Role of Muscle and Fat in Physical Function and Survival

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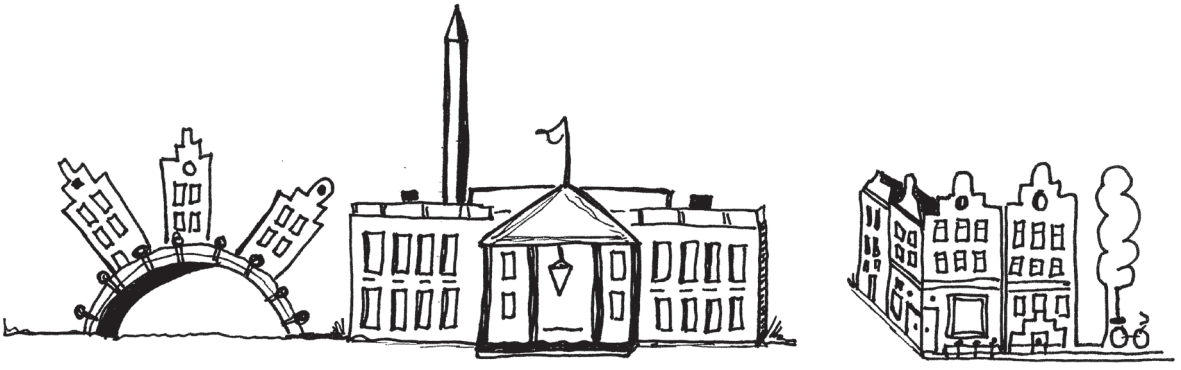

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PhD-thesis, VU University Amsterdam, the Netherlands.

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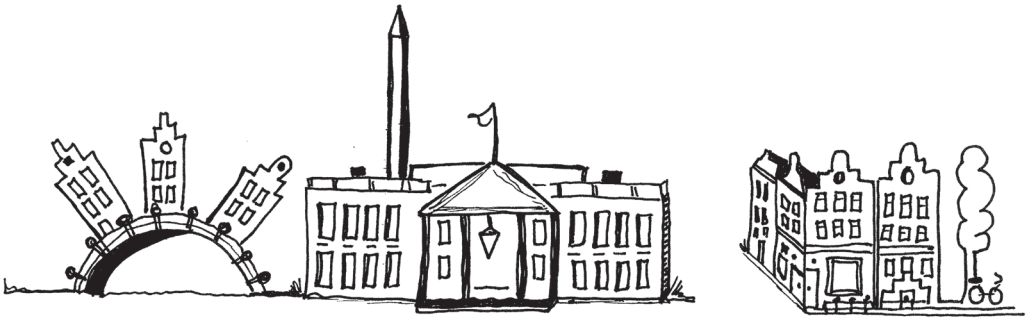

CHAPTER 1

General introduction and outline of the thesis

AGING POPULATION

The aging population is rapidly growing. For example, the U.S. population aged > 65 years grew 15.1% between 2000 and 2010, while the total population only grew 9.7% in that same time period (1). This is a result of two factors; 1) fallen fertility rates, and 2) increased life expectancy (2). Due to this so-called demographic transition, the relative and absolute number of older adults worldwide will increase. In the year 2000, 600 million people worldwide were > 60 years, while it is expected that in the year 2050 this number will increase up to 2 billion (3).

As a result of the aging population, the prevalence of people with mobility disability, decreased quality of life, and other physical or mental health problems will increase. Mobility disability is an adverse health outcome, however, it also increases the risk of subsequent difficulty in self-care tasks such as bathing, dressing and toilet hygiene, and household tasks such as preparing meals and transportation within the community (4). Thus, population aging will increase the burden on society since need for family care, institutionalization or hospitalization will grow. Even though little is known about the effect of the aging population on health care costs (5), it is expected that health care costs will rise excessively.

To address the societal challenges accompanied with an aging population, health promotion in older adults is important. A focus on improving physical function (i.e. mobility performance and activities of daily living), and thereby prevention of disability and maintenance of independence, will result in improving quality of life (6). Therefore, identifying factors that promote healthy aging is essential.

AGE-RELATED CHANGES IN BODY COMPOSITION

Aging is associated with loss of muscle strength and muscle mass, and a redistribution of fat, where subcutaneous fat relocates to more detrimental locations such as fat between and within muscles or organs (7-13). Body composition can be determined using simple anthropometric measurements as well as highly advanced techniques.

Body mass index

Body mass index (BMI, body weight (kg) divided by height (m) squared) is often used as an indicator of nutritional status and subsequent health risk. The World Health Organization (WHO) cut-off values for being underweight, normal weight, overweight and obese are sex

and age-independent and for those up to the age of 70 years (14). However, according to the European Society for Clinical Nutrition and Metabolism (ESPEN) guideline, the cut-off value for underweight geriatric patients (> 70 years) should be < 22.0 kg/m^2 instead of < 18.5 kg/m^2 (15) (**Table 1.1**).

Table 1.1. The classification of adult underweight, normal weight, overweight and obesity according to BMI (14, 15).

	BMI (kg/m^2) cut-off	
	Ages 18-70 years	**Ages > 70 years**
Underweight	< 18.5	< 22.0
Normal weight	18.5 – 24.99	22.0 – 24.99
Overweight	≥ 25.0	≥ 25.0*
Obese	≥ 30.0	≥ 30.0

* Cut-off values for overweight among older adults are controversial.

The cut-off value of ≥ 25.0 kg/m^2 to classify overweight older adults is controversial. Previous research has shown a U-shaped relationship between BMI and risk of major mobility disability (16) and mortality risk, whereas increased mortality risk starts to rise significantly for BMIs greater than 31 to 32 kg/m^2 (17). This indicates that being overweight (BMI 25.0 – 29.9 kg/m^2) might be protective for disability and mortality risk compared to being under/normal weight or obese. Based on these results, researchers have suggested that the optimum BMI for older adults is higher compared with young and middle-aged populations (17).

BMI is an easy and applicable measure in clinical practice, however, a single measurement of BMI does not provide sufficient information to determine people at risk for negative health outcomes. For example, BMI does not provide information regarding body and muscle composition. Even when BMI is stable over time (18), body composition changes can occur, such as decreased muscle mass and increased fat. Consequently, BMI is likely to underestimate adiposity since older adults are more likely to have less muscle mass and more fat compared to younger adults with the same body weight, and may thus be less useful for indicating body composition in older adults (19-21). In addition, when people age, body height declines and when weight is stable, BMI will thus increase. Therefore, applying BMI cut-off values might lead to misclassification. Previous research has shown that, based on recall data, early onset as well as the duration of obesity increased the risk of disability (22). Therefore, tracking (changes in) BMI over time provides information on initial body weight, changes over time, and overall weight trajectories, which could be

more informative than a single measure of BMI. However, more accurate methods to assess adipose tissue depots and muscle composition are also required.

BMI provides an indication of nutritional status. Multiple measurements are informative to identify people at risk of functional decline and mortality. However, BMI provides no information on body composition.

Body fat

As mentioned, the distribution of body fat changes with aging, where fat relocates from appendicular subcutaneous fat to abdominal fat. In addition, subcutaneous fat declines and total fat increases (23). The majority of previous studies investigating the relation between body composition and disability or mortality risk used BMI as an indicator of overall adiposity. However, other simple measures of adiposity, such as waist circumference, have been shown to be a better predictor of disability compared to BMI (24, 25). Waist circumference is a proxy measure for visceral fat, which is the fat between and around the organs. More abdominal fat, as determined by dual energy X-ray absorptiometry (DXA) or computed tomography, has been associated with increased disease or disability risk (26-28) independent of overall fatness. Visceral fat is also associated with increased mortality risk in most (29-32), but not all (28, 32) studies. In addition to areas of visceral fat, more dense visceral fat has also been associated with increased mortality risk (33). Previous studies mainly focussed on total body fat and visceral fat, though limited research has been performed regarding the role of other specific fat depots and the characteristics of fat in skeletal muscle tissue.

Muscle composition

Muscle tissue consists of muscle mass and fat infiltrated into the muscle. Muscle mass represents the actual muscle fibers with the primary function to establish locomotion and remain posture. There are two types of muscle fibers, namely type I and type II. Type I muscle fibers are the ‘slow’ muscle fibers, mainly responsible for aerobic activity, where type II fibers are considered to be the ‘fast’ muscle fibers accountable for short, anaerobic burst of activity (34). Muscle fat infiltration can be divided into intermuscular adipose tissue and intramuscular adipose. Intermuscular adipose tissue is the visible fat within the fascia surrounding skeletal muscles (10). Intramuscular adipose tissue is the lipid, in the form of cytosolic triacylglycerols, within skeletal myocytes (muscle fibers) (10, 35). Concurrent with the accumulation and relocation of body fat in older adults, muscle

composition also changes, where muscle mass decreases and muscle fat infiltration increases. The age-related loss of muscle mass represents a decrease in the total number of both type I and type II fibers, with greater losses in the size of type II fibers (36, 37). As a result of the increased losses in size of type II fibers, a larger proportion of slow type muscle mass in aged muscle is observed, which may results in slower contraction and reduced muscle strength (38). Regarding muscle fat infiltration, the increase in muscle fat infiltration is most noticeable among older adults who increase in body weight, but even among older people who lose weight, muscle fat infiltration may increase (12).

Muscle quality (the ratio of muscle strength to muscle mass) is a measure of muscle's functional properties, defined as strength per unit of mass (7, 39). It has been shown that older adults yearly lose approximately 1% of muscle mass, while mean muscle strength losses are 3% (40). This indicates that the loss of muscle strength is more rapid than the loss of muscle mass, suggesting a significant decline in muscle quality with aging. This decline in muscle quality might be (partly) due to muscle fat infiltration, however, it is not clear whether this is due to intermuscular adipose tissue and/or intramuscular adipose tissue.

Methods to assess body composition

Body composition can be estimated using simple methods such as BMI and waist circumference, and by using advanced, accurate and precise techniques. DXA is one of the most commonly used techniques for estimating skeletal muscle mass. It measures the relative attenuation of two different energy X-rays by the body (41). It is based on a three-compartment model dividing the body into bone, fat and lean mass. Lean mass from the arms and legs represents appendicular skeletal muscle mass, i.e. the non-fat and non-bone mass of the arms and legs. **Figure 1.1** shows a DXA image. On the left image, the white areas represent bone tissue. On the right image, the dark grey areas in the arms and legs represent appendicular skeletal muscle mass and the white areas represent fat.

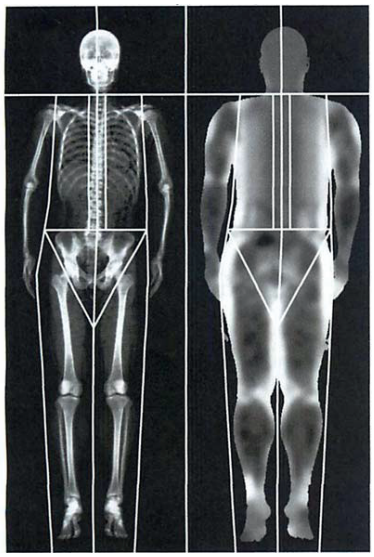

Figure 1.1. Whole body dual energy x-ray absorptiometry image

Dual-energy X-ray absorptiometry assesses whole-body and regional bone mass and soft-tissue (fat mass and lean mass).

Computed tomography (CT) imaging can identify all different kinds of tissue, such as muscles, organs, arteries, and connective tissue. Regarding body composition assessment it is used to distinguish muscle tissue, bone and different fat depots, such as intermuscular adipose tissue or intramuscular adipose tissue. CT is highly precise and accurate. It is based on the tissue density of pixels (42), and each image represents a 'slice' of the body. On a continuum of density where bone tissue is the most dense and fat is the least dense, muscle tissue falls between those two extremes. The Hounsfield Unit (HU) of muscle pixels, or muscle attenuation, represents the density of the lean tissue, where lower HUs indicate less dense muscle and greater fat infiltration (43). The density of adipose tissue can also be determined using CT, where greater attenuation of adipose tissue represents denser, and thus more fatty. With CT, muscle mass is indicated by cross-sectional muscle area. **Figure 1.2** shows CT images of the mid-thigh of women with similar thigh cross-sectional area, but different levels of muscle mass and muscle fat infiltration; reflecting muscle tissue (light grey), adipose tissue (purple) and bone (white).

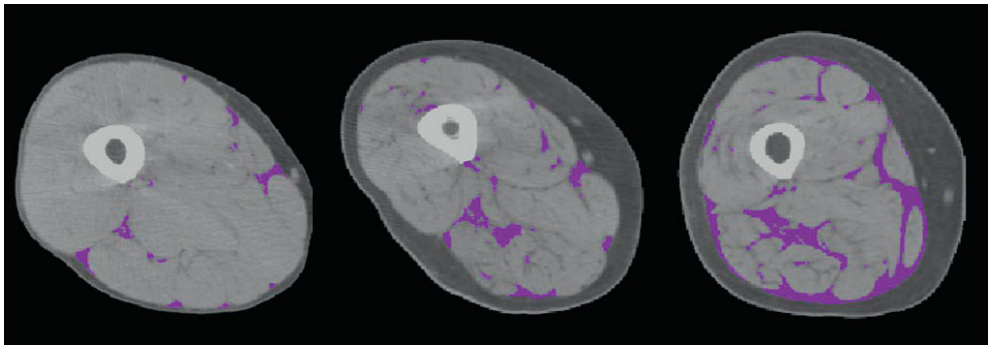

Figure 1.2. Computer tomography image of the mid-thigh muscle

Computed tomography imaging can distinguish bone tissue, muscle tissue and fat in and surrounding the muscle.

Consequences of age-related changes in body and muscle composition

Several studies based on BMI have shown that both underweight and obese older adults, but not overweight older adults, have higher morbidity and mortality risk (17, 44-47). However, a previous study has shown that the weight throughout adult life is also important in relation to incident mobility limitation, where an earlier onset of overweight and obesity in earlier life (using recalled weight) contributes to a higher risk of mobility limitation later in life (48). This shows the importance of monitoring peoples weight during lifetime. However, no large cohort study has prospectively investigated the trajectory of measured weight over time (advanced statistics performed to derive BMI trajectories), and related these patterns to contemporaneous changes in appendicular lean mass and physical function.

In addition to (multiple) measurements of BMI, more specific body composition measures identifying the location, distribution and quality of fat depots, and in addition the amount and quality of muscle, might be relevant to provide insight into disability relationships (49, 50). Therefore, determining associations between different adiposity measures and muscle composition in relation to physical function in older adults is required.

Body composition and physical function

Previous studies have investigated several measures of muscle composition in relation to physical function. A meta-analysis showed that low muscle mass (determined by bio-electrical impedance, DXA, or CT) was weakly related to functional decline (51). Inconsistencies in results may be explained by different study designs (cross-sectional vs. longitudinal) and different techniques to determine muscle mass. Based on CT imaging, thigh intramuscular adipose tissue has cross-sectionally been associated with poorer physical function (52) and lower knee extension strength (11) among older adults. To date, few studies have investigated prospective associations between muscle composition determined by CT in relation to physical function in a general older population. These studies show that thigh intramuscular adipose tissue was associated with increased risk of incident mobility limitation, independent of muscle mass and strength among initially well-functioning older men and women (13). In addition, higher and increasing thigh intramuscular adipose tissue was associated with greater gait speed decline (53). Lower calf muscle density, which represents higher levels of muscle fat infiltration, and lower calf muscle mass (cross-sectional muscle area) were associated with increased mobility loss defined as inability to walk 1/4 mile or walk up and down one flight of stairs without assistance among patients with peripheral arterial disease (54). In contrast to muscle mass and muscle fat infiltration, numerous studies have investigated the association between muscle strength (measured as grip or knee extension strength) with functional decline. Pooled results provided strong evidence that low muscle strength is associated with functional decline (51). While the authors state that the role of muscle fat infiltration and muscle strength in relation to the development of functional decline is more pronounced compared to the role of muscle mass with functional decline (51), to date only few studies used CT imaging to determine muscle mass, which might provide new insights.

Body composition and mortality risk

Previous studies investigating body composition in relation to mortality risk mainly focused on anthropometric measures such as BMI or waist circumference (45, 55-57). However, as mentioned, the use of BMI as a measure of overweight is controversial in older adults (19, 55, 58). Furthermore, older persons with similar BMI may have very different body composition. Therefore, as with physical function, it is important to assess body and muscle composition to determine mortality risk.

A well-studied fat depot is visceral fat. Previous studies have shown a positive association between visceral fat and mortality risk (29- 32). Regarding the density of different fat depots, one study has shown that greater density of visceral adipose tissue and

subcutaneous adipose tissue was associated with higher mortality risk (33). Few studies have investigated the association between muscle composition and mortality risk using dual energy X-ray absorptiometry for assessing muscle mass. One study has shown an inverse association between fat percentage and mortality risk among older women, however, the muscle mass index (muscle mass divided by height squared) was not associated (59). In addition, leg skeletal muscle mass was not associated with mortality risk among older men and women (60).

Even though several studies have shown a prospective role of muscle fat infiltration in physical function (13, 53), few studies have used CT imaging to determine the association between muscle composition with mortality risk, particularly in a general older population. Independent of calf muscle mass and whole body fat, increased calf muscle fat infiltration was associated with increased all-cause and cardiovascular disease mortality risk among older men from the Osteoporotic Fractures in Men (MrOS) Study (61). In addition, muscle density, muscle mass or fat area of the calf were not associated with mortality risk in men and women aged 65 years or older from the InCHIANTI study (62).

Additional studies, using accurate and precise techniques to assess body composition are warranted to elucidate potential associations between specific adipose depots and muscle composition in relation to disability and mortality risk.

THE ROLE OF POLYUNSATURATED FATTY ACIDS ON MUSCLE COMPOSITION AND PHYSICAL FUNCTION

More studies are needed to determine the associations between muscle composition based on CT with physical function and mortality. Besides clarifying these associations, it is of interest to investigate whether modifiable factors, such as diet (fatty acids), are associated with better muscle composition, physical function and mortality.

Saturated fatty acids vs. unsaturated fatty acids

Dietary fat can be classified as saturated fatty acids and unsaturated fatty acids, depending on the number of double bonds between carbon atoms. Important sources of saturated fatty acids (no double bonds) are meat and dairy products. Unsaturated fatty acids have one or more double bonds in their configuration and can therefore be classified as monounsaturated fatty acids (MUFAs) (one double bond), or polyunsaturated fatty acids (PUFAs) (multiple double bonds). The main dietary source of MUFAs is olive oil, while PUFAs are mainly found in fatty fish, sea products and sunflower oil and corn oil.

Depending on the location of the double bonds, PUFAs can be sub-divided into n-3 and n-6 PUFAs. The n-3 and n-6 PUFAs have their first double bond in the n-3 and n-6 position, respectively, counted from the methyl end (**Figure 1.3**). Previous research has mainly focused on the n-3 PUFAs eicosapentaenoic acid (EPA) (20:5n-3), docosapentaenoic acid (22:5n-3), docosapentaenoic acid (DHA) (22:6n-3), and alpha-linolenic acid (ALA) (18:3n-3). The main n-6 PUFAs of interest are linoleic acid (18:2n-6) and arachidonic acid (20:4n-6).

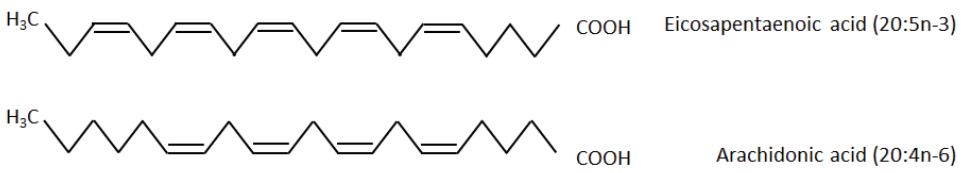

Figure 1.3. Chemical structures of eicosapentaenoic acid (n-3) and arachidonic acid (n-6)

Higher dietary and higher circulating omega-3 (n-3) polyunsaturated fatty acids (PUFAs) are known for their positive influence on cardiovascular health (63), lower risk of fatal ischemic heart disease (64), and lower risk of total and coronary heart disease death (65). With regard to n-6 PUFAs, trials have shown a positive effect of n-6 PUFAs intake on blood lipid levels (66). Large cohort studies have also shown inverse associations between intake or circulating n-6 PUFAs with coronary heart disease risk (67-69), and total and cardiovascular disease mortality (70, 71). To date, limited studies have investigated the potential role of PUFAs in relation to muscle composition and physical function in a general older population.

PUFAs in relation to muscle and physical function

Incorporation of n-3 and n-6 PUFAs in muscle membranes may improve muscle characteristics, and thereby, potentially improve physical function. Results from a systematic literature review showed that n-3 PUFA supplementation in cancer patients might be associated with maintenance of muscle mass (72). One study in lung cancer patients showed that low concentrations of n-3 PUFAs were associated with accelerated loss in muscle mass, and that despite the fact that patients were receiving palliative treatment, patients with high concentrations of plasma n-3 PUFAs gained muscle (73). In healthy older adults, supplementation of n-3 PUFAs increased the rate of muscle protein synthesis (74). In addition, in older women the combination of n-3 supplementation with strength training resulted in greater improvements in muscle strength and functional

capacity compared to strength training alone (75). A positive association between PUFA intake and grip strength was also observed in a large study of community-dwelling older adults (76). One study investigated the prospective relation of PUFAs with physical function, and showed that higher plasma n-3 PUFAs, but not n-6 PUFAs, were associated with lower risk of poor physical function (77). Even though there is some evidence regarding a positive association between PUFAs with enhanced muscle composition and/or physical function, studies were limited by their study design, size, or PUFAs were based on intake questionnaires instead of measurements of circulating PUFAs. Therefore, more prospective studies in the general older population are needed to determine the potential role of PUFAs in muscle composition and function.

AIM AND OUTLINE OF THIS THESIS

The general aim of this thesis is to investigate the relation of various measures of body composition, including fat depots and muscle composition, with physical function and mortality risk. In addition, we investigate whether polyunsaturated fatty acids are associated with muscle composition and physical function. The outline of this thesis is represented in **Figure 1.4.**

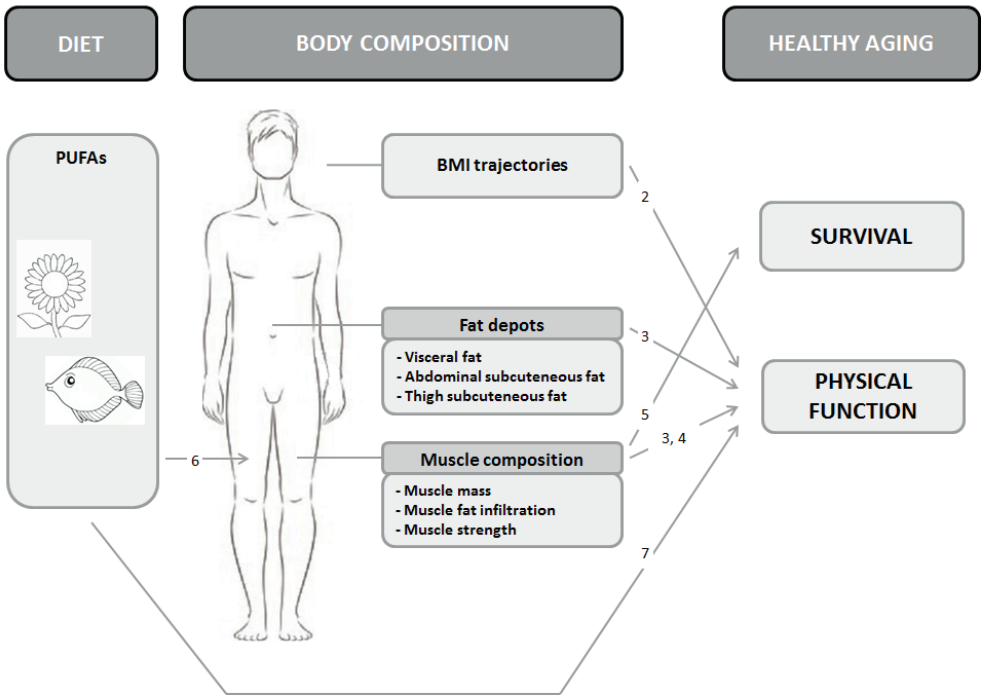

Figure 1.4. Schematic representation of the outline presented in this thesis. Numbers indicate the corresponding chapters.

Research questions

The specific research questions for this thesis are:

1) What are the trajectories of BMI over time, and what are the associations between these trajectories with change in appendicular lean mass and physical function in old age (**Chapter 2**);
2) Which fat depots are associated with physical function among older adults (**Chapter 3**);
3) Which muscle composition measures are associated with physical function and mortality in older adults (**Chapter 4 and 5**);
4) Are circulating polyunsaturated fatty acids associated with muscle composition and physical function (**Chapter 6 and 7**)?

In **Chapter 8** the key findings of the studies are summarised, methodological issues are discussed and recommendations for further studies are provided.

To address these objectives, data from two large prospective cohort studies were used: the Health, Aging and Body Composition (Health ABC, USA) Study, and the Age, Gene/Environment Susceptibility–Reykjavik (AGES-Reykjavik, Iceland) Study.

The Health, Aging and Body Composition Study

The Health ABC Study is an ongoing prospective study of 3075 community-dwelling, initially well-functioning black and white men and women aged 70 to 79 years. The aim of the study is to investigate interrelationships among health conditions, body composition, social and behavioral factors, and functional change (78). Participants were recruited from a random sample of white Medicare beneficiaries and all age-eligible black residents from the Memphis, Tennessee, and Pittsburgh, Pennsylvania, areas. Individuals were eligible if they reported no difficulty in walking one-quarter of mile, climbing 10 steps without resting, or performing mobility-related activities of daily living. Exclusion criteria were a history of active cancer treatment in the prior three years, planning to move out of the study area in the next three years, or current participation in a lifestyle intervention. The baseline home interview and clinic-based examination took place between April 1997 and June 1998. Clinic visits were repeated annually for 10 years, with the exception of years 7 and 9. Data of the Health ABC Study were used in **Chapter 2 and 3**.

The Age, Gene/Environment Susceptibility–Reykjavik Study

The AGES-Reykjavik Study is a single-center, prospective, ongoing population study and was designed to examine risk factors, including genetic susceptibility and gene/environment interaction, in relation to disease and disability in old age among survivors from the Reykjavik Study (79, 80). The Reykjavik Study was initiated in 1967. A random sample of men and women born between 1907-1935 and living in Reykjavik in 1967 was drawn; of which 30.795 participated. Of the 11.549 participants still alive in 2002, 7995 individuals were randomly chosen and invited to the AGES-Reykjavik Study. Baseline examination took place from September 1, 2002, through February 28, 2006 among 5764 (58% women) individuals (81). Follow-up took place between April 1, 2007 through September 30, 2011 in 3316 participants (mean follow-up about five years) reflecting losses due to death (n=1039), and study attrition (n=1409). In **Chapter 4, 5, 6 and 7**, data from AGES-Reykjavik Study participants who took part in both the baseline and follow-up examination were used.

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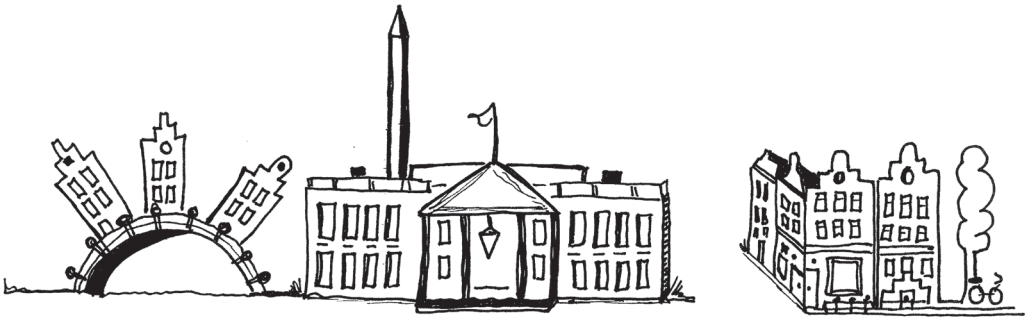

CHAPTER 2

Body mass index trajectories in relation to change in lean mass and physical function: the Health, Aging and Body Composition Study

Ilse Reinders, Rachel A. Murphy, Kathryn R. Martin, Ingeborg A. Brouwer, Marjolein Visser, Daniel K. White, Anne B. Newman, Denise K. Houston, Alka M. Kanaya, Daniel S. Nagin, and Tamara B. Harris, for the Health, Aging and Body Composition Study.

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ABSTRACT

Objectives: To examine body mass index (BMI) trajectories with change in lean mass and physical function in old age.

Design: Prospective cohort study.

Setting: Health, Aging and Body Composition Study.
Participants: Black and white men (n=482) and women (n=516) aged 73.1 ± 2.7 years and initially free of disability.

Measurements: A group-based trajectory model was used to determine BMI trajectories; the path a person's BMI followed over 9 years. Lean mass, gait speed, grip strength, and knee extension strength were assessed at baseline and after 9 years, and relative changes were calculated. Multivariable linear regression was used to determine associations between trajectories and relative change in lean mass and physical function.

Results: Four BMI trajectories were identified for men and four for women. Although all demonstrated a decline in BMI, the rate of decline differed according to trajectory for women only. Men in Trajectory 4 (mean BMI at baseline 33.9 ± 2.3 kg/m^2) declined more than those in Trajectory 1 (mean BMI at baseline 22.9 ± 1.6 kg/m^2) in gait speed (-9.91%, 95% confidence interval (CI)= -15.15% to -4.67%) and leg strength (-8.63%, 95% CI= -15.62% to -1.64%). Women in Trajectory 4 (mean BMI at baseline 34.9 ± 3.0 kg/m^2) had greater losses than those in Trajectory 1 (mean BMI at baseline 20.5 ± 1.6 kg/m^2) in lean mass in the arms (-3.19%, 95% CI= -6.16% to -0.23%). No other associations were observed.

Conclusion: Obese men had the highest risk of decline in physical function despite similar weight loss between trajectories, whereas overweight and obese women who lost the most weight had the greatest risk of lean mass loss. The weight at which a person enters old age is informative for predicting loss in lean mass and physical function, illustrating the importance of monitoring weight.

INTRODUCTION

Aging is associated with changes in body composition (1) and deterioration of physical function (2). Weight changes may be related to impaired physical function. One study showed a failure to conserve lean mass with weight loss (3). Approximately one-third of weight loss reflects loss of lean mass (4, 5), so greater weight loss may accelerate loss of skeletal muscle and subsequent physical function.

It is unclear whether different patterns of weight loss relate to changes in body composition or physical function. Previous studies investigating changes in weight in relation to function have defined weight change groups using cut points or used mixed-effect models, which may be prone to misclassification. An advantage of group-based trajectory modeling is that it is data driven; it identifies distinctive clusters of individual trajectories that follow similar developmental trajectories (6-8). Trajectories track a measurement (e.g. weight) over time and thus provide information on starting weight, changes between measurements and overall weight patterns that could be more informative than a simple measure such as initial weight.

No large cohort study has used trajectory modeling to describe weight trajectories (expressed as body mass index (BMI)) over time in community-dwelling older adults. The purpose of this study was to examine BMI trajectories over a 9 year period in initially well-functioning older men and women using group-based trajectory modeling. The relationship between BMI trajectories and contemporaneous changes in lean mass and physical function was also investigated. It was hypothesized that men and women who lost weight at a faster rate would lose more lean mass and physical function than those who lost weight at a lower rate.

METHODS

Study population

The Health, Aging and Body Composition (Health ABC) Study is a prospective longitudinal study of 3075 community-dwelling, initially well-functioning black and white men and women aged 70 to 79 years (9). Participants were recruited from a random sample of white Medicare beneficiaries and all age-eligible black residents from the Memphis, Tennessee, and Pittsburgh, Pennsylvania, areas. Individuals were eligible if they reported no difficulty in walking one-quarter of mile, walking up 10 steps without resting, or performing mobility-related activities of daily living. Exclusion criteria were a history of active cancer treatment in the prior three years, planning to move out of the study area in

the next three years, or current participation in a lifestyle intervention. Baseline data were collected between April 1997 and June 1998. Clinic visits were repeated annually for 10 years, with the exception of years 7 and 9. The institutional review boards of the study sites and the coordinating center approved the study, and written informed consent was obtained from all participants. After 9 years of follow-up, 514 men (34.5%) and 368 (23.2%) women had died. Of the survivors (n=2193), participants with fewer than three measures of BMI over the 9 year period were excluded (35 men, 58 women), because fewer than three data points results in unstable estimates, as were those without baseline or year 10 measurements of lean mass and physical function (452 men, 629 women). Of participants with three or more measures of BMI and with baseline and year 10 measures of lean mass and physical function, those with missing data on covariates were excluded (8 men, 13 women), resulting in a final sample of 998. The majority of excluded participants were excluded because a method of contact was used that did not enable the body composition or physical function measurements (e.g., home visit, proxy interview, telephone interview); 7.9% of participants who were alive at year 10 were missed because of refusal or other reasons. Excluded participants were older and more likely to be black, less educated, former or current smokers, and less physically active and to have more comorbidities, slower baseline gait speed, and weaker grip and leg strength. BMI was higher in excluded women but did not differ between included and excluded men ($P < 0.05$ for all).

Assessment of body composition

BMI was calculated from body weight measured annually using a standard balance beam scale and body height measured at year 1 using a Harpenden Stadiometer (Holtain Ltd, Crosswell, UK). Total lean mass (excluding bone mineral content) was determined at baseline and year 10 from total body scans using fan-beam dual-energy X-ray absorptiometry (DXA; QDR 4500A, Hologic, Bedford, MA). Appendicular lean mass was calculated as the sum of the lean mass of the arms and legs (kg). The validity and reproducibility of the DXA scanner have been reported previously (10, 11).

Assessment of physical function

Physical function was assessed at baseline and Year 10 from gait speed, grip strength, and leg strength. Usual 20-m gait speed (m/s) was determined during the first lap of the 400-m long-distance corridor walk (12). Participants were allowed to use walking aids during the test, such as canes or walkers, if needed. Grip strength (dominant hand (kg)) was assessed using a Jamar dynamometer (Sammons Preston Rolyan, Bolingbrook, IL) (13). The maximum strength of two trials was analyzed. Leg strength was based on knee extension

strength, which was measured concentrically at 60°/s on an isokinetic dynamometer (125 AP, Kin-Com, Chattanooga, TN). The right leg was tested unless there was a contraindication such as joint replacement or knee pain (n=10 at baseline, n=52 at year 10). The maximum muscle torque (Nm) was selected from the three reproducible and acceptable trials from a maximum of six.

Covariates

Demographic characteristics, smoking status (never, current, former), and physical activity (kcal/kg per week) of all self-reported activity in the week before baseline (14) were ascertained using an interviewer-administered questionnaire at baseline. Prevalent hypertension, diabetes, cardiovascular disease (percutaneous transluminal coronary angioplasty, angina pectoris, myocardial infarction, bypass surgery, stroke, transient ischemic attack, cerebral vascular accident), heart failure, edema, and cancer were determined from self-report, medication use, and clinical assessments, similar to the Cardiovascular Health Study (15).

Statistical analysis

Because of differences with regard to body composition (16) and physical performance (17), analyses were stratified according to sex. Trajectories were estimated using group-based trajectory modeling (18, 19) using proctraj for Stata (Stata Corp., Chicago, IL), which allows individuals to be grouped based on their longitudinal trajectories rather than mean values at a particular time-point. The trajectories were derived by modeling BMI as a function over time (participant age at each follow-up measurement). The best-fitting model was based on the Bayesian Information Criterion and the presence of a minimum of 5% of participants per trajectory to ensure stable estimates per trajectory (6, 8). After the number of trajectories was defined, nonsignificant quadratic and cubic terms were removed, but linear parameters stayed in the model, regardless of significance. Posterior probabilities of group membership were estimated to assess the adequacy of the selected model (6). Specifically, higher-probability values indicate greater likelihood that an individual's trajectory pattern fits within the broader trajectory group. The posterior probability of allocating each participant to the BMI trajectory groups was greater than 0.97, indicating good fit of the models. A Wald test was performed to determine whether slopes of trajectories were significantly different.

