Age related changes in skeletal muscle mass and function

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Abstract

The loss of muscle mass with age (Sarcopenia) has received growing attention over the past decade. Despite efforts to provide a universal definition with clinically meaningful cut-off points for diagnosis, there is no clear consensus on how to best quantify and assess the impact of loss of muscle mass and function on functional limitations. Whilst most previous studies have used dual energy x-ray absorptiometry (DXA) to quantify this loss, chapter 2 of this thesis shows that DXA underestimates the loss of muscle mass with age in comparison to the gold standard MRI.

Muscle mass per se is not enough to determine whether a person has an exceptionally low muscle mass, as it can be readily seen that a healthy tall person will have a larger muscle mass than a small person. Clinicians and researchers thus need an index of muscle mass that takes differences in stature into account and also gives an objective cut off point to define low muscle mass. In Chapter3, we show that femur volume does not significantly differ between young and old. We used this observation to introduce a new index: thigh muscle mass normalised to femur volume, or the muscle to bone ratio. This index allows the examination of the true extent of muscle atrophy within an individual.

In previous studies the appendicular lean mass (determined with DXA) divided by height squared appeared to be a relatively poor predictor of functional performance. In Chapter 4, the index introduced in Chapter 3, the muscle to bone ratio, proved to be a somewhat better predictor of functional performance in the overall cohort. This was, however, not true when examining the intra-group relationships. A similar situation applied to the maximal muscle strength. In older adults, the parameter which predicted functional performance best was muscle power per body mass, measured during a counter-movement jump.

Chapter 5 shows that part of the larger loss power and force than muscle mass is attributable to a left-ward shift of the torque-frequency relationship, indicative of a slowing of the muscle, and reduction in maximal voluntary activation, as assessed using the interpolated twitch technique in older adults.

Chapter 5 also shows that the fatigue resistance during a series of intermittent contractions was similar in young and older adults. However, older adults could sustain a 50% maximal voluntary contraction force longer than young people. Part of this discrepancy maybe due to an age-related slowing of the muscle.

Table of Contents

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Additional Publications

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McPhee JS; Narici MV; **Maden-Wilkinson TM**; Bijlsma AY; Meskers CGM; Maier AB; Sipila S; Stenroth L; Sillanpää E; Gapeyeva H; Pääsuke M; Seppet E; Barnouin Y; Hogrel JY; Bottinelli R; Butler-Browne G and Jones DA. (2013). Physiological and Functional Evaluation of Healthy Young and Older Men and Women: Design of the European Myoage Study. Biogerontology. 14(3):325-337

Ireland A, **Maden-Wilkinson TM,** Degens H, Cooke K, Rittweger J. (2013) Upper limb muscle-bone asymmetries in elite junior tennis players. Med Sci Sports Exerc. 45(9):1749-58

E Sillanpää; L Stenroth; AY Bijlsma; T Rantanen; JS McPhee; **TM Maden-Wilkinson**; DA Jones; MV Narici; H Gapeyeva; M Pääsuke; Y Barnouin; JY Hogrel; G Butler-Browne; AB Maier, S Sipilä (2013). Muscle power, strength and physical performance are associated with ventilatory function in healthy older people (**Submitted**)

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Abstracts

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List of Tables

Chapter 1*:* Introduction and Literature Review

Chapter 2: Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles.

Chapter 3: Thigh muscle volume in relation to age, sex and femur volume

List of Figures

Chapter 1*: Introduction and Literature Review*

Chapter 2: Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles.

Chapter 3: Thigh muscle volume in relation to age, sex and femur volume

Chapter 4: Age related loss of muscle mass, strength and power and their effect on mobility in recreationally active UK older adults

Chapter 1

General Introduction and Literature Review

1.1 Muscle Structure and Function

Skeletal muscle constitutes about 40% of body mass. Skeletal muscle is required for movement, but also for maintenance of posture, breathing and even communication. Besides these roles that are realised by the contractile properties of the muscle fibres, skeletal muscle tissue is also important for metabolism, such as storage of glucose, and even serves as an endocrine organ (Pedersen 2009).

Overview of Muscle

The structure of the muscle helps to understand how the muscle is able to generate force and shorten at the same time. Skeletal muscle is made up of bundles of muscle fibres (Figure 1.1), which are usually joined by tendons (though they may also directly attach to the bone) at the proximal and distal ends of the muscle. When the muscle contracts the forces are transmitted to the bones and when the forces are larger than the resistance to movement, the bones will move. Contraction the Quadriceps Femoris muscles on the anterior thigh, for instance, results in the extension of the knee.

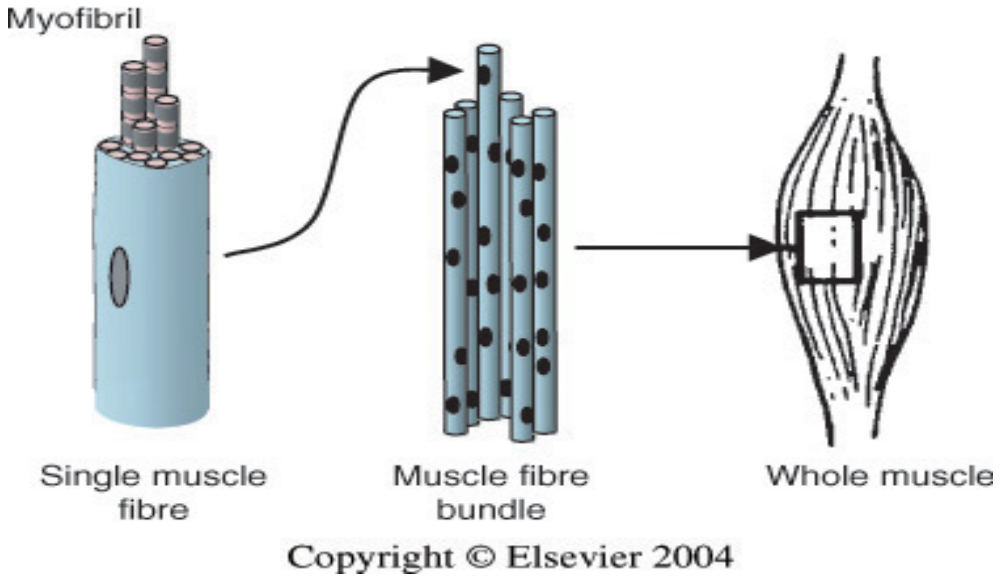

Figure 1.1- Illustration of the ultrastructure of skeletal muscle. Adapted from (Jones et al. 2004)

Skeletal muscle fibres are multinucleated elongated cell. In mammalian muscles, the nuclei are located beneath the sarcolemma. The thin (actin) and thick (myosin) contractile filaments are arranged in sarcomeres that run from Z line to Z line (Figure 1.2). The contraction of skeletal muscle is controlled by the motor neurons in the spinal cord. Each motor neuron innervates a group of muscle fibres within a given muscle, and thus when a motor neuron is active it will cause contraction of all the muscle fibres it innervates. One could this say that they act as a unit, and it is therefore that the motor neuron plus it's innervated fibres are called the motor unit.

The contraction of a muscle fibre is initiated by the depolarisation of the motor end plate that causes the fusion of acetylcholine (ACh) vesicles with the presynaptic membrane and the consequent release of ACh in synaptic cleft. The ACh interacts with the ACh receptors on the post-synaptic membrane that increases the permeability of the muscle membrane for Na^+ resulting in a rapid depolarisation of the muscle membrane. This depolarisation propagates rapidly across the muscle membrane into the transverse tubules (T-tubules). The voltage-gated dihydropiridine receptors (DHPR) change their conformation and as a consequence the calcium channels, known as ryanodine receptors (RyR) on the sarcoplasmic reticulum, open and Ca^{2+} is released from the sarcoplasmic reticulum (Huang et al. 2011). When the Ca2+ binds to troponin C on the actin filament it causes a conformational change in Tropomyosin. This conformational change in Tropomyosin exposes the myosin binding sites on actin enabling the subsequent binding of myosin with actin. The hydrolysis of adenosine 5' tri-phosphate (ATP) in several steps causes conformational changes in the myosin molecule that pull the actin filament along the myosin filament, the so-called 'sliding' of the filaments. During the sliding of the filaments the myosin molecules not only produce force, but also cause shortening of the muscle. The whole process from electrical excitation to contraction of the muscle is known as Excitation-contraction coupling and was originally proposed by Huxley (Huxley 1957).

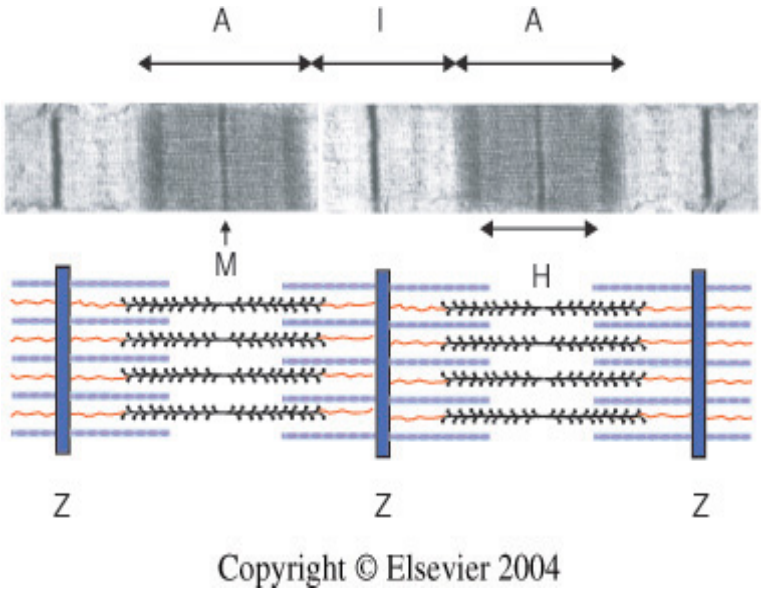

Figure 1.2 – Electromicrograph of skeletal muscle showing striations and illustration of Z, M, A and I bands as discussed above, Adapted from (Jones et al. 2004).

Skeletal Muscle Fibre Types

Skeletal muscle is composed of different types of motor units, which are characterised depending on their contractile properties. In Human skeletal muscle, fibres can be broadly classified into 3 fibre types (I, IIa and IIx) (Brooke & Kaiser 1970). There is of course the added complication that some fibres can contain more than one myosin heavy chain isoform, but they usually do not comprise more than 5% of the total fibre population; such fibres are called hybrid fibres.

The requirement of the muscle to be able to generate force is evident during locomotion and moving around objects. Force alone is, however, not enough, as locomotion also requires muscle shortening. Since force x velocity equals power, one can imagine that the power generating capacity of a muscle has a major impact on the ability to perform daily life activities.

While adequate power and force generating capacities are important to perform daily life activities, they are not the whole story. One must also be able to perform some

tasks, such as repeatedly placing one foot in front of the other during walking, for prolonged periods. In this case, it is important that the muscle maintains as much as possible the ability to generate adequate force and power; that is show little fatigue. The fatigue resistance of a muscle is therefore another important aspect of maintaining mobility and preventing falls. This resistance to fatigue is dependent on the characteristics of the motor unit (See Fatigue).

Contractile Properties of Skeletal Muscle

The maximal speed at which a muscle can shorten is not only dependent on muscle length but also on the fibre type composition of the muscle In 1873, Ranvier recognised that red and white muscles exhibited different contractile properties (Ranvier 1873). The red fibres described by Ranvier are predominantly type I fibres that have relatively slow contractile properties with a high fatigue resistance as a result of a high oxidative capacity. Type II fibres have faster contractile properties, but are more fatigable than the type I fibres and rely more on glycolysis for ATP replenishment during activity than aerobic metabolism.

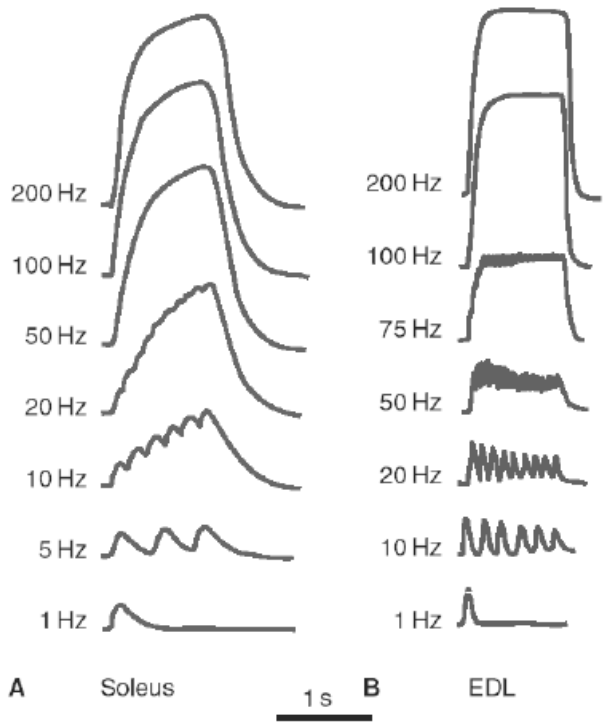

Figure 1.3. Illustration of force generated during electrically evoked contractions in skeletal muscle in the (A) Soleus and (B) Extensor Digitorum Longus (EDL). Adapted from (Jones et al. 2004).

The time it takes for a muscle to achieve its maximal force (contraction time) and fully relax is largely dependent on the fibre type composition of the muscle. In rodents, for example, the soleus muscle is predominantly type I whereas the extensor digitorum longus (EDL) is predominantly Type II. When examining the contractile properties of these muscles using a low stimulation frequency (10-20 Hz) the EDL muscle contraction and relaxation time are short enough to return to baseline before the next stimulation. In the soleus muscle on the other hand, the relaxation time is so long that at these stimulation frequencies the next stimulus arrives before the muscle could fully relax. As a result, the next contraction is superimposed upon the remaining force and the force during the next stimulus is higher than that during the first stimulus in the 10- or 20-Hz train. The relationship between stimulation frequency and force generated can thus tell us something about the contractile properties of the muscle.

The difference in contractile properties between fibre types is attributable to differences in myosin heavy chain (MyHC) composition (Harridge et al. 1996) as well as factors that control calcium flow and activation (Allen et al. 2008a). For example, Type II fibres have a higher density of both DHPR (Hollingworth & Marshall 1981) and RyR (Block et al. 1988) allowing for a faster release of calcium from the SR in faster muscle fibres (Briggs et al. 1977), while the higher density of calcium pumps in the SR (Everts et al. 1989) results in much quicker reuptake of calcium into the SR following contraction than in type I fibres.

Voluntary Activation

People may not able to fully activate their muscle and voluntary activation is defined as “the level of voluntary drive during an effort” (Gandevia 2001). The level of muscle activation depends on the number of motor units recruited and on the firing frequency of the motor neurons associated with the motor unit (Rate coding). Smaller motor neurons are predominantly recruited at low force levels while at higher force levels the larger motor neurons containing fast fibres are also recruited (Henneman et al. 1965).

There is some discussion in the literature as to whether it is physiologically possible to maximally activate the muscle (Häkkinen et al. 1998) or not (Jones & Rutherford

1987). Voluntary activation is often assessed by the use of interpolated twitches evoked before and during a maximal effort. The additional force produced by these evoked stimulations is indicative of sub-maximal voluntary activation and reflects the reserve capacity of the motor unit in terms of recruitment (Merton 1954).

There is an on-going argument within the literature as to whether the use of interpolated twitches is a valid measure to determine muscle activation (Horstman et al. 2009; DeHaan et al. 2009; Taylor 2009). Currently there are no good alternatives to assess voluntary activation and we therefore choose to use the interpolated twitch technique to get some idea of the level of voluntary activation.

1.2 Fatigue and in relation to skeletal muscle contractile properties.

Muscle fatigue can be defined as “any contraction-induced reduction in the capacity to generate force” (Degens & Veerkamp 1994; Jones 1996; Vøllestad et al. 1997). This loss of force and the slowing of a muscle contribute to the reduction in muscle power output during the development of fatigue (Fitts & Holloszy 1978; DeHaan & Koudijs 1994; Jones et al. 2006). The causes of muscle fatigue can be central or peripheral.

Central fatigue has been defined as “a progressive reduction in the voluntary activation of muscle during exercise” (Gandevia 2001) and may reflect a loss of motivation or an inhibitory influence of sensory afferent feedback from active muscle, affected joints, tendons or skin (Bigland-Ritchie et al. 1978).

Peripheral fatigue is considered to occur at, or distal to, the neuromuscular junction (NMJ). Once an action potential originating in the motor neuron reaches the NMJ, ACh is released, crosses the NMJ and causes the generation of an action potential at the post-synaptic membrane. Bigland-Ritchie et al. (1982) and Sacco et al., (1994) argued that the fatigue during voluntary contractions is due to neither an impaired release or re-synthesis of neurotransmitter, nor a reduced sensitivity of the postsynaptic membrane. It is more likely that accumulation of metabolites, such as K^+ in the extracellular space and Pi in the cytoplasm, impairs action potential propagation along the sarcolemma, and calcium release from the sarcoplasmic

reticulum and/or cross bridge function to the detriment of force generating capacity, respectively (reviewed in (Allen et al. 2008b).

Extracellular K^+ increases approximately two fold following a maximal voluntary contraction (Vyskocil et al. 1983) and the concentration may be increased even more in the T-tubules. The effect of the accumulated K^+ is to reduce the electrical excitability of the plasma membrane, slowing, or even blocking the propagation of the action potential along the surface of the fibre and down the T- tubular network although whether this is an important factor during voluntary activity is debateable (Jones 1996).

There are two main causes for muscle fatigue at the level of the fibre. The first concerns the release of calcium from the sarcoplasmic reticulum and subsequent activation of the contractile proteins by the binding of Ca^{2+} to troponin. The other concerns the interaction of actin and myosin to generate force and movement, where metabolites might change the rate constants for attachment and detachment. A decrease in the ATP concentration could affect cross-bridge cycling but more important is probably the accumulation of metabolites, such as inorganic phosphate, ADP, AMP and H^+ that not only could affect cross-bridge cycling but also the release of Ca^{2+} from the sarcoplasmic reticulum (Fitts & Balog 1996; Martyn & Gordon 1992). The result of this is not only a reduction in force, but also a reduction in the shortening velocity of the muscle (fibres), (Cooke & Pate 1985; Metzger & Moss 1987) and this has implications for the development of power.

During metabolically demanding contractions there is a decrease in the release of Ca^{2+} (Allen et al. 2008a; Westerblad & Lannergren 1991) probably as a result of the accumulation of Pi within the sarcoplasmic reticulum. The consequence of this is that the number of attached cross bridges, and thus the force, is reduced.

The rate at which a muscle fatigues depends on the balance between the rate at which ATP is consumed by cross-bridge interactions and ion pumping and by the rate at which ATP is re-synthesised and metabolite accumulation is minimised. Fast glycolytic muscles have high rates of ATP consumption and re-synthesis, but metabolite accumulation is high as they generate ATP primarily via glycolysis. It is this accumulation of metabolites and increases in NAD+ from glycolysis that slows

down ATP replenishment. Slow, oxidative fibres on the other hand, have slower rates of ATP turnover but work aerobically. It is not surprising, therefore, that fast muscles, or single fibres, fatigue more rapidly than their slower counterparts.

Electrical stimulation can be used to bypass voluntary activation of the muscle by evoking an action potential within the nerve either through direct stimulation of the nerve or through percutaneous stimulation via electrodes. It is therefore useful in the study of skeletal muscle contractile properties, activation levels and fatigue while bypassing central factors. For example, a fatigue protocol as used in chapter 5 where muscle contractions are elicited by percutaneous stimulation removes the participants' motivation to complete the task, allowing the measure of peripheral muscle fatigue (resistance).

Electrical Stimulation

There are two main ways to electrically stimulate quadriceps muscle contraction, either through the femoral nerve or, as in this study, with percutaneous stimulation electrodes. Eliciting muscle contractions using stimulation of the femoral nerve (Bigland-Ritchie et al. 1978) is not user and participant friendly (Rutherford et al. 1986). A study by Rutherford et al. (1986) found that although percutaneous electrical stimulation does not activate the entire muscle it provides similar functional data to those obtained with direct nerve stimulation. A limitation of using percutaneous electrodes is the assumption that the muscle group of interest, here the Quadriceps muscle, is homogenous in terms of the muscle fibre phenotype. Lexell et al., (1983) showed the proportion of type II fibres was marginally greater in the superficial than the deep regions of the Vastus Lateralis muscle. This is not much of a problem as Adams et al., (1993) examined the regions of the Quadriceps that were activated during stimulation by surface electrodes and showed that the activation was "not exclusively superficial but rather dispersed and different amongst subjects."

1.3 Age-related changes in skeletal muscle

Ageing is associated with a "progressive remodelling of the neuromuscular system with a number of implications for muscle strength, power and ultimately quality of life"

(Degens 2010). The loss of muscle mass with advancing age and accompanying debilitation are already known since antiquity (For review please refer to: Narici & Maffulli 2010; Degens & Mcphee 2013). The age-related loss of muscle mass and strength and its role in disability is estimated to have cost the health service in the USA approximately $18.5 Billion in 2000 (Janssen et al. 2004). The financial burden is likely to rise even further as the number of people over 60 years of age in the world is estimated to increase to 2 billion people by 2050 (WHO 2012).

Age-related changes in Muscle Mass

Cross-sectional data shows that the age-related loss of whole body muscle mass is around 27% between the age of 18 and 88 (Janssen et al. 2000) with the loss of skeletal muscle greatest in the upper and lower limbs between the ages of 18-34 and over 75 years (Kyle et al. 2001). In addition, the muscles of the lower limb have been demonstrated to undergo greater age-related atrophy than the muscles of the upper limb (Candow & Chilibeck 2005; Janssen et al. 2000) and extensor muscles have been reported to atrophy more during ageing than flexors (Candow & Chilibeck 2005; Ogawa et al. 2012). The Quadriceps muscle, for instance may have lost 30% of its mass by the age of 80 (Young et al. 1985; Macaluso et al. 2002; Klitgaard et al. 1990) while the mass of the elbow flexors does not significantly differ between young and old (Klein et al. 2001; Candow & Chilibeck 2005). However longitudinal data suggests the rate of the loss of muscle mass is greater than these cross sectional estimates (Hughes et al. 2001) with approximately 1% per annum observed (Delmonico et al. 2009; Frontera et al. 2000; Greig et al. 1993; Koster et al. 2011), with the start of loss occurring after the age of 30 (Gallagher et al. 1997), 45 (Janssen et al. 2000) or indeed 60 years of age (Kyle et al. 2001).

Whether the rate of atrophy is greater in men than women (Delmonico et al. 2009; Hughes et al. 2001) or similar (Frontera et al. 2000; Koster et al. 2011) is a matter of controversy. However, with men naturally having greater absolute muscle mass (I Janssen et al. 2000) and women living longer, the prevalence of sarcopenia and associated mobility issue are more likely to be greater in older women (Narici & Maffulli 2010; Degens & Mcphee 2013).

The muscle atrophy during ageing has been termed “Sarcopenia” (from σαρκός (sarkos) meat and πένης (peneis) poor) (Rosenberg 1989). The use of the term Sarcopenia within the data chapters of this thesis refers to the age-related loss of muscle mass, rather than the inclusion of functional parameters as discussed below. Sarcopenia is associated with a decrease in number (43%) and size (57%) of in particular Type II fibres (Larsson et al. 1979; Lexell et al., 1983; Coggan et al. 1992; Andersen 2003; Cristea et al. 2010 Verdijk et al. 2010; Nilwik et al. 2013). As a consequence, there is an associated increase in the proportion of smaller slower oxidative muscle fibres (type I) (Klitgaard et al. 1990; Larsson et al. 1979; Lexell et al. 1988). The accompanying muscle weakness may increase the risk of injury, impair mobility, result in frailty and hence reduce the quality of life of older people (Murton & Greenhaff 2009; Clark & Manini 2010).

1.4 Quantification of Skeletal Muscle Mass

The earlier discussions about the definition of Sarcopenia show that it is crucial to have an accurate estimate of skeletal muscle mass (Jubrias et al. 1997; Mathur et al. 2008; Lukaski 1997). The age associated changes in body composition and in particular the loss of muscle mass (Shaw et al. 2007) may not only have a significant impact on locomotion, but may also decrease the metabolic rate (Tzankoff & Norris 1977) and increase drug toxicity (Nawaratne et al. 1998). Therefore, the accurate determination of skeletal muscle mass is essential across the age range and with different conditions. Of particular interest in this thesis is that all current functional and clinical definitions of Sarcopenia include a measure of muscle or lean mass quantity (Cruz-Jentoft et al. 2010; Fielding et al. 2011).

Muscle mass can be estimated using a variety of techniques including Dual Energy X-ray Absorptiometry (DXA), Magnetic Resonance Imaging (MRI), Computer tomography (CT), Bioelectrical Impedance (BIA) and Anthropometry. Briefly, DXA provides a 2-dimensional estimate of lean body mass, fat mass and bone mineral content. More detailed estimates can be achieved using MRI and CT scanning, which provide muscle cross-sectional areas and can be used to estimate muscle volume. MRI and CT have also been used to quantify subcutaneous and intramuscular fat infiltration. MRI and CT are considered the “gold standard” to

estimate skeletal muscle size and body composition in populations including children (DeRidder et al. 1992), healthy adults (Ross et al. 1996) and the elderly (Baumgartner et al. 1992). However, the acquisition of data from MRI and CT scanning is an expensive and time-consuming process. There might also be issues with scanning very large individuals and it may be difficult to determine intramuscular lipids and water (See Table 1.1 for advantages and disadvantages of each method)

Dual-Energy X-Ray Absorptiometry (DXA)

DXA was originally developed to assess bone mineral density and is often used for the diagnosis of osteopenia and osteoporosis. It also can provide information of whole body and regional body composition.

DXA utilises a "X-Ray tube which produces a low and a high pseudomonoenergetic beam," (Andreoli et al. 2009) along with a detector and a User Interface. DXA works on the principle of photon attenuation, where the photon attenuation of pixels measured at the 2 different energies provides an R-value. This R-Value corresponds to 3 components; Fat-Free Mass, Fat Mass and Bone Mineral. A fundamental assumption with the use of DXA is that the hydration of the Fat-Free Mass is 73%, but it is known to vary between 67-85% depending on the hydration status of the individual (Pietrobelli et al. 1998). This appears to be not too much of a problem as it has been demonstrated that for hydration ranges from 68 to 78% there is no significant effect on total fat percentage (Kelly et al. 1998).

Another assumption lies in the quantification of "fat free mass" or "lean mass" with the inclusion of non-mineral components of bone (Heymsfield et al. 1990), non-adipose components of fat tissue (Wang et al. 1999) and connective tissue. When considering studies of ageing, these assumptions could potentially lead to increased measurement errors consequent to the age-related increases in intramuscular fat, connective and subcutaneous tissue.

The main advantages of using DXA are its ease of use, interpretation and accessibility. The substantially lower costs than MRI scanners is one of the reasons that there is a greater accessibility in both clinical and research settings (Table 1.1).

A disadvantage of DXA is the radiation exposure. For a full body DXA scan ranges from 0.04 to 0.86 mrem (5-7 uSv), dependent on the size of the person and the make and model of the machine (Lee & Gallagher 2008). This dose is, however, small in comparison to the daily radiation dose (1.69 mrem). Another disadvantage is the inability to differentiate between different muscle groups. Thus, when one wishes to examine muscle quality (force per unit mass) or differential atrophy between muscles the use of DXA is limited and in large individuals, the fat and bone mineral content are often overestimated (Shaw et al. 2007).

The initial validation studies for DXA where done by Heymsfield and colleagues (Heymsfield et al. 1990) who observed a strong correlation between the combined lean mass of both the upper and lower limbs, termed appendicular lean mass, and total body potassium (r=0.94) in 44 middle aged individuals (52 ± 20 years). Total body potassium is an indication of an individual's lean mass (Boddy et al. 1972).

The development of fan beam DXA scanners as opposed to the previous pencil beam scanners drastically shortened the time required for a full body scan (~5 minutes compared to ~25minutes). Visser et al. (1999) examined the validity of using fan beam DXA to estimate leg lean mass using multi-slice CT scans as a standard in 60 older adults (30 men, 70-79years). They normalised the DXA lean mass values correcting for the inclusion of non mineral aspects of bone inclusion in lean mass estimates (Heymsfield et al. 1990) and observed a strong relationship between the two measures of muscle mass for both the lower limb and the thigh (both R^2=0.96). They did note, however, that muscle mass estimated by DXA was always higher than that found by CT; when pooling men and women there was a mean difference of 0.9 ± 0.9 kg in the whole lower limb and a mean difference of 0.7 ± 0.6 kg in the thigh region alone. The overestimation of muscle mass with DXA increased with increasing muscle mass (r=0.69,p<0.001). However, when BMI and age were included as co-variants the impact of muscle mass on the overestimate was not evident (Shih et al. 2000).

The overestimate of lean mass by DXA is probably a result of the assumptions underlying the inclusion in DXA estimates of lean mass of aspects of bone and fat (Heymsfield et al. 1990). In terms of bone, DXA provides an estimate of bone mineral content, which only comprises of around 50-55% of total bone mass (Heymsfield et al. 1990). Therefore the additional 45% of bone mass is accounted in the lean mass estimate, it is possible to correct for this by multiplying bone mineral content (BMC) estimates by 1.82 (Heymsfield et al. 1990). Whilst correction for the non-mineral aspect of bone does improve the relationship between lean mass measures in DXA and other imaging techniques as MRI and CT, it is not the full story. DXA also includes the non-fat fractions within adipose tissue as lean mass (Tylavsky et al. 2003; Tylavsky et al. 2003; Salamone et al. 2000). Normalising for this fraction is, however, difficult due to a wide range (14-32%) of non-fat fractions of total adipose tissue. Any significant changes in this fraction will have a significant effect on lean mass estimate by DXA.

Previous studies by Wang (Wang et al. 1996) and Fuller (Fuller et al. 1999) produced a series of formulas to correct for the inclusion of non-fat aspects of adipose, non-mineral aspects of bone, as well as connective and skin components. These formulas are, however, difficult to utilise in a clinical setting (Shih et al. 2000). One potentially simpler method to exclude the non-fat fraction of adipose tissue is the subtraction of skin mass and subcutaneous tissue via use of skinfold values (Fuller et al. 1999). So far it is not clear whether this is a valid approach in different populations, besides the fact that there is a large inter-tester variability in skinfold assessments.

Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) works on the basis that body tissue has a high component of water and hence protons. It uses a magnet to orient the spin of the protons that can be excited with electromagnetic waves. During the relaxation, or return to the original spin orientation, radio frequency waves are emitted and picked up by receiver coils. Protons in different tissues emit different radio frequencies. The pattern of radio frequencies is then used to generate single slice images of the

region of interest using a computer allowing the differentiation between different tissue components with fat being white, connective tissue dark grey and muscle tissue light grey.

MRI and computer tomography (CT) are considered the “gold standard” for quantification of body composition. They allow the accurate estimation of skeletal muscle (Engstrom et al. 1991; Narici et al. 1992; Baumgartner et al. 1992), adipose tissue (Kullberg et al. 2009), bone (Woodhead et al. 2001) and connective tissue areas in a slice. MRI allows the production of 3D volume estimates using multiple slice acquisition (Narici et al. 1992; Erskine et al. 2009).

The main advantages of using MRI is the high accuracy and reproducibility allowing for the accurate portrayal of changes in muscle mass over a time period even when the changes are small. It is also possible to distinguish individual muscles or muscle groups. Whilst these advantages also apply to computer tomography (CT), the main advantage of using MRI is that in contrast to CT scans MRI does not involve radiation, making it safe to use in both acute and chronic studies. There are also some disadvantages to MRI. First, MRI machines are very expensive and as a consequence accessibility is limited. Scanning and analysis of the MRI scans require skill and are often time consuming (Table 1.1). Finally, scanning of larger people may not be possible in all MRI scanners.

The Knee Extensor muscles have been of particular interest because of their role in locomotion. MRI scans allow the investigator to differentiate the different component muscles of the thigh (Trappe et al. 2001; Buford et al. 2012; Narici et al. 1992; Ogawa et al. 2012). Using MRI, Ogawa and colleagues (Ogawa et al. 2012) observed a 20% lower Quadriceps volume and 16% lower average cross-sectional area in older (60-78) than young men (20-28), similar to observations by others (Janssen et al. 2000; Macaluso et al. 2002; Overend et al. 1992). In addition, they observed no differential atrophy of hamstrings or adductor muscles (Ogawa et al. 2012). Buford et al. (2012) observed a good relationship between knee extensor muscle mass and functional limitation, concluding that thigh muscle mass is a major determinant of physical limitation.

Despite its use in muscle research, there are only few suggested cut-off points for low total muscle mass or volume determined with MRI or CT. The cut-off points that do exist are primarily relating to single-slice cross-sectional areas, probably due to its ease and time efficiency in comparison to volume determinations.

Using this approach, Visser et al., (2005) observed in the Health ABC study that the lowest quartiles of mid-thigh CSA (50% Femur Length) in white men was <230cm^2 and in women <152cm^2 and that those quartiles were more likely to develop mobility problems. The cross-sectional area might, however, not be the most appropriate way to accurately estimate muscle mass (Akagi et al. 2009; Cotofana et al. 2010).

Bioelectrical Impedance

Bioelectrical impedance (BIA) can provide estimates of total body water and fat free mass. It works by applying an electrical current through electrodes at the extremities (Ankle, Wrist). This small electrical signal is then passed through the body and back to the device where a measurement of impedance is quantified. The principle behind this is that lean mass with ions dissolved in water conducts electricity better than fat. The accuracy of BIA can be improved by using equations that include factors such as height, weight and sex (Janssen et al. 2000). Although this approach has been validated against MRI the error in the measurement is approximately 9% (Janssen et al. 2000).

