, 121, pp. 71–78. Available at: https://doi.org/10.1016/j.exger.2019.04.002.

Soendenbroe, C. *et al.* (2022) 'Preserved stem cell content and innervation profile of elderly human skeletal muscle with lifelong recreational exercise', *The Journal of physiology*, 600(8), pp. 1969–1989. Available at: https://doi.org/https://doi.org/10.1113/JP282677.

Soendenbroe, C., Andersen, J.L. and Mackey, A.L. (2021) 'Muscle-nerve communication and the molecular assessment of human skeletal muscle denervation with aging', *American Journal of Physiology-Cell Physiology*, 321(2), pp. C317–C329. Available at: https://doi.org/10.1152/ajpcell.00174.2021.

Sözen, T., Lale, Ö. and Başaran, N.Ç. (2017) 'An overview and management of osteoporosis', *European Journal of Rheumatology*, 4, pp. 46–56. Available at: https://doi.org/10.5152/eurjrheum.2016.048.

Stensvold, D. *et al.* (2015) 'A randomised controlled study of the long-term effects of exercise training on mortality in elderly people: study protocol for the Generation 100 study', *BMJ Open* [Preprint]. Available at: https://doi.org/10.1136/bmjopen-2014-007519.

Stensvold, D. *et al.* (2020) 'Effect of exercise training for five years on all cause mortality in older adults-the Generation 100 study: randomised controlled trial', *BMJ*, 371. Available at: https://doi.org/10.1136/bmj.m3485.

Stunes, A.K. *et al.* (2022) 'Acute Effects of Strength and Endurance Training on Bone Turnover Markers in Young Adults and Elderly Men', *Frontiers in Endocrinology*, 13. Available at: https://doi.org/10.3389/fendo.2022.915241.

Suetta, C. *et al.* (2019) 'The Copenhagen Sarcopenia Study: lean mass, strength, power, and physical function in a Danish cohort aged 20–93 years', *Journal of Cachexia, Sarcopenia and Muscle*, 10(6), pp. 1316–1329. Available at: https://doi.org/10.1002/jcsm.12477.

Takács, J. and Nyakas, C. (2021) 'The role of social factors in the successful ageing – Systematic review', *Developments in Health Sciences*, 4(1), pp. 11–20. Available at: https://doi.org/10.1556/2066.2021.00044.

Thomas, A.G. *et al.* (2012) 'The effects of aerobic activity on brain structure', *Frontiers in Psychology*. Available at: https://doi.org/10.3389/fpsyg.2012.00086.

Thompson, L. V (1994) ‘Effects of Age and Training on Skeletal Muscle Physiology and Performance’, *Physical Therapy*, 74(1), pp. 71–81. Available at: https://doi.org/10.1093/ptj/74.1.71.

Tiainen, K. *et al.* (2004) ‘Heritability of maximal isometric muscle strength in older female twins’, *Journal of Applied Physiology*, 96(1), pp. 173–180. Available at: https://doi.org/10.1152/japplphysiol.00200.2003.

Tieland, M., Trouwborst, I. and Clark, B.C. (2018) ‘Skeletal muscle performance and ageing’, *Journal of Cachexia, Sarcopenia and Muscle*, 9(1), pp. 3–19. Available at: https://doi.org/10.1002/jcsm.12238.

Tokmakidis, S.P. *et al.* (2009) ‘Effects of detraining on muscle strength and mass after high or moderate intensity of resistance training in older adults’, *Clinical Physiology and Functional Imaging*, 29(4), pp. 316–319. Available at: https://doi.org/10.1111/j.1475-097X.2009.00866.x.

Torres, E.R. *et al.* (2015) ‘Physical activity and white matter hyperintensities: A systematic review of quantitative studies’, *Preventive Medicine Reports*, 2, pp. 319–325. Available at: https://doi.org/10.1016/j.pmedr.2015.04.013.

Trappe, S., Williamson, D. and Godard, M. (2002) *Maintenance of Whole Muscle Strength and Size Following Resistance Training in Older Men*, *Journal of Gerontology*. Available at: https://academic.oup.com/biomedgerontology/article/57/4/B138/600128.

Uusi-Rasi, K. *et al.* (2017) ‘A 2-Year Follow-Up After a 2-Year RCT with Vitamin D and Exercise: Effects on Falls, Injurious Falls and Physical Functioning Among Older Women’, *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, 72(9), pp. 1239–1245. Available at: https://doi.org/10.1093/GERONA/GLX044.

Vasikaran, S. *et al.* (2011) ‘Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards’, *Osteoporosis International*, 22(2), pp. 391–420. Available at: https://doi.org/10.1007/s00198-010-1501-1.

Villa, J.K.D. *et al.* (2017) ‘Effect of vitamin K in bone metabolism and vascular calcification: A review of mechanisms of action and evidences’, *Critical Reviews in Food Science and Nutrition*, 57(18), pp. 3959–3970. Available at: https://doi.org/10.1080/10408398.2016.1211616.

Vincent, K.R. and Braith, R.W. (2002) *Resistance exercise and bone turnover in elderly men and women*, *Med. Sci. Sports Exerc*. Available at: http://journals.lww.com/acsm-msse.

Walker, S. (2021) ‘Evidence of resistance training-induced neural adaptation in older adults’, *Experimental Gerontology*, 151. Available at: https://doi.org/10.1016/j.exger.2021.111408.

Wang, B. *et al.* (2023) ‘Exercise Regulates Myokines in Aging-Related Diseases through Muscle-Brain Crosstalk’, *Gerontology*, pp. 1–17. Available at: https://doi.org/10.1159/000535339.

Warburton, D.E.R., Nicol, C.W. and Bredin, S.S.D. (2006) ‘Health benefits of physical activity: The evidence’, *CMAJ*, pp. 801–809. Available at: https://doi.org/10.1503/cmaj.051351.

Warming, L., Hassager, C. and Christiansen, C. (2002) ‘Changes in Bone Mineral Density with Age in Men and Women: A Longitudinal Study’, *Osteoporosis International*, 13, pp. 105–112.

Warriner, A.H. *et al.* (2011) ‘Which fractures are most attributable to osteoporosis?’, *Journal of Clinical Epidemiology*, 64(1), pp. 46–53. Available at: https://doi.org/10.1016/j.jclinepi.2010.07.007.

Washburn, R.A. *et al.* (1993) *THE PHYSICAL ACTIVITY SCALE FOR THE ELDERLY (PASE): DEVELOPMENT AND EVALUATION*, *J Clin Epidemiol*.

Washburn, R.A. *et al.* (1999) *The Physical Activity Scale for the Elderly (PASE): Evidence for Validity*, *J Clin Epidemiol*.

Westerterp, K.R. (2018) ‘Changes in physical activity over the lifespan: impact on body composition and sarcopenic obesity’, *Obesity Reviews*, 19, pp. 8–13. Available at: https://doi.org/10.1111/obr.12781.

Wetmore, A.B. *et al.* (2020) ‘The Effect of Training Status on Adaptations to 11 Weeks of Block Periodization Training’, *Sports*, 8(11), p. 145. Available at: https://doi.org/10.3390/sports8110145.

Weuve, J. *et al.* (2004) *Physical Activity, Including Walking, and Cognitive Function in Older Women*. Available at: https://jamanetwork.com/.

Wewege, M.A. *et al.* (2022) ‘The Effect of Resistance Training in Healthy Adults on Body Fat Percentage, Fat Mass and Visceral Fat: A Systematic Review and Meta-Analysis’, *Sports Medicine*. Springer Science and Business Media Deutschland GmbH, pp. 287–300. Available at: https://doi.org/10.1007/s40279-021-01562-2.

WHO (2022) *World Health Organization - Ageing and Health*. Available at: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health (Accessed: 30 October 2023).

Wilckens, K.A. *et al.* (2021) ‘Exercise interventions preserve hippocampal volume: A meta-analysis’, *Hippocampus*, 31(3), pp. 335–347. Available at: https://doi.org/10.1002/hipo.23292.

Wood, T.M., Maddalozzo, G.F. and Harter, R.A. (1993) *Accuracy of Seven Equations for Predicting 1-RM Performance of Apparently Healthy, Sedentary Older Adults*, *MEASUREMENT IN PHYSICAL EDUCATION AND EXERCISE SCIENCE*. Lombardi.

Xiao, J. *et al.* (2020) ‘Association of Low Muscle Mass and Low Muscle Radiodensity With Morbidity and Mortality for Colon Cancer Surgery’, *JAMA surgery*, 155(10), pp. 942–949. Available at: https://doi.org/10.1001/jamasurg.2020.2497.

Xue, B. *et al.* (2018) *Effect of retirement on cognitive function: the Whitehall II cohort study*, *Source: European Journal of Epidemiology*. Available at: https://www.jstor.org/stable/44849917.

Yarasheski, K.E., Campbell, J.A. and Kohrt, W.M. (1997) *Effect of resistance exercise and growth hormone on bone density in older men*, *Clinical Endocrinology*.

Yardley, L. *et al.* (2007) *Attitudes and Beliefs That Predict Older People’s Intention to Undertake Strength and Balance Training*. Available at: https://academic.oup.com/psychsocgerontology/article/62/2/P119/548948.

Zheng, J.J.J. *et al.* (2012) ‘Brain white matter hyperintensities, executive dysfunction, instability, and falls in older people: A prospective cohort study’, *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, pp. 1085–1091. Available at: https://doi.org/10.1093/gerona/gls063.

Zia, A. *et al.* (2021) ‘Molecular and cellular pathways contributing to brain aging’, *Behavioral and Brain Functions*. BioMed Central Ltd. Available at: https://doi.org/10.1186/s12993-021-00179-9.

Zong, G. *et al.* (2016) ‘Total and regional adiposity measured by dual-energy X-ray absorptiometry and mortality in NHANES 1999-2006’, *Obesity*, 24(11), pp. 2414–2421. Available at: https://doi.org/10.1002/oby.21659.

PAPERS

Paper 1

Mads Bloch-Ibenfeldt, Anne Theil Gates, Karoline Karlog, Naiara Demnitz, Michael Kjaer, Carl-Johan Boraxbekk
Heavy resistance training at retirement age induces 4-year-lasting beneficial effects in muscle strength: a long-term follow-up of an RCT.

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Heavy resistance training at retirement age induces 4-year lasting beneficial effects in muscle strength: a long-term follow-up of an RCT

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ABSTRACT

Objectives Muscle function and size decline with age, but long-term effects of resistance training in older adults are largely unknown. Here, we explored the long-lasting (3 years) effects of 1 year of supervised resistance training with heavy loads.

Methods The LIve active Successful Ageing (LISA) study was a parallel group randomised controlled trial at a university hospital in Denmark. Older adults (n=451) at retirement age were randomised to 1 year of heavy resistance training (HRT), moderate-intensity training (MIT) or a non-exercising control group (CON). Primary outcome measure was leg extensor power. Secondary outcomes included maximal isometric quadriceps torque (isometric leg strength) and body composition (dual-energy X-ray absorptiometry (DXA)). Participants completed test procedures at baseline, following the 1-year intervention, and 2 and 4 years post study start.

Results At the 4-year assessment, 369 participants attended (mean age=71 years, 61% women). The main finding was that across all four time points, there was a significant group×time interaction in isometric leg strength ($F_{6,1049}=8.607$, $p<0.001$, $\eta^2=0.05$). Individuals in HRT maintained baseline performance in isometric leg strength (Baseline: 149.7±51.5 Nm, 4 years: 151.5±51.1 Nm, t(1050)=1.005, p=1.00) while participants in CON and MIT decreased.

Conclusion In well-functioning older adults at retirement age, 1 year of HRT may induce long-lasting beneficial effects by preserving muscle function.

Trial registration number NCT02123641.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Worldwide, the ageing population is growing. Unfortunately, skeletal muscle function and autonomy decrease with increased age. Thus, a challenge for society is to promote a healthy lifespan without age-related diseases and loss of autonomy.

WHAT THIS STUDY ADDS

⇒ Despite relatively healthy and well-functioning participants, 1 year of heavy resistance training at retirement age resulted in maintained strength 4 years after the study started. We propose that higher load resistance training may play an important role to induce long-lasting adaptations.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study provides evidence that resistance training with heavy loads at retirement age can have long-term effects over several years. The results, therefore, provide means for practitioners and policy-makers to encourage older individuals to engage in heavy resistance training.

INTRODUCTION

Skeletal muscle function declines with advancing age.[1–3] Although resistance training may partly counteract loss of muscle mass and function, shorter training studies (6–9 months duration) only show somewhat preserved muscle mass and function at 6–12 months follow-up.[4 5] Unfortunately, long-term follow-ups are sparse.[6] In one study, strength gains following high-intensity resistance training, and not low-intensity training, were preserved after 48 weeks of detraining.[7] The LIve active Successful Ageing (LISA) study, a large-scale randomised controlled trial (n=451), showed that strength can be maintained over 12 months following 1 year of heavy resistance training (HRT), but not after moderate training.[8] Thus, to gain long-lasting effects of resistance training in ageing one could speculate that high intensity or heavy loads are required. Here, we investigated whether there would be long-lasting effects of a 1-year supervised resistance training regimen with heavy loads, 3 years following the training in older individuals at retirement age.

METHODS

Intervention

The current manuscript is an interim analysis of the LISA study, and additional follow-ups

Table 1 Sample characteristics (mean±SD), n=369 unless otherwise specified

	Baseline	4 years	T-test
Sex (men/women, %)	39/61	39/61	–
Age (years)	66.4±2.5	70.5±2.5	–
Body weight (kg)	75.7±13.6	75.3±14.0	P=0.09
BMI (kg/m^2)	25.8±4.0	25.9±4.3	P=0.10
Waist circumference (cm) (n=367)	92.7±11.5	92.5±12.2	P=0.66
Daily physical activity (steps/day) (n=349)	9548±3446	9590±3387	P=0.79

BMI, body mass index.

are planned (7-year and 10-year follow-ups). For details of intervention, recruitment and power calculations, see previous publications.[9 10] Briefly, 451 older adults were stratified according to sex, body mass index (BMI) and chair-rise test performance and randomised to 1 year of training with either heavy loads (HRT, n=149), moderate-intensity training (MIT, n=154) or a control condition (CON, n=148). At a commercial gym, HRT performed a supervised full body programme three times per week, with 6–8 weeks of initial habituation. The periodisation programme was machine based and each exercise included 3 sets of 6–12 repetitions at ~70%–85% of 1 RM, which was estimated using the prediction equation according to methods by Brzycki.[11 12] The moderate training in MIT was performed as circuit training with body weight and resistance bands once per week at the hospital and two times per week at home. Exercises in MIT progressed with the load of resistance bands (Thera-Band, Akron, Ohio, USA) and mimicked the exercises in HRT but were performed with 3 sets of 10–18 repetitions at ~50%–60% of 1 RM. Both training programmes were created to comply with recommended guidelines[13] and included nine exercises—see published study protocol for full details.[10] Individuals in CON were encouraged to maintain their habitual physical activity level and were invited to regular cultural and social activities. In general, participants did not receive advice on healthy behaviour but were aware of the study timeline and planned follow-ups.

Test procedures

Day 1 included a health screening. On day 2, participants were dual-energy X-ray absorptiometry (DXA) scanned. Visceral fat mass was estimated by scanner software (Lunar iDXA, GE HealthCare—enCORE software V.16). Isometric leg strength (quadriceps) was assessed in a Good Strength chair (Bluetooth V.3.14, Metitur) and maximal isometric quadriceps torque (Newton metres) was measured during a minimum of 3 attempts per leg.[14 15] Day 3 included MRI of the brain and thigh (two-dimensional T1-weighted, 3.0 Tesla Phillips Achieva). Blinded assessors determined CSA of m. vastus lateralis using JIM software (Xinapse systems).

Daily physical activity was assessed as daily step count between days 2 and 3, by an accelerometer (activPAL micro, PAL Technologies) worn by the participants for five consecutive days. The test procedures were performed at baseline, postintervention (year 1) and at 2-year and 4-year follow-ups.

Patient and public involvement

Participants were informed of study progress through newsletters, and received overviews of personal results after tests at each time point. Additionally, participants were invited to an information evening, where the general study results at the time were presented.