Linear regression analyses were performed to tests for linear trend across trajectories by modelling the mean baseline values of continuous variables for each trajectory. By testing linear trend across trajectories you test whether there is an overall increase (or decrease) in the dependent variable as the trajectory group increases.

Chi-square tests were used for baseline categorical variables. Absolute and relative changes (%) between Year 1 and Year 10 of BMI, lean mass, and physical function were calculated. Multivariable linear regression analyses were used to determine the association between BMI trajectories and relative change in lean mass and physical function. Unstandardized regression coefficients (B) and 95% confidence intervals (CIs) were reported for each BMI trajectory after adjustment for covariates. Model 1 was adjusted for age, race, study site, and education. Model 2 was additionally adjusted for smoking status, physical activity, hypertension, diabetes, cardiovascular disease, heart failure, edema, and cancer. Differences between trajectories were tested for, with Trajectory 1 (participants with the lowest mean BMI over time) as the reference group. Differences across trajectories were also tested for, using *P*-values for trend analyses. Because hospitalization did not differ between BMI trajectories, and adjustment for hospitalization did not attenuate associations in men or women, it was not included in the model. All *P*-values were two-tailed (α=0.05). Data were analyzed using Stata version 12.1.

RESULTS

Four BMI trajectories were identified for 482 men and 516 women. Baseline demographic characteristics, lifestyle factors, and comorbidities are presented according to trajectory in **Table 1**. All BMI trajectories in men followed a quadratic relationship and showed a consistent decline over time (**Figure 1**), but the slopes were not significantly different between trajectory groups (*P*=0.26). Mean BMI at baseline for men was 22.9 ± 1.6 kg/m^2 for Trajectory 1, 26.3 ± 1.3 kg/m^2 for Trajectory 2, 29.7 ± 1.7 kg/m^2 for Trajectory 3, and 33.9 ± 2.3 for Trajectory 4. The absolute change in BMI over the 9 year period in men was –0.85 ± 1.94 kg/m^2 for Trajectory 1, –1.02 ± 1.92 kg/m^2 for Trajectory 2, –0.79 ± 2.46 kg/m^2 for Trajectory 3, and –0.15 ± 2.48 kg/m^2 for Trajectory 4. For women, all trajectories declined, and the slopes of trajectories were significantly different between groups (*P*=0.03). The lowest three trajectories were linear trajectories, while Trajectory 4 was a quadratic trajectory (**Figure 1**). Mean BMI at baseline was 20.5 ± 1.6 kg/m^2 for Trajectory 1, 24.8 ± 1.5 kg/m^2 for Trajectory 2, 28.8 ± 1.7 kg/m^2 for Trajectory 3, and 34.9 ± 3.0 for Trajectory 4. Change in BMI was –1.00 ± 1.67 kg/m^2 for Trajectory 1, -1.31 ± 2.22 kg/m^2 for Trajectory 2, -0.81 ± 2.44 kg/m^2 for Trajectory 3, and -1.75 ± 3.27 kg/m^2 for Trajectory 4.

Men had greater lean mass in the arms and legs and better physical function measures than women at baseline and year 10 (*P* < 0.001) (**Supplement Table S1**). **Table 2** depicts absolute and relative changes in lean mass and physical function and associations between trajectories and relative changes in lean mass and physical function. Men in Trajectory 4 had a significantly greater decrease in gait speed than those in Trajectory 1

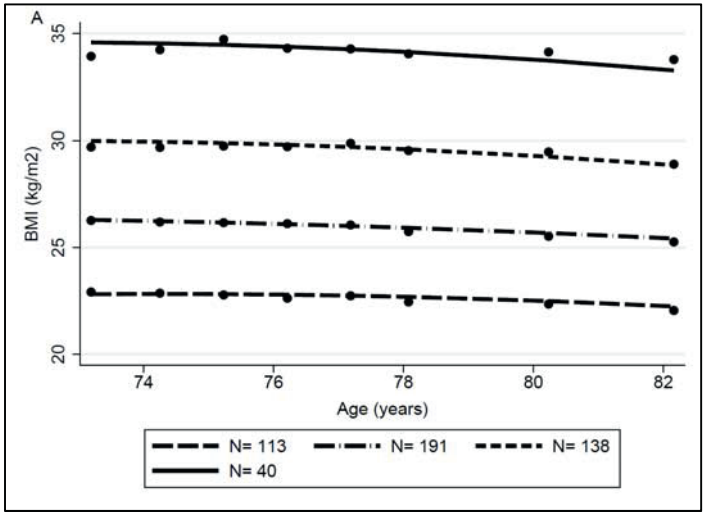
Table 1. Baseline participant characteristics according to body mass index (BMI) trajectory.

Characteristic	BMI Trajectory									
	Men, n=482					Women, n=516				
	1, n=113 (23.3%)	2, n=191 (39.8%)	3, n=138 (28.5%)	4, n=40 (8.4%)	*P*-for trend	1, n=77 (15.1%)	2, n=183 (34.9%)	3, n=171 (33.6%)	4, n=85 (16.4%)	*P*-for trend
BMI at Year 1, kg/m^2, range	18.0–26.8	23.6–30.3	26.7–34.4	30.5–44.2		16.8–24.2	21.5–28.8	23.9–34.1	28.8–46.1	
Age, mean±SD	73.5±2.7	73.4±2.8	72.9±2.7	72.6±2.5	0.04	73.3±2.9	73.2±2.8	72.8±2.6	72.8±2.7	0.15
White, n (%)	80 (71)	149 (78)	92 (67)	26 (65)	0.09	61 (79)	140 (77)	89 (52)	28 (33)	< 0.001
Memphis site, n (%)	65 (58)	89 (47)	64 (46)	20 (50)	0.25	47 (61)	102 (56)	81 (47)	42 (49)	0.16
BMI, kg/m^2										
Year 1	22.9±1.58	26.3±1.28	29.7±1.70	33.9±2.27	< 0.001	20.5±1.64	24.8±1.51	28.8±1.72	34.9±3.01	< 0.001
Year 10	22.0±2.00	25.3±1.66	28.9±1.90	33.8±3.07	< 0.001	19.5±1.84	23.4±1.88	28.0±2.34	33.2±3.36	< 0.001
Change	–0.85±1.94	–1.02±1.92	–0.79±2.46	–0.15±2.48	0.05	–1.00±1.67	–1.31±2.22	–0.81±2.44	–1.75±3.27	0.14
Education, n (%)										
< High school	15 (13)	32 (17)	31 (22)	7 (18)		9 (11)	28 (15)	32 (19)	16 (19)	
High school graduate	26 (23)	48 (25)	39 (29)	10 (25)	0.40	27 (35)	70 (38)	64 (37)	37 (44)	0.52
Postsecondary	75 (64)	111 (58)	68 (49)	23 (58)		41 (53)	85 (46)	75 (44)	32 (38)	
Smoking status, n (%)										
Never	44 (39)	69 (36)	47 (34)	10 (25)		52 (68)	109 (60)	106 (62)	53 (62)	
Current	12 (11)	14 (7)	10 (7)	1 (3)	0.31	10 (13)	12 (7)	6 (4)	2 (2)	0.02
Former	57 (50)	108 (57)	81 (59)	29 (73)		15 (19)	62 (34)	59 (35)	30 (35)	
Physical activity, kcal/kg per week	95±84	92±69	88±63	83±97	0.35	90±49	96±74	100±75	85±60	0.79
Hypertension, n (%)	57 (50)	119 (62)	74 (54)	1251 (63)	0.15	26 (34)	95 (52)	110 (64)	63 (74)	< 0.001
Diabetes, n (%)	10 (9)	21 (11)	26 (19)	5 (13)	0.08	5 (6)	10 (5)	17 (10)	13 (15)	0.04
Cardiovascular disease, n (%)	23 (20)	51 (27)	31 (22)	16 (40)	0.08	4 (5)	25 (14)	19 (11)	7 (8)	0.19
Coronary heart failure, n (%)	12 (11)	24 (13)	20 (14)	9 (23)	0.28	5 (6)	15 (8)	17 (9)	16 (19)	0.03
Edema, n (%)	7 (6)	16 (8)	11 (8)	7 (18)	0.17	2 (3)	6 (3)	10 (6)	7 (8)	0.23
Cancer, n (%)	25 (21)	31 (16)	32 (23)	10 (26)	0.30	19 (25)	43 (24)	19 (11)	10 (12)	0.003
Number of hospital admissions, n (%)										
0	10 (9)	19 (10)	17 (12)	4 (10)		12 (16)	43 (24)	30 (18)	20 (24)	
1–3	63 (56)	101 (53)	63 (47)	20 (50)	0.91	49 (64)	85 (47)	88 (52)	39 (46)	0.11
4–5	24 (21)	36 (19)	30 (22)	9 (23)		10 (13)	29 (16)	30 (18)	8 (9)	
≥ 6	16 (14)	35 (18)	28 (20)	7 (18)		6 (8)	26 (14)	23 (13)	18 (22)	

Baseline characteristics are presented according to BMI trajectories, which were derived by modeling BMI as a function over time. Linear regression analyses were used to calculate *P*-values for trend.

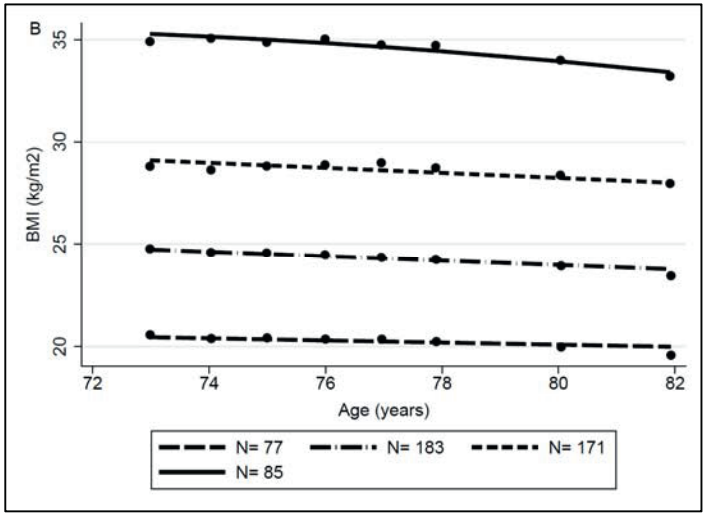
Figure 1: Body mass index (BMI) trajectories of the Health, Aging and Body Composition Study participants during 9 years of follow-up in men (n=482) and women (n=516).

Men



In men, all four trajectories were quadratic. The decline in kg/m^2 (slope) per year was 0.00933 kg/m^2 for Trajectory 1, 0.00509 kg/m^2 for Trajectory 2, 0.01108 kg/m^2 for Trajectory 3, and 0.01325 kg/m^2 for Trajectory 4. The lines are mean BMI.

Women



In women, Trajectories 1, 2, and 3 were linear, and Trajectory 4 was quadratic. The decline in kg/m^2 (slope) per year was 0.05165 kg/m^2 for Trajectory 1, 0.10820 kg/m^2 for Trajectory 2, 0.12283 kg/m^2 for Trajectory 3, and 0.01030 kg/m^2 for Trajectory 4. The lines are mean BMI.

Table 2. Associations between body mass index (BMI) trajectory and relative changes from baseline to 9 Year follow-up in body composition and physical function.

Trajectory	Men					Women				
	n	Absolute	Relative	Model 1[a]	Model 2[b]	n	Absolute	Relative	Model 1[a]	Model 2[b]
Body composition										
Total appendicular lean mass, kg										
1	113	−1.29±1.57	−5.8±7.1	Reference	Reference	77	−0.44±0.86	−3.1±6.0	Reference	Reference
2	191	−1.36±1.53	−5.6±6.3	−0.40 (−1.90; 1.10)	−0.45 (−1.95; 1.06)	183	−0.61±1.09	−3.9±7.1	−0.89 (−2.79; 1.01)	−0.85 (−2.80; 1.11)
3	138	−1.33±1.91	−4.9±7.1	0.40 (−1.21; 2.01)	0.31 (−1.31; 1.93)	171	−0.64±1.30	−3.5±7.2	−0.45 (−2.41; 1.51)	−0.38 (−2.42; 1.67)
4	40	−1.34±2.33	−4.3±8.1	1.04 (−1.27; 3.36)	1.24 (−1.11; 3.60)	85	−0.78±1.64	−3.7±8.5	−0.35 (−2.65; 1.94)	−0.15 (−2.56; 2.26)
P for trend		0.90	0.18	0.28	0.22		0.08	0.66	0.86	> 0.99
Lean mass in arms, kg										
1	113	−0.45±0.50	−7.3±8.1	Reference	Reference	77	−0.12±0.22	−3.4±6.3	Reference	Reference
2	191	−0.50±0.51	−7.3±7.4	−0.87 (−2.52; 0.77)	−0.97 (−2.64; 0.70)	183	−0.17±0.36	−4.1±9.5	−0.94 (−3.28; 1.39)	−0.98 (−3.39; 1.42)
3	138	−0.53±0.62	−7.0±8.0	−0.56 (−2.33; 1.20)	−0.55 (−2.35; 1.24)	171	−0.19±0.41	−4.0±9.5	−1.07 (−3.48; 1.34)	−1.15 (−3.67; 1.37)
4	40	−0.63±0.74	−7.5±9.5	−0.90 (−3.44; 1.65)	−0.67 (−3.28; 1.94)	85	−0.33±0.49	−6.3±10.1	−3.07(−5.89; −0.25)	−3.19(−6.16; −0.23)
P for trend		0.07	0.98	0.55	0.70		< 0.001	0.05	0.04	0.04
Lean mass in legs, kg										
1	113	−0.85±1.18	−5.2±7.3	Reference	Reference	77	−0.31±0.75	−2.9±7.0	Reference	Reference
2	191	−0.86±1.17	−4.9±6.7	−0.22 (−1.85; 1.41)	−0.25 (−1.88; 1.39)	183	−0.45±0.85	−3.8±7.3	−0.88 (−2.93; 1.16)	−0.83 (−2.93; 1.28)
3	138	−0.80±1.43	−4.1±7.5	0.78 (−0.97; 2.54)	0.66 (−1.10; 2.42)	171	−0.46±0.98	−3.3±7.3	−0.27 (−2.38; 1.84)	−0.16 (−2.36; 2.04)
4	40	−0.72±1.77	−3.1±8.4	1.79 (−0.72; 4.31)	2.00 (−0.56; 4.56)	85	−0.45±1.40	−2.8±9.6	0.46 (−2.01; 2.93)	0.74 (−1.85; 3.34)
P for trend		0.54	0.07	0.10	0.08		0.39	0.86	0.61	0.48

[a] Model 1 adjusted for age, race, study site, and education.

[b] Model 2 adjusted for factors in Model 1 plus smoking status, physical activity, hypertension, diabetes, cardiovascular disease, heart failure, edema, and cancer.

Table 2. (continued)

Trajectory	Men					Women				
	n	Absolute	Relative	Model 1[a]	Model 2[b]	n	Absolute	Relative	Model 1[a]	Model 2[b]
Physical function										
Gait speed, m/s										
1	113	−0.37±0.23	−23.5±14.2	Reference	Reference	77	−0.37±0.20	−25.5±12.1	Reference	Reference
2	191	−0.43±0.24	−27.3±13.6	−4.00 (−7.33; −0.67)	−3.58 (−6.94; −0.23)	183	−0.33±0.21	−23.6±14.5	1.49 (−2.43; 5.41)	2.33 (−1.68; 6.34)
3	138	−0.36±0.24	−23.7±14.7	−1.12 (−4.70; 2.46)	−0.77 (−4.36; 2.83)	171	−0.33±0.22	−24.1±15.6	0.22 (−3.82; 4.27)	1.20 (−3.00; 5.39)
4	40	−0.50±0.26	−33.4±17.4	−11.11(−16.26; −5.97)	−9.91(−15.15; −4.67)	85	−0.31±0.22	−25.2±16.7	−1.11 (−5.84; 3.62)	−0.24 (−5.18; 4.70)
P for trend		0.02	0.001	< 0.001	0.001		0.07	0.98	0.54	0.81
Grip strength, kg										
1	113	−6.87±6.30	−16.3±16.5	Reference	Reference	77	−2.94±4.31	−9.4±26.0	Reference	Reference
2	191	−6.19±6.48	−14.4±16.2	0.97 (−2.88; 4.81)	0.49 (−3.45; 4.35)	183	3.56±4.65	−12.8±18.0	−3.99 (−9.92; 1.95)	−3.02 (−9.08; 3.04)
3	138	−5.70±6.97	−12.6±17.3	2.54 (−1.59; 6.68)	2.42 (−1.76; 6.62)	171	−2.98±5.28	−9.0±26.6	−1.28 (−7.40; 4.85)	−0.01 (−6.35; 6.34)
4	40	−7.00±6.67	−15.2±16.5	0.07 (−5.86; 6.01)	−0.01 (−6.07; 6.06)	85	−3.34±4.79	−11.2±18.2	−3.63(−10.79; 3.54)	−3.04 (−10.51; 4.43)
P for trend		0.98	0.59	0.85	0.84		0.79	0.88	0.47	0.61
Leg strength, Nm										
1	113	−36.8±27.4	−26.1±21.9	Reference	Reference	77	−19.7±14.9	−24.9±22.4	Reference	Reference
2	191	−35.5±29.2	−24.0±19.9	−0.07 (−4.49; 4.35)	−0.44 (−4.91; 4.03)	183	−19.0±18.1	−20.9±23.3	3.21 (−3.30; 9.73)	3.94 (−2.68; 10.56)
3	138	−41.0±33.7	−26.1±20.9	−2.46 (−7.21; 2.29)	−2.40 (−7.20; 2.41)	171	−22.4±23.6	−22.5±24.5	0.70 (−6.03; 7.42)	1.41 (−5.52; 8.34)
4	40	−56.0±52.4	−32.8±20.9	−8.59(−15.42; −1.76)	−8.63(−15.62; −1.64)	85	−24.5±23.8	−23.0±33.2	1.09 (−6.78; 8.95)	1.83 (−6.32; 9.99)
P for trend		< 0.001	0.06	0.009	0.01		0.08	0.74	0.95	0.82

[a] Model 1 adjusted for age, race, study site, and education.

[b] Model 2 adjusted for factors in Model 1 plus smoking status, physical activity, hypertension, diabetes, cardiovascular disease, heart failure, edema, and cancer.

(-9.91%, 95% CI= -15.15% to -4.67%, *P*-for trend 0.001; Model 2). Men in Trajectory 4 lost more leg strength than those Trajectory 1 (–8.63%, 95% CI= -15.62% to -1.64%, *P*-for trend=0.01; Model 2). There were no significant differences between trajectories in relative change in grip strength or change in lean mass in men, nor was there a significant trend across trajectories. Women in Trajectory 4 lost relatively more lean mass in the arms than women in Trajectory 1 (-3.19%, 95% CI= -6.16% to -0.23%; Model 2). BMI trajectories were not associated with change in total appendicular lean mass or leg lean mass. No significant differences for physical function were observed for trajectories in women.

DISCUSSION

This study identified four distinctive BMI trajectories for men and women over 9 years. All trajectories were characterized by a decline in BMI. Participants with the highest mean BMI over time (Trajectory 4) lost more lean mass or had greater decline in physical function, although significant sex differences were observed. In men, there were no differences in relative loss of lean mass between the four trajectory groups, although men in Trajectory 4 had greater loss in leg strength and gait speed than those in Trajectory 1. Conversely, women in Trajectory 4 lost relatively more lean mass in the arms than those in Trajectory 1 but had no difference in physical function.

Duration and type of physical activity may explain the sex differences observed in this study. Results from the National Health and Nutrition Examination Survey showed that, even though men and women had similar activity counts per minute, older women had more light-intensity activity than men and spent more overall time in nonsedentary activity (20). Based on self-report questionnaire data, at baseline men were less physically active (91 ± 74 kcal/kg per week) than women (95 ± 69 kcal/kg per week) (Mann-Whitney test; *P*=0.06). Additionally, sex differences in activity diversity and level of activity were observed in a nationally representative British birth cohort. Men more often reported greater-intensity activity at early old age, yet changes in activity type may occur more in men than women with increasing age (21) (e.g., reduced running or cycling, which could increase loss in leg strength and perhaps explain reduction in gait speed). Women are more likely to report more lighter-intensity activity such as household activities than men (22), which may explain why there were no differences in physical function but was loss in lean mass.

Little is known about household activities in older adults and how they might affect changes in body composition and areas of lean tissue loss. More studies are warranted to

investigate whether differences in type and duration of activities in older age might prevent loss in function.

Despite being an initially well-functioning cohort, all participants lost weight, lean mass, and physical function. This is comparable with results from a cross-sectional study that showed that men and women aged 60 to 80 become less physically active with age, which results in a reduction in physical function (e.g., 8 foot up and go test, 2 minute step test) (23). In addition, recent findings from the Baltimore Longitudinal Study of Aging showed that physical activity counts, measured using an accelerometer, were 1.3% lower for each year increase in age, which was especially due to lower afternoon and evening activity in older individuals (24). Although loss of function is a common occurrence of aging, the results of the current study show that loss of physical function varies according to BMI trajectory, with Trajectory 1 most closely representing healthy aging.

Men in Trajectory 4 had greater decline in gait speed and greater loss of leg strength than men in Trajectory 1, which health and lifestyle variables could not explain. The data indicate that the BMI with which a person enters into old age is an important determinant of the trajectory he or she follows in later life. The Health ABC Study has previously shown that the onset of overweight and obesity in midlife or earlier contributes to risk of incident mobility limitation (25). Participants in Trajectory 4 were obese (men: mean 33.9 kg/m^2, range 30.5 – 44.2 kg/m^2; women: mean 34.9 kg/m^2, range 28.8 – 46.1 kg/m^2), so these findings are also in line with results of a recent meta-analysis that showed that obesity is associated with greater risk of functional decline in old age than normal weight or overweight (26). In addition, adding exercise training to energy restriction results in preservation of lean mass and better physical function (27). The results of the current study provide further evidence of the importance of preventing obesity before entering old age.

This study had several strengths. First, the large study sample of community-dwelling black and white men and women followed over 9 years. Second, group-based trajectory modeling was used to determine distinctive BMI trajectories within the study population and enabled differences in participant characteristics within trajectories to be investigated (28). Third, using BMI categories can result in similar people being placed in different risk categories e.g. BMI of 24.9 kg/m^2 is normal weight while a BMI of 25.0 kg/m^2 is overweight.

In contrast trajectories model an individual's path and then groups with similar trajectories, thus minimizing misclassification. Fourth, the use of DXA provides accurate measurement of appendicular lean mass. Fourth, physical function was assessed using multiple indicators including gait speed and grip and leg strength. Finally, reverse causation was minimized because participants were initially free from mobility disability. Some limitations need to be acknowledged. Although the statistical methods allowed differences in lean mass and physical function changes to be investigated according to BMI trajectory, fewer people were allocated to Trajectory 1 and 4, which might have resulted in low statistical power to detect differences between groups. Nevertheless, the analyses met the criteria outlined in the proctraj method for the presence of a minimum of 5% of participants per trajectory to ensure a meaningful description of characteristics per trajectory, which makes sure it provides stable estimates (6, 8). Nevertheless, larger studies with a wider BMI range are warranted. For the 2,193 participants who were alive at follow-up, 998 (45.5%) with at least three BMI measurements, data on change in lean mass and physical function and complete data on baseline covariates were included in the analyses because long-term weight change was of interest. This may have resulted in a healthier analytical sample than the original cohort. Differences in declines from Year 1 and Year 10 measurements in lean mass and physical function were observed by trajectory group, although the magnitude of change may be even greater in less-healthy populations, so observed associations are likely to be underestimated.

In conclusion, despite a well-functioning population at baseline, all BMI trajectories showed a decline over 9 years. Participants in BMI Trajectory 4 had greater decreases in arm lean mass and physical function, although results differed according to sex. Declines in lean mass did not consistently reflect declines in physical function suggesting the importance of other pathways in functional decline. Finally, the decline in BMI was modest, and the rate of decline was not different between trajectory groups in men, suggesting that, although BMI may change over time, those changes tend to be modest and change in BMI does not depend on where you start. A person's trajectory of disability is set before they enter old age, indicating the importance of healthy weight during mid-life and earlier and of monitoring weight.

SUPPLEMENTS

Supplemental Table 1. Gender differences in body composition and physical function measures

	Men	**Women**	***P*-value**
	n=482	n=516	
Total appendicular lean mass (kg)			
Year 1	24.1 ± 3.5	16.4 ± 2.9	< 0.001
Year 10	22.8 ± 3.5	15.8 ± 2.8	< 0.001
Lean mass in the arms (kg)			
Year 1	6.7 ± 1.1	4.0 ± 0.8	< 0.001
Year 10	6.2 ± 1.0	3.8 ± 0.7	< 0.001
Lean mass in the legs (kg)			
Year 1	17.4 ± 2.5	12.4 ± 2.2	< 0.001
Year 10	16.5 ± 2.5	12.0 ± 2.2	< 0.001
Gait speed (m/s)			
Year 1	1.50 ± 0.24	1.35 ± 0.22	< 0.001
Year 10	1.09 ± 0.22	101 ± 0.22	< 0.001
Grip strength (kg)			
Year 1	40.7 ± 8.4	25.2 ± 6.0	< 0.001
Year 10	34.4 ± 8.0	21.9 ± 5.4	< 0.001
Leg strength (Nm)			
Year 1	140 ± 36	85 ± 22	< 0.001
Year 10	101 ± 29	64 ± 21	< 0.001

Values are mean ± standard deviation

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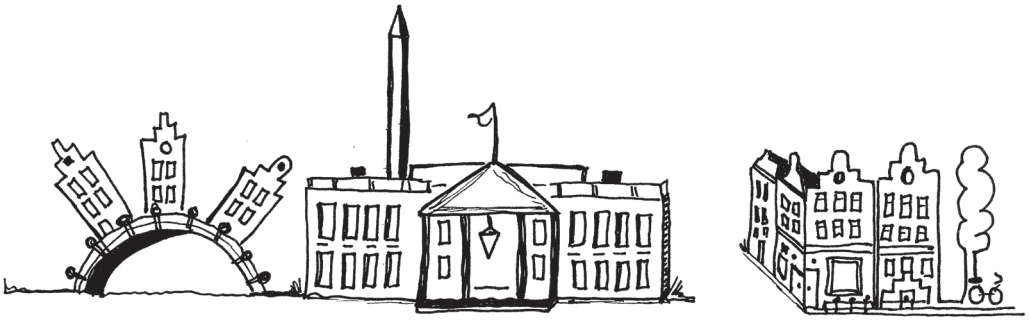

CHAPTER 3

Associations of BMI and adipose tissue area and density with incident mobility limitation and poor performance in older adults

Rachel A. Murphy, Ilse Reinders, Thomas C. Register, Hilsa N. Ayonayon, Anne B. Newman, Suzanne Satterfield, Bret H. Goodpaster, Eleanor M. Simonsick, Stephen B. Kritchevsky, and Tamara B. Harris.

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ABSTRACT

Background: Obesity is a risk factor for disability, but risk of specific adipose depots is not completely understood.

Objective: We investigated associations between mobility limitation, performance, and the following adipose measures: body mass index (BMI) and areas and densities of visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and intermuscular adipose tissue (IMAT) in older adults.

Design: This was a prospective population-based study of men (n=1459) and women (n=1552) initially aged 70 to 79 years and free from mobility limitation. BMI was determined from measured height and weight. Adipose tissue area and density in Hounsfield Units were measured in the thigh and abdomen by using computed tomography. Mobility limitation was defined as two consecutive reports of difficulty walking one-quarter mile or climbing 10 steps during semiannual assessments over 13 year. Poor performance was defined as a gait speed < 1.0 m/s after 9 years of follow-up (n=1542).

Results: In models adjusted for disability risk factors, BMI, and areas of VAT, abdominal SAT, and IMAT were positively associated with mobility limitation in men and women. In women, thigh SAT area was positively associated with mobility limitation risk, whereas VAT density was inversely associated. Associations were similar for poor performance. BMI and thigh IMAT area (independent of BMI) were particularly strong indicators of incident mobility limitation and poor performance. For example, in women, the HR (95% CI) and OR (95% CI) associated with an SD increment in BMI for mobility limitation and poor performance were 1.31 (1.21; 1.42) and 1.41 (1.13; 1.76), respectively. In men, the HR (95% CI) and OR (95% CI) associated with an SD increment in thigh IMAT for mobility limitation and poor performance were 1.37 (1.27; 1.47) and 1.54 (1.18; 2.02), respectively.

Conclusions: Even into old age, higher BMI is associated with mobility limitation and poor performance. The amount of adipose tissue in abdominal and thigh depots may also convey risk beyond BMI.

INTRODUCTION

Disability rates have achieved modest declines in the elderly US population (1). However, steady increases in obesity in older adults (2) may mitigate these improvements (3–5). Studies from recent years have provided convincing evidence that links obesity and disability (6–9), including 30–150% greater risk of incident disability relative to normal weight (8, 9). Similarly, a systematic review concluded that obesity is consistently and positively associated with disability in older adults (10).

Most studies have classified individuals as obese by using BMI, which is an indicator of overall adiposity. Additional adiposity measures may provide insight into disability relationships. For example, studies have reported that abdominal adipose tissue estimated from waist circumference is a better predictor of disability than BMI (11, 12). However, little is known regarding specific adipose depots (ie, measured with radiographic imaging) or other characteristics of adipose tissue such as adipose tissue density.

Previously, we identified adipose density measured from the Hounsfield units (HU) (4) of computed tomography images as a novel marker of mortality risk in older adults (13). Denser adipose tissue was associated with increased mortality risk independent of adipose tissue area and BMI. We propose that adipose tissue density may provide insight into relations between adiposity and disability indicators.

The aims of this study were to provide a comprehensive assessment of adiposity and risk of mobility limitation and poor performance in older adults. Specifically, we explored associations with BMI and areas and densities of adipose depots in the abdomen (visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT)) and thigh (SAT and intermuscular adipose tissue (IMAT)).

SUBJECTS AND METHODS

Subjects

We used data from the Health, Aging, and Body Composition Study (Health ABC). Health ABC is a prospective, longitudinal study of 3075 community-dwelling black and white men and women aged 70 to 79 years. Participants were recruited from a random sample of white Medicare beneficiaries and all black Medicare-eligible residents in the Memphis, Tennessee, and Pittsburgh, Pennsylvania, areas. All participants were initially free of mobility limitation defined as no difficulty walking one-quarter mile or climbing 10 steps and free of difficulty performing basic activities of daily living. Exclusion criteria were

active cancer treatment in the previous three year, participation in a lifestyle intervention, or planned move from the study area within three year. Baseline data from interviews and a clinic-based examination were collected between April 1997 and June 1998.

Adiposity measures

BMI was calculated from height measured with a stadiometer and weight measured with a calibrated scale. Computed tomography (CT) imaging of the abdomen at the L4/L5 vertebrae and midthigh was performed in Memphis (Somatom Plus 4 scanner (Siemens) or PQ 200S (Picker)) and Pittsburgh (9800 Advantage; General Electric). Tissue areas (cm^2) were calculated by multiplying the number of pixels of a given tissue by the pixel area by using Interactive Data Language software (RSI Systems). Tissue types were identified on the basis of radiographic density (HU), which was calibrated to distilled water (0 HU) and air (−1000 HU). Thus, higher HU indicated more-dense tissue. Adipose tissue areas were defined as voxels between −150 and −30 HU. After distinguishing fat from lean and bone tissues, VAT was distinguished from SAT by tracing along the fascial plane defining the internal abdominal wall. Adipose tissue density was assessed from the mean HU. Adipose measures were missing or outside of the image-viewing field for n=114 (VAT area), n=191 (abdominal SAT area), n=64 (thigh SAT area, IMAT area, thigh SAT density, and IMAT density), n=119 (VAT density), and n=209 (abdominal SAT density), which resulted in differing numbers of participants by measure.

Outcomes

Self-reported physical function was assessed during annual clinic visits and telephone interviews every six months over 13 years of follow-up. Mobility limitation was defined as two consecutive reports of having difficulty walking one-quarter mile or climbing 10 steps. Reports must have involved the same function (i.e., two reports of difficulty walking one-quarter mile or two reports of difficulty climbing stairs). If participants missed a study visit, target dates for when the visit should have been completed were used to calculate the time to event or censorship. When questions were not answered, missing data were imputed by interpolating between the most-recent previous visit with data and the first following visit with data. A final determination of limitation status was made from an interview or, if needed, a proxy interview and hospital records.

Gait speed was examined as an objective measure of performance. Usual gait speed over six meter was assessed nine years after the baseline study visit in n=1542. Participants were instructed to walk at their normal pace for the duration of the test. Timing was

started with the first footfall and stopped with the first footfall after crossing the end line. Gait speed < 1.0 m/s was used to identify poor performance (14, 15).

Covariates

Baseline covariates related to adiposity or mobility limitation were chosen a priori including age, education, race, study site, smoking status, prevalent diabetes, cancer, coronary heart disease from self-report and medications (coronary bypass, angioplasty, myocardial infarction, or angina), pulmonary disease (asthma, chronic bronchitis, emphysema, or chronic obstructive pulmonary disease), physical activity, self-reported midlife weight, and knee pain. Education was categorized as less than high school, high school, or postsecondary education. Smoking was categorized as never, former, or current. Prevalent disease was determined from self-report, medications, and clinical assessments. Physical activity was assessed as the activity spent walking or exercising in the seven days before baseline (16). Pain was determined from self-reported presence or absence of pain in either knee.

Statistical analysis

Differences between groups were compared by using two-sided *t*-tests or chi-square tests. Correlations between BMI and adipose area and density were examined by using Spearman's correlation coefficient (*r*). Sequentially adjusted Cox proportional hazards models were used to estimate Hazard Ratios (HRs) and 95% CIs for risk of mobility limitation. HRs were expressed per sex- and race-specific SDs of BMI and adipose area and density. Models were stratified by sex because of known differences in body composition and physical performance (6, 17). The proportional hazards assumption was tested by using Schoenfeld residuals and was not met for physical activity, which was modeled as time varying. Model 1 was adjusted for age, race, and study site. Model 2 was additionally adjusted for education, smoking status, prevalent disease, physical activity, midlife weight, and pain. Model 3 was further adjusted for BMI to determine whether adipose measures were associated with mobility limitation beyond risk attributable to BMI. Collinearity assessment within models revealed mean variance inflation factors < 1.7. Sensitivity analyses were conducted with the exclusion of participants with BMI (in kg/m^2) < 20 because of possible relationships between underweight and limitation (18).

Logistic regression was used to estimate ORs and 95% CIs for poor performance per SD increment in adipose measures. Models were sequentially adjusted for the same risk factors as in Cox models. All analyses were performed with STATA version 12.1 (StataCorp, College Station, Texas, USA). Significance was determined at $P < 0.05$.

Table 1. Characteristics at baseline of participants in the Health ABC Study with thigh adipose tissue measures (n=3011) according to final mobility-limitation classification[1,2].

