Muscle mass and physical function in ageing: the effects of physical activity and healthy diet
“Science is a way of thinking much more than it is a body of knowledge.”

— Carl Sagan
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Physical function and muscle mass in ageing: 
the effects of physical activity and healthy diet

by

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Abstract


Ageing is associated with a gradual deterioration in physical function, accompanied by a decrease in muscle mass, leading to loss of independency. In this respect, physical activity and healthy diet represent key lifestyle factors with potential to delay onset of age-related physical disability. The overall aim of the present thesis was to explore the effects of physical activity behaviours in general and resistance training (RT) in particular, with or without addition of a healthy diet (HD), on muscle mass and physical function in older community-dwelling women. A main finding was that physical activity of at least moderate intensity at old age infers beneficial effects on physical function, even in individuals with a previously sedentary lifestyle. Additionally, engagement in exercise-related activities during middle age years is linked to better physical function and higher muscle mass at old age, regardless of present physical activity level. This thesis further highlights that in older women RT combined with HD rich in omega-3 polyunsaturated fatty acids elicits significant gains in muscle mass, whereas no corresponding gain was induced by RT alone. Likewise, larger improvements in muscle strength and physical function were evident in response to combined effects by RT and HD compared to RT alone. Taken together, findings from this thesis support public health efforts aiming to promote physical activity of at least moderate intensity together with a healthy diet rich in omega-3 polyunsaturated fatty acids in order to combat age-related decline in muscle mass and physical function.

Keywords: Healthy ageing, Sarcopenia, Dynapenia, Functional capacity, Resistance training, Omega-3 fatty acids, Muscle mass, Body fat

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List of publications

This doctoral thesis is based on the following original papers, which will be referred to in the text by their Roman numerals.


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Other publications by the author not included in the thesis:


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Introduction

Ageing

The average life expectancy of the human population worldwide is increasing rapidly, and older adults now represent the fastest growing age group. The United Nations predicts that people aged 65 and over will triple, from 11% in 1950 to no less than 33% (equivalent to ca 2 billion) in 2050 (UN, 2015). A similar trend has been observed in Sweden where the number of older adults (>64 years of age) is predicted to rise from 1.5 million in 2000 to 3 million in 2060 (SCB, 2017). Given that the number of young and middle-aged adults (20–64 years of age) is foreseen to increase by a meagre 0.5 million during the same time, the proportion of older adults in Sweden will increase dramatically from approximately 15% in 2000 to 25% in 2050. These major demographic changes are primarily due to an increased life expectancy combined with a decline in birth rate (Harper, 2014). As ageing is associated with deteriorating health and an increased need for hospitalization, this demographic shift presents major societal challenges, not least an increased burden on the health care system and increased care costs. While increases in life expectancy can be seen as a triumph of medical, economic and social advancements, future progression will depend on whether we are successful in adding healthy years, and years without disability, to our lives. In this context, one must separate primary ageing, i.e. the innate maturational processes, from secondary ageing, i.e. effects of disease and the environment. While the first is an inevitable process, the latter represents physiological changes that are reversible and sensitive to physical activity behaviour and nutritional intake (Busse & Pfeiffer, 1969). Therefore, in order to address ageing-related societal challenges, health promotion among older adults is
essential. Accordingly, more research is needed to identify factors that prevent disability and improve physical function and thereby promote a healthy ageing.

**Physical function**

One of the most striking effects of ageing is the progressive deterioration in physical performance, which represents the ability to perform both basic and instrumental activities of daily living (Kalache & Kickbusch, 1997). This decline in physical performance leads to an impaired ability to perform daily tasks, loss of independence, development of disability and frailty and an increased all-cause mortality (Cooper, Kuh, & Hardy, 2010). In the European Union, prevalence of reported functional limitations in daily activities affects 40% of adults aged 65 years and older and >60% of those aged 75 years and older (EU, 2015). Notably, the prevalence of functional limitations and frailty is higher in older women than in men, which is likely due to lower physical capacity in women (Katz et al., 1983). For example, a study on disabled adults (55 years and older) in the Netherlands between 1990 and 1999 reports that 33.2% of women and 19.7% of men were disabled at the follow-up 6 years later, (Tas et al., 2007). Therefore it has been suggested that special attention should be paid to the design of preventive approaches aiming to delay the loss of physical function in older women (Katz et al., 1983).

Deteriorations in physical function may not be directly apparent as older adults rarely perform exercises that tax their maximal functional capacity. However, as time passes, the margins between the maximal capacity and the capacity needed to perform normal everyday tasks, i.e. the critical threshold necessary for maintained physical function, are narrowed (Fig 1). As a result, with increased age and diminished physical function, everyday activities such as climbing stairs or getting up on a stool become increasingly difficult or even impossible to perform (Young, 1997).
The annual loss in physical function has been reported to be 1–3% in adults aged 60–85 years and these declines seem to accelerate with increased age (Alcock, O’Brien, & Vanicek, 2015). The relationship between loss of skeletal muscle strength and physical function (e.g. walking speed) seems to be curvilinear (Buchner, Larson, Wagner, Koepsell, & de Lateur, 1996). This supports the idea of a functional threshold that acts as a lower limit, after which further decreases result in significant impairments in physical function and consequently a reduced ability to carry out everyday tasks (Byrne, Faure, Keene, & Lamb, 2016).

Measurement of physical functioning can be complicated. It ranges from self-report questionnaires to performance measures of specific tasks to vigorous laboratory measures. There are advantages and limitations to each of the measurement methods; however, validated standardized tests such as the Timed Up and Go (TUG) test, Chair Stand Test, Squat Jump (SJ) test, Single-Leg Stance balance test and 6-minute walk test (6MW) are commonly used in research and clinical practise. These tests assess different dimensions of lower extremity physical function, including strength, balance and aerobic capacity, which is of importance to perform activities of daily life. In addition to single tests, it is also common to generate different physical function scores based on a number of different functional tests in combination.
Given that aggregated functional scores encompass several aspects of function, these test batteries are generally considered better in capturing overall physical function (Desjardins-Crepeau et al., 2014; Sardinha et al., 2015). Additionally, subjective measures of physical function can be assessed using self-report questionnaires (e.g. the Short Form 12 Health Survey (SF-12), or the SF-36). Such questionnaires have the advantage, that they may be distributed to a large number of subjects and that they are associated with a relatively low burden on the participants. However, as with all self-reported data, the outcome is generally less reliable compared to objective measurements. Nevertheless, inclusion of different methods that evaluate different aspects of physical function is currently recommended in order to capture a more representative picture of physical function in older adults (Branch & Meyers, 1987).

**Muscle mass and strength**

An important contributor to compromised physical function among older adults is age-related loss of skeletal muscle mass and muscular function (Doherty, 2003). Muscle mass declines by an average of 0.5–1.0% per year after the fourth decade of life in both men and women (Holloszy, 2000) (Lindle et al., 1997). This age-related loss in muscle mass has been shown to be more pronounced in the lower extremities compared with the upper extremities and trunk (Baumgartner, 2000; Doherty, 2003; Janssen, Heymsfield, & Ross, 2002). At the cellular level, the most consistent finding in old skeletal muscle is a reduction in type-II muscle fibre cross-sectional area (D’Antona et al., 2003; Grimby, 1995; Larsson, Sjodin, & Karlsson, 1978; Lexell, 1995; Lexell, Taylor, & Sjostrom, 1988; Singh et al., 1999).

The age-related decline in muscle mass is accompanied by reduced maximal and explosive muscle strength. Maximal strength and explosive strength differ in that the former represents an individual’s ability to generate maximal muscle force, while the latter represents the ability to quickly and forcefully generate muscle power (i.e. force per time). During ageing, explosive muscle strength appears to decline more rapidly than maximal muscle strength (Bassey et al., 1992; Hakkinen et al., 1996; Izquierdo, Aguado, Gonzalez, Lopez, & Hakkinen, 1999; Skelton, Greig, Davies, & Young, 1994). For example, a decline in maximal muscle strength of 1–2%
and in muscle power of 3–5% per year has been reported in the knee extensors, a muscle group instrumental in everyday activities (Fig 2) (Delmonico et al., 2009; Frontera et al., 2000; Goodpaster et al., 2006; Lauretani et al., 2003; Skelton et al., 1994). The accelerated loss in explosive strength can at least in part be explained by the selective atrophy of type II muscle fibres, which are characterized by their ability to generate high muscle force in a very short time.

Figure 2. The relationship between age and knee-extension torque (A), lower extremity power (B) and calf muscle cross-section area (C). Figure adapted from Lauretani et al., (2003)
Merged data from 50 well-conducted randomized controlled trials provide strong evidence that low levels of muscle strength and low muscle mass are related to reduced physical function in older adults. Moreover, the role of muscle strength in relation to physical function is more pronounced compared with the role of muscle mass (Rolland et al., 2008; Schaap, Koster, & Visser, 2013). Fiatarone and colleagues (Fiatarone et al., 1990) were among the first to investigate the relationship between muscle strength and physical function in elderly individuals. They reported an inverse association between maximal leg strength, walking speed and chair rising ability. Since their work, others have provided supportive evidence for a link between lower limb muscle strength and different measurements of physical function including stair climbing, balance performance, and the ability to recover from a trip or a slip (Pijnappels, Bobbert, & van Dieen, 2005; Pisciottano, Pinto, Szejnfeld, & Castro, 2014; Winters-Stone et al., 2012).

While earlier studies focused primarily on the relationship between maximal muscle strength and physical function, later studies have revealed an even greater role for explosive muscle strength. For example, one study reports that leg extensor peak power explained between 12% and 45% of the variance in various functional performances (Bean et al., 2002). Likewise, a recent review (Byrne et al., 2016) showed explosive muscle strength to be one of the strongest individual predictors of functional status in the elderly. Taken together, these studies provide evidence for the importance of maintaining explosive muscle strength to preserve physical function.

There are a wide range of tests available to assess muscle strength. For example, the handgrip strength test is frequently used, as it is easy to perform, inexpensive and highly correlated with most health parameters including mortality (Leong et al., 2015). However, as lower limb strength is more related to physical function, including gait speed, chair stand, and stair climbing ability, different tests for leg strength are often included in research studies. Common leg strength tests include the one repetition maximum test (1RM) during leg press and seated knee extension. Even though traditionally used by coaches and athletes to evaluate training progression, there is strong evidence that the assessment of 1RM is also associated with physical function in the general population (Artero et al., 2011). Other methods commonly used to evaluate muscle strength in the lower limbs include assessment of knee extension peak torque and maximal concentric force with
isokinetic and isometric dynamometers. These methods can give additional information regarding explosive muscle capacity, i.e. how fast the person can recruit and develop muscle power/force, which, as stated earlier, is regarded as a key predictor of functional capacity during ageing.

**Aerobic capacity**

Aerobic capacity reflects the ability to perform work by using oxygen and is limited by maximal oxygen consumption (VO$_2$max). As VO$_2$max quantitates an individual’s ability to perform work over sustained periods of time it is an important determinant of functional status. For example, reduced VO$_2$max have been shown to limit the speed of walking and stair climbing in both middle aged and old adults (Jette, Sidney, & Blumchen, 1990; Wagner, LaCroix, Buchner, & Larson, 1992). Moreover, large increases in VO$_2$max and physical function are generally observed in older adults after aerobic exercise training (McGuire et al., 2001). VO$_2$max declines at a rate of 3–8% per decade after the age of 30 years (Fig 3) (Paterson, Cunningham, Koval, & St Croix, 1999; Rosenberg, 1997). This means that from 30 to 80 years of age, about 50% of aerobic capacity is lost. Nevertheless, the variation in VO$_2$max decline is large enough that the range in older adults overlaps that of younger adults (Buchner, Beresford, Larson, LaCroix, & Wagner, 1992; Plowman, Drinkwater, & Horvath, 1979). Moreover, the absolute rates of VO$_2$max decline is considerably higher in sedentary adults compared to physically active adults (Dehn & Bruce, 1972). Indeed, physically active older adults lose about 0.25 ml/kg/min in VO$_2$max each year, which is merely one-third the yearly loss rate of 0.75 ml/kg/min for sedentary peers (Kasch, Boyer, Van Camp, Verity, & Wallace, 1990).

Interestingly, age-related loss of muscle mass also seems to be an important contributor to the diminished VO$_2$max seen during ageing. For example, one study showed that the age-related effect on VO$_2$max was completely attenuated after adjusting for changes in muscle mass (Fleg & Lakatta, 1988). This again highlights the importance of maintaining muscle mass to preserve physical function during ageing.
Figure 3. The relationship between maximal oxygen consumption (VO\textsubscript{2}max) and increasing age. Rate of decline is not different between sex. Figure adapted from Paterson et al., (1999)

Adiposity

In the western society, age-related loss of muscle mass and strength is often accompanied by a simultaneous increase in adiposity (St-Onge & Gallagher, 2010). In older women, increased adiposity has been shown to be related to impaired physical function independently of changes in muscle mass and strength (Batsis, Mackenzie, Lopez-Jimenez, & Bartels, 2015; Kim, Leng, & Kritchevsky, 2017; Visser et al., 2005). Increased adiposity especially affects the ability to perform weight-bearing everyday activities, such as walking, carrying loads and climbing stairs as it is associated with a reduced strength capacity in relation to body weight (Bouchard, Heroux, & Janssen, 2011). Increased adiposity is also related to increased systemic low-grade inflammation, which has been suggested to be an important mechanism behind age-related loss of muscle mass (Dupont, Dedeyne, Dalle, Koppo, & Gielen, 2019). Even more concerning than increased adiposity with ageing is the combination of low muscle mass and a high fat mass, a phenomenon termed “sarcopenic obesity”. Indeed, there is compelling evidence that physical limitations related to sarcopenic obesity are much greater than those of low muscle mass and high adiposity alone, which again suggests that low muscle mass and high adiposity have independent and synergetic adverse effects on physical function in older adults (Lee, Shook, Drenowatz,
The concurrent increases in fat tissue and loss of muscle mass in older individuals often occur without changes in total body weight. Therefore, the absence of weight fluctuation itself cannot be regarded as a reliable way to assess body composition during ageing.