The main advantage of using BIA is its ease of use and simplicity, making it an attractive choice for clinicians and fieldwork. It is also cheap in comparison to other methods of body composition such as DXA and MRI. The disadvantage of BIA is the inaccurate estimate of fat free mass, which includes not only lean mass but also bone and similarly to DXA, organs and is greatly affected according to hydration levels of the individual and also temperature.

In addition, despite the use of co-variate equations to improve accuracy it remains unsuitable to use these across different populations (Janssen et al. 2000).

Anthropometry

Anthropometric assessment of muscle mass is based on measurements of circumferences with a simple tape measure combined with skinfold measures to correct for subcutaneous fat (Jones & Pearson 1969; Durnin & Womersley 1974). Whilst offering a low-cost practical estimate of muscle mass, the accuracy of anthropometric assessment is limited, even with skilled experience operators. Muscle volume estimates of the thigh have been shown to be 30% greater than MRI (Tothill & Stewart 2002). These discrepancies are primarily due to the inaccuracies of skinfold fat assessment and the assumption that there is a constant proportion of fat and bone within the circumference (Tothill & Stewart 2002). The assumption concerning fat in particular makes the technique unsuitable for use in older adults where both subcutaneous and intra- and inter muscular fat is increased (See below).

Table 1.1- Summary of Advantages and Disadvantages of Muscle Mass

Method	Measure	Accuracy	Radiation	Cost	Accessibility	Ease of Use
MRI	Mid-Thigh CSA Muscle Volume	★★★★★	★	★★★★	★★	★★
CT	Mid-Thigh CSA	★★★★★	★★★★★	★★★★★	★	★
DXA	Whole body ASMM	★★★	★★	★★★	★★★	★★★
BIA	Estimated FFM Estimated ASMM	★★	★	★	★★★★★	★★★★★
Anthropometry	Circumference Skinfold	★	★	★	★★★★★	★★★★

Table 1.1- MRI= Magnetic resonance imaging; CT= Computer Tomography; DXA= Dual energy x-ray absorptiometry; BIA= Bioelectrical impedance. ★ = low; ★★★★★ = high.

1.5 Changes in the force generating capacity of muscle

As expected from the different rates of age-related atrophy, the strength of the muscles of the lower limb is decreased more than that of the upper limb (Candow & Chilibeck 2005; Lynch et al. 1999). It is equivocal whether the strength of the extensors is reduced to a greater extent than that of the flexors (Candow & Chilibeck 2005), or not (Hughes et al. 2001).
Although muscle strength during isometric contractions remains strongly associated with muscle mass or its surrogates in older adults (Callahan & Kent-Braun 2011), the loss of muscle strength with age is greater than the loss of muscle mass in both cross-sectional and longitudinal studies (Visser et al. 2000; Visser et al. 2005; Goodpaster et al. 2001; Johannesdottir et al. 2012; Delmonico et al. 2009). The reduced muscle strength to muscle mass ratio is a reflection of a reduced 'muscle quality' or ability of the remaining muscle tissue to generate force and power. Potential factors that contribute to this loss in specific tension are an increase in co-activation of antagonist muscles which increases with age (Macaluso et al. 2002), an increase in muscle fat infiltration, a reduction in force generating capacity of the muscle and/or reduced levels of voluntary activation.

To describe the age-related loss of strength Clark & Manini (2008) coined the term "Dynapenia", but the use of this term has yet to find particular favour amongst the literature. Low muscle strength is twice as powerful a predictor of future loss of mobility and independence status than muscle mass (Visser et al. 2005), warranting its inclusion in any definition to describe and diagnose sarcopenia. Muscle strength is a predictor of loss of mobility and independence, it can also tell something about the present quality of life. Using data from the Health ABC cohort, Manini et al., (2007) found that for men a torque less than 1.13 Nm/kg muscle was associated with a greater future risk of functional limitation, while values above 1.71 Nm/kg were associated with a significantly lower risk. In women these cut off points were 1.01 Nm/kg for high risk and 1.34 Nm/kg for low risk.

Handgrip strength

Both grip strength and knee extensor strength decrease with advancing age (Mathiowetz et al. 1985) and a low relative grip strength is strongly associated with low muscle mass (Castillo et al. 2003), poor balance (Stevens et al. 2012), old age disability, low gait speed (Rantanen, et al. 1999a; Rantanen et al. 1999b) and life expectancy (Rantanen et al. 2011). Data from the Hertfordshire cohort study of older adults, for instance, demonstrated that a 1 kg higher grip strength was associated with a better performance in timed get up and go, 3-m walk-time and chair-rise tasks in both men and women (Stevens et al. 2012). Because of these observation and the ease of measurement, only requiring a hand held dynamometer (Bohannon et al. 2012), hand grip strength measures have been the most popular in the literature to obtain indications of dynapenia (Cruz-Jentoft et al. 2010; Fielding et al. 2011).

1.6 Age related changes in Muscle Power

It has been reported that the decline in power generating capacity is even more pronounced than that of force generating capacity (Metter et al. 1999; Thom et al. 2005; Power et al. 2012; Skelton et al. 1994; Macaluso & De Vito 2003; Ditroilo et al. 2011; Degens 2007) with some estimates of up to 3.5% decrement in power per year after the age of 65 (Skelton et al. 1994). The loss of muscle power with age is thought to be caused by both a decrease in force generating capacity and a decrease in the maximum shortening velocity (Yu et al. 2007; Petrella et al. 2005; Power et al. 2012; Thom et al. 2007). The reduction in the maximal velocity of shortening is at least partly the consequence of loss and/or atrophy of fast type II fibres (Pearson et al. 2006). Muscle power is mostly measured with dynamic contractions, with peak muscle power occurring at approximately 70% 1RM (Cuoco et al. 2004; Izquierdo et al. 1999). However there has not been universal agreement in a standardised methodology and it is thus difficult to define specific cut-points for low muscle power (Macaluso & De Vito 2004; Reid & Fielding 2012). Bassey et al.,(1992) used a custom-built power rig and found that leg extensor power correlated with performance in chair rising, stair climbing and a 6 metre walk test (13). Other studies, using different physical function outcome measures such as short performance battery (SPPB) or 6-minute walk distance have reported similar

correlations in different cohorts (Suzuki et al. 2001; Bean et al. 2002; Bean et al. 2003; Puthoff & Nielsen 2007; Hicks et al. 2012). When considering the force-velocity relationship one can understand that an older adult with the same body mass as a young person would require a similar force for take-off during a countermovement jump, but will have to do so at a lower velocity, resulting in poorer jump performance (Harridge et al. 1997; DeVito et al. 1998). This is exactly what Izquierdo et al., (1999) found and as a consequence older men had ~29% lower jump height than young men.

Given the even larger decrements in power than force, it is not too surprising that muscle power is a stronger predictive risk factor of developing functional limitations than muscle strength (Bean et al. 2002; Bean et al. 2003), where increases in muscle power improve functional measures more than increases in muscle strength (Pereira et al. 2012; Bean et al. 2010; Bean et al. 2003; Bean et al. 2002). Conversely, Storer et al., (2008) observed significant increases in muscle mass, strength and power in response to a 20-week testosterone treatment, which was, however, not accompanied with an improved performance of the 6 and 400m walk, stair climbing or time to perform timed up and go. This thus raises questions as to whether improvements in muscle power can lead to improvements in functional tasks.

A recent 3-year longitudinal study by Hicks et al., (2012) in 934 adults from the InCHIANTI cohort examined how the loss of muscle strength and power over the follow-up period was related to disability. Interestingly, they observed much stronger associations with the baseline data of muscle strength (Grip and Knee Extension) and leg power in predicting future mobility problems, than between the magnitude of the decline in both strength and power over the follow-up period with mobility problems. This suggests that low muscle strength and power at baseline or a single time point is sufficient to predict future functional decline.

1.7 Diagnosis of Sarcopenia

There is widespread discussion in the literature concerning the definition of sarcopenia, with arguments to whether to include measures of low muscle strength and functionality to that of low muscle mass or not. These discussion are fed by the weakening of the relationship between muscle strength and mass during ageing (Clark & Manini 2008).

For the quantification of Sarcopenia, the index that has received the most interest and use is the skeletal muscle mass index (SMMI), defined as the appendicular lean mass (ALMM) divided by height squared (Gallagher et al. 1997). Using this index In the Elder health study in New Mexico, Baumgartner and colleagues (15) derived a cut-off value for sarcopenia as an SMMI 2 standard deviations below the mean from a young population. The cut-off points for older adults were 7.26 $kg.m^{-2}$ for men and 5.45 $kg.m^{-2}$ for women. Participants with a SMMI lower than the cut-off value were more likely to suffer from greater than 3 physical disabilities as assessed by Questionnaire (Activities of Daily Living Scale) particularly in Men.

The cut-off values reported in this article (Baumgartner et al. 1998) have often been used in subsequent studies (Table 1.2). The general validity may, however, be limited as there may well be differences between different ethnicities (Kelly et al. 2009) and young and older participants. For example, in a Chinese study by Lau et al., (Lau et al. 2005), cut-off values of 5.72 $kg.m^{-2}$ and 4.82 $kg.m^{-2}$ were reported for sarcopenia, clearly much lower than that reported by Baumgartner et al., (1998). There are a number of flaws in the original study by Baumgartner (1998). Firstly the appendicular lean mass was derived via an anthropometric equation based upon DXA values from a random subsample of 199 participants (out of a total of 883 participants). The normalisation to height squared was used as it “eliminated the difference in appendicular lean mass associated with greater height in younger adults as well as sex and ethnicity.” However other studies have shown this isn’t always the case (Melton et al. 2000; Tanko et al. 2002). There is also the problem that the age-related muscle wasting is much less in the arms than in the legs, thus casting some doubt on the functional significance of its use in the definition of sarcopenia (Candow & Chilibeck 2005; Janssen et al. 2000; Kubo et al. 2003; Reimers et al. 1998).

As part of the Health ABC study, Newman et al. (2003) introduced a new approach to define cut-off values as it had been found that applying the cut-off values proposed by Baumgartner et al., (1998) people with a normal body weight had a high prevalence of sarcopenia, whereas heavier participants had a substantially reduced prevalence. This does not mean that the obese individuals hadn't lost muscle mass with age, just that they had a substantially higher muscle mass in relation to their height. Clearly, one has to consider also the body mass and the muscle to body mass ratio might well be lower in these than normal-weight people. The overweight people may thus well be sarcopenic and this led to the term Sarcopenic Obesity (Stenholm et al. 2008; Zamboni et al. 2008). The method proposed by Newman et al., (2003) similarly normalised ALMM to Height squared, but also normalised to fat mass, producing residuals based on the combined regression effect of these on SMMI, using the lowest 20^{th} percentile as a cut-off value. These lowest 20^{th} percentiles using the residual method were described as -2.29 kg and -1.73 kg for men and women respectively. The authors plotted the residual values for participants against SMMI with these cut off points corresponding to 7.23 $kg.m^{-2}$ for Men and 5.67 $kg.m^{-2}$, which are very similar to those originally proposed (Baumgartner et al. 1998).

When examining these cut-off values and low functioning participants, sarcopenic men by both definitions suffered from low function, however in women only the residual definition increased the risk of low function, with the authors concluding that this was due to the greater amount of excess fat found in older women, who according to SMMI would have been non-sarcopenic. This finding was confirmed in a 5 year follow up study by Delmonico et al. (2007) who found the residuals incorporating fat mass to provide a much better predictor of physical function than the Baumgartner method.

As a result of similar cut-off points between the SMMI and residual methods, the more convenient SMMI method has found greater prevalence within the literature (Dufour et al. 2012; Estrada et al. 2007; Gillette-Guyonnet et al. 2003; Iannuzzi-Sucich et al. 2002; Kenny et al. 2003; Lima et al. 2009; Melton et al. 2000; Tanko et al. 2002; Morley et al. 2001) as can be seen in Table 1.2. A recent publication by

Bijlsma et al., (2012) reported that there was a significant deviation in the prevalence of Sarcopenia ranging from 4-50% based on grip strength and DXA-determined measures of skeletal muscle mass in 70-85 year olds. Not only are there inherent differences in Sarcopenia prevalence according to definition and methods used, but there are also differences between populations and ethnicities (Table 1.2).

While sarcopenia is defined as poorness of flesh, and as such indicators of muscle mass are appropriate to determine whether someone is sarcopenic or not, in practise we are more interested in how function is impaired or limited. Above it was discussed that force and power generating capacity are affected more than muscle mass during the ageing process. Therefore, the functional significance of measuring muscle mass alone is questionable (Clark & Manini 2008). In fact, the value of muscle strength and power is greater than muscle mass in predicting low physical performance (Clark & Manini 2010; Goodpaster et al. 2006; Visser et al. 2000).

Cruz-Jentoft et al., (2010), on behalf of the European working group on sarcopenia in older people (EWGSOP), defined sarcopenia as "*a syndrome characterised by progressive loss of skeletal muscle mass and strength with a risk of adverse outcomes, such as physical disability, poor quality of life and death*." Based on this definition they came up with a decision tree to define sarcopenia (Figure 1.4).

Table 1.2- Overview of prevalence of sarcopenia in different populations and ethnicities determined with various indicators of muscle mass.

Reference	Sex	Study	Age	Method	Measure	Defined Cut-Off point	N	Reference Pop.	Normalisation	Prevalence
Baumgartner et al., 1998	M F	US (NMEHS)	61-70 71-80 80+	DEXA	aLM/ht^2	7.26 kg.m^{-2} 5.45 kg.m^{-2}	883	18-40	Height^2	13% 24% 50%
Morley et al., 2001	M F	US (NMEHS)	<70 >80	DEXA	aLM/Ht^2	7.26 kg.m^{-2} 5.45 kg.m^{-2}	199	18-40	Height^2	12% 30%
Janssen et al., 2002	M F	US (NHANES III)	60+ 60+	Impedence	Muscle mass/body mass	M<31% F<22.1%	2224 2278	NHANES III	Total body mass	7% 10%
Newman et al., 2003	M F	US (Health ABC)	70-79	DEXA	aLM/Ht^2	7.23 kg.m^{-2} 5.67 kg.m^{-2}	1435 1549	Baseline Residuals	Height^2	20% 20%
Iannuzzi-Sucich et al, 2002	M F	US	65+	DEXA	aLM/Ht^2	7.26 kg.m^{-2} 5.45 kg.m^{-2}	142 195	18-40	Height^2	27% 23%
Janssen et al, 2004	M F	US (NHANES III)	60+	Impedance	tMuscle/ht^2	8.5 kg.m^{-2} 5.75 kg.m^{-2}	2223 2276	NHANES III	Height^2	11% 9%
Janssen et al., 2004	M F	US (CHS)	65+	Impedance	tLean Mass/ ht^2	8.5 kg.m^{-2} 5.75 kg.m^{-2}	2196 2840	CHS	Height^2	17% 11%
Visser et al., 2003/ Schaap et al., 2006	M F	Dutch (LASA)	74.6 ± 6.2	DEXA	aLM	> 3 % per annum	158 170	Baseline		15.7%
Gillette-Guyonnet et al., 2003	F	France (EPIDOS)	75+	DEXA	aLM/ht^2	5.45 kg.m^{-2}	1311	18-40	Height^2	9.5%
Tanko et al., 2002	F	Denmark	70+	DEXA	aLM/ht^2	5.45 kg.m^{-2}	67	18-40	Height^2	12%

Table 1.2 Continued

Reference	Sex	Study	Age	Method	Measure	Defined Cut-Off point	N	Reference Population (Study)	Normalisation	Prevalence
Melton et al., 2000	M F	US (Rochester)	70+	DEXA	aLM/Ht^2	7.26 kg.m^{-2} 5.45 kg.m^{-2}	100 99	18-40 (Rosetta)	Height^2	28% 52%
Estrada et al., 2007	F	US	59-76	DEXA	aLM/Ht^2	5.45 kg.m^{-2}	189	18-40 (Rosetta)	Height^2	25.9%
Lima et al., 2009	F	Brazil	66.5 ± 6.37	DEXA	aLM/Ht^2	5.45 kg.m^{-2}	246	18-40 (Rosetta)	Height^2	
Woods et al., 2011	F	Australian	Mean 86years	DEXA	aLM/Ht^2 %SMMI	4.85 kg.m^{-2}	63	18-40 (n=62)	Height^2	3.2%
Chien et al., 2008	M F	Taiwan	65+	Bioelectrical Impedance	FFM/Ht^2	>2 SD from Young	302	18-40 (n=200)	Height^2	23.6% 18.6%
Lau et al., 2005	M F	China	70+	DEXA	tLean/Ht^2	>2 SD from young	262 265	18-40 (n=111)	Height^2	12.3% 7.6%
Patil et al., 2012	F	Finland	70-80	DEXA	5.5 kg.m^{-2}	EWGSOP Algorithm	409	18-40 (Rosetta)	Height^2	0.9%
Patil et al., 2012	F	Finland	70-80	DEXA	5.67 kg.m^{-2}	IWG Algortihm	409	Residual (Newman et al., 2003)	Height^2	2.7%
Dufour et al., 2012	M F	US (Framington)	72-92	DEXA	aLM/Ht^2	7.26 kg.m^{-2} 5.45 kg.m^{-2}	274 493	18-40 (Rosetta)	Height^2	19% 13%
Dufour et al., 2012	M F	US (Framington)	72-92	DEXA	aLM/Ht^2	7.23 kg.m^{-2} 5.67 kg.m^{-2}	274 493	Residuals (Newman et al., 2003)	Height and Fat Mass Residuals	25% 24%
Kenny et al., 2003	F	US	59-78	DEXA	aLM/Ht^2	5.45 kg.m^{-2}	189	18-40 (Rosetta)	Height^2	22.6% No-ERT 23.8% ERT

M=Male; F=Female; CT= Computer Tomography; DXA= Dual energy x-ray absorptiometry; alm/ht2= appendicular lean mass/height^2 ; FFM= Fat Free Mass; ERT= Oestrogen replacement therapy; SD= standard deviation

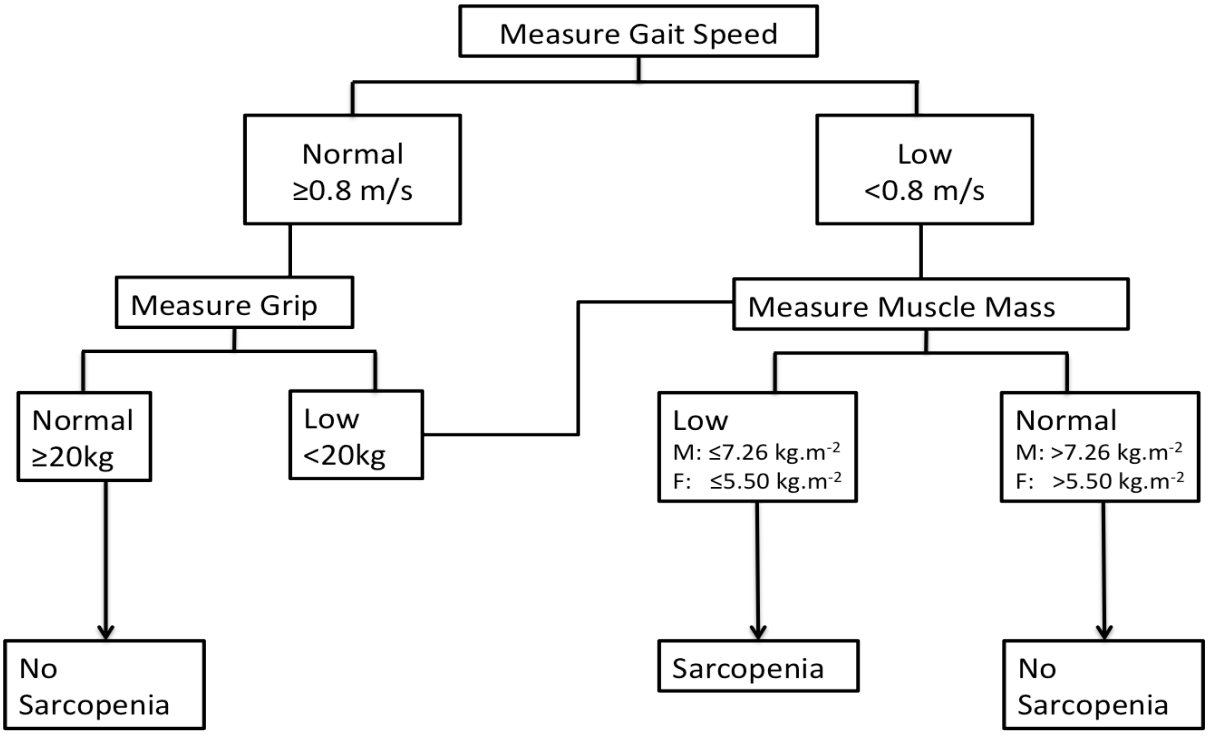

Figure 1.4. Algorithm for the diagnosis of sarcopenia as described by Cruz-Jentoft et al., (Cruz-Jentoft et al. 2010) and adapted from (Patil et al. 2012)

In addition, the EWGSOP consensus provides a staging of sarcopenia severity similar to that done previously by Janssen et al., (2002). However the observation of low muscle mass is still key to all stages (Table 1.2).

Stage	Low Muscle Mass	Low Muscle Strength	Low Physical Performance
Presarcopenia	✔		
Sarcopenia	✔	✔ Or	✔
Severe Sarcopenia	✔	✔	✔

Table 1.3- Stages of Sarcopenia as suggested by the EWGSOP consensus paper (Cruz-Jentoft et al. 2010)

Fielding et al., (2011), writing on behalf of the International working group on Sarcopenia (IWGS), provide another decision tree for the diagnosis of sarcopenia, incorporating muscle mass and strength, the Short Physical Performance Battery (SPPB) or 6 minute walk distance. The cut-off points for low muscle function, were a 4-m gait speed of <1 m/s, a 6-minute walk distance <400 m, or a score <9 on the SPPB scale.

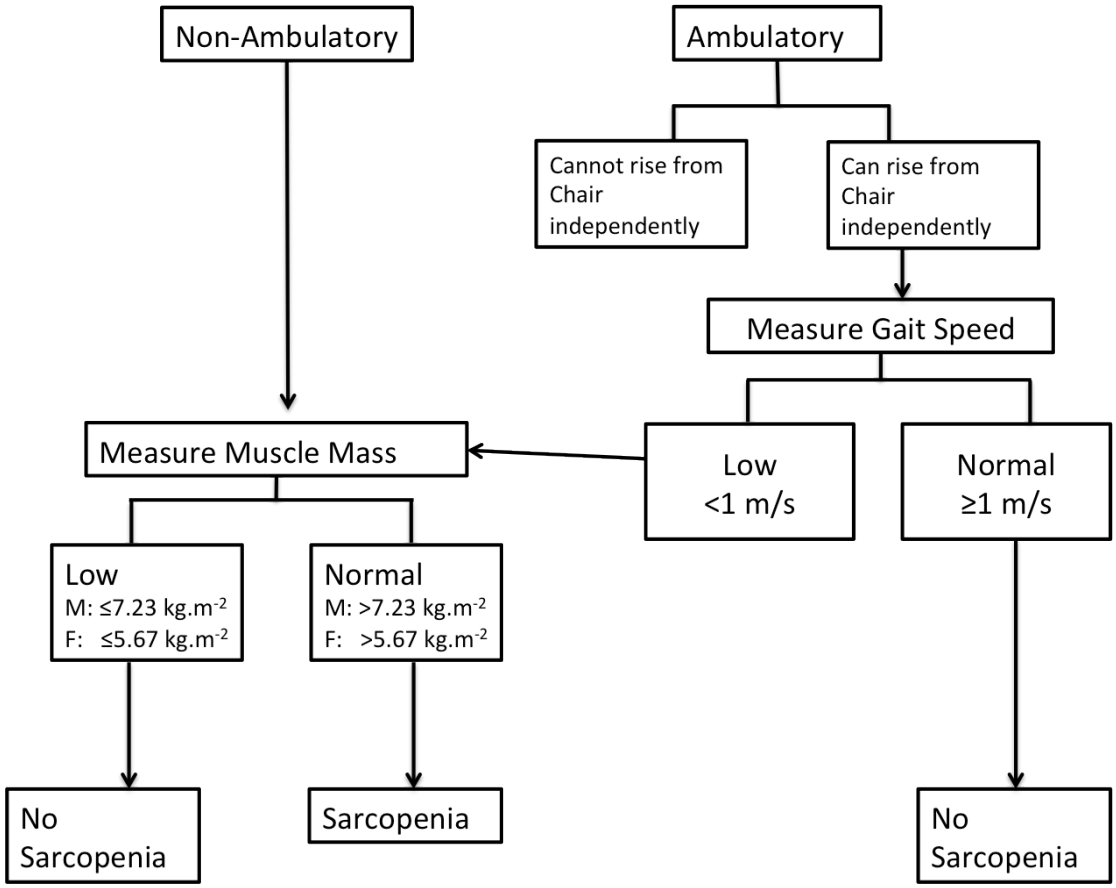

Figure 1.5. Algorithm for the diagnosis of sarcopenia based on (Fielding et al. 2011) and adapted from (Patil et al. 2012).

Prevalence of sarcopenia

At first inspection, there is a relatively good agreement between the two proposed definitions, however a recent study by (Lee et al. 2013) in Taiwan observed that according to the IWGS definition 14.9% and 16.6% of men and women were sarcopenic, while only 5.8% and 4.1% were so when using EWGSOP. Similarly, Patil et al.,(2012) observed that the prevalence of sarcopenia, in 409 70-80-year-old Finnish women, was 0.9% and 2.7% when applying the EWGSOP and IWGS, respectively. Nevertheless, applying the EWPSOP criteria, in the Hertfordshire sarcopenia studies (mean age 73 and 67 years) the prevalence of sarcopenia was 7.9% in women and 4.6% in men, while 21.8% of the ilSIRENTE cohort of participants aged between 80 and 85 were sarcopenic (Patel et al. 2013). This confirms that there is a substantial increase in sarcopenia prevalence with advancing age. It is interesting, however, that the prevalence of sarcopenia determined in this way is much lower than in studies that have solely used muscle mass in the diagnosis of sarcopenia. This lower prevalence is due to the inclusion of both strength and physical performance parameters (Landi et al. 2013).

1.8 Aetiology of Sarcopenia

The loss of muscle mass with advancing age and therefore the ability of the muscle to produce strength and power is a complex process. It is the net result of a higher rate of muscle protein breakdown (MPB) than muscle protein synthesis (MPS). There are a number of factors that play a key role in increasing the rate of MPB and/or decreasing MPS which are known to change with ageing (Figure 1.6).

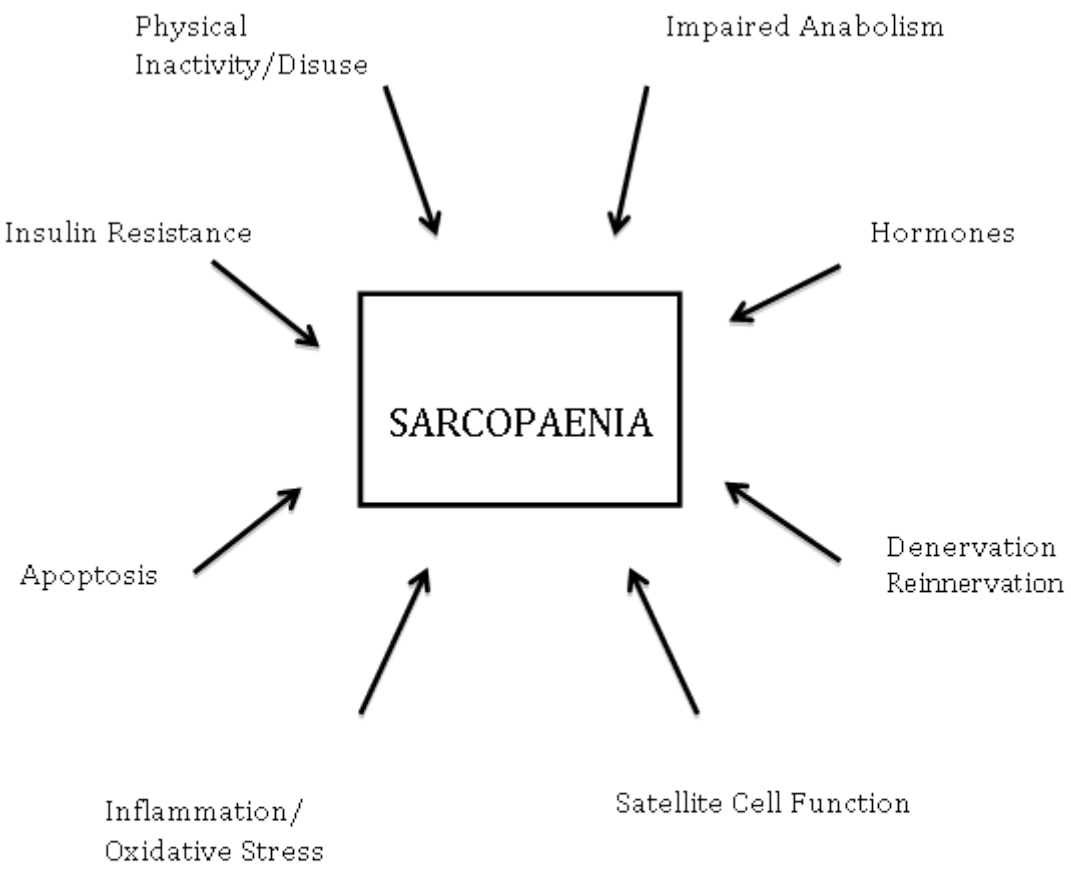

Figure 1.6- Summary of aetiology of Sarcopenia highlighting its multi-factorial causes.

Skeletal muscle is highly responsive to mechanical, hormonal and nutrient anabolic stimuli. Many studies have demonstrated that the response to anabolic stimuli is attenuated in older adults (Cuthbertson et al. 2005; Slivka et al. 2008; Katsanos et al. 2005; Katsanos et al. 2006). This attenuated effect could be a result of a reduction in insulin sensitivity (Guillet et al. 2004; Rasmussen et al. 2006) or insulin availability to the muscle tissue due to endothelial dysfunction (Fujita et al. 2009; Dillon et al. 2011) leading to a diminished blood supply to the muscle. Another factor that might contribute to anabolic blunting is the altered hormonal environment. (Horstman et al. 2012; Sipilä et al. 2013).

In men, for instance, there is a gradual drop of circulating testosterone levels (termed “Andropause”) from the age of 40 (Dillon et al. 2010) matching the start of muscle mass decline (Janssen et al. 2000). Although several studies do indeed show that

the lower serum testosterone level is associated with a lower muscle mass, cross-sectional area and strength (Baumgartner et al. 1999; Iannuzzi-Sucich et al. 2002; Szulc et al. 2004; Verdijk et al. 2010), others have not observed such an association (Stenholm et al. 2010). In women, low serum Estradiol concentrations are associated with low muscle strength (Greising et al. 2009; Horstman et al. 2012). Others, however, did not find a significant correlation between Oestrogens concentrations and muscle mass or strength with age (Baumgartner et al. 1999; Kenny et al. 2003).

Insulin like growth factor (IGF) and growth hormone (GH) are both implicated in the hypertrophic response and maintenance of muscle mass (Erskine & Degens 2013). The reduction in androgen levels is thought to contribute to the age-related decrease in circulating IGF-1 and GH (Sherlock & Toogood 2007) and low IGF-1 and GH is related to a decrease in muscle mass and function (Sherlock & Toogood 2007; Ryall et al. 2008). Although one might thus consider to provide GH and IGF-I supplementation, this is not without risk as IGF-1 supplementation is strongly linked to increased cancer risk (Juul 2003).

Anabolic resistance, muscle wasting and a decrease in muscle quality may also be a consequence of **c**hronic low grade systemic inflammation (Degens 2010). A pro-inflammatory environment has been shown to negatively affect the regenerative capacity of muscle and at the same time activate catabolic pathways (Greiwe et al. 2001). It is thus not surprising that chronic low-grade systemic inflammation is independently associated with a higher risk of disability and low physical performance (Miller et al. 2008; Goodpaster et al. 2008; Verghese et al. 2011).

The increase in pro-inflammatory cytokines in old age is potentially a consequence of an increase in adipose tissue, that is known to produce pro-inflammatory cytokines such as IL-1, IL-6 and TNFa (Roubenoff et al. 1998; Cesari et al. 2005). Therefore, the condition of Sarcopenic Obesity, where an individual has low muscle mass as well as an increase in fat mass is of particular clinical concern (Schrager et al. 2007). Other sources may include contraction-induced injuries (Faulkner et al. 1995) or chronic diseases such as Chronic obstructive pulmonary disease and heart failure (Yende et al. 2006); Cancer (Mantovani et al. 2008); Type II diabetes (Donath & Shoelson 2011) etc.

Physical Activity Levels

Physical activity levels are important to maintain muscle mass and strength in both sexes (Baumgartner et al. 1999). There is generally a decrease in the amount and intensity of physical activity in advancing age (Ingram 2000). This inactivity/disuse is thought to play at least some role in sarcopenia, with muscles of the lower limb being more affected than those of the upper limb (Narici & Maffulli 2010).

The World health organisation recommends 30 minutes of moderate intensity aerobic exercise on most days combined with strength training 2-3 times per week. In line with these recommendations several studies have demonstrated that an increase or maintained physical activity level in adulthood and in later life is associated with reduced risk of all-cause mortality (Bijnen et al. 1999; Woodcock et al. 2011; Kilander et al. 2001; Hrobonova et al. 2011) and greater walking speed and grip strength (Tikkanen et al. 2012). In addition, an 8-year follow-up study showed that those with the greatest loss of gait speed were those with the lowest physical activity levels (White et al. 2013).