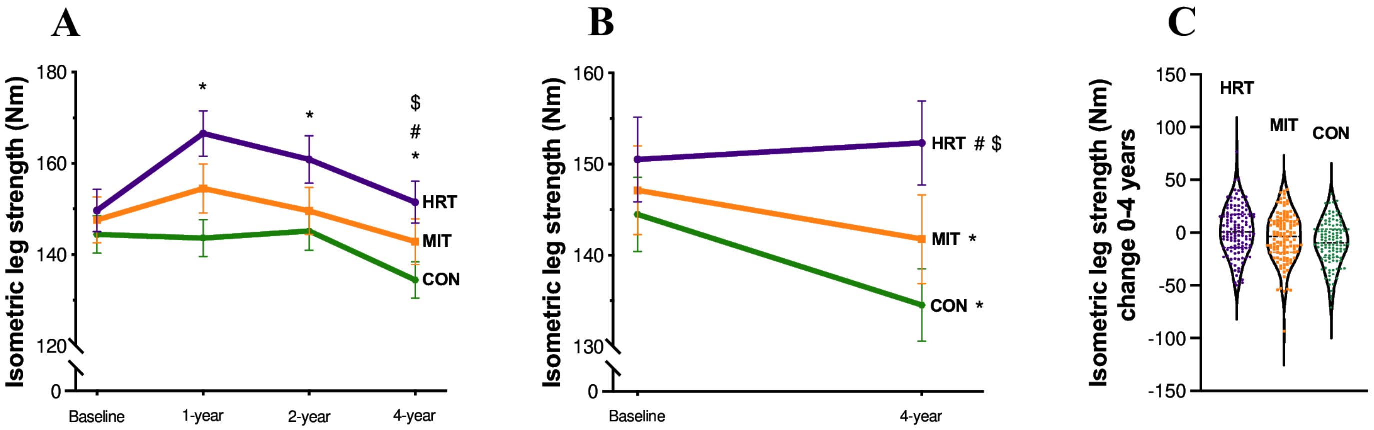

Figure 1 (A–C) Isometric strength (mean±SEM) across 4 years for the different groups (heavy resistance training, HRT, moderate-intensity training, MIT and control group, CON). (A) (n=353) Isometric leg strength (Nm) trajectories for all time points separated by group. (B) Baseline and 4-year follow-up data (n=362), each group shown separately. (C) Individual data points showing the distribution of change from baseline to year four separated by group. *Significantly different from baseline (A): HRT 1 year, p<0.001; MIT 1 year, p=0.01; HRT 2 years, p<0.001; CON 4 years, p<0.001) (B): MIT 4 years, p=0.01; CON 4 years, p<0.001). #Change from baseline significantly different from change in MIT (A): HRT 4 years, p=0.003) (B): HRT 4 years, p=0.03). $Change from baseline significantly different from change in CON (A): HRT 4 years, p<0.001) (B): HRT 4 years, p<0.001).

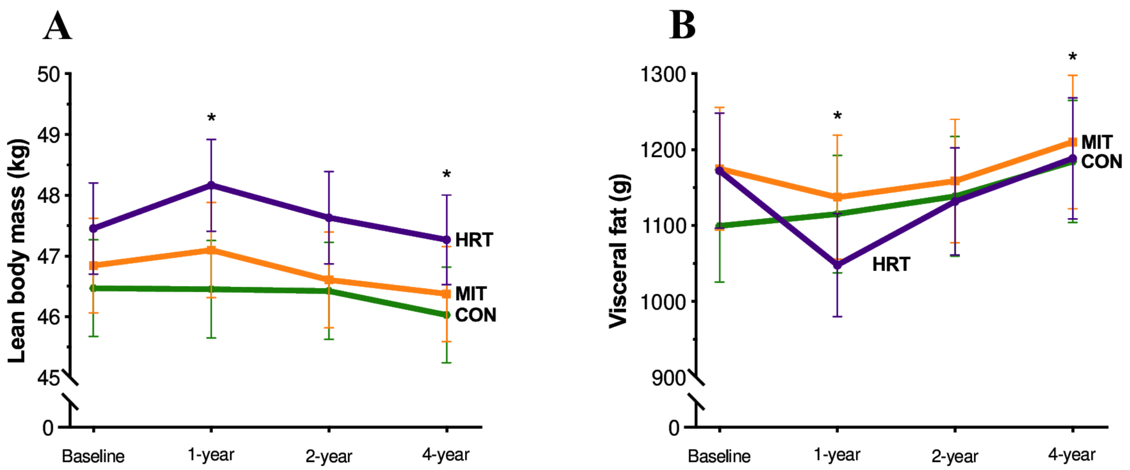

Figure 2 (A–B) Lean body mass and visceral fat (mean±SEM) across 4 years for the different groups (heavy resistance training, HRT, moderate-intensity training, MIT and control group, CON). (A) (n=365) Lean body mass (kg) trajectories for all time points separated by group. (B) Visceral fat (g) trajectories (n=365), for all time points separated by group. *Significantly different from baseline (A): HRT 1 year, p<0.001; MIT 4 years, p<0.001; CON 4 years, p=0.003) (B): HRT 1 year, p=0.01; CON 4 years, p=0.04).

Statistical analysis

Statistical analyses were performed in R V.4.1.1 and Rstudio 2021.09.0 using 'psych',[16] 'emmeans'[17] and 'sjstats'[18] packages. Figures were created in GraphPad Prism V.10.0.3.

Descriptive data are presented as mean±SD. Student's paired t-tests tested changes from baseline to year 4 in sample characteristics. Two-way mixed-model analyses of variance (ANOVAs), adjusted for age and sex, were used to test group×time interaction effects on strength (power, isometric leg strength and handgrip), and on body composition (lean body mass, lean leg mass, CSA of m. vastus lateralis, fat percentage and visceral fat content) including all four available time points. For Δchanges from baseline to year 4, one-way mixed-model ANOVAs were used to test group differences. The ANOVAs were controlled for sex and age. Significant interactions were further examined with post hoc tests. Effect sizes are reported in the form of eta squared (η^2). The significance level was p<0.006 after Bonferroni correction for multiple comparisons (eight tests).

RESULTS

At year 4, 369 participants attended follow-up assessments (HRT, n=128; MIT, n=126; CON, n=115). 82 older adults dropped out primarily due to lack of motivation or severe illness. These individuals had higher body weight, BMI and waist circumference at baseline compared with participants who were still part of the study at year 4. However, there was no difference in the response to the intervention in all outcomes at year 1 assessments between participants and individuals subsequently lost

Table 2 Outcome variables (mean±SD) at baseline and at 4 years separated by group

	HRT		MIT		CON			
	Baseline	4 years	Baseline	4 years	Baseline	4 years	F (group×time)	F (time)
Power (W)	195.3±65.1	185.3±60.2	193.2±64.0	179.4±64.1	187.7±61.8	171.2±57.2	$F_{6,1067}$=1.054 p=0.39, η^2=0.006	$F_{3,1067}$=43.651 p<0.001, η^2=0.11
Handgrip (kg)	35.6±10.5	34.3±9.9	34.3±10.4	32.7±10.2	34.9±10.2	32.8±9.9	$F_{6,1064}$=0.554 p=0.77, η^2=0.003	$F_{3,1064}$=27.789 p<0.001, η^2=0.07
LLM (kg)	17.0±3.3	16.3±3.1	16.7±3.5	15.9±3.3	16.4±3.4	15.7±3.2	$F_{6,1085}$=1.841 p=0.09, η^2=0.01	$F_{3,1085}$=249.742 p<0.001, η^2=0.41
CSA (mm^2)	1403.7±339.7	1306.2±328.4	1371.4±358.5	1284.3±352.1	1359.5±330.7	1243.1±293.8	$F_{6,908}$=3.654 p=0.001, η^2=0.02	$F_{3,908}$=36.068 p<0.001, η^2=0.11
Body fat (%)	34.0±7.9	33.8±8.2	33.5±7.6	33.7±6.4	32.7±8.3	33.1±8.5	$F_{6,1085}$=3.813 p<0.001, η^2=0.02	$F_{3,1085}$=16.964 p<0.001, η^2=0.04

Leg extensor power (power), handgrip strength (handgrip), lean leg mass (LLM), CSA of m. vastus lateralis (CSA) and total body fat (body fat). F-statistics for group×time interaction and effect of time.
CON, control group; HRT, heavy resistance training; MIT, moderate intensity training.

to follow-up. On average, participants were 71 years old (range: 64–75 years), 61% women and still active based on the daily physical activity (table 1). There was no difference in sample characteristics between groups at baseline or at follow-up.

For isometric leg strength, there was a significant group×time interaction across the four time points ($F_{6,1049} = 8.607$, p<0.001, $\eta^2 = 0.05$; figure 1A). In HRT, the strength was unaltered after 4 years (Baseline: 149.7±51.5 Nm, 4 years: 151.5±51.1 Nm, t(1050)=1.005, p=1.00), unlike the CON group in which strength decreased (Baseline: 144.4±43.3 Nm, 4 years: 134.5±42.2 Nm, t(1050)=−5.261, p<0.001). The decrease in MIT was not significant (Baseline: 147.6±54.9 Nm, 4 years: 142.9±54.6 Nm, t(1050)=−2.594, p=0.28). For the Δchanges over the 4 years, HRT significantly differed from MIT and CON (HRT>MIT, t(350) = 3.273, p=0.003; HRT>CON, t(350) = 3.655, p<0.001).

In the change from baseline to year 4 (figure 1B), muscle strength was decreased in MIT (t(122)=1.98, p=0.01) and in CON (t(113)=1.98, p<0.001), whereas it was maintained in HRT (t(124)=1.98, p=0.37).

There was a significant group×time interaction for lean body mass ($F_{6,1085} = 5.353$, p<0.001, $\eta^2 = 0.03$), see figure 2A, again in favour of HRT (Baseline: 47.5±8.5 kg, 4 year: 47.3±8.3 kg, t(1086)=−1.813, p=0.81), compared with MIT (Baseline: 46.8±8.7 kg, 4 years: 46.4±8.6 kg, t(1086)=−4.506, p<0.001) and CON (Baseline: 46.5±8.5 kg, 4 years: 46.0±8.5 kg, t(1086)=−4.075, p=0.003).

Additionally, there was a significant group×time interaction for visceral fat ($F_{6,1085} = 3.120$, p=0.005, $\eta^2 = 0.02$), see figure 2B. HRT (Baseline: 1172.4±854.6 g, 4 years: 1188.5±898.3 g, t(1086)=0.676, p=1.00) and MIT (Baseline: 1175.0±897.4 g, 4 years: 1210.1±972.9 g, t(1086)=1.450, p=0.95) did not change over the 4 years, while in CON, there was an increase in visceral fat content (Baseline: 1099.6±794.7 g, 4 years: 1184.4±862.5 g, t(1086)=3.387, p=0.04).

Significant group×time interactions for CSA of m. vastus lateralis and the percentage of total body fat (table 2) were driven by 1-year and 2-year changes, which have been reported previously.[8 9]

For leg extensor power, handgrip strength and lean leg mass, there was a main effect of time, with decreases over the 4 years across all groups, but no interaction effects or significant group differences for the Δchange over 4 years (table 2).

DISCUSSION

Resistance training with heavy loads induced long-lasting beneficial effects on muscle strength in a sample of older adults. We observed a difference between groups in leg strength, whereas handgrip strength, a measure of overall muscle strength,[19] was not influenced by any of the training regimes. Notably, benefits in leg strength were present despite lowered leg lean mass. Neural adaptations influence the response to resistance training.[20 21] The present results suggest that these adaptations might play a role even as lean leg mass and thigh CSA decrease. This is in line with a recent report showing that prolonged training across the lifespan is associated with permanently elevated acetylcholine receptors and improved neuromuscular function.[22] Resistance training may, therefore, be beneficial for function beyond the influence of muscle size itself.

Despite no group effects in lean leg mass, HRT maintained total lean mass, yet differences were minor. Interestingly, leg muscle strength was maintained from baseline in HRT, indicating that among individuals who already seemed to have a high physical activity level but were previously resistance training naive, implementing resistance training with heavy loads for 1 year may at group-level induce long-term health effects. Considering that muscle strength has been shown to predict mortality in apparently healthy populations,[23] these results may be of particular relevance. It is somewhat surprising that there was no muscular effect of the moderate training at year 4, as the intervention improved both lean mass and function in MIT, although to a lesser extent than HRT.

Interestingly, the amount of visceral fat was maintained from baseline to year 4 in both training groups, implying that some parameters may not be load-dependent or intensity-dependent in the long term. Recent research suggests that visceral fat is positively affected by resistance training.[24] Like visceral fat, the decrease over time in leg extensor power (primary outcome measure) was in line with our previous studies.[8 9]

The present study benefited from its large sample size, long intervention and multiple follow-ups. Further, study attendance remained high (82% at year 4). Of note, with almost 10 000 daily steps, the study sample is likely to be healthier and more active than the average ageing population. Even so, ≈80% of the participants had at least one chronic medical disease.[9] In age-matched older individuals living in residential care facilities, high-intensity functional training has proven effective in improving independence in activities of daily living. Although over 4 months, these results show further evidence of the effectiveness of high-intensity training in older adults.[25]

In conclusion, we showed that in a group of well-functioning older adults around retirement age, 1 year of HRT may induce long-lasting beneficial effects by preserving muscle function.

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Contributors Conceptualisation: MK; Methodology: MK and C-JB; Validation: MB-I, ATG, KK, ND, MK and C-JB; Formal analysis: MB-I and ND; Investigation: MB-I, ATG and KK; Visualisation: MB-I; Supervision: MK and C-JB; Project administration: MB-I and ATG; Writing–original draft: MB-I; Writing–review and editing: MB-I, ATG, KK, ND, MK and C-JB. All authors approved the final manuscript to be published.

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Ethics approval This study involves human participants and was approved by Regional ethics committee: Capital Region, Copenhagen, Denmark, No. H-3-2014-017. Participants gave informed consent to participate in the study before taking part.