**Mechanisms underlying age-related changes in muscle mass and determinants of physical function**

There is compelling evidence that multiple factors and mechanisms contribute to the development and progression of a loss of muscle mass and different determinants of physical function in older adults. Interaction among some of the most common factors are graphically presented in Fig 4 (Beas-Jiménez et al., 2011). The following section describes some of the factors and mechanisms generally acknowledged to be responsible for these age-related changes. At a cellular level, all loss of skeletal muscle mass originates from an imbalance between muscle protein synthesis and muscle protein breakdown. Compared with younger adults, upregulation of muscle protein synthesis after feeding and exercise is reduced in the elderly. Indeed, while muscle protein synthesis is maximally stimulated by ≈ 0.24 g of protein per kg body weight per meal in young adults, older adults require closer to ≈ 0.4 g protein/kg body weight per meal to maximize the same (Moore et al., 2015). However, this reduction in muscle protein synthesis may in part be caused by a sedentary lifestyle rather than by the normal ageing process itself (Chaput et al., 2007; Lord, Chaput, Aubertin-Leheudre, Labonte, & Dionne, 2007). For example, reductions in muscle protein synthesis have been reported in subjects participating in bed rest studies that include a dramatic decrease in habitual physical activity levels (Ferrando, Lane, Stuart, Davis-Street, & Wolfe, 1996). Nevertheless, given the fact that protein synthesis often is downregulated in older adults and that a higher protein intake has been associated with overall positive health effects, especially in older individuals consuming relatively small amounts of calories per day, it has been suggested that the recommended daily intake of protein...
Another important factor that most likely contributes to reduced muscle mass and physical function is chronic low-grade systemic inflammation, which is very common in the older population and often termed “inflammageing”. Chronic low-grade systemic inflammation is defined as a two to threefold elevation of circulating inflammatory mediators including acute-phase proteins such as C-reactive protein (CRP) and pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and IL-6 (Roubenoff, 2000; Schaap et al., 2009; Schaap, Pluijm, Deeg, & Visser, 2006b; Visser et al., 2002). The associations between the occurrence of chronic low-grade systemic inflammation and loss in muscle mass and physical function have been elucidated in a large number of papers (Schaap, Pluijm, Deeg, & Visser, 2006a; Strasser et al., 2018; Wahlin-Larsson,
Carnac, & Kadi, 2014; Visser et al., 2002). However, the exact mechanistic link between age-related low-grade systemic inflammation and changes in muscle mass and physical function is currently unresolved.

In addition to the abovementioned factors, it is well recognised that lifestyle factors affect age-related changes in body composition and physical function. Firstly, ageing is often associated with a decline in nutritional intake, especially in very old adults (Doherty, 2003). Changes in physical activity behaviour, reduced taste sensation and increased social isolation have been suggested as important factors leading to a reduced dietary intake and energy expenditure. A number of studies have linked this impaired energy intake to a progressive loss in body weight including muscle mass (Roberts et al., 1996; Wilson & Morley, 2003). Therefore, even if specific guidelines for dietary intake in older adults are currently lacking, it is evident that a less than optimal food intake is a contributing factor to age-related loss in muscle mass, strength and physical function.

Secondly, ageing is generally accompanied by an increasingly sedentary lifestyle, which reduces anabolic processes derived from repetitive mechanical loading during physical activity (Hagstromer, Troiano, Sjostrom, & Berrigan, 2010; Rolland et al., 2008). Hence, physically inactive older adults experience a more rapid decrease in skeletal muscle mass and function compared with their physically active peers (Doherty, 2003). Therefore it has been suggested that physical activity may be an important contributor to loss of physical function among older adults (Kortebein, Ferrando, Lombeida, Wolfe, & Evans, 2007; Lee et al., 2007; Martin, Spenst, Drinkwater, & Clarys, 1990). In support of this, data from bed rest studies indicate that the decline in muscle function occurs before the decline in muscle mass (Heymsfield, Olafson, Kutner, & Nixon, 1979). This suggests that there is a downward spiral in which reduced physical activity leads to muscle weakness, leading to a loss in muscle mass and subsequently a decrease in physical function leading to a further decrease in physical activity. However, changes in muscle mass and physical function occur also in older adults with a life-long history of exercise training, although at a much slower rate (Crane, Macneil, & Tarnopolsky, 2013). For example, older master athletes competing in weight lifting show a remarkably small change in skeletal muscle mass and strength compared with healthy young individuals (Klein, Rice, & Marsh, 2001). Nevertheless, when compared with young and healthy resistance exercise-trained athletes, an age-related decline in muscle mass and muscle function is evident even in older athletes.
Moreover, like their inactive peers, physically active older adults have an increased infiltration of fat into the muscle, which has been shown to independently contribute to loss in muscle strength and physical function (Addison, Marcus, Lastayo, & Ryan, 2014; Reinders, Murphy, Koster, et al., 2015). It has been suggested that increased muscle fat infiltration reduces the muscle fibres’ contractility and thereby their strength output. It has also been hypothesized that increased infiltration of fat tissue into the muscle inhibits blood flow and thereby hampers muscle contraction (Lee, Kehlenbrink, Lee, Hawkins, & Yudkin, 2009). Nevertheless, the general consensus is that the age-related changes in body composition and function are not merely a consequence of an increased sedentary lifestyle, but also represent the result of ageing itself. Moreover, several different mechanisms seem to contribute to the age-related changes in muscle mass and physical function. However, the degree to which each of these factors contributes to these changes likely varies from individual to individual.

**Methods to counteract age-related changes in muscle mass and determinants of physical function**

**Effects of physical activity**

Habitual physical activity behaviour can play an important role in the preservation of muscle mass and physical function in older adults and can thereby contribute to healthy ageing. Physical activity levels generally decline with age, making the elderly a target population for physical activity interventions (Nelson et al., 2007). A number of studies have investigated the association between habitual physical activity behaviour and physical function in older adults. However, findings have been inconclusive, with some studies reporting a positive association between physical activity level and physical function (Corcoran et al., 2016; Davis et al., 2014; Keevil et al., 2016; Morie et al., 2010; Reid et al., 2016) while others have failed to observe such a relationship (Daly et al., 2008; Manini et al., 2009; Wannamethee, Ebrahim, Papacosta, & Shaper, 2005). This discrepancy may be explained in part by methodological differences related to the assessment of physical activity and physical function (e.g. objective vs. self-reported data) and differences in the health status of participants. Although physical activity is likely to exert beneficial effects on physical function, the
optimal amount of daily physical activity needed to infer such effects remains to be clarified. Moreover, the importance of time spent in different intensity levels of physical activity in order to preserve physical function is a matter of debate (Davis et al., 2014; Keevil et al., 2016; Reid et al., 2016). For example, do older adults who fulfil the current physical activity recommendations of 150 minutes or more of moderate to vigorous physical activity (MVPA) per week (WHO, 2010) have better physical function than their less active peers?

Additionally, it is debated whether time spent in sedentary behaviour may have detrimental effects on physical function independently of time spent in physical activity (Keevil et al., 2016; Nilsson, Wahlin-Larsson, & Kadi, 2017; Rosenberg et al., 2016; Santos et al., 2012; Troiano et al., 2008). The effects of sedentary behaviour on physical function in older adults is of particular importance as older individuals spend more time in inactivity and less time in MVPA compared with younger age groups (Harvey, Chastin, & Skelton, 2013).

Finally, current knowledge regarding the effects of physical activity on physical function in older adults is mainly based on studies investigating present physical activity behaviour. Therefore, to what extent physical function at old age is further influenced by physical activity performed throughout adulthood has often been overlooked. Currently, there is some evidence that physical inactivity and, to a lesser degree, decreased physical activity during adulthood may play a negative role on preserved physical function and quality of life (Booth, Roberts, & Laye, 2012). For example, (Akune et al., 2014; Leino-Arjas, Solovieva, Riihimaki, Kirjonen, & Telama, 2004; Stenholm et al., 2016) have reported positive associations between leisure-time physical activity during adulthood and physical function at old age. Regarding occupational physical activity, studies present conflicting outcomes, with some reporting deleterious effects on physical function while others report neutral or even positive effects (Andersen, Thygesen, Davidsen, & Helweg-Larsen, 2012; Heneweer, Staes, Aufdemkampe, van Rijn, & Vanhees, 2011; Hoogendoorn, van Poppel, Bongers, Koes, & Bouter, 1999; Schmidt, Tittelbach, Bos, & Woll, 2017; van der Windt et al., 2000).
These differences in outcome may be due to methodological issues (e.g. self-reported vs. objectively assessed physical activity and functional capacity) and differences in the investigated population (e.g. blue-collar vs. white-collar workers). Nevertheless, an important limitation in previous studies is that they have not taken the participants’ current present physical activity behaviour into account. Therefore, it is still unknown whether the effects of physical activity on physical function accumulate over time.

Effects of resistance training
Resistance training is currently considered as the most effective form of physical activity to slow the age-related decline in muscle mass, strength and physical function (Peterson, Sen, & Gordon, 2011). Numerous studies have shown that older adults are still capable of increasing their muscle mass and strength by performing resistance training and that these adaptations often translate into improved physical function (Binder et al., 2005; Brown, McCartney, & Sale, 1990; Charette et al., 1991; Fiatarone et al., 1990; Fiatarone et al., 1994; Frontera, Meredith, O’Reilly, Knuttgen, & Evans, 1988; Hakkinen, Kallinen, et al., 1998; Hakkinen, Pakarinen, et al., 2001; Hanson et al., 2009; Holviala et al., 2014; Izquierdo et al., 1999; Kosek, Kim, Petrella, Cross, & Bamman, 2006). Resistance training has shown to increase both myofibrillar content (Welle, Totterman, & Thornton, 1996) and muscle electromyographic (EMG) activity (Hakkinen, Kallinen, et al., 1998; Hakkinen, Kraemer, Newton, & Alen, 2001), which indicates that gains in muscle strength in older adults result from a combination of muscle hypertrophy and neuromuscular adaptation. While both young and older adults seem to experience similar relative increases in muscle strength in response to resistance training (Lemmer et al., 2000), gains in skeletal muscle mass are dampened or even lacking in older adults. Among others, (Greig et al., 2011; Kosek et al., 2006; Tieland et al., 2012; Vincent et al., 2002) did not observe any significant gains in skeletal muscle mass in older adults despite large increases in muscle strength after 16 weeks of resistance training. Moreover, (Hanson et al., 2009) reported a significant increase in muscle mass in men, but not women, following 22 weeks of resistance training. The so-called “anabolic resistance” has been put forward as a putative mechanism behind this blunted hypertrophic response in older adults.
and a possible explanation is the decreased muscle protein synthesis in relation to both nutritional intake and exercise observed in older compared with young adults (Breen & Phillips, 2011).

In addition to resistance training induced improvements in maximal muscle strength in older adults, increased ability to rapidly generate muscle force (i.e. explosive capacity) has also been reported by some (Ferri et al., 2003; Hakkinen, Kallinen, et al., 1998; Hakkinen, Pakarinen, et al., 2001; Holviala et al., 2014; Jozsi, Campbell, Joseph, Davey, & Evans, 1999) but not in all previous studies (Frontera et al., 1988; Hakkinen, Newton, et al., 1998; Harvey et al., 2013). Therefore, compared with changes in maximal muscle strength, improvements in explosive strength are less frequently reported in aged populations. This is an important issue as the age-related decrease in explosive strength is generally greater than that in maximal muscle strength (Bassey et al., 1992; Hakkinen et al., 1996; Izquierdo et al., 1999; Skelton et al., 1994). And as explosive strength is more strongly correlated to the ability to perform normal daily activities such as stair climbing and the ability to recover from a trip or slip than is maximal muscle strength (Bassey et al., 1992; Pijnappels, van der Burg, Reeves, & van Dieen, 2008; Skelton et al., 1994). Therefore, maintaining explosive muscle strength seems to be especially important for preserved physical function during ageing.

While resistance training is recommended to older adults primarily based on its positive effect on muscle mass and strength, there is also evidence for its preventive effect regarding weight gain and the onset of diseases. Indeed, cross-sectional studies reported an inverse relationship between muscle mass and the prevalence of metabolic syndrome and all-cause mortality in the elderly, that is independent of cardiorespiratory fitness level (Jurca et al., 2005). Furthermore, skeletal muscle is also the most important location for glucose and triacylglycerol disposal and therefore skeletal muscle mass is an important determination of resting metabolic rate. As we lose muscle mass with age, our resting metabolic rate decreases, which initiates a chain reaction leading to a reduced capacity to oxidize lipids, reduced insulin-mediated glucose uptake (Hunter et al., 1997) and increased adiposity. These factors all contribute to an increased risk for the development of type II diabetes and cardiovascular diseases (Braith & Stewart, 2006; Hurley et al., 1988; Williams et al., 2007). Although resistance training generally in-
increases energy expenditure to a lesser extent than does intensive aerobic exercise training, resistance training elevates the resting metabolic rate as a result of a substantially greater protein turnover (Evans, 2001). Theoretically, each kilogram of muscle mass increases the resting metabolic rate by ca 21 kcal per day. This means that a gain of 5 kg muscle mass translates to an increased daily basal energy expenditure of ca 100 kcal, which over 1 year equals the energy stored in 4.7 kg fat (Wolfe, 2006). Therefore, when performed properly, sustained resistance training over several years can have the potential to translate into clinically important differences in energy expenditure and associated fat gains among older adults.