The significance of maintaining physical activity levels for maintenance of muscle mass and health was shown by Breen et al. (2013). They demonstrated that even a 76% reduction in physical activity (step count) for only 2 weeks resulted in increased insulin resistance, cytokine production and a significant reduction in postprandial MPS (Paddon-Jones et al. 2006).

It thus seems that the loss of muscle mass, anabolic resistance and the inflammatory environment in old age are largely the result of disuse and inactivity. Although physical inactivity undoubtedly plays an important role, it does not explain all the age-related changes in the muscle. There is, for instance, a preferential phenotypic shift towards type II muscle fibres during disuse, whereas during ageing there is, if anything, a type II to type I transition (Degens & Alway 2006).

Increase in non-contractile tissue

Ageing is associated with an increase in intramuscular adipose tissue (IMAT) (Goodpaster et al. 2001; Jubrias et al. 1997; Overend et al. 1992; Song et al. 2004; Visser et al. 2002; Forsberg et al. 1991; Boettcher et al. 2009; Zoico et al. 2010). This increase in IMAT is termed “Myosteatosis” and can account for up to 15% of the total CSA (Kent-Braun et al. 2000; Macaluso et al. 2002). The adipose tissue in the thigh muscles has been reported to be increased by 59% in the quadriceps and 127% in the hamstrings in older participants (Overend et al. 1992), with greater deposits of IMAT in older women than men (Forsberg et al. 1991). Interestingly, despite a 20% increase in IMAT between young and older participants, Buford et al., (Buford et al. 2012) showed no significant difference in subcutaneous fat Increases in IMAT have been associated with an increase in disease prevalence (Goodpaster et al. 2000) and also impaired muscle strength and function (Marjolein Visser et al. 2005; Cesari et al. 2005; Delmonico et al. 2009; Marcus et al. 2012) accounting for up to 36% the variance in strength in older participants (Marcus et al. 2012). Part of the increase in IMAT in old age may be due to lower physical activity levels, as IMAT deposits are inversely related to the levels of physical activity (Kent-Braun et al. 2000). It is promising to see that decreases in IMAT can be achieved in response to resistance training (Taaffe et al. 2009). An increase in IMAT does not only decrease muscle quality, but is also associated with a blunted adaptive response to a training stimulus (168), potentially due to increase in systemic inflammation (Neels & Olefsky 2006).

Excitation-contraction uncoupling

As discussed above, excitation-contraction coupling is a complex process in which electrical signals from the motor neuron cause contraction of the muscle through calcium release from the sarcoplasmic reticulum. Many stages in this process potentially change with age, leading to excitation-contraction uncoupling. This, in addition to changes to contractile proteins can all affect the force a muscle can generate.

The first potential site for uncoupling is the linkage between the t-tubule DHPR and RyR. It has been observed that the DHPR:RyR was lower in both fast *extensor*

digitorum longus and slow *soleus* of old than young rats (Renganathan et al. 1997). The consequence may be a decrease in the voltage-gated calcium release from the sarcoplasmic reticulum and hence a lower muscle activation for a given excitation (Delbono et al. 1995). This was confirmed by Wang et al., (2012) who, using voltage clamping techniques, showed that despite a substantial depolarisation period (~2 seconds) the amount of calcium remaining within the SR was greater in older than young mice, though there is no change with age in the maximum sarcoplasmic reticulum calcium release (Jiménez-Moreno et al. 2008).

Age related changes in the nervous system

Part of the muscle wasting during ageing is thought to be attributable to loss of motor neurons and a consequent loss of muscle fibres (Degens & Mcphee 2013). The cause of motor neuron loss is yet to be established although reductions in endocrine factors such as IGF-1 as well as chronic low-grade systemic inflammation may play a role (Lee et al. 2013). In addition to the loss of motor neurons there is also a slowing of the conduction velocity after approx. 40 years of age (Metter et al. 1998; Norris et al. 1953). This loss of conduction velocity along with other factors such as altered E-C coupling (see above) and the slowing of the contractile properties of the muscle (fibres) contribute to an overall slowing of the older person, which may be a major cause of preventable falls. It is also of interest to note that the type I muscle fibres in older adults tend have a more pronounced angular shape which is characteristic of denervated tissue (Bazzucchi et al. 2004; Lexell et al. 1988; Lexell et al. 1983).

Is there an age related change in muscle voluntary activation?

Since the nervous system controls muscle activation, it is possible that changes in the nervous system cause problems with voluntarily activating the muscle in old age. This then might explain part of the greater loss of strength than muscle mass with age. To date there is, however, no consensus as to whether there is a decrease in the ability to voluntarily activate the muscle in old age or not (see Table 1.3). In the muscle group of interest in this thesis, the knee extensors, the majority of studies show no significant difference in activation capacity between young and older participants (Roos et al. 1999; Callahan et al. 2009; Cannon et al. 2007; Knight &

Kamen 2001; Wilder & Cannon 2009). In a larger study (Stevens et al. 2003), however, older participants were able to activate 94% of the muscle in comparison to 98% in young individuals. In the oldest old the ability to activate the muscle is (further) reduced as it has been reported that 85-97-year-old people were able to only activate 81% of their muscles (Harridge et al. 1999) (Table 1.3).

Table 1.4 Summary table of quantification of voluntary activation between young and old adults

Study	Muscle	Young Age (yrs)	N	Old Age (yrs)	N	Method	Finding	%Activation
Bilodeau et al., 2001	Elbow Flexors	25 ± 3	10	76 ± 6	11	ITT	Y>O	Y:96.6 ± 2.7 O: 91.0 ± 5.7
Callahan et al., 2009	Knee Extensors	26 ± 1	16	71 ± 1	16	CAR	Y=O	Y:98.0 ± 1.0 O: 100 ± 0.0
Cannon et al., 2007	Knee Extensors	23 ± 4	9	69 ± 4	9	ITT	Y=O	Y: 95.1 ± 1.7 O: 96.2 ± 1.8
Connolly et al., 1999	Ankle DF	21 ± 2	6	82 ± 4	6	ITT	Y=O	Y: 99.1 ± 0.1 O: 99.3 ± 0.2
Harridge et al., 1999	Knee Extensors			85-97	11	ITT		O 81 ± 7 at baseline
Hurley et al., 1998	Knee Extensors	23 (Mean)	20	72(Mean)	15	ITT	Y=O	Y:88.4 O:90.6
Kent-Braun et al., 1999	Ankle DF	32 ± 1	24	72 ± 1	24	CAR	Y=O	Y: 96 ± 2 O: 99 ± 1
Klein et al., 2001	Elbow Flexors	23 ± 3	20	81 ± 6	13	ITT	Y=O	Range 92-100%
Klein et al., 2001	Elbow Extensors	23 ± 3	20	81 ± 6	13	ITT	Y=O	Range 94-100%
Knight and Kamen 2001	Knee Extensors	21 ± 1	8	77 ± 2	8	ITT	Y=O	Y: 95.7 ± 7 O: 97 ± 6
Roos et al., 1999	Knee Extensors	26 ± 4	13	80 ± 5	12	ITT	Y=O	Y:93.6 O: 95.5
Stackhouse et al., 2000	Knee Extensors	23 ± 4	20	71 ± 6	17	CAR	Y>O	Y:98.0 O: 94.0
Stevens et al., 2003	Knee Extensors	18-32	46	64-84	46	CAR	Y>O	Y: 98.3 ± 0.3 O: 93.8 ± 0.8
Wilder and Cannon,2001	Knee Extensors	23 ± 4	18	69 ± 4	12	ITT	Y=O	Y: 97.7 ± 2.3 O: 98.2 ± 1.5

Table 1.4 DF= Dorsiflexors; ITT= Interpolated twitch technique; CAR= Central activation ratio; Y=Young; O=Old.

1.9 Fatigue and Sarcopenia

As discussed above, sarcopenia is accompanied by a preferential loss and/or atrophy of type II fibres and an increase in the proportion of slow oxidative type I fibres (Deschenes 2004). These slower oxidative motor units are more resistant to fatigue than faster motor units (Burke et al. 1971; Edström & Kugelberg 1968). Although it might thus be expected that elderly muscles will be more fatigue resistant than those of a younger person, the elderly frequently complain of excessive fatigue. Several articles support the hypothesis that old muscle is more fatigue resistant (Chan et al. 2000; Chung et al. 2007; Hunter et al. 2005), but others have found no such change (LaForest et al. 1990; Lindstrom et al. 1997). Part of this discrepancy might be related to differences in the fatigue protocols used and different contributions of central and peripheral factors to the observed fatigue (Allman & Rice 2002).

When using intermittent or sustained isometric contractions the majority of, but not all, studies indicate that older muscle has a greater fatigue resistance than young (Table 1.10). The discrepancies between studies could be due to differences in muscle groups tested and the intensity of the contraction. For example, when examining the knee extensors, the studies that have observed no significant difference between young and old have used contractions of a much lower intensity (20-25% MVC) (Allman & Rice 2004; Stackhouse et al. 2001; Stevens et al. 2001) than studies that showed an age related decrease in fatigue resistance (Callahan et al. 2009; Callahan & Kent-Braun 2011).

Chung et al., (Chung et al. 2007) examined the age-related differences in muscle fatigue during both normal and ischaemic intermittent isometric contractions of the ankle dorsiflexors in young and older men. They observed that the older adults had a superior fatigue resistance in both conditions, concluding that the age-related changes in muscle perfusion or oxidative metabolism do not explain the superiority, but rather that the improvements in fatigability are a result of neuromuscular changes in peripheral or central factors.

Whether older people appear more fatigue resistant than young people or not is very much dependent on the speed of contraction. With intermediate-velocity dynamic

contractions there is no significant difference between young and older women (Callahan & Kent-Braun 2011), but during high-velocity dynamic contractions young women had significantly greater fatigue resistance than older women (52.1 ± 6.9 vs 28.0 ± 3.9%) replicating the findings of the same group and others previously (Callahan et al. 2009; Lindstrom et al. 1997). This probably is a result of the slower muscle phenotype in older adults (Callahan & Kent-Braun 2011)

Not only are there significant differences in fatigability with age, but there are also substantial differences between men and women (Hunter 2009). Hunter et al., (Hunter et al. 2004) observed, for instance, that there was no significant difference between time to task failure between young and older women (Wüst et al. 2008). In addition, others have observed that older men were more fatigue resistant when performing dynamic contractions than older females (Katsiaras et al. 2005), while during intermittent isometric contractions women have a higher fatigue resistance than men (Wüst et al. 2008).

Table 1.5: Summary of observations for changes in age related muscle fatigability

Study	Muscle	Young Age (yrs)	N	Old Age (yrs)	N	Contraction Type	Fatigue Protocol	Finding
Allman & Rice 2001	Elbow Flexors	24 ± 2	7M	84 ± 2	7M	Isometric	3s on 2s off, 60% MVC until failure	Y=O
Allman & Rice 2004	Knee Extensors	27 ± 1	9M	78 ± 1	9M	Isometric Intermittent	1. ES 25% MVC 180x 2. ES 60% F-F	1. Y=O 2. Y<O
Baudry et al., 2007	Ankle DR	30.5 ± 2.5	8M 8F	77.2 ± 1.4	8M 8F	Concentric Eccentric	5 Sets 30x MVC	Conc: Y>O Ecc: Y>O
Bilodeau et al., 2001a	Elbow Flexors	25 ± 3	5M 5F	76 ± 6	7M 4F	Isometric Sustained	Sustained MVC until <50% MVC	Y<O*
Bilodeau et al., 2001b	Elbow Flexors	26 ± 3	5M 5F	71 ± 4	6M 4F	Isometric Sustained	35% MVC	Y<O
Callahan et al., 2009	Knee Extensors	26.1 ± 0.9	8M 8F	70.9 ± 1.1	8M 8F	1.Isometric 2.Dynamic	1. Max 5 s on 5 s off, 4 mins 2. 0.5s on 2 s Rest, 4mins (120°/s)	Y<O Y=O
Callahan & Kent Braun, 2011	Knee Extensors	23.5 ± 0.9	11F	68.9 ± 4.3	10F	1.Isometric 2. Dynamic 3. Dynamic	1. MVC 5s on 5s off, 24reps 2. 120reps (270°/s) 3. 120reps (120°/s)	1. Y<O 2. Y>O 3. Y=O
Chan et al., 2000	Thenar	30 ± 6	5M 5F	70 ± 9	5M 4F	Isometric sustained	1. 90s maximal isometric sustained	1. Y<O
Chung et al., 2007	DorsiFlexors	26 ± 5	12M	72 ± 4	12M	Isometric Intermittent	1. 6 mins MVC 2. 6m MVC + Ischaemia	1. Y<O 2. Y<O
Hunter et al., 2004	Elbow Flexors	22 ± 4 23 ± 4	14M 13F	73 ± 4 71 ± 4	10M 8F	Isometric Sustained	20% MVC	Overall: Y<O, YF=OF,OM
Katsiaris et al., 2005	Knee Extensors Knee Flexors	Old Only		75.6 ± 2.8 75.3 ± 2.8	713 799	Isokinetic	30x MVC (180°/s)	OM>OF OM>OF
Kent-Braun et al., 2002	Ankle Plantar Flexors	33 ± 6	10M 10F	75 ± 6	11M 11F	Isometric-Incremental Intermittent	4s Contract, 6 secs relax	Y<O
Lindstrom et al., 1997	Knee Extensors	28 ± 6	14M 8F	73 ± 3	8M 8F	Dynamic (90°/s)	100x MVC (90°/s)	Y=O
Mademli et al., 2008	Plantar Flexors	32 ± 7	10M	64 ± 4	11M	Isometric	65% MVC 3s on 4s Rest	Y<O
McNeil and Rice, 2007	Dorsi Flexors	25.9 ± 3.8	12M	64.3 ± 2.8 83.7 ± 3.3	12M 12M	Dynamic	25 x 20% MVC 1 per 1.6s	Y>O
Petrella et al., 2005	Knee Extensors	26.9 ± 0.7	13M 11F	63.6 ± 0.8	12M 16F	Dynamic	10reps 40% MVC	Y>O
Stackhouse et al., 2001	Knee Extensors	22.7 ± 4.1	11M 9F	71.5 ± 5.9	8M 9F	Isometric	25 x MVC 5s On 2s Rest	Y=O
Stevens et al., 2001	Knee Extensors	23.7 ± 2.1	10M 10F	73.2 ± 4.6	10M 10F	Isometric	150 contractions at 20% MVC	Y=O

1.10 Aim and outline of the thesis

The participants used in the work presented are part of a much larger European wide cohort study known as MYOAGE. The overall aim of the study and this thesis is to characterise and observe the consequence of the age-related loss of muscle mass and strength on mobility and function in European older adults. The use of the term Sarcopenia within the data chapters of this thesis refers to the age-related loss of muscle mass, rather than the inclusion of functional parameters as discussed above.

As discussed above, there is a substantial discrepancy between DXA and MRI measurements of skeletal muscle mass. With the increased use of DXA in the diagnosis of sarcopenia, it is important to examine how muscle mass estimates of DXA differ from that obtained with the 'gold standard', MRI. This was the subject of **Chapter 2**. It was shown that DXA overestimates thigh muscle mass.

Muscle mass per se may not give a valid indication of a low muscle mass in a given individual. One can readily understand this shortcoming when comparing the muscle mass of a 2-m tall person with that of a 1.50-m tall person. To account for this many studies use the skeletal muscle mass index (SMMI; body mass per height squared; $kg \cdot m^{-2}$). This is not without problems as ageing is associated with a reduction in height, resulting in an underestimation of the loss of muscle mass. In **Chapter 3**, the thigh muscle mass to femur volume is explored as a new index. It incorporates femur volume, and assumes that femur volume 1) does not change with age and 2) is largely determined by the muscle forces acting upon it around puberty. It then also potentially eliminates secular changes that are often an associated limitation of cross sectional studies.

As discussed above, the SMMI is a poor predictor of muscle function. In **Chapter 4,** the muscle:bone ratio, muscle strength measures (grip and knee extensors) and muscle power as quantified by counter movement jump correlated with measures of mobility. In older adults, muscle power appeared to be the greatest predictor of low function.

Chapter 5 examines the age-related changes in muscle contractile properties and muscle fatigue. It appeared that while during an intermittent fatigue test old and

young show the same fatigue resistance, the time to exhaustion during a sustained contraction at 50% maximal voluntary contraction was significantly longer in older than young people. It should be noted that despite the author of the published manuscript being another member of the research group, the current author of this thesis contributed to the study design, data collection analysis and drafting of the manuscript and author order was determined by group decision.

In the general discussion in **Chapter 6**, the findings of the above chapter are briefly summarised and discussed in context of the literature with suggestions for future work and some final conclusions.

Chapter 2

Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles

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2.1 Abstract

Magnetic resonance imaging (MRI) and dual-energy x-ray absorptiometry (DXA) were used to examine the thigh lean mass in young and old men and women. A whole-body DXA scan was used to estimate thigh lean mass in young (20 men; 22.4 ± 3.1y; 18 women; 22.1 ± 2.0y) and older adults (25 men; 72.3 ± 4.9y; 28 women; 72.0 ± 4.5y). Thigh lean mass as determined by DXA and full thigh MRI scans were compared. Although the thigh lean mass quantified by DXA and MRI in young and older participants were correlated (r^2=0.88; $p<0.001$) the magnitude of the differences in thigh lean mass between young and old was smaller with DXA than MRI (old vs. young men 79.5±13.1% and 73.4±11.2%; old vs. young women 88.6±11.8% and 79.4±12.3%, respectively). Detailed analysis of MRI revealed 30% smaller quadriceps muscles in the older than young individuals, while the other thigh muscles were only 18% smaller. DXA underestimates the age-related loss of thigh muscle mass in comparison to MRI. The quadriceps muscles were more susceptible to age-related atrophy compared with other thigh muscles.

2.2 Introduction

The age-related loss of muscle mass, often known as sarcopenia, contributes to reduced physical function and vitality in old age (Doherty 2003). There is, therefore, great interest in understanding the mechanisms of muscle loss and finding ways to prevent or minimise this loss of muscle mass. However, any such research depends on accurate and reliable measurements of muscle size.

There are several imaging techniques available to measure muscle size, including magnetic resonance imaging (MRI), computer axial tomography (CT), ultrasonography, bio-electrical impedance analysis (BIA) and dual energy X-ray absorptiometry (DXA) (Heymsfield et al. 1990; Narici et al. 1992; Visser et al. 1999; Wang et al. 1996). Of these, sarcopenia is most often defined from measurements obtained using BIA (Janssen et al. 2000) or DXA (Baumgartner et al. 1998), both being convenient and quick methods that allow the collection of data from large cohorts. Cut-off values for diagnosis of sarcopenia are generally given as a defined deviation (usually 2SD) from the mean muscle size of a young-adult reference population. When examining a whole limb or other large body segment in a homogeneous group of subjects (of similar age) there is a good correlation between the muscle sizes estimated with DXA, Computer tomography (CT) and MRI, the latter being considered the gold standard (Visser et al. 1999; Fuller et al. 1999; Levine et al. 2000; Segal et al. 2009). It is not known, however, whether this correlation between muscle sizes determined by DXA and MRI changes with age.

A good correlation between two methods does not necessarily mean the methods show similar values because a systematic error (under- or overestimation) in one measurement would still lead to a good correlation. In a 10-week unilateral training study (Delmonico et al. 2008), for instance, it was found that at baseline DXA overestimated muscle mass in the thigh region by ~2 kg in comparison to CT despite a strong correlation between the measures ($r=0.88$, $p<0.001$). In this study it was also observed that DXA underestimated training-induced hypertrophy by around 25% compared with CT-derived measurements. If such an error also applies to

atrophy, it could have implications in assessment of the degree of sarcopenia and muscle wasting in other clinical conditions.

Another drawback of DXA is that it does not permit a distinction to be made between individual muscles within a single muscle group, such as the quadriceps, or between agonists and antagonists. This may be important because various muscles may be affected differently with ageing (Candow & Chilibeck 2005; Abe et al. 2011) and after bed rest (Belavý et al. 2009), although a 12-yr longitudinal follow-up of 7 elderly subjects showed no differences between knee extensors and flexors (Frontera et al. 2000).

The best way of assessing muscle-specific atrophy is by examination of multiple axial-plane MR images along the length of the limb. Consequently, the purpose of the present study was two-fold; first, to examine the level of agreement between MRI and DXA in estimating thigh muscle size in young and older men and women and, secondly, to examine age and sex differences in size of individual thigh muscles.

2.3 Methods

Participants and ethical approval

The study was carried out on a sub-group of 91 volunteers participating in a larger study designed to understand age-related muscle weakness (www.myoage.eu) (McPhee et al. 2013). The study was approved by the ethics committee of Manchester Metropolitan University and conformed to the Declaration of Helsinki. Written informed consent was obtained from each participant prior to participating in the study. Young-adult participants (20 men, 18 women) were recruited from amongst the university student population and older participants (25 men, 28 women) from the local community. Participant characteristics are presented in Table 2.1. All participants were healthy, not known to suffer from any musculoskeletal or cardiovascular disease, nor had suffered any limb fractures within the last 5 years. Other exclusion criteria were; not being able to walk 250m unassisted, institutionalisation, co-morbidities such as neurological disorders (e.g. Parkinson's Disease), heart failure, chronic obstructive pulmonary disease, chronic pain syndrome or metabolic disease. In addition participants were excluded if they had undergone hip or knee replacement in the previous 2 years, or had a period of immobilisation greater than 1 week in the 3 months prior to testing. Older participants were all socially active and community dwelling and their medical doctor (General Practitioner) confirmed there was no medical reason why they should not participate.

Anthropometrics

Body mass was recorded on a digital scale with participants in light indoor clothing. Standing height was measured with a portable Stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated by dividing the participant's body mass in kilograms by their height-squared in metres.

Magnetic Resonance Imaging (MRI)

A 0.25 T MRI scanner (G-Scan, Esaote, Genova, Italy) was used to measure thigh muscle volume. The participant was positioned supine in the scanner. A turbo 3D-

T1-weighted protocol was used (matrix 256 x 256, TR 40 ms, TE 16 ms) and multiple 3.1 mm thick serial transverse sections were obtained every 25 mm from the distal to the proximal heads of the femur. Computing imaging software (OsiriX medical imaging software, OsiriX, Atlanta, USA) was used to determine the cross-sectional area of each of the four muscles of the quadriceps group and other thigh muscles (adductors, hamstrings and abductors) in each slice (Figure 2.1A).

The total thigh volume was estimated by summation of the cross-sectional area of each head of the individual quadriceps muscles and other muscles in each slice multiplied by the distance between slices, as previously described (McPhee et al. 2009; Morse et al. 2007). To aid comparison between the two measures, MRI volumes were converted to mass by multiplying by 1.04 $g.cm^{-3}$ (the density of muscle tissue) (Snyder WS et al. 1975).

To examine changes in muscle cross-sectional area along the length of the femur, scan locations were normalised to % Femur Length to allow comparisons between participants of different height. The length of the femur was defined as the distance between the greater trochanter and the distal lateral condyle at the knee. The locations of the slices along the femur length were rounded to the nearest 5% Femur Length. The muscle cross-sectional areas at a given % femur length were used for comparison between individuals.

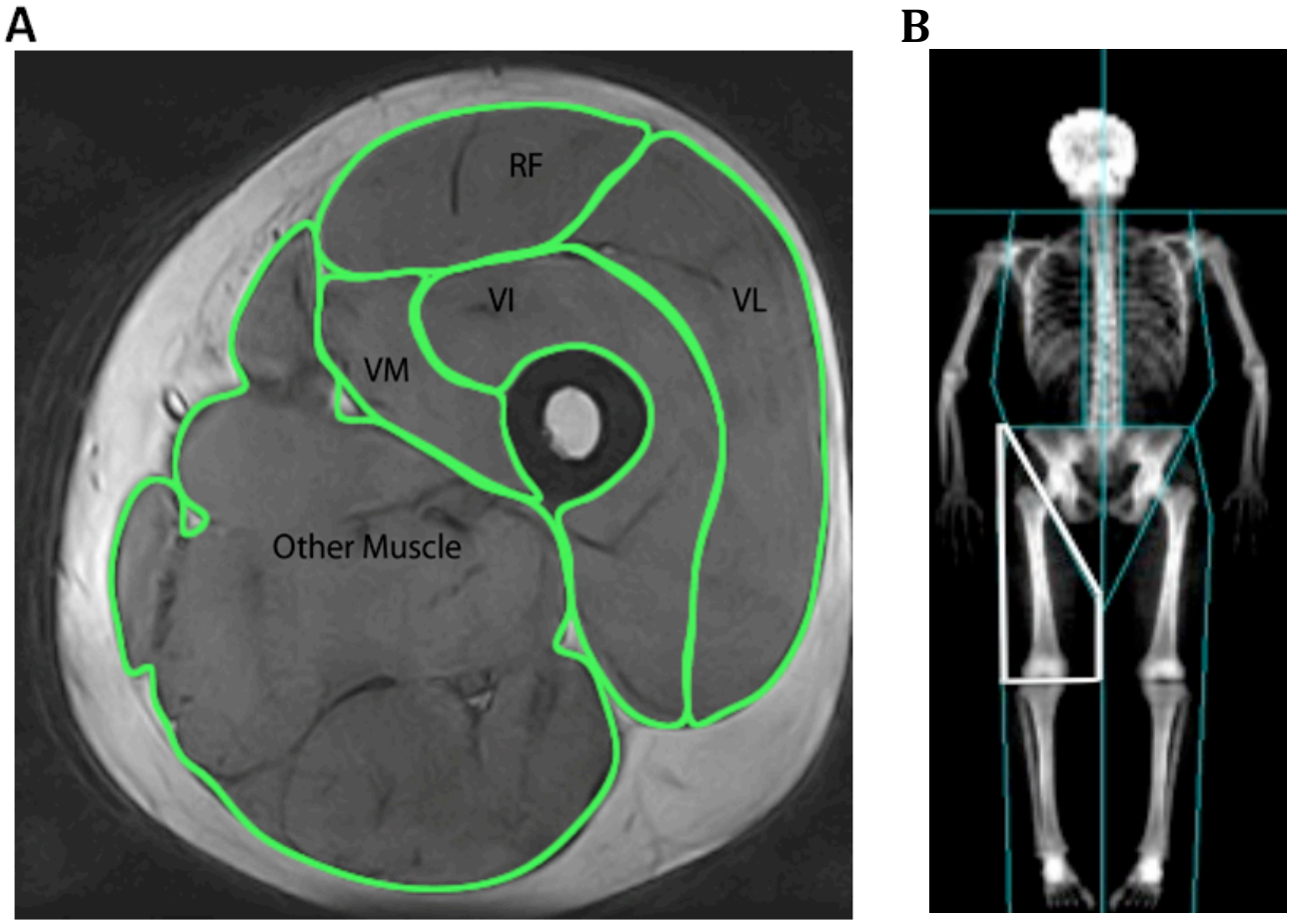

Figure 2.1 (A) Representative magnetic resonance image of the thigh with *rectus femoris (RF), vastus lateralis (VL), vastus intermedius (VI)* and *vastus medialis (VM)* labelled and highlighted. **(B)** Diagram showing the region of interest in DXA scans highlighted.

Dual Energy X-ray Absorptiometry (DXA)

A total body DXA (Lunar Prodigy Advance, GE Healthcare) scan was performed to measure total body composition and bone mineral density. Participants lay supine on the scanning bed. Computer software (Prodigy, Encore 2006 v 10.50.086, GE healthcare) was used to provide estimations of total body lean mass and fat mass. The thigh was identified as a region of interest using previously reported borders from the femoral neck (Segal et al. 2009) to the knee joint (Figure 2.1B). In the thigh of the dominant leg lean mass,, fat mass and bone mineral content were estimated. All DXA analyses were performed by the same experienced investigator (TMW). In

estimating “lean mass” the typical DXA machine (including the Lunar Prodigy used in this study) includes not just muscle mass but also connective tissue and the non-mineral components of bone. Bone mineral content accounts for approximately 55% of total bone mass with the rest being made up by protein and water. For this reason, an adjusted lean mass was calculated as follows (Heymsfield et al. 1990):

Lean mass = total mass - fat mass - (1.82 * BMC)

DXA is also includes non-adipose components of fat tissue, such as protein, in the lean mass but the contribution this makes is unclear, so no further adjustments were applied.

Statistics

Data were analysed using SPSS v18 (IBM, 2011). A univariate two-way ANOVA with “as between factors” Age and Gender, was used to examine differences between groups. Significant interactions indicate that the effects of age differed between men and women. Pearson’s product moment correlation was used to determine the relationships between variables. Repeated-measures ANOVA, with distance as “within” factor and Age and Gender as “between” factors, was used to determine differences along the femur and also sex-related differences in the atrophy in individual muscles. One-way ANOVA with Bonferroni post-hoc analysis was used to determine age-related differences in individual muscle atrophy. Data were expressed as mean ± standard deviation unless stated otherwise. Statistical significance was accepted as $p<0.05$.

2.4 Results

Participant Characteristics

It can be seen from Table 2.1 that the older subjects were shorter than the young ($p<0.001$), but had a similar body mass ($p=0.114$) and thus a higher BMI ($p<0.001$). The older participants had higher total-body fat mass ($p<0.001$) and a lower total-body lean mass ($p<0.001$).

Table 2.1 - Participant Characteristics

	YM (n=20)	OM (n=25)	YF (n=18)	OF (n=28)	Significant differences
Age (years)	22.4 ± 3.1	72.3 ± 4.9	22.1 ± 2.0	72.0 ± 4.5	Y<O
Height (m)	1.81 ± 0.05	1.73 ± 0.08	1.67 ± 0.05	1.60 ± 0.06	Y>O; M>F
Mass (kg)	72.8 ± 9.8	77.9 ± 13.2	61.7 ± 9.5	64.1 ± 11.2	M>F
BMI ($kg.m^{-2}$)	22.2 ± 3.3	25.8 ± 2.7	22.0 ± 3.2	25.0 ± 3.9	Y<O
Lean Mass (kg)	59.2 ± 5.8	53.0 ± 7.5	40.1 ± 3.4	37.4 ± 3.8	Y>O; M>F
Total Fat Mass (kg)	12.7 ± 6.3	22.1 ± 8.5	19.4 ± 8.1	23.9 ± 8.4	Y<O; M<F

Body Mass Index (BMI); Young Men (YM); Older Men (OM); Young Women (YF) and Older Women (OF); Significant differences between young and old, men and women; $p<0.05$.

Relationship between MRI and DXA to determine thigh muscle size

Total thigh muscle size measured by MRI and by DXA was significantly greater in young participants than old ($p<0.001$) and in men than in women ($p<0.001$). The age x gender interaction ($p=0.006$) is reflected by a larger age-related decrease in muscle mass in men than women (Table 2.2). Women had higher thigh fat mass than men ($p<0.0005$), but there was no significant difference between young and old in thigh fat mass ($p=0.144$).

Table 2.2- Measurements of thigh muscle size by MRI and DXA

	YM (n=20)	OM (n=25)	YF (n=18)	OF (n=28)	Significant differences
MRI thigh muscle volume (cm^3)	4546 ± 740	3328 ± 509	2911 ± 4.11	2312 ± 359	Y>O; M>F
MRI Quad Volume (cm^3)	2237 ± 351	1523 ± 301	1374 ± 207	991 ± 180	Y>O; M>F
MRI Other Muscle volume (cm^3)	2309 ± 431	1805 ± 276	1537 ± 238	1321 ± 212	Y>O; M>F
DEXA Thigh Fat Free Mass (kg)	6.33 ± 0.74	5.03 ± 0.85	4.01 ± 0.5	3.55 ± 0.45	Y>O; M>F
DEXA Thigh Lean Mass (kg)	5.81 ± 0.68	4.56 ± 0.76	3.61 ± 0.47	3.21 ± 0.43	Y>O; M>F
DEXA Thigh Fat Mass (kg)	2.06 ± 1.02	2.43 ± 1.04	2.91 ± 1.09	3.10 ± 0.98	M<F

Fat free mass (DXA) was adjusted for bone mineral content (Heymsfield et al. 1990). Volume represented the sum of muscle volume for the four Quadriceps muscles including other muscle volumes of the hamstring, adductors and abductors in the region of interest. Significant differences between young and old, men and women; $p<0.05$.

The use of the above correction factor for BMC assumes that BMC accounts for 55% of total bone mass. During diseases such as osteopenia/osteoporosis the BMC may be closer to 52% (Heymsfield et al. 1990). As a consequence, this could result in an further overestimation of lean mass in participants suffering from osteopenia/osteoporosis. Assuming that this is the case, the correction factor could be corrected, for example a loss of BMC to 54% of total bone mass would need a correction factor of 1.86 to calculate the remaining 46%. If using this correction factor for older adults it would alter the mean thigh lean mass for older men to 4.54 ± 0.76 kg and 3.20 ± 0.45 kg for older women from 4.56 ± 0.76 kg and 3.21 ± 0.43 kh respectively, a difference of approximately 0.4% of what we reported here.