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REFERENCES

1. Fielding RA, Vellas B, Evans WJ, *et al*. Sarcopenia: an Undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. *J Am Med Dir Assoc* 2011;12:249–56.
2. Cruz-Jentoft AJ, Bahat G, Bauer J, *et al*. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;48:16–31.
3. Suetta C, Haddock B, Alcazar J, *et al*. The Copenhagen Sarcopenia study: lean mass, strength, power, and physical function in a Danish cohort aged 20–93 years. *J Cachexia Sarcopenia Muscle* 2019;10:1316–29.
4. Fernández-Lezaun E, Schumann M, Mäkinen T, *et al*. Effects of resistance training frequency on cardiorespiratory fitness in older men and women during intervention and follow-up. *Exp Gerontol* 2017;95:44–53.
5. Snijders T, Leenders M, de Groot L, *et al*. Muscle mass and strength gains following 6 months of resistance type exercise training are only partly preserved within one year with autonomous exercise continuation in older adults. *Exp Gerontol* 2019;121:71–8.
6. Kennis E, Verschueren SM, Bogaerts A, *et al*. Long-term impact of strength training on muscle strength characteristics in older adults. *Arch Phys Med Rehabil* 2013;94:2054–60.
7. Fatouros IG, Kambas A, Katrabasas I, *et al*. Strength training and Detraining effects on muscular strength, anaerobic power, and mobility of inactive older men are intensity dependent. *Br J Sports Med* 2005;39:776–80.
8. Gylling AT, Bloch-Ibenfeldt M, Eriksen CS, *et al*. Maintenance of muscle strength following a one-year resistance training program in older adults. *Exp Gerontol* 2020;139:111049.
9. Gylling AT, Eriksen CS, Garde E, *et al*. The influence of prolonged strength training upon muscle and fat in healthy and chronically diseased older adults. *Exp Gerontol* 2020;136:110939.
10. Eriksen CS, Garde E, Reislev NL, *et al*. Physical activity as intervention for age-related loss of muscle mass and function: protocol for a randomised controlled trial (the LISA study). *BMJ Open* 2016;6:e012951.
11. Brzycki M. Scholastic coach and athletic director. In: *Strength training -- A practical approach to strength training*. 1995: 65. 86.
12. Wood TM, Maddalozzo GF, Harter RA. Accuracy of Seven Equations for Predicting 1-RM Performance of Apparently Healthy, Sedentary Older Adults. Lombardi, 1993.
13. American College of Sports Medicine. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 2009;41:687–708.
14. Bieler T, Magnusson SP, Kjaer M, *et al*. Intra-Rater Reliability and agreement of muscle strength, power and functional performance measures in patients with hip osteoarthritis. *J Rehabil Med* 2014;46:997–1005.
15. Tiainen K, Sipilä S, Alen M, *et al*. Heritability of maximal Isometric muscle strength in older female twins. *J Appl Physiol (1985)* 2004;96:173–80.
16. Revelle W. R package version 2.3.6. Psych: Procedures for Psychological, Psychometric, and Personality Research. Evanston, Illinois: Northwestern University, 2023. Available: https://CRAN.R-project.org/package=psych
17. Searle SR, Speed FM, Milliken GA. Population marginal means in the linear model: an alternative to least squares means. *The American Statistician* 1980;34:216–21.
18. Lüdecke D. [10.5281/zenodo.1284472]. sjstats: Statistical Functions for Regression Models (Version 0.18.2), 2022. Available: https://CRAN.R-project.org/package=sjstats
19. Rijk JM, Roos PR, Deckx L, *et al*. Prognostic value of Handgrip strength in people aged 60 years and older: A systematic review and meta-analysis. *Geriatr Gerontol Int* 2016;16:5–20.
20. Häkkinen K, Kallinen M, Izquierdo M, *et al*. Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. *J Appl Physiol (1985)* 1998;84:1341–9.
21. Walker S. Evidence of resistance training-induced neural adaptation in older adults. *Exp Gerontol* 2021;151:111408.
22. Soendenbroe C, Dahl CL, Meulengracht C, *et al*. Preserved stem cell content and Innervation profile of elderly human Skeletal muscle with lifelong recreational exercise. *J Physiol* 2022;600:1969–89.
23. García-Hermoso A, Cavero-Redondo I, Ramírez-Vélez R, *et al*. Muscular strength as a Predictor of all-cause mortality in an apparently healthy population: A systematic review and meta-analysis of data from approximately 2 million men and women. *Arch Phys Med Rehabil* 2018;99:2100–13.
24. Wewege MA, Desai I, Honey C, *et al*. The effect of resistance training in healthy adults on body fat percentage, fat mass and visceral fat: a systematic review and meta-analysis. *Sports Med* 2022;52:287–300.
25. Toots A, Littbrand H, Lindelöf N, *et al*. Effects of a high-intensity functional exercise program on dependence in activities of daily living and balance in older adults with dementia. *J Am Geriatr Soc* 2016;64:55–64.

Paper 2

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No long-term benefits from resistance training on brain grey matter volumes in aging.

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No long-term benefits from resistance training on brain grey matter volumes in aging.

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ABSTRACT

BACKGROUND Resistance training and other forms of physical exercise are commonly suggested to promote brain health, yet the relationship between resistance training and brain structure in aging is poorly understood. We examined the short- and long-term influence of one year of supervised resistance training at two different loadings on brain structure in aging.

METHODS In the LISA (LIve active Successful Ageing) study, well-functioning older adults at retirement age (mean age: 66 ± 2 years) were randomized to one year of heavy resistance training (HRT), moderate intensity training (MIT), or a non-exercising control group (CON). Magnetic resonance imaging (MRI) of the brain was performed at baseline, 1-, 2-, and 4-years follow ups. Trajectories of total grey matter, hippocampus, dorsolateral prefrontal cortex (dlPFC), ventrolateral prefrontal cortex (vlPFC), and white matter hyperintensities were analyzed in relation to changes in muscle strength.

RESULTS Individuals (n = 276) with MRI scans at all 4 timepoints were included (HRT, n = 96; MIT, n = 95; CON, n = 85). Total grey matter volume decreased with time across all groups ($F_{3,819}$ = 231.549, $p < 0.001$, $\eta^2 = 0.46$), as did hippocampal ($F_{3,819}$ = 310.07, $p < 0.001$, $\eta^2 = 0.53$), vlPFC ($F_{3,818}$ = 74.380, $p < 0.001$, $\eta^2 = 0.21$), and dlPFC ($F_{3,818}$ = 3.640, $p = 0.013$, $\eta^2 = 0.01$) volumes. White matter hyperintensity volumes increased ($F_{3,819}$ = 101.876, $p < 0.001$, $\eta^2 = 0.27$). There were no significant group x time interactions for any of the brain structures. Change in isometric leg strength was weakly associated with change in white matter hyperintensity volume across all individuals ($r^2 = 0.01$, $p = 0.048$).

CONCLUSIONS One year of resistance training in well-functioning older adults at retirement age did not influence volume changes in selected brain regions over a 4-year period.

TRIAL REGISTRATION The study was approved by the regional ethics committee and registered on clinicaltrials.gov 2014-04-24 (NCT02123641).

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KEYWORDS

Resistance training; Exercise; Older adults; Aging; Brain health; Magnetic resonance imaging; Grey matter volume; White matter hyperintensity; Muscle strength

BACKGROUND

The beneficial effects of resistance training on general physical health and aging are well known and have been studied substantially [1,2]. In relation to brain health, there are, surprisingly, only a few studies, although it has been encouraged for more than a decade [3]. In a small sample of healthy young adults, 4 weeks of unilateral leg resistance training induced some changes of white matter microstructure and putamen volume [4]. In older adults, improved muscle function following 12 weeks of resistance training seemed to preserve brain metabolism [5], and prolonged resistance training (52 weeks) attenuated brain atrophy [6]. It has also been proposed that prolonged resistance training could be beneficial for cognitive functions [7–9]. Moreover, in the oldest old (85 years old or above) it has been speculated that combining aerobic and resistance training could provide a synergistic effect [10]. Despite of these initial positive results, the knowledge on brain structural changes following resistance training is much sparser in comparison to what is known of other modes of exercise, such as cardiorespiratory training [11].

Regardless of training modality, less focus has been on the potential long-term effects of an exercise intervention on brain health. One exception is the Generation 100 study [12]. In this large long-term study, the effects of a 5-year exercise intervention at different intensities were investigated. The brain imaging results suggested that a high cardiorespiratory fitness level at study start and following the national activity guidelines was related to preserved brain structure rather than training at high intensity [13]. Similar observations were made for the neurochemical profile of the hippocampus [14] as well as the development of white matter hyperintensities, which was not attenuated by taking part in the prolonged supervised training [15]. The Generation 100 study underlines how important it is to follow individuals over time also in relation to physical training interventions. In a similar attempt the LISA study was initiated [16]. Here, a 1-year resistance training intervention at two different loadings (moderate vs heavy) has been compared to a non-exercising control condition. So far, the results have shown that muscle strength was improved [17] and maintained at the 2-year follow up [18] but there was no effect of the training intervention on hippocampal atrophy at either of these time-points. Initially, hippocampal volume was used as the primary brain outcome, due to its importance in healthy brain aging [19,20] as well as previously showing a relationship with aerobic fitness [21]. Considering that age-related atrophy occurs throughout the brain [22,23], and that it has previously been shown that also prefrontal cortex volume [24] and white matter structure [25] may be related with fitness, the aim of this study was twofold. First, we examined how age-related structural brain changes were influenced by one year of resistance training at two different loadings. Second, we explored whether the long-term maintenance of muscle strength that was present at both the 2-years follow up [18] and at the 4-years follow up (in review) was associated with brain structural changes.

METHODS

The LISA (LIve active Successful Ageing) study is a large-scale randomized controlled trial based at a university hospital, with a 1-year intervention and longitudinal follow-ups at years 2, 4, 7 and

10. The full study protocol has previously been published [16]. To outline, 1026 older adults were screened after recruitment through advertisements in local media. Of these, 451 individuals underwent pre-testing and were subsequently included in the study. The participants were volunteering, home-dwelling, older adults around retirement age, 62-70 years (mean age: 66 years; 61% women). The participants were rather active, with an average daily physical activity level of almost 10.000 steps/day, although ≈80% had at least one chronic disease [17]. Participants were stratified based on age, body mass index (BMI), and performance in the chair-rise test and randomized to one of three intervention groups: heavy resistance training (HRT, n = 149), moderate intensity training (MIT, n = 154), or a non-exercising control group (CON, n = 148). HRT and MIT trained 3 times per week. HRT trained in two local commercial gyms, where training was supervised in small groups and performed as a machine-based full body programme, with a focus on the lower extremities. Training was progressed and linearly periodized, with one week of rest every 9th week. In the moderate intensity training, exercises were performed with bodyweight and resistance bands once weekly at a facility at the hospital and twice weekly at home, with training progressed similarly to HRT. When the 1-year intervention was completed, no further training was offered to the participants. Training-compliance was relatively high, 77% and 78%, respectively. For specific exercises, intensity, and volume in the two training groups, see previous publications [16,17]. Individuals in the non-exercising control group were asked to maintain their habitual physical activity level and were allowed to perform a maximum of 1 hour of systematic strenuous physical activity per week during the 1-year intervention. Individuals randomized to this group were offered cultural and social activities, e.g., bridge sessions, lectures, or walks, on average once per month.

In total, three separate days were allocated for assessments. On day 1, a short and basic medical examination was performed including anthropometric measures and blood samples in the fasted state. An accelerometer was attached and worn for 5 days, and photographs of body and face for perception of age were collected. The second day, which was scheduled a minimum of 6 days after the first day, started with dual-energy x-ray absorptiometry (DXA) scans for body composition. Cognitive ability was assessed with a shortened Danish version of the intelligence structure test IST-2000-R [26], containing 3 subtests of 6, 7 and 10 minutes respectively, as previously used in three different Danish cohorts [27]. Finally, tests of physical function including 400m walking time, 30 second chair-rise test, leg extensor power, handgrip strength, and maximal isometric leg strength were performed. For the third and last day of assessments, magnetic resonance imaging (MRI) of the brain and thigh was acquired using a 3.0 Tesla scanner (TX Phillips Achieva Scanner, Philips Healthcare) at the Danish Research Centre for Magnetic Resonance (DRCMR) at Hvidovre Hospital, Denmark. Participants were asked to refrain from strenuous physical activity in the preceding 72 hours. All scans were performed by an experienced radiographer. The images were used to estimate the cross-sectional area (CSA) of m. vastus lateralis as well as to segment the brain into selected regions of interest (ROI) using the FreeSurfer software (version 6.0).

The automatically generated volumes were used to obtain total brain grey matter, white matter hyperintensities, and hippocampal volume. For the volume of dorsolateral prefrontal cortex (dlPFC) the ‘caudal-middle-frontal’ ROI was used [23] while ‘pars opercularis’, ‘pars orbitalis’, and ‘pars triangularis’ ROIs were combined for the ventrolateral prefrontal cortex (vlPFC) volume [24]. For all regions, the sum of the left and right hemisphere was used.

Due to contraindications for MRI (e.g., pacemakers, other metallic components, claustrophobia) not everyone included in the LISA-study took part in the MRI. For the present study, 276 individuals had MRI scans at all 4 timepoints (HRT, n = 96; MIT, n = 95; CON, n = 85). See table 1 for characteristics at baseline and year 4.

STATISTICAL ANALYSIS

All statistical analyses were performed in R version 4.1.1 and Rstudio 2021.09.0 using "psych" [28] , "dplyr" [29], "emmeans" [30] and "sjstats" [31] packages. Figures were created in GraphPad Prism version 10.0.3.

Student's paired t-tests were used to test potential changes over the 4 years in participant characteristics for each group. For these participant characteristics two-way ANOVAs were computed to test for group x time interactions. Two-way mixed model ANOVAs were used to test for group x time interaction on brain structure (total grey matter volume, hippocampus, dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, and white matter hyperintensity volume). These analyses were corrected, in the model, for estimated total intracranial volume and sex. Associations between Δ-change in muscle strength (baseline to year 4) and Δ-change in brain structures (baseline to year 4) were tested with partial correlation analysis. The significance level was $p < 0.01$ after Bonferroni correction for multiple comparisons across the five brain outcomes. Effect sizes are reported in the form of eta squared (η^2) for interactions and r^2 for change-change correlations.

RESULTS

As shown in **Table 1**, at the 4-year follow-up assessments, participants were on average 71 years old. The amount of daily physical activity was still high with an average daily step count of nearly 10,000 in each of the groups.

PARTICIPANT CHARACTERISTICS, n = 276

	HRT n = 96			MIT n = 95			CON n = 85			
	Baseline	4-year	T-test	Baseline	4-year	T-test	Baseline	4-year	T-test	F (group x time)
Sex (m/w, %)	41/59	41/59	-	37/63	37/63	-	38/62	38/62	-	-
Age (years)	66.4 ± 2.6	70.5 ± 2.6	t(95) = 1.99 $p < 0.001$	66.4 ± 2.4	70.5 ± 2.5	t(94) = 1.99 $p < 0.001$	66.6 ± 2.5	70.7 ± 2.4	t(84) = 1.99 $p < 0.001$	$F_{2,273} = 14.21$ $p < 0.001$, $\eta^2 = 0.09$
Weight (kg)	76.3 ± 13.0	75.5 ± 13.4	t(95) = 1.99 p = 0.051	74.6 ± 11.9	73.8 ± 12.3	t(94) = 1.99 p = 0.035	74.4 ± 12.7	74.8 ± 13.8	t(84) = 1.99 p = 0.401	$F_{2,273} = 2.685$ p = 0.070, $\eta^2 = 0.02$
BMI (kg/m^2)	25.8 ± 3.9	25.8 ± 4.0	t(95) = 1.99 p = 0.986	25.6 ± 3.6	25.5 ± 3.8	t(94) = 1.99 p = 0.577	25.4 ± 3.5	25.7 ± 3.9	t(84) = 1.99 p = 0.058	$F_{2,273} = 1.911$ p = 0.150, $\eta^2 = 0.01$
Waist circum. (cm)	92.5 ± 11.1	92.2 ± 11.3	t(95) = 1.99 p = 0.551	91.7 ± 10.7	91.2 ± 11.1	t(93) = 1.99 p = 0.342	91.9 ± 10.6	92.0 ± 12.0	t(83) = 1.99 p = 0.847	$F_{2,271} = 0.343$ p = 0.710, $\eta^2 = 0.003$
Daily physical activity (steps/day)	9604 ± 3256	9562 ± 3403	t(93) = 1.99 p = 0.877	9737 ± 2859	9761 ± 2907	t(88) = 1.99 p = 0.941	9661 ± 3923	9524 ± 3560	t(79) = 1.99 p = 0.681	$F_{2,260} = 0.066$ p = 0.936, $\eta^2 = 0.0005$

Table 1. Sample characteristics (mean ± SD) at baseline and year 4 for each group: HRT, heavy resistance training; MIT, moderate intensity training; CON, non-exercising control group.

Brain changes over the 4 years in the specified brain regions are displayed for each group respectively in **Figure 1A-E**.

For total grey matter volume, there was a significant effect of time, with a decrease over the four years ($F_{3,819} = 231.549$, $p < 0.001$, $\eta^2 = 0.46$). This was similar for hippocampal volume ($F_{3,819} = 310.07$, $p < 0.001$, $\eta^2 = 0.53$), where the decrease from 2-year follow-up to 4-year follow up (~1.7%) was at a rate similar to what has previously been described from baseline to year 2 (0.8% during each of the first two years). Likewise, there was a decrease in volume over time for vlPFC ($F_{3,818} = 74.380$, $p < 0.001$, $\eta^2 = 0.21$), as well as for dlPFC ($F_{3,818} = 3.640$, $p = 0.013$, $\eta^2 = 0.01$). The increase in white matter hyperintensities ($F_{3,819} = 101.876$, $p < 0.001$, $\eta^2 = 0.27$) corresponded to ~7-8% annually across all groups. There were no significant group x time interactions for any of the brain structures.