Effects of dietary intake alone and in combination with resistance training

It is well known that dietary intake directly impacts body composition and physical function. Since a direct relationship exists between daily protein intake and the age-related loss of muscle mass, protein intake among older adult has received a lot of attention. (Houston et al., 2008; Nilsson, Montiel Rojas, & Kadi, 2018). As described previously, all loss of skeletal muscle mass originates from an imbalance between muscle protein synthesis/breakdown and protein intake has a direct impact on the rate of muscle protein synthesis, independently of exercise (Churchward-Venne et al., 2012). In addition to protein intake, muscle protein synthesis is stimulated by physical activity, especially resistance type exercise training (Bennet, Connacher, Scrimgeour, & Rennie, 1990). Therefore, a large number of studies have investigated the potential preventive effects of increased protein intake alone and in combination with resistance training on changes in muscle mass, strength and physical function in the older population. Nevertheless, in healthy older adults who have an adequate dietary intake (≈1.0 g protein/kg bodyweight/day), several studies have found no additive effect of increased protein intake on muscle mass and function, even when combined with resistance training (Campbell, Crim, Young, & Evans, 1994; Campbell, Crim, Young, Joseph, & Evans, 1995; Campbell, Johnson, McCabe, & Carnell, 2008; Courtney-Martin, Ball, Pencharz, & Elango, 2016; Fiatarone et al., 1994; Leenders, Verdijk, Van der Hoeven, Van Kranenburg, Nilwik, Wodzig, et al., 2013; Thomas, Quinn, Saunders, & Greig, 2016; Tieland et al., 2012; Welle & Thornton, 1998; Verdijk et al.,
Taken together, this suggests that, while adequate protein intake is essential for maintenance of muscle mass and physical function during ageing, more protein does not seem to induce further benefits. In fact, the impact of protein supplementation on gains in muscle mass seems to be reduced with increasing age (Morton et al., 2018).

More recently, interesting evidence has emerged suggesting that dietary fatty acid composition may have profound effects on preservation of body composition and physical function in older adults. Fatty acids are divided into saturated fatty acids and unsaturated fatty acids, depending on the number of double bounds between their carbon atoms. Saturated fatty acids have no double bonds, while unsaturated fatty acids have one (monounsaturated fatty acids, (MUFAs) or more double bonds (polyunsaturated fatty acids, (PUFAs)). Following consumption, fatty acids are used in many different processes, such as β-oxidation for energy release, storage in lipid droplets or incorporation as phospholipids to form the major component of cell membranes. The biochemical structure of the fatty acids incorporated into the cell membrane, such as the length of the carbon chain and the number and position of eventual double bonds, will greatly influence the physiological effects on the cell (Los & Murata, 2004). For example, cell membranes that are rich in phospholipids from SFAs (i.e. with no double bonds) will result in a membrane that is tightly packed, with low fluidity, while incorporation of a large amount of phospholipids obtained from PUFAs (i.e. with two or more double bonds) will give a less tightly packed membrane and more membrane fluidity (Holte, Peter, Sinnwell, & Gawrisch, 1995). The fluidity of the cell membrane is important as it alters the ability of the cell to signal and communicate within itself and with other cells. Therefore, alteration of the cell membrane by changes in the dietary composition of SFAs and PUFAs can have an important effect on many of the cell’s physiological and metabolic functions, including those in skeletal muscle cells.

Additionally, PUFAs can affect metabolic processes more directly by the regulation of several key enzymes and by acting as signal molecules (Burdge & Calder, 2015). Of particular interest are the so-called “long-chain PUFAs”, which consist of two major families: omega-3 and omega-6 PUFAs. Omega-3 and omega-6 PUFAs differ biochemically from each other by the position of the first double bond, counted from the methyl group at the terminal end of the chain. Important long-chain omega-3 and omega-6
PUFAs are 20:5 n3 (eicosapentaenoic acid (EPA)), 22:6 n3 (docosahexaenoic acid (DHA)) and 20:4 n6 arachidonic acid. Omega-3 and omega-6 are essential FAs and must be derived directly from the diet as humans lack the metabolic function required to synthesize them from other fatty acids. Dietary \( \alpha \)-linolenic acid, however, can be converted into EPA, DPA and DHA and linolenic acid can be converted into arachidonic acid. Nevertheless, this conversion is very inefficient, with only approximately 5% of \( \alpha \)-linolenic acid being converted to DHA and even less into EPA and DPA (Burdge & Calder, 2015; Burdge, Jones, & Wootton, 2002; Burdge & Wootton, 2002). As a consequence of this ineffective conversion, consumption of \( \alpha \)-linolenic acid and linolenic acid-rich food will result in negligible levels of DHA, DPA, EPA and arachidonic acid in the muscle tissue (Surette, 2008).

From a cellular perspective the most important PUFA in the omega-6 family is arachidonic acid. When activated, cells release arachidonic acid from the membrane, after which it is transformed into powerful cellular inflammatory mediators (Funk, 2001). Critically, omega-3 fatty acids counteract the inflammatory effects of arachidonic acid by displacing arachidonic acid from membranes and competing with arachidonic acid for important enzymes necessary to catalyse the inflammatory process (Calder, 2006). Therefore, the net ratio intake of dietary omega-3 versus omega-6 PUFAs, known as the omega-6/3 ratio, has an important role in the regulation of inflammation and consequently affects whole-body metabolic health (Jeromson, Gallagher, Galloway, & Hamilton, 2015). For example, supplementation with omega-3 PUFA-rich fish oil, which contains the key fatty acids DHA and EPA, has been shown to decrease the levels of pro-inflammatory cytokines in subjects suffering from chronic inflammatory conditions such as rheumatoid arthritis and bowel disease (Barbalho, Goulart Rde, Quesada, Bechara, & de Carvalho Ade, 2016). Likewise, the consumption of a Mediterranean-type diet, which is rich in fish and sea products and has a low omega-6/3 ratio, reduces systemic inflammation and risk factors associated with cardiovascular disease (CVD) in adults with metabolic syndrome (Rees et al., 2019). In addition, it is well established that skeletal muscle cells are sensitive to changes in dietary FA composition and that clinically relevant changes in muscle lipid composition (e.g. omega-3 and omega-6 PUFA) may occur within 2 weeks (Andersson, Nalsen, Tengblad, & Vessby, 2002; McGlory et al., 2014). As the age-related loss in muscle
mass and function is associated with chronic low-grade inflammation, the idea of an increased dietary intake of omega-3 PUFAs to counteract these changes has raised scientific attention.

Indeed, several large cross-sectional studies have suggested a potential effect of omega-3 PUFA in general, and EPA and DHA in particular, on muscle mass, muscle strength and physical function in older adults. One of these studies investigated the relationship between food intake and muscle function in nearly 3,000 community-dwelling older adults (59–73 years old) (Robinson et al., 2008). Robinson and colleagues (2008) reported that for each additional intake of fatty fish which is very rich in omega-3 PUFAs, grip strength increased by 0.48 kg in women and 0.43 kg in men. In another study, a positive relationship between omega-3 PUFA intake and leg strength and physical function in older adults was reported (Rousseau, Kleppinger, & Kenny, 2009). However, this association deteriorated and ended up non-significant after adjusting for protein intake. More recently Reinders and colleagues (2015) investigated the association between the plasma concentration of PUFAs and muscle mass and strength in nearly 6,000 Icelandic older adults. They found that high plasma concentrations of PUFA, which is a biological marker strongly associated with PUFA intake, were associated with larger muscle size and greater knee extension strength (Reinders, Song, et al., 2015). Moreover, while greater concentrations of the omega-6 PUFA arachidonic acid were associated with smaller muscle size, higher concentrations of both total omega-3 PUFAs and omega-3 DHA were associated with greater muscle strength. In a 5-year follow-up of the same cohort, a positive association was seen between concentration of omega-3 α-linolenic acid and changes in muscle strength. No similar association was seen for other PUFAs and no effect of changes in muscle size was observed. This illustrates the complex relationship between intake of PUFA and muscle mass and strength. However, as the Icelandic population in general has a high intake of fatty fish rich in omega-3 PUFA, the authors acknowledge that relatively high omega-3 PUFA levels in the reference group may have blurred some of the potential myotropic effects. Interestingly in another study by the same group, high intake of omega-3 PUFA was associated with reduced mobility disability in women but not men (Reinders, Murphy, Song, et al., 2015). This highlights a potential sex difference that warrants further research.
The potential therapeutic role of omega-3 PUFA in older adults has also been investigated in a number of clinical trials. Smith and colleagues were the first to report randomized control trial evidence that 8 weeks of fish oil supplement, which is rich in omega-3 PUFA, diminished the age-related decline in both muscle mass and strength in healthy older adults (Smith et al., 2011a; Smith et al., 2015). These changes were accompanied by increased muscle protein synthesis, which explains, at least in part, the increased anabolic muscle response. Similarly, (Hutchins-Wiese et al., 2013) showed that 24 weeks of fish oil supplementation induced a small but clinically relevant increase in walking speed in older women. However, in contrast to these two studies, (Krzyminska-Siemaszko et al., 2015) found no myotrophic effects of omega-3 PUFA supplementation on muscle mass, strength or gait speed in older adults. Overall, randomized controlled trials provide some evidence for a positive effect of omega-3 PUFA intake as an effective method of combating muscle wasting and loss of muscle strength in older adults. However, results are scarce and somewhat inconsistent.

Given that the anabolic effect of resistance training is blunted in older adults and that omega-3 PUFA has shown promising effects as a therapeutic agent, combining resistance training with high omega-3 PUFA intake has been suggested as a potential method to counteract the age-related decline in muscle mass and physical function. When this thesis was initiated, only one study had investigated this method (Rodacki et al., 2012). In that study it was shown that 12 weeks of resistance training combined with omega-3 PUFA supplementation led to significantly better improvements in maximal muscle strength and chair rising performance in older adults, compared with resistance training alone. However, the study by Rodacki et al. (2012) had some major limitations including lack of a control group and no assessment of muscle mass.

Thus, by the start of this thesis some interesting novel findings indicating a positive link between omega-3 PUFA intake muscle strength and physical function in older adults had emerged. However, more prospective randomized controlled trials in older populations were needed to determine the potential effect of PUFA on muscle mass and function, especially when combined with resistance training.
Aims

The overall aim of this thesis was to study the effects of physical activity behaviours in general and resistance training in particular, with or without addition of a healthy diet rich in omega 3 PUFA, on physical function and muscle mass in older, community-dwelling women.

The specific objectives were to examine:

- the influence of present physical activity behaviour on physical function at old age
- the influence of past physical activity behaviour on muscle mass and physical function at old age
- the effects of resistance training alone or in combination with a healthy diet rich in omega-3 PUFA on changes in muscle mass and maximal muscle strength
- the effects of resistance training alone or in combination with a healthy diet rich in omega-3 PUFA on changes in explosive muscle strength and physical function
Methods

Study designs and participants

Studies I and II are based on a cross-sectional design including 60 and 112 older women between 65 and 70 years of age respectively. Exclusion criteria for study I and II were smoking, occurrence of pulmonary, cardiovascular, metabolic or rheumatologic diseases. In addition, all participants in study I had normal levels of fasting blood glucose, cholesterol and triglycerides. Studies III and IV are based on a three-armed controlled trial in which 63 older women were recruited and randomized into three groups: control (CON), resistance training (RT) and resistance training plus healthy diet (RT+HD). During the randomized controlled trial, eight subjects withdrew from the study (three CONs, and four participants from the RT and one from the RT+HD group) and did so for reasons not related to the intervention. All subjects enrolled in the studies III-IV were between 65 and 70 years of age and were healthy i.e. they had a BMI <30, fasting glucose <6 mmol/L, fasting cholesterol <8 mmol/L and resting blood pressure of <140/90 mmHg. Additionally, participants had to be recreationally physically active. Exclusion criteria were: smoking, history of pulmonary, cardiovascular, metabolic, or rheumatologic disease.

Body composition

Body composition mass was assessed between 07.00 and 09.00 AM in fasted state using either dual X-ray absorptiometry (DXA) (LUNAR Prodigy, GE Medical Systems, Waukesha, WI, USA) and Hologic Apex software, version 2.3, Waltham, MA, USA (Studies I and III–IV), or bioelectrical impedance analysis (BIA) (TANITA BC-420MA; Tanita Corporation, Tokyo,
Japan) (Study II). In Study II, skeletal muscle mass was calculated using the equation from Janssen et al. (2002):

$$\text{skeletal muscle mass (kg)} = [(\text{height}^2/\text{BIA resistance} \times 0.401) + (\text{gender} \times 3.825) + (\text{age} \times -0.071)] + 5.102,$$

where height is in cm; BIA resistance is in ohms; gender = 0 for women; and age is in years (Janssen et al., 2002). Skeletal muscle mass index (SMI %) was calculated as skeletal muscle mass/body mass x 100. This BIA equation has been validated against magnetic resonance imaging measures of whole-body muscle mass ($R = 0.93$) in a sample of both men and women with varying age (18–86 years) and adiposity (BMI = 16–48 kg/m$^2$) (Janssen, Heymsfield, Baumgartner, & Ross, 2000). The standard error of the estimate for predicting skeletal muscle mass from BIA using this method has been reported to 9% (Janssen, Heymsfield, Baumgartner, & Ross, 2000). Body mass index was calculated as body weight (kg) divided by the square of height (m$^2$).

**Physical activity**

In all studies, present physical activity behaviours were assessed by accelerometry (Actigraph model GT3x; ActiGraph LLC, Pensacola, FL, USA). Participants were instructed to wear the accelerometer on the hip with an elastic belt during awake times, except during water activities. Inclusion criteria for a valid monitoring were at least 4 days with at least 10 hours recorded per day. Non-wear time was defined as periods of at least 60 consecutive minutes of zero values. Daily average physical activity levels were expressed as counts per minute (CPM). In addition, daily average times spent in sedentary behaviour (<100 counts/min), light-intensity physical activity (LPA) (100–2,019 counts/min) and moderate-to-vigorous intensity of physical activity (MVPA) (>2,020 counts/min) were derived according to a previous study by (Troiano et al., 2008). In study III, an accelerometer count cut point of >760 counts/min (Matthew, 2005) was used to assess time spent in physical activity at the start, in the middle, and at the end of the 24 week trial. This cut point allows for lifestyle physical activities (e.g. grocery shop-
ping, vacuuming) ranging between light to moderate intensity to be included, and has been previously used to describe physical activity behaviours in older Swedish women (Orsini et al., 2008).