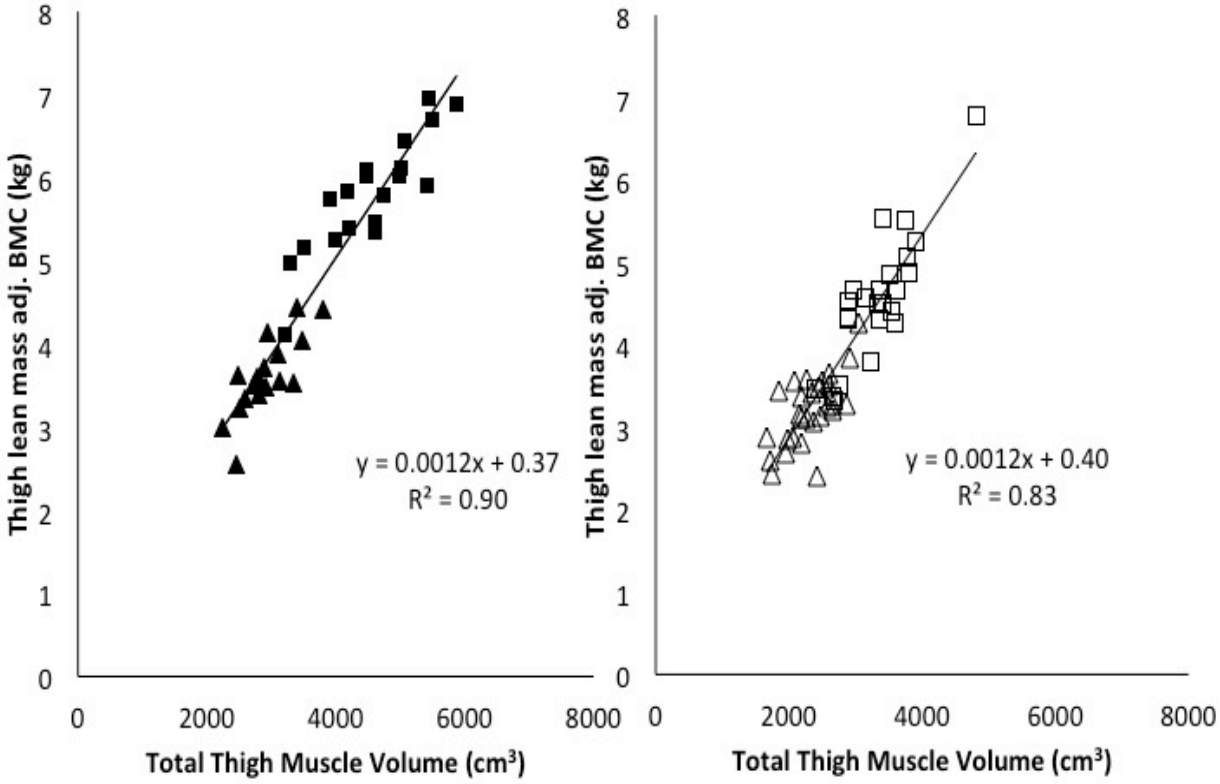

Figure 2.2. The relationship between total thigh muscle volume as assessed by magnetic resonance imaging and thigh lean mass as quantified by DXA. **A**, Young men are depicted with filled squares; young women are represented by filled triangles. **B**, Older men are depicted with open squares; older women are represented by open triangles.

Figure 2.2 shows a strong relationship between thigh muscle size measured by MRI and DXA. In young adults the correlation was very strong (R^2 = 0.90, p<0.001), with a positive y-axis (DXA) intercept of 0.37 kg (Figure 2.2A). In older subjects, the correlation was also very strong (R^2 = 0.83, p<0.001), again, with a positive intercept on the y-axis (0.40 kg)(Figure 2.2B). This intercept is also evident in the Bland-Altman plot (Altman & Bland 1983) which illustrates that DXA overestimated muscle size in all conditions (Figure 2.3).

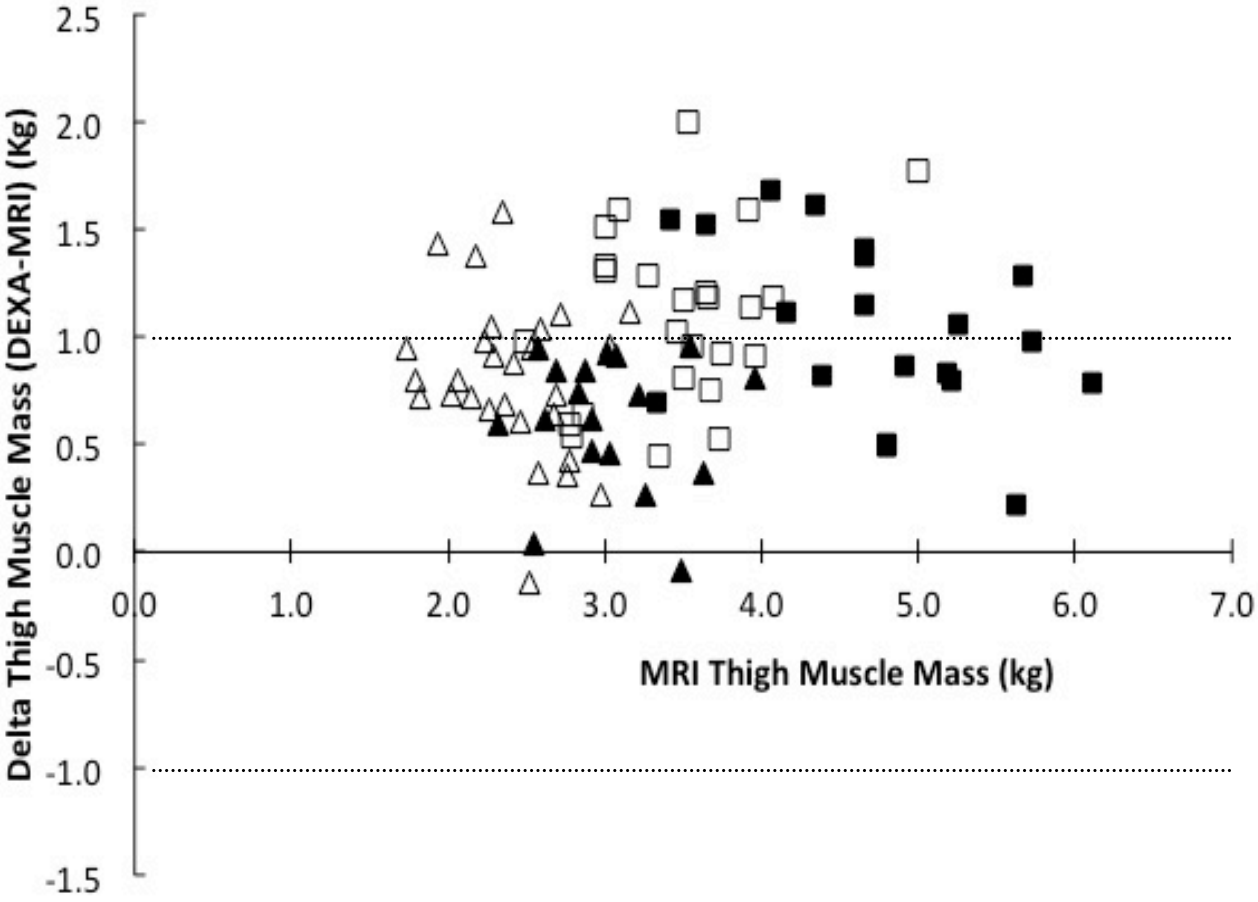

Figure 2.3- A Bland-Altman plot of thigh muscle mass determined with MRI on the x-axis and the difference between DXA-estimated muscle mass with MRI. Horizontal dotted lines represent one standard deviation above and below the average of MRI. Young men are depicted with filled squares; young women are represented by filled triangles. Older men are depicted with open squares; older women are represented by open triangles.

The positive intercept on the y-axis (DXA) contributes a relatively larger proportion of the older muscle compared with young and as a consequence, DXA underestimates the difference of thigh muscle size between young and older participants in comparison to differences detected with MRI. In men, the MRI showed older thigh muscles to be 73.4% ± 11.2, while the DXA scan showed thigh lean mass of older men to be 79.5% ± 13.1 of young men (Figure 2.4). In women, the thigh muscle volume of older women measured using MRI was 79.4% ± 12.3 of young women, while the DXA-derived measurement showed older women to have thigh lean mass that was 88.6% ± 11.8 of younger women (Figure 2.4). The discrepancy between the MRI and DXA in determining the extent of the difference between young and old in muscle size was significant ($p<0.001$). There was no significant discrepancy between the MRI and DXA in determining muscle size differences between men and women ($p=0.718$, interaction $p=0.268$).

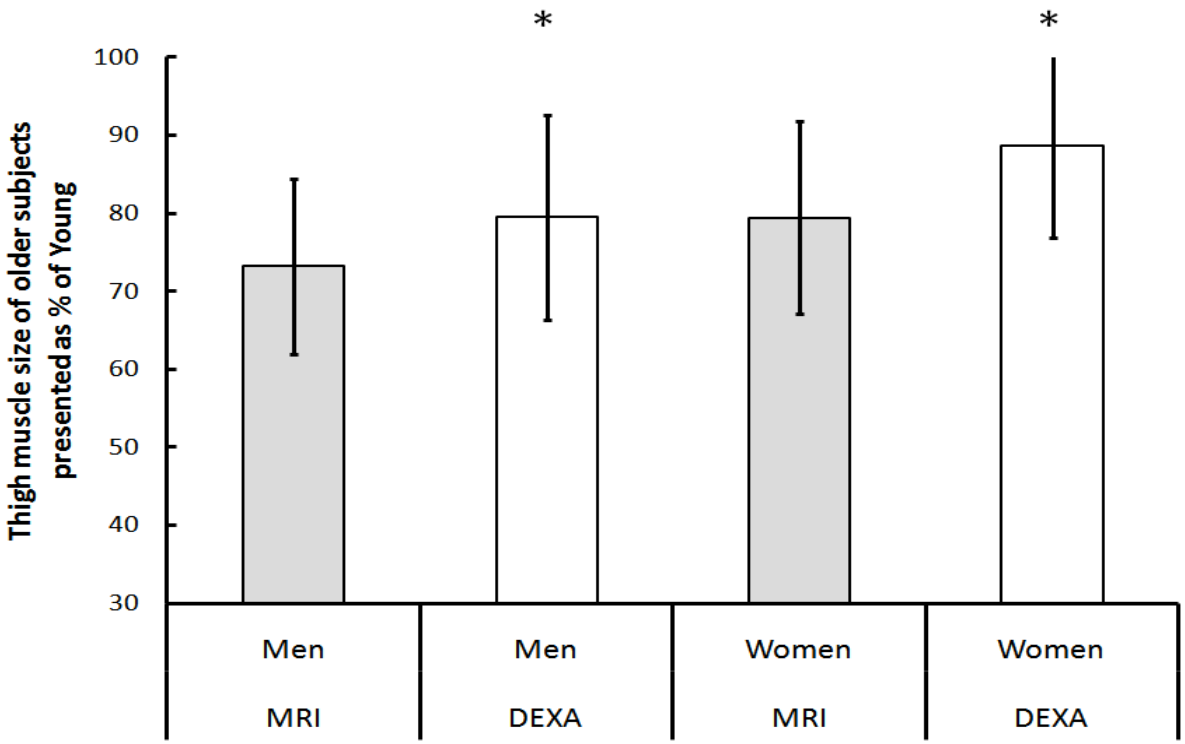

Figure 2.4- Muscle mass of older individuals expressed as % of young. * Indicates significant difference between the DXA- and MRI-determined measurements of muscle size $p<0.001$.

Quadriceps in relation to other muscles of the thigh

Quadriceps volume, determined from MRI serial axial-plane scans, showed the difference in volume between old and young men to be 1218 cm^3 and between young and old women to be 599 cm^3 (Table 2.2; $p<0.001$). When expressed as percentage of the young values the differences were 68% for older men and 72% for older women (Table 2.2). The other thigh muscles (knee flexors, hip abductors and adductors) were also smaller in old compared with young, although the extent of the difference was not as great as seen for the quadriceps. In older men, the "non-quadriceps" muscles of the thigh were 78% of young men while those of the older women were 86% of young women (Table 2.2). Thus, the proportion of the quadriceps to the total thigh muscle volume was significantly lower in old than young individuals ($p<0.001$).

Component muscles of the quadriceps

In Figures 2.5 & 2.6 it can be seen that each of the individual quadriceps muscles were smaller in old compared with young and for women compared with men (both $P<0.001$). The atrophy of the older muscle was evident across the entire muscle length, with the exception of the most proximal and distal muscle-tendon insertions.
In older women, the *rectus femoris* was more atrophied, at 37%, than the other three muscles (*medialis*, 23.5%; *intermedius*, 25.5%; *lateralis*, 29.6%) with the differences between *rectus femoris* and *vastus intermedius* as well as between *rectus femoris* and *vastus medialis* being significant ($p=0.01$). In older men, there were no significant differences in the degree of atrophy between the individual Quadriceps muscles.

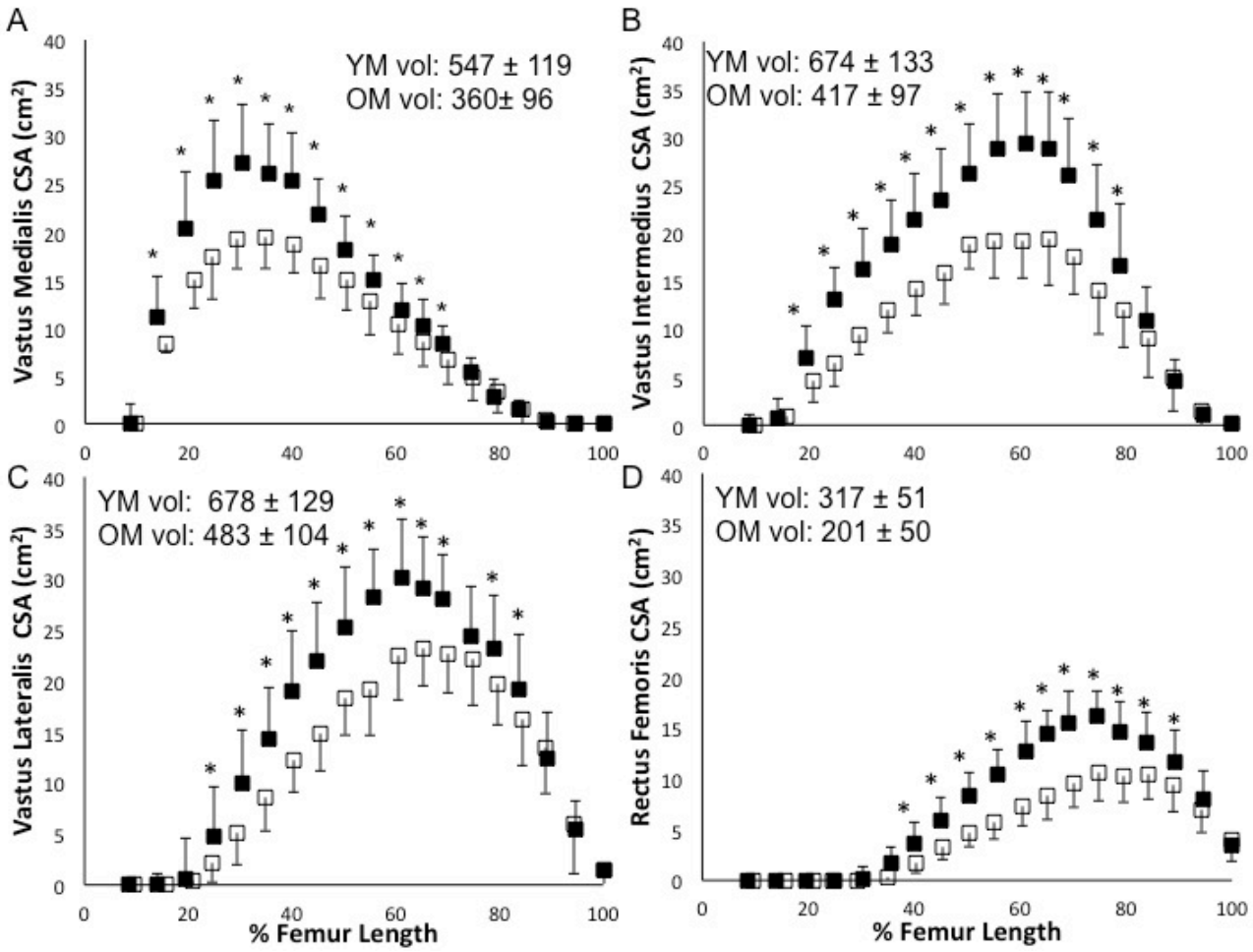

Figure 2.5. Cross sectional areas of the constituent muscles of the quadriceps along the length of the femur in men. Filled symbols represent young men, open symbols are older men. (A) *vastus medialis* (B) *vastus intermedius* (C) *vastus lateralis* (D) *rectus femoris*. * indicates a significant difference between young and old ($p<0.05$). The volume (cm^3) is given for each muscle.

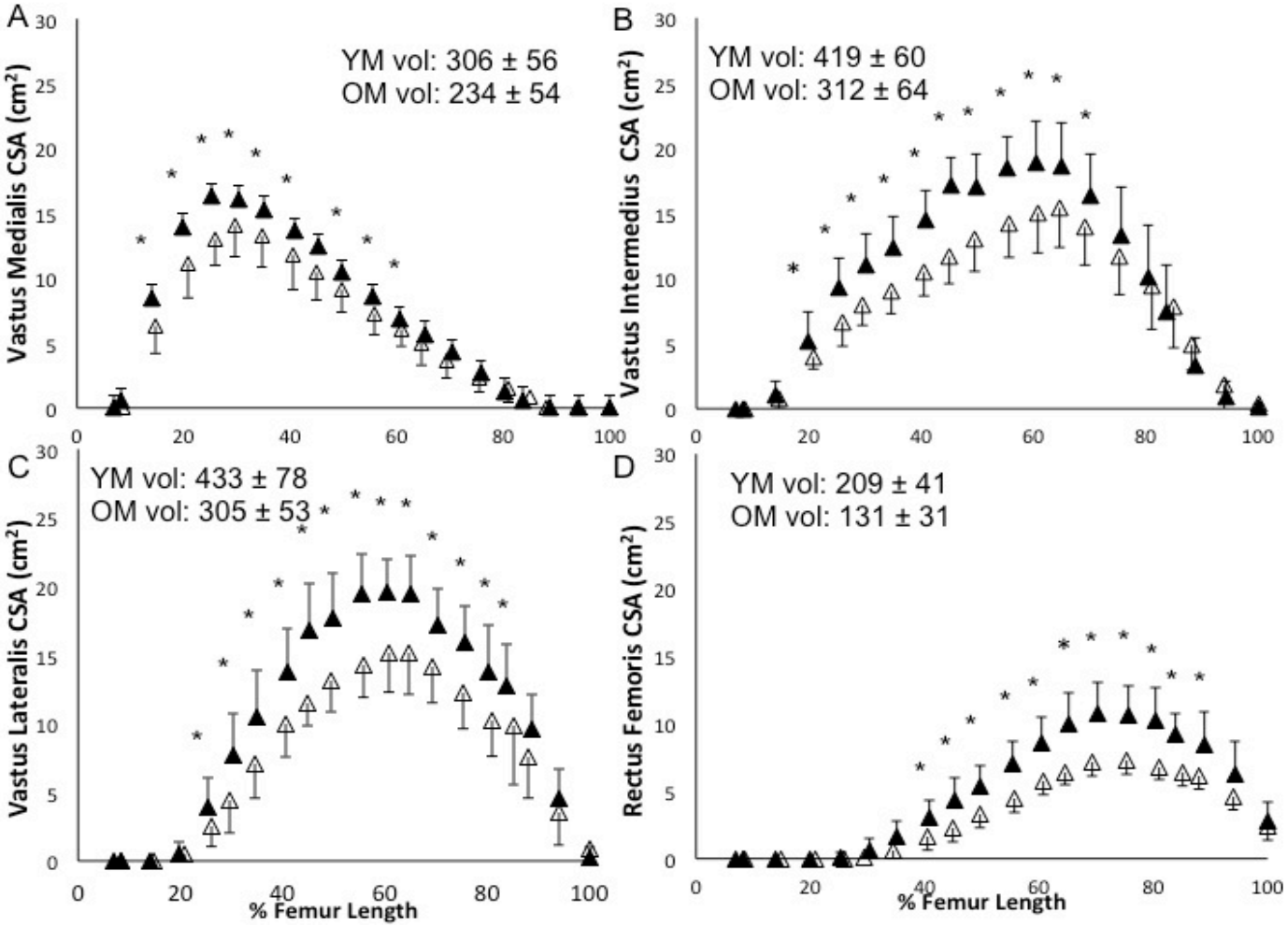

Figure 2.6. Cross sectional areas of the constituent muscles of the quadriceps along the length of the femur in women. Filled symbols represent young women and open symbols are older women. (A) *vastus medialis* (B) *vastus intermedius* (C) *vastus lateralis* (D) *rectus femoris*. * indicates a significant difference between young and old ($p<0.05$). The volume (cm^3) is given for each muscle.

2.4 Discussion

Sarcopenia is associated with muscle weakness and mobility problems in old age. With the global rise in life expectancy the need to understand the progression and causes of sarcopenia becomes more pressing. Dual-energy x-ray absorptiometry is often used in large clinical and epidemiological studies of sarcopenia. However the results of the present study show that DXA underestimates the incidence of sarcopenia in thigh muscles compared with more detailed measurements derived from MRI. The major advantage of MRI over DXA is that it enables a distinction to be made between individual muscles. The detailed analysis of thigh muscle sizes in MRI revealed that the knee extensors are particularly susceptible to age-related atrophy (Table 2.2). The other muscles of the thigh (flexors, adductors and abductors) also experience loss of muscle mass with ageing, but to a significantly lesser extent than the quadriceps, especially in women. Our results also confirm that men are more affected by age-related muscle wasting than women.

The wide accessibility of DXA, with its low radiation dose, ease of use and better accuracy compared with most other common measurements, such as bioelectrical impedance, has enabled its use in large cohort studies of sarcopenia and body composition (Baumgartner et al. 1998; Delmonico et al. 2007; Gillette-Guyonnet et al. 2003; Kullberg et al. 2009; Rolland et al. 2003; Visser et al. 2003). We observed a strong correlation between DXA and MRI measurements of thigh muscle size (Figure 2.2), similar to that reported in previous studies (Delmonico et al. 2008; Fuller et al. 1999; Levine et al. 2000; Visser et al. 1999), suggesting that DXA can detect differences in muscle size between people. However the results from DXA showed a lesser degree of sarcopenia of the thigh muscles than when using MRI, with older muscle being 21% and 11% smaller than young in men and women, respectively, while MRI measurements were 27% and 21% smaller for older men and women, respectively (Figure 2.4). This disparity is evident from the data shown in Fig 2 where the regression line has an intercept of approximately 0.4 kg on the DXA axis. It is not clear why the positive intercept exists, but it is also evident in other similar studies (Visser et al. 1999; Levine et al. 2000; Shih et al. 2000). The positive

intercept makes up an increasingly large proportion of the total thigh mass of smaller muscles such as in the older people. Consequently, the tendency will be for DXA to underestimate the extent of sarcopenia. One possibility is that the algorithms used by the computer software (Prodigy, Encore 2006 v 10.50.086, GE healthcare) to obtain lean mass may need some adjustment, taking better account of the protein content of bone and fat tissue and the age-related changes in proportions of these non-muscle tissues.

There are reports that the extent of muscle ageing may be greater in men than women (Castillo et al. 2003; Iannuzzi-Sucich et al. 2002), although others suggest women are affected more than men (Janssen et al. 2002). The discrepancy is most likely due to differences in the definition of sarcopenia, with some defining sarcopenia only as loss of muscle mass (Visser et al. 2002), others normalising lean mass to total body mass (Janssen et al. 2000) or using muscle mass and function (Goodpaster et al. 2006) (e.g. walking speed or strength). Our own results are not conclusive, but tend towards a greater loss of muscle in men (Figures 2.4 and 2.5). Changes to habitual physical activity levels as well as altered hormonal status will affect both men and women and contribute to loss of muscle size, but it is possible that the reduced testosterone experienced by older men removes the anabolic "advantage" seen in younger men and leads to relatively greater loss of muscle size (Ryall et al. 2008).

In the MRI analysis, around 20 equidistant axial-plane cross-sectional slices from the most proximal insertion of *vastus medialis* through to the proximal origin of the *rectus femoris* on the anterior inferior iliac spine were analysed per subject. For every slice, the four individual quadriceps muscles were identified while all the other thigh muscles (knee flexors, and hip adductors and abductors) were grouped together. The cost of MRI as well as the time needed to analyse images can restrict its use. If access is limited, our comprehensive analysis suggests that a single scan taken at 55-65% femur length (the distal lateral condyle being 0% and the proximal end at the greater trochanter being 100%) provides the optimal location to examine differences between groups. At 55-60% femur length the quadriceps cross sectional area is largest. Of the thigh muscle groups, the quadriceps were affected most by age-related atrophy; in older men they were 32% smaller than those of young men and in

older women they were 28% smaller than younger women. This difference of around 30% between young and old is similar to those reported in other studies that used MRI to measure muscle volume in young and elderly (Macaluso et al. 2002; Ogawa et al. 2012). It is notable, however, that the other muscles of the thigh showed less of a difference between young and old. These muscles were 22% and 14% smaller for older men and women, respectively, compared to the younger subjects. It remains to be determined why the knee extensors should be more affected by ageing than other muscles of the thigh.

The quadriceps muscles have a complex anatomical arrangement not only does the whole group consist of four muscles, but the *vastus lateralis* muscle, for instance, can be subdivided into 4 compartments, with each being innervated by individual nerve branches and having different fascicular arrangement (Waligora et al. 2009). The *rectus femoris* is bipennate and biarticular and the *vastus medialis* muscle belly is located more distally along the thigh length. The different portions of the quadriceps probably have slightly different functions and might thus be subjected to different metabolic or mechanical stimuli that affect ageing. While each of the four-quadriceps muscles was smaller in old than young participants, in older women the degree of atrophy in the *rectus femoris* was larger than that of the *vastus medialis* and *vastus intermedius*. However, in men, no differences were observed in the extent of age-related atrophy between different portions of the quadriceps.

Limitations:
In some subjects it was difficult to distinguish between the *vastus medialis* and the *vastus lateralis* muscles in the MRI images of the proximal thigh, possibly due to fusion of these muscles, as seen in cadaveric specimens (Waligora et al. 2009). Furthermore, we were not able to clearly distinguish between hip abductor and adductor muscles and the knee flexors, so these muscles were grouped and labelled “other muscles”. Therefore, we could not determine age or sex differences in these other muscles individually.

To enable a direct comparison between MRI-measured muscle volume (cm^3) and DXA-derived estimations of mass (kg), the MRI data was converted to mass using a previously reported conversion of 1.04 $g.cm^{-3}$ (Snyder et al. 1975). A further

correction was used to adjust DXA values to account for the non-mineral component of bone (Heymsfield et al. 1990). However, the bones of older people may have a higher non-mineral content than those from young people and this correction may thus result in an overestimation of muscle density in older age and hence underestimate the connective tissue and fat infiltration (Kent-Braun et al. 2000). This, however, is not a major factor as conclusions remained even when the data were not adjusted for the non-mineral component of bone.

In conclusion, there was a good overall correlation between DXA and MRI-derived measurements of thigh lean mass. However, there was a discrepancy between the two methods when estimating the extent of age-related loss of muscle mass. Compared with MRI, the DXA underestimates the difference between young and old in thigh lean mass, which could lead to an underestimate of the loss of muscle mass with age. The MRI data indicate that the age-related decrease in muscle size was greater for the quadriceps than the other muscles of the thigh.

Chapter 3

Thigh muscle volume in relation to age, sex and femur volume

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Age (2013) (In Press)
Data presented at The Physiological Society Annual Meeting 2011

In Chapter 2, it was shown that DXA underestimates the age-related muscle loss. In addition there are discussions within the literature whether correction factors such as Height are valid in comparisons between young and old. Even so, the use of muscle mass alone is not sufficient, since muscle size differences between individuals will partly depend on body shape and size and confound cross-sectional studies of muscle ageing. A new method to quantify sarcopenia is thus required to accurately define the extent of sarcopenia within an individual.

3.1 Abstract

Normalising muscle mass to height squared is often suggested as a solution for these individual differences. We hypothesised that normalisation of muscle volume to femur volume is a way of determining the extent of muscle lost with ageing (sarcopenia). Thigh and femur muscle volumes were measured from serial MRI sections in 20 recreationally active young men (Mean Age 22.4 yrs), 25 older men (72.3 yrs), 18 young women (22.1 yrs) and 28 older women (72.0 yrs). There were no age-related differences in femur volume. The relationship between thigh muscle volume and femur volume (R^2=0.76; exponent of 1.12; P<0.01) was stronger than that with height (R^2=0.49; exponent of 3.86; P<0.01) in young participants. For young subjects the mean muscle:bone ratios were 16.0 and 14.6 for men and women, respectively. For older men and women the mean ratios were 11.6 and 11.5, respectively. The Z-score for the thigh muscle:bone volume ratio relative to young subjects was -2.2 ± 0.7 for older men and -1.4 ± 0.8 for older women. The extent of sarcopenia judged by the muscle:bone ratio was approximately twice that determined when normalising to height squared. These data suggest that the muscle:bone ratio captures the intra-individual loss of muscle mass during ageing, and that the age-related loss of muscle mass may be underestimated when normalised to height squared. The quadriceps seems relatively more affected by ageing than other thigh muscles.

3.2 Introduction

Frailty, decreased mobility and the consequent loss of independence are common features of old age and there are compelling reasons to understand the underlying causes of these problems. One aspect that has received considerable attention is the age-related loss of muscle bulk and strength, often referred to as “sarcopenia” (Rosenberg 1989), that has been linked to reduced mobility, disability, decreased quality of life and mortality (Hairi et al. 2010; Janssen et al. 2002; Lauretani et al. 2003). It is important, therefore, to be able to accurately document the extent of muscle wasting with age and to identify possible differences in the extent of atrophy between muscle groups that may have different metabolic profiles or be used in particular ways.

It is notable, however, that while the time course and extent of muscle changes over many decades are widely discussed, the evidence is almost entirely based on cross-sectional data for the obvious reasons that it is almost impossible to fund and undertake a longitudinal study lasting 50-70 years. When interpreting cross-sectional data as evidence for longitudinal change the assumption is often made that old people measured today were of the same stature and physical development in their youth, some 50 to 70 years ago, as the young people of today. However there are well known secular changes in height, mass and rates of maturation, possibly associated with changing diet, levels of physical activity and general health care. In addition, Europeans in their seventh and eighth decade today probably had a much-restricted diet during and immediately after the Second World War (Heijmans et al. 2008; Lumey et al. 2007) as well as higher levels of habitual physical activity than people born in the latter part of the last century (Prentice & Jebb 1995). For these reasons we cannot be certain that a direct comparison of the muscle mass in today’s older population with that of younger adults gives an adequate reflection of the age-related muscle loss. In judging the extent of sarcopenia, either from a single measurement of an individual or in a cross-sectional study, it would be valuable to have a reference measure of body shape or size that could be used to normalise the data.

Mass and height, the two most obvious indicators of body size, have equally obvious disadvantages. Mass has the disadvantage that body fat is a significant component which can vary independently of muscle mass and since the majority of people tend towards higher BMI as they age, using body mass as a standard would overestimate muscle loss with age. Height, or height squared, has been proposed as a way of normalising lean body mass (Baumgartner et al. 1998) but there are two objections to this. First, height squared has the wrong dimensions for normalising a volume, which might be better reflected by height cubed. In addition, height can decrease by as much as 1 cm per year from the age of 30 on (Sorkin et al. 1999), mainly as a result of increased spinal curvature and vertebral compaction (Moayyeri et al. 2008; Sorkin et al. 1999) and normalising muscle mass to height will thus underestimate changes in muscle mass.

An alternative rationale starts with the observation that bones and muscles are adapted to each other at young age (Rittweger et al. 2000; Schiessl et al. 1998). Whilst bone mass seems to be lost during ageing from the upper extremities and from the spine, such bone losses seem to be moderate in the lower extremities (Riggs et al. 2004; Wilks et al. 2009). In the shafts, those small changes are conveyed through thinning of the cortex, with the total bone cross section undergoing no or only small changes (Garn et al. 1967; Wilks et al. 2009). Another option would be to use femur length since this does not change after growth plate fusion and the cessation of longitudinal growth, and femur length cubed might be used to provide the correct dimensions for normalising thigh muscle volume and provide a better estimate of changes in muscle mass. However, multiplying femur length by its cross-sectional area to give a nominal femur volume may be more appropriate, as muscle forces on the bone are important factors determining bone cross-sectional area during the critical growth period (Rittweger 2008; Schoenau et al. 2002). Jones et al. (Jones et al. 1983) used total bone cross-sectional area as a standard against which to judge muscle development in boys with muscular dystrophy, and growth of bone relative to muscle has been used as a way of gauging bone development in children with underlying skeletal problems (Schoenau et al. 2002).

The aim of the work described here was to develop a more valid indicator of sarcopenia than the commonly used muscle mass per height squared. We hypothesized that femur muscle volume would be a better measure with which to normalise muscle volume than height squared when comparing young and older subjects. To substantiate this argument it was first necessary to show that muscle volume correlates well with bone volume in young subjects. Having established this we have assessed muscle and bone size in the thigh of young and older participants by Magnetic Resonance Imaging (MRI).

3.3 Methods

Participants and ethical approval

The study was approved by the local ethical committee of Manchester Metropolitan University and conformed to the Declaration of Helsinki. Written informed consent was obtained from each volunteer prior to participating. Young participants (20 men, 18 women) were recruited from amongst the university student population and older participants (25 men, 28 women) from the local community: participant characteristics are presented in Table 3.1. These volunteers were recruited in the UK as part of a larger study of ageing (the MYOAGE study, EU-FP7 nr: 223576) (McPhee et al. 2013). All the subjects were healthy and participated in recreational physical activities but none were training to compete in athletic competitions. Older participants were all socially active and their General Practitioner confirmed there was no medical reason not to take part in the study.