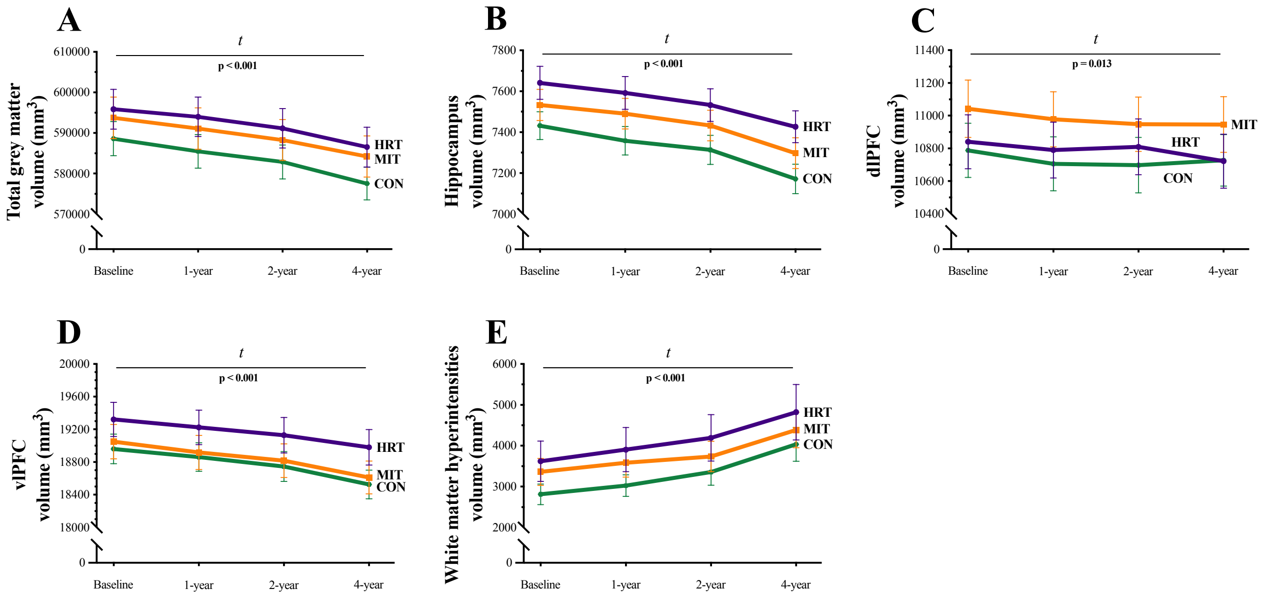

Figure 1. Brain structure volumes (mean ± SEM) for baseline, 1-year, 2-year, and 4-year, across each group. **A** Total grey matter volume (mm^3) **B** Hippocampus volume (mm^3) **C** Dorsolateral prefrontal cortex volume (mm^3) **D** Ventrolateral prefrontal cortex volume (mm^3) **E** White matter hyperintensities volume (mm^3).

t, significant effect of time (**A**, **B**, **D**, **E**: $p < 0.001$; C: $p = 0.013$).

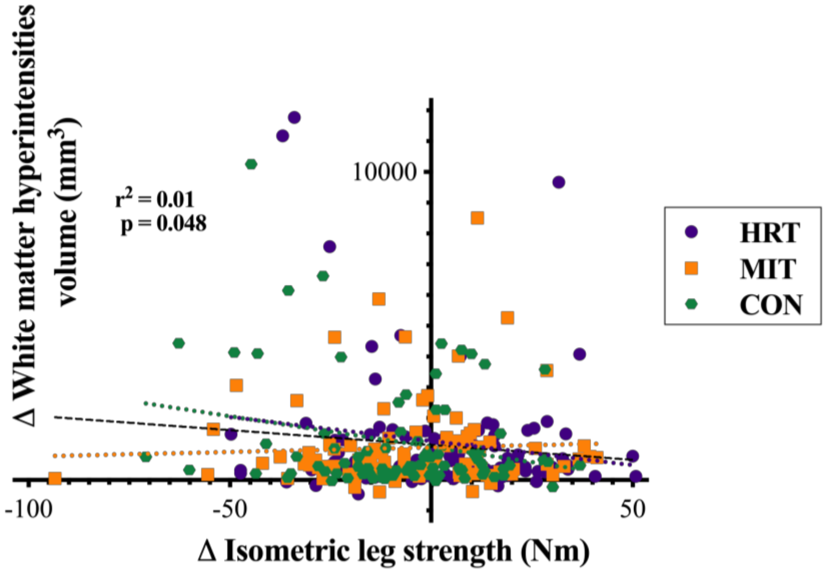

Figure 2. Association between Δ white matter hyperintensity volume (change from baseline to 4-year follow-up) and Δ isometric leg strength (change in leg muscle strength from baseline to 4-year follow-up in the dominant leg). HRT, heavy resistance training; MIT, moderate intensity training; CON, non-exercising control group. For each group, regression slopes are displayed with dotted lines in corresponding colors. The overall regression slope is displayed with a block dotted line.

The association between leg muscle strength and white matter hyperintensity volume is shown in **Figure 2**. Changes over the four years were negatively correlated ($r^2 = 0.01$, $p = 0.048$). When the same association was tested for each group separately, changes over the four years were negatively correlated for CON ($r^2 = 0.06$, $p = 0.029$), but not for HRT ($r^2 = 0.03$, $p = 0.119$) or MIT ($r^2 = 0.002$, $p = 0.653$). However, associations were not significant after correcting for multiple comparisons ($p > 0.01$). Other structural changes in the brain were not correlated with the change in strength.

DISCUSSION

We found that one year of resistance training did not result in immediate or long-term (4 years) structural brain changes in older individuals around retirement age. Using the same MR scanner and protocol, repeated structural brain mapping showed that resistance training at neither heavy nor moderate loading influenced grey matter volumes. Rather, each group displayed the expected decline for this specific age group [22,32]. These results were thus in line with what has previously been shown for hippocampus volume [17,18]. Further, there were no associations between change in leg muscle strength and change in volume of the pre-specified brain regions despite the fact that leg muscle strength was maintained from study start in the group of individuals who had performed the resistance training with heavy loads (in review). The progression of white matter hyperintensities, however, may be linked to muscular strength, as the change in volume was weakly associated with change in leg muscle strength.

There has been some evidence that brain structure may be associated with muscle function or structure [33], and there has been support of a long-term relationship between physical function and brain structure [34–36]. Here, we did not observe any resistance-training specific link to brain volumes, and it remains unclear what, if any, the specific effects of resistance training would be on brain structure in aging. However, we did observe a small association between change in leg muscle strength and change in white matter hyperintensity volume. Considering that white matter hyperintensities are increasing with age [37], associated with many other negative health outcomes [38] and related to falls and impaired gait performance [37,39], this association could be of potential relevance for older individuals. Aerobic exercise has shown to influence white matter structural integrity in older adults [36,40], thus a potential influence from resistance training should be directly addressed in future studies.

The relationship between physical training and brain health in aging is complex, with some studies showing positive associations and some studies showing no effects [41]. One suggested mechanism for the crosstalk between muscle and brain is the release of myokines during exercise and the suggested existence of a muscle–brain endocrine loop [42]. One key myokine for brain plasticity is brain-derived neurotrophic factor (BDNF), which is produced by skeletal muscle during contraction [43]. Improvements in maximal oxygen consumption have been linked to increased levels of BDNF and hippocampus volume change [44]. With resistance training BDNF levels, as well as other myokines, should be increased, which so far has been observed in some intervention studies (mostly low- to moderate-intensity and short-term training), with the idea that finding the optimal exercise prescription for myokine expression is the key question for brain plasticity [45–47]. Unfortunately, the present study did not have any measures of myokine expression, hence we do not know if the resistance training evoked the hypothesized release of plasticity-inducing myokines. A deeper mechanistical understanding is necessary to further understand if resistance training plays a key role in lifelong learning and neuroplasticity, and what the active ingredient would then be.

A limitation in the current study was that the included participants were relatively well functioning and active. Measures of handgrip strength, for example, which is widely considered a reliable measure of overall muscle strength and functional status, compared well to normative values from age-matched peers [48,49]. Thus, perhaps it is a question about whether adding more exercise to an already active population will further benefit brain health, which was also recently addressed in the Generation 100 exercise study performed in a similar Nordic country. In that study, long-term trajectories of brain structure and white matter hyperintensity development did not seem to be altered by two different training intensities [15,50]. Rather, it was in the control situation, that the lowest atrophy rate in hippocampus was observed, and not after high intensity training, which was otherwise hypothesized [13]. Noteworthy, participants in the control group were encouraged to follow the rather demanding national activity guidelines of at least 30 minutes of moderate intensity exercise almost every day [12]. Combined with the general physical activity level of nearly 10,000 steps/day that was observed for the LISA-participants, one could speculate that the level of physical activity was already too high to induce any further benefits, or to influence the small changes in volume of different brain regions seen during early aging. However, it should be noted that we cannot exclude that the resistance training had some beneficial effects upon the brain that goes beyond mere changes in volume of the selected brain regions. Despite this, there are several strengths in the present study. First, with a large sample size the LISA-study has so far been the largest study of resistance training and brain structure in humans. Second, the drop-out rate was low, and third the participants were followed over several years.

CONCLUSIONS

In conclusion, we did not observe any influence of resistance training on brain structure measured with MRI in older adults around retirement age. We observed a small association between changes in leg muscle strength and development of white matter hyperintensities. Considering the many negative influences white matter hyperintensities have on an individual's life, this finding should be further investigated in future studies.

LIST OF ABBREVIATIONS

BMI: body mass index
HRT: heavy resistance training
MIT: moderate intensity training
CON: non-exercising control group
DXA: dual-energy x-ray absorptiometry
MRI: magnetic resonance imaging
CSA: cross-sectional area
ROI: region of interest
dlPFC: dorsolateral prefrontal cortex
vlPFC: ventrolateral prefrontal cortex
BDNF: brain-derived neurotrophic factor

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the regional ethics committee (Capital Region, Copenhagen, Denmark, No. H-3-2014-017), all participants gave written informed consent before participating, and the trial was registered on clinicaltrials.gov (NCT02123641).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this article are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

HRS has received honoraria as speaker and consultant from Lundbeck AS, Denmark, and as editor (Neuroimage Clinical) from Elsevier Publishers, Amsterdam, The Netherlands. He has received royalties as book editor from Springer Publishers, Stuttgart, Germany, Oxford University Press, Oxford, UK, and from Gyldendal Publishers, Copenhagen, Denmark.

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AUTHORS' CONTRIBUTIONS

MK and **HRS** conceptualized the study. **MK** and **CJB** supervised the project. **MBI** and **ATG** administered the project, performed the experiments, and collected the data. **MBI** performed the statistical analysis of data and created the visuals. **MBI**, **CJB**, and **ND** made the initial interpretation of data. **MBI** wrote the original draft. All authors reviewed and edited the manuscript for scientific content. Prior to submission, all authors read and approved the final manuscript.

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REFERENCES

1. Fragala MS, Cadore EL, Dorgo S, Izquierdo M, Kraemer WJ, Peterson MD, et al. Resistance Training for Older Adults: Position Statement From the National Strength and Conditioning Association. J Strength Cond Res [Internet]. 2019; Available from: www.nsca.com

2. Latham NK, Bennett DA, Stretton CM, Anderson CS. Systematic Review of Progressive Resistance Strength Training in Older Adults. The Journals of Gerontology: Series A [Internet]. 2004; Available from: https://academic.oup.com/biomedgerontology/article/59/1/M48/533579

3. Liu-Ambrose T, Donaldson MG. Exercise and cognition in older adults: Is there a role for resistance training programmes? Br J Sports Med. 2009. p. 25–7.

4. Palmer HS, Håberg AK, Fimland MS, Solstad GM, Iversen VM, Hoff J, et al. Structural brain changes after 4 wk of unilateral strength training of the lower limb. J Appl Physiol [Internet]. 2013;115:167–75. Available from: http://www.jappl.org

5. Sheoran S, Vints WAJ, Valatkevičienė K, Kušleikienė S, Gleiznienė R, Česnaitienė VJ, et al. Strength gains after 12 weeks of resistance training correlate with neurochemical markers of brain health in older adults: a randomized control 1H-MRS study. Geroscience. 2023;45:1837–55.

6. Best JR, Chiu BK, Liang Hsu C, Nagamatsu LS, Liu-Ambrose T. Long-Term Effects of Resistance Exercise Training on Cognition and Brain Volume in Older Women: Results from a Randomized Controlled Trial. Journal of the International Neuropsychological Society. 2015;21:745–56.

7. Liu-Ambrose T, Nagamatsu LS, Graf P, Beattie BL, Ashe MC, Handy TC. Resistance training and executive functions: A 12-month randomized controlled trial. Arch Intern Med. 2010;170:170–8.

8. Liu-Ambrose T, Nagamatsu LS, Voss MW, Khan KM, Handy TC. Resistance training and functional plasticity of the aging brain: A 12-month randomized controlled trial. Neurobiol Aging. 2012;33:1690–8.

9. Landrigan JF, Bell T, Crowe M, Clay OJ, Mirman D. Lifting cognition: a meta-analysis of effects of resistance exercise on cognition. Psychol Res. Springer; 2020. p. 1167–83.

10. Ho BD, Gullett JM, Anton S, Franchetti MK, Bharadwaj PK, Raichlen DA, et al. Associations between physical exercise type, fluid intelligence, executive function, and processing speed in the oldest-old (85 +). Geroscience. 2023;

11. Thomas AG, Dennis A, Bandettini PA, Johansen-Berg H. The effects of aerobic activity on brain structure. Front Psychol. 2012.

12. Stensvold D, Viken H, Rognmo Ø, Skogvoll E, Steinshamn SL, Vatten LJ, et al. A randomised controlled study of the long-term effects of exercise training on mortality in elderly people: study protocol for the Generation 100 study. BMJ Open. 2015;

13. Pani J, Reitlo LS, Evensmoen HR, Lydersen S, Wisløff U, Stensvold D, et al. Effect of 5 years of exercise intervention at different intensities on brain structure in older adults from the general population: A generation 100 substudy. Clin Interv Aging. 2021;16:1485–501.

14. Reitlo LS, Mihailovic JM, Stensvold D, Wisløff U, Hyder F, Håberg AK. Hippocampal neurochemicals are associated with exercise group and intensity, psychological health, and general cognition in older adults. Geroscience. 2023;45:1667–85.

15. Arild A, Vangberg T, Nikkels H, Lydersen S, Wisløff U, Stensvold D, et al. Five years of exercise intervention at different intensities and development of white matter hyperintensities in community dwelling older adults, a Generation 100 sub-study. Aging (Albany, NY). 2022;14:596–622.

16. Eriksen CS, Garde E, Linde Reislev N, Lawaetz Wimmelmann C, Bieler T, Kraag Ziegler A, et al. Physical activity as intervention for age-related loss of muscle mass and function: protocol for a randomised controlled trial (the LISA study). BMJ Open. 2016;6.

17. Gylling AT, Eriksen CS, Garde E, Wimmelmann CL, Reislev NL, Bieler T, et al. The influence of prolonged strength training upon muscle and fat in healthy and chronically diseased older adults. Exp Gerontol. 2020;136.

18. Gylling AT, Bloch-Ibenfeldt M, Eriksen CS, Ziegler AK, Wimmelmann CL, Baekgaard M, et al. Maintenance of muscle strength following a one-year resistance training program in older adults. Exp Gerontol. 2020;139.

19. Bussy A, Patel R, Plitman E, Tullo S, Salaciak A, Bedford SA, et al. Hippocampal shape across the healthy lifespan and its relationship with cognition. Neurobiol Aging. 2021;106:153–68.

20. Driscoll I, Hamilton DA, Petropoulos H, Yeo RA, Brooks WM, Baumgartner RN, et al. The Aging Hippocampus: Cognitive, Biochemical and Structural Findings. Cerebral Cortex. 2003;13:1344–51.

21. Wilckens KA, Stillman CM, Waiwood AM, Kang C, Leckie RL, Peven JC, et al. Exercise interventions preserve hippocampal volume: A meta-analysis. Hippocampus. 2021;31:335–47.

22. Fjell AM, Walhovd KB, Fennema-Notestine C, McEvoy LK, Hagler DJ, Holland D, et al. One-year brain atrophy evident in healthy aging. Journal of Neuroscience. 2009;29:15223–31.

23. Nyberg L, Andersson M, Lundquist A, Baaré WFC, Bartrés-Faz D, Bertram L, et al. Individual differences in brain aging: heterogeneity in cortico-hippocampal but not caudate atrophy rates. Cerebral Cortex. 2023;33:5075–81.