In Studies I and II, the amount of past leisure-time physical activity performed during adulthood was assessed using the Historical Adulthood Physical Activity Questionnaire (HAPAQ). The HAPAQ has demonstrated sufficient validity for ranking individuals according to their past physical activity behaviour compared with objectively assessed physical activity (Besson et al., 2010). The participants were asked to report on leisure-time physical activities requiring at least moderate intensity (≥3 metabolic equivalents (METs)) and performed at least once a week within four discrete time periods throughout adulthood (20–34 years, 35–49 years, 50–59 years and 60–65 years). Activity duration and frequency for each activity within the four time periods were derived. Based on the compendium of physical activities (Ainsworth et al., 2011) a MET score was allocated to each type of activity expressed in MET-minutes by multiplying total time reported in each activity with the allocated MET score. Finally, the total number of MET-minutes was averaged per week. We also separated time spent walking from all other types of recreational activities, thereby distinguishing engagement in exercise-related activities from walking. All participants also reported on past occupations throughout adulthood and were classified into two categories: (1) sedentary occupations, where participants spent most of the time sitting; (2) manual occupations, spending most of the time standing requiring moderate to vigorous physical efforts bouts. To be classified into the sedentary category, participants should have spent at least 30 years in sedentary occupations between ages 35 to 65 years.

**Muscle strength, aerobic capacity and physical function**

Muscle strength, aerobic capacity and physical function were assessed using common, validated and standardized tests. Subjects were familiarized with the equipment and procedures before each test and the tests were conducted by trained and experienced staff. In the randomized controlled trial, all performance measurements were performed at the same time of the day in all groups before and after the 24-week intervention. Tests utilized in this thesis are briefly described below.
Maximal dynamic muscle strength was assessed during knee extension and leg press exercise using one repetition maximum tests (1RM test). The initial load was set at 90–95% of the estimated 1RM and load was increased by ≈2.5–5% after each successful lift until failure. One repetition maximum was generally obtained within five attempts. Tests of 1RM strength were performed in Studies III and IV.

Maximal isometric strength was assessed in a seated position using an adjustable chair with a 90° angle of hip, knee and elbow joints and with restraining straps across the torso and the tested arm and leg. For the arm strength test, a force sensor (K. TOYO 333A, Toyo-Korea, Seoul, South Korea) was attached at one-fourth of the distance between the radial styloid process and the lateral epicondyle; for the leg strength test, the same force sensor was attached above the malleoli at one-third of the distance between the lateral femoral epicondyle and the lateral malleolus. All isometric strength measurements were performed on the dominant arm/leg, and each subject performed three arm flexions and three knee extensions at maximal voluntary effort separated by a rest period of 2.5 minutes between trials and 5 minutes between exercises. Subjects were instructed to exert maximal muscle force as “fast and forcefully” as possible and to maintain it for 3–5 seconds. Onset of muscle contraction was defined as the time point when the knee extension force exceeded 2.0 N above the baseline level, which corresponded to ≈1% of maximal peak force (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002).

Explosive muscle force during dynamic movement was assessed by measuring maximal power and time to reach peak power during seated knee extension exercise. A force sensor (K. TOYO 333A) and a linear encoder (Muscle Lab; Ergotest Technology, Oslo, Norway) were used to monitor force and vertical displacement generated during the concentric phase of a knee extension exercise. Subjects were instructed to perform a maximal bilateral knee extension as forcefully and quickly as possible. All tests were performed against a load corresponding to 70% of 1RM assessed at baseline.

Squat jump (SJ) test assesses lower limb explosive muscle strength during loaded multi-joint activity and was included in Studies I and IV. The test was performed on a force platform (Kistler 9281 B; Kistler Nordic AB, Jonsered, Sweden) and SJ maximal force (sjMAX) and SJ rate of force development (sjRFD) were calculated from the concentric phase of the SJ. The
two parameters, sjMAX and sjRFD, are directly correlated to jump performance and are not influenced by technical aspects such as landing technique (Linthorne, 2001).

The *single-leg stance test* measures balance performance and was included in Studies I and IV. The test was performed standing barefoot on one leg (the dominant leg) with arms crossed and eyes closed (Springer, Marin, Cyhan, Roberts, & Gill, 2007).

The *five sit-to-stand (5-STS) test* measures lower extremity strength and transfer skill. During the test, subjects are instructed to stand fully upright from a chair and sit down and repeat this sequence five times as fast as possible (Guralnik, Ferrucci, Simonsick, Salive, & Wallace, 1995). The 5-STS test was included in Study IV.

*Timed-up-and-go (TUG)* measures lower limb strength, balance, transfer and walking ability. For the TUG, the subjects were instructed to stand up from a chair, walk at a “comfortable speed” to the 3 m mark, turn around, walk back and sit down (Manson et al., 1999). The test was included in Study IV.

The *6-minute walk test* (6MW) is a submaximal exercise test that measures distance walked during 6 minutes. The 6MW provides a measure of overall cardiopulmonary and lower limb musculoskeletal function and was included in Study I. The test was performed by walking a corridor of 50 m in length according to the protocol outlined by the American Thoracic Society (ATS) (ATS, 2002).

*Maximal aerobic capacity (VO2max)* was estimated using the Åstrand cycle test on a mechanically braked ergometer bicycle (Monark 874E; Monark Exercise AB, Vansbro, Sweden) (Astrand & Ryhming, 1954). Briefly, participants cycled at 50 revolutions per minute for 6 minutes at an individually chosen workload. Steady-state heart rate was measured by a heart rate monitor (Polar RS400; Polar Electro Oy, Kempele, Finland) and predicted VO2max was obtained from the Åstrand nomogram with adjustment for age and sex (Astrand, 1960). The test was included in Study II.

*Physical function score*. In addition to the above tests an aggregated physical function score was created in Study I, based on participants’ 6MW, SJ and single-leg stance balance performance. First, standardized values (z-scores) for each outcome variable (6MW, SJ and balance) were expressed.
Thereafter the average z-score based on all standardized and outcome variables was calculated, thus providing a continuous variable weighing different aspects of physical function into one standardized score.

**Biochemical analysis**

Blood samples were collected between 7:00 and 9:00 AM after an overnight fast by venipuncture from an antecubital vein. Samples were centrifuged at 4,000 rpm for 10 minutes and stored at -80°C. Determination of high-sensitivity CRP was performed using a fully automated immunoturbidimetric assay (Advia 1800; Chemistry System, Siemens, Erlangen, Germany). Interleukin-6 level was assessed using an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine HS; R&D Systems, Minneapolis, MN, USA). Fasting blood glucose was assessed using Reflotron Plus (Roche, Risch-Rotkreuz, Switzerland), and cholesterol (high- and low-density lipoprotein (HDL and LDL), oxidised LDL (oLDL)) and triglycerides were assessed using the Vitros 5.1 clinical chemistry analyser (Ortho Clinical Diagnostics, Raritan, NJ, USA). Serum lipids (linolenic acid, EPA, DHA, linoleic acid, and arachidonic acid) were extracted with chloroform and phospholipids were separated from other lipids by thin-layer chromatography and transmethylated with methanol and sulphuric acid (Boberg, Croon, Gustafsson, & Vessby, 1985). The percentage composition of methylated fatty acids was determined by gas chromatography with flame ionization detection. Gas chromatography used for the analysis consisted of a 30 m capillary column coated with Thermo TR-FAME (Thermo Electron, Stockholm, Sweden) and an Agilent Technologies system consisting of GC 6890N, Autosampler 7683, and Agilent ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The temperature used was between 150°C and 260°C. Identification of the fatty acids was done by comparing the retention time of each peak with the methyl ester standard (Nu Check Prep, Elysian, MN, USA).
Resistance training

In the randomized controlled trial (Studies III and IV), supervised progressive resistance training was performed twice a week for 24 weeks by the RT and RT+HD groups. Subjects performed three sets per exercise, with a 2-minute rest between sets and 3 minutes of rest between exercises. The following exercises were performed: squat, seated knee extension, leg press, seated row, and pull down. During the first 2 weeks the workload was set to 12–15 repetitions per set at 50% of the participants’ 1RM. Thereafter the workload increased to 8–12 repetitions per set at 75–85% of 1RM for the rest of the intervention. All training sessions were preceded by a short warm-up, which included step-up and core exercises, and ended with 5 minutes of stretching.

Healthy diet

At the start of the randomized controlled trial, subjects in the RT+HD group attended a dietary consultation and were given a diet plan. Details of the prescribed diet are summarized in Table 1. Briefly, the prescribed diet was in line with the current dietary guidelines in Europe and the USA, i.e. a healthy diet rich in whole grain products, vegetables, fruits, fish and PUFAs from vegetable oils and nuts, with the following major adjustment: the omega-6/3 ratio was <2. In accordance with the general dietary goals, several numbered menus were suggested to the participants. The subjects were trained to prepare their meals according to the recommendations provided by a nutritionist. The daily calorie requirements for each participant in the RT+HD group were calculated based on predicted basal metabolic rate (BMR) using the Harris-Benedict equation (Roza & Shizgal, 1984), and estimated physical activity level. The dietary intake was adjusted in 200 kcal increments to match individual energy requirements. Subjects in the CON and RT groups were carefully instructed to maintain their habitual dietary intake throughout the study. The dietary intake was monitored using a food record over a period of 6 days at three time points (baseline, week 12 and week 24). Participants were instructed by a nutritionist on how to record their daily food intake by using a portion size guide developed by the Swedish National Food Agency (Livsmedelsverket, 2009). Dietist XP software (Kost och Näringsdata, Bromma, Sweden) was used to analyse dietary intake.
Table 1. Nutrient goals and prescribed key foods for the resistance training+healthy diet (RT+HD) group.

<table>
<thead>
<tr>
<th>Nutrient goals</th>
<th>Dietary intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate, E%</td>
<td>44</td>
</tr>
<tr>
<td>Fibre, g/day</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Protein, E%</td>
<td>20</td>
</tr>
<tr>
<td>Total fat, E%</td>
<td>36</td>
</tr>
<tr>
<td>Saturated fat, E%</td>
<td>10</td>
</tr>
<tr>
<td>Unsaturated fat</td>
<td>2/3 of total fat</td>
</tr>
<tr>
<td>Omega-6/3 ratio</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Prescribed foods</td>
<td></td>
</tr>
<tr>
<td>Fish and seafood</td>
<td>&gt;500 g/week</td>
</tr>
<tr>
<td>Vegetables/fruits/berries</td>
<td>&gt;600 g/day</td>
</tr>
<tr>
<td>Cereal products</td>
<td>High whole grain (rye, oats, barley)</td>
</tr>
<tr>
<td>Dietary fats</td>
<td>Rape seed oil, olive oil, nuts and seeds</td>
</tr>
<tr>
<td>Meat products</td>
<td>Lean meat</td>
</tr>
<tr>
<td>Dairy products</td>
<td>&lt;0.5 L/day (low fat)</td>
</tr>
<tr>
<td>Soft drinks/juice</td>
<td>To be avoided/≤1.5 dL juice/day</td>
</tr>
</tbody>
</table>

E%, energy percentage

**Ethical considerations**

This work was performed in accordance with the principles set out by the Declaration of Helsinki and was approved by the regional ethical review board of Uppsala, Sweden. Participants were informed about the aim, study procedures and potential discomfort related to investigations. All participants provided written informed consent.

Prior to the randomized controlled trial, participants were familiarized with the training equipment. All training sessions were supervised by an instructor to ensure proper lifting technique to prevent injuries. Blood and muscle samples were collected before and after the randomized controlled trial and may be associated with minor discomfort, a rapid transitory pain
and a small risk for infection. However, all samples were collected by experienced and legitimated staff (nurses and medical doctors) in a sterile environment, thus minimizing the risk for complication. Moreover, muscle samples are routinely taken both in our and other laboratories and the risks associated with the muscle biopsy methods are reported to be very small (Edwards, Round, & Jones, 1983; Ekblom, 2017). Of note, data from the muscle samples is presented in other studies that are not included in the present thesis. Measurement of body composition with DEXA exposes participants for a minor dose of radiation which may have negative effects on health. However, the effective patient dose of a whole body DEXA scan using modern equipment (≈ 4 µSv) is only 7-10% of that received during a normal chest X-ray scan (≈ 40-60 µSv) and comparable the daily background radiation dose (≈ 4 µSv) (Damilakis, Adams, Guglielmi, & Link, 2010). Finally, collected data were coded and safely stored with access granted only to authorized personnel, and are presented at a group level.

**Statistical analysis**

All statistical analyses were performed using SPSS (SPSS, Chicago, IL, USA) or SigmaStat software, version 12.0 (SYSTAT Software Inc., San José, CA, USA). The level of statistical significance was set at P<0.05. Data were tested for normality using the Shapiro-Wilks normality test and skewed data were log-transformed before further analysis was performed. Data are presented as means ± standard deviation (SD) (Studies I and II) and as means ± standard error (SE) (Studies III–IV) unless otherwise noticed.