Table 3.1 Participant Characteristics

	Young Men (n=20)	Older Men (n=25)	Young Women (n=18)	Older Women (n=28)	Significant difference.
Age (years)	22.4 ± 4.5	72.3 ± 4.9	22.1 ± 4.5	72.0 ± 4.5	Y<O
Height (m)	1.81 ± 0.05	1.73 ± 0.08	1.67 ± 0.06	1.60 ± 0.06	Y>O; M>F
Body mass (kg)	72.8 ± 9.8	77.9 ± 13.2	61.7 ± 9.5	64.1 ± 11.2	M>F
Femur Length (cm)	45.4 ± 1.6	43.8 ± 3.0	41.5 ± 1.7	40.6 ± 2.0	Y>O; M>F
Femur CSA 60% (cm^2)	6.3 ± 0.8	6.6 ± 0.8	4.9 ± 0.5	5.0 ± 0.6	M>F
Nominal femur volume (cm^3)	285 ± 38	289 ± 49	200 ± 20	205 ± 32	M>F

There were no significant age x gender interactions. Femur CSA 60%: Femur cross-sectional area at 60% femur length from proximal. Significant differences $p<0.05$.

Magnetic Resonance Imaging (MRI)

The volume of the quadriceps femoris muscle group was measured with a 0.25 T MRI scanner (G-Scan, Esoate, Genova, Italy) in the dominant leg. The participant was positioned supine in the scanner. A turbo 3D-T1-weighted protocol was used (matrix 256 x 256, TR 40ms, TE 16ms) and multiple 6-mm thick serial transverse sections were obtained along the entire length of the thigh with no inter-slice gap. Computing imaging software (OsiriX medical imaging software, OsiriX, Atlanta, USA) was used to determine the total cross-sectional area of each of the four muscles of the quadriceps group as well as total bone cross-sectional areas. This analysis was completed using manual tracing in MRI slices at distances of 24 mm along the entire length of the quadriceps muscles, from the most distal point of the vastus medialis to the most proximal origin of the rectus femoris. Obvious visible deposits of fat infiltration were subtracted from the cross-sectional areas (Figure 3.1).
Muscle volumes were obtained by summation of the cross-sectional areas in each slice (16-19, depending on femur length) multiplied by the distance between slices. Femur length was obtained from total-body DXA scans (Lunar Prodigy Advance, GE Healthcare) by using the computer software (Lunar EnCore version 10.50.086) tools to draw a straight line from the proximal point of the greater trochanter to the distal region of the lateral condyle. A nominal value for femur volume was obtained by multiplying femur cross-sectional area at 60% (from proximal) femur length by femur length, but the precise location is not critical since the femur cross-sectional area is relatively constant in this region.

Muscle data were normalised using Z-scores with individual data expressed as the number of standard deviations from the mean of the young men or women, calculated as:

$$z = \frac{\chi - \mu}{\sigma}$$

Where χ is the value for the individual subject, and μ and σ the mean and standard deviation, respectively, of the corresponding young population.

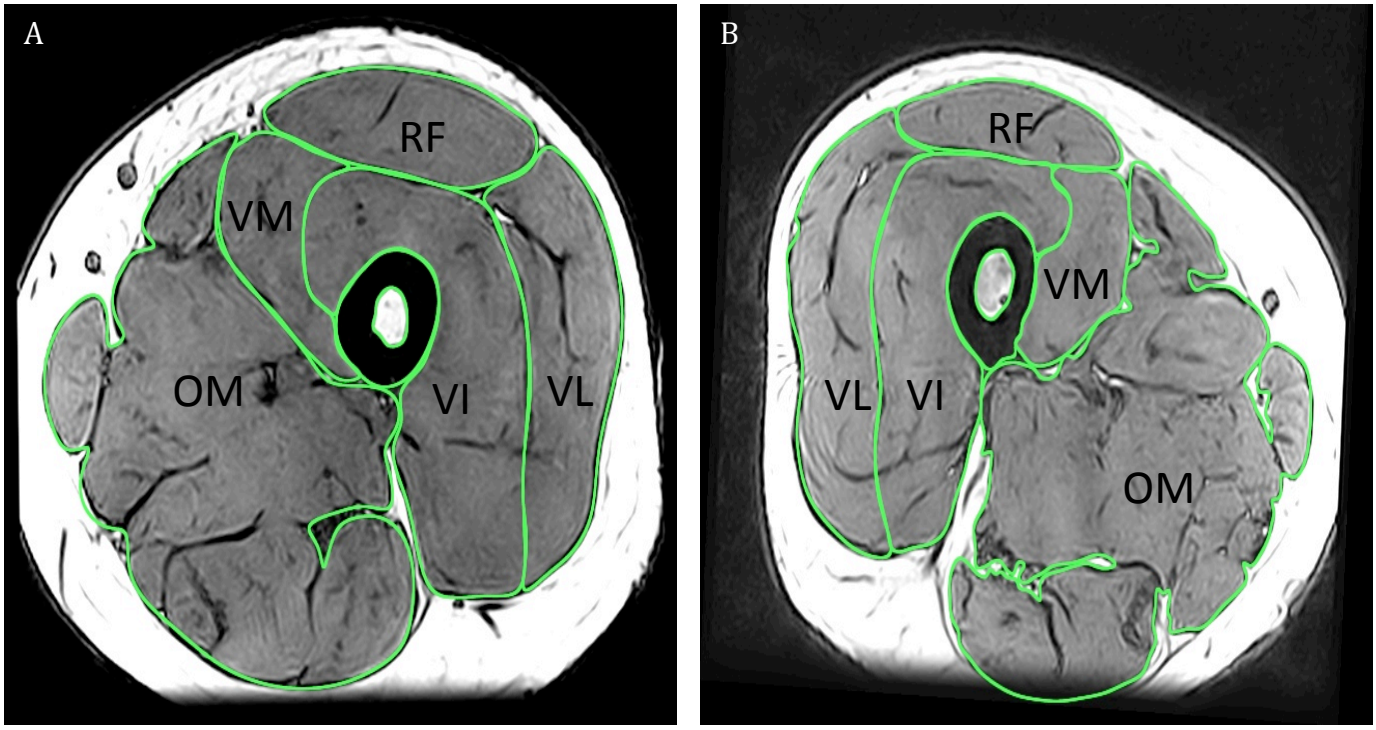

Figure 3.1. Magnetic Resonance Image of the thigh of **A,** a young man (22 years) and **B,** an older man (76 years) at 60% of femur length. Highlighted are the cross-sectional areas of the femur, RF, rectus femoris; VL, vastus lateralis; VI vastus intermedius; VM, vastus Medialis and OM Other Muscles.

Statistics

Data were analysed using SPSS v19 (IBM, 2011). Univariate two-way ANOVA was used with Age and Sex as "between factors" to examine differences between groups. Significant interactions indicate that the effects of age differed between men and women. Pearson's product moment correlation was used to determine the relationships between variables. Data are expressed as mean ± standard deviation unless stated otherwise. Differences were considered significant with p values ≤0.05.

3.4 Results

Participant Characteristics

Data for age, height, body mass and femur dimensions are given in Table 3.1. Both older men and women were ~7-8 cm shorter than their younger counterparts ($p<0.005$). Femur length was ~1.5 cm shorter in both older men and women than the younger people ($p=0.012$). Femur cross-sectional areas at 60% femur length were marginally, but not significantly ($p=0.11$), larger in the older subjects and there were no significant differences in the nominal femur volumes between young and old.

Muscle volumes

Figure 1 shows typical scans at 60% femur length for a young (Figure 3.1A) and older (Figure 3.1B) man indicating the measured areas of muscle and bone. Values for total thigh muscle volume and for the two major components, the Quadriceps and “Other muscles”, the latter including the hamstrings, adductor and abductor muscles, are given in Table 3.2. Total thigh muscle volumes of the older subjects were, respectively, 80 and 73% of the values for young women and men ($p<0.001$). Of the two components of the thigh muscle volume, the quadriceps group was more affected than the “Other muscles” for both men and women ($p<0.001$).

Table 3.2 Muscle volumes of the thigh in young and older men and women

	Young Men (n=20)	Older Men (n=25)	Young Women (n=18)	Older Women (n=28)	Significant Difference
Thigh Muscle Volume (cm^3)	4549 ± 740	3338 ± 512 (73% Young)	2905 ± 407	2314 ± 360 (80% Young)	Y>O; M>F §
Mean Z-Score	0 ± 1	-1.64 ±0.88	0 ± 1	-1.45± 0.88	Y>O
Quadriceps Volume (cm^3)	2240 ± 366	1533 ± 306 (68% Young)	1368 ± 204	993 ± 181 (73% Young)	Y>O; M>F §
Mean Z-Score	0 ± 1	-1.93 ± 0.84	0 ± 1	-1.84 ± 0.89	Y>O
Other Muscle Volume (cm^3)	2309 ± 431	1805 ± 276 (78% Young)	1536 ± 238	1321 ± 212 (86% Young)	Y>O; M>F §
Mean Z-Score	0 ± 1	-1.17 ± 0.64	0 ± 1	-0.91 ± 0.89	Y>O

Data for the old subjects are also expressed as a percentage of the young in brackets. In addition, the data for the older subjects are expressed as Z scores (for calculation see methods). Significant differences $p<0.05$. § Denotes significant age x gender interactions ($p<0.05$).

When expressed as Z scores (Figure 3.2 and Table 3.2) it can be seen that the mean score of the total thigh volume for the older subjects was about 1.5 SD below the mean value for the young. For the Quadriceps the Z score approached 2 and for the “Other muscles” it was closer to 1. The difference in Z score between Quadriceps and “Other muscles” was highly significant ($p<0.0001$).

It is notable that the distribution of Z scores for the old subjects, evident by eye in Figure 3.2 and numerically in Table 3.2, was as tight, if not slightly tighter (i.e. a standard deviation < 1), than the distribution of the young subjects.

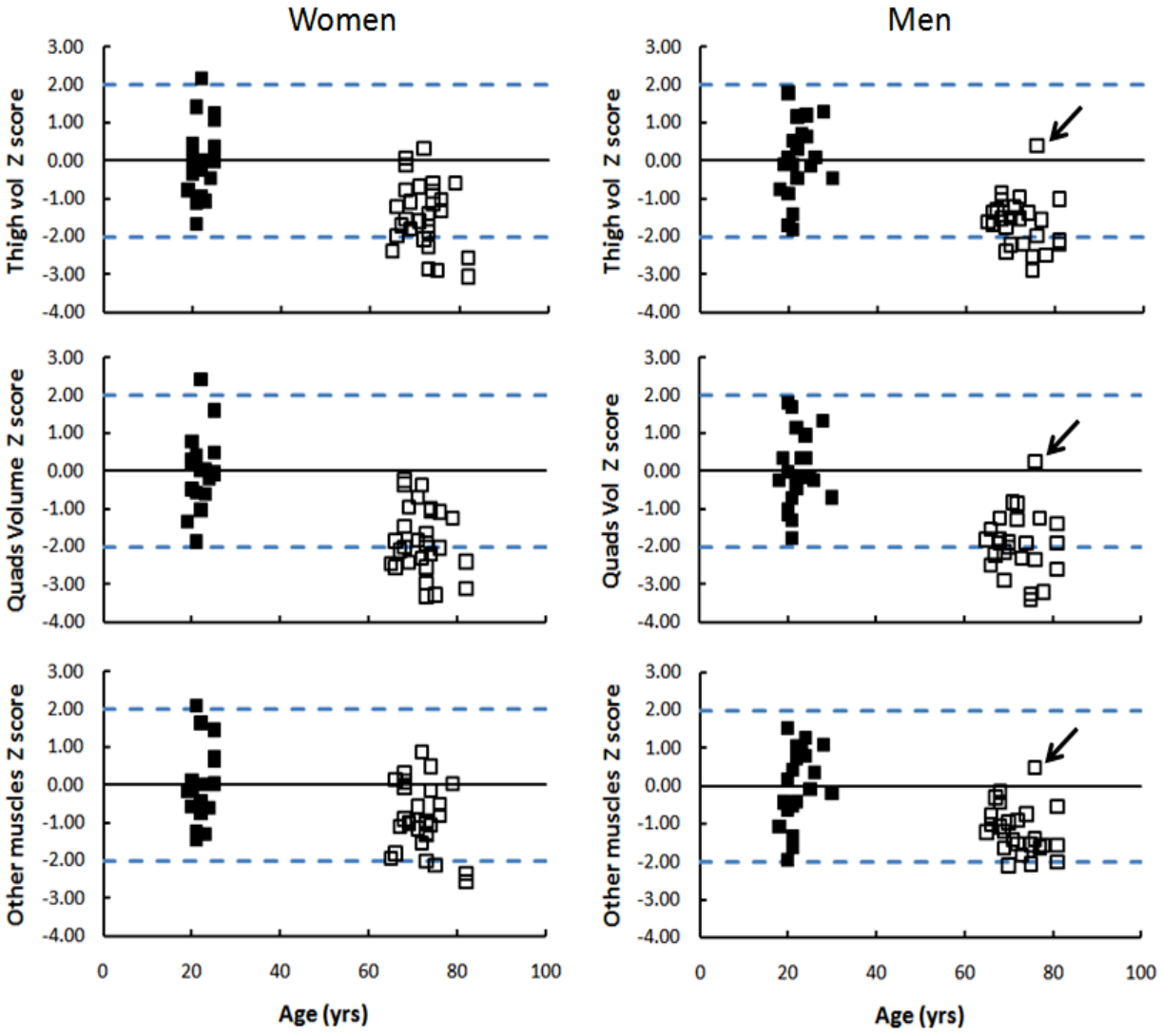

Figure 3.2. Muscle volume Z scores as a function of age. Z scores (for calculation see methods) for the total thigh muscle volume, quadriceps and hamstring muscles. The mean values for the young subjects (0, solid line) and ±2 SD (dashed lines) are shown.

In Figure 3.2, it can be seen that only one older man (indicated by the arrow) had Z scores that were above the mean Z scores for the young subjects, both for the total thigh muscle volume and the component parts. This subject, at the age of 76, was the tallest and heaviest of all the subjects and had the largest femur cross-sectional area. The obvious question is whether he had exceptionally large and strong muscles in his youth and had become merely average, compared to the young, as a result of ageing, or whether he was always this strong but had, as a consequence of lifestyle choices, good fortune or genetics, managed to avoid the effects of ageing. We will come back to this question below.

Normalising muscle volume

The relationships between height and thigh muscle volume for the combined young male and female subjects are shown in Figure 3.3A. The best fit to the data (R^2 = 0.49; $p<0.01$) had an exponent of 3.86. Plotting muscle volume against femur length (Figure 3.3B) gave a best fit with an exponent of 2.89 (R^2 = 0.38; $p<0.01$). However, the fit to the data was very much improved (R^2 = 0.76; $p<0.01$) when plotting muscle volume against the nominal femur volume (Figure 3.3C) with an exponent of 1.12.

It is evident in Figure 3.3A & 3.3C that muscle volume correlated better with femur volume than height in the young subjects. In Figure 3.3D comparison of the older and the young data normalised in this way shows that despite a similar range of femur volumes all the muscle volumes of the older people fell below the regression line for the young subjects.

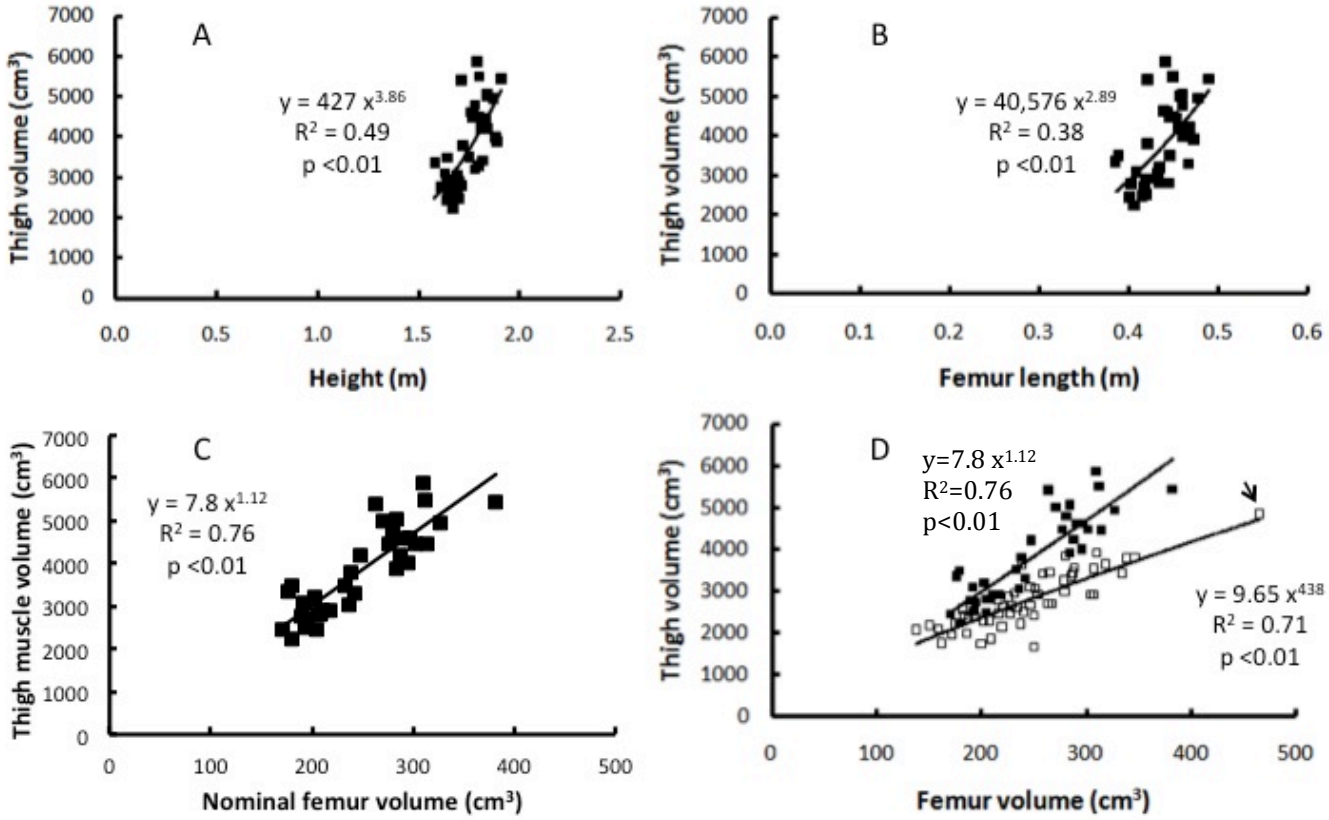

Figure 3.3. Relationship between thigh muscle volume as a function of **A**, height and **B**, femur length and **C,** nominal femur volume (cross sectional area multiplied by length) in young people and **D**, nominal femur volume for all young subjects (Solid symbols) and that of all older subjects (Open symbols) both $p<0.01$.

The one data point from an older subject on the extreme right of Figure 3.3D (indicated by arrow) is the older man referred to above, and indicated in Figure 3.2, whose Z score for muscle volume was just above the mean for the young men. It is evident that while he had a large muscle volume compared with all older subjects, and many of the young, his muscle to bone volume ratio was below that in the young men. This is evident in Figure 3.4 where muscle volumes are shown normalised for femur volume and expressed as Z scores.

Height squared is commonly used to normalise for body size when defining sarcopenia. Therefore, values for muscle volume divided by height squared are given in Table 3.3 next to data for muscle volume relative to nominal

femur volume. It can be seen that the latter approach indicates a greater degree of muscle loss associated with ageing than estimates based on muscle volume corrected for height squared; the mean Z score for the thigh volume of older men adjusted to femur volume was -2.2 while it was -1.17 when adjusted for height squared. The Z score for total thigh volume divided by femur volume for the one large older man (arrow in Figures 3.2, 3.3D & 3.4) was -2.85 suggesting he had even experienced somewhat greater muscle loss compared to the average older man (Table 3.3).

Table 3.3 Muscle volumes of the thigh normalised for femur volume or height squared in young and old men and women

	Young Men (n=20)	Older Men (n=25)	Young Women (n=18)	Older Women (n=28)	Significant Difference
Thigh Muscle Volume / Femur Volume	16.0 ± 2.0	11.6 ± 1.5	14.6 ± 2.2	11.5 ± 1.8	Y>O. M>F
Mean Z-Score	0 ± 1	-2.2 ± 0.7	0 ± 1	-1.4 ± 0.8	Y>O, M>F §
Quadriceps Volume/ Femur Volume	7.9 ± 1.0	5.3 ± 0.8	6.8 ± 0.9	4.9 ± 0.8	Y>O. M>F
Mean Z-Score	0 ± 1	-2.7 ± 0.8	0 ± 1	-2.2 ± 0.9	Y>O
Other Muscle Volume / Femur Volume	8.1 ± 1.2	6.3 ± 1.0	7.7 ± 1.5	6.6 ± 1.2	Y>O
Mean Z-Score	0 ± 1	-1.5 ± 0.8	0 ± 1	-0.8 ± 0.8	Y>O
Thigh Muscle Volume/ Ht^2 ($cm^3.m^{-2}$)	1390 ± 241	1108 ± 124	1040 ± 151	900 ± 112	Y>O, M>F §
Mean Z-Score	0 ± 1	-1.17 ± 0.5	0 ± 1	-0.92 ± 0.7	Y>O

Data are the different muscle groups together with the data expressed as Z scores (for calculation see methods). Significant differences $p<0.05$. § denotes significant age x gender interactions ($p<0.05$).

A similar difference between normalizing muscle volumes by height squared and femur volume was evident for the women and when considering the quadriceps and hamstring muscles separately. Finally, the Z-scores for the muscle volumes and muscle:bone ratio were larger in the quadriceps muscle group than the 'other muscles' (Figure 3.2; Tables 3.2 & 3.3), indicating that the quadriceps was relatively more affected by ageing than other thigh muscles.

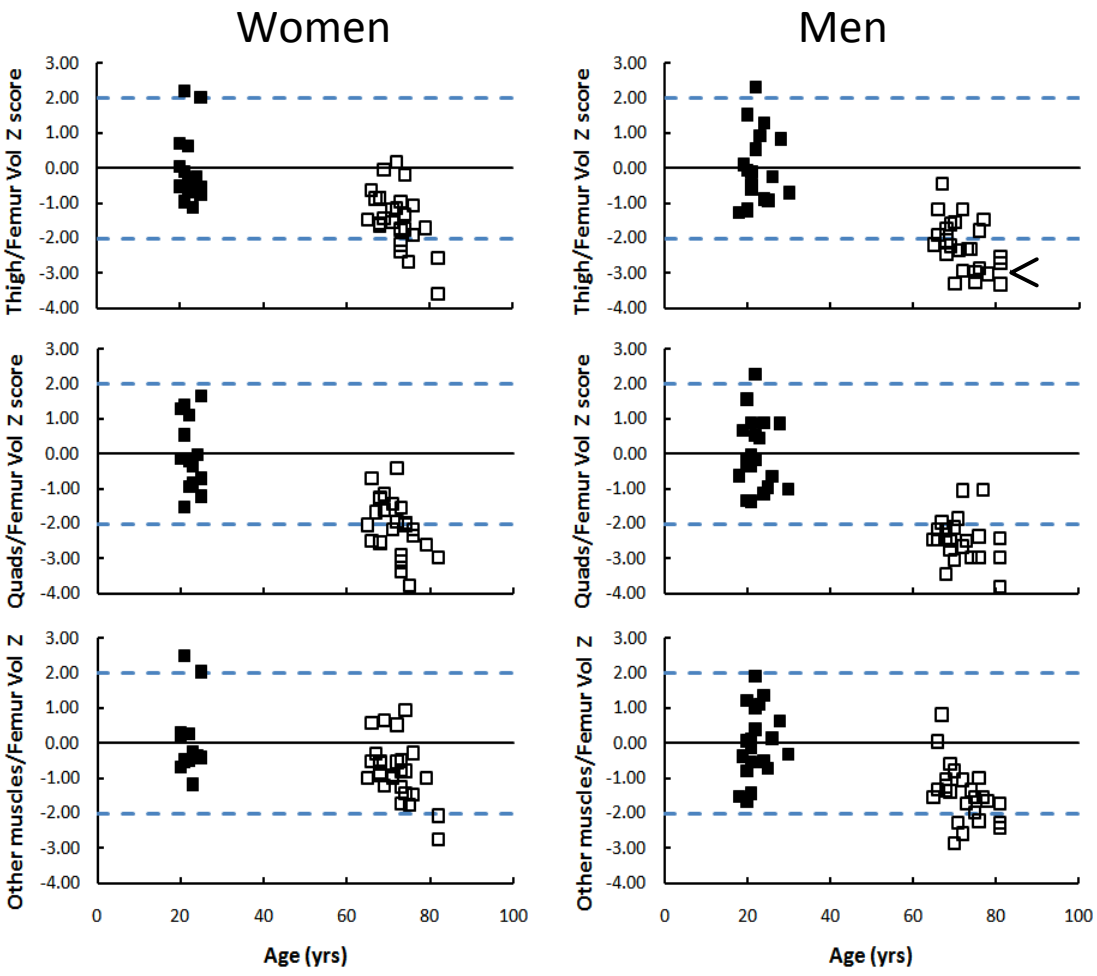

Figure 3.4. Z scores (for calculation see methods) for muscle volume divided by femur volume as a function of age. Left panels are the women, the right panels men. The Mean values for the young subjects (0, solid line) and ±2 SD (dashed lines) are shown.

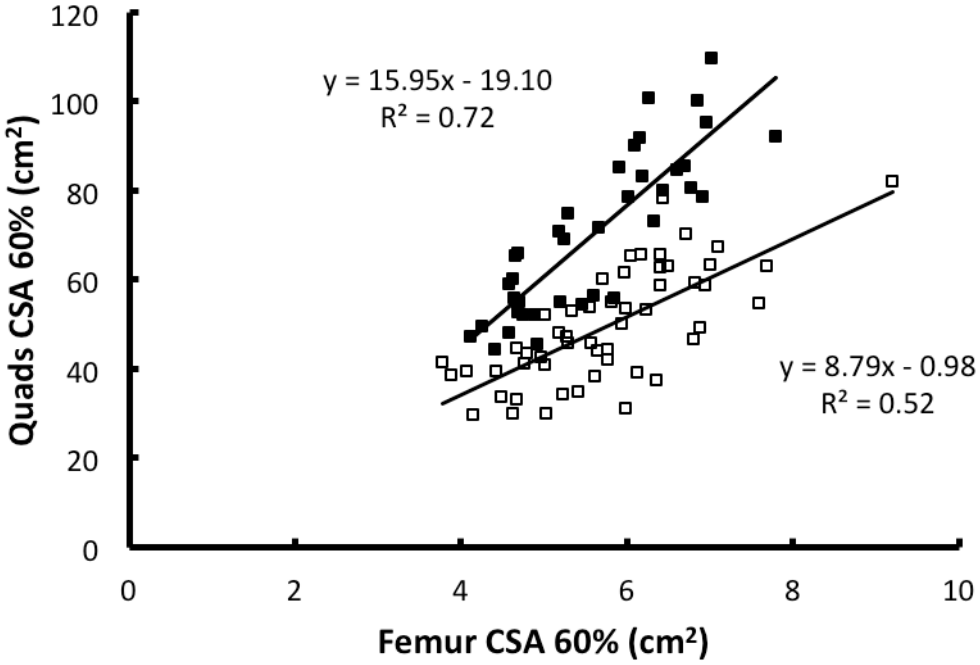

Figure 3.5 Relationship between quadriceps muscle cross-sectional area (Quads CSA) and femur cross-sectional area (Femur CSA) measured at 60% femur length. Young subjects: solid symbols; older subjects: open symbols.

3.5 Discussion

The loss of muscle mass that occurs with advanced age is a matter of considerable interest and concern but studies in this area have tended to be limited by two factors. One is the fact that almost all studies of muscle changes over several decades have, out of necessity, been cross sectional in design. There is no argument that 70-80-year-old people have a smaller muscle mass than people currently in their third decade. However, it is possible that this is a consequence of the older generation having had a smaller muscle mass in their youth, either as a consequence of secular changes or a lower protein and calorie rich diet in the years during and

immediately after WWII (Lumey et al. 2007). We have addressed this possibility by normalising muscle volumes to the volume of the femur and conclude that this is a better way of determining sarcopenia in an individual than the more commonly used method of dividing muscle mass by height squared. Judging by this muscle:bone ratio, thigh muscle mass was reduced by about 2 SD by the age of around 70 years with slightly greater differences seen in the extensor muscles than the other muscles of the thigh. There were no significant effects of age upon total bone cross-sectional area in the cohort studied here, so that any existing and possibly marginal periosteal expansion with age is unlikely to affect the muscle:bone ratio.

Leaving aside methodological problems and measurement errors, there are four reasons why the muscle mass may vary between people of different ages. First, subjects differ in body size and the larger the person the more muscle they are likely to have. Secondly, they may differ in somatotype where for a given body size mesomorphs will have a greater proportion of muscle than ectomorphs. Thirdly, there may have been secular changes with the phenotype of the population changing in the years over which the ageing process has its effects. For instance, the body height in the western world increased in the last generation by around 1 cm (Lissner et al. 2013). Finally, the ageing process may have affected some individuals more than others (Degens & Korhonen 2012). It is this latter ageing process that most research, including the present study, is concerned with. Longitudinal studies are the only certain way of revealing the true effects of ageing but practical issues make this impossible over a 50-year span. Given that most studies are cross-sectional and have relatively small sample sizes it is necessary to have some way of allowing for differences in body size and composition and, if possible, accounting for secular changes. Normalising muscle mass to height squared is the most common procedure (Baumgartner et al. 1998; Dufour et al. 2012; Estrada et al. 2007; Gillette-Guyonnet et al. 2003; Iannuzzi-Sucich et al. 2002; Kenny et al. 2003; Morley et al. 2001) but despite the fact that this is also the basis for calculating body mass index, it has no theoretical justification since volume would be expected to vary as the third power of a linear measurement. In fact, the data in Figure 3.3A show an exponent of 3.85.

There is an additional objection to using height to normalise muscle mass when comparing young and old since stature is well known to change with advancing years, mainly as a result of shrinkage and increased curvature of the spine, with up to 8 cm lost over the lifespan (Moayyeri et al. 2008; Sorkin et al. 1999), which is similar to the differences between the young and older cohorts reported here.

Thigh muscle volume in the young subjects was related to femur length with an exponent of 2.89, close to the third power that might be expected (Figure 3.3B). However, thigh muscle volume correlated even better with nominal femur volume with an exponent of 1.12 and R^2 = 0.76. It seems likely that taking femur cross-sectional area into account when calculating femur volume allows for variation in somatotype so that for a given femur length we might expect mesomorphic subjects to have both greater muscle mass and greater femur cross-sectional area. In addition to somatotype, adjusting muscle to femur volume may also allow for differences in muscle and bone development as a result of training or differences in habitual activity in the adolescent and early adult years.

Here we calculated a nominal femur volume as the cross-sectional area at 60% femur length multiplied by femur length. The cross-sectional area of the femur at 60% femur length is representative of the shaft of the femur and excludes the mass of bone at the two ends of the femur. One possible drawback to using femur volume to normalise muscle volume is that it assumes the outer dimensions of the mid-shaft region remain constant throughout life. Bone is constantly being remodelled and during ageing it is thought that the rate of periosteal apposition increases, with this increase being greater in men (Ahlborg et al. 2003; Rittweger 2008; Ruff & Hayes 1988). In line with previous studies (Feik et al. 1996; McNeil et al. 2009; Riggs et al. 2004) we found no significant change in total bone shaft CSA, and studies where an age-related increase was reported it was only 5% in a small population (Allen et al. 2011). Bone size is therefore a suitable internal standard against which to normalise muscle size.

There were considerable differences in the extent of age-associated muscle atrophy depending on whether muscle volume is normalised to height squared or to femur volume. When normalising to height squared, only one out of 25 older men fell two SD below the mean of the young men (giving a prevalence of just 4%), while when normalising for femur volume, 24 of the 25 were more than two SD below the corresponding young average (giving a 96% prevalence of sarcopenia). For the female subjects, 3 out of 28 older women were more than two SD below the young when thigh volume was adjusted for height squared (prevalence of 11%) but this rose to 16 out of 28 when normalised to femur volume (prevalence of 57%). It appears, therefore, that adjusting muscle volume for height squared may seriously underestimate the effect of age on muscle mass. It should also be noted that although every effort was made to exclude non-muscle components from the measured cross-sectional areas, it is impossible to account for small fat deposits and connective tissue that can infiltrate the muscles of older people. Consequently, the extent of the loss of contractile material must be greater than the extent of muscle atrophy we report. This is most likely to be at least part of the explanation of the commonly reported reduction in specific tension with ageing (Rutherford & Jones 1992; Hairi et al. 2010).

The few longitudinal studies of muscle ageing show a loss of muscle size of about 1% per year. Delmonico et al., (2009) report a 5% decrease in thigh muscle cross-sectional area of the knee extensors in men over a 5-year period in participants aged between 71-79 years and Frontera et al., (2000) found a 16% decrease in quadriceps and 14% decrease in knee flexors over a 12-year period in men aged around 65 yrs. at the start of the study. The data in Table 3.3 suggest a loss of around 0.5% per year of the original muscle volume over a 50-year period. Given that the observed rate of muscle wasting in longitudinal studies is higher this suggests that the loss of muscle mass may begin around the age of 45 year as is also suggested by cross-sectional data over the 18-88 yr. age range (Janssen et al. 2000), or simply be a reflection of the fact that a reduction in muscle mass in a year as a % of the muscle mass in young people is less than when the same loss is expressed as a % of the mass at the start of the year (Degens 2012).