24. Jonasson LS, Nyberg L, Kramer AF, Lundquist A, Riklund K, Boraxbekk CJ. Aerobic exercise intervention, cognitive performance, and brain structure: Results from the Physical Influences on Brain in Aging (PHIBRA) Study. Front Aging Neurosci. 2017;8.

25. Mukaetova-Ladinska EB, Liu Y, Venneri A. Editorial: The impact of physical activity on white matter during healthy aging. Front Aging Neurosci. 2023;15.

26. Liepmann D, Beauducel A, Brocke B, Amthauer R. Intelligenz-Struktur-Test 2000 R. 2nd ed. Hogrefe; 2007.

27. Mortensen EL, Flensborg-Madsen T, Molbo D, Fagerlund B, Christensen U, Lund R, et al. The relationship between cognitive ability and demographic factors in late midlife. J Aging Health. 2014;26:37–53.

28. Revelle W. psych: Procedures for Psychological, Psychometric, and Personality Research. Northwestern University, Evanston, Illinois. R package version 2.3.6, https://CRAN.R-project.org/package=psych. 2023.

29. Wickham H, François R, Henry L, Müller K, Vaughan D. dplyr: A Grammar of Data Manipulation. https://dplyr.tidyverse.org, https://github.com/tidyverse/dplyr. 2023.

30. Searle SR, Speed FM, Milliken GA. Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. Am Stat. 1980;34:216–21.

31. Lüdecke D. sjstats: Statistical Functions for Regression Models (Version 0.18.2). doi:10.5281/zenodo.1284472, https://CRAN.R-project.org/package=sjstats. 2022.

32. Scahill RI, Frost C, Jenkins R, Whitwell JL, Rossor MN, Fox NC. A Longitudinal Study of Brain Volume Changes in Normal Aging Using Serial Registered Magnetic Resonance Imaging. Arch Neurol. 2003;60:989.

33. Kilgour AH, Todd OM, Starr JM. A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse. BMC Geriatr. 2014;14:85–85.

34. Demnitz N, Gates AT, Mortensen EL, Garde E, Wimmelmann CL, Siebner HR, et al. Is it all in the baseline? Trajectories of chair stand performance over 4 years and their association with grey matter structure in older adults. Hum Brain Mapp. 2023;44:4299–309.

35. Demnitz N, Zsoldos E, Mahmood A, Mackay CE, Kivimäki M, Singh-Manoux A, et al. Associations between mobility, cognition, and brain structure in healthy older adults. Front Aging Neurosci. 2017;9.

36. Mendez Colmenares A, Voss MW, Fanning J, Salerno EA, Gothe NP, Thomas ML, et al. White matter plasticity in healthy older adults: The effects of aerobic exercise. Neuroimage. 2021;239.

37. Montero-Odasso M. Are falls a manifestation of brain failure? Revisited 40 years later. Age Ageing. Oxford University Press; 2023.

38. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ. 2010;341:c3666–c3666.

39. Zheng JJJ, Lord SR, Close JCT, Sachdev PS, Wen W, Brodaty H, et al. Brain white matter hyperintensities, executive dysfunction, instability, and falls in older people: A prospective cohort study. Journals of Gerontology - Series A Biological Sciences and Medical Sciences. 2012. p. 1085–91.

40. Sexton CE, Betts JF, Demnitz N, Dawes H, Ebmeier KP, Johansen-Berg H. A systematic review of MRI studies examining the relationship between physical fitness and activity and the white matter of the ageing brain. Neuroimage. 2016;131:81–90.

41. Ciria LF, Román-Caballero R, Vadillo MA, Holgado D, Luque-Casado A, Perakakis P, et al. An umbrella review of randomized control trials on the effects of physical exercise on cognition. Nat Hum Behav. 2023;7:928–41.

42. Pedersen BK. Physical activity and muscle–brain crosstalk. Nat Rev Endocrinol. Nature Publishing Group; 2019. p. 383–92.

43. Matthews VB, Åström MB, Chan MHS, Bruce CR, Krabbe KS, Prelovsek O, et al. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. Diabetologia. 2009;52:1409–18.

44. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A. 2011;108:3017–22.

45. Zhang M, Jia J, Yang Y, Zhang L, Wang X. Effects of exercise interventions on cognitive functions in healthy populations: A systematic review and meta-analysis. Ageing Res Rev. Elsevier Ireland Ltd; 2023.

46. Wang B, Liang J, Lu C, Lu A, Wang C. Exercise Regulates Myokines in Aging-Related Diseases through Muscle-Brain Crosstalk. Gerontology [Internet]. 2023;1–17. Available from: https://karger.com/doi/10.1159/000535339

47. Tyndall A V., Clark CM, Anderson TJ, Hogan DB, Hill MD, Longman RS, et al. Protective Effects of Exercise on Cognition and Brain Health in Older Adults. Exerc Sport Sci Rev. 2018;46:215–23.

48. Mayhew AJ, So HY, Ma J, Beauchamp MK, Griffith LE, Kuspinar A, et al. Normative values for grip strength, gait speed, timed up and go, single leg balance, and chair rise derived from the Canadian longitudinal study on ageing. Age Ageing. 2023;52.

49. Aadahl M, Beyer N, Linneberg A, Thuesen BH, Jørgensen T. Grip strength and lower limb extension power in 19-72-year-old Danish men and women: The Health2006 study. BMJ Open. 2011;1.

50. Pani J, Marzi C, Stensvold D, Wisløff U, Håberg AK, Diciotti S. Longitudinal study of the effect of a 5-year exercise intervention on structural brain complexity in older adults. A Generation 100 substudy. Neuroimage. 2022;256.

Paper 3

Mads Bloch-Ibenfeldt, Anne Theil Gates, Niklas Rye Jørgensen, Allan Linneberg, Mette Aadahl, Michael Kjær, Carl-Johan Boraxbekk

Heavy resistance training provides short-term benefits on bone formation in well-functioning older adults.

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Heavy resistance training provides short-term benefits on bone formation in well-functioning older adults.

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ABSTRACT

OBJECTIVES Maintained bone health is critical for independent living when aging. Currently, weight-bearing exercises with impact are prescribed as the optimal exercise modality for maintaining bone health, while there is less consensus on the effects of resistance training at different intensities upon bone. Here we examined whether bone health was positively influenced by 1 year of supervised resistance training.

METHODS Older adults at retirement age (mean age: 66 ± 3 years, n = 451) were randomized to either 1 year of heavy resistance training (HRT), moderate intensity training (MIT) or a non-exercising control group (CON) in the LISA (LIve active Successful Ageing) study. Bone mineral density (BMD) was assessed using Dual-energy X-ray absorptiometry (DXA). Bone degradation and formation were evaluated with blood C-terminal telopeptide of type I collagen (CTX) and procollagen type I N-propeptide (PINP). Dephosphorylated uncarboxylated matrix Gla-protein (dp-ucMGP) was used as a biomarker of functional vitamin K status. Participants were assessed at baseline, immediately following the intervention (year 1), and at longitudinal follow-ups at years 2 and 4. Two-way mixed model ANOVAs were used to assess group differences at all time points.

RESULTS At the 4-year follow-up n = 329 participants remained in the study. BMD was not influenced by training and decreased across all groups over the 4 years for total body ($F_{3,977}$ = 4.617, p = 0.003, η^2 = 0.01) and femoral neck both in the dominant ($F_{3,893}$ = 45.135, p < 0.001, η^2 = 0.13) and non-dominant leg ($F_{3,896}$ = 33.821, p < 0.001, η^2 = 0.10).

Independent of group, CTX increased ($F_{3,932}$ = 47.434, p < 0.001, η^2 = 0.13) over the 4 years. HRT resulted in an increased bone formation (PINP rise) only after the first year with systematic training (t(936) = 3.357, p = 0.04), and it was more pronounced than in CON (t(312) = 2.494, p = 0.04). Plasma dp-ucMGP remained unaltered over time in all groups. In general, women had significantly lower BMD and higher levels of CTX and PINP compared to men.

CONCLUSION We demonstrated that 1 year of heavy resistance training positively influenced short-term bone formation in well-functioning older adults, although the effect was not maintained at long-term follow ups. These minor changes in bone biomarkers were not reflected in changes in BMD measured with DXA.

TRIAL REGISTRATION clinicaltrials.gov (NCT02123641).

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KEY WORDS

Resistance training

Older adults

Aging

Dual Energy X-ray Absorptiometry

Bone formation

Bone mineral density

INTRODUCTION

The proportion of older individuals around the world is increasing at a rapid rate (WHO, 2022). One of the major components in aging is bone health, and it has been suggested that the incidence of osteoporosis, as well as the risk of falls and fractures, will markedly increase the burden on future health care system (Black and Rosen, 2016). Lifestyle factors including physical exercise and diet have been suggested to have beneficial effects on bone health (Muñoz-Garach, García-Fontana and Muñoz-Torres, 2020; Chen and Avgerinou, 2023). The current recommendations highlight the importance of weightbearing exercises with impact (NIH Consensus Statement, 2000). Still, despite a plethora of trials looking into the exercise effects on bone health, there seems to be no clear consensus on the effects of resistance training. For example, several studies have not been able to observe any beneficial effects upon bone mineral density (BMD) with resistance or resistance-like training (Pruitt *et al.*, 1992; McCartney *et al.*, 1995; Pruitt, Taaffe and Marcus, 1995; Fujimura *et al.*, 1997; Maddalozzo and Snow, 2000). Others have nevertheless found beneficial effects, although these were often minor or exclusively at regional bone sites in the spine or hip (Lohman *et al.*, 1995; Yarasheski, Campbell and Kohrt, 1997; Layne and Nelson, 1999; Rhodes *et al.*, 2000; Vincent and Braith, 2002; Hinton, Nigh and Thyfault, 2015; Shojaa *et al.*, 2020; Herda and Nabavizadeh, 2023). Notably, studies vary considerably in their sample size, age and sex of the participants, specific exercises performed, and inclusion of healthy or non-healthy individuals. Most studies have focused on postmenopausal women, due to hormonal changes related to menopause. However, it has also been suggested that bone health is not as age- or sex-specific as previously suggested, with male cases likely being under-reported (NIH Consensus Statement, 2000; Nguyen, 2023).

Besides the use of dual-energy x-ray absorptiometry (DXA) to examine manifested changes in bone density, blood-based biomarkers can measure both bone metabolism and bone turnover. The two markers, procollagen type I N-propeptide (PINP) and C-terminal telopeptide of type I collagen (CTX), have been recommended as the international reference markers (Vasikaran *et al.*, 2011). PINP is a marker of bone formation, has a low circadian and biological variation, and is not affected by food intake (Szulc *et al.*, 2017; Gillett, Vasikaran and Inderjeeth, 2021). CTX is a marker of bone resorption and has been shown to correlate with BMD (Bønløkke *et al.*, 2022). In addition to these two biomarkers, dephosphorylated-uncarboxylated Matrix Gla-Protein (dp-ucMGP) has more recently been linked to bone formation, through its reflection of vitamin K status, where high plasma concentrations of dp-ucMGP reflect low vitamin K status (Cranenburg *et al.*, 2010; Villa *et al.*, 2017; Akbari and Rasouli-Ghahroudi, 2018).

In the randomized controlled LISA trial, we have previously reported on beneficial effects of 1 year of resistance training upon muscle function and body composition; both short- (Gylling, Eriksen, *et al.*, 2020) and long-term ones (Bloch-Ibenfeldt *et al.*, in review). The aim of the present study was therefore to examine whether the positive effects of resistance training upon skeletal muscle were translated into changes in BMD and markers related to bone turnover both immediately after the 1-year intervention as well as during the long term follow-ups 2 and 4 years post study start.

METHODS

Full details on the LISA trial have been described previously (Eriksen *et al.*, 2016; Gylling, Eriksen, *et al.*, 2020). To summarize, in a large-scale randomized controlled trial 1026 older adults around retirement age were initially screened and 451 enrolled in an intervention targeting physical activity as a mean of successful aging. Participants were home-dwelling volunteers at retirement age, 62-70 years (mean age: 66 years at study start), recruited through local advertisements, and 61% were women.

The participants were stratified – based on sex, body mass index (BMI), and chair-rise test performance – and randomized to one of three conditions: 1 year of resistance training with either heavy loads (HRT, n = 149) or at moderate intensity (MIT, n = 154), or a non-exercising control condition (CON, n = 148). Additionally, individuals were followed through the next 3 years (follow-ups at year 2 and 4), with further planned assessments at years 7 and 10. The primary focus was physical function measures and brain imaging, and to a lesser degree cognitive function and physiological well-being in general. Results of the intervention and initial follow-up assessments have been reported (Gylling, Bloch-Ibenfeldt, *et al.*, 2020; Gylling, Eriksen, *et al.*, 2020). This paper will focus on markers of bone health.

INTERVENTION

The heavy resistance training was performed three times per week in a public gym and supervised by an experienced physiologist. Training was progressed and linearly periodized throughout the full year and designed as a classic full-body program. In contrast to the heavier training, the moderate intensity training was carried out at a training facility at the hospital, where one weekly session was supervised. The two additional weekly sessions were performed at home. Training consisted of progressive bodyweight exercises and the use of elastic bands. For details on specific exercises, intensity, and volume in the two training regimes, see Eriksen et al., 2016; Gylling, Eriksen, et al., 2020. The non-exercising control group was told to maintain their habitual physical activity level (less than 1 hour of systematic strenuous physical activity per week) and were offered participation in cultural and social activities on average once per month. After the 1-year intervention there was no further guidance to engage in exercise for any of the participants.

TEST PROCEDURES

Assessments and tests in the trial were performed over three days. The first day included a short and basic medical examination, anthropometric measures, and an accelerometer (activPAL™ micro, PAL Technologies Ltd) was attached to the dominant thigh. Questionnaires were also handed out to be completed at home. On the second day, body composition and BMD were measured with state-of-the-art dual-energy x-ray absorptiometry (DXA) scans, that provides only a low dose of radiation and is compatible with World Health Organization (WHO) Z- and T-score definitions (Blake and Fogelman, 2007). This was followed by tests for cognitive and physical function. The final day featured magnetic resonance imaging (MRI) of the brain and thigh.

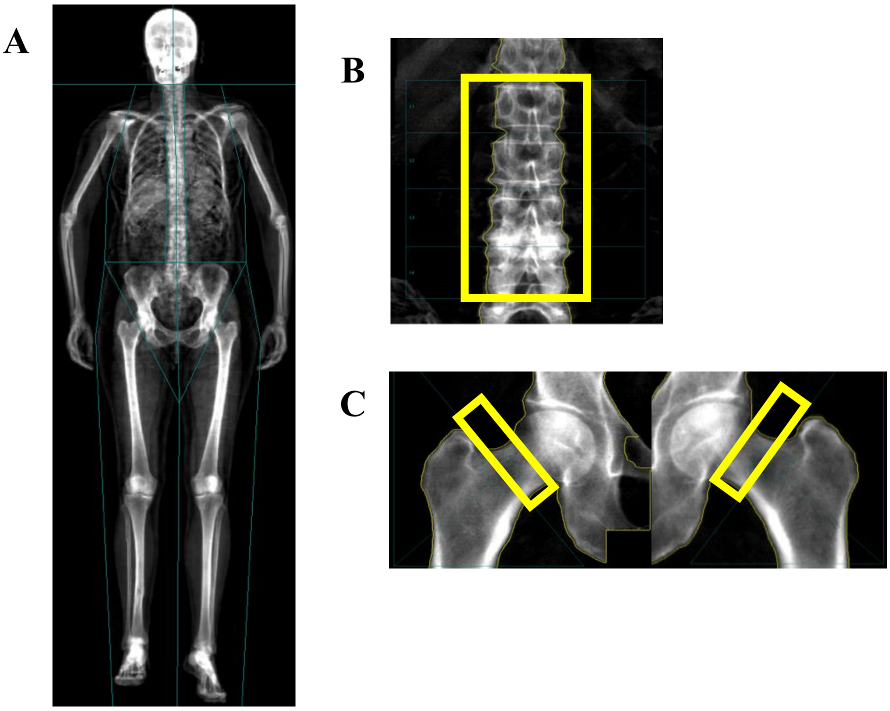

Figure 1. DXA-scan images from a sample participant. **A** Whole-body image. **B** Lumbar spine with the regions L1-L4 highlighted. **C** Right and left femur with femoral necks highlighted.