In Study I, data on present amounts of physical activity (CPM; MVPA, LPA), sedentary time, past leisure-time physical activity (between 20-65 years), and level of adiposity (fat mass %) were divided into tertiles. Factorial analysis of variance (ANOVA) was employed to investigate differences in physical function across tertiles of past and present physical activity behaviours, adjusted by level of adiposity. Differences in the aggregated functional score were first analysed across tertiles of physical activity behaviours, followed by separate analyses of each of the three physical function components in case significant main effects were observed. When analysing the influence of present physical activity behaviours on physical function, adjustment for total amount of past leisure-time physical activity was made. Similarly, when analysing the influence of past amounts of leisure-time
physical activity on physical function, adjustment for present physical activity level (measured in CPM) was made. In addition, when analysing the influence of one of the two parts of the past leisure-time physical activity (e.g. exercise-related activities) on physical function, any confounding influence from the remaining part (e.g. walking) was additionally adjusted for in the model. In case significant main effects were observed across tertiles, the Holm-Sidak post hoc procedure was further employed to determine between-tertile effects. The strength of relationships between the three components of physical function was determined by Pearson correlation coefficients (r). Based on the sample size, detection of effect sizes around 0.4 was possible with a power of ≥80%.

In study II, participants were classified into two groups based on whether or not they reported a weekly average of at least 600 MET-minutes of leisure-time PA during middle age years. The women were also categorized based on whether or not they reported a sedentary occupation during the middle age years. Factorial analysis of variance (ANOVA) was employed to investigate impact of having a physically active leisure-time [Yes/No] and a sedentary occupation [Yes/No] during middle age years on continuous outcomes of physical function and SMI. Multiple factorial analysis of variance (ANOVA) was performed to investigate the main effect of physical activity on physical function. Main and interaction effects (exercise X occupation group) were tested. All models were adjusted by present physical activity level (CPM) and level of adiposity. Analyses were conducted across the whole middle age period [35 to 65 years], followed by separate analyses of the early [35-50 years] and late [50-65 years] periods. When analysing the early middle age period, data on PA and occupation during late middle age period was accounted for and vice versa. Statistical analyses were performed using SPSS ver. 24.0 (SPSS, Chicago, IL). Level of statistical significance was set to P < 0.05, which allowed detection of medium effect sizes (≤ 0.30) with a power of ≥ 80% when performing all ANOVA models.

In Studies III–IV, one-way ANOVA was used to examine between-group differences at baseline. The effects of the randomized controlled trial were analysed using two-way repeated-measure ANOVA with one within-subject (time) and one between-subject (group) factor, followed by Holm-Sidak post hoc procedure. In study III an analysis of covariance (ANCOVA) was performed to control for possible confounding effects of total energy intake.
or relative protein intake on changes in muscle mass. In study IV, differences in amplitude of changes between RT and RT-HD were analysed using one-way ANOVA. Associations between variables were assessed using Pearson’s correlation coefficient. With an alpha level set at 0.05, the power of statistical tests ranged between 0.80 and 0.90. With a required minimum of 15 subjects in each group, 21 subjects per group were included to allow for a 20–25% drop-out rate.
Main results and discussion

Effects of physical activity on muscle mass and determinants of physical function

Influences of present physical activity behaviours (Study I)

In Study I, we demonstrated a significant main effect of present physical activity level (measured in CPM) on physical function. Women belonging to the two lower physical activity tertiles had a lower functional score (z-score) compared with those in the highest physical activity tertile (P<0.01) (Fig. 5A). These findings suggest that a low habitual physical activity level at older age is negatively related to physical function. This is in accordance with several other studies that investigated this issue by using objective methods to assess physical activity and physical function (Corcoran et al., 2016; Keevil et al., 2016; Morie et al., 2010). However, none of above-mentioned studies adjusted for past physical activity behaviour in their analysis. In Study I we demonstrated that the effect of physical activity on physical function remained after adjustment for past leisure-time physical activity behaviour. Therefore, the present work expands current knowledge as it shows the beneficial effects of present physical activity on physical function at old age, regardless of past physical activity behaviour. Therefore, adopting a physically active lifestyle at old age may prevent detrimental effects of a previously sedentary lifestyle on physical function in older adults.

In Study I, we further demonstrated that the effect of physical activity on the functional score was driven by time spent in MVPA (P<0.05) (Fig. 5B), while no influence on the functional score was observed across tertiles of either sedentary time or time spent in LPA. Moreover, women belonging to the first tertile with respect to time spent in MVPA and thus having the lowest functional score did not meet the current recommended amount of
at least 150 minutes of MVPA per week. This finding is in accordance with a previous investigation based on a large population of adults, which also showed that women who did not reach at least 150 minutes of MVPA per week had significantly lower physical function compared with those who met the recommendation (Keevil et al., 2016). Together the studies support the global health recommendation of at least 150 minutes of MVPA per week with the goal to maintain overall health and promote maintenance of physical function in older adults.

Figure 5. Mean (± standard error (SE)) functional score across tertiles of present physical activity level (Fig. 5A) and time in moderate to vigorous physical activity (MVPA) (Fig. 5B) after adjustment for body fat mass and amount of past leisure-time physical activity (PA).

*significant difference vs. tertile 1; #significant difference vs. tertile 2.

Notably, when investigating the influence of physical activity on physical function it is essential to adjust the analysis for adiposity. This is due to the fact that body fat percentage by itself is strongly related to both physical activity level and physical function, where older adults with a high body fat
percentage generally demonstrated a lower activity level and a lower physical function. Therefore, an association between physical activity and physical function could be due to the fact that lower adiposity in general is observed in physically active individuals rather than a true association between physical activity and physical function. However, in the current work, the effect of present physical activity (CPM and MVPA) on physical function remained after adjustment off adiposity. This suggests that being physically active at old age infers beneficial effects on physical function, regardless of amount of adiposity.

The lack of association between sedentary time and physical function, reported in Study I, is in coherence with some recent studies (Corcoran et al., 2016; Keevil et al., 2016; Reid et al., 2016). However, an inverse relationship between sedentary behaviour and physical function has also been reported (Cooper, Simmons, Kuh, Brage, & Cooper, 2015; McDermott et al., 2011; Santos et al., 2012). These differences in study outcomes may partly be explained by differences in health status among participants. For example, one study included older adults classified as frail and living in nursing homes (McDermott et al., 2011). In these groups of older adults even small amounts of physical activity can infer beneficial effects on physical function.

Additionally, differences in how the physical activity behaviours are assessed (self-report vs. objective assessment), and the choice of functional measurements, can affect the comparability between studies. Notably, in the present work the lack of association between sedentary behaviour and physical function was evident, even without controlling for present physical activity level. Therefore, we believe that a sedentary lifestyle is detrimental to physical function since it negatively affects the total volume of physical activity, rather than being directly related to aspects of physical function itself. Instead, physiological adaptations in response to habitual physical activity behaviours in general, with time spent in MVPA in particular, most likely explain the positive effects on physical function in older women.
Further analysis of the subcomponents of the functional score revealed a significant main effect of present physical activity level (in CPM) on both the 6MW and SJs, where older women in the highest physical activity tertile showed higher physical capacity compared with those in the lowest (Fig. 6) (6MW: tertile 1 vs. 3; SJ: tertile 1 vs. 3 and tertile 2 vs. 3), whereas no such effect was observed on balance.

**Figure 6.** Mean (± standard error (SE) performance in the 6-minute walk test (6MW) (Fig. 6A) and squat jumps (SJs) (Fig. 6B) across tertiles of present physical activity (PA) level after adjustment for body fat mass and amount of past leisure-time PA.

*significant difference vs. tertile 1; # significant difference vs. tertile 2.

This is in coherence with (Barone Gibbs et al., 2017) who reported that higher amounts of daily physical activity were associated with better overall physical function (Short Physical Performance Battery (SPPB) score), gait speed, and repeated sit-to-stand ability, but not balance performance. Therefore, in older adults it seems that balance performance is driven by
factors other than habitual physical activity. Nevertheless, as balance performance deteriorates with age, affecting locomotion and increasing fall incidence (Vellas et al., 1997), findings from the current work indicate that specific balance training should be promoted to all older adults including those with a high physical activity level.

Influences of past physical activity behaviours (Studies I and II)
In addition to investigating influences of present physical activity, the present work also sought to determine the relationships between past physical activity (leisure-time and occupational) and muscle mass and physical function at old age. In Study I, we showed a significant main effect of engagement in leisure-time exercise activities on the functional score, which was independent of present physical activity level (CPM) (P<0.05). Women belonging to the first tertile and reporting no, or only minor, engagement in exercise-related activities throughout adulthood had the lowest functional score (Fig. 7: tertile 1 vs. 2; P<0.05). No corresponding effect on functional score from leisure-time walking was observed (P = 0.426).

Figure 7. Mean (± standard error (SE) of the aggregated functional score across tertiles of engagement in past exercise-related activities after adjustment for body fat mass and present physical activity level (measured in counts per minute (CPM)).
*significant difference vs. tertile 2.
Previous studies (Akune et al., 2014; Leino-Arjas et al., 2004; Stenholm et al., 2016) have indicated positive associations between physical function at old age and engagement in physical activity during adulthood. However, in common with other previous work, these studies did not assess present physical activity levels. Hence, confounding influences of habitual physical activity level on physical function during old age cannot be ruled out. Therefore, the present study reveals a novel finding as it shows that engagement in exercise-related activities during adulthood has a positive impact on physical function at old age, even in women with a sedentary lifestyle at present. This was evident when considering time spent in exercise activities but not time spent walking, which suggests that physical activity at intensities corresponding to brisk walking pace may be insufficient to elicit physiological adaptations that impact on physical function at older age.

Study II shows that engagement in exercise-related activities during the age range 35–65 years influenced both cardiorespiratory fitness and muscle mass in later life since women who fulfilled the recommendation of >600 MET-minutes/week had a significantly higher VO$_2$max (P<0.01) and SMI % (P<0.05) at old age compared with those who did not meet the physical activity recommendation. This positive effect remained even when potential influences of past occupational and present physical activity level (in CPM) were considered (Fig. 8A and 9A). We further sought to determine the separate influences of exercise-related activities performed during the early (35–50 years) and late middle age (50–65 years) period and their effect on physical function and muscle mass. Interestingly, significant main effects of engagement in exercise-related activities during late middle age (50–65 years) on VO$_2$max (P<0.01) and SMI (P<0.01) were observed, even after adjustment for occupational physical activity and exercise-related activities performed during early middle age (35–50 years) (Fig. 8B and 9B). No corresponding effects of exercise-related activities during early middle age (35-50 years) on VO$_2$max or SMI were observed (Fig. 8C and 9C). Notably, previous occupation had no significant effect on either VO$_2$max or SMI at old age. Finally, middle-age engagement in exercise-related activities had no influence on maximal arm and leg isometric strength at old age.
Figure 8. Mean (± standard error (SE)) aerobic capacity (VO₂max) among older women who met (■) or did not meet (□) current physical activity recommendations of 600 MET-minutes/week during the age range 35–65 years (Fig. 8A), late middle-age (50–65 years (Fig. 8B) and early middle-age (35–50 years) (Fig. 8C). All data are adjusted for adiposity, current physical activity level and previous occupation. In addition, the effects of exercise-related activities performed during 50–65 years of age on VO₂max have been adjusted for amount of exercise-related activity during 35–50 years of age, and vice versa (Fig. 8B/8C). #P<0.01.

Figure 9. Mean (± standard error (SE)) skeletal muscle mass index (SMI %) among older women who fulfilled (■) and did not fulfil (□) current physical activity recommendations of 600 MET-minutes/week during middle-age
(35–65 years) (Fig. 9A), late middle-age (50–65 years) (Fig. 9B) and early middle-age (35–50 years) (Fig. 9C). All data are adjusted for current physical activity level and previous occupation. In addition, the effects of exercise-related activities performed during 50–65 years on maximum aerobic capacity ($\text{VO}_2\text{max}$) have been adjusted for amount of exercise activity during 35–50 years of age, and vice versa (Fig. 9B/9C). *P<0.05; # P<0.01.

These findings have several important implications: firstly, the present work shows that a physically active lifestyle during adulthood, is associated with a better physical function at old age also among those who are currently inactive. Among older adults, age-related diseases, frailty and mobility limitations are the commonly reported obstacles for a physically active life (Bethancourt, Rosenberg, Beatty, & Arterburn, 2014). The inability to remain physically active at old age can lead to a negative spiral, as a sedentary lifestyle is strongly associated with further impairments in physical function. Therefore, being active throughout adulthood and thus having a high functional capacity at old age, may at least partly protect against the detrimental effects of a sedentary lifestyle later in life. Moreover, although the beneficial effects of past physical activity behaviour on physical function have been reported before (Stenholm et al., 2016), the important question of how much physical activity that is needed throughout mid-life to infer such an effect was previously unanswered. In the current work, we show that adherence to current physical activity guidelines of at least 600 MET-minutes per week is associated with a better aerobic capacity and more skeletal muscle mass at old age compared to women who didn’t fulfil the same guidelines. Thus, our data support the current global physical activity recommendations and suggest that an active life corresponding to a least 600 MET-minutes per week provides a sufficient physiological stimulus to maintain cardiovascular function and muscle mass during ageing. Of note, the two percent unit difference in muscle mass between older women classified as physically active vs. inactive during middle age, is clinically relevant, as it corresponds to nearly 1.5 kg of skeletal muscle mass. Moreover, as the annual loss of muscle mass during middle age is approximately 0.5% per year (Deschenes, 2004), our observed difference between the physically active and the inactive group corresponds to ca. 4 years age related loss of muscle mass. Similarly, older women who were physically inactive during middle
age had an average aerobic capacity of about 27 ml/kg/min, which puts them at higher risk to reach the aerobic threshold of physical dependency, compared to those who were physically active (Young, 1997). In fact, the observed 3 ml/kg/min difference in VO2max between the women who were classified as physically active and those classified as inactive during middle age, would hypothetically correspond to approximately 6 years a retention of aerobic capacity (Paterson et al., 1999). Furthermore, though physical activity behaviour throughout middle age (35–65 years) is associated with physical function and skeletal muscle mass at old age, the current study shows that physical activity later in life (50–65 years), compared with earlier in life (i.e. at 35–50 years), has a greater effect on physical function and muscle mass. The lack of association at early middle age confirms previous data demonstrating weak links between engagement in leisure-time PA and lean mass in a cohort of adults younger than 50 years old (Bann et al., 2014). Interestingly, our findings clearly show that variations in muscle mass and aerobic capacity at old age seem independent of the physical demand of the former occupation. To our knowledge, only one study (Schmidt et al., 2017) has previously investigated the life course effects of both leisure time and occupational physical activity behaviour and its influence on physical function in an older-aged population. Similar to our results, its authors reported a positive effect of leisure-time physical activity on physical function, while no such associations were found for occupational physical activity (Schmidt et al., 2017). It is currently unclear why leisure-time physical activity has a positive effect on physical function and skeletal muscle mass at old age, whereas no such effect is seen for occupational physical activity. However, physical activity during leisure time and work differs in many ways. For example, leisure-time physical activity is freely chosen, and the individual decides what type of activity to perform, when, how often, and at which load. By contrast, occupational physical activity is characterized by monotonous, supervised work and generally involves more repetitions and a longer duration. Additionally, vigorous work is associated with an increased risk to develop musculoskeletal disorders and injuries, which in turn can negatively affect physical function (Andersen, Clausen, Mortensen, Burr, & Holtermann, 2012; Heneweer et al., 2011; Hoogendoorn et al., 1999; van der Windt et al., 2000). Nevertheless, our and Schmidt et a.,
(2017) results highlights the importance of participation in leisure time exercise-related activities during middle age years to infer beneficial effects on muscle mass and function at old age. Finally, in the present work, the effect of physical activity during mid-life remained after adjustment for adiposity. This suggests that being physically active during midlife infers beneficial effects on physical function, regardless of adiposity at old age.