Most large studies of sarcopenia have assessed muscle mass by dual energy X-ray absorptiometry (DXA), which cannot distinguish between different component muscle groups in, for instance, the thigh and thus cannot detect any differential effects of ageing on various muscle groups. Using MRI we were able to determine the size of different components of the thigh muscles; the knee extensors (quadriceps) and all the other muscles of the thigh, which includes the flexors, abductors and adductors, revealing that the quadriceps muscles were more affected by age than the other muscles. While the total thigh muscle volume was 20% lower in the older subjects, the quadriceps were 27-28% smaller and the other muscles about half this, at 14-15% smaller. The reason for this differential susceptibility is not obvious, but might reflect different patterns of activity of the various muscle groups or possibly differences in fibre type composition since studies have shown that type II fibres atrophy more during ageing than type I fibres (Andersen 2003). However, a study by Garrett et al., (1984) observed that the knee flexors have a greater proportion of type II fibres than Quadriceps or Adductor muscles suggesting this is not the explanation.

The definition of sarcopenia as muscle size falling below some lower limit, often defined as -2SD of young values, gives the impression that the extent of muscle loss with age is a phenomenon that affects some individuals to a greater extent than others; i.e. that some older people “suffer” from sarcopenia, while others are little or not affected at all by this condition. If this were the case, the effect would be a greater dispersion of the muscle data for old subjects in comparison with the young subjects. However, it is evident in Figures 3.2 & 3.4 that the variances of the muscle data for young and old subjects were similar in both male and female; the standard deviation of Z scores for the older subjects was less than 1. The most likely explanation is that, when young, the older subjects had a similar mean and range of muscle volumes as the present-day young, and the effect of ageing has been to lead to a similar loss of muscle volume in all subjects. The results suggest therefore that all the older subjects had age-related thigh muscle loss to a similar extent of around 20% for women and 27% for men. Those older subjects who were at the lower end of the distribution and had the lowest Z

scores probably had small muscle mass when young while those at the top of the range and who might have been thought to not have suffered the effects of ageing (such as Subject identified by the arrow in Figure 3.2 & 3.3) had, in fact, considerably larger muscles when young which with age had shrunk to what would be average for a young person. Overall, the effect is that the decrease with age is approximately 2 SD, taking the top of the range down to the mean and the mean to the bottom of the range of the corresponding young people. This observation may reflect the fact that our older sample were relatively homogeneous, remaining active and in good health. This can be considered a limitation of this study as in the population as whole, inter-individual differences in activity levels; diseases or strength training may increase the dispersion.

Measuring muscle volume with multiple MRI scans is demanding in terms of time and equipment and simply comparing muscle to bone cross-sectional areas from a single scan at 60% femur length showed a similar relationship between bone and muscle cross-sectional areas (Figure 3.5) to that obtained comparing bone and muscle volumes (Fig 3.3D). *Z* scores for the quadriceps: femur cross-sectional areas (-2.27 for the older female -3.2 for the older men) are similar, although slightly larger, than those for muscle:bone volumes (Table 3.3). We have previously shown that it is possible to estimate quadriceps muscle volume of young male subjects from a single MRI scan if the length of the femur is known (Morse et al. 2007) and it appears therefore that the same is true for women and older subjects. It also implies that sarcopenia affects all the components of the quadriceps to a similar extent, a conclusion we have reported elsewhere (Maden-Wilkinson et al. 2013).

In summary, the data presented here lead to the following conclusions. First, that normalising upper leg muscle volumes to height squared has little validity and leads to a substantial underestimation of the differences in muscle volume between young and old. Secondly, the differences in muscle volume between young and old are a consequence of the ageing process and have not arisen because the older subjects were of a smaller stature when they themselves were young; this removes one of the concerns about interpreting

cross-sectional data in terms of longitudinal changes. Thirdly, muscle changes with age appear to have affected all subjects to a similar degree, as there was no evidence of individuals who were protected from the ageing process. Fourth, the extent of muscle changes was greater in the quadriceps than in the other muscles of the thigh.

Chapter 4

Age related loss of muscle mass, strength and power and their effect on mobility in recreationally active UK older adults

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Jones DA,
Degens H

(Submitted)

In Chapter 3, a new method of quantifying sarcopenia in older adults was introduced and validated: normalising the muscle mass to the cross-sectional area of the femur at 60% femur length. In Chapter 4, this new method of quantifying sarcopenia is explored in relation to another index of muscle mass, the skeletal muscle mass index (appendicular lean mass divided by height squared), muscle strength and power and their association with functional performance.

4.1 Abstract

During ageing mass, force and power generating capacity of the muscle decrease. This decrease may well contribute to functional impairment in the old. However, in most studies relationships between these parameters and functional impairment are evaluated in mobility limited people, while it remains to be seen whether such relationships also exist in recreationally active older people without overt mobility limitations. Whole body DXA scans and serial MRI sections of the thigh were taken to estimate muscle and bone mass in 49 Young (28 Men; 22.4 ± 3.1y) and 66 older adults (31 Men; 72.3 ± 4.9y). Voluntary maximal isometric knee extensor torque was measured by dynamometry and muscle power by counter-movement jump and correlated with the 6-minute walk distance (6MWD) and timed get up and go tests (TUG) as measures of functional capacity. Heart rate (HR) was measured during the 6MWD. In the old group none of the indicators of muscle mass correlated significantly with the 6MWD and TUG, indicators of the ability to perform daily life activities. Muscle power correlated positively with the 6MWD and TUG and more so in the old (R^2=0.38 & 0.30) than the young (R^2=0.27 & 0.19) (all $p<0.01$). In the young (men R^2=0.45; W=0.29), but not the old people the 6MWD correlated with HR. Both worked at the same relative maximal heart rate (74 ± 11%). The lower performance in healthy old age appears to have a multifactorial origin, such as both a lower muscle power and a lower cardiorespiratory fitness, but not muscle mass per se.

4.2 Introduction

The loss of muscle mass (sarcopenia) and muscle strength (dynapenia) with age is associated with a progressive loss of mobility and consequent reduction in the quality of life in the older person (Burns et al., 2010; Janssen et al., 2002; Rantanen et al., 1999). The decline in muscle mass seems to start around the age of 45 (Janssen et al., 2000) and if below a certain threshold it causes functional impairment (Mithal et al. 2013).

The mass of the knee extensors has been shown to be a better indicator of function than the calf musculature (Abe et al. 2012). DXA derived measures have been demonstrated used to estimate appendicular lean mass (Levine et al., 2000; Visser et al., 1999) and within an older population a low appendicular muscle mass was associated with physical disability (Janssen 2004). Also a lower thigh muscle mass as estimated with Magnetic Resonance Imaging (MRI) was associated with a lower short physical performance battery score and gait speed in older adults (Buford et al., 2012). While such correlations are often observed within older people with mobility limitations (Barbat-Artigas et al., 2012), it is not known whether the age-related loss of muscle mass has any relationship with performance of daily life activities in a healthy older population.

When measuring muscle mass or muscle wasting one has to consider that taller people may have and require a larger muscle mass (Newham et al., 2003) to move the body around. To account for this inter-individual variation the majority of epidemiological studies have used the skeletal muscle mass index (SMMI), defined as appendicular lean mass divided by height squared (alm/ht^2) (Baumgartner et al., 1998), with cut-off points for sarcopenia 2 standard deviations below the young mean. While this appears reasonable and there is a good relationship between height squared and appendicular lean mass (Maden-Wilkinson et al. 2013) the problem is that people become smaller when they age and hence sarcopenia is underestimated with this

parameter. Another problem is the necessarily cross-sectional design of studies on sarcopenia, where ideally one would like to get an estimate of the loss of muscle mass of a given individual. Recently, we suggested a new parameter, the muscle volume to bone volume ratio, as determined with MRI to assess the age-related loss of muscle mass of a given individual, based on the observation that bone size is similar in young and older individuals (Maden-Wilkinson et al. 2013). The advantage of this approach is that it reduces bias related to the decrease in height and increases in body mass during ageing and secular changes in population over time. While this parameter may thus be useful to get insight into the (age-related) loss of muscle mass in a given individual it remains to be seen whether this parameter has any significance for the capacity to perform daily life activities.

The age-related loss of muscle force and power generating capacity is proportionally larger than the loss of muscle mass (Goodpaster et al. 2001; Rutherford & Jones 1992; Morse et al. 2005; Barbat-Artigas et al. 2013; Vandervoort & McComas 1986; Degens et al. 2009). Hence, isometric force is generally preferred above muscle size as a proxy for functional capacity (Clark & Manini, 2010; Goodpaster et al., 2006; Visser et al., 2005). In daily life isometric contractions are, however, rarely encountered and muscle force generating capacity as a predictor of physical function is not always ideal (Manini et al., 2013). Most contractions during daily life are shortening contractions during which the muscle generates power, given as Force x Velocity. It has been shown that the power generating capacity of the muscle decreases even more than force during ageing and is a strong predictor of physical performance (Barbat-Artigas et al. 2012; Reid & Fielding 2012; Bean et al. 2003).

We hypothesised that a lower muscle mass and function in healthy older people contributes to a reduced ability to perform daily life activities. To investigate this we correlated parameters of muscle mass and function with the distance covered in a 6-minute walk test (6MWD) and the time taken for the timed up and go (TUG) test, indicators of the ability to perform daily life

activities (Mathias 1986; Troosters et al. 1999; Enright & Sherrill 1998; Lipkin et al. 1986; Peeters & Mets 1996).

4.3 Methods

Participants and ethical approval

The study was approved by the local ethics committee and conformed to the Declaration of Helsinki. Written informed consent was obtained from each volunteer prior to participating in the study. Young participants (28 male, 21 female) were recruited from amongst the university student population and older participants (31 male, 35 female) from the local community; participant characteristics are presented in Table 4.1. All the subjects were healthy and participated in recreational physical activities but none were training to compete in athletic competitions. Older participants were all socially active and their General practitioner confirmed there was no medical reason not to participate.

Table 4.1 Participant Characteristics

	YM (28)	OM (31)	YF (21)	OF (35)	Sig. Dif.
Age	22.8 ± 3.3	72.2 ± 4.9	22.6 ± 2.0	72.1 ± 4.3	Y<O;
Height (m)	1.80 ± 0.06	1.74 ± 0.08	1.67 ± 0.05	1.59 ± 0.06	Y>O; M>F
Mass (kg)	72.1 ± 13.7	81.7 ± 15.9	62.2 ± 9.7	63.9 ± 11.5	M>F
BMI ($kg.m^{-2}$)	23.3 ± 4.0	27.0 ± 4.3	22.4 ± 3.6	25.2 ± 4.3	Y<O
FMI ($kg.m^{-2}$)	4.5 ± 2.4	8.0 ± 3.2	7.2 ± 2.9	9.2 ± 3.6	Y<O; M<F
Body Fat %	18. 2 ± 7.1	30.4 ± 7.5	32.6 ± 8.0	37.5 ± 8.7	Y<O; M<F
Resting HR (bpm)	60.1 ± 8.7	65.5 ± 9.1	69.3 ± 11.7	67.9 ± 7.7	M<F
aLM (kg)	27.0 ± 4.2	22.8 ± 3.5	16.9 ± 1.8	15.1 ± 2.1	Y>O; M>F
Quadriceps Volume (cm^3)	2237 ± 351	1522 ± 301	1374 ± 207	991 ± 180	Y>O; M>F
ACSA (cm^2)	85.6 ± 12.1	60.5 ± 10.2	55.4 ± 6.9	41.9 ± 7.0	Y>O; M>F

Table 4.1- Participant characteristics for Young Men (YM), Older Men (OM), Young Female (YF) and Older Female (OF). BMI= Body Mass Index, FMI= Fat Mass Index, aLM= Appendicular Lean Mass as defined by DXA, ACSA= Anatomical cross-sectional area. Data presented are mean ± standard deviation with significant differences defined as $p<0.05$.

Anthropometrics

Body mass was recorded on a digital scale (SECA, Switzerland) with participants in light indoor clothing. Standing Height was measured with a portable Stadiometer to the nearest 0.1 cm (SECA, Switzerland). Body mass index (BMI) was calculated by dividing the participant's body mass in kilograms by their height-squared in metres.

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Images were taken from 93 out of the 116 Participants. The volume of the quadriceps femoris muscle group was measured with a 0.2 T MRI scanner (G-Scan, Esaote, Genova, Italy) in the same leg as used for the measurements of maximal voluntary isometric torque. The participant was positioned supine in the scanner. A turbo 3D-T1-weighted protocol was used (matrix 256 x 256, TR 40 ms, TE 16 ms) and multiple 3.1-mm-thick serial transverse sections were obtained every 25 mm from the distal to the proximal heads of the femur. Computing imaging software (OsiriX medical imaging software, OsiriX, Atlanta, USA) was used to determine the total cross-sectional area of the four muscles of the quadriceps group as well as total bone cross-sectional areas in each MRI section as shown previously in Figure 3.1. Bone and muscle analyses were performed using manual trace functions by a single investigator (TMW).

Previously we have shown that the anatomical muscle cross-sectional area (ACSA) to femur cross-sectional area at 60% femur length gives essentially the same outcome as the muscle volume to bone volume ratio (Maden-Wilkinson et al., 2013) and therefore present here the muscle:bone ratio as the ACSA:femur cross-sectional area at 60% femur length For each individual a z-score was calculated as the number of SDs it deviated from the corresponding young control group. The relationship between the muscle:bone ratio at 60% Femur Length and muscle:femur volume was very strong, with an R^2=0.94.

Dual Energy X-ray Absorptiometry (DXA)

DXA was used to measure total body composition and bone mineral density (Lunar Prodigy Advance, GE Healthcare). Participants lay supine on the scanning bed with ankles strapped together to limit movement during the scan. Participants underwent a full body scan followed by a dual femur scan. Computing imaging software (Prodigy, Encore 2006 v 10.50.086, GE healthcare) was used to determine bone mineral content, bone mineral density and whole body composition. All DXA analyses were performed by the same investigator (TMW). It is important to understand that in estimating "lean mass" the typical DXA machine (including the Lunar Prodigy used in this study) includes not just muscle mass but connective tissue and components of bone that are not bone mineral. Bone mineral content accounts for approximately 55% of total bone mass with the rest being made up by protein and water (Heymsfield et al. 1990). Thus, a corrected lean mass was calculated as follows (Heymsfield et al. 1990):

Lean mass = total mass - fat mass - (1.82 * BMC)

DXA is also known to include non-adipose components of fat tissue, such as protein, in the lean mass but the contribution this makes is unclear. Appendicular lean mass was calculated using corrected lean mass estimates from DXA scans of the upper and lower limbs (Visser et al. 1999). The Upper limbs were defined as lean mass between the humeral head to phalange tips, avoiding contact with the ribs, pelvis or greater trochanter. The lower limb were defined from an angled line through the greater trochanter to the phalange tips (Figure 4.1) (Gallagher et al., 1997).

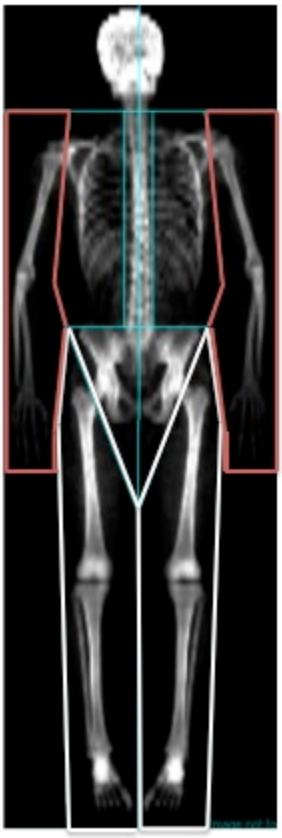

Figure 4.1 Representative image of DXA scan, with the region of interest in the upper and lower limbs highlighted

Muscle Power

To assess leg extension muscle power, a maximal-effort countermovement vertical jump was performed on a force platform (Leonardo, Novotec Medical, Pforzheim, Germany). The test was repeated three times with a rest interval of 60 sec. The maximum force (kN), maximum power of the concentric phase (Watts) and jump height (m) were measured using the vertical component of the ground reaction force (Caserotti *et al.*, 2001).

Isometric maximal voluntary contraction (MVC) torque

All knee extension torque measurements were performed on a custom-built isometric testing device (MMuscle, Amsterdam, NL). Participants were seated with the knee and hip joints at 90^{o} flexion with the hips strapped to minimise extraneous movements, and the knee joint set at 90^{0}, (full extension = 0^{o}), which was confirmed by goniometer (BIOPAC systems, Inc. USA). Before

determination of the maximal voluntary force (MVC) the participants performed a 30-s warm-up consisting of intermittent incremental sub-maximal isometric contractions. During the subsequent determination of MVC participants received visual feedback of the torque signal and strong verbal encouragement throughout the manoeuvre. The duration of each maximal voluntary contraction was 3 s. The participants performed 3 MVC with 30 s rest between each MVC to prevent the development of fatigue. The highest of the 3 contractions was presented as the MVC torque.

Grip Strength

Handgrip strength was measured to the nearest 0.1 kg using a JAMAR hand dynamometer (Sammons Preston, Inc., Bolingbrook, IL, USA). All participants were instructed to maintain an upright standing position, arms down by the side, and holding the dynamometer in the dominant hand without squeezing the arm against the body. The dynamometer was adjusted to the participants' hand size, with the 2nd phalanx resting against the inner handle. Participants performed three trials, with the highest value used for subsequent analysis.

Timed get up and Go (TUG)

Participants were instructed to get up from a standardised chair (44 cm high, no arm rests) and to walk forward as quickly as possible, without running, around a cone 3 m away and return to the sitting position on the chair. Participants began on the word 'go' and timing was concluded when seated again. Each participant performed the task three times with the quickest of the three trails being recorded; between tasks participants were at rest for a period of 1 minute.

Six-Minute Walk (6MW)

Participants were asked to walk a circuit between two cones 20 m apart as quickly as possible for a period of six minutes (Enright, 2003). Heart rate was recorded continuously during the test (Polar, USA). Readings taken during the

final 3 minutes of the test were averaged to provide steady state heart rate. Participants were instructed to “complete as many circuits as possible without running” and were given verbal encouragement after each minute of the task. The total distance walked in the six-minute period was recorded. The use of a walking aid was permitted however none of the participants required this. All Participants completed the six-minute walking period. Maximal heart rate was estimated as below (Tanaka et al. 2001):

$$\text{Maximal Heart Rate} = 208 - (0.7 \times \text{Age})$$

Statistics

Data were analysed using SPSS v18 (IBM, 2011). A univariate two-way ANOVA with as between factors Age and Sex was used to examine differences between groups. Significant interactions indicate that the effects of age differed between men and women. Pearson’s product moment correlation was used to determine the relationships between variables Data were expressed as mean ± standard deviation unless stated otherwise. Differences were considered significant at $p < 0.05$.

4.4 Results

Anthropometrics

Participant characteristics are shown in Table 4.1. Men were taller and heavier than women (both $p<0.005$) and the young were taller than the older participants ($p<0.005$). Body mass did not differ between young and old ($p=0.09$), but the BMI was larger in the old than the young ($p<0.05$).

Dual energy x-ray absorptiometry (DXA*)*

DXA was used to determine total and regional body composition (Table 4.1). The % body fat was larger in women than men and in the old than the young

(p<0.05). The same applied to the fat mass index (p<0.05). Appendicular lean mass was determined as the sum of the corrected lean mass for the upper and lower limbs. It was significantly greater in men than women (p<0.005) and in young than older participants (p<0.005). The age-related difference in appendicular lean mass was larger in men than women (age x gender interaction; p=0.045; Table 4.1). The lower limb lean mass and thigh lean mass were greater in men than women and in the young than old participants (all p<0.005; data not shown). In the thigh region the age-related difference was larger in men than women (age x gender interaction; p=0.005).

Normalising Lean Mass

Table 4.2 shows the normalised appendicular lean mass to height^2 (SMMI) and total lean mass divided by height^2. Young had a greater SMMI and total lean mass divided by height^2 than older participants (p<0.005). Men had higher values than women (p<0.005).

Magnetic Resonance Imaging (MRI)

The muscle:bone ratio provides an alternative way to normalise for an individual's stature and use of a Z-Score shows the loss of muscle mass in comparison to the young mean (T. M. Maden-Wilkinson et al. 2013). As shown previously the muscle:bone ratio is significantly greater in young c.f. older participants and in men than women (both p<0.005), with the age-related difference in absolute terms being larger in men than in women (age x gender interaction, p= 0.02). The Z-score of the muscle:bone ratio was lower in older men than women (older Men: -3.38 ± 1.09, older Women: -2.75 ± 1.25, p<0.005).

Young had significantly greater muscle:bone ratio than older participants (p<0.005) but there was no significant difference between men and women (p=0.38, Interaction p=0.242) (Table 4.2).

Table 4.2: Normalising for Lean Mass using Height, Fat Mass, muscle to bone ratio

	YM	OM	YF	OF	Sig. Dif.
SMMI ($kg.m^{-2}$)	8.4 ± 1.14	7.5 ± 0.8	6.2 ± 0.7	6.0 ± 0.1	Y>O; M>F
Total lean mass/ht^2	18.4 ± 2.0	18.1 ± 1.8	14.4 ± 1.1	14.6 ± 1.2	M>F
aLM/Fat Mass	2.5 ± 1.1	1.2 ± 0.6	1.0 ± 0.5	0.8 ± 0.4	Y>O; M>F
M:B Ratio	13.5 ± 1.3	9.0 ± 1.5	11.4 ± 1.3	8.2 ± 1.4	Y>O; M>F
KE MVC Torque (Nm)	282.0 ± 54.1	170.4 ± 55.2	182.8 ± 35.0	108.4 ± 24.4	Y>O; M>F
KE Torque/ ACSA ($Nm.cm^{-2}$)	3.2 ± 0.4	2.7 ± 0.3	3.3 ± 0.4	2.6 ± 0.4	Y>O
Grip Strength (kg)	50.4 ± 10.8	38.7 ± 7.6	34.5 ± 5.4	25.2 ± 4.5	Y>O; M>F
Power per body mass (w.kg-1)	48.7 ± 4.8	28.2 ± 4.7	36.5 ± 5.7	22.2 ± 4.9	Y>O; M>F
Power per Quad Vol ($w.kg^{-1}.cm^{-3}$)	0.022 ± 0.004	0.019 ± 0.004	0.027 ± 0.005	0.024 ± 0.005	Y>O; M<F
Timed up and go (s)	3.9 ± 0.4	5.2 ± 0.7	4.2 ± 0.3	5.8 ± 1.0	Y>O; M>F
6 MWD (m)	725 ± 54	564 ± 68	668 ± 47	528 ± 77	Y>O; M>F
6MW steady state HR (% Max)	68.9 ± 11.4	71.6 ± 10.1	75.6 ± 11.9	78.0 ± 11.1	M<F

Table 4.2- The loss of muscle mass, strength and function in older adults. SMMI= skeletal muscle mass index as calculated by appendicular lean mass/height^2, M:B ratio= muscle to bone ratio at 60% femur length. KE MVC Torque= Knee extensor maximal voluntary contraction torque. Data are expressed as mean ± standard deviations; significant differences are defined as $p<0.05$

Counter movement jump (CMJ)

Young participants generated more power per kg body mass during a counter movement jump than older participants (Table 4.2, $p<0.001$) and men were more powerful than women ($p<0.0005$). The age x gender interaction ($p=0.002$) is reflected by a larger difference in power between young and old in men than in women, but when expressed as a percentage of the young mean the changes were similar in men and women; older men 57.9% of young men, and older women 60.9% of young women.

Strength

Men had a higher grip strength and knee extensor MVC than women (Table 4.2; $p<0.0005$). In both men and women the MVC was approximately 60% and grip strength 75% of that observed in the young participants (all $p<0.0005$) (Table 4.2).

The grip strength per upper limb lean mass was lower in older than younger people ($p<0.0005$). In Figure 4.2A it can be seen that the MVC torque correlated well with ACSA of the Quadriceps muscle group and that the regression line of the older was parallel to, but below that of the young group. The same applied to the jumping power per quadriceps muscle volume (Figure 4.2B). The absence of significant age x gender interactions indicates that these age-related differences were similar in men and women.

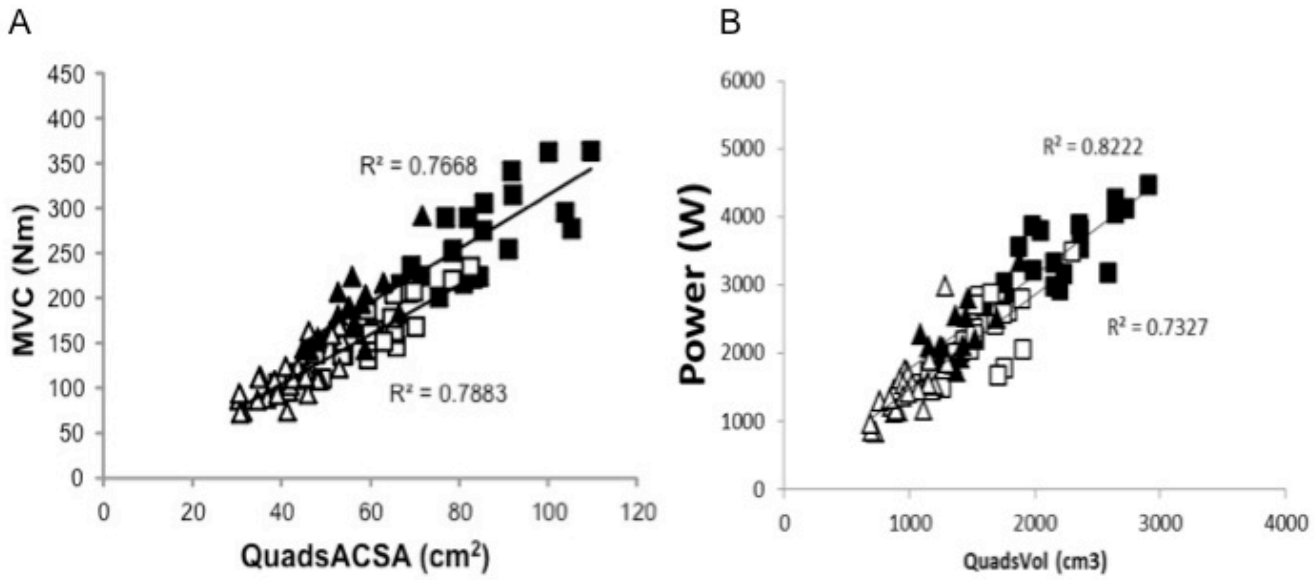

Figure 4.2- The relationship between **A,** Quadriceps anatomical cross-sectional area (QuadACSA) taken at 60% Femur Length and maximal voluntary isometric torque (MVC) and **B,** quadriceps volume (QuadVol) with jumping power in young men (filled Squares), Young women (filled triangles), older men (open Squares) and older women (open triangles).

Timed up-and-go test (TUG) and six-minute walking distance (6MWD)

The TUG and 6MWD are indicators of the ability to perform daily life activities. In table 4.2 it can be seen that men performed the TUG in a shorter time than women and that both older men and women took more time to complete the test than their younger counterparts ($p<0.05$). The 6MWD was larger in men than women and smaller in the older than the younger men and women (Table 4.2; $p<0.05$). While the resting heart was similar in all groups the younger participants performed the 6MWD at a higher absolute heart rate than the older people (Table 4.2; $p<0.05$). The result is that young and older people performed the 6MWD at a similar proportion of their maximal heart rate (Table 4.2) or heart rate reserve; (HR during test-resting HR)/(predicted maximal HR – resting HR) (data not shown). It is somewhat surprising that

women performed the test at a higher proportion of their maximal HR than men ($p<0.05$; Table 4.2)

Relationship between measures of Muscle Mass and function with Mobility

Table 4.3 shows the correlation coefficients and their statistical significance between several measures of muscle mass and function with the 6MWD and TUG. It can be seen that in almost all cases the correlation with the 6MWD and TUG is significant when the data of all groups are pooled. This gives the impression that indeed muscle mass and function have a significant impact on the performance in these tests. What we are particularly interested in is whether the performance in the older individuals is in any way related to measures of muscle mass and function. Under the assumption that the ability of a unit of muscle mass to generate force and power is similar in men and women we have also calculated the correlation coefficients for the pooled men and women in each age group (Table 4.3). It can be seen in figure 1A and B that there is no significant correlation between the TUG and 6MWD with the often-used SMMI in the old, yet there are significant correlations for the young group. The same applies to the muscle:bone ratio (Table 4.3). The larger body mass of the older person may place a higher demand on the available muscle mass and hence have an impact on the performance in TUG and 6MWD. Yet, when the muscle:bone ratio was corrected for body mass none of the correlations with 6MWD and TUG were significant (Fig. 4.2 A & B).

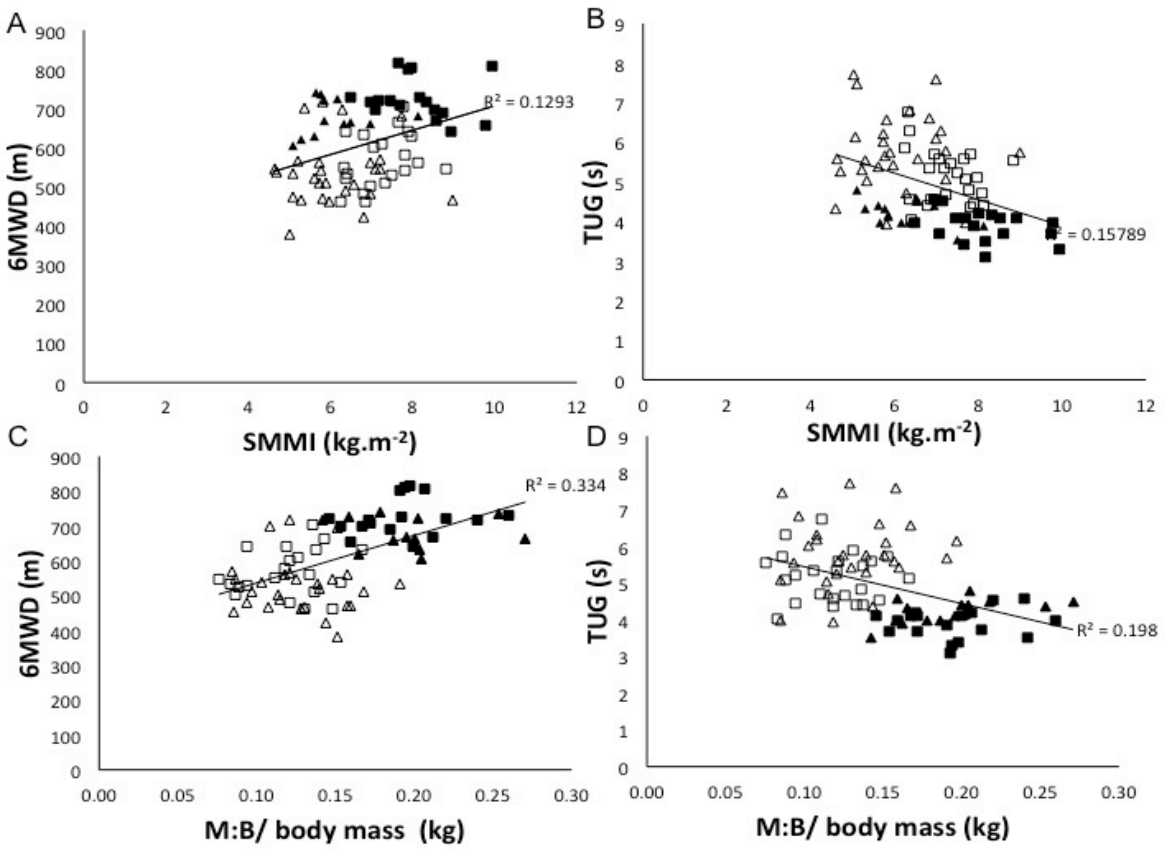

Figure 4.3- The relationship between skeletal muscle mass index (SMMI) (alm/ht^2) and **A, 6**-minute walk distance (6MWD) and **B,** timed get up and go (TUG), and between muscle to bone ration normalized to body mass (M:B/BM) and **C,** 6MWD and **D,** TUG in Young Men (Filled Squares), Old Men (Open Squares), Young Women (Filled Triangles) and Old Women (Open Triangles). Correlations are given in Table 4.3.

It was rather surprising that grip strength, which plays no obvious role in locomotion, correlated significantly with the 6MWD and TUG in both the young and old group (Table 4.3). As was found in other studies the MVC torque correlated significantly with the 6MWD and TUG within the group as a whole and in the young, but not the old group (Table 4.3). The best relationship between a muscle function measure and mobility was observed with muscle power per body mass as determined by counter movement jump (Table 4.3). The correlations with the TUG (Fig. 4.2A) and the 6MWD (Fig.

4.2B) were stronger in the old than in the young, and were even significant within the old men and women separately (Table 4.3)

Table 4.3 Partial Correlations between variables and function

Measure	Function	Overall	Young	Old	YM	OM	YF	OF
SMMI	6min	0.13	0.20*	0.05	0.02	0.11	0.07	0.002
	TUG	0.16*	0.34*	0.05	0.13§	0.05	0.23	0.00
M:B	6min	0.43§	0.13§	0.00	0.01	0.00	0.01	0.02
	TUG	0.42*	0.16§	0.03	0.14	0.00	0.00	0.02
M:B/Wt	6min	0.33*	0.02	0.00	0.03	0.04	0.01	0.01
	TUG	0.19*	0.06	0.00	0.01	0.01	0.01	0.02
M:B Z Score	6min	0.32§	0.01	0.01	0.01	0.02	0.02	0.09
	TUG	0.32*	0.02	0.00	0.14	0.001	0.001	0.03
MVC Torque	6min	0.43*	0.21*	0.06	0.01	0.003	0.00	0.16
	TUG	0.44*	0.30*	0.03	0.07	0.02	0.17	0.1
MVC/Wt	6min	0.44*	0.12§	0.12*	0.08	0.05	0.08	0.27
	TUG	0.43*	0.07	0.04	0.00	0.06	0.06	0.00
Grip strength	6min	0.36*	0.31*	0.12*	0.15	0.01	0.08	0.30
	TUG	0.34*	0.37*	0.16*	0.11	0.03	0.54	0.15
Power	6min	0.64*	0.27*	0.38*	0.01	0.23*	0.04	0.38§
	TUG	0.56*	0.19*	0.30*	0.01	0.13	0.51§	0.37*
HR rest	6min	0.04	0.19§	0.00	0.02	0.05	0.00	0.04
	TUG	0.05§	0.11	0.03	0.05	0.00	0.00	0.09
HR ss6m	6min	0.00	0.15§	0.02	0.44*	0.00	0.27§	0.08
	TUG	0.02	0.08	0.05	0.09	0.15§	0.34§	0.00

Table 4.3- Overall and inter-group correlations (values shown are r^2) between measures of muscle mass and function in Young Male (YM), Older Male (OM), Young Female (YF) and Older female (OF). Overall values are reprehensive of whole cohort correlations. § signifies significant correlation using two-tailed distribution test at $p<0.05$; * signifies significant correlation at $p<0.01$.