DXA-scans were performed in the following order: 1) full-body 2) lumbar spine 3) left femur 4) right femur. Participants emptied their bladder beforehand and were scanned in a supine position in underwear and a standardized hospital t-shirt. Scanner software (Lunar iDXA, GE HealthCare – enCORE software V.16) automatically determined regions of interest (ROI) on all scan images, i.e., arms, legs on full-body scans and specific lumbar vertebrae and femoral neck on regional bone specific scans. After the last 4-year follow-up, each scan image in the trial was manually re-assessed, analyzed, corrected, and standardized by the same person – see **Figure 1**.

For the left and right femur scans, femoral neck was chosen as the desired ROI, as compared to e.g., total femur. This location has been shown to better predict hip fractures (Cummings *et al.*, 1993) and be linked to osteoporosis-associated fractures (Warriner *et al.*, 2011).

In addition to BMD, the DXA-scanner provided T- and Z-scores, which were used to interpret the results in line with WHO definitions. While matched for gender and ethnic group, the T-score provides a result that expresses a relative difference in standard deviations (SD) from the mean BMD of a healthy young adult. The Z-score, unlike the T-score, takes age into account, and is instead expressed relative to the BMD of an age-matched healthy individual (Blake and Fogelman, 2007).

At the medical examination fasting venous blood samples were drawn from the cubital vein. Initial samples were analyzed at the biochemistry laboratory for traditional health parameters - e.g., markers of glucose and lipid metabolism, as well as inflammation and immune function. Two 9 ml samples were further drawn, and the plasma sample was placed on ice for 30 minutes while the serum sample was at room temperature. Samples were then centrifuged at 3970 RPM, 4°C, for 10 minutes (Centrifuge 5810 R, Eppendorf, Germany), to separate the plasma and serum from cells. Immediately after centrifugation plasma and serum samples were pipetted into Eppendorf tubes® and stored at -80°C until further analysis.

After the 4-year follow-up had been completed plasma samples from all timepoints were transported to the Department of Clinical Biochemistry, Rigshospitalet, Glostrup, Denmark and analyzed for CTX and PINP, and subsequently dp-ucMGP.

Plasma CTX and PINP were measured using the IDS-iSYS CTX (CrossLaps®) assay (Immunodiagnostic Systems, plc, Tyne and Wear, UK) and the IDS-iSYS intact P1NP assay (Immunodiagnostic Systems), respectively. Biochemical determination of vitamin K status was performed with the IDS-iSYS Ina*K*tif MGP assay (Immunodiagnostic Systems), which is intended for the quantitative determination of the inactive isoform of MGP, i.e. dp-ucMGP, in human plasma. All three assays were carried out on a dedicated automated analyzer, iSYS (Immunodiagnostic Systems), according to the manufacturer's instructions. For each assay the sample aliquots were kept frozen at - 80°C until the day of analysis. None of the samples had previously been thawed, and all analyses were performed immediately after thawing the samples. All samples were analyzed using one single batch of each assay.

The reportable range for dp-ucMGP is 300–12.000 pmol/L. Values below 300 pmol/L have been fixed to 299 pmol/L as previously suggested (Jespersen *et al.*, 2020). Correspondingly, CTX is reportable in the range of 33-12.000 ng/L, and values below 33 ng/L have been fixed to 0 ng/L.

The study was carried out in accordance with the Declaration of Helsinki, was approved by the regional ethics committee (Capital Region, Copenhagen, Denmark, No. H-3-2014-017) and all participants gave written informed consent before participating. In addition, the trial was registered on clinicaltrials.gov (NCT02123641).

STATISTICAL ANALYSIS

In general, all data from individuals with a DXA-scan performed at all four timepoints were included in the analysis. Individuals that were on medication for osteoporosis were excluded (n = 38 in total). For some of the measures the number of datapoints is reduced, either due to a specific bone scan not being performed (e.g., due to hip arthroplasty) or due to problems with the blood sampling. The exact number of participants for each analysis is specified in figures and tables. All statistical analyses were performed in R version 4.1.1 and Rstudio 2021.09.0 using "psych" (Revelle, 2023) , "dplyr" (Wickham *et al.*, 2023), "emmeans" (Searle, Speed and Milliken, 1980)and "sjstats" (Lüdecke, 2022) packages. Figures were created in GraphPad Prism version 10.0.3. Student's paired t-tests were used to test potential changes in sample characteristics over the 4 years. One-way mixed model ANOVAs were used to test differences at study start between those who remained in the study and individuals that either dropped out or started with bone-related medical treatments during the four years (n = 104). Two-way mixed model ANOVAs were used to test for group x time interactions on bone mineral densities (whole body, dominant leg femoral neck, non-dominant leg femoral neck, and lumbar spine L1-L4), bone markers (CTX and PINP), and dp-ucMGP. Additionally, one-way mixed model ANOVAs were used to test for changes over time in these outcomes, within each sex separately. For Δchanges between all timepoints one-way mixed model ANOVAs were used to test group differences. Regression analyses were used to test the associations between BMD, bone markers, dp-ucMGP, and muscular strength measures. Significant interactions were further examined with post hoc tests. Analyses were controlled for sex and age and the significance level was corrected (Bonferroni) for multiple comparisons, $p < 0.005$ (10 tests). Effect sizes are reported as eta squared (η^2) and r-squared (r^2).

RESULTS

The participants of the study were all relatively active and well-functioning with a daily physical activity level of nearly 10,000 steps/day, however ~85% had at least one chronic disease. At the 4-year follow-up, the participants were 70 years on average. There was no change of the physical activity level, measured as steps/day, over the 4-year period. A slight decrease in body weight from baseline to year 4 was observed (Baseline: 76.2 ± 12.8 kg; 4-year: 75.8 ± 13.1 kg, t(328) = 2.12, p = 0.03). See **Table 1** for full characteristics at baseline and year 4.

The drop-out analysis showed that in comparison to those that remained in the study, both whole body BMD ($F_{1,429} = 5.504$, $p = 0.02$, $\eta^2 = 0.01$) and femoral neck BMD in the dominant leg ($F_{1,417} = 5.609$, $p = 0.02$, $\eta^2 = 0.01$) were lower at baseline for individuals that subsequently dropped out.

Table 1. Sample characteristics (mean ± SD) for baseline and year 4.

	Baseline	**4-year**
Sex (men/women, %)	42/58	42/58
Age (yrs)	66.4 ± 2.5	70.5 ± 2.5
Weight (kg)	76.2 ± 12.8	75.8 ± 13.1
BMI (kg/m^2)	25.9 ± 3.8	26.0 ± 4.0
Waist circumference (cm)	93.0 ± 11.1	92.8 ± 11.5
Daily physical activity (steps/day)	9523 ± 3385	9547 ± 3424

Note: n = 329 for all variables except waist circumference (n = 327) and daily physical activity (n = 310).

For the DXA-measures there were no significant interactions with group, see **Figure 2A-F**. Rather, all groups changed equally over time. Decrease over time was observed in: whole-body BMD ($F_{3,977} = 4.617$, $p = 0.003$, $\eta^2 = 0.01$), T-score ($F_{3,977} = 5.499$, $p = 0.001$, $\eta^2 = 0.02$), femoral neck BMD in the dominant leg ($F_{3,893} = 45.135$, $p < 0.001$, $\eta^2 = 0.13$), and non-dominant leg ($F_{3,896} = 33.821$, $p < 0.001$, $\eta^2 = 0.10$). Increase over time was observed in BMD for L1-L4 ($F_{3,743} = 10.113$,

$p < 0.001$, $\eta^2 = 0.04$) and in Z-scores ($F_{3,977} = 4.225$, $p = 0.006$, $\eta^2 = 0.01$). However, the increase in Z-scores was not significant after correcting for multiple comparisons ($p > 0.005$).

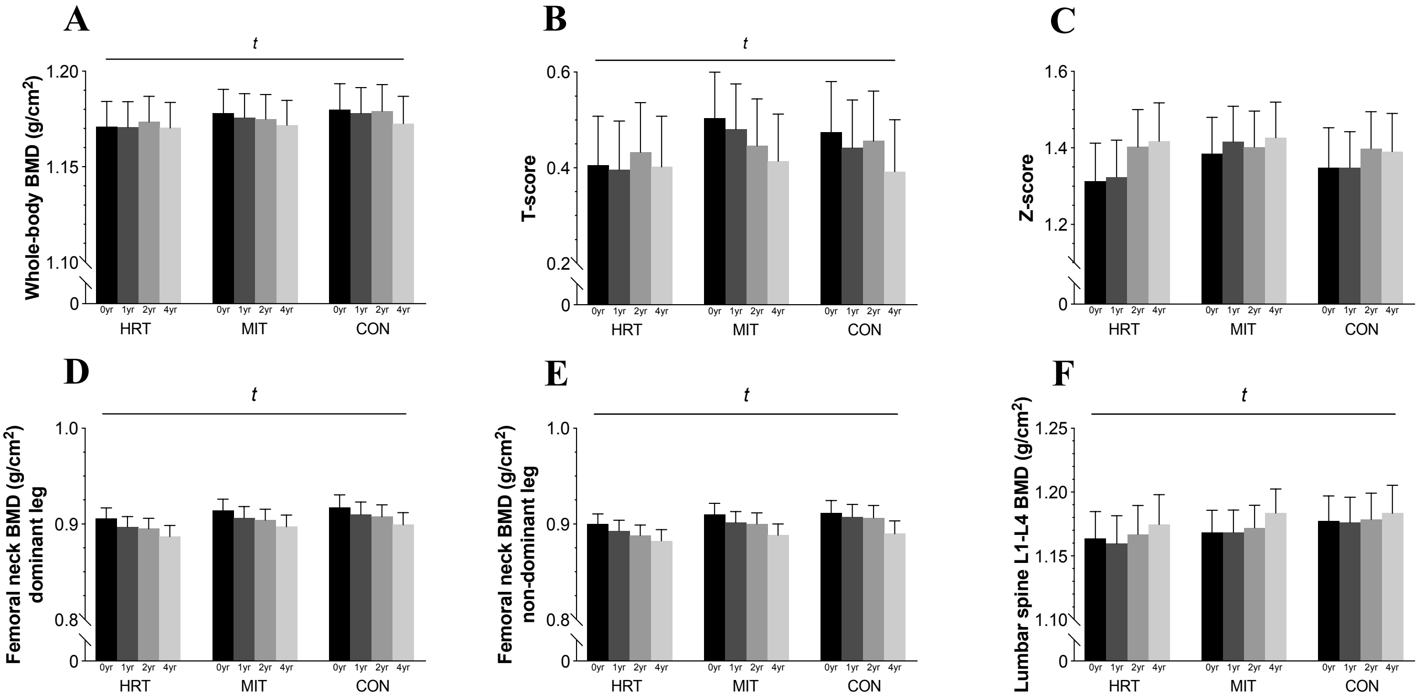

Figure 2. DXA-measures. Mean ± SEM for baseline (black bar - 0yr), 1-year (dark grey - 1yr), 2-year (grey - 2yr) and 4-year (light grey - 4yr), across each group: HRT, heavy resistance training; MIT, moderate intensity training; CON, non-exercising control group. **A** Whole-body bone mineral density (g/cm^2), n = 329. **B** T-score for whole-body bone mineral density, n = 329. **C** Z-score for whole-body bone mineral density, n = 329. **D** Bone mineral density (g/cm^2) in the femoral neck of the dominant leg, n = 301. **E** Bone mineral density (g/cm^2) in the femoral neck of the non-dominant leg, n = 302. **F** Bone mineral density (g/cm^2) in the lumbar spine (L1-L4), n = 251.

t, significant effect of time (**A**, $p = 0.003$; **B**, $p < 0.001$; **D**, $p < 0.001$; **E**, $p < 0.001$; **F**, $p < 0.001$) across all groups.

In general, men had larger bone specific values than women. When sex-differences were analyzed separately, in relation to the effects of the intervention or change over time, whole-body BMD, T-score, and Z-score all decreased over time in women, but not in men. In contrast, BMD for L1-L4 increased in men, but did not change over time in women. See **Supplementary table 1** for sex-specific values.

Bone biomarkers are shown in **Figure 3A-D**. For bone formation measured as PINP there was a significant group x time interaction ($F_{6,935} = 3.733$, $p = 0.001$, $\eta^2 = 0.01$) with an increase in HRT from baseline to year 1 (HRT, Baseline: 58.9 ± 20.9; 1-year: 64.6 ± 24.1, $t(936) = 3.357$, $p = 0.04$).

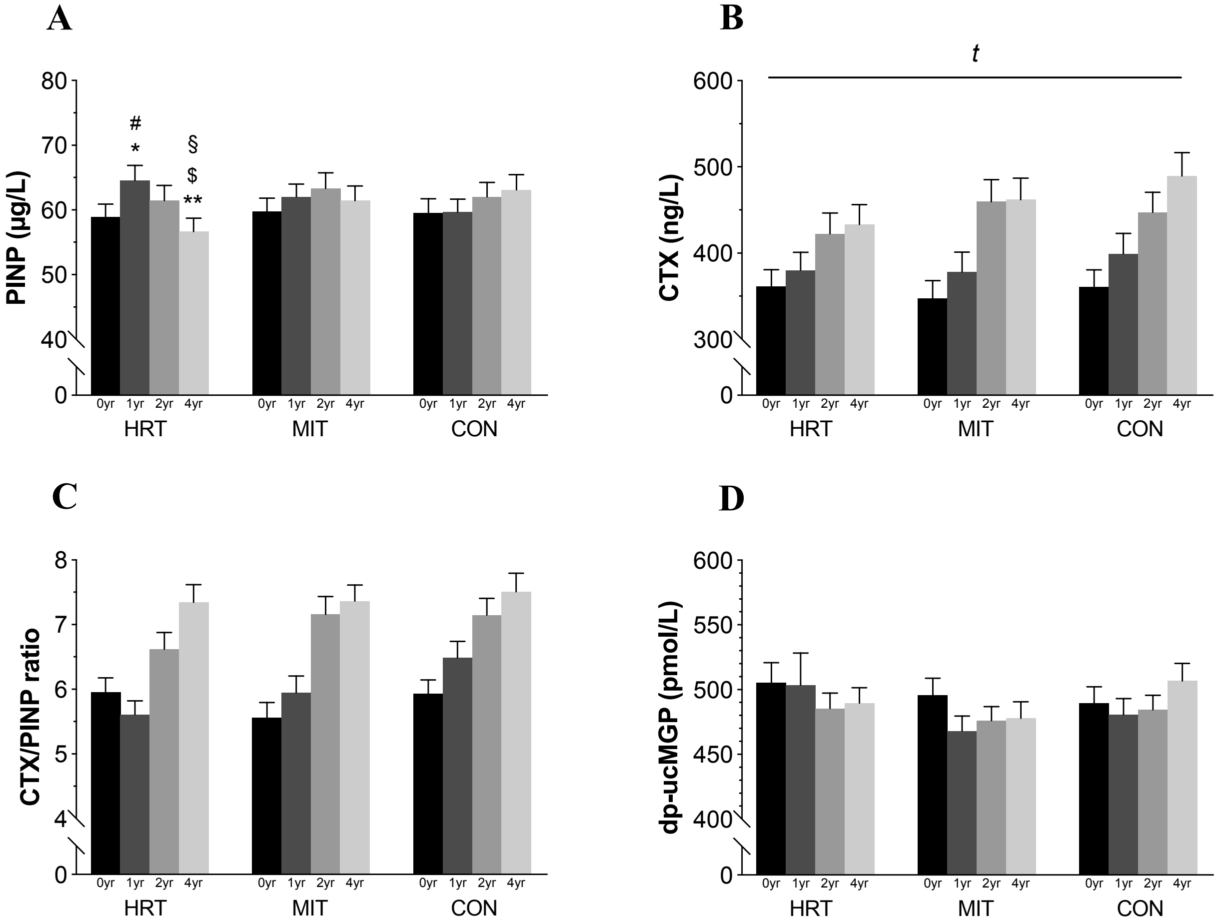

Figure 3. Mean ± SEM for baseline (black bar - 0yr), 1-year (dark grey - 1yr), 2-year (grey - 2yr) and 4-year (light grey - 4yr), for each group: HRT, heavy resistance training; MIT, moderate intensity training; CON, non-exercising control group. **A** Procollagen type I N-propeptide (PINP, µg/L), n = 315. **B** C-terminal telopeptide of type I collagen (CTX, ng/L), n = 314. **C** CTX/PINP ratio, n = 314. **D** dephosphorylated-uncarboxylated Matrix Gla-Protein (dp-ucMGP, pmol/L), n = 313.

t, significant effect of time ($p < 0.001$) across all groups. *, significantly different from baseline (HRT, $p = 0.04$). **, significantly different from 1-year (HRT, $p < 0.001$). #, increase from baseline to year 1 significantly bigger than in CON (HRT, $p = 0.035$). $, decrease from year 1 to 4 significantly bigger than decrease in MIT (HRT, $p = 0.005$). §. decrease from year 1 to 4 significantly bigger than decrease in CON (HRT, $p < 0.001$).

