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„Quadriceps fat infiltration, strength and neural activation in frail elderly individuals determined by computed tomography and voluntary muscle activation“

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I. Abstract

Background: Fat infiltration within the muscle or intermuscular adipose tissue (IMAT) has been associated with, impaired motor unit (MU) drive and skeletal muscle function. Combined with muscle atrophy these changes reduce muscle strength and quality, and in consequence may lead to loss of physical function. As non-invasive method, computed tomography (CT) provides a rapid, accurate information on muscle cross-sectional area (CSA) and enables the measurement of fat infiltration between and within the lean tissue. The interpolated twitch method (ITT) presents a percentage measure of voluntary muscle activation of the innervated muscle.

Methods: In this study frail elderly females (N=6) and males (N=8) (aged 86.2 ± 5.5 years, BMI 25.6 ± 3.3 kg/m²) with a short physical performance battery (SPPB) score of 4.2 ± 2.3 points were included. To examine the effects on muscle CSA and voluntary muscle activation, participants were assigned into a training group (TG: N=7, 10-weeks of resistance training twice a week) or a control group (CG: N=7). Maximum voluntary isometric contraction (MVC), maximum concentric strength and voluntary activation were assessed during knee extension while the CT scans taken at the mid-thigh were analysed with ImageJ. Measured in Hounsfield units (HU), IMAT was determined alongside muscle density which was divided into areas consisting of normal-density lean tissue (NDM) and low-density lean tissue (LDM) based on its attenuation characteristics. All test and measurements were assessed at baseline and post-intervention.

Result: A strong correlation was found between concentric knee extensor strength and NDM muscle (r=0.857, P<0.001), whereas no correlation was observed for LDM (r=-0.119, P=0.679) or IMAT (r=-0.185, P=0.544). Concentric force production per unit muscle size was correlated with muscle attenuation (r=0.640, P=0.019). The training intervention increased NDM by 8.6% (P=0.040) while LDM remained unchanged (-0.9%, P=0.965). The concentric knee extensor strength was improved by 18.0% (P=0.025) and the comparison between groups for muscle attenuation showed a difference in favour of the TG (4.8%, P=0.049). The baseline level of voluntary muscle activation was at 91.7%. Following the training intervention, no significant changes in the level of voluntary muscle activation were observed.

Discussion: The findings displayed that in frail elderly individuals, muscle density as defined with CT scans is related to force generating-capacity. The segmentation of NDM and LDM demonstrated that higher m. quadriceps density is positively related to force production. IMAT, identified in the m. quadriceps, was obviously too small to negatively influence muscle force production. Moreover, it was shown that 10-weeks of resistance

training was sufficient time to elicit improvements in strength and NDM in frail elderly individuals. In addition, nearly all individuals were able to fully activate the muscle, thus voluntary muscle activation was not detected as an inhibiting factor in strength generation.

I. Abstract (German)

Hintergrund: Fettinfiltration im Muskels oder intermuskuläres Fettgewebe wird mit beeinträchtigter Ansteuerung der motorischen Einheit (MU) sowie gestörter Muskelfunktion assoziiert. In Kombination mit Muskelatrophie führen diese Veränderungen zu einer Minderung der Muskelkraft sowie Muskelqualität und folglich zu einer Abnahme der körperlichen Mobilität. Als nicht invasive Methode liefert die Computertomographie (CT) schnelle und präzise Information über den Muskelquerschnitt (CSA) und ermöglicht die Messung von Fettinfiltration zwischen- und im Muskelgewebe. Die Interpolated-Twitch Methode (ITT) präsentiert hingegen eine Methode zur Messung der willkürlichen Muskelaktivierung.

Methoden: Die Studie umfasste gebrechliche ältere Frauen (N=6) und Männer (N=8) im Alter von 86.2 ± 5.5 Jahre, einem BMI von 25.6 ± 3.3 kg/m² und einem durchschnittlichen Short Physical Performance Battery (SPPB) Testergebnis von 4.2 ± 2.3 Punkten. Um die Auswirkungen auf den Muskel CSA und die willkürliche Muskelaktivierung zu untersuchen, wurden die Studienteilnehmer einer Trainingsgruppe (TG: N=7, 10-Wochen Krafttraining, 2-mal wöchentlich) oder eine Kontrollgruppe (CG: N=7) zugeteilt. Die maximale willkürliche isometrische Kontraktion (MVC), maximale konzentrische Kraft sowie Muskelaktivierung wurde während der Kniestreckung erfasst. Die Analyse der Oberschenkel-CT Bilder erfolgte mittels ImageJ. Neben dem IMAT wurde auch die Muskeldichte mithilfe von Hounsfield Einheiten (HU) bestimmt. Weiters wurde der Muskel basierend auf seiner Dichte in sehr dichtes Muskelgewebe (NDM) und weniger dichtes Muskelgewebe (LDM) unterteilt. Alle Messungen wurden vor und nach der Intervention durchgeführt.