How does physical activity promote muscle mass and physical function in older adults?
Physical activity induces a myriad of physiological adaptations that may transfer to improvements in physical function (Manini & Pahor, 2009). Firstly, repetitive muscle contractions directly stimulate muscle anabolism, which prevents age-related loss of muscle mass and muscle function and thereby has a protective effect against disability. Secondly, physical activity also reduces the risk of becoming overweight and increases insulin sensitivity and is therefore preventive against developing metabolic syndrome and cardiovascular disease. These are severe medical conditions which are associated with a reduced physical function. Thirdly, physical activity can also reduce the occurrence of chronic systemic inflammation and its damaging effects. Furthermore, physical activity also has benefits on the executive control and cognitive function, which may facilitate the performance during challenging tasks. Finally, physical activity can provide psychological and social benefits. This is especially true for older adults since undesired social loneliness is common in this age group.

Resistance training alone and in combination with a healthy diet rich in omega-3 polyunsaturated fatty acids in older women

Effects on muscle mass (Studies III and IV)
To investigate the effects of resistance training combined with a healthy, omega-3 PUFA-rich diet on body composition, systemic inflammation, muscle strength and physical function, we performed a 24-week randomized controlled trial. In Study I, we showed that the diet intervention was successful, as dietary intake of PUFA significantly increased (+76%; P<0.05) and the omega-6/3 ratio decreased (-42%; P<0.05) in RT+HD participants. In addition, serum omega-3 DHA levels (+8.3%; P<0.05) increased and the
pro-inflammatory precursor omega-6 AA (-5.3%; P<0.05) decreased in participants from the RT+HD group, which further confirms dietary compliance. Participants in the CON and RT groups did not alter their nutritional habits throughout the 24-week intervention and in these two groups no changes in serum fatty acid composition were found.

A main finding in the randomized controlled trial was that, despite large increases in maximal muscle strength in both training groups, leg and whole-body lean mass increased significantly only when resistance training was combined with the healthy, omega-3 PUFA-rich diet (RT+HD); (Fig. 10A) +1.7%; P<0.05, for leg lean mass (Study III), and (Fig. 10B). +1.5%; P<0.05, for whole-body lean mass (study IV).

![Figure 10. Relative changes in leg (Fig. 10A) and whole-body (Fig. 10B) lean mass in older women following the 24-week intervention (Study I). □ = control (CON) group; ■ = resistance training (RT), and ● = RT+HD group. *significant changes; P<0.05.](image)

Although somewhat surprising, lack of gain in muscle mass after prolonged resistance training alone has been reported in several previous studies. This suggests that increases in muscle mass are more difficult to obtain than are gains in muscle strength in healthy older adults (Kosek et al., 2006; Vincent et al., 2002) and older women in particular (Hanson et al., 2009). Cur-
Currently, the reason for the lack of resistance training-induced muscle hypertrophy in healthy older adults remains unknown and nutritional strategies to optimize the impact of resistance training have been proposed (Phillips, 2015). Our findings that lean mass increased significantly when resistance training was combined with an omega-3 PUFA-rich diet are therefore important. Currently, no other study has investigated the combined effect of resistance training and a healthy diet high in omega-3 PUFAs and its effect on muscle mass in older adults. Moreover, studies investigating the effect of omega-3 PUFA supplementation alone provides contractionary results, with six reporting unchanged muscle mass (lean body or fat-free mass) (Couet, Delarue, Ritz, Antoine, & Lamisse, 1997; Crochemore, Souza, de Souza, & Rosado, 2012; Hill, Buckley, Murphy, & Howe, 2007; Krzyminska-Siemaszko et al., 2015; Sneddon et al., 2008; Tardivo et al., 2015), while an additional three studies reported gains of 1–4% in lean body mass (Logan & Spriet, 2015; Noreen et al., 2010; Smith et al., 2015). This discrepancy in output may be due to several different causes. Firstly, the studies in which no changes in muscle mass were reported may simply have been too short (3–12 weeks) to detect any meaningful change. Indeed, even if 8 weeks of omega-3 supplementation leads to increases in muscle protein synthesis in both young (McGlory et al., 2014; Smith et al., 2011b) and older adults (Smith et al., 2011a) it may take several more weeks for this increased anabolic environment to be transformed into measurable changes in muscle tissue. In addition, it can be speculated that an amount of omega-3 PUFA equivalent to >2 g DHA and EPA per day may be the minimal dose required to induce myotropic effects. Indeed, in studies where low amounts of omega-3 PUFA (0.5–1.8 g of DHA and EPA) were provided no significant effects were reported (Hill et al., 2007; Krzyminska-Siemaszko et al., 2015), while significant gains in muscle mass were shown in studies where larger amounts of omega-3 PUFA were given (2.0–3.0 DHA and EPA) per day (Logan & Spriet, 2015; Noreen et al., 2010; Smith et al., 2015). This observation is in coherence with our findings, in which approximately 2.0 g of DHA and EPA per day were consumed by participants in the RT+HD group, leading to a muscle mass increase of 1.5%.

Nevertheless, the gains in lean mass (whole body + 1.5% and leg + 1.8%) reported here are relatively modest and in the lower range compared with those previously reported in similar-length resistance training studies in
older adults (+2–6%). For example, Leenders et al. (2013) reported an increase of 2.9% in leg lean mass in older women after a similar resistance training regimen (24 weeks, supervised progressive resistance training twice a week, with a load at 80% of 1RM) (Leenders, Verdiijk, van der Hoeven, van Kranenburg, Nilwik, & van Loon, 2013). Differences in participants’ physical status prior to the intervention and intra-individual anabolic responses to the resistance training may explain the differences between the studies. Indeed, subjects in our study had a higher baseline maximal muscle strength and a higher whole-body lean mass compared with physically inactive and frail older women (Binder et al., 2005; Zaslavsky et al., 2017). Even so, in Study II we showed that the whole-body lean mass of older women in the CON group decreased by 0.4% (non-significant) during the 24 weeks’ duration of the trial. This decline is in line with the annual rate of loss of muscle mass in older women over 65 (Deschenes, 2004). Therefore, the increase in whole-body lean mass (+1.5%) reported by us after 24 weeks of combined resistance training and a healthy, omega-3 PUFA-rich diet is of clinical relevance as this corresponds to almost twice the annual rate of loss of muscle mass in older women (> 65 years of age) (Deschenes, 2004).

Effects on muscle strength and determinants of physical function (Studies III and IV)

Another major finding of the current work was that the healthy, omega-3 PUFA-rich diet enhanced resistance training-induced gains in explosive knee extension force capacity and physical performance. For example, knee extension peak power and time to reach peak power, both of which are important indicators of dynamic explosive force capacity, improved significantly more in the RT+HD intervention (+24.6±2.6%, -20.3±2.7%) than as a result of RT alone (+15.7±2.6%, -11.0±3.8%) (Fig. 11A and 11B). In addition, significant increases in maximal knee extension and leg press 1RM strength were observed in both RT (+20% and +52%) and RT+HD (+21% and +60%) (Studies III and IV). However, compared with improvements in explosive capacity the magnitude of changes in 1RM maximal muscle strength did not differ significantly between the RT and RT+HD intervention, even if gains in maximal leg press strength tended to be larger in the RT+HD group.
The fact that the healthy, omega-3 PUFA-rich diet increased improvements in explosive capacity is of importance given that explosive muscle strength decreases more than maximal muscle strength in older adults (Bassey et al., 1992; Hakkinen et al., 1996; Izquierdo et al., 1999; Skelton et al., 1994). Moreover, age-related changes in the explosive capacity have been linked to a reduced ability to perform normal daily activities such as stair climbing and chair rising, as well as to an increased prevalence of fall injuries (Bassey et al., 1992; Pijnappels et al., 2008; Skelton et al., 1994). The ability of resistance training to improve explosive muscle capacity in older adults has been reported in some (Ferri et al., 2003; Hakkinen, Kallinen, et al., 1998; Hakkinen, Pakarinen, et al., 2001; Holviala et al., 2014; Jozsi et al., 1999) but not all studies investigating this (Frontera et al., 1988; Hakkinen, Newton, et al., 1998; Skelton, Young, Greig, & Malbut, 1995). Therefore,
compared with changes in maximal muscle strength, improvements in explosive capacity are less frequently reported in aged populations. It is therefore interesting that the current work indicates that combining a healthy diet rich in omega-3 PUFA with resistance training increases the effects of resistance training with respect to gains in muscle power.

Physical function measured by SJ performance (i.e. sjMAX and sjRFD) also improved more in the older woman after combined resistance training and healthy diet compared with resistance training alone (RT+HD +58.5±8.4% and +185.4±32.9% vs. RT +35.7±6.9% and +105.4±22.4%) (see Fig 12). A representative force-time curve during an SJ exercise is shown in Figure 12C.

Moreover, correlations analysis revealed that improvements in sjMAX and sjRFD were associated with increases in knee extension peak power (R = 0.55; P<0.001). Squat jump is a demanding functional test that requires rapid increases in limb muscle force under weight-bearing, multi-joint conditions. Therefore, compared with knee extension exercise, the SJ test evaluates the dynamic explosive force capacity during a complex and coordinated motor task and provides salient information about the physical performance of the elderly individual (Caserotti, Aagaard, Larsen, & Puggaard, 2008).
Figure 12. Maximal ground reaction force (sjMAX) (Fig. 12A) and rate of force development (sjRFD) (Fig. 12B) during the concentric phase of the squat jump (SJ) test in the control (CON), resistance training (RT), and RT and healthy diet (RT+HD) groups. PRE-intervention = white bar and POST-intervention = black bar. Figure 12C presents a representative force-time curve during maximal SJ showing changes (PRE = solid line; POST = dotted line) in sjMAX and sjRFD in one participant. *Denotes significant difference vs. PRE (P<0.05); #denotes significant a difference in amplitude of changes between RT and RT+HD (P<0.05). Data are presented as means ± standard error (SE); n = 52.
Only two other studies have investigated the effects of combined resistance training and intake of omega-3 PUFAs on muscle strength and physical function in older adults. Rodacki et al. (2012) reported an enhanced effect of resistance training induced gains in muscle torque, rate of torque development and chair rising performance in healthy older adults after omega-3 PUFA supplementation. Likewise, (Da Boit et al., 2017) reported greater gains in maximal isometric strength following combined resistance training and omega-3 PUFA supplementation compared with resistance training alone in older women but not in men. Altogether, these results clearly support the use of a healthy, omega-3 rich diet to optimize gains in muscle function in healthy older women.

Additionally, the present work shows a weak but significant association between changes in whole-body lean mass and changes in explosive strength capacity (Study IV). This suggests that the additive effects of the healthy, omega-3 PUFA-rich diet on resistance training induced gains in dynamic explosive muscle performance are at least partly explained by increased hypertrophic effects. However, as this association was fairly weak in our population, neuromuscular adaptations most likely also contributed. The latter is supported by Rodacki et al. (2012) who detected larger increases in muscle electromyographic activity in older adults following resistance training combined with omega-3 PUFA supplementation, compared with resistance training alone. Unfortunately, the study by Rodacki et al. (2012) did not measure muscle mass, which makes it impossible to determine whether the improvements in muscle performance were caused by increases in muscle mass or improvements in neuromuscular function. Notwithstanding, current data indicates that the additive effect of omega-3 PUFA on resistance training induced gains in muscle function is caused by both enhanced muscle hypertrophy and neuromuscular adaptations.
Mechanism behind the effect of the healthy polyunsaturated fatty acid-rich diet on muscle mass and determinants of physical function

Potential mechanisms explaining the additive effects of the healthy diet on muscle mass and muscle function include an omega-3 PUFA-stimulated anabolic effect. Indeed, in a paper not included in the present thesis our research group showed that the observed gain in lean mass after combined resistance training and healthy omega-3 rich diet in study I, were accompanied by up-regulation in gene expression of mammalian target of rapamycin signalling pathway (mTOR), which is a key regulator of muscle cellular growth (Strandberg, Ponsot, Piehl-Aulin, Falk, & Kadi, 2019). In line with our findings a novel study by Smith et al., (2011) reported an mTOR-induced increase in protein synthesis accompanied by a decreased age-related loss of muscle mass after 24 weeks of omega-3 PUFA supplementation. Moreover, in-vitro models and animal studies have consistently shown a stimulatory effect of omega-3 PUFA on muscle protein synthesis mediated by the mTOR pathway (Kamolrat & Gray, 2013; Wang, Lin, Zheng, Zhang, & Huang, 2013; Wei et al., 2013). In addition, high concentrations of omega-6 PUFA arachidonic acids have been associated with smaller muscle size and increased muscle protein degradation in older adults (Reinders, Song, et al., 2015; Whitehouse, Khal, & Tisdale, 2003). Therefore, even if the exact molecular mechanisms still remain to be determined, current evidence suggests a positive effect of omega-3 PUFA on muscle protein synthesis and increased signalling for protein breakdown of omega-6 PUFA.