	No mobility limitation	Mobility limitation	*P*-value
Women [*n* (%)]	381 (45.9)	1171 (53.7)	< 0.001
Age (y)	73.8 ± 2.85	74.3 ± 2.87	< 0.001
Black race [*n* (%)]	290 (34.9)	959 (44.0)	< 0.001
Pittsburgh site [*n* (%)]	443 (53.3)	1056 (48.4)	< 0.001
Education, [*n* (%)]			< 0.001
Less than high school graduate	154 (18.6)	599 (27.5)	
High school graduate	257 (31.1)	727 (33.4)	
Postsecondary	416 (50.3)	850 (39.1)	
Smoking status, [*n* (%)]			
Never smoker	397 (48.0)	929 (42.7)	
Current smoker	70 (8.45)	239 (11.0)	0.01
Former smoker	361 (43.6)	1010 (46.4)	
Cancer [*n* (%)]	122 (14.8)	399 (18.4)	0.02
Diabetes [*n* (%)]	78 (9.39)	376 (17.3)	< 0.001
Hypertension [*n* (%)]	286 (34.4)	877 (40.2)	0.003
Coronary heart disease [*n* (%)]	118 (14.4)	429 (20.1)	0.001
Physical activity ($kcal/kg^{-1}/wk^{-1}$)	1394 ± 2205	919.4 ± 1760	< 0.001
Midlife weight (kg)	70.0 ± 12.9	72.4 ± 14.0	< 0.001
Weight (kg)	72.7 ± 13.6	76.8 ± 15.1	< 0.001
BMI (kg/m^2)	26.0 ± 3.89	27.9 ± 4.93	< 0.001
< 20.0 kg/m^2 [*n* (%)]	39 (4.69)	81 (3.72)	< 0.001
20.0 to < 25.0 kg/m^2 [*n* (%)]	307 (36.9)	544 (25.0)	
25.0 – 29.9 kg/m^2 [*n* (%)]	366 (44.0)	916 (42.0)	
≥ 30.0 kg/m^2 [*n* (%)]	119 (14.3)	639 (29.3)	
VAT area (cm^2)	131 ± 60.9	148 ± 68.8	< 0.001
Abdominal SAT area (cm^2)	255 ± 103	296 ± 125	< 0.001
Thigh SAT area (cm^2)	134 ± 71.0	165 ± 98.6	< 0.001
Thigh IMAT area (cm^2)	17.0 ± 9.75	22.0 ± 13.6	< 0.001
VAT density (HU)	−85.7 ± 10.2	−87.2 ± 9.67	< 0.001
Abdominal SAT density (HU)	−96.3 ± 9.48	−97.1 ± 8.95	0.04
Thigh SAT density (HU)	−105 ± 11.6	−106 ± 11.2	0.003
Thigh IMAT density (HU)	−71.8 ± 11.0	−74.3 ± 10.3	< 0.001

[1] Differences between groups were tested by using two-sided *t*-tests for continuous variables or chi-square tests for categorical variables. Coronary heart disease was determined as any of the following: coronary bypass, angioplasty, myocardial infarction, or angina. Health ABC, Health, Aging, and Body Composition Study; HU, Hounsfield Units; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

[2] Mean ± SD (all such values).

RESULTS

The mean (±SD) age of the analytic sample at baseline was 74.2 ± 2.87 years, 51.5% of subjects were women, and 41.5% of subjects were black. Baseline characteristics for participants (n=3011) with measures of thigh adipose tissue are presented in **Table 1**. Participants who developed mobility limitation were predominately women, older, black, less educated, and more likely to report current or former smoking in addition to being heavier, more obese, and having more comorbid conditions.

BMI was positively associated with all adipose areas and inversely correlated with all measures of adipose density ($P < 0.001$; **Table 2**). The mean follow-up for mobility limitation was ~six year. During follow-up, 2243 participants (129 participants/1000 person-years) developed mobility limitation.

Table 2. Spearman correlations (*r*) of BMI and adipose measures in the Health ABC Study participants[1].

	BMI	VAT area	Abdominal SAT area	Thigh SAT area	Thigh IMAT area	VAT density	Abdominal SAT	Thigh SAT density	Thigh IMAT
BMI	1.00								
VAT area	0.58	1.00							
Abdominal SAT area	0.75	0.35	1.00						
Thigh SAT area	0.51	0.07	0.79	1.00					
Thigh IMAT area	0.66	0.48	0.58	0.38	1.00				
VAT density	-0.46	-0.63	-0.42	-0.21	-0.35	1.00			
Abdominal SAT density	-0.31	-0.11	-0.63	-0.56	-0.22	0.60	1.00		
Thigh SAT density	-0.19	-0.03	-0.38	-0.52	-0.19	0.17	0.47	1.00	
Thigh IMAT density	-0.35	-0.18	-0.57	-0.58	-0.54	0.37	0.58	0.82	1.00

[1] Data are for all participants with nonmissing data for each measure of adiposity (n=2855). $P < 0.001$ for all correlations except the VAT area and thigh SAT density (P=0.13). Health ABC, Health, Aging, and Body Composition Study; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Relationships between adipose measures and risk of mobility limitation are shown in **Table 3**. In minimally adjusted models (Model 1), BMI and all adipose depot areas were positively associated with risk of mobility limitation in men and women. Associations remained significant with adjustment for covariates in Model 2 except for thigh SAT area in men. With additional adjustment for BMI (Model 3), the following adipose depots remained associated with risk: VAT area in men (HR: 1.10; 95% CI 1.01; 1.19), IMAT area in men and women [HRs: 1.37 (95% CI 1.27; 1.47) and 1.08 (95% CI 1.00; 1.16), respectively], abdominal SAT area (HR: 1.13; 95% CI 1.02; 1.26), and thigh SAT area (HR: 1.18; 95% CI 1.08; 1.28) in women.

In Model 1 (**Table 3**), VAT and IMAT density were inversely associated with risk of mobility limitation in men and women. With adjustment for covariates (Model 2), thigh IMAT density remained marginally associated with risk in men, whereas VAT and IMAT density remained associated in women. After adjustment for BMI (Model 3), abdominal SAT density became positively associated with mobility limitation (HR: 1.10; 95% CI 1.02; 1.18) in men, whereas all relations were attenuated in women. The exclusion of participants with BMI < 20 did not appreciably change relations for any adipose measure (not shown).

BMI and areas of VAT, abdominal SAT, thigh SAT, and thigh IMAT were all associated with increased odds of having poor performance in Model 1 (**Table 4**) in men and women. Associations for VAT area were attenuated for men and women with additional adjustment for covariates (Model 2). Thigh SAT area in men was marginally associated with poor performance in Model 3 (OR: 1.22; 95% CI: 1.00; 1.50), whereas thigh IMAT area was associated in men and women [ORs: 1.54 (95% CI: 1.18; 2.02) and 1.25 (95% CI 1.01; 1.54), respectively].

IMAT density in men and VAT density in women were associated with poor performance in Model 1 (**Table 4**). With adjustment for additional covariates (Model 2), only IMAT density in men persisted and remained associated with poor performance even with adjustment for BMI (OR: 0.80; 95% CI: 0.67; 0.97; Model 3).

Table 3. Associations between BMI and adipose measures at baseline and risk of mobility limitation over 13 years follow-up in the Health ABC Study participants stratified by sex[1,2].

	n. at risk	n. of events	Event rate per 1000 person-years	Model 1	*P*-value	Model 2	*P*-value	Model 3	*P*-value
Men									
BMI	1491	1031	108	1.25 (1.17, 1.33)	< 0.001	1.18 (1.09, 1.27)	< 0.001		
VAT area	1434	989	106	1.24 (1.17, 1.32)	< 0.001	1.14 (1.06, 1.21)	< 0.001	1.10 (1.01, 1.19)	0.02
Abdominal SAT area	1405	970	106	1.18 (1.11, 1.26)	< 0.001	1.09 (1.01, 1.18)	0.03	1.01 (0.91, 1.12)	0.81
Thigh SAT area	1459	1009	107	1.11 (1.05, 1.19)	0.001	1.05 (0.98, 1.13)	0.14	0.97 (0.89, 1.06)	0.53
Thigh IMAT area	1459	1009	107	1.39 (1.31, 1.47)	< 0.001	1.36 (1.27, 1.46)	< 0.001	1.37 (1.27, 1.47)	< 0.001
VAT density	1431	988	106	0.92 (0.85, 0.98)	0.02	0.99 (0.92, 1.06)	0.76	1.04 (0.96, 1.13)	0.35
Abdominal SAT density	1395	962	106	1.00 (0.94, 1.07)	0.90	1.06 (0.98, 1.13)	0.13	1.10 (1.02, 1.18)	0.01
Thigh SAT density	1459	1009	107	1.02 (0.95, 1.08)	0.62	1.03 (0.96, 1.11)	0.36	1.06 (0.99, 1.13)	0.13
Thigh IMAT density	1459	1009	107	0.89 (0.84, 0.95)	0.001	0.93 (0.87, 1.00)	0.05	0.97 (0.90, 1.04)	0.35
Women									
BMI	1584	1199	136	1.46 (1.37, 1.55)	< 0.001	1.31 (1.21, 1.42)	< 0.001		
VAT area	1527	1152	135	1.27 (1.20, 1.35)	< 0.001	1.10 (1.03, 1.17)	0.004	1.02 (0.94, 1.09)	0.70
Abdominal SAT area	1479	1114	134	1.33 (1.25, 1.41)	< 0.001	1.22 (1.13, 1.31)	< 0.001	1.13 (1.02, 1.26)	0.02
Thigh SAT area	1552	1171	135	1.34 (1.26, 1.42)	< 0.001	1.25 (1.16, 1.34)	< 0.001	1.18 (1.08, 1.28)	< 0.001
Thigh IMAT area	1522	1171	135	1.27 (1.21, 1.34)	< 0.001	1.14 (1.07, 1.22)	< 0.001	1.08 (1.00, 1.16)	0.04
VAT density	1525	1151	135	0.84 (0.78, 0.89)	< 0.001	0.91 (0.84, 0.97)	0.007	0.98 (0.90, 1.05)	0.52
Abdominal SAT density	1471	1107	134	0.97 (0.91, 1.03)	0.26	0.95 (0.89, 1.02)	0.17	1.00 (0.93, 1.07)	0.95
Thigh SAT density	1522	1171	135	0.97 (0.92, 1.03)	0.36	0.96 (0.90, 1.02)	0.15	0.97 (0.91, 1.03)	0.26
Thigh IMAT density	1522	1171	135	0.89 (0.84, 0.95)	< 0.001	0.92 (0.87, 0.98)	0.01	0.96 (0.90, 1.02)	0.18

Abbreviations: Health ABC, Health, Aging, and Body Composition Study; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

[1] Multivariate Cox proportional hazards models were used to assess risk of incident mobility limitation for each SD increment in BMI and adipose measure. Model 1 was adjusted for age, race, and study site. Model 2 was adjusted as for Model 1 and for education, smoking status, prevalent disease, physical activity, midlife weight, and pain. Model 3 was adjusted as for Model 2 and for BMI.

[2] HR; 95% CI in parentheses (all such values).

Table 4. Associations between BMI and adipose measures at baseline and odds of poor performance after 9 years in the Health ABC Study participants stratified by sex[1,2].

	N	No. with gait speed < 1.0 m/s	Model 1	*P*-value	Model 2	*P*-value	Model 3	*P*-value
Men								
BMI	726	314	1.30 (1.11, 1.53)	0.001	1.31 (1.07, 1.61)	0.01		
VAT area	707	305	1.25 (1.06, 1.48)	0.008	1.20 (0.99, 1.44)	0.06	1.14 (0.92, 1.40)	0.23
Abdominal SAT area	699	299	1.30 (1.11, 1.52)	0.001	1.29 (1.06, 1.57)	0.01	1.29 (0.99, 1.67)	0.06
Thigh SAT area	714	308	1.25 (1.07, 1.47)	0.004	1.26 (1.06, 1.50)	0.008	1.22 (1.00, 1.50)	0.05
Thigh IMAT area	714	308	1.46 (1.20, 1.76)	< 0.001	1.54 (1.22, 1.93)	< 0.001	1.54 (1.18, 2.02)	0.001
VAT density	706	305	0.87 (0.72, 1.05)	0.15	0.93 (0.77, 1.14)	0.50	0.99 (0.80, 1.23)	0.91
Abdominal SAT density	697	299	0.95 (0.79, 1.14)	0.57	1.02 (0.84, 1.23)	0.87	1.06 (0.87, 1.30)	0.55
Thigh SAT density	714	308	0.87 (0.74, 1.03)	0.11	0.86 (0.72, 1.03)	0.10	0.88 (0.73, 1.05)	0.16
Thigh IMAT density	714	308	0.78 (0.66, 0.92)	0.003	0.78 (0.65, 0.93)	0.006	0.80 (0.67, 0.97)	0.02
Women								
BMI	843	467	1.71 (1.45, 2.02)	< 0.001	1.41 (1.13, 1.76)	0.002		
VAT area	816	451	1.47 (1.24, 1.73)	< 0.001	1.12 (0.93, 1.36)	0.24	0.96 (0.77, 1.20)	0.74
Abdominal SAT area	792	432	1.51 (1.28, 1.77)	< 0.001	1.31 (1.06, 1.61)	0.01	1.07 (0.81, 1.42)	0.62
Thigh SAT area	828	456	1.46 (1.23, 1.74)	< 0.001	1.30 (1.04, 1.62)	0.02	1.10 (0.84, 1.44)	0.49
Thigh IMAT area	828	456	1.66 (1.40, 1.97)	< 0.001	1.36 (1.12, 1.64)	0.002	1.25 (1.01, 1.54)	0.04
VAT density	815	451	0.80 (0.68, 0.95)	0.01	0.93 (0.77, 1.12)	0.44	1.03 (0.84, 1.26)	0.81
Abdominal SAT density	789	432	1.05 (0.89, 1.23)	0.57	1.04 (0.87, 1.24)	0.71	1.08 (0.90, 1.30)	0.41
Thigh SAT density	828	456	1.05 (0.90, 1.22)	0.55	1.00 (0.84, 1.18)	0.96	1.00 (0.84, 1.19)	0.97
Thigh IMAT density	828	456	0.88 (0.76, 1.03)	0.11	0.92 (0.78, 1.09)	0.35	0.96 (0.81, 1,14)	0.66

Abbreviations: Health ABC, Health, Aging, and Body Composition Study; IMAT, intermuscular adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

[1] Multivariate logistic regression models were used to assess odds of poor performance for each SD increment in BMI and adipose measure. Model 1 was adjusted for age, race, and study site. Model 2 was adjusted as for Model 1 and for education, smoking status, prevalent disease, physical activity, midlife weight, and pain. Model 3 was adjusted as for Model 2 and for BMI. [2]OR; 95% CI in parentheses (all such values).

DISCUSSION

To the best of our knowledge, these findings provide new insight into associations between obesity, mobility limitation, and poor performance by exploring multiple radiographic measures of adipose tissue including adipose density, which is a novel indicator of adipose tissue characteristics. Our results suggest that BMI as well as adipose depot area are robustly associated with risk of mobility limitation and poor performance. In addition, adipose area may convey risk beyond BMI for select depots. For every SD increment in VAT and thigh IMAT area, there was 10% and 37%, respectively, increased risk of mobility limitation in men. In women, for every SD increment in abdominal SAT, thigh SAT, and thigh IMAT area, there was 13%, 18%, and 8%, respectively, increased risk of mobility limitation. Adipose tissue density may also convey risk of incident mobility limitation and poor performance, although risk relations were less convincing than for adipose area and generally not independent of BMI.

Our findings align with those of previous studies on obesity and risk of disability or limitation in older adults (8–10, 19). Of the measures assessed in the current study, thigh IMAT area and BMI appeared to be particularly strong risk factors for mobility limitation and poor performance. The positive association between IMAT area and mobility limitation was previously reported in the Health ABC Study after 2.5 years of follow-up (20). Our data suggest that IMAT continues to be an important risk factor for future mobility limitation even into old age (participants 83–92 years old after follow-up).

Similarly, risk relations persisted for BMI despite BMI generally being a weaker correlate of adiposity in old age (21). Also of note, thigh SAT area was positively associated with risk of mobility limitation in women and poor performance in men and women. This result contrasts with the notion that a greater leg fat accumulation may have metabolic and cardioprotective properties (22–24) that reflect a low fatty acid release and more favorable inflammatory profile relative to abdominal and portal adipose tissue (25–28). The potential opposing risk relations between thigh SAT and health outcomes should be examined in future research.

Although obesity is a well-established risk factor for disability, less is known regarding potential mechanisms. Obesity is a risk factor for many chronic diseases (i.e., diabetes, heart disease, and osteoarthritis) that would be expected to contribute to disability; however, relations between adipose tissue and mobility limitation persisted with adjustment for prevalent disease in our study and other studies (29). Previous studies have suggested that a direct physical burden (strain on the skeletal system) (30), adipose

infiltration of organs such as in liver and muscle (20), inflammation (31), and general deconditioning (32) may contribute to relations between obesity and disability. In our analysis, adjustment for indicators of deconditioning (physical activity and knee pain) did not attenuate associations between BMI, adipose area, and limitation or function, which suggested that these factors incompletely explain risk.

To our knowledge, this is the first examination of adipose tissue density in relation to functional outcomes, although parallels can be drawn with studies of muscle density that also assessed from the HU of computed tomography images. Studies within the Health ABC (20, 33) and other cohorts (34) have suggested associations between muscle density, strength, and a decline in performance, possibly more so than muscle area. These associations are in contrast with our results, which suggested stronger relations with BMI and adipose area than adipose tissue density. It is unclear why adipose tissue and muscle densities appear to have divergent relations. However, the fact that BMI and adipose tissue area were more consistently related to mobility limitation and poor performance provides a potential avenue for intervention because the modification of BMI and adipose depots may be more clinically feasible. Indeed, clinical trials of weight loss in older adults have suggested that weight and fat loss can improve mobility (35, 36).

Potential mechanisms that underlie relationships between adipose tissue density and mobility limitation are unclear because there are few studies of computed tomography–measured adipose density. Our previous work suggested that the density of adipose tissue is not related to inflammation but is positively related to adiponectin (13). Higher adiponectin in older adults has been associated with greater physical disability (37), but this variable likely represents a marker of disability not a causal relation. Rather, it appears that associations between adipose tissue density and risk of mobility disability largely reflect risk attributable to heavy BMI. Adipose tissue density was inversely correlated with BMI, and risk estimates for mobility limitation and poor performance were generally attenuated with adjustment for BMI. Additional research into clinical and biological correlates of adipose tissue density may provide additional insight into relations.

A strength of the study was that the population was initially free from mobility limitation, which minimized potential reverse causation from preexisting mobility limitation. Additional strengths included the biracial population, frequent assessment of mobility limitation over an extended follow-up period, and availability of computed tomography images, which permitted the precise assessment of abdominal and thigh adipose depots and adipose tissue density. We also used two outcomes related to mobility limitation and

function. Risk associations with an objective measure of function showed markedly similar relations as self-reported mobility limitation. However, the study cohort was restricted to initially well-functioning individuals and excluded individuals older than 79 years. Associations may differ for less-healthy populations or different ages. We also used indicators of disability that were centered on lower extremity function, and it is possible our results may have varied with more global measures of disability.

In conclusion, more than three-quarters of participants developed mobility limitation and upwards of 40% of participants had poor performance despite being initially well functioning. These results show the importance of identifying factors that place an individual at increased risk of future limitation. Our results show that, despite controversy over BMI as an indicator of overall adiposity in old age (21), heavier BMI continues to be associated with risk of mobility limitation and performance into late life. IMAT is also robustly associated with risk, suggesting that an investigation into interventions that target IMAT (38) in addition to the promotion of healthy body weight may be warranted.

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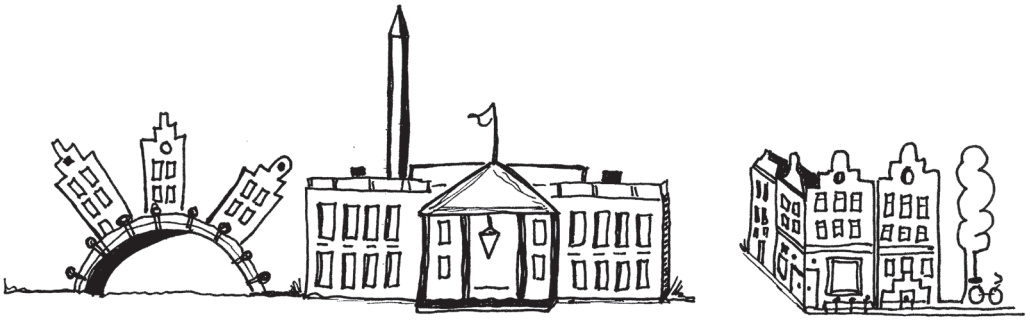

CHAPTER 4

Muscle quality and muscle fat infiltration in relation to incident mobility disability and gait speed decline; the Age, Gene/Environment Susceptibility-Reykjavik Study

Ilse Reinders, Rachel A. Murphy, Annemarie Koster, Ingeborg A. Brouwer, Marjolein Visser, Melissa E. Garcia, Lenore J. Launer, Kristin Siggeirsdottir, Gudny Eiriksdottir, Palmi V. Jonsson, Vilmundur Gudnason, and Tamara B. Harris.

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ABSTRACT

Background: Aging is associated with increased risk of reduced mobility. However, data on muscle components in relation to subjective and objective indicators of disability is limited.

Methods: Data were from 2,725 participants (43% men) aged 74.8 ± 4.7 years from the AGES-Reykjavik Study. At baseline, maximal isometric thigh strength (dynamometer chair), and midthigh muscle area and muscle fat infiltration were assessed with computed tomography. Usual six m gait speed and mobility disability were assessed at baseline and after 5.2 ± 0.3 years. Incident mobility disability was defined as having much difficulty or unable to walk 500 meter or climb-up 10 steps. A decrease of ≥ 0.1 m/s in gait speed was considered clinically relevant.

Results: Greater strength and area were protective for mobility disability risk and gait speed decline. After adjustment for other muscle components, greater strength was independently associated with lower mobility disability risk in women; OR; 0.78 (95% CI 0.62; 0.99), and lower decline in gait speed risk among both men; 0.64 (0.54; 0.76), and women; 0.72 (0.62; 0.82). Larger muscle area was independently associated with lower mobility disability risk in women; 0.67 (0.52; 0.87), and lower decline in gait speed risk in men; 0.74 (0.61; 0.91).

Conclusions: Greater muscle strength and area were independently associated with 15-30% decreased risk of mobility disability in women and gait speed decline in men. Among women, greater muscle strength was also associated with lower risk of gait speed decline. Interventions aimed at maintaining muscle strength and area in old age might delay functional decline.

INTRODUCTION

Aging is associated with loss of muscle strength and muscle mass, and greater muscle fat infiltration (1-5). A recent meta-analysis showed that low muscle strength was strongly associated with functional decline. However, low muscle mass was weakly related to functional decline, reflecting inconsistent results across individual studies (6) for reasons that are unclear. It is possible that more precise measures of muscle like computed tomography (CT) imaging that also measure the physical and biochemical composition of the muscle, i.e. attenuation in Hounsfield Units (HU) of muscle and intermuscular adipose tissue area can provide insight into relationships between muscle components and function.

Muscle fat infiltration has been associated with poorer performance (7) and lower muscle strength (5) in cross-sectional studies among older adults. Muscle fat infiltration was also associated with increased risk of incident mobility limitation, independent of muscle mass and strength (8). The latter is the only study to date that investigated associations between muscle fat infiltration and subsequent development of self-reported mobility disability. Investigating these associations in a different study population and with objective measures of physical function measures is important. Gait speed is one such measure. Slow gait speed likely represents disturbances in multiple organ systems and even small declines in gait speed are predictive of mortality (9) suggesting that gait speed may be a sensitive indicator of early functional decline.

Given the multi-dimensional nature of age-related changes in function, we used a comprehensive approach to examine associations between muscle strength, muscle area, muscle quality (strength/area), and two measures of muscle fat infiltration with incident mobility disability and gait speed decline over five years of follow-up in a large cohort of older adults. We hypothesized that participants with greater muscle strength, area and quality, and lower muscle fat infiltration would have lower odds of incident mobility disability and decline in gait speed.

METHODS

Study population

We used data from the Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) Study, a single-center, prospective, ongoing population study of survivors from the Reykjavik Study (10, 11). Details of the study design are previously published (12). Baseline data collection among 5764 men and women took place from 2002-2006. Follow-up took

place between 2007 and 2011 in 3316 participants (mean follow-up; 5.2 ± 0.3 years) reflecting losses due to death (n=1039), and study attrition (n=1409).
All participants provided written informed consent. The study was approved (VSN 00-063) by the National Bioethics Committee in Iceland and the Institutional Review Board of the National Institute on Aging, Intramural Research Program.

Measures

Computed tomography

CT imaging of the mid-thigh was performed with a 4 row detector system (Sensation; Siemens Medical Systems, Erlangen, Germany) (13). Thigh cross-sectional area (cm^2) was determined from a single 10 mm thick trans-axial section (14). Muscle cross-sectional area was segmented using the outline along the fascial plane between the muscle and subcutaneous fat. Muscle fat infiltration represents intermuscular adipose tissue (IMAT) and muscle attenuation. IMAT (cm^2) is the visible fat within the fascia surrounding skeletal muscles (4); lakes of adipose between and within muscle were determined as the number of pixels with HU between -200 and -50 multiplied by the area of a pixel. Muscle attenuation was calculated as the mean attenuation coefficient (HU) of the muscle area after subtraction of IMAT. The HU of distilled water is 0 and of air is -1000; lower HU indicates less dense muscle and greater fat infiltration (15) and is inversely associated with muscle strength (5). An operator used a manual contouring program to draw the contours of the hamstring, sartorius, and quadriceps muscles of the thigh as well as the contours of the total muscle bundle with the thigh. Within each region, a threshold was chosen to select voxels with a CT density greater than the maximal density of fat, as documented in Lang et al. (16). The lean muscle cross-sectional area of each region was calculated as the number of voxels above the threshold, and the lean tissue attenuation was the mean CT density of the thresholded voxels. Twenty-six randomly selected participants underwent a second CT scan after repositioning. The coefficient of variation was 3.5% for thigh muscle cross-sectional area. There was no significant difference between the repeated measurements (14). The average of the mean values for the left and right leg was used; if one leg was missing or incomplete, then the non-missing thigh was used. The ratio of muscle strength and muscle area was calculated as an indicator of muscle quality (17).

Maximal isometric knee extension strength

Maximal isometric muscle strength of the dominant side of the thigh was measured according to a standardized protocol in a sitting position in an adjustable dynamometer chair (Good Strength, Metitur, Palokka, Finland) (14, 18). Knee extension strength was measured with the knee angle at 60° from flexion toward full extension. The ankle was

fastened by a belt to a strain-gauge system and with the participant's hands gripping the edge of the seat. Before the measurement participants completed one trial to ensure they understood the standardized instructions. Three maximal efforts, separated by 30 seconds rest, were conducted. During the measurements, participants were encouraged verbally to produce at their maximal capacity, and the highest value was used (14). Previous studies have shown high reliability for the strength chair in older individuals (19, 20).

Self-reported mobility disability

Self-reported mobility disability was assessed at baseline and follow-up. Mobility disability was defined as having much difficulty or unable to walk 500 meter and/or climb 10 steps. Participants with prevalent mobility disability at baseline were excluded (see description of analytical cohort below).

Gait speed

Gait speed was used as an objective measure of physical performance. Gait speed (m/s) was assessed at both baseline and follow-up over a six meter long course according to a standardized protocol (21). A stopwatch was used to measure the time it took the participant to complete the six meter walk. Participants were instructed to walk at their usual walking pace. Change in gait speed over time was calculated. A decline of ≥ 0.1 m/s in gait speed was used as a clinically meaningful change (9) and is herein referred to as decline in gait speed.

Confounding variables

All confounders were assessed at baseline. BMI (kg/m^2) was calculated from measured height and weight, and waist circumference (cm) was measured using standardized protocols (12). Education (primary, secondary, college and university education), smoking status (never, former and current), and physical activity (frequency of moderate to vigorous activity) were assessed by questionnaire. Blood pressure was assessed from the mean value of two measurements with a large-cuff mercury sphygmomanometer. Medical conditions (hypertension, diabetes, congestive heart failure, coronary heart disease, myocardial infarction, stroke, disease of the lungs, disease of the kidneys, and cancer) were determined from self-report, medications and clinical assessments.

Analytical cohort

The number of participants included in the analyses differed by physical function measure. Regarding mobility disability, we only included participants with complete data at baseline and follow-up (n=3228). As the primary outcome was incident mobility disability,

participants who reported much difficulty (n=91) or were unable (n=69) to walk 500 meter, and/or had much difficulty (n=65) or were unable (n=9) to walk up 10 steps at baseline were excluded. Participants without muscle strength, CT imaging data or covariates (n=266) were excluded, resulting in 2,728 participants with complete data for muscle measures in relation to incident mobility disability. Regarding change in gait speed, 3,139 participants had baseline and follow-up gait speed. Exclusion of participants without muscle strength and/or CT imaging data or covariates (n=280) resulted in 2,859 participants for the gait speed analyses.

Statistical analysis

Baseline characteristics are presented according to incident mobility disability and decline in gait speed. Two-sided *t*-tests were performed for continuous variables and chi-square tests for categorical variables. Correlations between muscle parameters were examined using the Spearman correlation coefficient (r). Multiple logistic regression analyses were performed to determine associations between baseline muscle strength, muscle area, muscle quality, and muscle fat infiltration with incident mobility disability and decline in gait speed. Effect estimates were expressed as odds ratios (OR) and 95% confidence intervals (CI). Men and women differ with regard to body composition (22) and physical performance (23). Analyses were therefore stratified by sex and modeled per sex-specific SD of muscle parameters. Two models were fitted; Model 1 was adjusted for age, BMI and education. Model 2 was additionally adjusted for waist circumference, smoking status, hypertension, diabetes, coronary heart disease, stroke, lung disease, kidney disease, and physical activity. All models of gait speed decline were adjusted for baseline gait speed.

To investigate which muscle measures (muscle strength, muscle area, and muscle fat infiltration) were independently associated with mobility disability and gait speed decline, we mutually adjusted a model for each muscle measure with the exception of muscle attenuation and IMAT which are collinear. Collinearity assessment within models revealed mean variance inflation factors < 1.2. All *P*-values are two-tailed (α=0.05) and data was analyzed with STATA version 12.1 (StataCorp, College Station, Texas, USA).

RESULTS

Mean age was 74.7 ± 4.7 years, varying slightly depending on the outcome measures (i.e. 74.8 ± 4.7 years for mobility disability, 74.8 ± 4.8 years for decline in gait speed). Differences between participants' characteristics with or without mobility disability or decline in gait speed are shown in **Supplement Table 1**. Excluded participants, were older, had larger waist circumference, were more likely to be current smoker, were less educated, less physically active, and were more likely to have comorbidities than the

Table 1. Muscle measures in relation to incident mobility disability and decline in gait speed over five years of follow-up[*].

	Mobility Disability				Decline in Gait Speed			
	n. at risk	n. events	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))	n. at risk	n. events	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))
Men								
Muscle strength (N)	1190	74	0.61 (0.46;0.80)[§]	0.67 (0.50;0.90)[‡]	1208	504	0.55 (0.48;0.64)[§]	0.57 (0.49;0.66)[§]
Muscle area (cm^2)	1190	74	0.55 (0.39;0.77)[§]	0.66 (0.46;0.95)[†]	1208	504	0.57 (0.48;0.68)[§]	0.62 (0.52;0.75)[§]
Muscle quality (strength/area)	1190	74	0.79 (0.61;1.02)	0.81 (0.62;1.07)	1208	504	0.72 (0.63;0.82)[§]	0.71 (0.62;0.81)[§]
Muscle attenuation (HU)	1190	74	0.84 (0.66;1.09)	0.88 (0.68;1.15)	1208	504	0.83 (0.72;0.95)[‡]	0.86 (0.74;0.99)[†]
IMAT (cm^2)	1190	74	1.17 (0.90;1.52)	1.08 (0.81;1.42)	1208	504	1.10 (0.94;1.28)	1.05 (0.90;1.23)
Women								
Muscle strength (N)	1538	142	0.64 (0.52;0.78)[§]	0.67 (0.54; 0.82)[§]	1651	710	0.67 (0.59;0.76)[§]	0.68 (0.60;0.77)[§]
Muscle area (cm^2)	1538	142	0.56 (0.45;0.71)[§]	0.57 (0.45;0.73)[§]	1651	710	0.85 (0.74;0.97)[†]	0.86 (0.75;0.99)[†]
Muscle quality (strength/area)	1538	142	0.79 (0.65;0.96)[†]	0.81 (0.67;0.99)[†]	1651	710	0.72 (0.64;0.81)[§]	0.73 (0.64;0.82)[§]
Muscle attenuation (HU)	1538	142	0.81 (0.67;0.97)[†]	0.85 (0.71;1.03)	1651	710	0.90 (0.81;1.01)	0.92 (0.82;1.04)
IMAT (cm^2)	1538	142	1.08 (0.90;1.28)	1.03 (0.86;1.23)	1651	710	1.08 (0.96;1.21)	1.07 (0.95;1.20)

Abbreviations: HU; Hounsfield Units, IMAT; intermuscular adipose tissue, N; Newton.

[*]Logistic regression analyses were used to compute odds ratios (OR) and 95% confidence intervals (CI).

Model 1 was adjusted for age, BMI, and education. Model 2 was additionally adjusted for waist circumference, smoking status, hypertension, diabetes, coronary heart diseases, stroke, lung diseases, kidney diseases, and physical activity. Models of gait speed decline were additionally adjusted for baseline gait speed.

[†]$P < 0.05$

[‡]$P < 0.01$

[§]$P < 0.001$

analytical sample ($P < 0.05$). Excluded participants also had lower muscle strength, lower muscle area, lower muscle attenuation, and greater muscle fat infiltration compared to participants who were included ($P < 0.001$).
Muscle strength was positively correlated with muscle area, quality and muscle attenuation. Muscle area was negatively correlated with quality, but positively correlated with IMAT. Quality was positively correlated with muscle attenuation, and IMAT was negatively correlated with both quality and muscle attenuation (**Supplement Table 2**).

Muscle measures in relation to mobility disability

At follow-up, 52 (4.4%) men and 105 (6.8%) women reported having much difficulty or unable to walk 500 meter. In addition, 42 (3.5%) men and 66 (4.3%) women reported having much difficulty or unable to climb 10 steps at follow-up. Of those participants reporting having difficulty/unable to perform those two tasks, 20 men and 29 women reported both and 216 (7.9%) participants were classified as having mobility disability. Associations between SD increments in muscle measures with risk of mobility disability are presented in **Table 1**. Greater muscle strength and larger muscle area were both associated with a lower risk of mobility disability in both men and women in a model that was adjusted for age, BMI, and education (Model 1). With additional adjustment for covariates (Model 2), muscle strength and muscle area remained associated with lower risk of mobility disability in men OR 0.67 (0.50; 0.90) and 0.66 (0.46; 0.95), respectively, and in women OR 0.67 (0.54; 0.82), OR 0.57 (0.45; 0.73), respectively. Higher muscle quality (greater strength per unit muscle area) was associated with lower mobility disability risk in women OR 0.81 (0.67; 0.99), but not in men OR 0.81 (0.62; 1.07), although the results were still suggestive of a protective association (Model 2). Higher values of muscle attenuation (HU) among women were associated with lower risk of mobility disability in Model 1; OR 0.81 (0.67; 0.97), but further adjustments for covariates attenuated the associations and became non-significant; OR 0.85 (0.71; 1.03) (Model 2). Muscle attenuation (HU) was not associated with mobility disability in men. IMAT (cm^2) was not associated with risk of mobility disability in men or women.