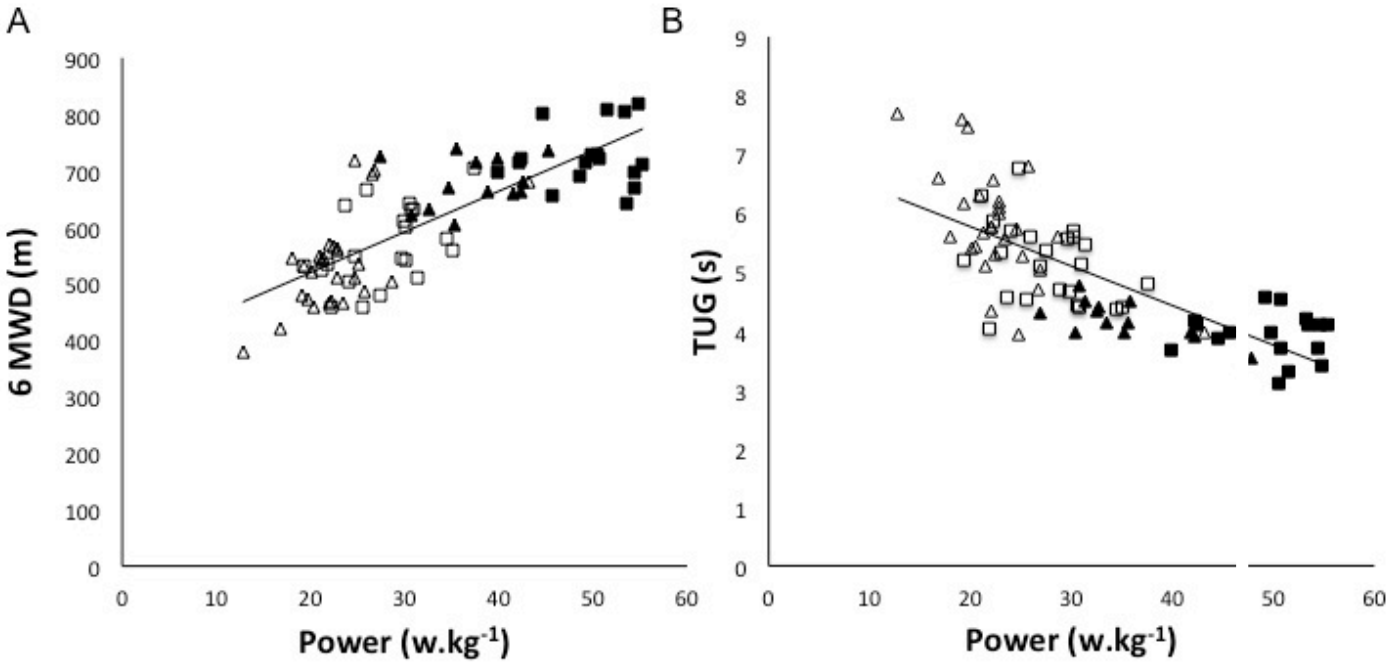

Figure 4.4- The relationships between muscle power normalised to body mass as determined with a countermovement jump and **A,** 6-minute walk distance (6MWD) and **B,** timed up and go (TUG) in Young Men (filled squares), Old Men (open squares), Young Women (filled triangles) and Old women (open triangles). Correlations are given in Table 4.3.

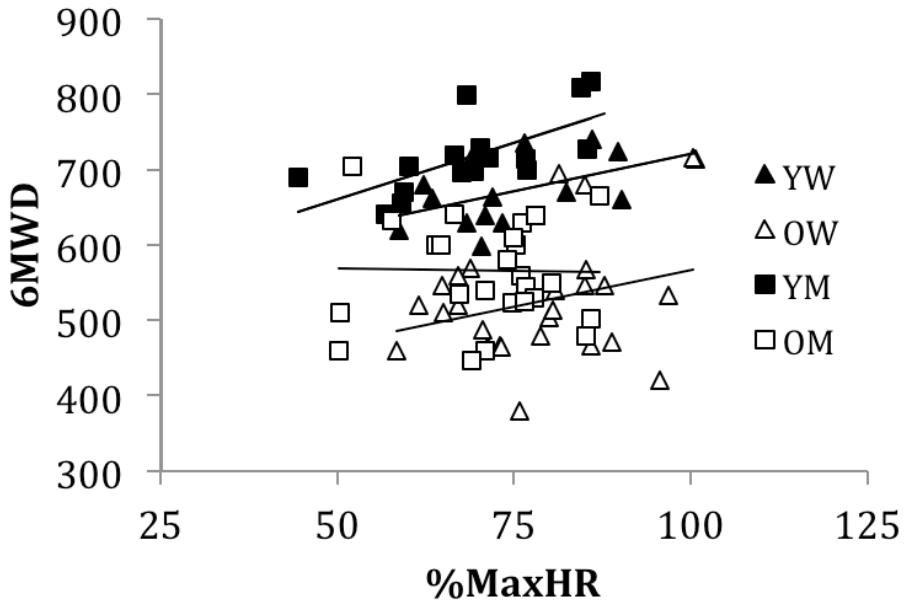

Figure 4.5- The relationship between Heart Rate (HR) expressed as % estimated maximum and 6-minute walk distance (6MWD) in Young Men (r^2=0.45), Old Men (r^2=0.00), Young Women (r^2=0.27) and Old Women (r^2=0.07). Correlations are given in Table 4.3.

4.5 Discussion

The aim of the present study was to assess whether the loss of muscle mass and function do contribute to the slowing and reduced mobility in healthy ageing. We did observe that even in healthy ageing the performance of the 6-minute walk test and the timed up-and-go test is less than in young people. Indices of muscle mass did not correlate with the 6MWD and the TUG in the older people. In old people, however, the correlation between muscle (jumping) power and the 6MWD and TUG was stronger than in young people. This may be a reflection of the reduced power generating capacity of the

remaining muscle tissue and/or an inability to fully recruit muscle mass during the countermovement jump in the older people. In young people the 6MWD correlated more strongly than muscle power with the HR during the test. Since maximal heart rate decreases with age and young and old and men and women performed the 6MWD at the same percentage maximal heart rate it is tempting to speculate that the velocity of walking and TUG is limited by a 'comfortable' stress on the cardiovascular system rather than muscle mass or function. Again, it was not this simple, as there was no correlation between HR and the 6MWD in older people and older people with a similar heart rate as the young still had a lower 6MWD (Fig. 4.5). Overall it thus appears that in young people the performance is primarily determined by the HR and to a lesser extent the power generating capacity of the muscles, while in older people the determinant of performance in these tests is shifting to a greater importance of muscle power.

The six-minute walk test provides a functional task similar to that of daily living and has previously been validated for use in both healthy (Trooster et al., 1999; Enright et al., 1998) and clinical populations (Peeters and Wets, 1996). The 6 minute walking distance (6MWD) was smaller in the older than the young men and women, and comparable to that observed previously (Bautmans et al. 2004). Only 1 participant walked less than 400 m, which is considered the cut-off for functional limitation (Abellan Van Kan, Cedarbaum, & Cesari et al., 2011).

An increased duration of the timed up and go (TUG) test has been associated with a decline in physical function and increases in the risk of falling and frailty index (Viccaro et al. 2011; Beauchet et al. 2011; Savva et al. 2013). Like the 6MWD the performance in the TUG test was less in the old than young men and women, but none of the participants took more than 12 s to perform the TUG task, a cut-off point indicative of poor physical function (Bischoff et al. 2003). Thus, while the performances in the 6-minute walk and TUG test was less in the old than young groups, the older adults in this cohort were free of significant physical impairment and can be considered a health ageing population.

The age-related loss of muscle mass will ultimately result in impaired mobility (Rantanen et al., 1996) It is therefore surprising that the reported relationships between muscle mass and functional limitations are rather weak (Lauretani et al. 2003). Most of the studies assessing correlations between muscle mass or function and the capacity to perform daily life activities used measures of appendicular lean mass and total lean mass obtained with DXA. DXA, however, underestimates the age-related loss of muscle mass in comparison to the gold standard MRI/CT (Delmonico et al. 2008; Maden-Wilkinson et al. 2013) and the poor relationship between muscle mass and functional limitations in old age may be at least partly attributable to this underestimation of age-related loss of muscle mass.

Another factor that may contribute to a weak relationship between muscle mass and functional capacity is the difference in stature between individuals. In the majority of epidemiological studies this is taken into account by normalising muscle mass to height squared (Baumgartner et al., 1998). There is the concern, however, that height decreases with age (Rolland et al. 2008; Proctor et al. 1999), and to get a better reflection of the age-related differences in muscle mass we also normalised the thigh muscle cross-sectional area to femur cross-sectional area, which does not change significantly with age (Maden-Wilkinson et al., 2013 AGE), but even the M:B ratio in the older group did not correlate with the 6MWD and TUG. While in theory correlations might be obscured by differences in body mass any correlation that did exist vanished when correcting for body mass. .

In the end force and power are required for locomotion and muscle force and power may thus be more relevant than just muscle mass for daily life activities. They do in fact correlate more strongly than muscle mass with functional measures such as gait speed and the short physical performance battery, and fall and fracture risks in old age (Hairi et al. 2010; Clark & Manini 2008; Maki et al. 2001; Carty et al. 2012; Lang et al. 2009; Visser et al. 2005). Similar to previous studies (Larsson et al., 1979; Murray et al., 1980, 1985; Young, Stokes, & Crowe, 1984, 1985) we observed that the isometric

quadriceps MVC and muscle power per unit body mass ($w.kg^{-1}$), as assessed by counter-movement jump, were 40% lower in old than young men and women.

In the present cohort muscle power per unit body mass had the strongest relationship with physical performance (Bassey et al. 1992; Bean et al. 2003), and even more so in the old (R^2=0.38 & 0.30; 6MWD & TUG) than the young (R^2=0.27 & 0.19) men and women (all $p<0.01$). The significance of muscle power for the performance of daily life activities is further emphasised by the significant correlations between power and 6MWD and TUG within individual groups. Since the main determinant of muscle force and power generating capacity is muscle mass it is surprising that the positive relationship between muscle power and maximal isometric force with performance is not also seen with muscle mass in the older population. The explanation for this apparent discrepancy is at least partly attributable to the age-related dissociation of the muscle mass with force and power, where the power and force in old age are much less than expected from muscle mass (Figure 1) (Degens et al. 2009; Barbat-Artigas et al. 2013). Further support for this notion comes from the observation that in young, but not in old people, also the SMMI correlated positively with performance,

Our primary focus was the contribution of muscle mass and function to performance of the 6MWD and TUG but we also measured HR during the 6MWD. Both young and old people did the test at a similar proportion of their predicted maximal heart rate and the smaller distance covered by the older people may thus be related to their lower heart rate. That this might be the case is reflected by the observation that in young people we found a positive relationship between HR and 6MWD. Yet, in older people no such relationship was found and even at a similar HR as younger people they covered a shorter distance (Fig 4.5). It thus appears that even though older people walk at a similar proportion of the maximal HR as the young people, the age-related reduction in maximal heart rate does not limit their performance in this test. Stepwise linear regression confirmed this and showed that both HR and power are related to the 6MWD in young people, but only power and not HR

correlated with the 6MWD in old people. Previously we have shown that the maximal oxygen uptake in young women is limited by the cardiovascular system and not by the maximal oxygen uptake of the legs (McPhee et al., 2009). Although speculative it is tempting to speculate that the shift from correlation of the 6MWD with HR in the young to a correlation with muscle power only in the old indicates a transition from cardiovascular to muscular limitations of exercise capacity.

In conclusion, it appears that in young people the performance is primarily determined by the HR and to a lesser extent the power generating capacity of the muscles, while in older people the determinant of performance in these tests is shifting to a greater importance of muscle power

Chapter 5

Knee extensor fatigue resistance of young and older men and women performing sustained and brief intermittent isometric contractions

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In Chapter 4, the strongest association with functional performance was with muscle power generated during a counter movement jump. The reduction in muscle power with age is often attributed to the slowing of muscle contractile properties. Along with the loss of muscle power, a common complaint amongst older adults is an increase in fatigue and tiredness. Therefore, in Chapter 5 the muscle contractile properties of the cohort and how they relate to muscle fatigability in young and old are studied.

5.1 Abstract

Skeletal muscle fatigue is determined by rates of energy turnover and replenishment by aerobic or anaerobic pathways, which in turn are influenced by muscle fibre characteristics and contraction and relaxation time intervals. Two fatiguing protocols that differently stress contraction and recovery times to were used to examine age and sex differences in fatigue resistance. Young and older (mean age 22 and 70 yr., respectively) men and women (*n* = 37) completed assessments of contractile properties and two fatigue tests of the knee extensor muscles. A combination of voluntary and electrically stimulated contractions was used to distinguish central and peripheral aspects of fatigue. It was found that young men had the fasted contractile properties, followed by young women; older men and older women were slowest. Older adults were able to hold the sustained contraction for around 20 s longer than young ($P<0.0005$). Fatigue was almost entirely due to peripheral muscle fatigue in all groups. However, during intermittent contractions when the activation was reduced to that more typical of usual activities, the older subjects no longer showed superior fatigue resistance, but women fatigued less than men ($P=0.001$). In both tests, superior fatigue resistance was associated with slower contractile properties. It is concluded that older muscles are more fatigue resistant when performing sustained isometric contractions, but not during brief, intermittent contractions. Women fatigue less than men during intermittent contractions and this was the case for young and old.

5.2 Introduction

Older people have been shown to have greater fatigue resistance during sustained voluntary or electrically stimulated isometric contractions of upper limb or lower leg muscles (Bilodeau & Henderson 2001; Bilodeau et al. 2001; Hunter et al. 2004; Narici et al. 1991; Griffith et al. 2010). During sustained contractions where the intensity is greater than around 40% maximal isometric strength the blood supply to the active muscle becomes occluded by the pressure generated within the muscle (DeRuiter et al. 2007) and this can restrict the oxidative energetic recovery. The rate of fatigue in this instance is mainly due to the rate of energy utilisation. For oxidative recovery to occur there needs to be a period of relaxation between contractions. Typical patterns of muscle activation usually involve intermittent contraction/relaxation cycles, allowing blood to perfuse the muscle during the relaxation phase. Thus, the rate of fatigue that occurs following intermittent muscle activation reflects the balance between energy expenditure during contraction and regeneration during relaxation. Some studies have reported less-fatigable muscles in older compared with young adults when performing intermittent contractions (Lanza et al. 2004; Kent-Braun et al. 2002; Chung et al. 2007; Rubinstein & Kamen 2005; Ditor & Hicks 2000). However, all of these studies utilized relatively long contraction durations (~5 – 30-s contraction with similar rest interval) and these studies were carried out in peripheral muscles acting on the ankle joint or hand.

Studies of the quadriceps femoris have provided conflicting reports that might be explained by methodological differences in contraction/relaxation protocols, the use of voluntary or electrically stimulated contractions and possible differences between the sexes. Callahan et al (2009) found that older men and women were more fatigue resistant when using repeated intermittent, voluntary 5-s contraction / 5-s recovery cycles. However, in other studies that utilized a recovery time between contractions of ≤ 2-s, no difference was seen in fatigue profiles of young and older men (Stackhouse et al. 2001; Allman & Rice 2004; Stevens et al. 2001). It is unclear whether age-related differences in fatigue would be seen if the contraction and relaxation

duration were both reduced to ≤ 2-s. It also remains to be determined whether sex differences similar to those seen in young individuals following intermittent fatiguing contractions (Wüst et al. 2008) are also present in old, or whether sex differences diminish, as was the case for sustained contractions of the arm flexors (Hunter et al. 2004).

Quadriceps muscle fatigue following intermittent activation has been studied using voluntary (Callahan et al. 2009; Stackhouse et al. 2001) as well as electrically-stimulated activation (Allman & Rice 2004; Stevens et al. 2001; Wüst et al. 2008). Electrical stimulation protocols have the advantage that they by-pass possible bias related to the central activation of muscle, such as the possibility that older people show greater decline in central activation (Stackhouse et al. 2001; Mademli & Arampatzis 2008) or a different response of the motor unit firing rate during fatiguing contractions (Rubinstein & Kamen 2005). They therefore provide a greater focus on factors at, or after, the neuromuscular junction and in the muscle itself as causes of fatigue. Stimulated contractions have also been used to study age-related differences in fatigue following prolonged, sustained contractions of small peripheral muscles (Narici et al. 1991). Sustained stimulated contraction of the quadriceps can be very unpleasant and cause muscle soreness lasting several days (unpublished observations). Therefore, to distinguish the effects of voluntary activation failure or motivation from peripheral muscle fatigue during a sustained contraction of quadriceps, brief electrically stimulated-contractions can be applied on to the sustained voluntary activation (Rutherford et al. 1986).

In light of the questions raised here, the present study was undertaken with the objective to examine age and sex differences in fatigue resistance following two different types of contractile activity, one a sustained voluntary isometric contraction to task failure and the other an intermittent series of brief electically stimulated contractions.

5.3 Methods

Participants and ethical approval

The study conformed to the latest revisions of the Declaration of Helsinki and received approval from the ethics committee of the Faculty of Science and Engineering at Manchester Metropolitan University. Data were collected from 10 young men (YM), 8 young women (YF), 9 older men (OM) and 10 older women (OF), who were participating in the MYOAGE Study (McPhee et al. 2013). All participants provided written informed consent after the procedures had been fully explained. Young participants were recruited from amongst the university student population and older participants were recruited from the local community. Participant characteristics are shown in Table 5.1. All were healthy and recreationally active, but none were engaged in sporting competitions. Participants were excluded if they had a history of neuromuscular or skeletal disease or injury, cardiovascular, metabolic or other major disease, and if they were suffering pain or injury that would prevent them from performing maximal effort contractions of the leg muscles. Older participants received clearance from their medical doctor to take part in the study.

Table 5.1 Participant Characteristics

Variable	YM (10)	OM (9)	YF (8)	OF (10)
Age (years)	22.4 ± 3.1	68.9 ± 4.4	22.3 ± 1.9	71.1 ± 3.4
Height (m)	1.81 ± 0.07	1.74 ± 0.07	1.69 ± 0.03	1.58 ± 0.06
Body Mass (kg)	78.7 ± 9.3	73.2 ± 5.0	61.9 ± 3.9	59.3 ± 10.6
BMI ($kg.m^{-2}$)	24.0 ± 3.3	24.1 ± 1.9	21.6 ± 1.5	23.9 ± 4.4

Body Mass Index (BMI); Young Men (YM); Older Men (OM); Young Women (YF) and Older Women (OF); Significant differences between young and old, men and women; $p<0.05$.

Overview of study procedures

Participants completed two fatigue protocols, one on each leg. All assessments were of the knee extensor muscles using a custom built isometric testing dynamometer with torque signals recorded via a computer interface running customised Labview (National Instruments Corporation, Texas, USA) and Matlab software (Matlab, the Mathwork Inc., S Natik, MA, USA). The leg and the fatigue protocol to be used first were chosen at random. On the first leg, participants completed assessments of maximal voluntary isometric contraction torque (MVC) with assessment of the voluntary activation capacity using the interpolated twitch technique (Rutherford et al. 1986). After a short rest of around 2 min, contractile properties were assessed using a series of electrically stimulated contractions (Wüst et al. 2008). After another short rest period, the first fatigue test was performed. Participants were then allowed to rest for around 6 min while the testing equipment was set up to test the second leg. On the second leg, MVC was assessed and after a short rest of around 2 min the second fatigue test was completed. None of the participants reported pain (e.g. in the knee) that interfered with the ability to perform contractions and the protocols were well tolerated.

Isometric maximal voluntary contraction torque

Knee extensor MVC was assessed at a knee angle of 90° (where full extension is 0°) and a back support at 85° (where lying supine is 0°). A strap was firmly secured across the hip joint and the lower leg was firmly secured to the force transducer 2 cm above the ankle malleolus. Participants were familiarised to knee extension exercise by performing three contractions at around 50% of maximal effort, each lasting 3 s and another two contractions at around 80% maximal effort. After 2 min rest, participants were instructed to perform a maximal effort by increasing force rapidly and sustaining the maximal effort for around 3 s. This maximal effort was repeated twice more or until the highest two values were within 10%, the highest value being taken as MVC. Visual feedback was available throughout and strong verbal encouragement was provided.

Voluntary Activation

The capacity to activate the knee extensor muscles during isometric contractions was assessed using the interpolated twitch technique (Rutherford et al. 1986; VanLeeuwen et al. 2012). The procedure was fully explained and the participants were slowly accustomed to the electrical stimulation. Muscle stimulation electrodes (AmericanImex, CA, USA) were placed over the proximal (anode) and distal (cathode) heads of the quadriceps femoris muscles. The electrodes were connected to a Digitimer DS7AH (Digitimer Ltd, Herts, UK) via anode and cathode leads. Voltage was set at 400 V and pulse width at 200 μs. A "doublet" stimulus (two pulses separated by 10 ms) inducing ≥ 30% MVC was applied to the relaxed muscle. Approximately 1-s later the participant performed a maximal voluntary effort and a doublet was applied again at the highest point of the MVC. This test was performed twice and the percentage voluntary activation was calculated as:

$$\textbf{Voluntary activation} = 100 * (1 - t/T)$$

Where t is the amplitude of the superimposed doublet (i.e. the size of the additional peak) and T the value of the resting doublet.

Torque-Frequency relationship and rates of contraction and relaxation

The torque-frequency relationship was determined using pre-set pulse width, voltage and current (200 μs, 400 V and current, which differed between participants, that elicited 25% MVC at 30 Hz). This stimulation intensity was chosen as higher intensities were not tolerated well, especially not by older women, as also seen in previous studies (Allman & Rice 2002; Allman & Rice 2004). The muscle was stimulated at seven different frequencies (1, 10, 15, 20, 30, 50 and 100 Hz) in random order, separated by 60-s, and with the tetani lasting 2-s. The maximal torque output at each frequency was recorded and expressed as a percentage of torque elicited at 100 Hz (Figure 5.2). Relaxation rate (delta force/delta time), normalised relaxation rate (relaxation

rate/peak force) and half relaxation time of the 30 Hz contraction were also calculated (Wüst et al. 2008).

Sustained Fatigue test

The procedure for the sustained isometric contraction fatigue test was fully explained to the participants. A computer interface was set to show the target force at 50% MVC and the participant was allowed to practice holding the torque at the target line for 3 – 5 s. After a 2 min rest the participant was asked to perform a voluntary contraction at 50% MVC for as long as possible. The end point was taken as the time at which the torque fell below 45% MVC for more than 3-s despite strong verbal encouragement to regain the required 50% target. The end point of this fatigue test may be determined by central or peripheral muscle fatigue. Thus, to assess the extent of central activation stimulated doublets (30% MVC) were superimposed at 10-s intervals throughout the test in a subgroup of 13 young and 16 older participants (similar split for men and women). The superimposed stimuli were normalised to a doublet applied to the resting muscle prior to commencement of the test, as described for the calculation of voluntary activation, above. Figure 5.1 shows an example of raw data in a young and older man.

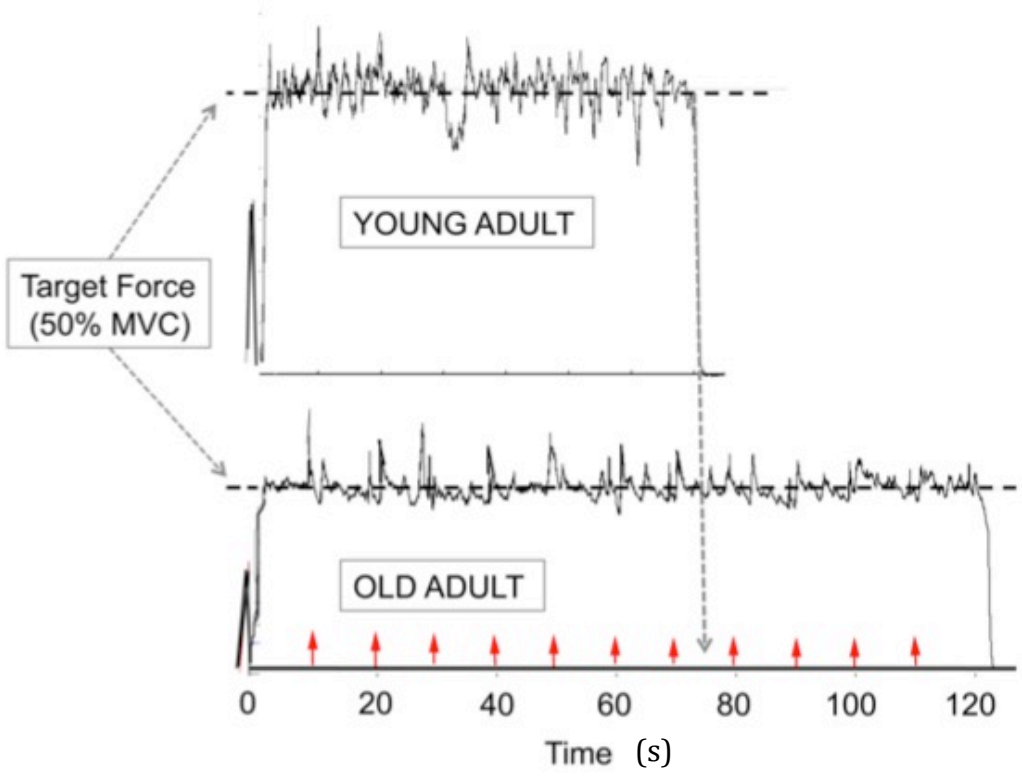

Figure 5.1: Example raw data from a sustained fatigue test in a young and older man. The test began with a stimulated doublet to approximately 30% MVC and immediately after, the voluntary contraction began. Electrically-stimulated doublets were superimposed every 10-s throughout the test, indicated by the arrows. The twitch-interpolation technique was used to assess the level of voluntary activation following each stimulus.

Electrically stimulated intermittent contraction fatigue test

The procedures of this test were fully explained to the participant, emphasising the need to remain relaxed throughout the test, avoiding any voluntary contractions of muscles acting around the knee. The test was carried out after setting pulse width, voltage and current at: 200 μs, 400 V and current that elicited around 25% MVC at 30 Hz, respectively. A total of 60 stimuli of 1-s duration were applied, each separated by 1-s rest interval over the course of two minutes. Participants received visual and verbal feedback throughout the test that helped them stay relaxed. This intermittent, stimulated protocol allowed relaxation and hence metabolic recovery, between contractions and bypassed the possible complications of motivation and differences in voluntary activation. The test was well tolerated and all participants remained relaxed (i.e. no additional voluntary contraction) and all completed the full 2 min protocol.

Statistics

Data were analysed using SPSS v18 (IBM, USA). A two-way ANOVA was used to assess sex and age effects and possible interactions between sex and age. An age x sex interaction would indicate that the effects of age differ between men and women. Pearson's product moment correlation coefficient

was used to determine the relationships between variables. Descriptive data in Table 5.1 are expressed as mean ± standard deviation (SD), while all other data relating to comparisons between groups are presented as mean ± standard error of the mean (SEM). Differences between groups were considered significant at $P<0.05$.

5.4 Results

The older participants were weaker than young and women weaker than men (Table 5.2; P<0.0005). The young group had a slightly higher voluntary activation than old during maximal isometric knee extension (P = 0.033; Table 5.2).

Table 5.2- Muscle contraction and activation characteristics

	YM (10)	OM (9)	YF (8)	OF (10)	Significant Differences
MVC (Nm)	286.7 ± 12.0	167.5 ± 10.8	175.2 ± 6.4	101.2 ± 6.2	Y>O, M>F
% Activation	89.2 ± 1.5	86.9 ± 1.4	92.9 ± 1.1	87.2 ± 2.9	Y>O
Normalised relaxation rate (s^{-1})	16.8 ± 0.9	12.9 ± 0.7	14.0 ± 0.8	12.1 ± 0.5	Y<O
½ Relaxation time (ms)	65.4 ± 3.6	77.0 ± 9.6	84.3 ± 4.6	96.1 ± 5.9	Y>O, M>F

Table 5.2 Mean (SEM) in young men (YM), young women (YF), older men (OM) and older women (OW). MVC: maximal voluntary contraction; % activation: percentage of muscle activated during MVC; Normalized relaxation rate and ½ relaxation time have been normalized to the peak torque. ½ relaxation time is the time taken to relax from the final pulse of a 30 Hz electrically-stimulated contraction to 50% peak torque. Data are mean (SEM).

Muscle contractile properties

Significant sex and age differences were found in rates of relaxation following a brief 30-Hz stimulus, with men having faster relaxation than women, and young faster than old (Table 5.2). The torque/frequency relationships (Figure 5.2) show that older participants produced relatively more force than young at 1 Hz (P = 0.017), 10 Hz (P = 0.005), 15 Hz (P = 0.007) and 20 Hz (P = 0.028). The torque/frequency relationships did not differ between men and women.

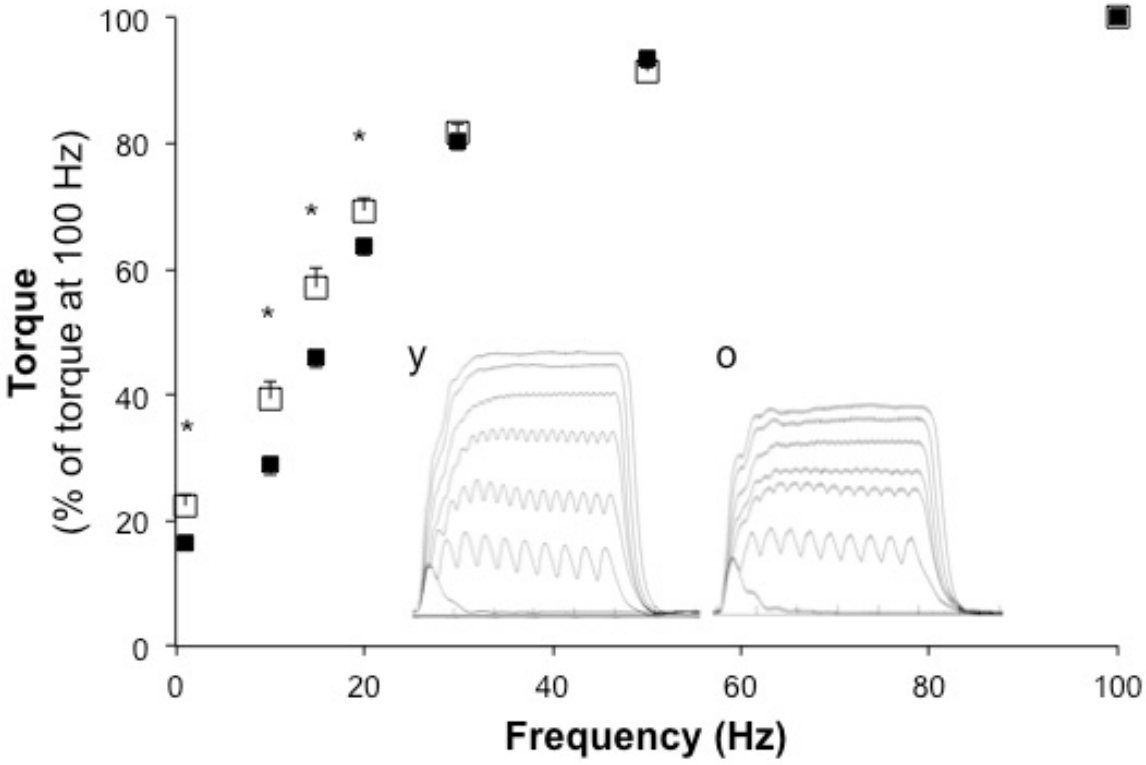

Figure 5.2 Torque-frequency relationship. Mean ± SEM are shown for young (filled squares) and older participants (open squares). Subplots show typical torque traces for a young (y) and older man (o) at each stimulation frequency. * indicates significant difference between age groups. No significant differences were seen between sexes.

Sustained Contraction Fatigue Test

Figure 5.3 shows individual data points for the time to task failure as a function of age in men and women. There was no significant difference between sexes (P=0.416), but the older participants were able to sustain the contraction on average for around 20-s longer than young (71.2 ± 5 vs 91.5 ± 5 seconds in young and old, respectively; P = 0.001).