Further, there was a significant decrease in HRT from year 1 to 4 (HRT, 1-year: 64.6 ± 24.1: 4-year: 56.7 ± 21.7, t(936) = 4.723, $p < 0.001$). The increase in HRT during the intervention was larger than in CON (t(312) = 2.494, $p = 0.04$). Similarly, HRT experienced a larger decrease than both MIT (t(312) = -3.154, $p = 0.005$) and CON (t(312) = -4.751, $p < 0.001$) from year 1 to 4. Women had higher PINP values than men ($F_{1,310} = 40.679$, $p < 0.001$), but their levels did not change over time. For men, PINP increased over the four years. See **Supplementary table 1** for sex-specific bone biomarker values.

There was a larger resorption of bone, demonstrated by an increase in CTX over all four years ($F_{3,932} = 47.434$, $p < 0.001$, $\eta^2 = 0.13$) with no group interaction. CTX was higher in women than in men ($F_{1,309} = 44.637$, $p < 0.001$).

In the CTX/PINP ratio there was a group x time interaction ($F_{6,932} = 2.187$, $p = 0.04$, $\eta^2 = 0.01$) across the 4 years, which was driven by the changes in HRT following the intervention. However, after correcting for multiple comparisons ($p > 0.005$) the interaction was not significant. Women had greater ratios than men ($F_{1,309} = 19.753$, $p < 0.001$).

The levels of dp-ucMGP did not change over time ($F_{3,931} = 1.530$, $p = 0.21$, $\eta^2 = 0.005$) and there were no sex differences. There was no group interaction ($F_{6,931} = 1.178$, $p = 0.32$, $\eta^2 = 0.008$), although levels seemed to go in opposite directions in the two training groups and CON, respectively.

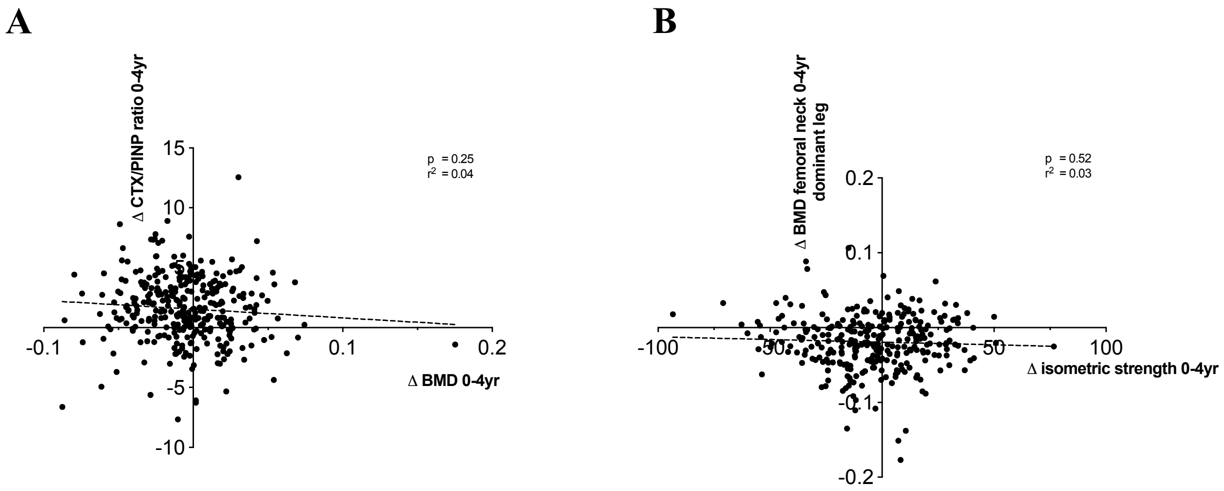

Figure 4. Associations between changes in bone turnover markers and strength from baseline to 4-year follow-up. In **A** Δ Whole-body BMD (g/cm^2) and Δ CTX/PINP ratio, n = 325. In **B** Δ isometric strength (Nm) and Δ femoral neck BMD (g/cm^2) in the dominant leg, n = 294. Plots include data from individuals across all groups. Linear regression slopes are displayed with a dotted line.

The associations between changes from baseline to year 4 in total BMD and CTX/PINP ratio, as well as in femoral neck BMD of the dominant leg and isometric leg strength are illustrated in **Figure 4A-B.** There were no associations between BMD and other measures (T- and Z-scores, bone markers, dp-ucMGP, and muscular strength) at whole body level or at specific regions at either baseline or measured as change over the four years.

DISCUSSION

The present study examined how resistance training influenced BMD and bone markers in older adults at retirement age, and our results showed that whole-body and regional BMD decreased over time. This is in line with other studies, that demonstrate a decline in bone health as a consequence of aging (Colón *et al.*, 2018). However, the age-matched Z-score revealed that the decrease in BMD was not as big as initially expected. Further, changes in BMD were not influenced by training in this study, neither immediately after the two intervention training regimes nor in the more long-term setting at year four. Considering the previously reported beneficial effects of both training regimes in the dominant leg at the muscular level (Gylling, Eriksen, *et al.*, 2020), including long-term beneficial effects of the heavy resistance training (Bloch-Ibenfeldt *et al.*, in review), we originally speculated that there could be a potential link between positive effects at the muscular level and at the bone level. However, such translation was not observed in the present study. This contrasts with findings from a Korean longitudinal study, in which change in muscle strength was the most significant independent factor associated with bone loss for both men and women (Kim *et al.*, 2018).

Contradictory to what was observed at the whole-body level and at the selected regional sites, BMD in the lumbar spine in fact increased over the four years. This is likely explained by either the increase in osteophytes that is affecting many individuals above 60 years of age, significantly impacting and skewing lumbar spine BMD measures by DXA (Liu *et al.*, 1997; Rand *et al.*, 1997), or by spinal osteoarthritis which is a known age-related local factor (Burger *et al.*, 1994). Thus, the use of lumbar spine BMD measures may not be ideal when examining older individuals over time. The present study does not support that resistance training with either moderate or heavy loading influences BMD. These findings need to be interpreted in light of the fact that participants in the LISA-study had mean T-scores higher than the reference value for young healthy adults and mean Z-scores markedly higher than averagely age-matched individuals, thus the participants appeared to have higher BMD to start with. Moreover, compared to other normative values or reference data for BMD in similar populations, participants in our study had higher values than age-matched individuals (Karlsson *et al.*, 1993; Lunt *et al.*, 1997; Rondanelli *et al.*, 2022). When compared to other ethnicities, these values were also higher (Larijani *et al.*, 2005; Makker *et al.*, 2008). The increase across groups in Z-score emphasizes that the participants were well-functioning and probably healthier than the average age-matched population. Thus, we cannot exclude the possibility that resistance training would influence BMD in a population that are less healthy. Unfortunately, the drop-out analysis showed that individuals with a poorer starting condition in terms of bone health, and thus perhaps the most interesting participants to follow, did not continue in the present study. Literature on longitudinal changes at bone sites by DXA is available, although these have predominately been reported in different ethnic populations (Ding *et al.*, 2008; Chen *et al.*, 2013; Kim *et al.*, 2018), with only sparse information on similar populations like ours. When the longitudinal change in BMD in both sexes was assessed in a Danish study, both men and women at a similar age increased their total body and lumbar spine BMD over a 2-year period. Additionally, the estimated changes from baseline cross-sectional data did not perfectly agree with actual longitudinal changes, with the authors further concluding that a longer follow-up period was needed for more reliable data (Warming, Hassager and Christiansen, 2002).

In line with previous studies, men had higher bone mineral densities than women at whole-body level and all regional sites (Warming, Hassager and Christiansen, 2002; Kim *et al.*, 2018). All female participants in the LISA study were post-menopausal, yet there were some sex-related differences in the rates of decline. However, these differences were primarily observed at the whole-body level. As previously described, BMD at the femoral neck has been linked to osteoporosis and fractures, and at these sites density decreased similarly in men and women.

The main finding from the analysis of the blood-based biomarkers for bone health was the increase in bone formation following 1 year of heavy resistance training. Notably, when the intervention stopped, the individuals in HRT experienced a steeper decrease in PINP the following years compared to the other two groups. In the LISA-study approximately 25% of the participants reported that they continued with regular resistance training following the intervention. In an exploratory analysis (data not shown), we therefore examined whether there was any difference in decline for those individuals who reported having continued with training compared with those who stopped, and no differences were observed. Considering that the sample size was significantly reduced, no firm conclusions should be made and whether maintaining training is also reflected in maintained PINP levels should be addressed in future studies.

In general, physical activity seems to positively influence markers of bone turnover (Ardawi, Rouzi and Qari, 2012), and an acute bout of resistance exercise has shown to have a positive effect on bone turnover, although the beneficial effects are suggested to be attenuated with age (Gombos *et al.*, 2016; Smith *et al.*, 2021; Stunes *et al.*, 2022). Studies, in which muscle strength and BMD were increased, showed that resistance training positively influenced some bone markers only (Sartorio *et al.*, 2001; Huovinen *et al.*, 2016). Nevertheless, in another study bone turnover was increased, in favor of bone formation, after 6 weeks of two different resistance training regimes (Karabulut *et al.*, 2011). We add to these studies by showing somewhat comparable results in our sample of active older adults, where bone formation, but not bone degradation, was beneficially influenced by a prolonged training setting with heavy loads. The results further confirmed what others have shown on predominately cross-sectional data, that women in general have higher absolute values of CTX and PINP, as well as higher CTX to PINP ratios (Michelsen *et al.*, 2013; Vasikaran, Chubb and Schneider, 2014; Chubb *et al.*, 2015; Jørgensen *et al.*, 2017; Yoo *et al.*, 2018; Bønløkke *et al.*, 2022). We show that despite these magnitude differences between men and women, the change over time in older adults follow a similar trajectory.

We did not observe any effect of training nor of time on dp-ucMGP values, despite the fact that dp-ucMGP is expected to increase with age (Shea *et al.*, 2011). Our values are comparable to age-matched values in the Danish general population (Jespersen *et al.*, 2020), and considering that the sample in the LISA-study is relatively healthy, an even longer follow-up period may be required to observe changes. Our results did not support a clear association of dp-ucMGP with bone markers or actual bone measures.

In conclusion, the present study provided evidence of short-term, but no long-term, benefits on markers of bone-formation following 1 year of heavy resistance training in well-functioning older adults. Other bone health markers where however not influenced by resistance training at either moderate or heavy loading in active older individuals at retirement age.

DECLARATION OF INTEREST

Declarations of interest: none.

AUTHOR STATEMENT

Mads Bloch-Ibenfeldt: Writing – Review & Editing, Writing – Original Draft, Project administration, Investigation, Formal analysis, Visualization, Validation. **Anne Theil Gates:** Writing – Review & Editing, Project administration, Investigation, Validation. **Niklas Rye Jørgensen:** Writing – Review & Editing, Resources, Conceptualization, Validation. **Allan Linneberg:** Writing – Review & Editing, Validation. **Mette Aadahl:** Writing – Review & Editing, Validation. **Michael Kjaer:** Writing – Review & Editing, Supervision, Methodology, Conceptualization, Validation. **Carl-Johan Boraxbekk:** Writing – Review & Editing, Supervision, Methodology, Validation.

All authors approved the final manuscript to be published.

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REFERENCES

Akbari, S. and Rasouli-Ghahroudi, A.A. (2018) ‘Vitamin K and Bone Metabolism: A Review of the Latest Evidence in Preclinical Studies’, *BioMed Research International*, 2018. Available at: https://doi.org/10.1155/2018/4629383.

Ardawi, M.S.M., Rouzi, A.A. and Qari, M.H. (2012) ‘Physical activity in relation to serum sclerostin, insulin-like growth factor-1, and bone turnover markers in healthy premenopausal women: A cross-sectional and a longitudinal study’, *Journal of Clinical Endocrinology and Metabolism*, 97(10), pp. 3691–3699. Available at: https://doi.org/10.1210/jc.2011-3361.

Black, D.M. and Rosen, C.J. (2016) ‘Clinical Practice. Postmenopausal Osteoporosis.’, *The New England journal of medicine*, 374(3), pp. 254–62. Available at: https://doi.org/10.1056/NEJMcp1513724.

Blake, G.M. and Fogelman, I. (2007) ‘The role of DXA bone density scans in the diagnosis and treatment of osteoporosis’, *Postgraduate Medical Journal*, pp. 509–517. Available at: https://doi.org/10.1136/pgmj.2007.057505.

Bønløkke, S.E. *et al.* (2022) ‘Baseline bone turnover marker levels can predict change in bone mineral density during antiresorptive treatment in osteoporotic patients: the Copenhagen bone turnover marker study’, *Osteoporosis International*, 33(10), pp. 2155–2164. Available at: https://doi.org/10.1007/s00198-022-06457-0.

Burger, H. *et al.* (1994) ‘The association between age and bone mineral density in men and women aged 55 years and over: The Rotterdam Study’, *Bone and mineral*, 25(1), pp. 1–13. Available at: https://doi.org/10.1016/S0169-6009(08)80203-6.

Chen, C.H. *et al.* (2013) ‘Longitudinal changes in bone mineral density of healthy elderly women in southern Taiwan’, *Taiwanese Journal of Obstetrics and Gynecology*, 52(1), pp. 61–65. Available at: https://doi.org/10.1016/j.tjog.2012.08.001.

Chen, H. and Avgerinou, C. (2023) ‘Association of Alternative Dietary Patterns with Osteoporosis and Fracture Risk in Older People: A Scoping Review’, *Nutrients*. Multidisciplinary Digital Publishing Institute (MDPI). Available at: https://doi.org/10.3390/nu15194255.

Chubb, S.A.P. *et al.* (2015) ‘Reference Intervals for Bone Turnover Markers and Their Association With Incident Hip Fractures in Older Men: The Health In Men Study’, *The Journal of Clinical Endocrinology & Metabolism*, 100(1), pp. 90–99. Available at: https://doi.org/10.1210/jc.2014-2646.

Colón, C.J.P. *et al.* (2018) ‘Muscle and Bone Mass Loss in the Elderly Population: Advances in diagnosis and treatment’, *Journal of Biomedicine*, 3, pp. 40–49. Available at: https://doi.org/10.7150/jbm.23390.

Cranenburg, E.C.M. *et al.* (2010) ‘Characterisation and potential diagnostic value of circulating matrix Gla protein (MGP) species’, *Thrombosis and haemostasis*, 104(4), pp. 811–822. Available at: https://doi.org/10.1160/TH09-11-0786.

Cummings, S.R. *et al.* (1993) ‘Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group.’, *Lancet.*, 341(8837), pp. 72–75. Available at: https://doi.org/10.1016/0140-6736(93)92555-8.

Ding, C. *et al.* (2008) ‘Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: A longitudinal study’, *Journal of Clinical Endocrinology and Metabolism*, 93(5), pp. 1952–1958. Available at: https://doi.org/10.1210/jc.2007-2325.

Eriksen, C.S. *et al.* (2016) ‘Physical activity as intervention for age-related loss of muscle mass and function: protocol for a randomised controlled trial (the LISA study)’, *BMJ Open* [Preprint]. Available at: https://doi.org/10.1136/bmjopen-2016.

Fujimura, R. *et al.* (1997) 'Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism', *Journal of Bone and Mineral Research*, 12(4), pp. 656–662. Available at: https://doi.org/10.1359/jbmr.1997.12.4.656.