Muscle measures in relation to decline in gait speed

Usual six meter gait speed at baseline was 1.04 ± 0.18 m/s for men, and 0.97 ± 0.19 m/s for women. At the follow-up measurement, both men and women had a slower gait speed compared to the baseline examination: 0.96 ± 0.20 m/s and 0.89 ± 0.20 m/s, respectively. Of those participants, decline in gait speed (≥ 0.1 m/s) occurred for 704 (41.7%) men and 710 (43.0%) women.

Table 2. Muscle measures in relation to incident mobility disability and decline in gait speed over five years of follow-up in mutually adjusted models[*].

	Mobility Disability		Decline in Gait Speed	
	Men	Women	Men	Women
n (events)	1093 (62)	1366 (129)	1155 (477)	1495 (635)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Muscle strength (N)				
Model 2	0.83 (0.60;1.16)	0.68 (0.55;0.84)[§]	0.59 (0.51;0.69)[§]	0.71 (0.63;0.81)[§]
Model 2 + muscle area	0.94 (0.66;1.34)	0.77 (0.61;0.97)[†]	0.64 (0.54;0.76)[§]	0.72 (0.62;0.82)[§]
Model 2 + muscle attenuation	0.86 (0.62;1.21)	0.69 (0.55;0.86)[§]	0.69 (0.51;0.70)[§]	0.71 (0.62;0.81)[§]
Model 2 + IMAT	0.84 (0.60;1.17)	0.68 (0.55;0.84)[§]	0.59 (0.50;0.69)[§]	0.71 (0.63;0.81)[§]
Model 2 + all	0.98 (0.68;1.40)	0.78 (0.62;0.99)[†]	0.64 (0.54;0.76)[§]	0.72 (0.62;0.82)[§]
Muscle area (cm^2)				
Model 2	0.66 (0.45;0.98)[†]	0.61 (0.48;0.78)[§]	0.62 (0.51;0.75)[§]	0.87 (0.75;1.00)
Model 2 + muscle strength	0.68 (0.44;1.04)	0.68 (0.52;0.88)[‡]	0.74 (0.61;0.91)[‡]	0.98 (0.84;1.15)
Model 2 + muscle attenuation	0.67 (0.45;0.99)[†]	0.61 (0.48;0.78)[§]	0.62 (0.52;0.76)[§]	0.87 (0.75;1.00)
Model 2 + IMAT	0.67 (0.45;0.99)[†]	0.61 (0.48;0.78)[§]	0.61 (0.50;0.74)[§]	0.87 (0.75;1.00)
Model 2 + all	0.68 (0.44;1.04)	0.67 (0.52;0.87)[‡]	0.74 (0.61;0.91)[‡]	0.98 (0.84;1.15)

Abbreviations: HU; Hounsfield Units, IMAT; intermuscular adipose tissue, N; Newton.

[*]Logistic regression analyses were used to compute odds ratios (OR) and 95% confidence intervals (CI).

Models were adjusted for age, BMI, education, waist circumference, smoking status, hypertension, diabetes, coronary heart disease, stroke, lung disease, kidney disease, physical activity + different muscle components or strength. Models of gait speed decline were additionally adjusted for baseline gait speed.

[†]$P < 0.05$

[‡]$P < 0.01$

[§]$P < 0.001$

Table 2. (continued)

	Mobility Disability		Decline in Gait Speed	
	Men	Women	Men	Women
n (events)	1093 (62)	1366 (129)	1155 (477)	1495 (635)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Muscle attenuation (HU)				
Model 2	0.83 (0.62;1.11)	0.88 (0.72;1.06)	0.89 (0.77;1.02)	0.96 (0.84;1.08)
Model 2 + muscle strength	0.86 (0.64;1.15)	0.94 (0.77;1.15)	0.98 (0.84;1.14)	1.01 (0.89;1.14)
Model 2 + muscle area	0.85 (0.64;1.14)	0.88 (0.72;1.07)	0.92 (0.79;1.06)	0.96 (0.85;1.08)
Model 2 + all	0.86 (0.64;1.15)	0.92 (0.75;1.12)	0.99 (0.85;1.15)	1.01 (0.89;1.14)
IMAT (cm^2)				
Model 2	1.14 (0.84;1.54)	1.00 (0.82;1.20)	1.02 (0.87;1.20)	1.08 (0.96;1.22)
Model 2 + muscle strength	1.13 (0.83;1.52)	0.99 (0.82;1.20)	0.96 (0.81;1.13)	1.08 (0.96;1.23)
Model 2 + muscle area	1.09 (0.81;1.47)	1.01 (0.83;1.22)	0.94 (0.80;1.11)	1.08 (0.96;1.22)
Model 2 + all	1.09 (0.80;1.47)	1.00 (0.82;1.22)	0.92 (0.78;1.09)	1.08 (0.96;1.23)

Abbreviations: HU; Hounsfield Units, IMAT; intermuscular adipose tissue, N; Newton.

[*]Logistic regression analyses were used to compute odds ratios (OR) and 95% confidence intervals (CI).

Models were adjusted for age, BMI, education, waist circumference, smoking status, hypertension, diabetes, coronary heart disease, stroke, lung disease, kidney disease, physical activity + different muscle components or strength. Models of gait speed decline were additionally adjusted for baseline gait speed.

[†]$P < 0.05$

[‡]$P < 0.01$

[§]$P < 0.001$

Table 1 also shows the association between muscle measures and decline in gait speed. Greater muscle strength, area and quality were all associated with lower risk of decline in gait speed among men and women (Model 2). The strongest associations were observed for muscle strength among both men and women; OR 0.57 (0.49; 0.66) and OR 0.68 (0.60; 0.77), respectively. With regards to muscle attenuation (HU) among men was inversely associated with risk of decline in gait speed; OR 0.86 (0.74; 0.99) (Model 2). IMAT was not significantly associated with risk of decline in gait speed.

Associations between all muscle measures and incident mobility disability or decline in gait speed using mutually adjusted models are shown in **Table 2**. Higher muscle strength and muscle area were independently associated with lower risk of mobility disability in women OR 0.78 (0.62; 0.99), and OR 0.67 (0.52; 0.87), respectively, but not in men. In men, adjustment for muscle strength attenuated the association between muscle area with mobility disability OR 0.68 (0.44; 1.04). In both men and women, adjustment for muscle attenuation did not appreciably change associations between muscle strength and muscle area with mobility disability nor was muscle fat infiltration associated with mobility disability. With regard to decline in gait speed, muscle strength remained associated with decreased risk, even after adjustment for other muscle measures in both men OR 0.64 (0.54; 0.76) and women OR 0.72 (0.62; 0.82). Muscle area was also inversely associated with decline in gait speed independent of other muscle measures, but only in men OR 0.74 (0.61; 0.91). In both genders, muscle attenuation and IMAT were not significantly associated with gait speed decline.

DISCUSSION

This study investigated associations between multiple muscle measures and subsequent development of incident mobility disability and gait speed decline. We confirm prior findings of Visser et al. (8); greater thigh muscle strength and muscle area were independently associated with decreased risk of mobility disability in men and women. We further showed that among men and women muscle strength was associated with gait speed decline independent of muscle area and muscle attenuation. Among men muscle area remained associated with lower odds of gait speed decline after adjustments for muscle strength and muscle attenuation. Interestingly, muscle fat infiltration was not associated with either mobility disability or decline in gait speed.

The associations for muscle strength and muscle area with both measures of physical function suggest that greater muscle strength and larger muscle area are independent predictors of less functional decline. Our results are supported by the Health, Aging, and

Body Composition (Health ABC) Study (8, 24) and other studies. Within the Cardiovascular Health Study and NHANES, low muscle mass was associated with greater risk of developing disability (25, 26). In addition, low muscle strength was strongly associated with inability to walk one kilometer and low walking speed among InCHIANTI participants (27). In contrast, low skeletal muscle mass was not cross-sectionally associated with self-reported physical disability among participants from the Framingham Heart Study (28). However, muscle was assessed with DXA which is less precise than CT and the study population was primarily healthy older people.

Although our study is observational and does not show causation, several clinical trials illustrate the effect of modifying muscle parameters and strength on function. Result from a randomized controlled trial in older men and women showed that a moderate-intensity physical activity program compared with education reduced the risk of major mobility disability over 2.6 years (29). A randomized controlled trial of physical activity and weight loss in overweight/obese older adults showed reduction in body fat and increase in lean mass, which in turn was associated with improved mobility (30). Others have shown that regular physical activity prevents age-associated loss of muscle strength and increase of muscle fat infiltration in older adults with moderate functional limitations (31). Combined, this evidence illustrates the importance of muscle mass and muscle strength in old age to maintain physical function, which is also recognized in sarcopenia definitions (32).

Despite prior indications of associations between muscle fat infiltration and incident mobility limitation (8), and decline in gait speed (24), our study found no evidence of similar associations. These inconsistent findings raise the question whether muscle fat infiltration influences physical function. The previous mentioned associations (8, 24) were observed among participants from the Health ABC Study. It is possible that the lack of associations in our study population might be a true finding or reflects important population differences. For example muscle fat infiltration is lower in AGES-Reykjavik than Health ABC, however, direct comparison is limited due to use of different CT scanners and methodology. The age range is wider (66–92 years) in AGES-Reykjavik than Health ABC (70–79 years), ethnic backgrounds differ (Health ABC Study included blacks and whites), as do diet and baseline physical function. In our analyses, 21.8% of the participants reported some difficulty in walking 500 meter, and/or walking up 10 steps at baseline, whereas Health ABC participants were selected to be initially well functioning. Restricting our study population to participants who were well functioning using the Health ABC definition (n=2108) still resulted in no significant associations between muscle fat infiltration and risk

of mobility disability or gait speed decline (data not shown). Further studies are needed to clarify the role of muscle fat infiltration in physical function.

In our study population, the incidence of mobility disability (7.9%) was low compared to the incidence of gait speed decline (42.6%). This difference might be related to elderly being more likely to give disproportionately positive health assessments compared to younger adults (33, 34). Older adults also tend to overestimate their physical function (35). Another explanation might be that decline in gait speed occurs before people perceive this decline. Previously gait speed has been shown to be a sensitive and informative measure of physical function (9, 36). Despite the difference in incidence, associations between muscle measures and measures of physical function were generally consistent.

Strengths and limitations

Strengths of our study include the large sample size and the detailed body composition measures from CT imaging, including two measures of muscle fat infiltration which has seldom been reported. Physical function was assessed using both self-reported and objectively measured mobility. Our analytic sample for incident mobility disability was restricted to participants who reported no or some mobility disability at baseline, which minimizes the possibility of reverse causation. Some limitations of the study need to be addressed. Like all observational studies, it precludes any conclusions on causality. Participants who did not have complete data at follow-up were older at baseline, had higher prevalence of comorbidities, and had lower gait speed at baseline, therefore, survival bias cannot be excluded. No muscle biopsies were available to directly measure the muscle fat infiltration, however, CT provides accurate images of the muscle and previous research has shown good correlations between CT and muscle biopsies (15, 37). The external validity of our findings may be limited to older white adults since our study only included individuals of European ancestry.

In summary, this study shows that greater thigh muscle strength and muscle area were associated with lower odds of mobility disability and decline in gait speed among an older population after five years of follow-up. In view of global ageing and the considerable prevalence of older persons with functional limitations, interventions aimed at maintaining both muscle strength and area in old age might be important to prevent functional decline. Of note, muscle strength can be assessed in the clinic and may be a particularly important clinical risk factor for functional decline in older adults.

SUPPLEMENTS

Supplemental Table 1. Baseline characteristics of the AGES-Reykjavik Study according to incident mobility disability and gait speed decline determined over five years of follow-up[*].

	No mobility Disability	Mobility Disability	*P*-value[†]	No Decline in Gait Speed	Decline in Gait Speed	*P*-value[†]
Participant no.	2512	216		1645	1214	
Men, n (%)	1116 (44)	74 (34)	0.004	704 (43)	504 (42)	0.493
Age (y)	74.5 ± 4.6	77.7 ± 5.0	< 0.001	74.3 ± 4.5	75.5 ± 5.0	< 0.001
Body mass index (kg/m^2)	27.0 ± 3.9	28.6 ± 4.8	< 0.001	27.2 ± 4.0	27.3 ± 4.3	0.381
Waist circumference (cm)	99.9 ± 11.0	104.8 ± 12.9	< 0.001	100.4 ± 11.3	101.0 ± 11.7	0.180
Education, n (%)						
< High School	1773 (71)	171 (79)		1195 (73)	880 (70)	
High School	414 (16)	28 (13)	0.022	272 (17)	193 (16)	0.059
Postsecondary	325 (13)	17 (8)		178 (11)	167 (14)	
Smoking, n (%)						
Never	1080 (43)	98 (45)		715 (44)	522 (43)	
Former	1166 (46)	88 (41)	0.161	756 (46)	560 (46)	0.953
Current	266 (11)	30 (14)		174 (11)	132 (11)	
Moderate to vigorous physical activity, n (%)						
Rarely to Never	1324 (53)	163 (75)		896 (55)	697 (57)	
Occasionally	201 (8)	9 (4)	< 0.001	128 (8)	93 (8)	0.269
Moderate to High	987 (39)	44 (20)		621 (38)	424 (35)	

Abbreviations: HU; Hounsfield Units, IMAT; intermuscular adipose tissue, N; Newton. Mobility disability is defined as having much difficulty or unable to walk 500 meter and/or climb 10 steps at follow-up. Gait speed decline is defined as a decrease in gait speed ≥ 0.1 m/s.

[*] Values are presented as mean ± SD for continuous variables, and number (%) for categorical variables.

[†] *P*-value for difference between groups (two-sided *t*-tests for means, chi^2 for percentage).

Supplemental Table 1. (continued)

	No mobility Disability	Mobility Disability	*P*-value†	No Decline in Gait Speed	Decline in Gait Speed	*P*-value†
Systolic blood pressure (mmHg)	141 ± 19	144 ± 21	0.069	141 ± 19	142 ± 20	0.656
Hypertension, n (%)	1932 (77)	186 (86)	0.002	1268 (77)	967 (80)	0.090
Diabetes, n (%)	180 (7)	25 (12)	0.018	119 (7)	99 (8)	0.359
Coronary heart diseases, n (%)	464 (18)	44 (20)	0.491	309 (19)	224 (18)	0.821
Stroke, n (%)	110 (4)	22 (10)	< 0.001	83 (5)	63 (5)	0.863
Myocardial infarction, n (%)	120 (5)	8 (4)	0.474	79 (5)	56 (5)	0.813
Lung diseases, n (%)	198 (8)	28 (13)	0.009	133 (8)	113 (9)	0.249
Kidney diseases, n (%)	96 (4)	17 (8)	0.004	64 (4)	61 (5)	0.143
Cancer, n (%)	342 (14)	31 (14)	0.762	224 (14)	170 (14)	0.767
Thigh muscle strength (N)	348 ± 115	289 ± 107	< 0.001	348 ± 120	328 ± 110	< 0.001
Thigh muscle area (cm^2)	112.0 ± 24.4	103.8 ± 24.0	< 0.001	111.6 ± 25.0	109.6 ± 23.6	0.034
Muscle quality (strength/area)	3.10 ± 0.7	2.77 ± 0.7	< 0.001	3.09 ± 0.7	2.98 ± 0.7	< 0.001
Thigh muscle attenuation (HU)	41.6 ± 4.7	39.0 ± 4.8	< 0.001	41.4 ± 4.9	40.9 ± 4.9	0.017
Thigh IMAT (cm^2)	17.7 ± 7.5	20.1 ± 8.9	< 0.001	17.9 ± 7.6	18.3 ± 8.0	0.137

Abbreviations: HU; Hounsfield Units, IMAT; intermuscular adipose tissue, N; Newton. Mobility disability is defined as having much difficulty or unable to walk 500 meter and/or climb 10 steps at follow-up. Gait speed decline is defined as a decrease in gait speed ≥ 0.1 m/s

* Values are presented as mean ± SD for continuous variables, and number (%) for categorical variables.

† *P*-value for difference between groups (two-sided *t*-tests for means, chi^2 for percentage).

Supplemental Table 2. Spearman correlations (*r*) of thigh muscle strength, muscle area, muscle quality, and muscle fat infiltration.

	Muscle strength (N)	Muscle area (cm^2)	Quality (strength/ area)	Muscle attenua-tion (HU)	IMAT (cm^2)
Men (n=1155)					
Muscle strength (N)	1.00				
Muscle area (cm^2)	0.5030*	1.00			
Quality (strength/area)	0.7647*	-0.1103*	1.00		
Muscle attenuation (HU)	0.2013*	0.0010	0.2392*	1.00	
IMAT (cm^2)	0.0428	0.2064*	-0.0891*	-0.3447*	1.00
Women (n=1495)					
Muscle strength (N)	1.00				
Muscle area (cm^2)	0.4004*	1.00			
Quality (strength/area)	0.8252*	-0.1376*	1.00		
Muscle attenuation (HU)	0.2407*	-0.00030	0.22624*	1.00	
IMAT (cm^2)	0.0404	0.2535*	-0.1041*	-0.2520*	1.00

Abbreviations: HU; Hounsfield Units, IMAT; intermuscular adipose tissue, N; Newton.

* $P < 0.01$

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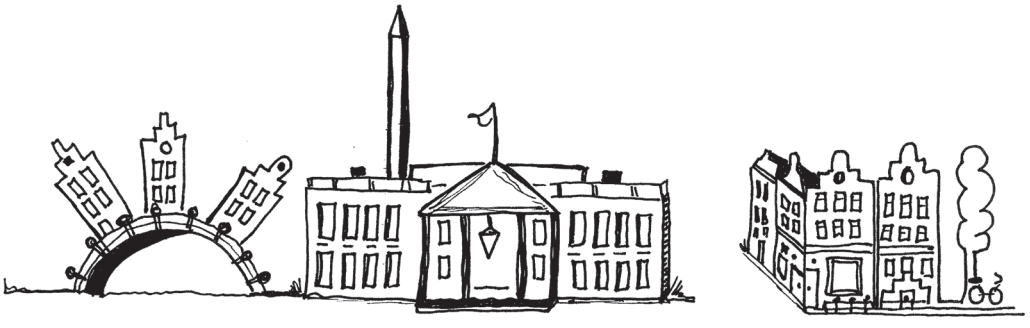

CHAPTER 5

Muscle quality and myosteatosis: novel associations with mortality risk; the Age, Gene/Environment Susceptibility-Reykjavik Study

Ilse Reinders, Rachel A. Murphy, Ingeborg A. Brouwer, Marjolein Visser, Lenore J. Launer, Kristin Siggeirsdottir, Gudny Eiriksdottir, Vilmundur Gudnason, Palmi V. Jonsson, Thomas F. Lang, and Tamara B. Harris, for the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik).

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ABSTRACT

Muscle composition may affect mortality risk, but prior studies have been limited to specific samples or less precise determination of muscle composition. We determined associations of thigh muscle composition, determined using computed tomography imaging, and knee extension strength with mortality risk among 4,824 participants aged 76.4 (standard deviation (SD), 5.5) years from the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study (2002–2006). Cox proportional hazards models were used to estimate hazard ratios. After 8.8 years of follow-up, there were 1,942 deaths. For men, each SD-increment increase in muscle lean area, muscle quality, and strength was associated with lower mortality risk, with decreases ranging between 11% and 22%. Each SD-increment increase in intermuscular adipose tissue and intramuscular adipose tissue was associated with higher mortality risk (hazard ratio (HR) = 1.13 (95% confidence interval (CI): 1.06, 1.22) and HR = 1.23 (95% CI: 1.15, 1.30), respectively). For women, each SD-increment increase in muscle lean area, muscle quality, and strength was associated with lower mortality risk, with decreases ranging between 12% and 19%. Greater intramuscular adipose tissue was associated with an 8% higher mortality risk (HR = 1.08, 95% CI: 1.01, 1.16). This study shows that muscle composition is associated with mortality risk. These results also show the importance of improving muscle strength and area and lowering muscle adipose tissue infiltration.

INTRODUCTION

Loss of muscle strength and muscle mass occurs with aging (1). Low muscle strength has consistently been associated with increased mortality risk (2-8), whereas muscle mass, based on bioelectrical impedance (BIA) or dual-energy X-ray absorptiometry (DXA), shows inconsistent associations with mortality risk (9-14). The use of computed tomography (CT) imaging can provide more precise estimates of muscle mass.

Besides the loss of muscle mass and strength, aging also results in a redistribution of adipose tissue, where subcutaneous adipose tissue relocates to more detrimental locations, as intramuscular and intermuscular adipose tissue or adipose tissue moves between and within muscles or organs (15). The result is that older people have greater levels of intramuscular or intermuscular adipose tissue compared with younger people with the same body mass index (BMI). The presence of intramuscular and intermuscular adipose tissue, defined as myosteatosis, is in turn inversely associated with loss of muscle strength (16) or mobility disability (17). The redistribution of adipose tissue can also be measured using CT imaging. However, few studies have used CT imaging to determine the association between muscle composition measures and mortality risk, particularly in a general older population. In the Health, Aging, and Body Composition Study, thigh muscle strength, but not muscle mass from CT, was associated with mortality risk after 4.9 years among 2292 older participants aged 70 to 79 years (18). In the InCHIANTI Study, cross-sectional muscle or fat areas of the calf assessed with peripheral quantitative CT were also not associated with mortality after 5.1 years among 934 participants aged ≥ 65 years (19).

Investigation of novel muscle composition measures, muscle quality, and myosteatosis in a large study population with a longer follow-up period is needed to clarify associations between muscle composition measures and mortality risk. Therefore, our aim in this study was to examine the relationship of thigh muscle composition, including muscle lean area, knee extension strength, muscle quality (defined as the ratio between knee extension strength and muscle lean area), and myosteatosis (defined as intermuscular adipose tissue and intramuscular adipose tissue), with all-cause mortality risk after 8.8 years of follow-up in a large study of older men and women.

METHODS

Study population

We used data from the AGES-Reykjavik Study, a prospective population study of survivors from the Reykjavik Study (20, 21). The baseline examination of 5764 men and women took place in Iceland between 2002 and 2006 (22). Participants with missing data on CT (n=457), muscle strength (n=396), and covariates (n=87) were excluded, resulting in 4824 participants with complete data in the analytical cohort. Male participants who were excluded were older, were more likely to be nonsmokers, had slower gait speed, had higher C-reactive protein (CRP) values, and were more likely to have diabetes than men included in the analytical cohort. Excluded women were older, had less education, were more likely to be nonsmokers, reported less moderate-to-vigorous physical activity in the previous 12 months, had slower gait speed, had higher CRP values and were more likely to have diabetes and coronary heart diseases (CHD) than women included in the analytical cohort ($P < 0.05$ for all). All participants provided written informed consent. The study was approved (VSN 00-063) by the National Bioethics Committee in Iceland, as well as the Institutional Review Board of the Intramural Research Program at the National Institute on Aging, US National Institutes of Health.

Measures

Computed tomography

CT imaging of the midthigh was part of the baseline examination (2002-2006) and was performed with a 4-row detector system (Sensation; Siemens Medical Systems, Erlangen, Germany) as previously described (23). Thigh muscle cross-sectional area (cm^2) represents muscle lean area, and was determined from a single 10-mm transaxial section, using a 120-kVp (24). Prior to the transaxial imaging, the right position for imaging at the midfemur was determined by measuring the maximum length of the femur on an anterior-posterior scout image and then finding the center of the long axis of the femur. Muscle lean area was segmented using the outline along the fascial plane between the muscle and subcutaneous fat.

Myosteatosis was defined as the presence of intermuscular adipose tissue and intramuscular adipose tissue. Intermuscular adipose tissue is the visible fat within the fascia surrounding skeletal muscles (25); lakes of adipose tissue between and within muscle were determined as the number of pixels with Hounsfield units (HU) between -200 and -50 multiplied by the area of a pixel. Intramuscular adipose tissue was calculated as the mean attenuation coefficient of the muscle lean area after subtraction of intermuscular adipose tissue, expressed in HU. Higher HU values indicate lower muscle adipose tissue infiltration and higher muscle strength (26). To facilitate interpretation on

study results of intermuscular adipose tissue and intramuscular adipose tissue in relation to mortality risk, we multiplied the values for intramuscular adipose tissue by -1.

An operator used a manual contouring program to draw the contours of the total muscle bundle. Within each region, a threshold was chosen to select voxels with a CT density greater than the maximal density of fat, as documented in the paper by Lang et al. (27). The muscle lean area of each region was calculated as the number of voxels above the threshold, and the lean tissue attenuation was the mean CT density of the thresholded voxels. The average of the values for the left and right legs was used; if data for one leg were missing or invalid, then the nonmissing thigh was used. This was the case for 34 participants (0.70%).

Visceral adipose tissue was determined with CT imaging of the abdomen at the L4/L5 vertebrae. Visceral adipose tissue was distinguished from subcutaneous adipose tissue by tracing along the facial plane defining the internal abdominal wall.

Analysis of the CT images was performed using specialized software developed at the University of California, San Francisco. Twenty-six randomly selected participants underwent a second CT scan after repositioning. The coefficient of variation was 3.5% for muscle lean area, 1.3% for intermuscular adipose tissue, and 5.9% for intramuscular adipose tissue. There was no significant difference between the repeated measurements (24).

Maximal isometric knee extension measurement and muscle quality

Maximal isometric knee extension strength was measured on the dominant side using an adjustable dynamometer chair. Knee extension strength was measured (in newtons) with the knee angle at 60° from flexion toward full extension (24, 28). The ankle was fastened by a belt to a strain-gauge system, and the participant was positioned with both hands gripping the side edge of the seat. Before the measurement, participants completed one trial to ensure that they understood the standardized verbal instructions. Three maximal efforts, separated by 30 seconds' rest, were conducted, and the highest value was used. Muscle quality was ascertained by taking the ratio of strength to muscle lean area.

Mortality

Mortality was ascertained from the Icelandic National Roster (http://www.statice.is/Statistics/Population/Births-and-death), an adjudicated registry of deaths. Person-years of follow-up were calculated from the date of the baseline examination to the date of death or September 30, 2014, for persons who were censored. Information on cause-specific mortality was available only through December 31, 2009.

Among participants with cause-specific mortality (n=143), the main causes of death were cardiovascular disease (n=68 (47.6%)) and cancer (n=40 (28.0%)). Eight deaths (5.6%) were attributed to diabetes. These numbers were too small to investigate cause specific mortality.

Baseline covariates

All confounders were assessed at baseline. BMI (kg/m^2) was calculated from measured height (cm) and weight (kg), and waist circumference (cm) was measured using standardized protocols (22). Information on education (less than high school, high school, and postsecondary), smoking status (never, former or current), and physical activity was self-reported. The physical activity variable was based on the following question on activity during the past year: "How often did you participate in moderate or vigorous physical activities?" Participants could choose from these answers: never, rarely, weekly but less than 1 hour per week, 1-3 hours per week, 4-7 hours per week, or more than 7 hours per week. Because of low numbers in some categories we combined "never" with "rarely", and "4-7 hours per week" with "more than 7 hours per week". Normal six meter gait speed (m/s) was used to assess mobility functioning (29, 30). Blood pressure was assessed from the mean value of two measurements with a large-cuff mercury sphygmomanometer. High-density lipoprotein (HDL) cholesterol, CRP, and glucose were analyzed from fasting blood samples using reagents from Roche Diagnostics (Mannheim, Germany) on a Hitachi 912 analyzer (Hitachi Ltd., Tokyo, Japan) according to the manufacturer's instructions. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. Current medication use was determined from medication containers brought to the clinic and the questionnaire. Medical conditions (diabetes, chronic obstructive pulmonary disease (COPD), CHD) were defined according to self-report, medications or clinical assessments.

Statistical analysis

Analyses were stratified by sex because of a significant interaction of muscle composition measures and strength with sex in relation to mortality risk. Baseline characteristics are presented as mean values and standard deviations (SDs) for continuous variables and numbers and percentages for categorical variables. Differences between men and women were assessed using *t*-tests and chi-square tests. Correlations between muscle composition measures and strength were examined using Spearman's correlation coefficient (r).

Cox proportional hazards analyses were performed to determine associations of muscle composition measures and strength (determined at baseline) with time to death.

The proportional hazards assumption was tested using Schoenfeld residuals. The assumption was not met for age and CRP among men, these variables were therefore modeled as time-varying covariates. Hazard Ratios and 95% confidence intervals were estimated for the association of muscle composition, per SD increment, with mortality risk. Model results were adjusted for demographic variables and potential confounders selected a priori on the basis of available literature. Model 1 adjusted for age, BMI, educational level, smoking status, and physical activity level. Additionally, we investigated whether the associations between muscle composition measures and mortality risk were influenced by factors that reflect potential pathways or factors that could mediate these associations. In Model 2, we adjusted for gait speed, diabetes, COPD, CHD and CRP.

To investigate linearity for continuous variables, we divided muscle composition measures and potential confounding variables into quartiles and used these as single determinants for mortality risk. We explored whether the regression coefficients were increasing or decreasing at the same rate for every quartile as compared with the previous quartile. This was the case for all of the variables except BMI among men and intermuscular adipose tissue among women; however, the direction remained the same. Therefore, we analyzed age, BMI, gait speed and CRP as continuous variables.

In sensitivity analyses, we adjusted for visceral adipose tissue as a more precise measure of adiposity instead of BMI. Complete data for those analyses were available for 4790 men and women. We also tested whether associations were driven by participants with extreme BMI values. Persons who are underweight are likely to have sarcopenia (age-related loss of skeletal muscle mass), which in turn is associated with an increased risk of mortality (2, 31). Extremely obese individuals also have increased risk of mortality (32-34). Therefore, we excluded participants with BMIs indicative of underweight (< 18.5 kg/m^2) (20 men and 44 women) or extreme obesity (> 35.0 kg/m^2) (61 men and 154 women), because they might have influenced the results. All *P*-values were two-tailed (α=0.05), and data were analyzed with STATA version 12.1 (StataCorp, College Station, Texas, USA).

RESULTS

Baseline characteristics of the 4824 participants (2065 men and 2759 women) are shown in **Table 1**. Compared with women, men were more likely to have comorbid conditions, more likely to be former smokers, to have higher education, to report more moderate-to-vigorous physical activity, and to have had a faster six meter gait speed. Men also had significantly greater values for muscle lean area, muscle quality and strength, while women had more myosteatosis ($P < 0.05$ for all). Correlations between muscle composition measures and strength are presented in Web **Table 1**.

During a mean follow-up period of 8.8 (SD, 2.8) years, 978 (47%) men died (56.5 per 1000 person-years), and 964 (35%) women died (38.6 per 1000 person-years). **Table 2** depicts mortality risk per SD increment increase in thigh muscle composition and strength. All of the muscle composition measures in men were associated with mortality in the model that adjusted for age, BMI, educational level, smoking status and physical activity level (Model 1). Associations were not substantially changed after additional adjustment for gait speed, diabetes, COPD, CHD and CRP (Model 2). Greater muscle lean area and strength showed an approximate 20% lower mortality risk for each SD increment increase, whereas greater muscle quality (strength/area) was associated with an 11% decreased risk. Greater intermuscular adipose tissue and intramuscular adipose tissue were associated with increased mortality risks (hazard ratio (HR) = 1.13 (95% confidence interval (CI): 1.06, 1.22) and HR = 1.23 (95% CI 1.15, 1.30), respectively) (Model 2). In women, each SD-increment increase in muscle lean area, muscle quality, and strength was associated with lower mortality risk, with the lowest hazard ratio being that for strength (HR = 0.81, 95% CI: 0.75, 0.88) (Model 2). Intramuscular adipose tissue, but not intermuscular adipose tissue, was associated with mortality among women (HR = 1.08, 95% CI: 1.01, 1.16).

To assess how the associations of muscle composition and strength with mortality risk were affected by other muscle composition measures, we additionally adjusted for other muscle composition measures separately in one model. Given that muscle quality is based on muscle lean area and knee extension strength, we did not put these three components in one model, that is we only adjusted for intermuscular adipose tissue or intramuscular adipose tissue within the association between muscle quality and mortality. Another example would be adjusting for strength within the association between intermuscular adipose tissue and mortality (**Table 3**). These models showed that muscle lean area and strength influenced each other, but the associations were modest and did not materially alter the results. The coefficient for muscle lean area was weakened by the addition of strength with a similar but less dramatic change being seen after adjustment for intermuscular adipose tissue or intramuscular adipose tissue, however, associations between muscle lean and mortality risk remained significant in men. Among men, risk associated with intermuscular adipose tissue was diminished by adjustment for muscle lean area, while strength had little influence.

Table 1. Baseline characteristics of 4824 participants from the AGES-Reykjavik Study[a].

	Men		Women		*P*-value
	n=2065		n=2759		
		%		%	
Demographics					
Age at baseline (years)	76.5 ± 5.4		76.3 ± 5.6		0.094
Education					
< Secondary	1431	69	2104	76	
College	257	12	488	18	< 0.001
University	377	18	167	6	
Diabetes	314	15	259	9	< 0.001
Chronic obstructive pulmonary disease	63	3	96	3	0.409
Coronary heart disease	633	31	353	13	< 0.001
Lifestyle factors					
BMI (kg/m^2)	26.8 ± 3.8		27.2 ± 4.7		0.015
Waist circumference (cm)	102.3 ± 10.5		99.3 ± 12.6		< 0.001
Smoking status					
Never	584	28	1451	53	
Former	1240	60	953	35	< 0.001
Current	241	12	355	13	
Moderate to vigorous physical activity					
Rarely to Never	1196	58	1799	65	
Occasionally	145	7	18	7	< 0.001
Moderate to High	724	35	771	28	
Gait speed (m/s)	0.98 ± 0.20		0.92 ± 0.20		<0.001
Metabolic variables					
Systolic blood pressure (mmHg)	143 ± 20		142 ± 20		0.059
Diastolic blood pressure (mmHg)	76 ± 9		72 ± 9		< 0.001
LDL cholesterol (mmol/l)	3.25 ± 0.98		3.68 ± 1.04		< 0.001
HDL-cholesterol (mmol/l)	1.41 ± 0.38		1.73 ± 0.45		< 0.001
C-reactive protein (mg/l)	3.65 ± 6.58		3.67 ± 6.16		0.929
Thigh muscle parameters					
Muscle lean area (cm^2)	127 ± 21		93 ± 15		< 0.001
Muscle quality (strength/area)	3.16 ± 0.70		2.79 ± 0.75		< 0.001
Knee extension strength (N)	401 ± 107		257 ± 76		< 0.001
Intermuscular adipose tissue (cm^2)	18 ± 7.9		19 ± 7.9		< 0.001
Intramuscular adipose tissue (HU)	42 ± 5.2		39 ± 5.1		< 0.001

[a]Continuous data are presented as mean (SD) and categorical traits as numbers and percentages.

Table 2. Associations between thigh muscle composition and strength (per SD increment) with all-cause mortality in participants from the AGES–Reykjavik Study[a,b].

	Men				Women			
	n=2065				n=2759			
	Model 1[a] HR	95% CI	Model 2[b] HR	95% CI	Model 1[a] HR	95% CI	Model 2[b] HR	95% CI
Muscle lean area (cm^2)	0.77	0.64, 0.76	0.78	0.71, 0.85	0.80	0.72, 0.85	0.85	0.78, 0.93
Thigh muscle quality (strength/area)	0.83	0.78, 0.89	0.89	0.83, 0.95	0.81	0.76, 0.87	0.88	0.82, 0.94
Knee extension strength (N)	0.72	0.67, 0.77	0.79	0.73, 0.86	0.73	0.68, 0.79	0.81	0.75, 0.88
Intermuscular adipose tissue (cm^2)	1.14	1.06, 1.22	1.13	1.06, 1.22	1.00	0.93, 1.07	0.99	0.92, 1.07
Intramuscular adipose tissue (HU)	1.28	1.21, 1.36	1.23	1.15, 1.30	1.16	1.08, 1.25	1.08	1.01, 1.16

Abbreviations: CI; confidence intervals, HU; Hounsfield units, N; Newton.