The ability of omega-3 PUFAs to alter the chronic low-grade systemic inflammation common in older adults also has to be considered (Bruunsgaard & Pedersen, 2003; Schaap et al., 2006a). In the present work, circulatory levels of the anti-inflammatory omega-3 DHA increased and the level of the pro-inflammatory omega-6 precursor arachidonic acid decreased after combined resistance training and healthy diet (Strandberg et al., 2015). However, it is important to acknowledge that no changes in systemic inflammatory markers C-reactive protein and interleukin-6 were observed (Strandberg et al., 2015), presumably due to low inflammatory levels in participants already at the start of the intervention. The lack of changes in systemic inflammation despite clear myotropic effects is in coherence with Smith (2011; 2015), who reported decreased age-related loss of muscle mass and strength but unchanged serum C-reactive protein, interleukin-6 and
tumor necrosis factor-α (Smith et al., 2011a; Smith et al., 2015). This raises the question whether the mechanisms by which omega-3 PUFA affects muscle mass and strength really are connected to the PUFA’s anti-inflammatory properties. Nonetheless, we still cannot rule out anti-inflammatory properties of the healthy omega-3 PUFA-rich diet as a potential mechanism behind the enhanced myotrophic effects, as reduction in the local inflammatory signal within the muscle cell itself may still occur. Indeed, in the aforementioned study (Strandberg et al., 2019), our research group found a significant downregulation in skeletal muscle gene expression of the pro-inflammatory cytokine IL-1β of RT-HD only. However, the fact that members of the NF-κB and Toll-like receptor family remained unchanged by the intervention, highlights the complexity of mechanisms regulating local muscle inflammatory processes. This warrants further research.

The additive effect of the healthy, omega-3 PUFA-rich diet on muscle function may also be related to improved neuromuscular aspects (Stiefel et al., 1999). For example, Rodacki et al. (2012) reported superior gains in muscle peak torque and muscle EMG activity following 12 weeks of combined resistance training and omega-3 PUFA supplementation, compared with resistance training alone, in healthy older adults. In animal models, improved neuromuscular function following omega-3 PUFA supplementation has been related to increased sensitivity for the neurotransmitter acetylcholine (Patten, Abeywardena, McMurchie, & Jahangiri, 2002). Increased acetylcholine sensitivity may enhance the action potential transmission rate at the neuromuscular junction and thereby increase the muscle EMG activity and force output.
Strength and limitations

Strengths of the present thesis include the use of objective assessment of present physical activity level by accelerometry as well as the use of a validated questionnaire for assessment of past physical activity level. Moreover, when the influence of physical activity on physical function was investigated, the analysis was adjusted for individual variations in adiposity by DXA (Study I) or BIA (Study II) derived fat mass percentage, instead of the more commonly used BMI. As the BMI reflects body weight in relation to body height rather than amount of fat mass, confounding effects of adiposity on observed links between physical function and PA are readily adjusted for in the present work. Another notable strength is that all resistance training sessions in the randomized controlled trial were supervised by experienced staff. Moreover, increases in training load was based on individual improvement in maximal muscle strength and test of new 1RM strength were conducted at least every fourth week. Finally, participants habitually physical activity level outside the training intervention was controlled by accelerometry and assessment of dietary intake occurred several times during the intervention.

Limitations of the present thesis include the cross-sectional design utilized in study I and II, which precludes causality between physical activity and physical function. Moreover, it is possible that other confounding factors than those included in our analysis may affect the observed relationships between physical activity and physical function. Even if a validated questionnaire was used for assessment of past physical activity level, assessment of behaviours retrospectively is prone to recall bias. Importantly, given that such bias likely results in a diluted effect size, the observed link between past PA and physical function would reflect a true relationship between these two variables. Of note, we specifically assessed leisure-time PA, which may be more accurately recalled than other PA behaviours related to transport and household chores. However, any added influence by time in PA performed outside the leisure-time domain and with intensities above that of walking cannot be ruled out. The 24-week dietary intervention was self-administered and based on dietary counselling. This dietary design is less rigorous than controlled-nutrient diets. However, the strategy used in our study allows reducing participant burden given that one of the major challenges in any long-term dietary study is the retention of eligible study
members for the duration of the intervention. Importantly, the present work did not address the effect of healthy diet alone on muscle function. This precludes inference on the potential effects of healthy diet alone on explosive muscle performance. Given that findings from several previous studies suggest that diet alone may not trigger myotrophic effects in elderly (Fiatarone et al., 1994; Leenders et al., 2011; Verhoeven et al., 2009) and that participants included in the present study were healthy older women who adhered to general guidelines regarding recommended physical activity, we hypothesized that any changes in muscle function in this healthy group of older women would require exposure of skeletal muscle to heavy loads during resistance training. Finally, while our data provide novel insights into the influence of physical activity behaviours in general and resistance training in particular on physical function and body composition in older women, caution should be taken when generalizing study findings from our relatively healthy sample to a broader population of older men and women with different health conditions.
Methodological considerations

In the present thesis muscle and fat mass were assessed using DXA, and BIA which are a less accurate methods compared with computed tomography (CT) and magnetic resonance imaging (MRI). An important difference between the method is that while CT and MRI measures skeletal muscle mass, DXA measures lean tissue mass. Lean tissue includes total body protein, soft-tissue minerals, and carbohydrates, as well as water content. In other words, in addition to skeletal muscle, lean tissue also includes connective tissues, lean portions of adipose tissue, internal organ tissues and skin (Kim, Wang, Heymsfield, Baumgartner, & Gallagher, 2002). This means that, for a given assessment, lean tissue mass is generally larger than skeletal muscle mass. Nevertheless, lean tissue mass of the extremities is primarily skeletal muscle mass (Heymsfield et al., 1990). Despite these important differences in absolute values, DXA is considered a reasonable alternative to CT and MRI given the relatively high between-method agreement ($r = 0.94–0.98$) in the determination of both total and regional muscle mass and, further, given that the amount of radiation using DXA is far less than with CT (Kim et al., 2002; Levine et al., 2000; Roche, Heymsfield, & Lohman, 1996). Likewise, both total and abdominal assessments of fat mass have shown strong correlations between DXA, CT ($R = 0.77–0.95$) and MRI ($R = 0.97–0.99$) measurements in lean as well as obese subjects ($r = 0.77–0.95; P<0.0001$) (Borga et al., 2018; Pietrobelli, Formica, Wang, & Heymsfield, 1996). Under standardized conditions (including body position, hydration status, prior exercise and dietary intake), using an appropriate and validated equation, the BIA method is considered as an accurate measurement of muscle mass ($R \approx 0.90$, BIA vs. MRI (Janssen et al., 2000)) and fat mass ($R \approx 0.85$, BIA vs. MRI (Wang et al., 2013)) in clinical settings and studies with larger sample sizes (Wells & Fewtrell, 2006).

Physical activity is a complex behaviour, which makes accurate assessment and quantification a challenging task (Lamonte & Ainsworth, 2001). In the present thesis, accelerometers were used to objectively assess total physical activity level and time spent in different intensities of physical activity. Accelerometers are motion sensors that estimate physical activity by measuring accelerations of the body during movement. Accelerometers have the ability to capture both frequency and duration, as well as intensity of physical activity as a function of body movement. In contrast to more simple
pedometers, which only record forward vertical movement (steps), modern tri-axial accelerometers have the ability to capture accelerations and consequently movement in all three planes (vertical, mediolateral, and anterior-posterior). Therefore, accelerometers are currently regarded as the best available tool to objectively assess physical activity behaviour in free-living conditions (Strath et al., 2013). The activity monitor (Actigraph model GT3x, Pensacola, FL) that was used in this work has shown high validity compared with indirect calorimetry in laboratory settings ($R = 0.6$, $P<0.001$) (Swartz et al., 2000) and during free-living conditions ($R \approx 0.5$, $P<0.01$) (Matthew, 2005), and a high inter-device reliability ($R \approx 0.98$, $P < 0.001$) (Brage, Wedderkopp, Franks, Andersen, & Froberg, 2003; Cliff, Reilly, & Okely, 2009; Pate, O’Neill, & Mitchell, 2010; van Cauwenberghe, Labarque, Trost, de Bourdeaudhuij, & Cardon, 2011; Westerterp, 1999). In general it is recommended that accelerometers are worn during waking hours for a week except during water activities (Hills, Mokhtar, & Byrne, 2014). In several studies a minimum wear time of 10 hours/day for at least 4 days was required for inclusion in data analysis (Hills et al., 2014). Physical activity data derived from accelerometers are typically presented as time in light, moderate or vigorous-intensity physical activity based on standardized thresholds (i.e. counts/min) (Freedson, Melanson, & Sirard, 1998; Rowlands, Thomas, Eston, & Topping, 2004; Swartz et al., 2000). A major issue when assessing time spent in different physical activity intensities is the selection of accelerometer cut-off points. Currently a gold standard accelerometer cut-off for older adults remains to be determined. In this work, the most commonly used cut-off points for adults were used (Matthew, 2005; Troiano et al., 2008).

When assessing physical activity behaviours, there is also a growing interest in the objective measurement of sedentary behaviours (Reilly et al., 2008). Sedentary behaviours include prolonged engagement in activities with no or only minimal movement (e.g. watching TV) (Biddle, Gorely, Marshall, Murdey, & Cameron, 2004; Reilly et al., 2008). A limitation of accelerometers with respect to assessment of sedentary behaviour is that the motion sensor is unable to separate sitting activities from static standing activities (Bouten, Sauren, Verduin, & Janssen, 1997). Nevertheless, several validation studies have concluded that accelerometers provide an acceptable measurement of sedentary behaviour (Atkin et al., 2012). Major advantages
of accelerometry include that detailed information on both duration and intensity, as well as frequency of physical activity is provided. In addition, accelerometers can store data for a long time, have a low burden for participants and are relatively inexpensive. Finally, accelerometers do not give visual feedback to the individual wearing the device. Therefore, the lack of direct feedback to the wearer reduces the likelihood of an overestimation of physical activity due to manipulation. Weaknesses inherent to the use of accelerometers include the inability to accurately capture exercises involving static muscle contractions, and non-ambulatory and swimming activities. In addition, upper body work is neglected if the accelerometer is placed on the hip or waist (Hills et al., 2014; Strath et al., 2013).

While physical activity is notoriously difficult to measure in free-living situations, retrospective measurement of past physical activity behaviours poses an even greater challenge (Besson et al., 2010). Nevertheless, one retro perspective study investigating the ability of older adults to recall occupational and leisure-time physical activity 35 years earlier in life showed a remarkably high correlation with original physical activity measurements (Falkner, Trevisan, & McCann, 1999). Also, the reported intraclass correlation (ICC) of ≈ 0.4 is close to that found in studies where the recall interval was 10 years or less. This clearly demonstrates that older adults’ ability to recall physical activity behaviour throughout adulthood is satisfactory given that the method used is appropriate.

In the present work the historical adulthood physical activity questionnaire (HAPAQ) was used to assess past physical activity behaviour. The HAPAQ is specially designed to retrospectively collect data regarding physical activity behaviour through adulthood. The major advantages of the HAPAQ are that it is easy to administer to a large population at a low cost. However, as with all self-report instruments, the HAPAQ is vulnerable to recall bias, which may lead to over- or underestimation of physical activity level, and compared with objective methods, questionnaire-based information about physical activity is crude (Jacobs, Ainsworth, Hartman, & Leon, 1993). Nevertheless, the HAPAQ has been validated against objectively measured physical activity (using the accelerometry and individual calibrated heart rate monitors) and demonstrates moderate correlations (R ≈ 0.3-0.5) depending on which type of physical activity that been assessed (Besson et al., 2010; DuBose, Edwards, Ainsworth, Reis, & Slattery, 2007).
These correlations are in line with those reported in review of previous studies examination self-reported against objectively measured physical activity (Neilson, Robson, Friedenreich, & Csizmadi, 2008). Therefore it is considered that HAPAQ offers an acceptable validity for ranking individuals according to their past physical activity behaviour. It is noteworthy that, in the present work, we specifically focused on past leisure-time physical activity, which may be more accurately recalled than other physical activity behaviours related to transport and household chores. Thus, at present the HAPAQ is regarded as offering the most feasible and acceptable option for retro perspective assessment of adulthood physical activity (Besson et al., 2010).