Resultate: Zwischen konzentrischer Kniestreckung und NDM (r=0.857, P<0.001) wurde ein starker Zusammenhang gefunden, im Gegensatz zu LDM (r=-0.119, P=0.679) oder IMAT (r=-0.185, P=0.544), welche keine Zusammenhänge aufweisen konnten. Die konzentrische Kraftentwicklung pro Einheit Muskelmasse korrelierte mit der Quadrizeps Muskeldichte (r=0.640, P=0.019). Weiters führte die Trainingsintervention zu einer Zunahme in NDM von 8.6% (P=0.040) während LDM (-0.9%, P=0.965) unverändert blieb. Die konzentrische Kraft des Quadrizeps verbesserte sich um 18% (P<0.025) und auch der Vergleich zwischen den Gruppen bezüglich Muskelabschwächung zeigte eine Veränderung zugunsten der TG (4,8%, P=0.049). Das Ausgangsniveau der willkürlichen Muskelaktivierung war bei 91.7% und veränderte sich nicht durch die Trainingsintervention.

Diskussion: Die Resultate zeigten, dass es bei gebrechlichen älteren Menschen einen Zusammenhang zwischen der Muskeldichte und der Muskelkraft gibt. Die Unterteilung in NDM und LDM verdeutlichte hierbei, dass bei gebrechlichen älteren Menschen ein positiver

Zusammenhang zwischen höherer Muskeldichte im Quadrizeps und der Kraftentwicklung besteht. Das ermittelte IMAT im Quadrizeps war zu gering um die Kraftentwicklung negativ zu beeinflussen. Weiters wurde demonstriert, dass 10-Wöchen Krafttraining ausreichend waren um bei gebrechlichen älteren Menschen eine Steigerung in Kraft und NDM hervorzurufen. Da fast allen Probanden in der Lage waren ihren Quadrizeps vollständig zu aktiveren, wurde die willkürliche Muskelaktivierung nicht als limitierender Faktor in der Kraftentwicklung angesehen.

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II. Abbreviations

1RM	One repetition maximum
AT	Adipose tissue
EMCL	Extramyocellular lipid
BMI	Body mass index
CG	Control group
CSA	Cross sectional are
CT	Computed tomography
D	Difference between torque at stimulus and total torque
DXA	Dual X-ray absorptiometry
HU	Hounsfield unit
IMAT	Intermuscular adipose tissue
IMCL	Intramyocellular lipid
ITT	Interpolated twitch technique
LDM	Low-density muscle
MMSE	Mini mental status examination
MN	Motoneuron
MQ	Muscle quality
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MVC	Maximum voluntary isometric contraction
MU	Motor unit
NDM	Normal-density muscle
NIH	Norwegian School of Sport Sciences
RFD	Rate of force development
ROI	Region of interest
SD	Standard deviation
STAS	Strength Training, Aging and Sarcopenia
TG	Training group
TMVC	Torque at maximum voluntary isometric contraction
TStim	Torque at stimulus
TSTimRest	Torque at stimulus in rested muscle
μ	Linear attenuation coefficient

III. Preface

I would like to thank my main supervisor Barbara Wessner. You supported me during my stay in Oslo and afterwards back home in Vienna. I admire your knowledge and I would like to extent my gratitude to your help and feedback on my thesis, you always pushed me to do better.

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1 Introduction

In many cases the process of ageing from a clinical perspective can be associated with lowered mobility, negative health conditions, higher fall risk or frailty, factors which are all related to progressive loss of muscle mass and function (sarcopenia) (Cruz-Jentoft et al., 2010). Although the greater loss of muscle mass is described as the major factor of muscle weakness in elderly individuals (Rosenberg, 1997), investigations in the last two decades suggested that reduced muscle strength is caused by multiple factors and cannot be merely contributed to the loss of muscle mass (Delmonico et al., 2009; Goodpaster, Kelley, Thaete, He, & Ross, 2000; Karmen & Knight, 2004; Metter et al., 1999; Narici, Maganaris, Reeves, & Capodaglio, 2003; Ochala, Frontera, Dorer, Van Hoecke, & Krivickas, 2007). In this regard, declined neuromuscular function (Lexell, Henriksson-Larsén, Winblad, & Sjöström, 1983) and increased fat infiltration in the muscle have been associated with impaired skeletal muscle function, -strength, lowered mobility (Goodpaster et al., 2001), and mortality (Newman et al., 2006).