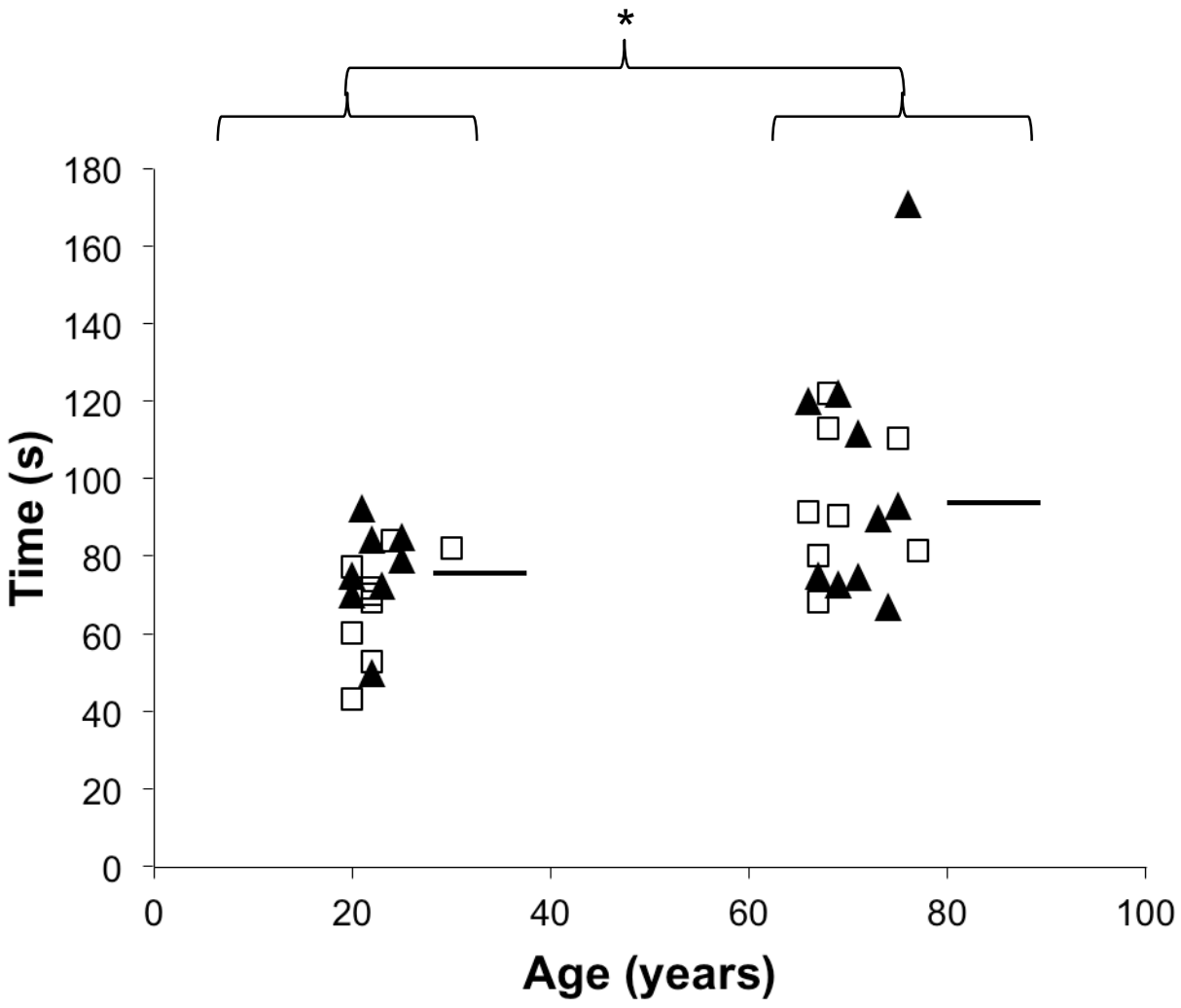

Figure 5.3 Individual data points for the sustained isometric contraction held at 50% MVC until task failure. The horizontal lines represent the mean time-to-task-failure in young and old. No significant difference was found between men (squares) and women (triangles), but old sustained the contraction for longer than young. *Indicates significant difference between young and old of P<0.0005.

Voluntary activation was assessed throughout the fatigue test by using the superimposed doublet stimulation and the results (Figure 5.4) show that at the start of the contraction the activation was close to 50%, as expected since the target was set at 50% MVC. There was then a steady increase in activation until at task failure the older participants were activating their muscles to approximately 84% and the young to 88% of their maximum, with no difference between ages (P = 0.181) or sexes (P = 0.908) at task failure.

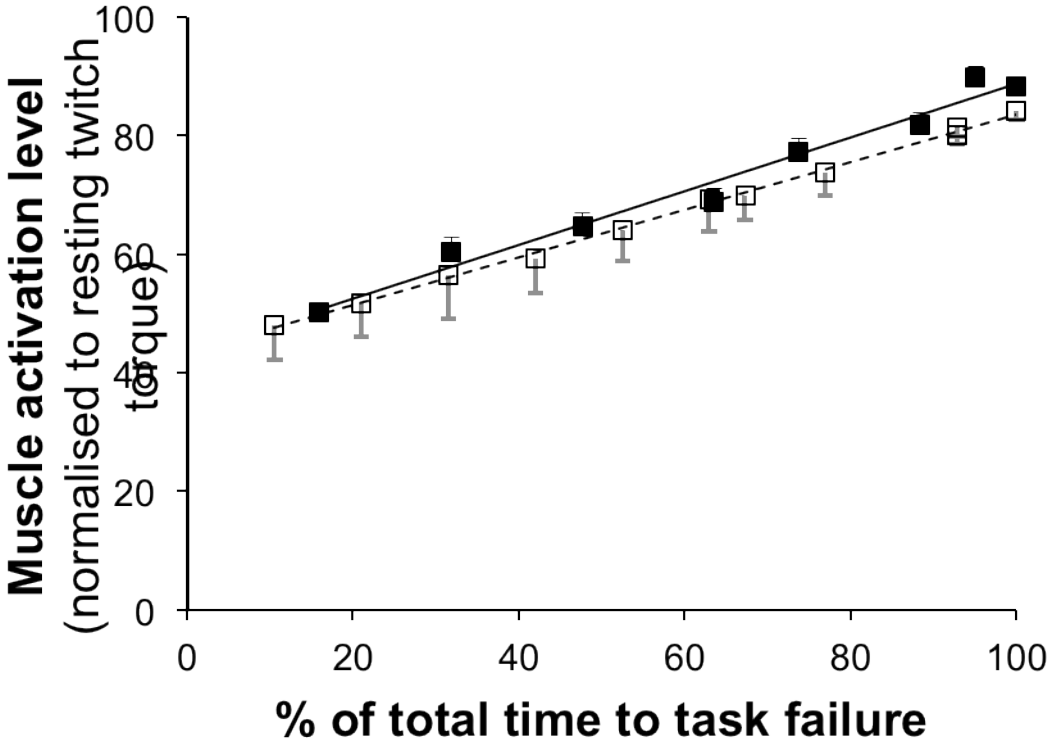

Figure 5.4 Progression of peripheral muscle voluntary activation during the sustained contraction fatigue test in young (filled squares, solid trend line) and older participants (open squares, dashed trend line).

When analysing the full dataset, time to task failure was significantly inversely related with MVC (r = -0.458; P = 0.005; Figure 5.5) and positively related to ½ relaxation time (r = 0.360, P = 0.034).

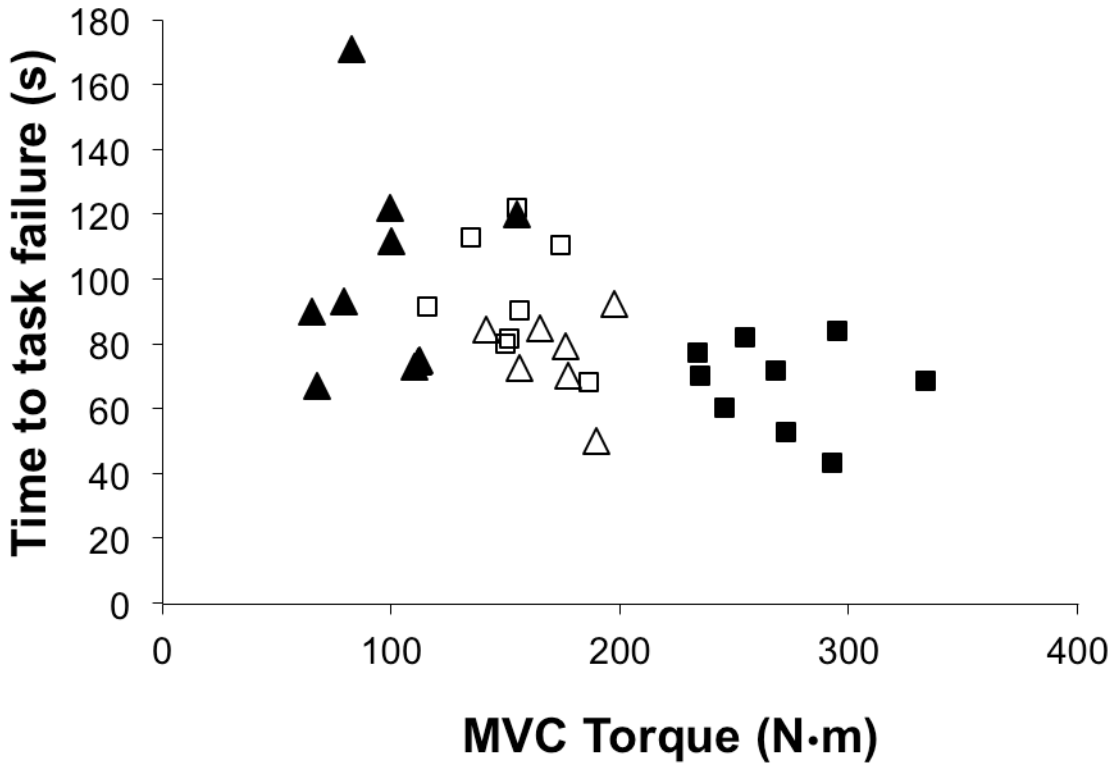

Figure 5.5 Time to task failure during the sustained contraction held at 50% MVC plotted as a function of MVC. Young men (filled squares); young women (open triangles); older men (open squares) and older women (filled triangles).

Intermittent contraction fatigue test

Intermittent electrically-induced 30-Hz tetani of 1-s duration and 1-s recovery between stimuli were applied to the knee extensors to elicit 60 cycles of contraction / relaxation, taking a total of 2 min. The 30-Hz stimulus applied to fresh muscle gave similar relative force in all groups, as can be seen on the torque-frequency relationship (Figure 5.2).

Figures 5.6a and 5.6b show the mean (SEM) data for men and women, respectively, for the full duration of the intermittent fatiguing protocol. Young

and old showed similar force loss through the duration of the test (P = 0.480), but women fatigued less than men (P=0.001). At the end of the test, the percentage force that remained (fatigue index) was 54.8% (±2.2), 56.4% (±2.6), 67.8% (±3.3) and 63.4% (±2.7) in young men, older men, young women and older women, respectively. When analysing the full dataset the fatigue index was inversely correlated with the MVC (r = -0.394, P = 0.016), but not with the ½ relaxation time (r = 0.290, P = 0.086).

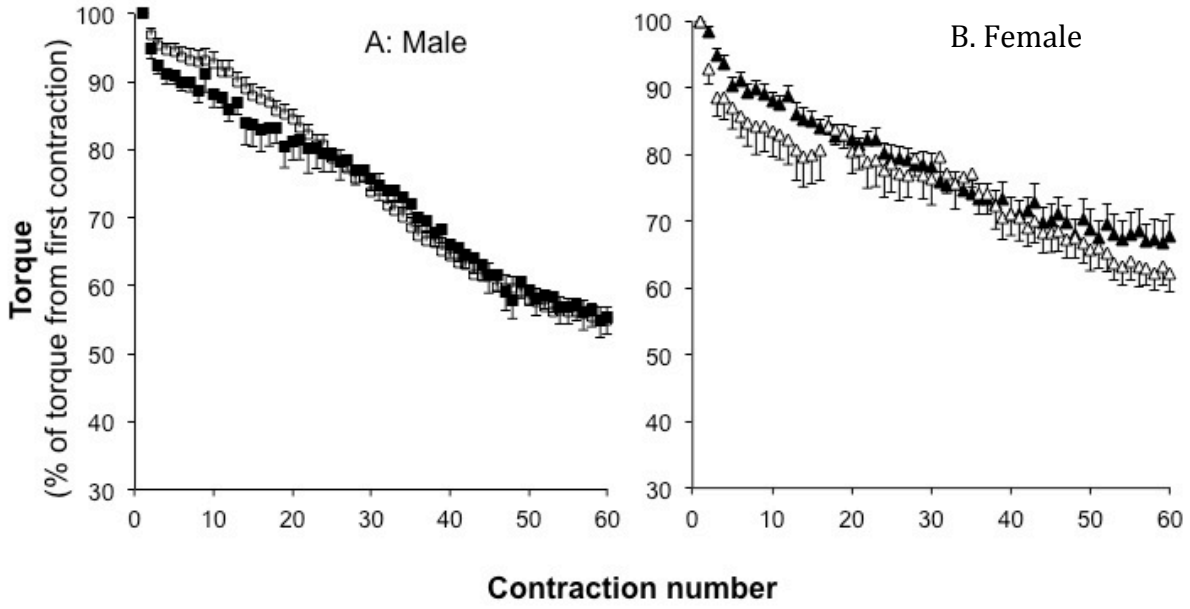

Figure 5.6: Decline in torque during the intermittent isometric contraction fatigue test. A: young men (filled squares) and older men (open squares). B: Young women (filled triangles) and older women (open triangles). The protocol included 60 stimulated 30-Hz contractions of 1-s, each separated by 1-s relaxation interval. Mean ± SEM are shown. There was no significant age effect, but women fatigued significantly less than men.

5.5 Discussion

We have utilized two different fatigue tests to differently stress the mechanisms of energy expenditure and regeneration to examine age and sex differences in skeletal muscle fatigue resistance. The results have shown that older men and women were able to hold a sustained contraction at 50% of MVC for around 20 s longer than young men and women. However, when the pattern of activation was changed to short, intermittent contractions with brief rest intervals, the young and older adults showed similar declines in force throughout a series of 60 contractions. The following discussion considers the contrasting findings in the two different fatigue tests as well as sex differences that were evident in the intermittent fatigue test.

Effects of ageing on the sustained isometric contraction

Our results show a longer endurance time to task failure in older compared with young adults during a sustained isometric contraction of the knee extensors, which extends previous work looking at smaller muscle groups (Bilodeau & Henderson 2001; Bilodeau et al. 2001; Hunter et al. 2004; Narici et al. 1991; Griffith et al. 2010). A sustained isometric contraction is a task that involves high voluntary effort and maintaining the contraction at 50% MVC becomes increasingly unpleasant, possibly due to build-up of ischaemic pain. One possible explanation for the lesser performance of the young participants is that they were more susceptible to central fatigue than the older participants. However the use of superimposed stimulation demonstrated that at the point of task failure both young and older participants were recruiting similar relative fractions of the muscle (84 and 88%, respectively) indicating that the difference in fatigability mainly reflects differences in peripheral mechanisms of fatigue. Progressive recruitment of motor units and lesser contribution of central fatigue at task failure in young and older adults has been reported in previous studies of the elbow flexor (Bilodeau et al. 2001; Hunter et al. 2004) and plantar flexor muscles (Mademli & Arampatzis 2008).

In all groups (young, old, men and women) there was approximately a two-fold inter-individual variation in time to task failure. One possible factor that has been discussed in relation to individual differences in muscle fatigue is that larger and stronger muscle might fatigue more rapidly in both sustained (Hunter et al. 2004) and intermittent contractions (Kent-Braun et al. 2002; Ditor & Hicks 2000; Katsiaras et al. 2005). We also observed an inverse relationship between time to task failure and muscle strength (Figure 5.5). Larger muscles might develop higher intramuscular pressures causing greater occlusion of the blood flow. However, this is unlikely to be the full explanation since the oxygenation of a muscle depends on the relative, rather than the absolute force, the muscle generates (DeRuiter et al. 2007) and even relatively small rabbit muscles produce intramuscular pressures sufficient to occlude the blood flow (Degens et al. 1998). In addition, another potential reason could be a change in pH resulting from greater extracellular hydrogen.

A reason for the superior performance of the older participants during the sustained isometric contraction probably lies in the different contractile properties of the old and young muscle. Slow contracting type I fibres constitute a greater proportion of the overall muscle in older compared with young participants (Lexell et al. 1988). Our examination of contractile properties showed muscles of young men to be fastest, followed by young women, with older men and women being the slowest, based on measurements of relaxation rates (Table 5.2) and the effect this has on the torque/frequency relationship (Figure 5.2). Slower muscles of older people have been shown to have a lower energy turnover during prolonged isometric contraction and accumulate metabolites at a slower rate (Kent-Braun et al. 2002; Tevald et al. 2010; Lanza et al. 2007), which is probably due to slower cross bridge kinetics, although there could also be fewer or slower cation pumps in the sarcoplasmic reticulum and plasma membranes which may play a role in fatigue (Green 1998).

Effects of age on the intermittent contraction fatigue test

The intermittent fatigue protocol reflects the balance between energy expenditure during contraction and regeneration during relaxation. While older muscles had a longer time to task failure during a sustained isometric contraction, there was no difference between young and older participants during a series of brief intermittent isometric contractions. This is consistent with two recent systematic reviews (Christie et al. 2011; Avin & Frey Law 2011) which suggested that older participants have superior fatigue resistance during sustained isometric contractions, but this advantage is not seen when performing dynamic (intermittent) contractions. During intermittent contractions the ability to regenerate ATP during the rest intervals may play a significant role in delaying the onset of fatigue. The mitochondrial content or activity as well as the microvascular supply in muscle can decrease with ageing (Crane et al. 2010; Safdar et al. 2010; Short et al. 2005; Kaczor et al. 2006; Degens 1998). Thus, a decrease in oxidative capacity may impair the performance of older muscle during intermittent contractions. In contrast, such a decrease in oxidative capacity would play a lesser role in the rate of fatigue during the sustained isometric contractions where blood flow is occluded (DeRuiter et al. 2007).

A second explanation for the failure of the slower, older muscle to translate to greater fatigue resistance during brief intermittent contractions might relate to the energetics of muscle contraction. The energy consumed by a muscle that is shortening and performing work is greater than when it is contracting isometrically (Fenn effect)(Fenn 1923). Furthermore, the differences between the energy demands of isometric and shortening contractions varies between fast and slow muscles. Barclay et al (Barclay et al. 1993) showed that slow mouse soleus muscles had relatively low rates of energy turnover during isometric contractions, but this increased up to four-fold during rapid shortening. In contrast, the fast extensor digitorum longus muscle had a higher energy turnover in the isometric state, but this increased only modestly during shortening contractions. Thus, the efficiency of slow muscles decreases to a greater extent than that of fast muscle as the velocity of

shortening increases. Extrapolating this to relatively slow older human muscles and faster young muscles, we may assume that during sustained isometric contractions the lower energy turnover of the old muscles conveys an advantage and permits superior endurance during prolonged contractions, as discussed above. However, during shortening contractions the energetic demands rise considerably for the slower older muscles and any advantage they had in the isometric state is lost. Although the brief intermittent contractions we have used are described as “isometric”, at the start of each contraction there is considerable shortening as the muscle stretches compliant structures such as tendons, connective tissue, joint structures and parts of the apparatus. Thus, in addition to developing tension, internal work is also done. This additional energy cost can be considerable as the energy costs of a series of short isometric contractions is nearly twice that of a continuous isometric contraction of the same duration and results in greater force loss and slowing of the muscle (Newham et al. 1995).

The energy cost of a single isometric contraction will be made up of a fast component at the start of the contraction where internal work is being done and a slower component when the muscle has reached the isometric phase. The proportions of these two components will depend on the duration of the contraction. For short contractions of 1 s, as used in the present study, the shortening phase might occupy one third to a half of the total contraction time and so contribute a great deal to the total turnover, offsetting the advantage that old muscle has during purely isometric contractions. As the duration of the contraction increases, so the relative contribution of the energy associated with the initial shortening will diminish and slower muscle will regain its energetic “advantage”. This may explain why several studies that utilized intermittent isometric contractions of 5 and up to 30-s, with similar recovery intervals, show that older muscle fatigues less than young (Lanza et al. 2004; Chung et al. 2007; Callahan et al. 2009; Lanza et al. 2007; Bazzucchi et al. 2004). However, where the duty cycle was changed so there was a shorter contraction / recovery interval such as in the present study and in other reports (Stackhouse et al. 2001; Allman & Rice 2004; Stevens et al. 2001), there was no difference in the extent of fatigue in young and old participants.

Sex differences

In the present study, men and women showed similar time to task failure when sustaining the contraction at 50% MVC. Although this is in agreement with the work of Maughan et al (1986), a larger sample size might be needed in future studies to account for the considerable inter-individual variability between people. In studies that utilized a lower target force in the knee extensors, women were found to have a longer time to task failure than men (Clark et al. 2005; Maughan et al. 1986). Interestingly, where sex differences were reported in young holding an isometric contraction of elbow flexors, they no longer existed in older men and women (Hunter et al. 2004). We have observed in the present study that the greater fatigue resistance of young women compared with men performing intermittent contractions continues into old age (Figure 5.6). We had previously attributed the greater fatigue resistance during intermittent contractions to a slower muscle phenotype in young women compared to men (Wüst et al. 2008) arguing that this led to a slower ATP turnover and thus slower fatigue. In the present study we again observed slower muscle in women compared with men, but another critical factor could be the difference in oxidative capacity, or the regeneration of ATP during the brief recovery interval between contractions (Ensbjornsson-Ligledahl et al. 2002). The fact that the difference in fatigue resistance between men and women during the intermittent contractions persists into old age indicates that the differences between men and women in rates of ATP use or its recovery also continue into old age.

Limitations

A limitation of this study into age- and sex-differences in muscle fatigue is that the models of muscle activation using electrical stimulation and isometric contractions of a single joint are not fully representative patterns of muscle activation *in vivo*, such as walking or stair climbing. In 'real-life' scenarios, fatigue is influenced by multi-systems that integrate human movement such as neural control, cardiorespiratory adjustments, motivation, perception of

effort and sensory feedback. When trying to understand the occurrence of fatigue during usual daily tasks it should be born in mind that, quantitatively, older muscles are smaller and weaker than young muscles and since, in general, body weight tends to increase with age, the older muscles will be working at a higher relative load. The tests of fatigability that we and most other groups have used have been standardised to the strength of the muscle, not to the tasks that they are required to perform in daily activities. Consequently, the commonly used tests of fatigability may underestimate the actual stress of daily activities on older people.

Conclusion

It is concluded that older muscle is, in general, more resistant to fatigue than young when performing sustained isometric contractions of the knee extensors. However, following a series of 60 brief, intermittent isometric contractions young and old did not differ in the extent of fatigue. Significant sex differences were observed following the brief, intermittent contractions, where young and older women had better fatigue resistance compared with men, but men and women showed similar fatigue during a sustained isometric contraction.

Chapter 6

General Discussion

The aim of this final chapter is to summarise the main findings of this thesis, providing an overview of the results and to discuss their relevance within the context of sarcopenia and the existing literature. In addition, suggestions for future research within the study field will be provided.

The main aim of the work reported in this thesis was to characterise the changes to muscle mass and neuromuscular control in older adults and determine the contribution of the changes to mobility limitations in a cohort of 'healthy' older people. .

All volunteers in the studies were independent living, socially active and free from disease that impaired mobility levels. Indeed, the detailed examination of healthy older people was an important aspect of the studies because the results are distinct from those of very large epidemiological studies of ageing that often included a wide range of co-morbidities and disabilities, and thus making it difficult to distinguish between ageing and disease. The original research findings reported in Chapters 2, 3, 4 and 5 will help to demonstrate the prevalence of sarcopenia within a healthy population, understand the consequences for mobility and inform future work aimed at understanding the underlying mechanisms of neuromuscular deterioration with ageing.

The population studied in this thesis gives an insight into healthy ageing with all older participants remaining physically active, relatively free of medications and independent (McPhee et al. 2013). It remains to be seen to what extend these observations also apply to other elderly populations such as those suffering from disease, the oldest-old or populations of dependant individuals.

Sarcopenia: the loss of muscle mass with ageing

There are several definitions and diagnosis criteria for sarcopenia commonly used in research. The most recent consensus definitions of sarcopenia both include an aspect of low muscle mass (Cruz-Jentoft et al. 2010; Fielding et al. 2011). Some definitions use muscle mass as the only variable; others include muscle mass and strength; some include total body mass or fat mass; while

others additionally include mobility. These different criteria do not consistently identify the same individuals as sarcopenic. The original definition of sarcopenia was *the loss of muscle mass with ageing* (Rosenberg 1989), and the diagnosis proposed by Baumgartner (1998) was people who were > 2 standard deviations lower than the mean of younger adults when measuring *Appendicular Lean Mass / Height*^{2}, measured using dual energy x-ray absorptiometry. As discussed in **Chapter 1** there are a number of limitations when using DXA in providing a lean mass estimate, namely in the inclusion of aspects of fat and bone mass within the estimation as well as questions to whether DXA can produce a reliable estimate of a change in muscle mass (Delmonico, 2008). One of the striking features of the data presented in **Chapter 2** was that total lean mass was reduced by just 5% in older subjects in our healthy older cohort. ***Using the definition of Baumgartner (1998), around 40% (32% Older women; 48% Older Men) of the cohort were sarcopenic.*** Previous reports indicate sarcopenia prevalence to be around 15% of men and 24% of women aged 65-70 yrs, rising to >50% in people aged over 80 yrs (Baumgartner et al 1998; Iannuzzi-Sucich et al 2002). Therefore our cohort and previous reports in cohorts of a similar age report a similar level of prevalence of sarcopenia even with the recruitment strategy in this cohort to specifically target healthy older people.

The functional deficit of our older participants, reported in **Chapter 4**, contrasts sharply with the *apparent* modest loss of muscle mass. This raises the very important and often controversial question of whether or not the conventional measurement of sarcopenia is appropriate. To address this question we included more detailed examination of the thigh using magnetic resonance imaging and defined regions of interest to measure the thigh on the DXA images. The thigh region was chosen in particular since it is often used for normalising muscle strength of the knee extensors as well as playing a key role in mobility (Buford et al. 2012). There was good agreement between the DXA and MRI-derived methods in both young and older adults (r^2=0.83-0.90). However, even when focussing attention onto the thigh muscles, the DXA-based measurements clearly underestimated the extent of muscle lost with ageing. The data reported in **Chapter 2** revealed that the quadriceps muscles of older people were on approximately 30% smaller than

in young. This is of similar magnitude to the loss of quadriceps muscle strength in older age (40%).

It is standard practice to normalise muscle mass to a measure of body size to allow comparisons to be made between people of different stature. Most often, muscle mass is normalised to height^2 or total body mass. The problem with these normalisations is that height can be reduced by as much as 1 cm/yr in older age due to curvature of the spine and compression of vertebrae, thereby cancelling out the loss of muscle mass and thus, underestimating the extent of sarcopenia. Total body mass can fluctuate independently of lean mass due to changes in fat mass, thereby shifting the focus of sarcopenia away from muscle tissue and more towards total body composition. To overcome these problems, we developed an approach to normalise the quadriceps muscle volume to the femur volume in young and older subjects, as previously done in studies of muscle and bone during pubertal growth (Ireland et al. 2013; Schoenau et al. 2002; Sumnik et al. 2006; Capozza et al. 2004; Högler et al. 2008). As reported in **Chapter 3**, the femur volume did not differ between young and old, thereby validating the use as an internal reference against which changes to muscle size can be related. The muscle:bone ratio does have a number of assumptions associated with its use, that femur volume 1) does not change with age and 2) is largely determined by the muscle forces acting upon it around puberty. It then also potentially eliminates secular changes that are often an associated limitation of cross sectional studies. ***Muscle mass normalised to bone volume revealed that the quadriceps muscles were more susceptible to age-related atrophy compared with the hamstrings, adductors and abductor muscles of the thigh. It also identified 96% of older men and 57% of older women to be sarcopenic, which contrasts strongly with the data showing only 40% of older people being classified as sarcopenic according to the traditional classification methods (Baumgartner et al. 1998).*** These results raise concerns over the 'consensus' definitions for sarcopenia and the apparent general acceptance of these definitions within the literature.

Association between sarcopenia and mobility

Demonstrating significant differences between young and old in neuromuscular characteristics is an important first step in understanding the deterioration of body systems evident even with 'healthy' ageing. However, it is of clinical interest to determine the level of causality between the observed structural and functional changes to the neuro-musculo-skeletal systems with the loss of mobility in older age.

Previous studies using the SMMI method observed that muscle mass was a relatively poor indicator of muscle function and physical disability (Visser et al. 2005; Goodpaster et al. 2001), with a recent study in a Chinese population, observing an ROC area under the curve of 0.53, concluding that muscle mass has a low predictive value for functional performance (Woo et al. 2009). However in light of the problems with using DXA highlighted in Chapter 2, it remains to be seen, whether this finding is due to the inherent inaccuracies.

In **Chapter 4**, it was observed that SMMI was indeed a poor predictor of functional performance as assessed by 6 minute walk distance and timed get up and go. When examining the whole cohort, the muscle to bone ratio substantially improved this relationship. However when examining the inter-group relationships individually, this relationship was lost. Muscle strength measures (grip and knee extensors) and muscle power as quantified by counter movement jump were significantly correlated with measures of mobility in older people, but not in young. In older adults, muscle power appeared to be the greatest predictor of low function. Neuro-muscular characteristics were not associated with mobility in groups of young, but this is not surprising since the mobility tests used in these studies were designed to assess function of elderly or diseased persons and not young, healthy populations in whom performance is probably limited by biomechanical constraints.

The use of the muscle to bone ratio in future studies should look into its use in other populations where the accurate estimation of muscle mass is crucial to the correct clinical decision being made for example rheumatoid arthritis patients and also whether its use remains valid in the oldest-old. In addition the incorporation of measures of intramuscular fat from MRI measures would introduce a perhaps more robust measurement, not only giving an accurate identification of an individuals extent of sarcopenia but also a measure of muscle quality.

In **Chapter 5**, the fatigability of quadriceps muscles was studied because fatigue is a common complaint in older people and can impact on ability or willingness to complete daily tasks as well as increasing the risk of falling. In one fatigue test, participants were asked to hold a sustained isometric voluntary contraction at 50% of their maximal strength until task failure. Older people held the contraction for longer than young on average, but there was very large variability between people. In this type of contraction, the intramuscular pressure rises enough to occlude blood flow into the muscle, so provides little opportunity for metabolic recovery during the task. Fatigue in this instance is probably dependent on the rate at which ATP is utilised. More detailed examination showed that the superior fatigue resistance of old compared with young was associated with having smaller muscles and slower contractile properties and thus, slower rate of ATP turnover. However, when contracting the quadriceps muscles using a series of 60 brief, intermittent stimulated contractions, the older and young subjects showed similar extent of fatigue, but women fatigued less than men. Again, larger muscles with faster contractile properties tended to fatigue more quickly than smaller muscles with slower contractile properties. These results suggest that older people use ATP at a slower rate than young, but this did not translate to any benefit during repeated brief, intermittent contractions. One reason could be that the older people may have had a reduced rate of ATP recovery during brief rest intervals, the effect being to cancel out the advantage of slower rate of ATP

utilisation. In order to resist fatigue, the ATP recovery needs to occur through oxidative pathways in the mitochondria. Another factor that might influence the intermittent contractions is that shortening of muscle fibres against series compliance might lead to reduced efficiency of the older, slower, more compliant muscles.

As highlighted in the limitations section of Chapter 5, the methods that were used to assess fatigability were standardised to the strength of the muscle for each individual. While this provides a valid assessment of the relative fatigability of the individual muscle group, it is not necessarily a reflection of the type of fatigue that would occur in everyday activities. It should be borne in mind that the older people had smaller leg muscles and relatively higher body fat and this means that their leg muscles must work proportionally harder during walking and other similar daily activities. Attempts should be made to provide a functional assessment of muscle fatigue in daily activities such as during stair climbing, which would truly examine how changes in muscle fatigue result in functional changes in mobility.

Future Directions

Further work should be carried out to further validate the use and accuracy of DXA to allow comparisons of muscle mass between young and old by appropriately adjusting DXA values for fat and connective tissue in these populations. This is important as the main advantages of DXA over MRI are its accessibility and lower relative cost.

The use of the muscle to bone ratio has been validated in the healthy older population (Chapter 3). It remains to be seen, however, whether this approach can be used in other distinct populations such as the oldest-old or patient groups. This is an area for further investigation.

Future directions should examine the functional changes with power training; some efforts to this end have been made, with some studies showing resistance exercise interventions generating improvements in muscle power

leading to improvements in functional performance. However the training of muscle power alone, has so far done little to change body composition (Pereira et al. 2012; Delmonico et al. 2005), as discussed in Chapter 1 the prevalence of sarcopenic obesity is on the rise, Therefore incorporation of some aspect of aerobic training concurrently with power training could potentially lead to beneficial improvements in muscle power, function and also body composition which theoretically should lead to greater functional performance benefits with greater muscle power per unit body mass.

Overall Conclusions

The overall aim of this thesis was to characterise the loss of muscle mass in a recreationally active UK population and to determine its consequence on functional performance. The novel results presented in this thesis show that the most commonly used assessments of muscle mass that use DXA to define sarcopenia might lead to an underestimation of the prevalence of sarcopenia. The muscle to bone ratio provides a better estimate of age-related atrophy. However, muscle mass quantified using DXA or MRI methods was a relatively poor predictor of functional mobility limitations in older people. Instead, mobility was more strongly associated with muscle power produced during a counter movement jump and normalised to body mass. This loss of muscle power can be attributed to the slower contractile properties within the muscle as well as a reduction in voluntary activation. Although the slower muscle might reduce power and mobility, we have shown that slower muscle might have the benefit of reducing the energy cost of muscle contractions. Slower muscle was associated with better fatigue resistance during prolonged isometric contractions. The problem is that such contractions are not common parts of everyday life. During intermittent contractions the older subjects with slower muscle did not show enhanced fatigue resistance compared with young. These findings have implications for the definition of sarcopenia and in demonstrating the clinical relevance of neuromuscular changes even in healthy ageing.

Chapter 7

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