[a] Model 1 was adjusted for age, BMI, education level, smoking status and physical activity level.

[b] Model 2 was adjusted as for Model 1 plus gait speed, diabetes, chronic obstructive pulmonary disease, coronary heart disease and C-reactive protein.

Among men, age and C-reactive protein did not meet the Schoenfeld criteria in all models and were therefore modeled as time-varying covariates.

In sensitivity analyses, adjustment for visceral adipose tissue instead of BMI resulted in similar hazard ratios in comparison with the main analyses, where greater values of muscle lean area, muscle quality, and strength, and lower values of myosteatosis were associated with lower mortality risk. The coefficients most affected were those for intermuscular adipose tissue in both men and women, whereas the association remained significant among men. Among women, adjustment for visceral adipose tissue resulted in a lower mortality risk for muscle lean area (HR 0.80 (95% CI 0.74, 0.87)) (**Supplemental Table 2**) as models adjusted for BMI. In addition, the strength and direction of muscle composition on mortality risk were comparable with the original associations after exclusion of participants with extreme BMI values. The coefficient most affected was muscle lean area among women and the association became borderline significant (HR = 0.92, 95% CI: 0.84, 1.01) (**Supplemental Table 3**).

DISCUSSION

In this study of older adults, we examined thigh muscle composition, and knee extension strength in relation to mortality risk. Our results indicate that the composition and function of muscle may determine mortality risk in older persons. Greater intramuscular adipose tissue was associated with higher mortality risk in both men and women, while greater intermuscular adipose tissue was associated with increased mortality risk among men only. We further showed that among both men and women, muscle quality and strength remained associated with lower mortality risk independently of other muscle composition measures. Associations between muscle composition measures were independent of visceral adipose tissue. In addition, associations remained after exclusion of participants with BMIs less than 18.5 and greater than 35.0 kg/m^2. This suggests that muscle composition measures may be important mortality risk factors regardless of weight or adiposity, that may be helpful for refining mortality estimates in older adults.

Muscle quality is an important measure, since it represents muscle strength relative to muscle size, and is associated with survival. Previous studies have shown that the loss of muscle strength is greater than the loss of muscle mass in older adults, leading to a decrease in muscle quality with age (35-38). This indicates that the decline in muscle mass alone cannot explain the decrease in muscle quality, and that other changes in muscle composition might explain the deterioration of muscle quality (37). Myosteatosis might contribute to the decrease in muscle quality due to the proinflammatory properties of the adipose tissue infiltrated into the muscle (39). However, in our analyses adjustment for a general indicator of inflammation did not attenuate results.

Table 3. Risk of mortality per SD increment of thigh muscle composition and strength: additional adjustments for other muscle parameters in participants form the AGES–Reykjavik Study[a].

	Men		Women	
	n=2065		n=2759	
	Model 3[a]		Model 3[a]	
	HR	95% CI	HR	95% CI
Muscle lean area (cm^2)	0.78	0.71, 0.85	0.85	0.78, 0.93
+ Knee extension strength	0.84	0.76, 0.92	0.91	0.83, 1.00
+ Intermuscular adipose tissue	0.80	0.73, 0.88	0.86	0.78, 0.93
+ Intramuscular adipose tissue	0.82	0.75, 0.90	0.86	0.79, 0.94
+ Intermuscular adipose tissue + Knee extension strength	0.86	0.78, 0.94	0.91	0.83, 1.00
+ Intramuscular adipose tissue + Knee extension strength	0.87	0.79, 0.95	0.91	0.83, 1.00
Thigh muscle quality (strength/area)	0.89	0.83, 0.95	0.88	0.82, 0.94
+ Intermuscular adipose tissue	0.89	0.83, 0.95	0.88	0.82, 0.94
+ Intramuscular adipose tissue	0.91	0.86, 0.97	0.89	0.82, 0.95
Knee extension strength (N)	0.79	0.73, 0.86	0.81	0.75, 0.88
+ Muscle lean area	0.84	0.77, 0.91	0.83	0.77, 0.90
+ Intermuscular adipose tissue	0.80	0.74, 0.87	0.81	0.75, 0.88
+ Intramuscular adipose tissue	0.83	0.77, 0.90	0.82	0.76, 0.89
+ Muscle lean area + Intermuscular adipose tissue	0.84	0.77, 0.91	0.83	0.77, 0.90
+ Muscle lean area + Intramuscular adipose tissue	0.86	0.79, 0.94	0.84	0.77, 0.91
Intermuscular adipose tissue (cm^2)	1.13	1.06, 1.22	0.99	0.92, 1.07
+ Muscle lean area	1.07	0.99, 1.15	1.00	0.93, 1.07
+ Knee extension strength	1.10	1.02, 1.18	0.99	0.92, 1.07
+ Intramuscular adipose tissue	1.04	0.96, 1.12	0.97	0.89, 1.04
+ Muscle lean area + Knee extension strength	1.06	0.98, 1.14	1.00	0.93, 1.07
+ Muscle lean area + Knee extension strength + Intramuscular adipose tissue	0.99	0.92, 1.08	0.98	0.91, 1.06
Intramuscular adipose tissue (HU)	1.23	1.15, 1.30	1.08	1.01, 1.16
+ Muscle lean area	1.18	1.11, 1.26	1.08	1.00, 1.16
+ Knee extension strength	1.18	1.11, 1.26	1.05	0.98, 1.13
+ Intermuscular adipose tissue	1.21	1.13, 1.29	1.09	1.01, 1.18
+ Muscle lean area + Knee extension strength	1.16	1.09, 1.24	1.05	0.98, 1.13
+ Muscle lean area + Knee extension strength + Intermuscular adipose tissue	1.16	1.08, 1.25	1.06	0.98, 1.14

Abbreviations: CI; confidence intervals, HU; Hounsfield units, N; Newton.

[a] Model 3 was adjusted for age, BMI, education level, smoking status, physical activity level, gait speed, diabetes, chronic obstructive pulmonary disease, coronary heart disease and C-reactive protein + additional adjustment for other muscle composition measures.

Among men, age and C-reactive protein did not meet the Schoenfeld criteria in all models and were therefore modeled as time-varying covariates.

As Newman et al. (40) mentioned, the prevention of muscle quality decline is likely to be accomplished through preservation of muscle mass and that effect on the maintenance of strength, however, body fat and myosteatosis also play a role in muscle quality. Since this was (to our knowledge) the first study investigating the association between thigh muscle quality and mortality risk, future studies are needed to corroborate our findings and mechanistic studies are warranted to determine how better muscle quality and myosteatosis are associated with survival. In addition, determining sex-specific cutpoints for muscle quality might be a useful tool in gerontology to identify persons at greater mortality risk, especially since sarcopenia definitions include muscle and strength/gait speed but do not consider other muscle measures that could potentially improve mortality estimates. Furthermore, more studies are needed to easily measure muscle composition measures in a clinical setting.

Greater strength was associated with decreased mortality risk, which is comparable to numerous other studies that have investigated strength (both grip and leg strength) in relation to survival (2-8, 18). The inverse association between muscle strength and mortality risk likely reflects the association between strength and better physical functioning and thereby lower mortality risk. Our results for other muscle composition measures are supported by several prior studies. However, the majority of previously published studies used bioelectrical impedance analysis or dual-energy X-ray absorptiometry imaging to measure muscle composition. Results from the National Health and Nutrition Examination Survey study (n=3659) showed that participants with the lowest muscle mass index (muscle mass/height^2) had higher mortality risk (13), which was also observed in a study carried out among 1512 Taiwanese elderly (14). In contrast, in a study of 4107 British men, fat mass index or fat-free mass index was not associated with mortality (9). Similar results were observed in a study with 3793 French women, where greater total lean mass was not associated with better survival (11). CT imaging provides more precise description of muscle composition and allows determination of muscle adipose tissue infiltration. Thereby it provides further understanding of relationships between muscle composition and mortality. Our results parallel those found in unhealthy populations (peripheral arterial disease), where lower calf skeletal muscle density (41), or leg strength (42) has been associated with higher mortality risk. However, Cesari et al. (19) observed no independent associations between calf muscle composition and mortality risk among 934 Italian community-dwelling older adults. Important study differences may have contributed to discrepancies between results. Compared with the study by Cesari et al., AGES-Reykjavik may have had greater power to detect risk, since there was a longer follow-up period (8.8 years vs. 5.1 years) and a greater number of deaths (1942/4824

(40.3%) vs. 263/934 (28.2%)). However, it is not possible to compare mean values for the muscle components, since the site of the CT imaging was not consistent between studies (thigh vs. calf). Additional studies are needed to clarify relationships between muscle composition measures and mortality.

Strengths and limitations

A major strength of this study was the availability of CT data, which enabled determination of muscle lean area, as well as myosteatosis. While there are many studies of body weight, body fat, and mortality risk, less focus has been put on the relationships of muscle size and muscle adipose tissue infiltration with mortality risk. In addition, the large sample size was a further benefit and allowed us to perform sex-specific analyses. A further strength was the well-characterized study population which allowed for adjustment of many potential confounders and provided evidence that muscle composition measures are associated with mortality independently of other lifestyle and demographic factors. Some limitations should be noted, however. First, a single time point for measurement of muscle composition and strength was used in this paper, thus, we were not able to investigate the role of gains or losses in these parameters. Second, information on cause-specific mortality was not available for all participants, and further studies are needed to determine if associations between muscle composition measures and mortality are driven by mortality from specific causes. Third, the study population consisted of survivors from the Reykjavik Study. A limitation is the nonparticipation of frail individuals or persons with more disease in the AGES-Reykjavik Study, which may have caused a bias towards healthier persons at baseline in this study. In addition, participants who were excluded from the analytical cohort due to missing measurements were older and more likely to have comorbidity at baseline, resulting in a healthier analytical sample, and this might have caused a potential survival bias, which may have led to underestimation of the associations observed. Finally, the AGES-Reykjavik Study populations consists of persons of European ancestry, which limits external validity for other ethnic groups.

In conclusion, in this study, the composition and strength of the thigh muscle were associated with mortality risk among 4824 older men and women. These results may be useful for refining mortality estimates, and they show the importance of improving muscle strength and area, and lowering muscle adipose tissue infiltration.

SUPPLEMENTS

Supplemental Table 1. Spearman correlations (*r*) of thigh muscle composition and strength.

	Thigh muscle area (cm^2)	Thigh muscle quality (strength/area)	Knee extension strength (N)	Intermuscular adipose tissue (cm^2)	Intramuscular adipose tissue (HU)
Men (n=2065)					
Thigh muscle area (cm^2)	1.00				
Thigh muscle quality (strength/area)	-0.0337	1.00			
Knee extension strength (N)	0.5421**	0.7890**	1.00		
Intermuscular adipose tissue (cm^2)	0.1688**	-0.1272**	-0.0063	1.00	
Intramuscular adipose tissue (HU)	-0.1144**	-0.2903**	-0.3125**	0.3771**	1.00
Women (n=2759)					
Thigh muscle area (cm^2)	1.00				
Thigh muscle quality (strength/area)	-0.0744**	1.00			
Knee extension strength (N)	0.4511**	0.8286**	1.00		
Intermuscular adipose tissue (cm^2)	0.2633**	-0.1050**	0.0450*	1.00	
Intramuscular adipose tissue (HU)	-0.0482*	-0.2836**	-0.2818**	0.2909**	1.00

Abbreviations: HU; Hounsfield Units, N; Newton.

* $P < 0.05$

** $P < 0.01$

Supplemental Table 2. Risk of mortality per SD increment of thigh muscle composition and strength: adjustments for visceral adipose area[a].

	Men		Women	
	n=2053		n=2737	
	Model 4[a]		Model 4[a]	
	HR	95% CI	HR	95% CI
Muscle area (cm^2)	0.81	0.75, 0.87	0.80	0.74, 0.87
Thigh muscle quality (strength/area)	0.89	0.84, 0.95	0.90	0.84, 0.97
Knee extension strength (N)	0.79	0.74, 0.86	0.80	0.74, 0.87
Intermuscular adipose tissue (cm^2)	1.08	1.02, 1.16	0.93	0.87, 1.00
Intramuscular adipose tissue (HU)	1.21	1.14, 1.29	1.05	0.98, 1.13

Abbreviations: CI; confidence intervals, HU; Hounsfield units, N; Newton.

[a] Model 4 was adjusted for age, visceral adipose tissue, education level, smoking status, physical activity level, gait speed, diabetes, chronic obstructive pulmonary disease, coronary heart disease and C-reactive protein.

Among men, age and C-reactive protein did not meet the Schoenfeld criteria in all models and were therefore modeled as time-varying covariates.

Supplemental Table 3. Associations between thigh muscle composition and strength (per SD increment) with all-cause mortality, excluding participants with BMI < 18.5 & > 35.0 $kg/m^{2\,a}$.

	Men		Women	
	n=1984		n=2561	
	Model 5[a]		Model 5[a]	
	HR	95% CI	HR	95% CI
Muscle area (cm^2)	0.79	0.72, 0.86	0.91	0.83, 1.00
Thigh muscle quality (strength/area)	0.88	0.82, 0.94	0.86	0.80, 0.93
Knee extension strength (N)	0.79	0.73, 0.85	0.82	0.76, 0.89
Intermuscular adipose tissue (cm^2)	1.13	1.06, 1.21	1.00	0.93, 1.08
Intramuscular adipose tissue (HU)	1.22	1.15, 1.30	1.11	1.03, 1.20

Abbreviations: CI; confidence intervals, HU; Hounsfield units, N; Newton.

[a] Model 5 was adjusted for age, BMI, education level, smoking status, physical activity level, gait speed, diabetes, chronic obstructive pulmonary disease, coronary heart disease, and C-reactive protein.

Among men, age and C-reactive protein did not meet the Schoenfeld criteria in all models and were therefore modeled as time-varying covariates.

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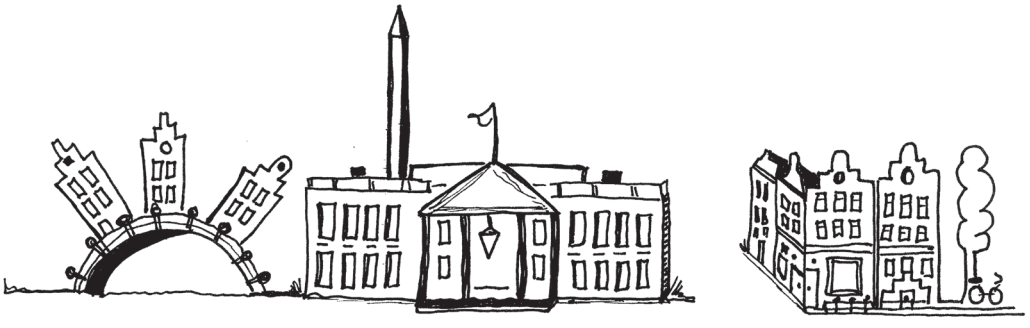

CHAPTER 6

Plasma phospholipid polyunsaturated fatty acids are associated with greater muscle and knee extension strength but not with changes in muscle parameters in older adults

Ilse Reinders, Xiaoling Song, Marjolein Visser, Gudny Eiriksdottir, Vilmundur Gudnason, Sigurdur Sigurdsson, Thor Aspelund, Kristin Siggeirsdottir, Ingeborg A. Brouwer, Tamara B. Harris, and Rachel A. Murphy.

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ABSTRACT

Background: Muscle mass, intermuscular adipose tissue and strength are important indicators of physical function. Dietary fatty acids have been associated with muscle parameters such as larger size and higher strength but large, population-based longitudinal data in older adults who are at risk of functional decline are lacking.

Objective: The objective was to investigate associations between plasma phospholipid polyunsaturated fatty acids (PUFAs) and measures of muscle size, intermuscular adipose tissue and muscle strength cross-sectionally and after five years of follow-up.

Methods: Data are from the Age, Gene/Environment Susceptibility-Reykjavik Study, a prospective cohort aged 66-96 years at baseline. The analytical sample included 836 participants with cross-sectional measures of muscle parameters, and 459 participants with data on change in muscle parameters. PUFAs were assessed at study baseline using gas chromatography. Thigh muscle parameters were assessed at baseline and after median of 5.2 years. Muscle area and intermuscular adipose tissue were assessed with computed tomography. Maximal grip strength and knee extension strength were assessed with dynometers. Relative changes in muscle parameters (%) were calculated. Multivariate linear regression was performed to calculate unstandardized regression coefficients and *P*-values for trends across tertiles of fatty acids reported.

Results: Higher concentrations of total PUFAs were cross-sectionally associated with larger muscle size (*P*-for trend=0.002) and with greater knee extension strength (*P*-for trend=0.038). Higher concentrations of arachidonic acid were associated with smaller muscle size (*P*-for trend=0.015). Greater linoleic acid was associated with less intermuscular adipose tissue (*P*-for trend=0.004), while the EPA was positively associated (*P*-for trend=0.047). Longitudinal analyses showed positive associations for alpha-linolenic acid with increased knee extension strength (*P*-for trend=0.014). No other associations were observed.

Conclusions: These data illustrate the complex relationship between PUFAs and muscle parameters; inconsistent cross-sectional relationships with muscle size, intermuscular adipose tissue and strength, and little evidence of a role in change in muscle parameters.

INTRODUCTION

The loss of muscle mass and/or muscle strength that occur with aging (1-3) are indicative of declining health, functional impairment (4), disability (4, 5), and mortality (6, 7). Aging is also accompanied by adipose infiltration of muscle tissue (greater intermuscular adipose tissue, IMAT), which is associated with low muscle strength (8) and insulin resistance (9). There have been numerous efforts to prevent or reverse loss of muscle mass and muscle strength (10, 11), but effective treatments are lacking (12), underscoring the importance of identifying modifiable factors related to muscle in old age.

Several studies suggest a potential role of fatty acids on muscle, particularly (n-3) polyunsaturated fatty acids (PUFAs) which predominately consist of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA). A study of cancer-related muscle loss, which shares features with age-related muscle loss (13), reported that individuals with low plasma (n-3) PUFAs had lower muscle mass and greater muscle mass loss than individuals with higher (n-3) PUFAs (14). A small study of older men and women reported a positive correlation between (n-3) PUFAs consumption and leg strength and inverse correlation with chair rise time, although not independent of protein consumption (15). In a study of nearly 3,000 community-dwelling older men and women, a positive association between fatty fish consumption and grip strength was reported (16). Clinical trials provide further evidence of a relationship with (n-3) PUFAs; supplemental (n-3) PUFAs enhance the rate of muscle protein synthesis in older adults (17), and improvements in muscle strength and functional capacity were observed when strength training was combined with fish oil supplementation in older women (18).

However prior studies are limited by their study size, cross-sectional study design, estimates of PUFAs consumption from food frequency questionnaire or non-representative sample, for example only women (18) or non-obese and free from chronic disease (17). Computed tomography (CT) derived measures of muscle size, which provide a more precise estimate of skeletal muscle than other imaging modalities (e.g. dual energy X-ray absorptiometry) (19), and also captures IMAT are also limited (20).

The objective of this study was to define relationships between two domains of PUFAs: plasma phospholipid PUFAs and lifetime fish oil consumption with thigh muscle size and IMAT from CT as well as muscle strength from knee extension and grip strength in a sub study of older men and women from the Age, Gene/Environment Susceptibility Study (AGES-Reykjavik). We further aimed to determine if PUFAs are associated with changes in these muscle parameters after five years of follow-up.

METHODS

Study population

The AGES-Reykjavik Study is a random sample of 5764 survivors from the Reykjavik Study, a single center population-based cardiovascular cohort begun in 1967 to study heart disease. At study baseline (2002-2006), participants were aged 66 to 96 years. A follow up examination was carried out between 2007 and 2011 for 3316 participants. Details of the study design are provided in Harris et al. (21). All participants provided written informed consent and the study was approved by the institutional review board (VSN: 00 063).

The analytical sample was drawn from two sub-studies in AGES-Reykjavik with plasma fatty acid data; ICELAND MI, a study of cardiac magnetic resonance imaging (22, 23) and a case cohort study of fracture (unpublished data). Participants in ICELAND MI and the fracture cohort were randomly selected AGES-Reykjavik participants who met eligibility for magnetic resonance imaging (i.e. no implanted devices or severe kidney disease) (n=1012). Participants who were fracture cases were excluded because of non-random sampling. Individuals missing data on fish oil consumption, thigh muscle size or IMAT, grip strength, knee extension strength, or covariates were excluded resulting in an analytical sample of 836 with baseline measures (referred to as the cross-sectional sample) and 459 with follow-up measures (longitudinal sample). Compared to the overall AGES-Reykjavik population, our sample had fewer women (cross-sectional only, P=0.009), were younger (longitudinal only, P <0.001), more educated (longitudinal only, P=0.010), less likely to be current smokers (cross-sectional only, P=0.011). Participants in our samples were also more likely to report more moderate-to-vigorous physical activity, less likely to have diabetes, and had greater muscle size and strength and less IMAT than the overall AGES-Reykjavik population (P < 0.05 for all).

Fatty acids

Plasma samples were collected following an overnight fast and stored at -80°C until analysis. Fatty acids were measured in the phospholipid fraction which provides a measure of short term dietary intake and fatty acids available to peripheral tissues. Analyses were carried out at the Biomarker Lab, Fred Hutchinson Cancer Research Center (Seattle, WA). Plasma lipids were extracted using the Folch method (24). Phospholipids were isolated from other lipids using thin layer chromatography (25). Fatty acid methyl esters were prepared by direct transesterification (26) and separated by gas chromatography (Agilent 7890 Gas Chromatograph FID detector; Supelco fused silica 100 m capillary column SP-2560; initial 160°C for 16 minutes, ramp 3.0°c/min to 240°C, hold for 15 minutes). Identification, precision, and accuracy were continuously evaluated using both model mixtures of known fatty acid methyl esters and established in-house control

pools. Fatty acids were expressed as a relative weight proportion (%). We focused on total polyunsaturated fatty acids (PUFAs), total (n-3) and total (n-6) PUFAs, and individual PUFAs previously associated with muscle size and/or strength: linoleic acid (LA, 18:2n6), arachidonic acid (AA, 20:4n6), alpha-linolenic acid (ALA, 18:3n3), EPA (20:5n3), and DHA (22:6n3). The coefficient of variation from pooled quality control samples for LA, AA, EPA and DHA were 0.43%, 0.62%, 2.05%, and 1.44%, respectively.

Fish oil

Fish oil consumption was assessed as a measure of long-term (n-3) PUFAs consumption by food frequency questionnaire. The questionnaire was administered at study baseline (late life) and participants recalled intake during early life (aged 14 to 19 years) and midlife (aged 40 to 50 years). The questionnaire has been validated for midlife and late life consumption (27, 28). The most commonly consumed fish in Iceland are cod and haddock (29) both of which contain low levels of n-3 PUFAs. Therefore, we focused on fish liver oil consumption (referred to as fish oil hereafter) which is rich in n-3 PUFAs and has been common in the Icelandic diet for many decades. Consumption was classified as never, <daily or daily.

Muscle parameters

Computed tomography measurements were performed in the mid-thigh using a 4 detector system (Sensation, Siemens Medical Systems, Erlangen, Germany) as described previously (30). Thigh cross-sectional muscle area (cm^2) was determined from a single 10 mm thick trans axial section (31). The fascial plane was used as an outline to segment muscle from subcutaneous fat. Adipose tissue between and within muscle (IMAT, cm^2) was determined as pixels with radiographic density between -150 and -30 Hounsfield Unit and multiplied by pixel area. The maximal isometric muscle strength, handgrip and knee extension, were measured on the dominant side using an adjustable dynamometer chair. Knee extension (N) was measured with the knee angle at 60° (31), and handgrip strength (N) was measured using a hand held dynamometer with the elbow flexed at 90° (32). Computed tomography, knee extension and grip strength were repeated at the follow up exam (median 5.16 years, interquartile range 5.06-5.21 years) using the same protocols as at baseline. Relative changes (%) in muscle size, IMAT, knee and grip strength were calculated.

Covariates

Covariates associated with muscle size and/or strength were assessed at baseline. Values in the text are means ± SDs. Age, education, smoking status, and physical activity were

assessed by questionnaire. BMI was determined from measured height and weight using standard protocols (21). Type 2 diabetes, chronic obstructive pulmonary disease and coronary heart disease were determined from self-report, medication use, and clinical assessment. Microalbuminuria was defined as urinary albumin/creatinine ratio ≥ 300 mg/g. Plasma C-reactive protein (CRP) was analyzed using reagents from Roche Diagnostics (Mannheim, Germany) on a Hitachi 912 analyzer according to manufacturer instructions.

Statistical analysis

Interaction terms for fatty acids by sex in relation to muscle parameters were non-significant and thus, data are presented for men and women together. The distribution of fatty acids varied, for example DHA met normal assumptions if log transformed while PUFAs would require cubic transformation and EPA 1/square root. To facilitate interpretation of results, fatty acids were examined in tertiles rather than continuous measures. To achieve an equal distribution of men and women across tertiles of PUFAs, and adequately adjust for potential confounding due to sex, we computed sex-specific tertiles. Multivariate linear regression was used to test associations of fatty acids and fish oil with cross-sectional measures of muscle size, IMAT, and muscle strength. For the longitudinal analyses we performed similar regression analyses with relative changes in muscle size, IMAT, and muscle strength as outcome measures. Tertile 1 and never consuming fish oil were the referent groups. Results are reported as regression coefficients with 95% confidence intervals (CI). Model 1 was adjusted for age, sex and physical activity. Model 2 was adjusted for Model 1 plus education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, CRP and time between assessments for the longitudinal sample. A *P*-value for trend was calculated to examine linear trends across the tertiles using the categorical variable in adjusted regression models. Analyses were performed with STATA version 12.1 (StataCorp, College Station, Texas, USA). A two-sided *P*-value < 0.05 considered statistically significant.

RESULTS

Baseline characteristics of participants in the cross-sectional and longitudinal samples are shown in **Table 1**. The age of the cross-sectional sample was 76.7 ± 5.60 years of which the majority were women. On average participants were overweight (BMI 27.1 ± 4.11 kg/m^2) with 1.35 ± 2.33 hours per week of moderate to vigorous physical activity. The distribution of plasma phospholipid fatty acids at AGES-Reykjavik Study baseline for the cross-sectional and longitudinal sample are shown in **Table 2**.

Table 1. Baseline characteristics of older men and women within the AGES-Reykjavik study[1].

	Cross-sectional cohort n=836	Longitudinal cohort n=459
Women, n (%)	448 (53.6)	249 (54.3)
Age at baseline (years)	76.7 ± 5.60	74.9 ± 4.98
Education, n (%)		
Primary school	191 (22.9)	89 (19.4)
Secondary	426 (51.0)	241 (52.5)
College	128 (15.3)	73 (15.9)
University	91 (10.9)	56 (12.2)
Smoking status, n (%)		
Never	334 (40.0)	195 (42.5)
Former	401 (48.0)	216 (47.1)
Current	101 (12.1)	48 (10.5)
BMI (kg/m^2)	27.1 ± 4.11	27.2 ± 3.93
Moderate to vigorous physical activity (hours per week)	1.35 ± 2.33	1.73 ± 2.59
Diabetes, n (%)	87 (10.4)	40 (8.7)
Chronic obstructive pulmonary disease, n (%)	23 (2.8)	11 (2.4)
Coronary heart disease, n (%)	177 (21.2)	89 (19.4)
Microalbuminuria, n (%)	64 (7.7)	27 (5.9)
Plasma C-reactive protein (mg/L)	4.05 ± 8.04	3.64 ± 8.11
Thigh muscle area (cm^2)	112 ± 25.6	116 ± 25.8
Thigh intermuscular fat area (cm^2)	17.3 ± 7.78	17.1 ± 7.99
Knee extension strength (N)	329 ± 117	353 ± 117
Grip strength (N)	294 ± 110	313 ± 114

[1] Continuous variables are presented as mean ± SD, and categorical variables are presented as number of observations and corresponding percent.

Table 2. Distribution of plasma phospholipid fatty acids at AGES-Reykjavik Study baseline[1,2].

	Tertile 1	Tertile 2	Tertile 3
Cross-sectional cohort	n=278	n=278	n=280
Total PUFAs	36.5 (30.0 – 38.0)	38.4 (37.6 – 39.2)	39.8 (38.8 – 42.8)
n-6 PUFAs	23.3 (14.9 – 26.4)	28.0 (26.3 – 29.6)	31.0 (29.2 – 37.4)
n-3 PUFAs	7.69 (4.36 – 9.16)	10.3 (8.70 – 12.3)	14.4 (11.3 – 22.6)
LA	14.4 (7.38 – 16.5)	17.6 (16.4 – 18.9)	20.6 (18.8 – 27.3)
AA	5.27 (3.81 – 6.05)	6.70 (5.96 – 7.46)	8.71 (7.33 – 16.5)
ALA	0.16 (0.09 – 0.19)	0.21 (0.18 – 0.24)	0.31 (0.23 – 0.66)
EPA	1.49 (0.69 – 2.05)	2.53 (1.87 – 3.49)	4.88 (3.13 – 10.9)
DHA	4.80 (2.50 – 5.73)	6.31 (5.49 – 7.21)	8.17 (6.82 – 12.1)
Longitudinal cohort	n=153	n=153	n=153
Total PUFAs	36.9 (32.0 – 38.2)	38.6 (37.9 – 39.3)	40.0 (39.0 – 42.8)
n-6 PUFAs	24.0 (14.9 – 27.2)	28.4 (26.6 – 29.8)	31.2 (29.3 – 37.4)
n-3 PUFAs	7.72 (4.36 – 9.16)	10.1 (8.77 – 12.0)	14.1 (10.8 – 22.6)
LA	14.8 (7.38 – 16.8)	17.9 (16.4 – 19.1)	20.8 (19.0 – 27.3)
AA	5.35 (3.82 – 6.18)	6.80 (6.08 – 7.47)	8.82 (7.38 – 16.5)
ALA	0.15 (0.09 – 0.19)	0.21 (0.19 – 0.24)	0.30 (0.24 – 0.63)
EPA	1.51 (0.69 – 2.06)	2.53 (1.87 – 3.41)	4.79 (2.94 – 10.9)
DHA	4.79 (2.50 – 5.69)	6.23 (5.48 – 7.10)	7.99 (6.63 – 12.1)

[1] Mean values are expressed as a relative weight proportion (%) and the range. Tertiles are sex-specific and therefore values may overlap.

[2] Abbreviations: AA; arachidonic acid (20:4n-6), ALA; alpha-linolenic acid (18:3n-3), DHA; docosahexaenoic acid (22:6n-3), EPA; eicosapentaenoic acid (20:5n-3), LA; linoleic acid (18:2n-6), PUFAs; polyunsaturated fatty acids.

PUFAs with cross-sectional muscle parameters

Table 3 shows the cross-sectional associations between PUFAs with muscle parameters. Total PUFAs were positively associated with thigh muscle size with adjustment for age, sex and physical activity (Model 1). Associations remained significant in Model 2 after additional adjustment for education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, and CRP; tertile 2: 2.86 cm^2 (95% CI 0.49; 5.22) and tertile 3: 3.83 cm^2 (95% CI 1.41; 6.25). Higher concentrations of AA were associated with lower thigh muscle size in fully adjusted models in tertile 2: -2.77 cm^2 (95% CI -5.14; -0.40) and tertile 3: -3.02 cm^2 (95% CI -5.45; -0.58). Tertiles 2 and 3 of LA were inversely associated with IMAT in both models; tertile2: -1.51 cm^2 (95% CI -2.67; -0.35) and tertile 3: -1.74 cm^2 (95% CI -2.91; -0.56) (Model 2). Tertile 3 of EPA was positively associated with IMAT after full adjustment for covariates; tertile 3: 1.20 cm^2 (95% CI 0.01; 2.38).

Greater knee extension strength was observed for participants in tertile 3 of total PUFAs: 15.2 N (95% CI 0.89; 29.6) (Model 2). Total n-3 PUFAs and DHA were positively associated with grip strength in Model 1, however associations attenuated in Model 2. EPA was positively associated with knee extension and grip strength in Model 1 but was no longer significant with adjustment for additional covariates (Model 2).

PUFAs with relative changes in muscle parameters

Loss of muscle size and strength and increased fatty infiltration of muscle was evident over follow-up. Change (%) in muscle size, IMAT, knee extension strength and grip strength were: -4.87 ± 7.87, 8.28 ± 25.5, -17.8 ± 21.2 and -3.42 ± 25.3, respectively. PUFAs in relation to changes in muscle size, IMAT, knee extension and grip strength are shown in **Table 4**. There were no associations with any of the fatty acids with change in muscle size or grip strength. Relative to tertile 1, tertile 3 of AA was associated with greater losses in knee extension strength relative to tertile 1: -5.31% (95% CI -10.1, -0.54) (Model 1). This association became non-significant in Model 2. Higher concentrations of ALA were associated with greater IMAT relative to tertile 1 in tertile 2: 8.07% (95% CI 2.47, 13.7) and in tertile 3: 6.01% (95% CI 0.40, 11.6) (Model 1). However, further adjustments attenuated the associations. Tertile 3 of ALA was associated with less loss in knee extension strength relative to tertile 1: 5.97% (95% CI 1.17, 10.8) (Model 2). No other associations between fatty acids and change in muscle parameters or strength were observed.

Table 3. Plasma phospholipid fatty acids in relation to baseline thigh muscle parameters and muscle strength among 836 participants[1-4].

	Muscle size, cm^2		Intermuscular adipose tissue, cm^2		Knee extension strength, N		Grip strength, N	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Total PUFAs								
T2	2.93 (0.05,5.81)	2.86 (0.49,5.22)	-0.83 (-2.12,0.47)	-0.92 (-2.10,0.25)	11.1 (-3.13,25.3)	11.9 (-2.10,25.9)	-1.93 (-14.1,10.2)	-1.58 (-13.8,10.6)
T3	4.31 (1.42,7.20)	3.83 (1.41,6.25)	-1.11 (-2.41,0.19)	-1.16 (-2.36,0.05)	16.5 (2.26,30.8)	15.2 (0.89,29.6)	11.0 (-1.21,23.2)	9.72 (-2.75,22.2)
P-trend	0.04	0.002	0.10	0.06	0.024	0.038	0.08	0.13
n-6 PUFAs								
T2	-0.46 (-3.34,2.41)	-2.29 (-4.65,0.08)	0.80 (-0.49,2.09)	0.16 (-1.02,1.33)	2.99 (-11.2,17.2)	0.39 (-13.6,14.4)	8.68 (-3.41,20.8)	8.82 (-3.33,21.0)
T3	2.53 (-0.39,5.45)	0.86 (-1.57,3.28)	-0.29 (-1.60,1.03)	-1.00 (-2.21,0.21)	3.32 (-11.1,17.8)	3.61 (-10.8,18.0)	-6.40 (-18.7,5.90)	-5.86 (-18.3,6.63)
P-trend	0.09	0.49	0.67	0.10	0.65	0.62	0.31	0.36
n-3 PUFAs								
T2	0.01 (-2.86,2.88)	-0.70 (-3.07,1.67)	0.67 (-0.61,1.96)	0.57 (-0.61,1.74)	6.59 (-7.55,20.7)	2.71 (-11.3,16.7)	18.1 (6.06,30.1)	16.6 (4.51,28.7)
T3	-1.38 (-4.27,1.50)	0.03 (-2.38,2.45)	-0.09 (-1.38,1.20)	0.66 (-0.53,1.86)	7.11 (-7.12,21.3)	5.18 (-9.06,19.4)	12.3 (0.20,24.4)	10.1 (-2.21,22.5)
P-trend	0.35	0.98	0.89	0.28	0.33	0.48	0.047	0.11
LA								
T2	-1.20 (-4.07,1.67)	-0.70 (-3.05,1.65)	-1.75 (-3.03,-0.47)	-1.51 (-2.67,-0.35)	2.71 (-11.4,16.9)	2.49 (-11.4,16.4)	8.07 (-4.00,20.1)	6.80 (-5.27,18.9)
T3	0.65 (-2.23,3.54)	1.41 (-0.97,3.79)	-2.07 (-3.36,-0.79)	-1.74 (-2.91,-0.56)	1.67 (-12.6,15.9)	0.90 (-13.2,15.0)	-2.73 (-14.9,9.40)	-3.88 (-16.1,8.33)
P-trend	0.66	0.25	0.002	0.004	0.82	0.90	0.66	0.54

[1] Multivariable linear regression analyses were performed to compute regression coefficient (95% confidence intervals).