Gillett, M.J., Vasikaran, S.D. and Inderjeeth, C.A. (2021) 'The Role of PINP in Diagnosis and Management of Metabolic Bone Disease', *Clinical Biochemist Reviews*, 42(1), pp. 3–10. Available at: https://doi.org/10.33176/AACB-20-0001.

Gombos, G.C. *et al.* (2016) 'Direct effects of physical training on markers of bone metabolism and serum sclerostin concentrations in older adults with low bone mass', *BMC Musculoskeletal Disorders*, 17(1). Available at: https://doi.org/10.1186/s12891-016-1109-5.

Gylling, A.T., Bloch-Ibenfeldt, M., *et al.* (2020) 'Maintenance of muscle strength following a one-year resistance training program in older adults', *Experimental Gerontology*, 139. Available at: https://doi.org/10.1016/j.exger.2020.111049.

Gylling, A.T., Eriksen, C.S., *et al.* (2020) 'The influence of prolonged strength training upon muscle and fat in healthy and chronically diseased older adults', *Experimental Gerontology*, 136. Available at: https://doi.org/10.1016/j.exger.2020.110939.

Herda, A.A. and Nabavizadeh, O. (2023) 'Effect of six weeks of resistance training on bone preservation in older adults: a randomized control trial.', *Aging clinical and experimental research.* [Preprint]. Available at: https://doi.org/10.1007/s40520-023-02575-9.

Hinton, P.S., Nigh, P. and Thyfault, J. (2015) 'Effectiveness of resistance training or jumping-exercise to increase bone mineral density in men with low bone mass: A 12-month randomized, clinical trial', *Bone*, 79, pp. 203–212. Available at: https://doi.org/10.1016/j.bone.2015.06.008.

Huovinen, V. *et al.* (2016) 'Bone mineral density is increased after a 16-week resistance training intervention in elderly women with decreased muscle strength', *European Journal of Endocrinology*, 175(6), pp. 571–582. Available at: https://doi.org/10.1530/EJE-16-0521.

Jespersen, T. *et al.* (2020) ‘Uncarboxylated matrix Gla-protein: A biomarker of vitamin K status and cardiovascular risk’, *Clinical Biochemistry*, 83, pp. 49–56. Available at: https://doi.org/10.1016/j.clinbiochem.2020.05.005.

Jørgensen, N.R. *et al.* (2017) ‘Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population’, *Osteoporosis International*, 28(7), pp. 2103–2113. Available at: https://doi.org/10.1007/s00198-017-4026-z.

Karabulut, M. *et al.* (2011) ‘Effects of high-intensity resistance training and low-intensity resistance training with vascular restriction on bone markers in older men’, *European Journal of Applied Physiology*, 111(8), pp. 1659–1667. Available at: https://doi.org/10.1007/s00421-010-1796-9.

Karlsson, M.K. *et al.* (1993) ‘Bone mineral normative data in Malmo, Sweden Comparison with reference data and hip fracture incidence in other ethnic groups’, *Acta Orthopaedica Scandinavica*, 64(2), pp. 168–169.

Kim, K.M. *et al.* (2018) ‘Longitudinal changes in muscle mass and strength, and bone mass in older adults: Gender-specific associations between muscle and bone losses’, *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, 73(8), pp. 1062–1069. Available at: https://doi.org/10.1093/gerona/glx188.

Larijani, B. *et al.* (2005) ‘Normative data of bone Mineral Density in healthy population of Tehran, Iran: A Cross sectional study’, *BMC Musculoskeletal Disorders*, 6. Available at: https://doi.org/10.1186/1471-2474-6-38.

Layne, J.E. and Nelson, M.E. (1999) ‘The effects of progressive resistance training on bone density: a review.’, *Medicine and science in sports and exercise.*, 31(1), pp. 25–30. Available at: https://doi.org/10.1097/00005768-199901000-00006.

Liu, G. *et al.* (1997) ‘Original Article Effect of Osteoarthritis in the Lumbar Spine and Hip on Bone Mineral Density and Diagnosis of Osteoporosis in Elderly Men and Women’, *Osteoporosis International*, 7, pp. 564–569.

Lohman, T. *et al.* (1995) ‘Effects of resistance training on regional and total bone mineral density in premenopausal women: a randomized prospective study.’, *Journal of bone and mineral research : JBMR.*, 10(7), pp. 1015–1024. Available at: https://doi.org/10.1002/jbmr.5650100705.

Lüdecke, D. (2022) *sjstats: Statistical Functions for Regression Models (Version 0.18.2). doi:10.5281/zenodo.1284472, https://CRAN.R-project.org/package=sjstats.*

Lunt, M. *et al.* (1997) ‘Population-Based Geographic Variations in DXA Bone Density in Europe: The EVOS Study’, *Osteoporosis International*, 7, pp. 175–189.

Maddalozzo, G.F. and Snow, C.M. (2000) ‘Clinical Investigations High Intensity Resistance Training: Effects on Bone in Older Men and Women’, *Calcified Tissue International*, 66, pp. 399–404.

Makker, A. *et al.* (2008) ‘Normative bone mineral density data at multiple skeletal sites in Indian subjects’, *Archives of Osteoporosis*, 3(1–2), pp. 25–37. Available at: https://doi.org/10.1007/s11657-008-0019-z.

McCartney, N. *et al.* (1995) ‘Long-term resistance training in the elderly: effects on dynamic strength, exercise capacity, muscle, and bone.’, *The journals of gerontology.*, 50(2), pp. B97-104. Available at: https://doi.org/10.1093/gerona/50a.2.b97.

Michelsen, J. *et al.* (2013) ‘Reference intervals for serum concentrations of three bone turnover markers for men and women’, *Bone*, 57(2), pp. 399–404. Available at: https://doi.org/10.1016/j.bone.2013.09.010.

Muñoz-Garach, A., García-Fontana, B. and Muñoz-Torres, M. (2020) ‘Nutrients and dietary patterns related to osteoporosis’, *Nutrients*. MDPI AG, pp. 1–15. Available at: https://doi.org/10.3390/nu12071986.

Nguyen, V.H. (2023) ‘Making a Move on the Mark of Osteoporosis in Men’, *Gerontology and Geriatric Medicine*, 9. Available at: https://doi.org/10.1177/23337214231204729.

NIH Consensus Statement (2000) *CONSENSUS CONFERENCE From the National Institutes of Health*. Available at: http://consensus.nih.gov.

Pruitt, L.A. *et al.* (1992) ‘Weight-training effects on bone mineral density in early postmenopausal women.’, *Journal of bone and mineral research : JBMR.*, 7(2), pp. 179–185. Available at: https://doi.org/10.1002/jbmr.5650070209.

Pruitt, L.A., Taaffe, D.R. and Marcus, R. (1995) ‘Effects of a one-year high-intensity versus low-intensity resistance training program on bone mineral density in older women.’, *Journal of bone and mineral research : JBMR.*, 10(11), pp. 1788–1795. Available at: https://doi.org/10.1002/jbmr.5650101123.

Rand, T. *et al.* (1997) ‘Influence of osteophytic size on bone mineral density measured by dual x-ray absorptiometry’, *Acta Radiologica*, 38, pp. 210–213.

Revelle, W. (2023) *psych: Procedures for Psychological, Psychometric, and Personality Research. Northwestern University, Evanston, Illinois. R package version 2.3.6, https://CRAN.R-project.org/package=psych.*

Rhodes, E.C. *et al.* (2000) *Original articles EVects of one year of resistance training on the relation between muscular strength and bone density in elderly women*, *Br J Sports Med*.

Rondanelli, M. *et al.* (2022) ‘Bone Mineral Density Reference Values in 18- to 95-Year-Old Population in Lombardy Region, Italy’, *American Journal of Men’s Health*, 16(5). Available at: https://doi.org/10.1177/15579883221119363.

Sartorio, A. *et al.* (2001) ‘Effects of a 16-week progressive high-intensity strength training (HIST) on indexes of bone turnover in men over 65 years: A randomized controlled study’, *Journal of endocrinological investigation*, 24, pp. 882–886. Available at: https://doi.org/10.1007/BF03343946.

Searle, S.R., Speed, F.M. and Milliken, G.A. (1980) ‘Population Marginal Means in the Linear Model: An Alternative to Least Squares Means’, *The American statistician*, 34(4), pp. 216–221. Available at: https://doi.org/10.1080/00031305.1980.10483031.

Shea, M.K. *et al.* (2011) ‘Circulating uncarboxylated Matrix Gla protein is associated with vitamin K nutritional status, but not coronary artery calcium, in older adults’, *Journal of Nutrition*, 141(8), pp. 1529–1534. Available at: https://doi.org/10.3945/jn.111.139634.

Shojaa, M. *et al.* (2020) ‘Effect of exercise training on bone mineral density in post-menopausal women: A systematic review and meta-analysis of intervention studies’, *Frontiers in Physiology*, 11. Available at: https://doi.org/10.3389/fphys.2020.00652.

Smith, C. *et al.* (2021) ‘The effects of acute exercise on bone turnover markers in middle-aged and older adults: A systematic review’, *Bone*. Elsevier Inc. Available at: https://doi.org/10.1016/j.bone.2020.115766.

Stunes, A.K. *et al.* (2022) ‘Acute Effects of Strength and Endurance Training on Bone Turnover Markers in Young Adults and Elderly Men’, *Frontiers in Endocrinology*, 13. Available at: https://doi.org/10.3389/fendo.2022.915241.

Szulc, P. *et al.* (2017) ‘Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability’, *Osteoporosis International*, 28(9), pp. 2541–2556. Available at: https://doi.org/10.1007/s00198-017-4082-4.

Vasikaran, S. *et al.* (2011) ‘Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards’, *Osteoporosis International*, 22(2), pp. 391–420. Available at: https://doi.org/10.1007/s00198-010-1501-1.

Vasikaran, S.D., Chubb, S.A.P. and Schneider, H.G. (2014) ‘Towards optimising the provision of laboratory services for bone turnover markers’, *Pathology*, 46(4), pp. 267–273. Available at: https://doi.org/10.1097/PAT.0000000000000092.

Villa, J.K.D. *et al.* (2017) ‘Effect of vitamin K in bone metabolism and vascular calcification: A review of mechanisms of action and evidences’, *Critical Reviews in Food Science and Nutrition*, 57(18), pp. 3959–3970. Available at: https://doi.org/10.1080/10408398.2016.1211616.

Vincent, K.R. and Braith, R.W. (2002) *Resistance exercise and bone turnover in elderly men and women*, *Med. Sci. Sports Exerc*. Available at: http://journals.lww.com/acsm-msse.

Warming, L., Hassager, C. and Christiansen, C. (2002) ‘Changes in Bone Mineral Density with Age in Men and Women: A Longitudinal Study’, *Osteoporosis International*, 13, pp. 105–112.

Warriner, A.H. *et al.* (2011) ‘Which fractures are most attributable to osteoporosis?’, *Journal of Clinical Epidemiology*, 64(1), pp. 46–53. Available at: https://doi.org/10.1016/j.jclinepi.2010.07.007.

WHO (2022) *World Health Organization - Ageing and Health*. Available at: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health (Accessed: 30 October 2023).

Wickham, H. *et al.* (2023) *dplyr: A Grammar of Data Manipulation. https://dplyr.tidyverse.org, https://github.com/tidyverse/dplyr.*

Yarasheski, K.E., Campbell, J.A. and Kohrt, W.M. (1997) *Effect of resistance exercise and growth hormone on bone density in older men*, *Clinical Endocrinology*.

Yoo, J.-I. *et al.* (2018) ‘Age-related Reference Intervals for Total Collagen-I-N-terminal Propeptide in Healthy Korean Population’, *Journal of Bone Metabolism*, 25(4), p. 235. Available at: https://doi.org/10.11005/jbm.2018.25.4.235.

SUPPLEMENTARY

Supplementary Table 1. Sex-specific values (Mean ± SD) for women and men at baseline (0yr), and years 1 (1yr), 2 (2yr), and 4 (4yr).

	Women					Men				
	0yr	1yr	2yr	4yr	F (time)	0yr	1yr	2yr	4yr	F (time)
Whole-body BMD (g/cm^2)	1.097 ± 0.101	1.094 ± 0.098	1.093 ± 0.102	1.088 ± 0.103	$F_{3,572} = 9.322$ $p < 0.001$, $\eta^2 = 0.05$	1.287 ± 0.101	1.287 ± 0.101	1.291 ± 0.101	1.289 ± 0.102	$F_{3,407} = 1.666$ $p = 0.17$, $\eta^2 = 0.01$
T-score	0.156 ± 1.000	0.136 ± 0.976	0.128 ± 1.010	0.073 ± 1.022	$F_{3,572} = 7.877$ $p < 0.001$, $\eta^2 = 0.04$	0.905 ± 1.038	0.880 ± 1.005	0.911 ± 0.993	0.880 ± 1.010	$F_{3,407} = 0.731$ $p = 0.53$, $\eta^2 = 0.005$
Z-score	0.094 ± 1.013	0.087 ± 0.979	0.085 ± 1.013	0.087 ± 1.024	$F_{3,572} = 2.749$ $p = 0.04$, $\eta^2 = 0.01$	1.295 ± 1.101	1.284 ± 1.023	1.339 ± 0.999	1.365 ± 1.014	$F_{3,407} = 2.251$ $p = 0.08$, $\eta^2 = 0.02$
Femoral neck BMD (g/cm^2) dominant leg	0.876 ± 0.105	0.868 ± 0.103	0.864 ± 0.104	0.854 ± 0.105	$F_{3,518} = 41.418$ $p < 0.001$, $\eta^2 = 0.19$	0.962 ± 0.118	0.954 ± 0.120	0.953 ± 0.117	0.950 ± 0.120	$F_{3,377} = 9.616$ $p < 0.001$, $\eta^2 = 0.07$
Femoral neck BMD (g/cm^2) non-dominant leg	0.868 ± 0.107	0.863 ± 0.106	0.862 ± 0.108	0.846 ± 0.105	$F_{3,521} = 41.768$ $p < 0.001$, $\eta^2 = 0.19$	0.957 ± 0.118	0.953 ± 0.118	0.949 ± 0.116	0.944 ± 0.125	$F_{3,377} = 5.129$ $p = 0.002$, $\eta^2 = 0.04$
Lumbar spine L1-L4 BMD (g/cm^2)	1.105 ± 0.149	1.099 ± 0.152	1.099 ± 0.156	1.101 ± 0.161	$F_{3,455} = 1.639$ $p = 0.18$, $\eta^2 = 0.01$	1.271 ± 0.172	1.275 ± 0.171	1.286 ± 0.174	1.305 ± 0.178	$F_{3,290} = 27.910$ $p < 0.001$, $\eta^2 = 0.22$
PINP (µg/L)	66.5 ± 22.1	67.0 ± 22.8	67.9 ± 24.8	65.6 ± 23.5	$F_{3,539} = 1.051$ $p = 0.37$, $\eta^2 = 0.006$	49.9 ± 15.3	55.7 ± 18.5	54.8 ± 20.7	53.2 ± 19.9	$F_{3,395} = 5.487$ $p = 0.001$, $\eta^2 = 0.04$
CTX (ng/L)	417.2 ± 212.8	433.2 ± 237.3	507.9 ± 256.2	536.9 ± 265.8	$F_{3,539} = 34.273$ $p < 0.001$, $\eta^2 = 0.16$	273.9 ± 153.9	320.1 ± 198.9	354.1 ± 206.9	356.2 ± 201.4	$F_{3,395} = 15.632$ $p < 0.001$, $\eta^2 = 0.11$
CTX/PINP ratio	6.2 ± 2.4	6.3 ± 2.5	7.4 ± 2.6	8.0 ± 2.6	$F_{3,539} = 43.969$ $p < 0.001$, $\eta^2 = 0.20$	5.3 ± 2.0	5.6 ± 2.5	6.4 ± 2.7	6.6 ± 2.7	$F_{3,395} = 15.857$ $p < 0.001$, $\eta^2 = 0.11$
dp-ucMGP (pmol/L)	497 ± 150	480 ± 124	483 ± 120	494 ± 126	$F_{3,536} = 2.342$ $p = 0.07$, $\eta^2 = 0.01$	496 ± 130	489 ± 242	481 ± 112	486 ± 138	$F_{3,393} = 0.328$ $p = 0.81$, $\eta^2 = 0.003$