Assessment of dietary behaviours is a complex task (Shim, Oh, & Kim, 2014). In the present thesis, detailed information on dietary intake among participants was obtained using the dietary record method. The dietary record is the most commonly used method for assessment of dietary intake in randomized clinical trials and cohort studies (Dauchet et al., 2007; Luke et al., 2011; Margetts, 1997). This dietary record asks for detailed information about food intake but may also include information about food preparation, brand names, etc depending on the research question. The amount of each food consumed is reported with the help of a common portion size guide, which the survey suggests, often by referring to a standard measuring container, glasses, cups and spoons; sometimes photographs of standard portions are also provided. Given that the survey respondents maintain their habitual dietary intake assessment over 5–7 days including at least 1 weekend day, this is considered to give a representative assessment of their overall dietary intake (Shim et al., 2014). Strengths of the method include the use of open-ended questions so that abundant information can be collected and analysed in various aspects. Compared with other methods for assessment of dietary intake, such as the 24-hour dietary recall and frequency food questionnaire (FFQ), the dietary record poses no risk of recall bias and no time-consuming interviews are needed. However, since all these three methods can be burdensome and affect normal eating habits, they may lead to under-reporting (Margetts, 1997).
Conclusions

This work explored muscle mass and physical function among older women and provided new knowledge regarding the impact of physical activity based on both cross-sectional and experimental study designs. The major conclusions are:

1) Being physically active at an older age is related to a better physical function, even in individuals with a previously sedentary lifestyle. In particular, increased time spent in MVPA is advocated for inferring beneficial effects on physical function in healthy older women.

2) Engagement in exercise-related activities at middle age is beneficially linked to muscle mass and aerobic capacity in women at old age. Importantly, these benefits are evident regardless of both physical demand of former occupation and present PA level.

3) Combining resistance training with a healthy diet rich in omega-3 PUFAs increases the effects of resistance training on gains in muscle mass and determinants of physical function in healthy older women.
Practical implications

Ageing is accompanied with a gradual decline in physical function, which reduces the ability to perform activities of daily living and leads to loss of independence. The present thesis demonstrates that habitual physical activity at old age infers beneficial effects on physical function, even in individuals with a previously sedentary lifestyle. Therefore, this work supports public health efforts aiming to increase physical activity levels in older age groups. In contrast to MVPA, neither time spent in sedentary behaviours nor time in LPA influence physical function. This emphasizes the importance of accumulating time in MVPA in order to promote a better physical function by advancing age. The present work also highlights the importance of engaging in regular exercise-related activities during middle age years in order to promote benefits on muscle mass and physical function at old age. Together, this work clearly supports the potential role of physical activity in delaying functional impairment and the occurrence of clinically manifest sarcopenia at old age.

Loss of skeletal muscle mass and muscle function are two major factors that contribute to the loss of physical function during ageing. Consequently, major health organizations including the World health organization (WHO) and the American College of Sports Medicine (ACSM, 2009; Nelson et al., 2007) recommend that older adults perform resistance training at least twice a week with a load corresponding to 75–85% 1RM (8–12 rep/set) in order to increase muscle mass and improve muscular function. However, in the present work, 24 weeks of supervised resistance training based on the recommendations from WHO/ACSM did not induce statistically significant gains in muscle mass in healthy older women, despite large increases in muscle function. This finding is in coherence with several other studies who also failed to induce gains in muscle hypertrophy in healthy older women following a similar resistance training regime (Hanson et al., 2009; Kosek et al., 2006; Vincent et al., 2002). The lack of gain in muscle mass after resistance training based on WHO/ACSM recommendations raises the question whether current recommendations are optimal for healthy older women? Nevertheless, the present work shows that when resistance training was combined with a healthy diet rich in omega-3 PUFA, muscle mass increased significantly. Furthermore, gains in dynamic explosive muscle
strength during both isolated lower limb movements and multi-joint exercises were larger compared with gains after resistance training alone. Thus, the present work demonstrates the need of integrated recommendation that takes both nutritional and exercise training aspects into account in order to maximize skeletal muscle adaptation in healthy older women.

Altogether, this thesis highlights the importance of a high habitual physical activity level both during middle age and at old age, as well as the positive effect of regular resistance training combined with intake of a healthy, omega-3 PUFA rich diet to maintain physical function and thereby promote a healthy ageing.
Future perspectives

Findings from the present thesis highlight the importance of a physically active lifestyle and intake of healthy diet in order to promote a healthy ageing. However, several important questions remain to be explored.

1) Results from the present work emphasize the importance of encouraging older adults to engage in physical activity of at least moderate intensity as a means to prevent age-related impairments in physical function. However, an important unresolved issue is the identification of the minimum dose of daily MVPA necessary for maintenance of physical function in older adults. Likewise, it is unknown whether there exists an upper threshold, after which more MVPA does not induce further benefits on physical function among the elderly.

2) Evidence for the positive effects of physical activity on physical function in older adults, including those presented in the current thesis, are almost exclusively based on cross-sectional studies. Consequently, there is a need for controlled long-term prospective studies to confirm a causal link between increased habitual physical activity and the rejuvenation of physical function.

3) Our results show that a healthy diet that is rich in omega-3 PUFAs augments the effect of resistance training on explosive muscle strength, physical function and muscle mass. However, the optimal intake of PUFAs to induce such effects remains unknown. Additionally, to optimize the anabolic effect of such a healthy diet, the exact signalling pathways involved in muscle adaptations need to be investigated.

4) The fact that muscle mass only increased when resistance training was combined to the healthy omega-3 PUFA rich diet highlights the need for more long-term studies that investigate the combination of training and diet using an integrated approach in order to facilitate training induced adaptations.
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Svensk sammanfattning

Åldrade är associerat med en gradvis försämrad arbetskapaclit, vilket på sikt påverkar individens rörelseförmåga och därmed möjlighet att självstän- digt utföra olika vardagsaktiviteter. Äldre kvinnor anses vara särskilt sår- bara för denna typ av åldersförändringar, då de generellt sett har en lägre fysisk arbetskapacitet och en högre förekomst av rörelsenedsättning jämfört med äldre män. Det är därför viktigt att identifiera modifierbara faktorer och utveckla effektiva träningsprogram som en del av det preventiva arbetet mot åldersrelaterad funktionsnedgång hos äldre kvinnor. Daglig fysisk aktivitetsnivå och framförallt motionsvanor har föreslagits vara sådana faktorer.

Sambandet mellan daglig fysisk aktivitetsnivå och fysisk funktion hos äldre individer är fortfarande oklart. Ett flertal men långt ifrån alla tidigare studier har föreslagit ett positivt samband mellan aktivitetsnivå och funktionell förmåga. Denna diskrepans i resultat beror troligtvis på metodolo- giska skillnader t.ex. huruvida aktivitetsnivå och funktionell förmåga har mätt med objektiva metoder eller via självskattning. Förutom daglig aktivitetsnivå har vissa forskare argumenterat för att stillasittande i sig skulle kunna ha en negativ effekt på funktionell förmåga, dvs. oberoende av individens övriga aktivitetsvanor. Huruvida detta är sant är dock även det oklart. Förutom nuvarande aktivitetsvanor har det även föreslagits att aktivitetsvanor och motionsvanor tidigare i livet skulle kunna ha en inverkan på funktionell förmåga vid äldre ålder. I vilken utsträckning nuvarande fysisk aktivitetsnivå påverkar funktionell förmåga vid äldre ålder, när hänsyn tagits till aktivitetsnivå tidigare i livet har dock inte undersöks tidigare.

Syftet med delstudie I var därför att undersöka sambandet mellan både nuvarande och tidigare fysiska aktivitetsvanor och objektivt uppmätt funktionell förmåga hos friska äldre kvinnor. Resultaten från studien visade att både nuvarande aktivitetsnivå och motionsvanor tidigare i livet, var positivt relaterade till funktionell förmåga hos de äldre kvinnorna. Detta samband drevs av hur mycket fysisk aktivitet av mer belastande karaktär som utförts. Medan lättare fysisk aktivitet (motsvarande promenad och mindre an- strängande) inte upptäcktes någon samband med funktionell förmåga, oav- sett om den utförts tidigare eller senare i livet. Efter att analysen justerats för deltagarnas nuvarande fysiska aktivitetsnivå sågs inget samband mellan tid i stillasittande och funktionell förmåga. Tillsammans indikerar detta att
det är avsaknad av fysisk aktivitet av mer belastande karaktär, snarare än för mycket stillasittande, som influerar negativt på den funktionella förmågan hos äldre kvinnor.

Åldersrelaterad förlust av muskelmassa, styrka och aerob kapacitet är tre starkt bidragande faktorer till den nedsatt funktionell rorelseförmåga hos äldre. Den preventiva effekten av daglig fysisk aktivitet med avseende på muskelmassa och styrka är idag oklar. Det är även oklart om fysiska aktiviteter utförda tidigare eller senare i livet har samma effekter på densamma. Slutligen har det även föreslagits att vilken typ av arbete som individen haft under sina yrkesverksamma år (dvs stillasittande eller fysisk ansträngande) kan påverka funktionell rorelseförmåga vid äldre ålder. Trots detta saknas i mycket forskning som analyserat effekten av fysisk aktivitetsnivå under både fritid och arbetsliv med avseende på funktionell förmåga vid äldre ålder.

Syftet med delstudie II var därför att undersöka sambandet mellan tidigare fysiska aktivitetsvanor både under fritid (idrottsrelaterade aktiviteter) och arbetsliv med avseende på muskelmassa och funktionell förmåga hos äldre kvinnor. Resultaten från studien visade att de kvinnorna som varit fysisk aktiva, dvs uppnått nuvarande rekommendationer motsvarande minst 150 min av mätlig till hög fysisk aktivitet per vecka, mellan 35 och 65 års ålder, hade mer muskelmassa och en bättre aerob kapacitet jämfört med individer som inte uppnådde samma rekommendation. Denna effekt drevs av individernas aktivitetsvanor under senare delen av medelåldern (50-65 år) och kvarstod även efter hänsyn tagits till tidigare yrke och nuvarande aktivitetsvanor. Slutligen noterades ingen samband mellan fysiska aktivitetsvanor tidigare i livet och ben eller armstyrka hos de äldre kvinnorna. Detta indikerar att motionsvanor under medelåldern och då framförallt senare medelåldern har en viktig effekt på funktionell kapacitet senare i livet och att detta samband är oberoende av hur aktiv individen är vid äldre ålder.

Styrketräning anses idag vara den främsta metoden för att minska förlusten av muskelmassa och muskelstyrka vid åldrande. Flera stora hälsoinstitutioner inklusive Världshälsoorganisationen (WHO) och Folkhälsomyndigheten rekommenderar därför att äldre individer utför styrketräning minst två gånger per vecka med fokus på de stora muskelgrupperna. Jämfört med yngre personer så är dock det anabola svaret efter styrketräning sämre hos äldre. Till exempel så rapporteras ofta mindre ökningar i muskelmassa hos äldre jämfört med yngre individer. Detta kan bero på ett flertal olika

Resultat från delstudie III visar att trots stora ökningar i maximal muskelstyrka efter både styrketräning och styrketräning i kombination med den hälsosamma omega-3 rika dieten, så var det bara i den senare interventional gruppen som signifikanta ökningar i muskelmassa kunde ses. Ett annat viktigt fynd var att den ökade anabol träningrespons inte tycktes vara direkt kopplad till omega-3 fettsyrornas presumtiva antiinflammatoriska egenskaper, då inga signifikanta skillnader i de vanligaste markörerna för systemisk inflammation (CRP och IL-6) kunde ses.

Delstudie IV visar att både explosiv muskelstyrka och funktionell förmåga mätt genom ett hoppsett, ökade mer efter kombinerad styrketräning och omega-3 rik diet jämfört med enbart styrketräning hos de äldre kvinnorna. Ökningarna i explosiv muskelstyrka och hoppkapacitet var signifikant korrelade med ökningar i muskelmassa, vilket indikerar att den additiva effekten av styrketräning kombinerat med omega-3 rik diet åtminstone delvis kan tillskrivas den ökade muskulära hypertrofin.

Sammantaget visar denna avhandling att både nuvarande och tidigare aktivitetssvanor, oberoende av varandra, har betydelse för funktionell förmåga och muskelmassa hos äldre. Avhandlingens resultat stödjer preventiva åtgärder som syftar till att öka den dagliga fysiska aktivitetsnivån, snarare än att minska tid i stillasittande. Avhandlingen visar även att en hälsosam diet, rik på omega-3 fettsyror kan förbättra anpassningar i muskelmassa och explosiv muskelkapacitet efter styrketräning hos äldre friska kvinnor. Detta är viktigt eftersom explosiv muskelkapacitet är en av de starkaste prediktatorerna för bibehållna funktionell kapacitet och ett oberoende leverne
hos äldre. Det faktum att den grupp som styrketräna de men bibehöll sina normala kostvanor inte ökade muskelmassan trots stora förbättringar i maximal muskelstyrka och funktionell förmåga reser frågetecken. Är nuvarande rekommendationerna avseende styrketräning optimala för friska äldre kvinnor? Bör kanske tränings- och kostrekom mendationer integreras i högre utsträckning för att nå en mer optimerad effekt? Resultaten från denna avhandling bidrar till en ökad förståelse för vikten av en fysisk aktiv livsstil, styrketräning och intag av en hälsosam diet för att bibehålla en hög funktionell förmåga och därmed främjandet av ett hälsosamt åldrande. Utifrån avhandlingens resultat dras slutsatserna att:

- Rekommendationer avseende fysisk aktivitet för äldre kvinnor bör fokusera på att öka tiden i fysisk aktivitet av minst moderat intensitet ( motsvarande rask promenad och mer ansträngande) snarare än reducera tiden stillasittande med avseende på bibehållen hög rörs elformåga.

- En fysisk aktiv livsstil under medelåldern, motsvarande nuvarande internationella rekommendationerna om > 150 min/vecka av fysisk aktivitet av minst moderat intensitet, är associerat med en större muskelmassa och en bättre funktionell förmåga hos äldre kvinnor. Detta samband var oberoende av om individen haft ett aktivt eller stillasittande yrke och oavsett hur aktiv individen var vid äldre ålder.

- Äldre kvinnor bör uppmuntras att regelbundet träna styrketräning och inta en hälsosam kost rik på omega-3 fettsyror med syftet att optimera anpassningar i muskelmassa, explosiv muskelstyrka och funktionell förmåga.
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