[2] Tertile 1 is reference group (Coefficient=0.00), n=278, n=278 for tertile 2 and n=280 for tertile 3.

[3] Model 1 was adjusted for age, sex and physical activity; Model 2 was adjusted for Model 1 plus education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, and C-reactive protein.

[4] Abbreviations: AA; arachidonic acid (20:4n-6), ALA; alpha-linolenic acid (18:3n-3), DHA; docosahexaenoic acid (22:6n-3), EPA; eicosapentaenoic acid (20:5n-3), LA; linoleic acid (18:2n-6), PUFAs; polyunsaturated fatty acids.

Table 3. (continued)

	Muscle size, cm^2		Intermuscular adipose tissue, cm^2		Knee extension strength, N		Grip strength, N	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
AA								
T2	-0.77 (-3.65,2.12)	-2.77 (-5.14,-0.40)	-0.39 (-1.69,0.91)	-1.12 (-2.30,0.06)	-5.78 (-20.0,8.47)	-7.69 (-21.7,6.36)	-5.04 (-17.2,7.12)	-4.30 (-16.5,7.92)
T3	0.64 (-2.25,3.52)	-3.02 (-5.45,-0.58)	0.81(-0.48,2.10)	-0.63 (-1.84,0.58)	-8.57 (-22.8,5.66)	-9.22 (-23.6,5.21)	-7.32 (-19.5,4.83)	-5.38 (-17.9,7.17)
P-trend	0.66	0.015	0.21	0.31	0.24	0.21	0.24	0.40
ALA								
T2	0.03 (-2.84,2.89)	0.54 (-1.81,2.90)	-1.02 (-2.30,0.26)	-0.88 (-2.05,0.28)	0.99 (-13.1,15.1)	2.13 (-11.8,16.0)	1.42 (-10.6,13.5)	1.34 (-10.8,13.4)
T3	-0.16 (-3.02,2.71)	0.87 (-1.49,3.24)	-0.93 (-2.21,0.35)	-0.51 (-1.68,0.66)	-5.50 (-19.6,8.63)	-4.36 (-18.3,9.59)	5.70 (-6.36,17.8)	5.28 (-6.85,17.4)
P-trend	0.92	0.47	0.16	0.39	0.45	0.54	0.35	0.39
EPA								
T2	2.01 (-0.86,4.87)	0.70 (-1.66,3.07)	0.58 (-0.71,1.86)	0.25 (-0.93,1.42)	12.0 (-2.14,26.1)	8.12 (-5.83,22.1)	18.3 (6.25,30.3)	17.1 (5.01,29.2)
T3	0.24 (-2.63,3.11)	0.81 (-1.58,3.20)	0.76 (-0.53,2.04)	1.20 (0.01,2.38)	15.5 (1.32,29.6)	13.3 (-0.81,27.4)	14.0 (1.98,26.0)	11.8 (-0.38,24.1)
P-trend	0.87	0.50	0.25	0.047	0.032	0.06	0.023	0.06
DHA								
T2	1.40 (-1.46,4.27)	0.58 (-1.78,2.95)	-0.01 (-1.30,1.28)	-0.17 (-1.34,1.00)	12.5 (-1.63,26.6)	9.79 (-4.17,23.7)	13.4 (1.39,25.5)	12.1 (-0.04,24.2)
T3	-0.62 (-3.52,2.27)	0.30 (-2.12,2.72)	-0.19 (-1.49,1.11)	0.42 (-0.78,1.62)	7.32 (-6.93,21.6)	4.91 (-9.37,19.2)	12.8 (0.69,25.0)	10.5 (-1.91,22.9)
P-trend	0.67	0.81	0.78	0.49	0.31	0.50	0.038	0.10

[1] Multivariable linear regression analyses were performed to compute regression coefficient (95% confidence intervals).

[2] Tertile 1 is reference group (Coefficient=0.00), n=278, n=278 for tertile 2 and n=280 for tertile 3.

[3] Model 1 was adjusted for age, sex and physical activity; Model 2 was adjusted for Model 1 plus education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, and C-reactive protein.

[4] Abbreviations: AA; arachidonic acid (20:4n-6), ALA; alpha-linolenic acid (18:3n-3), DHA; docosahexaenoic acid (22:6n-3), EPA; eicosapentaenoic acid (20:5n-3), LA; linoleic acid (18:2n-6), PUFAs; polyunsaturated fatty acids.

Table 4. Plasma phospholipid fatty acids in relation to relative change in muscle parameters and muscle strength among 459 participants over 5 years of follow-up[1-4].

	Muscle size, %		Intermuscular adipose tissue, %		Knee extension strength, %		Grip strength, %	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Total PUFAS								
T2	0.51 (-1.27,2.29)	0.88 (-0.88,2.65)	-1.98 (-7.63,3.68)	-2.18 (-7.87,3.52)	2.21 (-2.58,6.99)	2.57 (-2.27,7.41)	1.34 (-4.37,7.05)	1.58 (-4.21,7.38)
T3	0.04 (-1.77,1.84)	0.35 (-1.46,2.17)	-2.78 (-8.51,2.95)	-1.61 (-7.45,4.23)	2.54 (-2.31,7.38)	2.14 (-2.82,7.10)	0.74 (-5.04,6.52)	0.60 (-5.35,6.54)
P-trend	0.97	0.71	0.34	0.59	0.31	0.40	0.80	0.85
n-6 PUFAS								
T2	-0.81 (-2.58,0.97)	-0.29 (-2.05,1.48)	1.49 (-4.17,7.14)	0.73 (-4.95,6.41)	-1.34 (-6.12,3.45)	-0.33 (-5.16,4.51)	-0.16 (-5.86,5.53)	0.42 (-5.35,6.19)
T3	-0.97 (-2.79,0.84)	-0.50 (-2.33,1.34)	-0.05 (-5.83,5.73)	-1.09 (-6.98,4.79)	-1.22 (-6.11,3.67)	-0.21 (-5.22,4.80)	3.64 (-2.18,9.45)	3.88 (-2.10,9.86)
P-trend	0.29	0.60	0.99	0.72	0.62	0.93	0.22	0.20
n-3 PUFAS								
T2	0.39 (-1.38,2.16)	0.39 (-1.36,2.15)	-2.52 (-8.14,3.11)	-2.43 (-8.07,3.22)	-2.69 (-7.45,2.06)	-2.47 (-7.28,2.33)	-3.12 (-8.79,2.54)	-2.76 (-8.50,2.98)
T3	0.10 (-1.69,1.89)	-0.33 (-2.14,1.48)	-3.18 (-8.86,2.50)	-1.84 (-7.66,3.97)	-0.21 (-4.59,5.01)	-1.10 (-6.04,3.85)	-3.62 (-9.34,2.10)	-3.93 (-9.84,1.99)
P-trend	0.91	0.73	0.27	0.53	0.94	0.65	0.21	0.19
LA								
T2	0.11 (-1.66,1.89)	0.46 (-1.29,2.20)	-3.54 (-9.17,2.10)	-3.60 (-9.20,2.01)	-1.67 (-6.43,3.09)	-1.37 (-6.14,3.39)	-1.48 (-7.14,4.17)	-1.10 (-6.78,4.58)
T3	-0.30 (-2.08, 1.48)	-0.33 (-2.10,1.44)	-1.92 (-7.58,3.73)	-2.63 (-8.33,3.08)	2.54 (-2.23,7.32)	2.19 (-2.66,7.04)	5.03 (-0.65,10.7)	5.19 (-0.59,11.0)
P-trend	0.74	0.72	0.51	0.36	0.29	0.39	0.08	0.08

[1] Multivariable linear regression analyses were performed to compute regression coefficient (95% confidence intervals).

[2] Tertile 1 is reference group (Coefficient=0.00), n=153, n=153 for tertile 2 and n=153 for tertile 3.

[3] Model 1 was adjusted for age, sex, physical activity and time between assessments; Model 2 was adjusted for Model 1 plus education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, and C-reactive protein.

[4] Abbreviations: AA; arachidonic acid (20:4n-6), ALA; alpha-linolenic acid (18:3n-3), DHA; docosahexaenoic acid (22:6n-3), EPA; eicosapentaenoic acid (20:5n-3), LA; linoleic acid (18:2n-6), PUFAs; polyunsaturated fatty acids.

Table 4. (continued)

	Muscle size, %		Intermuscular adipose tissue, %		Knee extension strength, %		Grip strength, %	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
AA								
T2	0.47 (-1.30,2.25)	0.53 (-1.23,2.29)	1.26 (-4.41,6.92)	1.25 (-4.42,6.91)	-0.14 (-4.90,4.62)	0.52 (-4.28,5.32)	-4.61 (-10.3,1.09)	-4.38 (-10.1,1.37)
T3	-0.76 (-2.55,1.02)	-0.37 (-2.19,1.45)	-0.59 (-6.27,5.09)	-0.50 (-6.37,5.36)	-5.31 (-10.1,-0.54)	-4.02 (-8.99,0.95)	-1.29 (-7.00,4.42)	-0.97 (-6.93,4.98)
P-trend	0.40	0.70	0.84	0.87	0.029	0.12	0.66	0.74
ALA								
T2	-1.00 (-2.77,0.79)	-1.15 (-2.91,0.61)	8.07 (2.47,13.7)	7.32 (1.70,12.9)	-0.82 (-5.55,3.90)	-1.11 (-5.88,3.66)	-2.11 (-7.80,3.57)	-2.27 (-8.03,3.48)
T3	-0.37 (-2.15,1.40)	-0.49 (-2.26,1.28)	6.01 (0.40,11.6)	5.50 (-0.15,11.2)	6.51 (1.77, 11.2)	5.97 (1.17,10.8)	2.29 (-3.41,7.981)	1.88 (-3.91,7.68)
P-trend	0.68	0.60	0.037	0.06	0.007	0.014	0.43	0.51
EPA								
T2	-1.31 (-3.08,0.46)	-0.90 (-2.66,0.86)	5.06 (-0.56,10.7)	4.68 (-0.97,10.3)	-0.18 (-4.95,4.59)	0.68 (-4.14,5.50)	-3.81 (-9.45,1.86)	-3.27 (-9.03,2.49)
T3	-0.24 (-2.01,1.53)	-0.66 (-2.44,1.11)	1.18 (-4.45,6.81)	1.89 (-3.81,7.59)	0.01 (-4.77,4.79)	-0.86 (-5.72,4.00)	-2.96 (-8.64,2.73)	-3.08 (-8.89,2.72)
P-trend	0.79	0.46	0.68	0.50	0.99	0.74	0.31	0.29
DHA								
T2	0.21 (-1.56,1.98)	0.16 (-1.60,1.93)	-5.23 (-10.8,0.39)	-4.51 (-10.2,1.15)	-1.98 (-6.74,2.78)	-2.59 (-7.41,2.24)	-2.69 (-8.44,2.97)	-2.75 (-8.51,3.02)
T3	-0.02 (-1.81,1.77)	-0.23 (-2.05,1.60)	-3.05 (-8.71,2.62)	-2.10 (-7.95,3.76)	-0.61 (-5.41,4.20)	-2.09 (-7.08,2.90)	-4.55 (-10.3,1.16)	-4.89 (-10.8,1.07)
P-trend	0.98	0.81	0.29	0.47	0.80	0.41	0.12	0.11

[1] Multivariable linear regression analyses were performed to compute regression coefficient (95% confidence intervals).

[2] Tertile 1 is reference group (Coefficient=0.00), n=153, n=153 for tertile 2 and n=153 for tertile 3.

[3] Model 1 was adjusted for age, sex, physical activity and time between assessments; Model 2 was adjusted for Model 1 plus education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, and C-reactive protein.

[4] Abbreviations: AA; arachidonic acid (20:4n-6), ALA; alpha-linolenic acid (18:3n-3), DHA; docosahexaenoic acid (22:6n-3), EPA; eicosapentaenoic acid (20:5n-3), LA; linoleic acid (18:2n-6), PUFAs; polyunsaturated fatty acids.

Fish oil consumption, cross-sectional and relative changes in muscle parameters

Fish oil consumption across the lifetime in relation to cross-sectional muscle parameters and changes in muscle parameters is shown in **Supplemental Table 1** and **Supplemental Table 2**. Daily consumption of fish oil was common in our sample (old age 64.0%, midlife 45.2%, early life 33.5%), which is reflective of the Icelandic population (33). There were no associations between fish oil consumption in old age and any cross-sectional or prospective muscle parameters, confirming the largely null associations with plasma phospholipid (n-3) PUFAs. Further, there were no associations between fish oil consumption in midlife with any muscle parameters after full adjustment for covariates. There was a trend towards daily fish oil consumption in early life and lower baseline grip strength (P=0.019), but no association with change in grip strength (P=0.91).

DISCUSSION

This is one of the first population-based studies examining the association between circulating levels of fatty acids and comprehensive cross-sectional and longitudinal muscle parameters that are important indicators of physical function in older adults. Our results illustrate a complex relationship between fatty acids and muscle parameters. Total PUFAs were positively associated with cross-sectional muscle size and knee extension strength, but there were inconsistent associations with individual fatty acids, including the (n-3) PUFAs which are widely hypothesized to play a role in muscle mass and/or strength (17, 20). Of note, none of the cross-sectional associations were confirmed in our analysis of changes in muscle parameters.

Based on previous findings, we hypothesized that higher PUFAs levels would be associated with favorable muscle measures and higher/less diminution of muscle strength (15-18). For example, based on clinical trial evidence, it would have been reasonable to expect positive associations between PUFAs levels and muscle size, since PUFAs supplementation was associated with an enhanced rate of muscle protein synthesis (17) or higher muscle strength (18). In addition, results of the Hertfordshire Study, a large retrospective cohort study of nearly 3,000 participants aged 59 to 73 years, showed an increase in grip strength for each additional portion of fatty fish consumed per week (16). We did not find associations between PUFAs and grip strength. Differences between observed associations might be explained by different study design and/or age group.

The lack of associations between fatty acids and changes in muscle parameters casts doubt on the relevance of the cross-sectional associations we observed and those observed in prior cross-sectional studies (16). Further, the lack of consistent associations

between fatty acids and muscle parameters that are interrelated (e.g. total PUFAs were associated with knee extension strength but not grip strength or IMAT) does not provide convincing support for a role of PUFAs. Despite evidence from older adults of a stimulatory effect of fish oil supplementation on muscle protein synthesis (17), we did not find relationships between fish oil, total (n-3) or individual long chain (n-3) PUFAs with change in muscle size over a five year time period. It is important to note, that the trial by Smith et al. measured acute effects of high doses of fish oil (4 gram per day) in a small sample (7 in control, 8 in fish oil group) of individuals with different demographics than our population (BMI < 30kg/m^2 and free from disease). It is possible that our divergent results are due to a threshold effect of (n-3) PUFAs on muscle but a previous study, in cancer patients with a mean age of 62, reported a linear association between CT derived muscle size and circulating (non-supplementation) levels of phospholipid EPA (14).

(n-3) PUFAs have been investigated in a wide range of chronic conditions because of their anti-inflammatory potential whereby (n-3) derived lipid mediators (eicosanoids) inhibit formation of proinflammatory eicosanoids from n-6 PUFAs (34). Reports of the benefits of (n-3) PUFAs in chronic diseases with inflammatory processes, such as cardiovascular disease, cancer and bone loss were promising (35-38) but subsequent null studies have dampened enthusiasm (39, 40). We add to the debate over fatty acids and health outcomes by suggesting that phospholipid (n-3) PUFAs which reflect supplementation and dietary sources do not play a role in processes related to (change of) muscle size, IMAT or strength.

Strengths of the study include the measurement of circulating fatty acids in a relatively large sample of older adults, computed tomography assessment of muscle size and IMAT and assessments of both upper and lower body strength. The availability of self-reported data on fish oil supplementation at three key time points and longitudinal measures of muscle size, IMAT, and muscle strength, although in a smaller number of participants, uniquely enabled characterization of PUFAs in relation to change in muscle parameters. There are also study limitations to consider. PUFAs were only determined at baseline and therefore changes in exposure over time was limited to self-reported data on fish oil consumption. The self-reported data did not indicate any relationships between chronic PUFAs exposure and outcome. However, multiple measurements of phospholipid plasma PUFAs may be of additional value to investigate the role of dynamic circulating PUFAs in relation to muscle parameters. The Icelandic population is characterized by high fish oil consumption relative to the United States, although (n-3) PUFAs concentrations are lower than Asian countries (41). This may affect the generalizability of our results. It is also

possible that the likelihood of detecting associations in a population with relatively high n-3 PUFAs levels is reduced because the reference group includes individuals who have a quite favorable fatty acid profile. We did not have information on the composition of fish oil supplements and were limited to assessing muscle parameters in relation to the frequency rather than dose of (n-3) PUFAs. However, since phospholipid EPA and DHA were generally not related to muscle parameters, this does not limit the interpretation of our findings. Finally, participants in the longitudinal cohort had to be well enough to complete follow-up measurements. The limited associations we observed between baseline PUFAs and muscle parameters may have been driven by less healthy participants who did not survive long enough for follow-up muscle measures and inclusion in the longitudinal cohort. However, the longitudinal cohort was not typified by healthy aging; loss of muscle size, strength and gain of IMAT were the predominant features. Future studies should investigate whether changes in PUFAs profile are associated with muscle parameters. In addition, since this is the first longitudinal population-based studies, our results need to be confirmed by studies investigating the association between PUFAs and changes in muscle parameters in different study populations.

In conclusion, our results suggest inconsistent cross-sectional relationships between plasma phospholipid PUFAs with muscle size, IMAT and strength.

SUPPLEMENTS

Supplemental Table 1. Fish oil supplementation across the lifetime in relation to baseline thigh muscle parameters and muscle strength among 836 participants[1-3].

		Muscle size, cm^2		Intermuscular adipose tissue, cm^2		Knee extension strength, N		Grip strength, N	
	n	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Old age									
Never	208	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
< Daily	93	0.70 (-3.52,4.92)	2.55 (-0.92,6.01)	-1.73 (-3.62,0.16)	-1.07 (-2.79,0.65)	-12.9 (-33.6,7.92)	-10.3 (-30.7,10.1)	2.72 (-20.5,15.0)	-3.10 (-20.9,14.7)
Daily	535	-0.86 (-3.63,1.91)	0.44 (-1.86,2.75)	-0.31 (-1.55,0.93)	0.23 (-0.92,1.37)	6.10 (-7.54,19.7)	5.16 (-8.44,18.8)	5.57 (-6.09,17.2)	4.06 (-7.78,15.9)
P-trend		0.48	0.88	0.84	0.53	0.25	0.33	0.29	0.44
Mid life									
Never	234	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
< Daily	224	-0.88 (-4.06,2.31)	0.69 (-1.96,3.34)	-1.87 (-3.29,-0.44)	-1.18 (-2.49,0.13)	-9.09 (-24.8,6.61)	-9.89 (-25.5,5.74)	-0.34 (-13.7,13.1)	-3.08 (-16.7;10.5)
Daily	378	-1.71 (-4.54,1.11)	0.24 (-2.12,2.61)	-0.68 (-1.94,0.58)	0.12 (-1.05,1.29)	-6.39 (-20.3,7.56)	-7.17 (-21.1,6.78)	-3.05 (-15.0,8.87)	-5.57 (-17.7,6.57)
P-trend		0.23	0.89	0.45	0.62	0.42	0.37	0.60	0.37
Early life									
Never	325	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
< Daily	231	-0.31 (-3.23,2.61)	0.41 (-2.00,2.82)	-0.88 (-2.19,0.42)	-0.47 (-1.66,0.73)	-5.54 (-19.9,8.83)	-7.23 (-21.5,7.01)	-6.28 (-18.5,5.97)	-8.82 (-21.2,3.53)
Daily	280	-0.35 (-3.11,2.40)	1.06 (-1.22,3.34)	-0.35 (-1.91,0.55)	-0.18 (-1.31,0.95)	-8.22 (-21.8,5.36)	-8.03 (-21.5,5.42)	-12.9 (-24.5,-1.35)	-13.9 (-25.5,-2.18)
P-trend		0.80	0.36	0.26	0.74	0.23	0.24	0.029	0.019

[1] Multivariable linear regression analyses were performed to compute regression coefficient (95% confidence intervals).

[2] The never category is the reference group.

[3] Model 1 was adjusted for age, sex and physical activity; Model 2 was adjusted for Model 1 plus education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, and C-reactive protein.

Supplemental Table 2. Fish oil supplementation across the lifetime in relation to relative change in thigh muscle parameters and muscle strength among 459 participants over five years of follow-up[1-3].

		Muscle size, %		Intermuscular adipose tissue, %		Knee extension strength, %		Grip strength, %	
	n	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Old age									
Never	110	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
< Daily	58	-1.00 (-3.50,1.51)	-1.12 (-3.60,1.37)	-2.50 (-10.5,5.48)	-3.47 (-11.5,4.55)	2.56(-4.19,9.30)	1.82 (-5.00,8.63)	4.00 (-4.04,12.0)	3.63 (-4.53,11.8)
Daily	291	-1.02 (-2.77,0.72)	-1.36 (-3.11,0.39)	-2.15 (-7.70,3.40)	-2.05 (-7.69,3.59)	-0.78 (-5.47,3.91)	-1.49 (-6.28,3.31)	0.78 (-4.82,6.37)	1.13 (-4.61,6.87)
P-trend		0.27	0.14	0.48	0.55	0.62	0.44	0.92	0.81
Mid life									
Never	130	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
< Daily	129	0.87 (-1.06,2.79)	0.77 (-1.19,2.72)	-5.41 (-11.5,0.72)	-4.78 (-11.1,1.50)	-1.91 (-7.11,3.29)	-3.04 (-8.39,2.31)	2.76 (-3.44,8.95)	3.87 (-2.53,10.3)
Daily	200	1.76 (0.01,3.51)	1.44 (-0.35,3.22)	-4.62 (-10.2,0.95)	-3.92 (-9.65,1.81)	-0.35 (-5.07,4.37)	-1.19 (-6.07,3.70)	2.14 (-3.48,7.77)	3.05 (-2.79,8.89)
P-trend		0.047	0.11	0.13	0.23	0.96	0.75	0.50	0.36
Early life									
Never	183	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
< Daily	130	0.68 (-1.10,2.47)	0.70 (-1.10,2.50)	-6.85 (-12.5,-1.20)	-5.19 (-11.0,0.59)	3.00 (-1.80,7.80)	2.53 (-2.39,7.45)	2.04 (-3.69,7.77)	2.59 (-3.30,8.49)
Daily	146	1.02 (-0.69,2.73)	0.84 (-0.86,2.54)	-4.43 (-9.85,0.99)	-3.52 (-8.97,1.94)	-0.83 (-5.44,3.77)	-1.20 (-5.85,3.45)	-0.47 (-5.96,5.03)	0.20 (-5.37,5.77)
P-trend		0.24	0.32	0.09	0.19	0.79	0.65	0.91	0.91

[1] Multivariable linear regression analyses were performed to compute regression coefficient (95% confidence intervals).

[2] The never category is the reference group.

[3] Model 1 was adjusted for age, sex, physical activity and time between assessments; Model 2 was adjusted for Model 1 plus education, smoking status, BMI, diabetes, chronic obstructive pulmonary disease, coronary heart disease, microalbuminuria, and C-reactive protein.

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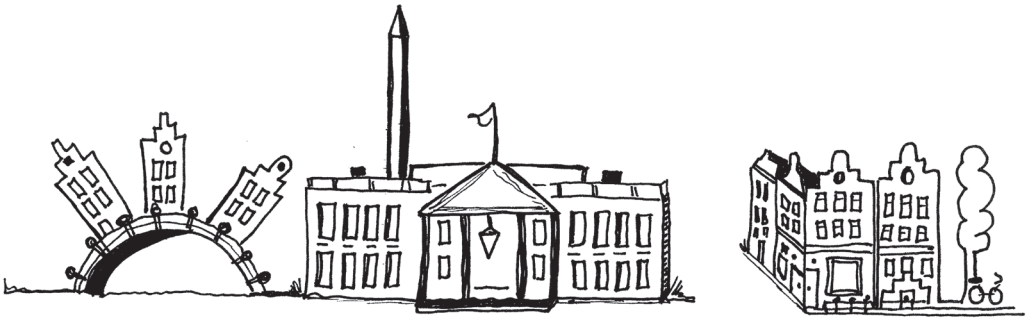

CHAPTER 7

Polyunsaturated fatty acids in relation to incident mobility disability and decline in gait speed; the Age, Gene/Environment Susceptibility-Reykjavik Study

Ilse Reinders, Rachel A. Murphy, Xiaoling Song, Marjolein Visser, Mary Frances Cotch, Thomas F. Lang, Melissa E. Garcia, Lenore J. Launer, Kristin Siggeirsdottir, Gudny Eiriksdottir, Palmi V. Jonsson, Vilmundur Gudnason, Tamara B. Harris, and Ingeborg A. Brouwer.

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ABSTRACT

Background/Objectives: Low intake of long chain polyunsaturated fatty acids (PUFAs) is associated with physical disability, however, prospective studies of circulating PUFAs are scarce. We examined associations between plasma phospholipid n-3 and n-6 PUFAs with risk of incident mobility disability and gait speed decline.

Subjects/Methods: Data are from a subgroup of the AGES-Reykjavik Study, a population-based study of risk factors for disease and disability in old age. In this subgroup (n=556, mean age 75.1 ± 5.0 years, 47.5% men), plasma phospholipid PUFAs were assessed at baseline using gas chromatography. Mobility disability and usual gait speed were assessed at baseline and after 5.2 ± 0.2 years. Mobility disability was defined as: having much difficulty, or being unable to walk 500 meter, or climb up 10 steps; decline in gait speed was defined as change ≥ 0.10 m/s. Logistic regression analyses were performed to determine associations between sex-specific SD increments in PUFAs with risk of incident mobility disability and gait speed decline. Odds ratios (95% confidence intervals) adjusted for demographics, follow-up time, risk factors and serum vitamin D were reported.

Results: In women, but not men, every SD increment increase of total n-3 PUFAs and docosahexaenoic acid was associated with lower mobility disability risk; OR 0.48 (0.25; 0.93) and OR 0.45 (0.24; 0.83), respectively. There was no association between n-6 PUFAs and the risk of incident mobility disability or gait speed decline.

Conclusions: Higher concentrations of n-3 PUFAs and, particularly, docosahexaenoic acid may protect women from impaired mobility, but does not appear to have such an effect in men.

INTRODUCTION

Aging is associated with loss of physical function (1). With the aging of the general population and the considerable prevalence of older persons with mobility disability, identifying modifiable factors that might delay or prevent loss of physical function is important to promote independence and quality of life for older persons.

Nutrient intake is a modifiable factor that may be important for maintaining the health of aging individuals. Long chain polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been associated with improved muscle composition (2) or muscle strength (2-4). In addition, lower intakes of long chain n-3 PUFAs are cross-sectionally associated with worse physical function (4, 5). Data from the InCHIANTI study showed that higher plasma n-3 PUFA levels were associated with lower risk of poor performance after three years of follow-up (6). Data on long chain n-3 PUFA supplementation (fish oil), although limited, suggest a benefit of 1.2 g of EPA and DHA on gait speed among post-menopausal women (7). Plasma n-6 PUFAs in relation to physical performance decline have only been investigated in one study. No associations were reported (6), but further studies are needed to confirm these results.

Since most previous studies were limited to cross-sectional measures of function and the majority of the studies estimated n-3 PUFAs using questionnaires rather than measurements of circulating PUFA, further studies are needed. In addition, prior studies focused on long chain n-3 PUFAs, and the role of long chain n-6 PUFAs on physical function is less well known.

The aim of the present study is to determine associations between plasma phospholipid n-3 and n-6 PUFAs with incident mobility disability and gait speed decline assessed over five years of follow-up in older adults. We hypothesized that participants with higher plasma phospholipid PUFAs would have lower risk of mobility disability and decline in gait speed.

SUBJECTS AND METHODS

Study population

Data are from the Age, Gene/Environment Susceptibility–Reykjavik (AGES-Reykjavik) Study, a single-center, prospective, ongoing population study of survivors from the Reykjavik Study (8, 9) Details of the study design were previously published (10). Briefly, baseline data collection among 5764 men and women took place from 2002-2006. During

a mean follow up of 5.2 ± 0.2 years 1039 participants died, 1198 were not willing to participate and 211 were lost to follow-up. Follow-up measurements took place between 2007 and 2011 in 3316 participants.
Participants were drawn from the random cohorts of two sub studies in the AGES-Reykjavik Study (n=1028) that had data on PUFAs. Participants who met criteria for MRI were identified and randomly selected to take part in the Iceland-MI study (n=702) (11) Participants who were identified as candidates for Iceland-MI but who did not participate were also randomly selected (n=326). Participants without baseline and follow-up data on mobility disability or gait speed (n=440) were excluded in the present analytic sample. Since we were interested in incident mobility disability, we excluded participants who reported difficulty walking 500 meter or climb 10 steps at baseline (n=32), resulting in 556 participants with complete data to assess incidence. Compared to the included sample, those who were excluded were older at baseline, were less moderate to vigorous physically active, and had lower serum 25OHD concentration (**Supplement Table 1**).

All participants provided written informed consent, and the study was approved (VSN 00-063) by the National Bioethics Committee in Iceland as well as the Institutional Review Board of the Intramural Research Program of the National Institute on Aging.

Determination of polyunsaturated fatty acids
Baseline blood samples were collected following an overnight fast and stored at -80°C. PUFAs were measured in plasma phospholipids which reflect short term dietary intake and fatty acids available to the periphery. Detailed description of determination has been previously described (2). In brief, phospholipids were separated from other lipids by one dimensional thin layer chromatography (12). Fatty acid methyl esters were prepared by direct transesterification (13) and separated using gas chromatography. PUFAs are expressed as a relative percent of the total phospholipid fatty acids analyzed. For this study, we focused on total and individual long chain n-3 PUFAs (EPA + DPA + DHA), ALA, total and individual long chain n-6 PUFAs (linoleic acid (LA) + arachidonic acid (AA)). The coefficient of variation from pooled quality control samples for EPA, DPA, DHA, ALA, LA and AA were all < 2.5%.

Determination of self-reported mobility disability
Self-reported mobility disability was assessed at baseline and follow-up with the following questions: “Because of health or physical problems do you have any difficulty walking 500 meter by yourself or without the use of aids?”, and “Do you have any difficulty climbing 10

steps without resting when you are by yourself and without the use of aids?" Mobility disability was defined as having much difficulty or being unable to walk 500 meter and/or climb 10 steps at follow-up.

Determination of gait speed

Gait speed was used as an objective measure of physical performance. Usual 6 meter walking speed was assessed using the same standardized protocol at both baseline and follow-up. Gait speed was calculated by dividing the distance with the walking time expressed in meter per second (m/s). Change in gait speed over time was calculated. A decline of ≥ 0.10 m/s in gait speed, a clinically meaningful change (14), was used to categorize participants according to whether or not they had a decline in gait speed.

Covariates

All covariates were assessed at the baseline examination. Body mass index (BMI) (kg/m^2) was calculated from measured weight and height, and waist circumference (cm) was measured using standardized protocols (10). Education (primary, secondary, college and university education), smoking status (never, former and current), and physical activity (hours per week of moderate to vigorous activity in prior year) were assessed by questionnaire. Circulating vitamin D, 25OHD, was determined using the Liaison chemiluminescence immunoassay as measured in nmol/L. Blood pressure was assessed from the mean value of two measurements with a large-cuff mercury sphygmomanometer. Medical conditions (hypertension, diabetes, coronary heart disease) were determined from self-report, medications and clinical assessments.

Dietary consumption was assessed by food frequency questionnaire in early life (aged 14 to 19 years), midlife (aged 40 to 50 years) and later life (AGES-Reykjavik Study baseline) (15). The food frequency questionnaire assessed frequency of intake of ten common foods and food groups, including fish and fish oil, using the same questions for all three time periods. The most commonly consumed fish in Iceland are cod and haddock (16) and both contain low levels of n-3 PUFAs. Therefore, we focused on fish liver oil consumption (referred to as fish oil hereafter) which is rich in n-3 PUFAs as well as vitamin D. Fish oil consumption was categorized as never, < daily (< once a month, 1-3 times a month, 1-2 times a month or 5-6 times a week) or daily.

Statistical analysis

Because of known differences in physical function in older men and women (17), we stratified analyses by sex. Differences in baseline characteristics were examined using ANOVA for continuous variables and chi-square tests for categorical variables.

Multivariate logistic regression analyses were used to examine baseline PUFAs in relation to odds of developing incident mobility disability and decline in gait speed. Effect estimates were expressed as odds ratios (OR) and corresponding 95% confidence intervals (CI) per sex specific SD increments of PUFAs. Three models were fit; Model 1 was adjusted for age, waist circumference, education, and time between baseline and follow-up examination. Model 2 was adjusted for all variables of Model 1 plus smoking status, physical activity, hypertension, diabetes, and coronary heart disease. The prevalence of daily fish oil consumption in the study population is high (62%). Besides being the main source of n-3 PUFAs, it is also rich in vitamin D. Higher concentrations of n-3 PUFAs (18-20) and vitamin D (21) are associated with lower cardiovascular disease events and cardiovascular disease risk factors. In turn, cardiovascular diseases are associated with mobility disability (22) or limited activity (23, 24). Therefore, we additionally adjusted for serum 25OHD in Model 3 to investigate associations between n-3 PUFAs and odds of developing mobility disability and decline in gait speed, independent of vitamin D status. All models of decline in gait speed were additionally adjusted for gait speed at baseline. All *P*-values are two-tailed (α=0.05). All analyses were performed using STATA version 12.1 (StataCorp, College Station, Texas, USA).

RESULTS

The mean age of the analytic sample was 75.1 ± 5.0 years, with a BMI of 27.3 ± 3.9 kg/m^2. Differences between men and women are shown in **Table 1**. Compared to women, men had a larger waist circumference, were more educated, less likely to report never smoking, more physically active, more likely to have coronary heart disease and had higher concentrations of vitamin D, total and individual long chain n-3 PUFAs ($P < 0.05$ for all).

Polyunsaturated fatty acids in relation to incident mobility disability and decline in gait speed

At follow-up, 17 (6.4%) men and 25 (8.6%) women reported mobility disability. Associations between SD increments in PUFAs with risk of mobility disability are presented in **Table 2**. In women, total long chain n-3 PUFAs were associated with lower risk of mobility disability with minimal (Model 1) and further adjustments for life style factors and diseases (Model 2): OR 0.49 (95% CI 0.26; 0.93). Associations remained after further adjustment for serum 25OHD (Model 3): OR 0.48 (95% CI 0.25; 0.93). The protective effect of total long chain n-3 PUFAs in relation to mobility disability mainly reflected associations between DHA and mobility disability risk. DHA was inversely associated with mobility disability risk in all models, with OR 0.45 (95% CI 0.24; 0.83) in Model 3. No other

associations were observed for PUFAs in relation to mobility disability risk for men or for women. During follow-up, 101 (38.3%) men and 104 (35.6%) women had a clinically significant decline in gait speed. **Table 3** depicts associations between SD increments in PUFAs with risk of gait speed decline. PUFAs were not associated with risk of gait speed decline in men or in women.