In advanced age above mentioned degenerative processes are often related to the term muscle quality (MQ) which is determined as muscle strength or power per unit of muscle mass (Moritani & DeVries, 1979). In this regard, muscle strength might be a key motor skill to not only maintain MQ but also physical function (Clark & Manini, 2010).

In regards to recent research, regular resistance training also demonstrated promising results counteracting fat accumulation within the muscle (Goodpaster et al., 2008; Narici & Maffulli, 2010) and improving motor unit (MU) activation capacity (Macaluso & De Vito, 2004). Nevertheless, not much is known whether these results would be achievable in highly aged frail individuals.

The purpose of this thesis was to examine the relations between muscle size, density and strength of the knee extensor and their responses to resistance training in frail elderly individuals. Therefore, non-invasive methods as computed tomography (CT) and voluntary muscle activation were conducted. While CT provides rapid and accurate information about the muscle cross-sectional area, voluntary muscle activation delivers insight into the muscles contractile properties. By means of these two methods, knee extensor characteristics concerning fat infiltration and neuronal activation were investigated and related to the muscles maximum static and dynamic force generating capacity. Peak force of the m. quadriceps was determined by one-repletion maximum (1RM) and maximum voluntary isometric contraction (MVC) tests. The outcomes of the thesis are expected to present a clearer picture of knee extensor strength and its association to MQ in higher aged individuals.

2 Background

2.1 Sarcopenia

The name sarcopenia was firstly proposed in a meeting by Irwin Rosenberg in 1988 and described as progressive loss of muscle mass and function during the process of ageing, independent of ethnical group, sex, health behaviour or socioeconomic status (Rosenberg, 1997). Years later Janssen, Heymsfield, & Ross (2002) additionally introduced a muscle mass index based on the dual-energy x-ray absorptiometry (DXA) to establish the prevalence of sarcopenia. When looking at the development of sarcopenia, it is suggested that it occurs around the age of 45 years (Janssen et al., 2000; Narici & Maffulli, 2010) and increases steadily until the age of 80 years, where up to 40% of the people are considered suffering from low relative muscle mass or sarcopenia (Baumgartner et al., 1998). Since this condition implies the loss of muscle mass and function it is often linked to the age-related loss of muscle strength (Narici & Maffulli, 2010), whereby strictly speaking the age-related loss of strength and power is defined as dynapenia (Clark & Manini, 2012). It neither implies the loss of muscle mass and function nor is it steadily correlated to it (Delmonico et al., 2009; Goodpaster et al., 2006).

2.2 Frailty

Frailty is a common clinical syndrome mostly occurring at advanced age which is in its origin multifactorial. It is associated with negative health conditions as lowered mobility, higher fall risk, hospitalization and mortality. (Xue, 2011). Fried et al. (2001) defined frailty as *“a biological syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems, and causing vulnerability to adverse outcomes”.* Since no standardised assessments have been established so far, a phenotype has been developed, where the presence of three out of five criteria indicates frailty: a) weight loss b) poor endurance d) slow walking speed c) weakness, and e) low physical activity. Although these criteria might seem applicable to sarcopenia as well, both frailty and sarcopenia are two separate conditions and should also be treated differently as concordance was only shown partly (Reijnierse et al., 2016). However, sarcopenia is a key contributor for the development of frailty (Phu, Boersma, & Duque, 2015; Xue, 2011; Yeolekar & Sukumaran, 2014).

2.3 Ageing and muscle quality

Ageing is characterized as a gradual decrease in muscle mass, neuromuscular function and physical performance (Janssen et al., 2000). In the past two decades, an increasing number of scientific literature arose, suggesting that age-related changes in MQ and performance may not merely be explained by changes in muscle mass. It consists of a complex system with many contributing factors resulting in reduced muscle function and impaired mobility (Correa-de-Araujo et al., 2017; Goodpaster et al., 2006). This chapter presents a brief overview about related terms concerning the quality of muscle.