DISCUSSION

In this study plasma phospholipid long-chain n-3 PUFAs, and in particular DHA, were associated with lower risk of mobility disability in women but not in men. We observed no associations for plasma phospholipid long-chain n-3 PUFAs with decline in gait speed. Plasma phospholipid long chain n-6 PUFAs were not associated with mobility disability or decline in gait speed.

Few studies have investigated relations of plasma phospholipid PUFAs with longitudinal measures of physical function in older populations. Consistent with our finding, results from the InCHIANTI study showed that baseline plasma n-3 PUFAs were inversely associated with the risk of developing impaired physical performance, but no associations for long chain n-6 PUFAs were observed (6). In a Japanese study, lower intake of long chain n-3 PUFAs was associated with shorter timed up and go tests in men but not in women (5). In contrast, another study showed no associations between self-reported n-3 PUFAs and physical performance measures such as chair rise, grip strength and walking speed (3), casting doubt over the role of n-3 PUFAs in physical function. In addition to our analyses between plasma phospholipid PUFAs with physical function, we determined associations for fish consumption in relation to physical function. Spearman correlations showed that current fish oil consumption was moderately correlated with EPA; r=0.42, and DHA levels; r=0.40 (both $P < 0.001$). Our results do not appear to support a major role for fish oil consumption in relation to mobility disability or decline in gait speed (**Supplement Table 2**). This may be explained by the limited number of events per fish oil intake group, which might have resulted in low statistical power to detect significant differences. Our study population is also typified by a high intake of fish and fish oil which may mean the reference group has a more favorable fatty acid profile than other studies.

Table 1. Baseline characteristics from a subgroup of the AGES-Reykjavik Study.

	Men	Women	*P*-value
	n=264	n=292	
Age (years)	75.1 ± 4.69	75.1 ± 5.26	0.993
Body mass index (kg/m^2)	27.0 ± 3.56	27.4 ± 4.12	0.232
Waist circumference (cm)	102.4 ± 9.74	98.0 ± 11.9	< 0.001
Education, n (%)			
< High School	178 (67)	219 (75)	
High School	42 (16)	51 (17)	0.004
Postsecondary	44 (17)	22 (8)	
Smoking status, n (%)			
Never	81 (31)	155 (53)	
Former	157 (59)	105 (36)	< 0.001
Current	26 (10)	32 (11)	
Moderate to vigorous activity (hours/week)	2.07 ± 2.73	1.43 ± 2.36	0.004
Vitamin D (nmol/L)	59.3 ± 26.4	54.4 ± 26.5	0.029
Systolic blood pressure (mmHg)	143 ± 20	141 ± 18	0.102
Hypertension, n (%)	214 (81)	223 (76)	0.178
Type 2 diabetes, n (%)	28 (11)	18 (6)	0.058
Coronary heart disease, n (%)	77 (29)	39 (13)	< 0.001
Current fish oil intake, n (%)			
Never	64 (24)	72 (25)	
< Daily	38 (14)	36 (12)	0.782
Daily	162 (61)	183 (63)	
Plasma polyunsaturated fatty acids (relative % of total fatty acids)			
Long chain n-3 PUFAs	10.8 ± 3.11	9.93 ± 2.93	< 0.001
Eicosapentaenoic acid	3.13 ± 1.68	2.74 ± 1.60	0.006
Docosapentaenoic acid	1.20 ± 0.21	1.11 ± 0.18	< 0.001
Docosahexaenoic acid	6.51 ± 1.51	6.08 ± 1.44	< 0.001
Alpha-linoleic acid	0.22 ± 0.07	0.22 ± 0.07	0.844
Long chain n-6 PUFAs	24.6 ± 3.29	25.0 ± 2.75	0.139
Linoleic acid	17.6 ± 3.10	17.9 ± 2.56	0.185
Arachidonic acid	7.02 ± 1.65	7.08 ± 1.70	0.672

Values are presented as mean ± SD for continuous variables, and number (%) for categorical variables

Table 2. Associations between plasma phospholipid PUFAs in relation to incident mobility disability risk.

	Men n=264			**Women** n=292		
	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))	Model 3 (OR (95% CI))	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))	Model 3 (OR (95% CI))
Long chain n-3 PUFAs	0.94 (0.49; 1.78)	1.07 (0.54; 2.14)	1.24 (0.57; 2.70)	0.47 (0.25; 0.89)	0.49 (0.26; 0.93)	0.48 (0.25; 0.93)
Eicosapentaenoic acid	0.83 (0.42; 1.64)	0.96 (0.45; 2.01)	1.05 (0.47; 2.32)	0.53 (0.28; 1.03)	0.55 (0.28; 1.07)	0.55 (0.28; 1.10)
Docosapentaenoic acid	0.79 (0.43; 1.45)	0.82 (0.41; 1.60)	0.85 (0.43; 1.70)	1.02 (0.67; 1.56)	1.07 (0.70; 1.66)	1.12 (0.71; 1.74)
Docosahexaenoic acid	1.11 (0.60; 2.08)	1.22 (0.65; 2.29)	1.46 (0.70; 3.04)	0.45 (0.25; 0.81)	0.46 (0.26; 0.84)	0.45 (0.24; 0.83)
Alpha-linoleic acid	1.16 (0.69; 1.93)	1.12 (0.65; 1.93)	1.11 (0.64; 1.94)	1.36 (0.94; 1.97)	1.42 (0.96; 2.09)	1.40 (0.95; 2.07)
Long chain n-6 PUFAs	0.92 (0.52; 1.65)	0.79 (0.42; 1.48)	0.72 (0.37; 1.41)	1.35 (0.83; 2.19)	1.32 (0.81; 2.16)	1.29 (0.78; 2.15)
Linoleic acid	1.11 (0.60; 2.06)	0.93 (0.46; 1.86)	0.90 (0.44; 1.83)	1.32 (0.83; 2.12)	1.29 (0.80; 2.07)	1.27 (0.78; 2.05)
Arachidonic acid	0.69 (0.34; 1.39)	0.69 (0.34; 1.39)	0.60 (0.28; 1.31)	1.05 (0.67; 1.64)	1.05 (0.65; 1.70)	1.03 (0.63; 1.67)

Mobility disability was defined as having much difficulty or unable to walk 500 meter and/or climb 10 steps at follow-up. 17 men and 25 women reported having mobility disability.

Model 1 was adjusted for age, education, waist circumference and follow-up time.

Model 2 was adjusted for Model 1 plus smoking status, physical activity, hypertension, diabetes and heart disease.

Model 3 was adjusted for Model 2 plus serum vitamin D.

Table 3. Associations between plasma phospholipid PUFAs in relation to risk of decline in gait speed.

	Men n=264			**Women** n=292		
	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))	Model 3 (OR (95% CI))	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))	Model 3 (OR (95% CI))
Long chain n-3 PUFAs	0.89 (0.67; 1.19)	0.89 (0.67; 1.19)	0.79 (0.57; 1.09)	0.86 (0.65; 1.12)	0.84 (0.63; 1.11)	0.90 (0.66; 1.23)
Eicosapentaenoic acid	0.85 (0.63; 1.14)	0.86 (0.64; 1.16)	0.76 (0.54; 1.06)	0.84 (0.64; 1.11)	0.82 (0.61; 1.10)	0.88 (0.65; 1.20)
Docosapentaenoic acid	1.06 (0.81; 1.39)	1.06 (0.81; 1.41)	1.04 (0.78; 1.37)	1.05 (0.81; 1.36)	1.05 (0.80; 1.38)	1.11 (0.84; 1.47)
Docosahexaenoic acid	0.94 (0.71; 1.24)	0.93 (0.70; 1.23)	0.83 (0.61; 1.14)	0.87 (0.67; 1.15)	0.86 (0.65; 1.14)	0.92 (0.68; 1.25)
Alpha-linoleic acid	0.94 (0.71; 1.24)	0.94 (0.70; 1.25)	0.94 (0.70; 1.25)	1.09 (0.85; 1.41)	1.12 (0.87; 1.46)	1.12 (0.86; 1.45)
Long chain n-6 PUFAs	1.14 (0.86; 1.50)	1.14 (0.86; 1.52)	1.22 (0.91; 1.65)	0.98 (0.75; 1.28)	0.99 (0.75; 1.32)	0.93 (0.70; 1.25)
Linoleic acid	1.22 (0.92; 1.62)	1.26 (0.94; 1.69)	1.30 (0.97; 1.75)	0.99 (0.76; 1.30)	0.99 (0.75; 1.30)	0.96 (0.72; 1.26)
Arachidonic acid	0.90 (0.68; 1.19)	0.86 (0.64; 1.15)	0.90 (0.66; 1.22)	0.98 (0.75; 1.28)	1.01 (0.76; 1.34)	0.97 (0.72; 1.29)

101 men and 104 women had a decline in gait speed (decline of ≥ 0.10 m/s).

Model 1 was adjusted for age, education, waist circumference, baseline gait speed and follow-up time.

Model 2 was adjusted for Model 1 plus smoking status, physical activity, hypertension, diabetes and heart disease.

Model 3 was adjusted for Model 2 plus serum vitamin D.

A potential mechanism for the observed associations between n-3 PUFAs in relation to lower mobility disability risk is the incorporation of PUFAs into membranes of the skeletal muscle. Several trials in animals (25) and humans (26-28) have shown that supplementation of EPA and DHA increased PUFA content in muscle phospholipids. This leads to improved muscle composition due to the anti-inflammatory properties of long chain n-3 PUFAs (29-31). Previously in this study population we showed that higher concentrations of total PUFAs cross-sectionally were associated with larger muscle size and with greater knee extension strength, and ALA was associated with increased knee extension strength over time (2) Since muscle size, composition, and strength are related to physical function (32, 33) this may help explain our finding of an inverse relationship between n-3 PUFA and risk of mobility disability among women. However the reason for the null relationship in men is unclear. One other study reported gender differences whereby there were relationships between PUFAs and faster time up and go test in men but not women (5). In our study concentrations of n-3 PUFAs were lower in women than men. It is possible that the odds of detecting associations is greater in women because the reference group includes individuals with a less favorable fatty acids profile than the reference group in men. However, further research is needed to fully understand the potential biological differences in these associations. There are also more indirect mechanisms whereby long chain n-3 PUFAs decrease risk of mobility disability such as through their cardioprotective effects, e.g. improved lipid profile, lower blood pressure, reduced heart rate, and less arrhythmia (18, 20). Those improvements, in turn, are associated with healthy aging (34) and lower risk of physical decline (35). In addition, n-3 PUFAs are also associated with decreased depression risk which can indirectly lead to a lower risk of physical inactivity. A recent meta-analysis showed that supplementation of n-3 PUFAs is beneficial in the treatment of patients with diagnosis of major depressive disorder (36) Since depression itself is associated with worse physical function (37-39), higher PUFAs levels in turn may contribute to more active life, engagement in more physical activity, and finally with better health status.

Interestingly, our results suggest divergent relationships between n-3 PUFAs and mobility disability and gait speed decline. In contrast to what we had hypothesized, we did not observe associations between plasma phospholipid PUFAs and decline in gait speed. Although, both mobility disability and gait speed provide indications of lower extremity function, mobility disability may capture more advanced decline in physical function since participants had to self-report much difficulty or being unable to walk a quarter mile or climb 10 steps. In comparison, a decline in gait speed of 0.10 m/s while walking on a straight, level surface, while clinically meaningful, may represent changes in function early

in the pathway to disability. In our study, there was a higher percentage of participants with a decline in gait speed compared to incident mobility disability; 36.9% vs 7.6%, respectively. It is possible that n-3 PUFAs are involved in processes that manifest later as a more severe mobility impairment. However, a recent randomized controlled trial performed in women showed that gait speed increased after fish oil supplementation of 1.2 gram of EPA and DHA per day (7). It is possible that discrepancies are due to dose effects of fish oil.

Strengths and limitations

A strength of our study is the objective determination of plasma phospholipids fatty acids. This approach offers an advantage in that it reflects the absorption and metabolism of fatty acids and provides a more precise measure of fatty acid status than dietary estimates. It also has the advantage of quantifying individual PUFAs and therefore facilitates identification of fatty acids that may be particularly important for functional outcomes. Further strengths were that physical function was assessed at two time points using both self-reported and objectively measured mobility and our analytic sample was restricted to participants who reported no or some mobility disability at baseline, which minimizes the possibility of reverse causation. However, our study was not without limitations. Plasma phospholipid PUFAs were determined at baseline only and therefore we were not able to determine changes in PUFAs levels over time. Having multiple measurements of PUFAs might be of additional value since it would allow us to examine the influence of dynamic circulating PUFAs in relation to risk of incident mobility disability or gait speed decline. In addition, our study is limited by a small sample size. Future large longitudinal studies with multiple measurements of PUFAs could investigate changes in exposure status over time in relation to the physical function measures. Another limitation is our limited external validity since fish oil intake in Iceland is high and, for several decades, has been extremely common; 62% of study participants consumed fish oil daily. Finally, since we excluded individuals with mobility disability at baseline, our sample was likely healthier than other populations of comparable age and only consisted of Caucasians, therefore, our results may not be applicable to a general population of older adults.

In conclusion, in this study of older men and women without mobility disability at baseline, we showed that higher concentrations of long chain n-3 PUFAs, especially DHA, are associated with lower mobility disability risk in women after five years of follow up. Other longitudinal studies are needed to confirm these associations and to examine mechanisms.

SUPPLEMENTS

Supplemental Table 1. Differences between included and excluded participants.

	Included	Excluded	*P*-value
	n=556	n=472	
Women, n (%)	292 (52)	262 (56)	0.338
Age (years)	75.1 ± 5.00	78.6 ± 5.57	< 0.001
Body mass index (kg/m^2)	27.3 ± 3.87	26.9 ± 4.57	0.203
Waist circumference (cm)	100.1 ± 11.1	100.4 ± 12.2	0.665
Education, n (%)			
< High school	397 (71)	346 (77)	
High school	93 (17)	61 (14)	0.109
Postsecondary	66 (12)	41 (9)	
Smoking status, n (%)			
Never	236 (42)	168 (37)	
Former	262 (47)	221 (49)	0.144
Current	58 (10)	61 (14)	
Moderate to vigorous activity (hours/week)	1.73 ± 2.56	0.80 ± 1.84	< 0.001
Vitamin D (nmol/L)	56.8 ± 26.5	52.4 ± 25.7	0.008
Systolic blood pressure (mmHg)	142 ± 19	143 ± 21	0.310
Hypertension, n (%)	437 (79)	393 (83)	0.059
Type 2 diabetes, n (%)	46 (8)	49 (10)	0.240
Coronary heart disease, n (%)	116 (21)	105 (22)	0.591
Current fish oil intake, n (%)			
Never	136 (25)	110 (25)	
< Daily	74 (13)	46 (10)	0.316
Daily	345 (62)	292 (65)	
Polyunsaturated fatty acids (%)			
Long chain n-3 PUFAs	10.36 ± 3.05	10.66 ± 3.22	0.126
Eicosapentaenoic acid	2.93 ± 1.65	2.99 ± 1.68	0.530
Docosapentaenoic acid	1.15 ± 0.20	1.17 ± 0.22	0.295
Docosahexaenoic acid	6.29 ± 1.49	6.51 ± 1.61	0.023
Alpha-linoleic acid	0.22 ± 0.07	0.23 ± 0.09	0.094
Long chain n-6 PUFAs	24.8 ± 3.02	24.0 ± 3.20	< 0.001
Linoleic acid	17.8 ± 2.84	17.2 ± 2.83	0.002
Arachidonic acid	7.05 ± 1.68	6.79 ± 1.55	0.011

Values are presented as mean ± SD for continuous variables, and number (%) for categorical variables.

Supplemental Table 2. Associations between fish oil intake across life in relation to mobility disability risk and decline in gait speed.

	Men									
	Mobility disability risk					Decline in gait speed				
	N at risk	N of events	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))	Model 3 (OR (95% CI))	N at risk	N of events	Model 1 (OR (95% CI))	Model 2 (OR (95% CI))	Model 3 (OR (95% CI))
Early life										
Never	98	11	Reference	Reference	Reference	98	42	Reference	Reference	Reference
< Daily	85	2	0.19 (0.03; 1.06)	0.16 (0.02; 1.11)	0.15 (0.02; 1.07)	85	28	0.58 (0.30; 1.12)	0.54 (0.27; 1.07)	0.53 (0.27; 1.07)
Daily	81	4	0.35 (0.09; 1.44)	0.47 (0.11; 1.93)	0.52 (0.12; 2.27)	81	31	0.83 (0.43; 1.61)	0.80 (0.41; 1.58)	0.76 (0.38; 1.51)
Midlife										
Never	64	9	Reference	Reference	Reference	64	26	Reference	Reference	Reference
< Daily	85	3	0.30 (0.05; 1.60)	0.28 (0.05; 1.63)	0.28 (0.05; 1.63)	85	36	1.30 (0.62; 2.71)	1.28 (0.61; 2.70)	1.22 (0.58; 2.59)
Daily	114	5	0.31 (0.08; 1.23)	0.42 (0.10; 1.80)	0.46 (0.10; 2.06)	114	39	0.83 (0.41; 1.69)	0.90 (0.43; 1.87)	0.77 (0.36; 1.65)
Late life										
Never	64	7	Reference	Reference	Reference	64	26	Reference	Reference	Reference
< Daily	38	4	1.51 (0.28; 8.24)	1.26 (0.22; 7.13)	1.16 (0.20; 6.77)	38	14	0.99 (0.40; 2.45)	1.00 (0.40; 2.49)	0.89 (0.35; 2.25)
Daily	162	6	0.24 (0.06; 0.96)	0.28 (0.07; 1.17)	0.24 (0.05; 1.22)	162	61	0.86 (0.44; 1.67)	0.92 (0.47; 1.83)	0.74 (0.35; 1.55)
	Women									
Early life										
Never	128	12	Reference	Reference	Reference	128	47	Reference	Reference	Reference
< Daily	70	3	0.68 (0.17; 2.69)	0.72 (0.18; 2.91)	0.72 (0.18; 2.90)	70	20	0.72 (0.37; 1.42)	0.68 (0.34; 1.37)	0.67 (0.33; 1.37)
Daily	94	10	1.69 (0.62; 4.61)	1.65 (0.60; 4.53)	1.69 (0.60; 4.68)	94	37	1.23 (0.68; 2.23)	1.25 (0.68; 2.31)	1.32 (0.71; 2.45)
Midlife										
Never	84	14	Reference	Reference	Reference	84	36	Reference	Reference	Reference
< Daily	78	1	0.08 (0.01; 0.69)	0.08 (0.01; 0.73)	0.08 (0.01; 0.72)	78	19	0.42 (0.20; 0.86)	0.43 (0.20; 0.91)	0.42 (0.20; 0.91)
Daily	130	10	0.57 (0.22; 1.48)	0.60 (0.22; 1.62)	0.63 (0.23; 1.71)	130	49	0.84 (0.46; 1.55)	0.88 (0.47; 1.68)	0.97 (0.51; 1.85)
Late life										
Never	72	9	Reference	Reference	Reference	72	30	Reference	Reference	Reference
< Daily	36	3	0.69 (0.16; 3.01)	0.70 (0.15; 3.22)	0.71 (0.15; 3.28)	36	7	0.30 (0.11; 0.84)	0.29 (0.10; 0.82)	0.30 (0.10; 0.84)
Daily	183	13	0.52 (0.19; 1.41)	0.55 (0.20; 1.51)	0.58 (0.20; 1.66)	183	66	0.68 (0.37; 1.25)	0.61 (0.33; 1.14)	0.69 (0.36; 1.33)

Mobility disability was defined as having much difficulty or unable to walk 500 meter and/or climb 10 steps at follow-up. A decline in gait speed of ≥ 0.10 m/s was considered clinically relevant.

Model 1 was adjusted for age, education, waist circumference and follow-up time.

Model 2 was adjusted for Model 1 plus smoking status, physical activity, hypertension, diabetes and heart disease.

Model 3 was adjusted for Model 2 plus serum vitamin D. All models of decline in gait speed were additionally adjusted for baseline gait speed.

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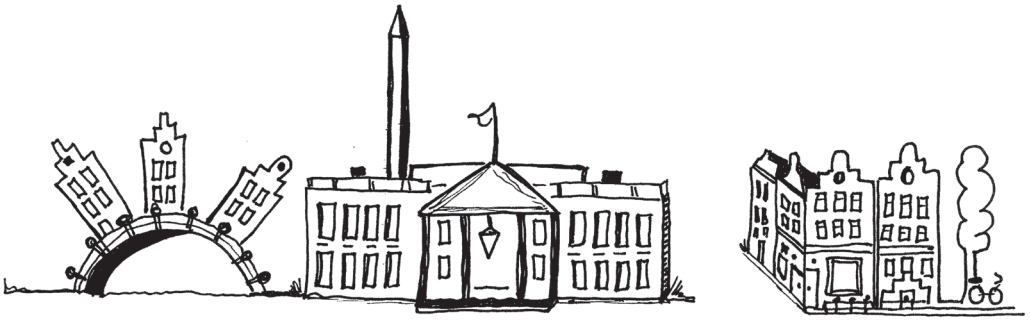

CHAPTER 8

General discussion

The overall objective of this thesis was to investigate the relation of various measures of body composition, including fat depots and muscle composition, with physical function and mortality risk in older adults. In addition, the association of polyunsaturated fatty acids with muscle composition and physical function in old age was examined. We have used two large prospective cohort studies to assess these associations. In this current chapter, we will summarize and discuss the answers to our research questions, methodological considerations will be addressed and recommendations for future research will be given. At the end of this chapter a general conclusion is presented.

RESEARCH QUESTIONS AND ANSWERS

What are the trajectories of BMI over time, and what are the associations between these trajectories with change in appendicular lean mass and physical function in old age?

We observed four distinctive BMI trajectories among black and white men and women aged 70 to 79 years. The trajectories differed in the BMI at baseline and over time, and all showed a modest decline in BMI over a time span of 9 years. Older men in the BMI trajectory with the highest mean BMI over time had greater decreases in gait speed and leg strength, whereas older women in the BMI trajectory with the highest mean BMI over time had a greater decrease in lean mass in the arms but not in the legs. Decline in lean mass did not consistently reflect decline in physical function, and vice versa, suggesting the importance of other pathways in determining functional decline. Based on these results we can conclude that a person's trajectory of disability might be determined before they reach old age, indicating the importance of healthy weight during life and of monitoring weight.

Which fat depots are associated with physical function among older adults?

We show that higher BMI is independently associated with increased risk of mobility limitation and greater reduction in gait speed. Regarding fat depots, mostly all fat depots areas were positively associated with higher risk of mobility limitation or greater reduction in gait speed. With additional adjustments for BMI, greater area of infiltrated thigh muscle fat and greater density of this fat remained associated with a higher risk, suggesting that muscle fat infiltration independently contributes to loss in physical function. A possible mechanism whereby muscle fat infiltration may contribute to relations between obesity and loss in physical function is via a decrease in muscle strength. Due to muscle fat infiltration, muscle fibers are less capable to contract (1), and thereby produce a lower strength output. It is also hypothesized that muscle fat infiltration inhibits blood flow to the muscle, and thereby hampers muscle contraction, or may contribute to insulin

resistance (2). Besides storage of energy, fat is an active metabolic and endocrine organ that secretes adipocytokines (1, 3). Increased adipocytokines such as interleukin-6 may have catabolic effects on muscle (4), which represents a more indirect mechanism. Our results endorse that high BMI is associated with increased risk of loss in physical function in older adults and shows the detrimental associations of higher and denser fat with physical function. Future studies are warranted to investigate the influence of reducing muscle fat infiltration in multiple fat depots on the risk for mobility disability.

Which muscle composition measures are associated with incident mobility disability and mortality in older adults?

Greater thigh muscle mass (cross-sectional muscle area) and greater knee extension strength were associated with lower risk of mobility disability, slower decline in gait speed and lower mortality risk among older adults, independent of BMI and other muscle composition measures. In contrast, more thigh muscle fat infiltration, in particular intramuscular adipose tissue, was associated with higher mortality risk. These associations were independent of BMI, muscle mass and muscle strength. These results show that different components of muscle are important determinants of physical function and mortality, suggesting that muscle composition may provide information beyond risk captured by BMI. Combined, these results confirm the importance of greater muscle mass, higher strength and lower muscle fat infiltration.

Are circulating polyunsaturated fatty acids associated with muscle composition and physical function?

To investigate potential modifiable factors influencing muscle composition, strength and physical function, we determined associations between plasma polyunsaturated fatty acids (PUFAs) and muscle composition, strength and physical function. We showed that plasma PUFAs were weakly associated with greater thigh muscle mass, lower thigh intermuscular fat area and greater grip or knee extension strength. In addition, higher plasma total n-3 PUFAs and docosahexaenoic acid were associated with lower mobility disability risk in women but not in men. Future observational studies including people with a larger age-range and different ethnic backgrounds are warranted to further investigate the potential effects of PUFAs with muscle composition, strength and physical function.

Figure 8.1 depicts the associations confirmed in this thesis. To summarize, our results show that 1) a higher BMI over time is associated with greater losses in physical function and losses in appendicular lean mass, 2) more and denser fat, especially infiltrated thigh muscle fat, is associated with incident mobility disability, 3) greater muscle mass, higher strength and lower muscle fat infiltration are independently associated with a decline in physical function and higher mortality risk, and 4) higher concentrations of PUFAs are weakly associated with better muscle composition and physical function.

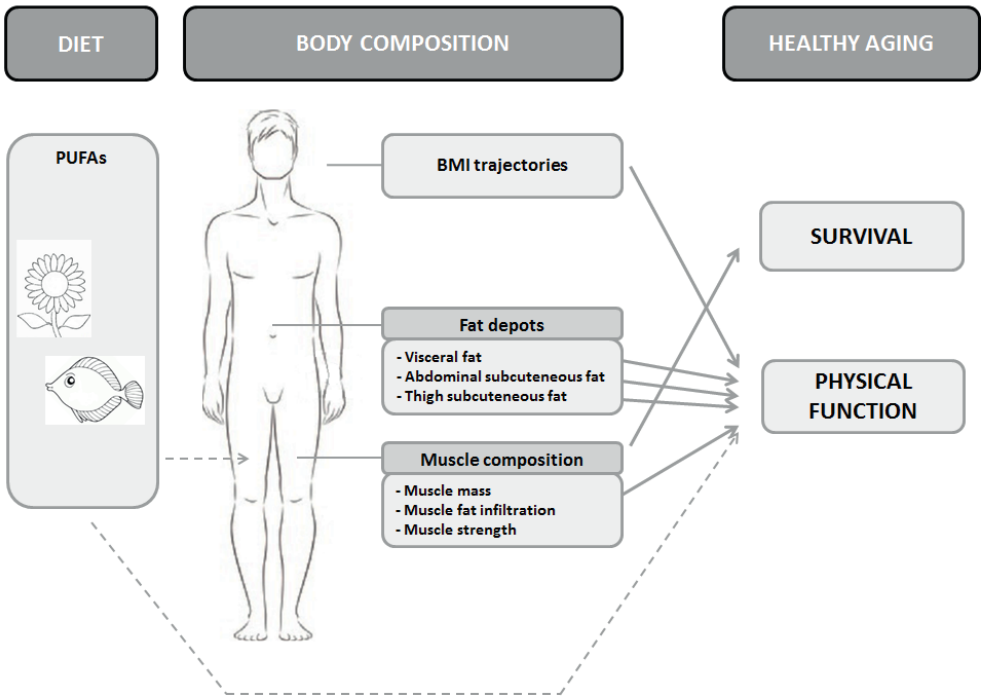

Figure 8.1. Summary of the associations observed between body composition and healthy aging, and of PUFAs with muscle composition and physical function. Observed associations that are likely to be robust are presented by bold arrows, whereas dotted arrows represent modest associations.

METHODOLOGICAL CONSIDERATIONS

It is important to take methodological considerations into account when interpreting the observations of this thesis. Specific strengths and limitations of the performed research have already been described per chapter. This section describes the methodological considerations most relevant for the overall thesis and will provide potential recommendations regarding study design and measurements for future studies.

Causality

A causal relationship or potential mechanism between exposure and outcome can only be determined by a randomized controlled trial. Even though this is not the case with observational studies, the combination of prospective findings with a long follow-up time and proper adjustment for confounding variables can approach robust results. In order to know which individuals will show greater deterioration in muscle composition or accelerated loss in physical function, a long follow-up period in combination with multiple assessments of muscle composition and physical function is desirable. This is the case in the Health, Aging and Body Composition (Health ABC) Study, and the Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) Study. Thus, although causality cannot be proven, these data can be used to determine which older adults are at greater risk of loss in physical function.

Characteristics of databases

Both prospective cohort studies used in this thesis have their own research focus and specialization. The focus of Health ABC Study is on changes in weight and body composition of initially well-functioning in older adults in relation to disability risk. With the Health ABC study data, it is also possible to identify other valuable indicators of persons at risk of disability (5). The AGES-Reykjavik Study was designed to examine risk factors, including genetic susceptibility and gene/environment interaction, in relation to disease and disability in old age (6). The major advantages of both studies include their large sample size, the long follow-up duration and the precise and accurate determination of body composition. In addition, the Health ABC Study includes a biracial sample, which made it possible to investigate whether associations were different for black and white participants.

Survival and selection bias

As with all observational studies, survival and selection bias are topics of concern. Survival bias represents the fact that healthier people will be more likely to survive, and that their inclusion in follow-up examinations will distort the actual health status of the overall study

population included at the beginning of the study. In other words, participants who are more frail or unhealthy are less likely to participate in a follow-up examination, which often results in an underestimation of the observed associations. This also might be the case within the Health ABC Study and the AGES-Reykjavik Study. Participants who were excluded from analyses (i.e. data on follow-up examination were missing) were older at baseline, had higher prevalence of comorbidities, and had worse physical function at baseline. Therefore, survival bias might have occurred. Observed associations may also have been biased due to the method used to select study participants or factors that influence study participation; this phenomenon is called selection bias (7). I.e. people who are more interested in health are more likely to participate in health-related studies. This results in a non-representative sample of the overall population, and external validity –the ability to extrapolate findings to different populations– may be limited.

Participants from Health ABC Study were initially free from mobility limitation, and in addition, we excluded participants from the AGES-Reykjavik Study who reported much difficulty at baseline, or were unable to walk and/or climb stairs at baseline when investigating incident mobility disability. This is an advantage when investigating factors related to incident mobility disability, or to see how 'healthy aging' proceeds. In addition, this importantly minimalized the possibility of reverse causation. However, the observed associations for both studies may not be generalizable to older adults who already have mobility limitations. It is possible that the influence of muscle composition on physical function is different among older adults who already experienced mobility disability at baseline. It is likely that poor muscle composition among people with a poor physical function (frail or unhealthy people) at baseline accelerates functional decline, however, future studies performed in different study populations are needed to confirm this hypothesis, and to be able to generalize observed results to the overall population.

The results about PUFAs in relation to muscle composition and physical function (Chapter 6 and 7) were obtained in the AGES-Reykjavik Study, where fish oil intake has been high and common for several decades. This might have resulted in a reference group including individuals with a quite favorable fatty acid profile, which may explain our null-findings. In addition, this affects the generalizability of our results, therefore it is difficult to compare our results with people with different dietary habits or background. It might be possible that higher fish oil intake is associated with muscle and physical function in other study populations. In addition, it is possible that the influence of PUFAs on muscle composition, strength and physical function may only become apparent in younger adults where deterioration of muscle composition has not been established yet.

Confounding variables

Confounding can be caused by covariates that are associated with both independent (exposure) and dependent (outcome) variables and are not on the causal pathway between exposure and outcome. Adjusting for these covariates (confounders) minimizes bias. Both cohort studies contain a well-characterized study population, which made it possible to adjust for many potential confounding variables. All confounding variables were determined at baseline which prevented recall bias, but associations did not take into account participants' health status at follow-up, which might have been associated with the outcome and thereby attenuate the actual associations. In addition, residual confounding due to measurement errors or unknown confounders might also be possible. On the other hand, adjustments for too many covariates may also lead to overadjustment bias (8). Furthermore, in our studies participants with missing data on confounders are omitted from analyses. This results in loss of information. Advanced multiple imputation techniques should be performed to reduce the influence of missing data (9).

Body composition

In Chapter 1 we have addressed some advantages and disadvantages for the use of different techniques to determine body composition. Body mass index (BMI) is an easy and applicable measure to determine nutritional status. On a group level BMI gives information about the overall adiposity of the population, however, BMI is a less accurate measure at the individual level. It can therefore be used as a screening tool, but it is not a diagnostic tool of the body fatness or health of an individual. The studies in this thesis have used two advanced techniques to determine body composition. In Chapter 2, appendicular skeletal muscle mass was determined by dual energy X-ray absorptiometry (DXA). DXA has the advantage of providing precise estimates of appendicular skeletal muscle mass and fat mass. However, it does not distinguish subcutaneous fat, intermuscular fat or intramuscular fat, which is especially of interest since the occurrence of age-related changes in these fat depots in our study population. The vast majority of our data on body composition is based on computed tomography (CT) imaging (Chapters 3-6). CT is a highly precise and accurate technique that can make the distinction in different fat depots and intermuscular fat infiltration. Magnetic resonance imaging (MRI) is another advanced technique that provides high-resolution images of body composition, inner body structures and tissues, and enables the evaluation of several fat depots, including muscle fat infiltration (10, 11). CT and MRI are both useful techniques to determine intermuscular adipose tissue. However, CT also assesses muscle attenuation, which is an indicator of intramuscular fat infiltration. Direct measurements of intramuscular fat infiltration are possible with muscle biopsies or magnetic resonance

spectroscopy (MRS). Muscle biopsies are less applicable in large epidemiological studies since they are expensive, time consuming, and cause a high burden for the study participant. MRS on the other hand is more applicable for research purposes and is able to distinguish intermuscular fat infiltration and intramuscular fat infiltration based on the molecular composition of the tissue. However, the quantification of the tissue is laborious (11). Therefore, MRI, CT and MRS are complementary techniques to further elucidate associations between muscle composition and healthy aging in research settings. Enhancements in technique, such as automatic processing of the images, would enable the use of these techniques for the assessment of body composition in the clinic.

Muscle strength

We have used grip and knee extension strength as measures of muscle strength (Chapter 4 and 5) or as outcome measures (Chapter 2 and 6). Multiple trials for strength were performed in both study populations, which increases the validity of diagnostic accuracy. Grip strength is a measure for upper body functionality, and is a strong determinant of functional limitations later in life (12), mobility disability (13), and mortality (14, 15). Knee extension strength is a valid measure of lower body strength, and it predicts e.g. risk of losing independence in activities of daily living (16), and mortality (17). However, the strength measures represent a specific muscle group, and do not provide information regarding other muscles and their relation to mobility. Yet, previous studies have shown high correlations between isometric muscle strength of multiple muscles (18).

The advantage of measuring grip strength compared to using advanced techniques to determine muscle strength is that it is very applicable in large epidemiological studies and in clinical practice. Using cut-off values will facilitate to identify older adults who may benefit from multimodel interventions, among which diet and resistance training. International normative values are based on a meta-analysis, however, no specific values are available for adults older than 75 years (19). The most commonly used cut-off values of grip strength to identify older adults at risk for mobility limitations are 30 kg for men and 20 kg for women (18). Recently, the Foundation of the National Institutes of Health (FNIH) Sarcopenia project determined cut-off values for appendicular lean mass and grip strength to diagnose older adults with low muscle mass and weakness. The FNIH cut-off values are based on nine large prospective cohort studies including predominantly Caucasian older adults with a mean age over 70 years. The FNIH cut-off values for low weakness are grip strength < 26.0 kg for men and < 16.0 kg for women (20). However, cut-off values from Lauretani et al. (18) and FNIH (20) are not BMI and age-dependent. One cross-sectional study has investigated sex and BMI-specific cut-off values to

determine an increased likelihood for mobility limitations, and showed that among men, the optimal cut-off value for normal-weight men was 33 kg, 39 kg for overweight men, and 40 kg for obese men. For women, a cut-off value of 21 kg was sufficient at any level of BMI (21).

Investigating leg strength in relation to physical function or other health outcomes could be of additional value, since it represents large muscle groups necessary for lower extremity function. When investigating knee extension strength, the lowest 20th percentile is often used as a measure of low muscle strength, however, this value is dependent on the specific study population and may therefore not be representative for the overall population. Based on current literature, in contrast to grip strength, no established cut-off values for knee extension strength are available.

Physical function

In both the Health ABC Study and the AGES-Reykjavik Study complementary measures of physical function were included. We have included gait speed as an objective measure of physical function. Poor gait speed (<1.0 m/s (22)) likely represents disturbances in multiple organ systems (23). In our study we had the advantage of a long follow-up period which made it possible to investigate meaningful changes in gait speed. Some individuals may have a proper baseline and follow-up gait speed which would not indicate poor physical function, however, investigating changes in gait can indicate a decline in physical function, which can be relevant in identifying people at risk for further physical decline. Therefore, we have used a decline of ≥ 0.1 m/s over time to indicate functional decline, which is considered a clinically meaningful change (23). As a subjective measure for physical function, we have used self-reported functional limitations defined as reports of having any difficulty walking a quarter mile or climbing 10 steps. The advantage of these measurements is that they are easy to determine in clinical practice, and, more importantly, they reflect the perceived physical function. Based on these questions, people with higher risk for developing mobility limitations can be identified. These persons can be advised to change diet or physical activity habits to improve body composition and thereby prevent or reduce the loss in physical function. A disadvantage of the subjective measures is, however, that the self-reported data might have been influenced by factors which could influence the current situation such as depression. This could result in potential misclassification and thereby less accurate associations. We tried to avoid this bias by defining mobility disability as two consecutive reports of having difficulty walking one-quarter mile or climbing 10 steps (Chapter 3), and therefore it is unlikely that our results are biased.

We conclude that a comprehensive assessment of physical function, including multiple measurements of objective and subjective measures, captures complementary aspects of physical function that are relevant for geriatric care to intervene according to patients' needs.

Determination of plasma polyunsaturated fatty acids

In Chapter 6 and 7, we have investigated the association between circulating (plasma) PUFAs with muscle composition and physical function. The advantage of measuring PUFAs in plasma is that it provides a more precise measure of PUFAs status compared to dietary estimates based on food frequency questionnaires or 24-h recall data. It is thereby not prone to recall bias. In addition, it allows ascertaining a wide spectrum of PUFAs. Regarding the correlation between intake and biomarkers, n-3 PUFA intake is in general moderately/good correlated with circulating PUFAs, especially for eicosapentaenoic acid and docosapentaenoic acid (24, 25). This is, however, not the case for n-6 PUFAs, where previous studies have shown low/weak correlations (26, 27). A low correlation means that the variance is mainly explained by other factors, such as measurement errors in both intake and plasma data, the range of intake (28), or the variation in metabolism (29). These factors should be taken into account depending on the purpose of the determination of PUFA status, e.g. assessing metabolic complications vs. describing dietary habits. Studies included in this thesis only had one single determination of PUFAs (baseline examination). Consequently, drawing conclusions about the influence of changes in plasma PUFAs on muscle composition and physical function is not possible. It would be of additional value to have multiple measurements of PUFAs to examine the potential role of dynamic plasma PUFAs in relation to muscle composition and physical function. Previous research has shown that dietary intake among older adults significantly changes with aging, with a substantial decrease in total energy intake (30), especially among older adults with a poor appetite (31, 32). However, seafood consumption (n-3 PUFAs) increases with aging (33-35). We did try to capture the limitation of a one-time measurement of PUFAs by investigating fish oil consumption determined at three different time points in life. However, no associations between fish oil consumption in relation to muscle composition and physical function were observed. We recommend that future studies should have multiple measurements of nutrients or dietary components to capture potential changes in consumption. In addition, studies among (younger) people with larger ranges in PUFA concentrations are warranted to determine these influences on muscle composition or physical function.

RELEVANCE OF CURRENT THESIS FOR FUTURE RESEARCH AND CLINICAL PRACTICE

Studies have shown that the number of obese older adults will grow excessively (36). This disproportionate increase is a result of the greater prevalence of younger obese people in the previous decades. Currently, seventeen percent of the youth, and over one-third of the adults in the United States are obese (37). Prevalences of obesity in Europe (38) and China (39) are lower, however, numbers still seem to increase. These numbers are alarming since we are aware of the detrimental effects of obesity, especially in combination with low muscle mass, low muscle strength, and greater muscle fat infiltration. It is important to provide effective interventions aiming at the prevention of obesity and/or reducing the current prevalence of obesity, in order to reduce poor physical function and early mortality in old age.

Future research

As an extension of our results from Chapter 2, it would be of interest for future studies to investigate long term changes in body weight with more specific changes in body composition, including muscle fat infiltration. The changes in body composition might in turn be associated with greater decline in physical function or increased mortality risk, however, future studies are warranted to confirm this hypothesis.

We did not observe racial differences in the association between BMI trajectories and change in lean mass or physical function in our study sample. However, it could be possible that associations between muscle composition measures with physical function are more pronounced in blacks compared to whites. This is likely since previous studies have found that African American people have greater amounts of total-body intermuscular fat compared to Asians or whites (40). These greater amounts might, therefore, explain the lower muscle quality, greater losses in strength or physical function among blacks (41, 42). Future studies investigating muscle composition measures with physical function should include individuals with diverse ethnical backgrounds to investigate potential differences.

In our study population, muscle strength and muscle mass showed comparable associations with physical function or mortality risk. Since muscle strength is easier to assess than muscle mass, it is important to determine sex, age, and BMI-specific cut-off values for grip and knee extension strength. These cut-off values can be used in clinical practice to identify older adults at risk for greater decline in physical function and who could potentially benefit from interventions aiming at improving muscle strength.

However, it should be mentioned that knee extension strength is more difficult to measure in clinical practice compared to grip strength. In addition to determination of cut-off values, secondary data analyses from intervention studies could help determine which diet and type of resistant training is the most effective. Furthermore, secondary data analyses could help identify which individuals respond to diet and resistance training intervention which might lead to more efficient treatment.

Limited studies have investigated the associations between body composition and muscle composition in relation to physical function in frail older adults. It could be possible that muscle strength is even stronger associated with physical function among older adults who are already coping with problems in physical function. Consequently, recommendations to improve body composition might be different. This is particularly of interest in the geriatric care where multiple comorbidities often occurs and the need for multiple health care providers is imperative (43). Future studies should include the oldest old, in addition to people with current problems with physical function or mobility disability.

Our results for PUFAs in relation to thigh-muscle composition and function were not convincing. However, it might still be possible that e.g. changes in PUFAs or PUFAs in study populations with different ethnic backgrounds, or with a larger diversity in blood levels do show associations. Therefore, future prospective studies with multiple measurements of circulating PUFAs investigating potential benefits of PUFAs on muscle composition and physical function are warranted. If positive associations will be observed, it is of interest to perform randomized controlled trial to investigate whether supplementation of PUFAs in a general older population will improve muscle composition and physical function.

Clinical practice

Our observed results in combination with previous findings from epidemiological and intervention studies have led to recommendations for clinical practice.

Monitoring weight in older adults

Monitoring weight in older adults is important, because fluctuations in weight may reflect declining health (44). We have shown that over a time span of 9 years all older men and women lost weight, however, no difference in rate of weight loss between trajectories was observed. In addition, differences in decline in gait speed and leg strength (men), and decline in grip strength (women) were observed for people with higher BMI at baseline and over time (Chapter 2). Previous studies have also shown that weight cycling (weight

loss and regain) was associated with a net loss of lean mass (45), or with a net gain of fat mass (46), thus indicating that weight cycling deteriorates body composition in old age. In addition, several studies have shown that weight loss (47-52) and weight cycling (49-51) are associated with increased mortality risk. This increased risk might be explained by unintentional weight loss due to underlying illness (52), since intentional weight loss has been associated with lower mortality risk (53). We recommend that weight in old age should be measured multiple times to acquire patterns of weight (loss), and detect older adults with potentially accelerated loss of muscle mass. Programs aimed at improving body composition could then be provided. Monitoring of weight could be done by a general practitioner. However, frequency of measuring and the feasibility of being measured by a general practitioner should be investigated.

Optimization of body composition in obese older adults

Previous trial studies have shown that diet-induced weight loss in obese older adults leads to metabolic and functional benefits (54). Unfortunately, it also results in an accelerated decrease in muscle mass (54-56). One randomized controlled trial among obese older adults investigated the effect of dietary restriction, exercise, and a combination of dietary restriction and exercise on physical function. Results show that the combination of diet and exercise leads to greater improvements in physical function, than diet or exercise alone. In addition, dietary restriction and exercise resulted in a lower decrease in muscle mass compared to the diet group, whereas the exercise group had an increase in muscle mass (57). Thus, in order to preserve or even increase muscle mass in obese older persons, the combination of diet-induced weight loss with physical activity (both endurance and resistance training) is important to retrieve a healthy body weight and body composition. Numerous randomized controlled trials have been performed to investigate the effect of dietary restriction with exercise on body composition and physical function in obese older adults. All show comparable results and have confirmed that dietary restriction in combination with resistance training among obese older adults is more effective for lowering body fat and preserving muscle mass (58-61). With regard to specific fat depots, dietary restriction and exercise-induced weight loss resulted in reductions in thigh muscle fat infiltration and visceral fat. However, exercise resulted in a twofold greater reduction in thigh muscle fat infiltration and visceral fat compared to dietary restriction (62). Based on the findings of these trials, and our findings of Chapter 4 and 5, we conclude that physical activity (both endurance and resistance training) in combination with dietary restriction in obese older adults may greatly contribute to healthy aging, particularly to preserve muscle mass and reduce fat mass and harmful fat depots such as intermuscular fat and visceral fat.

Optimization of body composition in normal weight older adults

As with obese older adults, it is important for normal weight older adults to maintain/improve body composition. This could be achieved by a healthy diet including physical activity (both endurance and resistance training) (63, 64).

CONCLUSIONS

Clear changes in body fat depots and muscle composition are observed with aging, including a loss of muscle mass, loss of muscle strength and increased muscle fat infiltration. Results from this thesis indicate that older adults with worse body composition (larger fat depots, poorer muscle composition and lower strength) are more likely to develop physical limitations and are at higher mortality risk. Our results do not support a major role of polyunsaturated fatty acids in muscle composition, strength and physical function. In view of global aging, increasing obesity prevalences in old age, and the considerable prevalence of older persons with functional limitations and thereby the concomitant greater dependence on society and health care, it is important to provide advice and effective programs aimed at improving body composition. Even in old age, strength training (combined with dietary energy restriction for those who are obese) will result in a reduction in detrimental fat depots and muscle fat infiltration, and will increase muscle mass and muscle strength. Future intervention trials are required to investigate the long-term effects of improving body composition in relation to the delay or prevention of (further) physical decline, and premature death.

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Summary

The central topic of the studies in this thesis is (changes in) body composition in relation to healthy aging. We investigated 1) different patterns of BMI in relation to change in lean mass and physical function, 2) which fat depots and the density of those specific fat depots are associated with physical function, 3) which thigh-muscle composition is associated with physical function and survival, and finally 4) whether polyunsaturated fatty acids are associated with thigh-muscle composition or physical function. This summary provides an overview of the study results described in this thesis.

Trajectories of BMI in old age and the associations with concomitant change in lean mass and physical function

Previous research has shown that obesity in old age is associated with greater risk of functional decline compared to normal weight or overweight. However, it is unclear whether different trajectories of BMI relate to changes in body composition or physical function. In **Chapter 2** we were interested in trajectories of BMI, and the contemporaneous changes in appendicular lean mass (determined by dual-energy X-ray absorptiometry) and physical function per specific BMI trajectory. We investigated this question among 998 black and white participants with a mean age of 73.1 years of the Health ABC Study. There was no interaction between race and BMI, and therefore results were presented for blacks and whites together. In both men and women, four distinctive trajectories with significantly different BMI at baseline were detected. All trajectories showed a modest decline in BMI over a time span of 9 years. The rate of decline was different between trajectory groups for women, but not for men. Based on solely the BMI trajectories, it is unclear whether this decline (weight loss) represents loss in fat mass and/or lean mass. We showed that women in the highest trajectory (mean BMI at baseline 34.9 kg/m^2) lost relatively more lean mass in the arms than those in the lowest trajectory (mean BMI at baseline 20.5 kg/m^2). There was no difference in body composition across trajectories among older men. In addition to changes in lean mass, changes in strength (grip strength and leg strength) and physical function (gait speed) were assessed. We observed that men in the highest trajectory (mean BMI at baseline 33.9 kg/m^2) had a greater decrease in gait speed and lost more leg strength than those in the lowest trajectory (mean BMI at baseline 22.9 kg/m^2). No differences in physical function across trajectories were observed for women. Our different results for men and women may be explained by duration and type of activity. I.e. older men are overall less sedentary and report more often greater-intensity activity compared to older women. An important conclusion of our results is that BMI changes modestly over time, and that, compared to older adults with a healthy weight, women with the highest weight at old age lose relatively more lean mass in the arms, and older men with the highest weight have

relatively greater decreases in gait speed and leg strength. This indicated the importance of healthy weight during life.

Type and location of fat depots with physical function

Obesity is associated with increased risk for physical dysfunction and mortality. However, limited research has been performed investigating specific fat depots in relation to mobility limitation and physical performance. In **Chapter 3** we examined the association of BMI and areas and densities of several fat depots (determined by computed tomography) with incident self-reported mobility disability and poor performance (gait speed < 1.0m/s). The results based on data of 3011 participants of the Health ABC Study suggest that even in old age, higher BMI, greater visceral adipose tissue, greater abdominal subcutaneous adipose tissue, greater thigh subcutaneous adipose tissue, and greater thigh intermuscular adipose tissue (IMAT) were associated with increased risk of mobility disability or poor performance in a basic model. However, adjustment for e.g. physical activity and midlife weight attenuated the associations for some areas, but BMI and thigh IMAT area remained robustly associated with risk of mobility limitation and poor performance into late life. Higher densities of different fat depots were also associated with an increased risk of incident mobility disability and poor performance, although risk relations were less convincing than for fat area and generally not independent of BMI. These results suggest that interventions aiming at lowering IMAT in addition to the promotion of healthy body weight are necessary.

Muscle composition, strength and physical function

Limited studies have examined the association between muscle composition (muscle mass and muscle fat infiltration) in relation to subjective and objective indicators of physical function. **Chapter 4** describes which thigh muscle composition (determined by computed tomography) were related to incident mobility disability and change in gait speed, using data from the AGES-Reykjavik Study. Among 2725 men and women, mean age 74.8 years, greater thigh muscle strength and thigh muscle mass (cross-sectional muscle area) were associated with decreased risk of mobility disability and a slower decline in gait speed. Muscle fat infiltration was not associated with either mobility disability or decline in gait speed. The contradictory results regarding IMAT in comparison to other studies requires additional studies to clarify the potential role of muscle fat infiltration in physical function. In view of clinical practice, it may be advised to determine leg strength in geriatric care since leg strength and thigh muscle mass (which only can be determined using advanced techniques) show comparable results. Using that data, cut-off values could be determined, which in turn could be used to identify older adults with an increased risk of functional

decline. These individuals could benefit from additional care, such as nutritional or physical support, to prevent further functional decline.

Muscle composition, strength and all-cause mortality

Previous studies investigating muscle composition with mortality risk mainly used bioelectrical impedance or dual-energy X-ray absorptiometry to determine muscle composition, and show inconsistent results. Computed tomography imaging gives more precise measures of muscle composition. In **Chapter 5** we determined mortality risk after 8.8 years of follow-up for different thigh muscle composition measures assessed by CT imaging, and leg strength. Results show that greater thigh muscle mass (cross-sectional muscle area), greater strength, and higher muscle quality (strength per area) were associated with lower mortality risk among 4824 men and women with a mean age of 76.4 years from the AGES-Reykjavik Study. In contrast, more muscle fat infiltration (both fat between and within the muscle) was associated with higher mortality risk. These results emphasize the importance of greater thigh muscle strength and thigh muscle mass, and lesser muscle fat infiltration to reduce the risk of premature death.

Polyunsaturated fatty acids in relation to muscle composition and strength

Previous studies in cancer patients have shown that supplementation of n-3 polyunsaturated fatty acids (PUFAs) may result in better maintenance of muscle mass during chemotherapy. It is possible that PUFAs are associated with improved muscle composition due to the incorporation of PUFAs in muscle membranes and thereby improve function. However, large, population-based longitudinal data in older adults who are at risk of functional decline are lacking. In **Chapter 6** we examined the association between plasma PUFAs with thigh muscle mass, muscle fat infiltration (both determined by computed tomography) and grip and knee extension strength. Cross-sectional associations were determined among 836 participants and longitudinal associations (after a median of 5.2 years) were determined among 459 participants from the AGES-Reykjavik Study. Cross-sectional results show that higher concentrations of total PUFAs were associated with larger thigh muscle mass and with greater knee extension strength. Higher concentrations of arachidonic acid were associated with lower muscle mass. Higher linoleic acid concentrations were associated with less muscle fat infiltration. In contrast, higher concentrations of eicosapentaenoic acid were associated with more intermuscular adipose. Longitudinal analyses only showed positive associations for alpha-linolenic acid concentrations with increased knee extension strength. In addition to plasma PUFAs and muscle composition, we also determined whether fish oil consumption was associated with muscle composition, however, no independent associations were observed. Our

results show inconsistent relationships of PUFAs with thigh muscle mass, muscle fat infiltration and strength, and little evidence of their role in change in muscle composition.

Polyunsaturated fatty acids in relation to physical function

Previous cross-sectional studies have shown positive associations between concentrations of PUFAs with physical function, however, longitudinal studies using data on circulating PUFAs are limited. **Chapter 7** describes the associations between plasma phospholipid n-3 and n-6 PUFAs with risk of incident self-reported mobility disability (having much difficulty, or being unable to walk 500 meters, or climb up 10 steps) and objectively measured gait speed decline after 5.2 ± 0.2 years. Data from the AGES-Reykjavik Study were used and included 556 men and women with a mean age of 75.1 years. Higher concentrations of total n-3 PUFAs and docosahexaenoic acid were associated with lower mobility disability risk among women only. No associations with gait speed were observed. We also investigated the association between fish oil consumption and physical function. Our results do not support a major role for fish oil consumption in preventing mobility disability or decline in gait speed. We cannot explain why associations were observed among women and subjective measures of physical function only.

CONCLUSION

In this thesis we examined various measures of body composition, fat depots and muscle composition, in relation to physical function and survival. Our results provide further evidence that a better body composition in old age, defined as greater muscle mass, higher muscle strength and lower muscle fat infiltration, is associated with better physical function and lower mortality risk. We recommend that intervention programs for obese older adults focus on weight loss by caloric restriction and resistance training in order to improve body composition and thereby lower the risk of functional impairments and premature death. Regarding normal weight older adults, resistance training alone should be recommended to improve body composition.

We also examined associations between polyunsaturated fatty acids with muscle composition and physical function. We did not observe strong associations for polyunsaturated fatty acids in relation to muscle composition and physical function, however, this might be due to our single measurement of polyunsaturated fatty acids or our population with relatively high fish intake. Therefore, more studies investigating changes in polyunsaturated fatty acids in different study populations are required to determine the potential role of polyunsaturated fatty acids on muscle composition and physical function.

Samenvatting

Het doel van dit proefschrift is het bepalen van de relatie tussen verschillende maten van lichaamssamenstelling en gezond ouder worden. We hebben onderzocht: 1) welke patronen van BMI er zijn en hoe deze patronen zich verhouden tot verandering in spiermassa en fysiek functioneren, 2) welke vetdepots in het lichaam geassocieerd zijn met fysiek functioneren, 3) welke eigenschappen van de dijspier geassocieerd zijn met fysiek functioneren en overlijdensrisico, en tot slot 4) welke meervoudig onverzadigde vetzuren geassocieerd zijn met eigenschappen van de dijspier en fysiek functioneren. Deze samenvatting bevat een overzicht van de resultaten besproken in dit proefschrift.

Patronen van BMI en de associaties met gelijktijdige verandering in spiermassa en fysiek functioneren bij ouderen

Eerder onderzoek heeft laten zien dat obesitas bij ouderen geassocieerd is met een hoger risico op fysieke achteruitgang in vergelijking tot ouderen met normaal gewicht of overgewicht. Het is echter niet bekend of verschillende patronen van BMI gerelateerd zijn aan veranderingen in lichaamssamenstelling of fysiek functioneren. In **Hoofdstuk 2** waren wij geïnteresseerd in patronen van BMI en de gelijktijdige verandering in spiermassa (bepaald met dual-energie X-ray absorptiometrie) en fysiek functioneren per BMI patroon. We hebben deze relatie bepaald in 998 Afro-Amerikaanse en blanke deelnemers van de Health ABC Study met een gemiddelde leeftijd van 73.1 jaar. Er was geen verschil in resultaten voor Afro-Amerikaanse en blanke deelnemers, vandaar dat de resultaten samengevoegd zijn. Bij zowel mannen als vrouwen werden vier verschillende patronen in BMI geobserveerd. Gedurende een periode van 9 jaar werden alle patronen gekenmerkt door een bescheiden afname in BMI. De snelheid in afname was verschillend per BMI patroon voor vrouwen, maar niet voor mannen. Op basis van alleen de BMI patronen is het niet duidelijk of de afname in BMI (gewichtsverlies) verklaard wordt door een verlies in vetmassa en/of spiermassa. Wij hebben aangetoond dat vrouwen met het BMI patroon gekarakteriseerd door een hoge BMI (hoogste gemiddelde BMI bij aanvang van de studie, gemiddelde was 34.9 kg/m^2) relatief meer spiermassa in de armen verloren dan vrouwen met het BMI patroon gekarakteriseerd door het laagste gemiddelde BMI (laagste gemiddelde BMI bij aanvang van de studie, gemiddelde was 20.5 kg/m^2). Er was geen verschil in verandering in lichaamssamenstelling voor mannen met verschillende BMI patronen. We hebben ook onderzocht of verandering in spierkracht (handknijpkracht en spierkracht in het bovenbeen) en fysiek functioneren (loopsnelheid) verschilt per BMI patroon. Onze resultaten lieten zien dat mannen met het BMI patroon gekarakteriseerd door een hoge BMI (hoogste gemiddelde BMI bij aanvang van de studie, gemiddelde was 33.9 kg/m^2) relatief een grotere afname hadden in loopsnelheid en spierkracht in het bovenbeen dan mannen met het BMI patroon gekarakteriseerd door het laagste

gemiddelde BMI (laagste gemiddelde BMI bij aanvang van de studie, gemiddelde was 22.9 kg/m^2). Er was geen verschil in verandering in fysiek functioneren bij vrouwen met verschillende BMI patronen. De verschillende resultaten voor mannen en vrouwen kunnen mogelijk verklaard worden door verschil in tijdsduur en type van lichaamsbeweging. Over het algemeen zijn oudere mannen minder sedentair en rapporteren zij vaker activiteiten met een hogere intensiteit dan oudere vrouwen. Op basis van onze studie kunnen wij concluderen dat BMI slechts gering afneemt en dat vergeleken met ouderen met een gezond gewicht, oudere vrouwen met het hoogste lichaamsgewicht relatief meer spiermassa in de armen verliezen, en oudere mannen met het hoogste lichaamsgewicht relatief een grotere afname hebben in loopsnelheid en spierkracht in het bovenbeen. Dit onderzoek toont het belang aan van een gezond gewicht gedurende het leven.

BMI, vetdepots en fysiek functioneren

Obesitas is geassocieerd met een verhoogd risico op fysiek disfunctioneren en overlijden. Beperkt onderzoek is gedaan naar de relatie tussen verschillende opslagruimtes voor vet en beperkingen in fysiek functioneren. In **Hoofdstuk 3** hebben wij gekeken naar BMI, de grootte en dichtheid van verschillende opslagruimtes voor vet (bepaald met computed tomography) in relatie tot beperkingen in zelf-gerapporteerde mobiliteitsbeperkingen en loopsnelheid. De resultaten zijn gebaseerd op gegevens van 3011 deelnemers van de Health ABC Study. Deze laten zien dat zelfs op oudere leeftijd, hoger BMI, meer visceraal vet (het vet in de buikholte rondom de organen), meer vet onder de huid van de buik, meer vet onder de huid van het bovenbeen, en meer vet in de bovenbeenspier geassocieerd is met een verhoogd risico op beperkingen in mobiliteit en slechte fysieke prestatie in een simpel statistisch model. Echter, bijna alle associaties verzwakten wanneer we in de analyses rekening hielden met fysieke activiteit en lichaamsgewicht tijdens het leven. De associatie tussen BMI en vet in de bovenbeenspier met beperkingen in mobiliteit of fysieke prestatie hield daarentegen stand. Een hogere dichtheid van het vetweefsel van de verschillende opslagruimtes voor vet was ook geassocieerd met een verhoogd risico op beperkingen in mobiliteit en slechte fysieke prestatie. Deze risico's zijn wel minder overtuigend dan de grootte van de opslagruimtes voor vet en over het algemeen niet onafhankelijk van BMI. Deze bevindingen suggereren dat programma's nodig zijn die verlaging van vet in de spier in combinatie met het stimuleren van een gezond lichaamsgewicht als doel hebben.

Spiersamenstelling, spierkracht en fysiek functioneren

Slechts een beperkt aantal studies heeft de associatie tussen spiersamenstelling (spiermassa, vetinfiltratie in de spier) met subjectieve en objectieve indicatoren van fysiek

functioneren bepaald. **Hoofstuk 4** beschrijft welke componenten van de bovenbeenspier (bepaald met computed tomography) gerelateerd zijn aan beperkingen in de mobiliteit en verandering in loopsnelheid. Dit hebben we onderzocht in de AGES-Reykjavik Study. Bij 2725 mannen en vrouwen met een gemiddelde leeftijd van 74.8 jaar, was meer spierkracht in het bovenbeen en meer spiermassa geassocieerd met een lager risico op beperkingen in de mobiliteit of langzame loopsnelheid. Vet in de spier was daarentegen niet geassocieerd met deze uitkomsten. De tegenstrijdige resultaten, wat betreft vet in de spier in vergelijking tot resultaten van andere studies, vereist extra onderzoek om de mogelijke rol van vet in de spier in relatie tot fysiek functioneren te verduidelijken. In het kader van de klinische praktijk kan geadviseerd worden om spierkracht in de benen te bepalen, vooral aangezien de resultaten voor spierkracht en spiermassa (welke alleen met behulp van geavanceerde technieken bepaald kunnen worden) vergelijkbaar zijn. Met behulp van die data zouden afkappunten bepaald kunnen worden, welke later bruikbaar zouden kunnen zijn om personen te identificeren met een verhoogd risico op functionele achteruitgang. Bij deze personen zouden dan door middel van extra zorg, zoals voedings- of fysieke ondersteuning, verdere fysieke achteruitgang beperkt kunnen worden.

Spiersamenstelling, kracht en overlijdensrisico

Eerdere onderzoeken waarbij de associatie tussen spiersamenstelling en overlijdensrisico bepaald is, hebben voornamelijk bio-elektrische impedantie of dual-energie X-ray absorptiometrie gebruikt om de spiersamenstelling te bepalen. Deze studies laten inconsistente resultaten zien. Computed tomography is een geavanceerde techniek waarbij de spiersamenstelling nog preciezer bepaald kan worden. In **Hoofdstuk 5** hebben we het overlijdensrisico na 8,8 jaar voor verschillende componenten van de bovenbeenspier (bepaald met computed tomography) en spierkracht bepaald. De resultaten lieten zien dat meer spiermassa, meer bovenbeenspierkracht en een grotere spierkwaliteit (kracht per massa) geassocieerd waren met een lager overlijdensrisico bij 4824 mannen en vrouwen met een gemiddelde leeftijd van 76.4 jaar van de AGES-Reykjavik Study. Daarentegen, meer vetinfiltratie in de spier (zowel vet tussen de spieren als in de spier) was geassocieerd met een hoger overlijdensrisico. Onze resultaten benadrukken het belang van meer spiermassa, meer kracht en minder vet in de spier om het risico op vroegtijdig overlijden te verkleinen.

Meervoudig onverzadigde vetzuren in relatie tot spiersamenstelling en spierkracht

Resultaten van onderzoek bij patiënten met kanker hebben laten zien dat suppletie met meervoudig onverzadigde n-3 vetzuren mogelijk resulteert in behoud van spiermassa tijdens chemotherapie. Het is mogelijk dat de spiersamenstelling verbetert door

incorporatie van vetzuren in de spiermembranen en dat daardoor de spierfunctie verbetert. Er zijn slechts weinig grote prospectieve studies bij ouderen met risico op fysieke achteruitgang uitgevoerd om deze hypothese te toetsen. In **Hoofdstuk 6** hebben we de relatie tussen concentraties van vetzuren in het bloed met massa van de bovenbeenspier, vet in de spier (beide bepaald met computed tomography), handknijpkracht en spierkracht in het bovenbeen bepaald. Cross-sectionele associaties zijn vastgesteld bij 836 deelnemers en longitudinale associaties (na gemiddeld 5.2 jaar) bij 459 deelnemers van de AGES-Reykjavik Study. De cross-sectionele resultaten laten zien dat hogere concentraties van het totaal aantal meervoudig onverzadigde vetzuren geassocieerd waren met meer spiermassa in het bovenbeen en met meer spierkracht in het bovenbeen. Een hogere concentratie van arachidonzuur was geassocieerd met minder spiermassa in het bovenbeen. Een hogere concentratie van linolzuur was geassocieerd met minder vet in de spier. Daarentegen, een hogere concentratie van eicosapentaeenzuur was geassocieerd met meer vet in de spier. Longitudinale associaties tonen aan dat alfa-linoleenzuur concentraties geassocieerd waren met meer spierkracht in het bovenbeen. Naast associaties tussen concentraties van vetzuren in het bloed is ook onderzocht of de inname van visolie geassocieerd was met spiersamenstelling. Er zijn geen onafhankelijke associaties gevonden. Onze resultaten laten inconsistente relaties en weinig bewijs voor een rol van vetzuren in de verandering in spiersamenstelling zien.

Meervoudig onverzadigde vetzuren in relatie tot fysiek functioneren

Eerder cross-sectioneel onderzoek heeft positieve associaties tussen meervoudig onverzadigde vetzuren en fysiek functioneren laten zien, echter data afkomstig van longitudinale studies zijn beperkt. **Hoofdstuk 7** beschrijft associaties tussen concentraties van meervoudig onverzadigde n-3 en n-6 vetzuren en het risico op zelf-gerapporteerde mobiliteitsbeperkingen (moeite hebben met, of verhinderd zijn om 500 meter te lopen of 10 traptreden te lopen) en objectief gemeten verandering in loopsnelheid, gemeten na 5.2 ± 0.2 jaar. We hebben gebruik gemaakt van gegevens van de AGES-Reykjavik Study en hebben 556 mannen en vrouwen met een gemiddelde leeftijd van 75.1 geïncludeerd. Hogere concentraties van totaal meervoudig onverzadigde n-3 vetzuren en docosahexaeenzuur in (de fosfolipidenfractie van) het bloed waren geassocieerd met lager risico op beperkingen in de mobiliteit bij vrouwen, maar niet bij mannen. Geen associaties met veranderingen in loopsnelheid zijn gevonden. Onze resultaten laten ook geen grote rol voor visolie-inname zien bij het voorkomen van beperkingen in de mobiliteit of verandering in loopsnelheid. We kunnen het verschil in waarneming tussen mannen vs. vrouwen en subjectieve vs. objectieve maat van fysiek functioneren niet verklaren.

CONCLUSIE

In dit proefschrift hebben we de relatie tussen verschillende maten van lichaamssamenstelling, opslagruimtes voor vet en spiersamenstelling met fysiek functioneren en overleving bepaald. Onze resultaten tonen aan dat betere lichaamssamenstelling, gedefinieerd als meer spiermassa, grotere spierkracht en minder vet in de spier, geassocieerd is met beter fysiek functioneren en lager overlijdensrisico. Wij raden aan dat interventieprogramma's voor obese ouderen moeten focussen op gewichtsverlies door middel van calorierestrictie en krachttraining, om zo de lichaamssamenstelling te verbeteren en daardoor het risico op functionele beperkingen en vroegtijdig overlijden te verkleinen. Wat betreft ouderen met een normaal gewicht adviseren wij alleen krachttraining om de lichaamssamenstelling te verbeteren.

We hebben ook de relatie tussen meervoudig onverzadigde verzuren in relatie tot spiersamenstelling en fysiek functioneren onderzocht. Wij hebben geen eenduidige associaties voor meervoudig onverzadigde verzuren met spiersamenstelling of fysiek functioneren gevonden. Dit is mogelijk te verklaren door de eenmalige meting van vetzuren, of doordat wij een steekproef hadden met een relatief hoge visconsumptie. Er zijn daarom meer studies nodig die veranderingen in meervoudig onverzadigde vetzuren in verschillende onderzoekspopulatie onderzoeken en daarbij de mogelijke rol met spiersamenstelling en fysiek functioneren kunnen bepalen.

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- Ilse

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1. **Reinders I.**, Murphy R.A., Brouwer I.A. *et al*. Muscle Quality and Myosteatosis: Novel associations with Mortality risk; the Age, Gene/Environment Susceptibility-Reykjavik Study. American Journal of Epidemiology. 2016: 183; 53-60.
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4. **Reinders I.**, Murphy R.A., Song X. *et al*. Polyunsaturated fatty acids in relation to incident mobility disability and decline in gait speed; the Age, Gene/Environment Susceptibility-Reykjavik Study. European Journal of Clinical Nutrition. 2015: 69; 489-493.
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About the author

Ilse Reinders was born on June 5th 1984 in Harderwijk, the Netherlands. After graduating from high school (VWO) at the R.S.G. het Slingerbos in Harderwijk in 2003, she moved to Amsterdam to study Human Movement Sciences at the VU University Amsterdam. Since she became more interested in nutrition, she started studying Nutrition and Dietetics at the Hogeschool van Amsterdam in 2005, where she graduated in 2009. Subsequently, she studied Health Sciences at the VU University from 2009-2011 and obtained her Master of Science degree (Cum Laude) in Nutrition and Health. Her MSc thesis was performed at the University of Kuopio, Finland where she wrote an article regarding polyunsaturated fatty acids and inflammation, using data from the Kuopio Ischemic Heart Disease Risk Factor Study.

She started working as a junior researcher at the department of Health Sciences, VU University Amsterdam in 2011. She focused on polyunsaturated fatty acids in relation to echocardiographic measures. In addition, she worked on projects related to parathyroid hormone in relation to cardiovascular events and mortality.

In 2013, she started her PhD-project on the role of muscle and fat in physical function and the relationship with survival at the National Institutes of Health, National Institute on Aging, Bethesda, Maryland, U.S.A. under supervision of prof.dr. Marjolein Visser, prof.dr. Ingeborg Brouwer and dr. Tamara Harris. During her stay in the U.S.A., she performed research described in this thesis, but was also involved in several other projects and co-authored multiple scientific articles.

Since March 2015 she works at the VU University Medical Center, Amsterdam as a scientific researcher investigating treatable malnutrition among older adults, under supervision of prof.dr. Marjolein Visser. In addition, Ilse works for the Longitudinal Aging Study Amsterdam, where she is focused on nutrition in relation to healthy aging.