Muscle quality is considered as a relative performance indicator and commonly described as the relation between strength or power per unit of muscle mass (Moritani & DeVries, 1979). In the last decades, MQ was interpreted in many different ways which are not always in line with the above-mentioned definition. The descriptions rank from muscle composition, density, muscle function per muscle mass to metabolic processes (Barbat-Artigas, Rolland, Zamboni, & Aubertin-Leheudre, 2012). Further, no standardised protocol to improve the inter-investigational reproducibility of MQ has been developed so far, neither for muscle mass (e.g. CT or Ultrasound) nor for force producing capacity (e.g. eccentric, isometric, isokinetic) (Barbat-Artigas et al., 2012). However, particularly in clinical research or rehabilitation, MQ could be an informative and powerful indicator to evaluate the treatment efficacy if regularly conducted under standardised conditions (Fragala et al., 2014; McGregor, Cameron-Smith, & Poppitt, 2014).

The loss of muscle mass is considered as a highly reasonable contributor for reduced muscle strength at advanced age and is reported to decline steadily after the fourth decade of life, independently of gender as shown in figures 1 A and 1 B. The research group of Narici & Maffulli (2010) determined the decline of lean body mass (LBM) between the second and the eighth decade of life by 18% in men and 27% in women. Although the decline in muscle mass is continuous, grade and location of the affected region of the body may alter; in general, greater muscle atrophy arises earlier in the lower than the upper body. In that case it is suggested that between the twentieth and seventieth year of age both men and women lose around 24% to 27% of thigh muscle mass. Considerations led to the assumption that a reduced activity level in the lower extremities (e.g. walking, stair climbing) could partially explain the greater loss in lower extremities (Janssen et al., 2000).

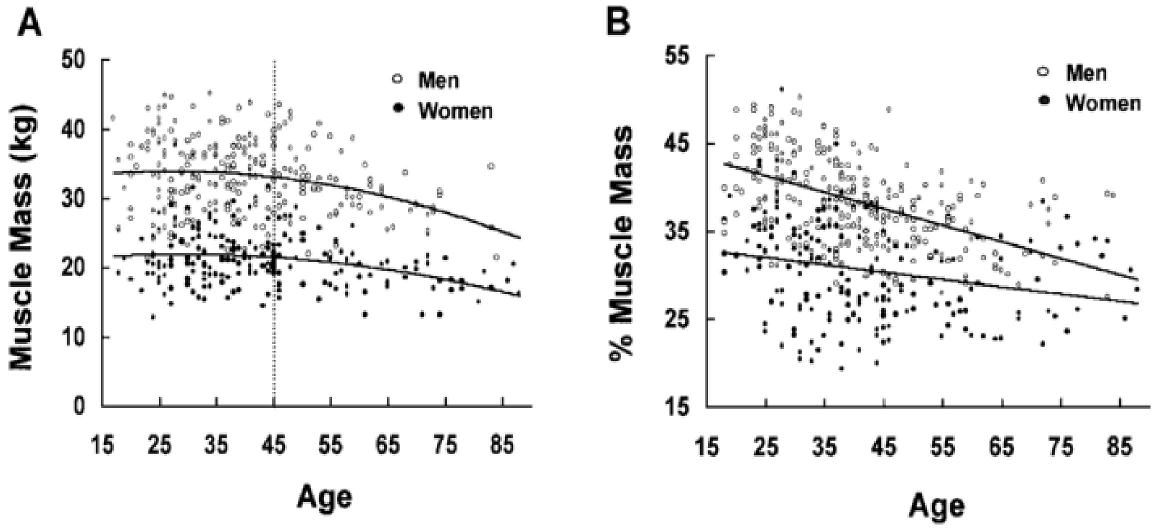


Figure 1: A) Association between absolute muscle mass and age in men and women. B) Association between relative muscle mass and age in men and women (Janssen et al., 2000).

Muscle strength detected as an essential component in maintaining MQ and physical function is suggested to decrease more rapidly at higher age (Goodpaster et al., 2006). In one of the most recent longitudinal studies of the past ten years Delmonico et al. (2009) demonstrated an annual decline in knee extensor strength over 5-years in individuals aged 70 to 79 years regardless of changes in muscle mass or body weight. Therefore, 1,578 individuals were stratified by their body weight change in groups losing (≥3%), maintaining (stable) and gaining (≥3%) body weight. Results showed an annual decline in maximal muscle torque in both groups, losing (-4.1%) and maintaining (-3.2%) weight and lean mass, but unexpectedly there was a strength decline (-2.9%) in the group that even gained weight and lean mass (Figure 2), which indicates that even an increase in body weight and lean mass does not prevent progressive loss of strength in higher age.

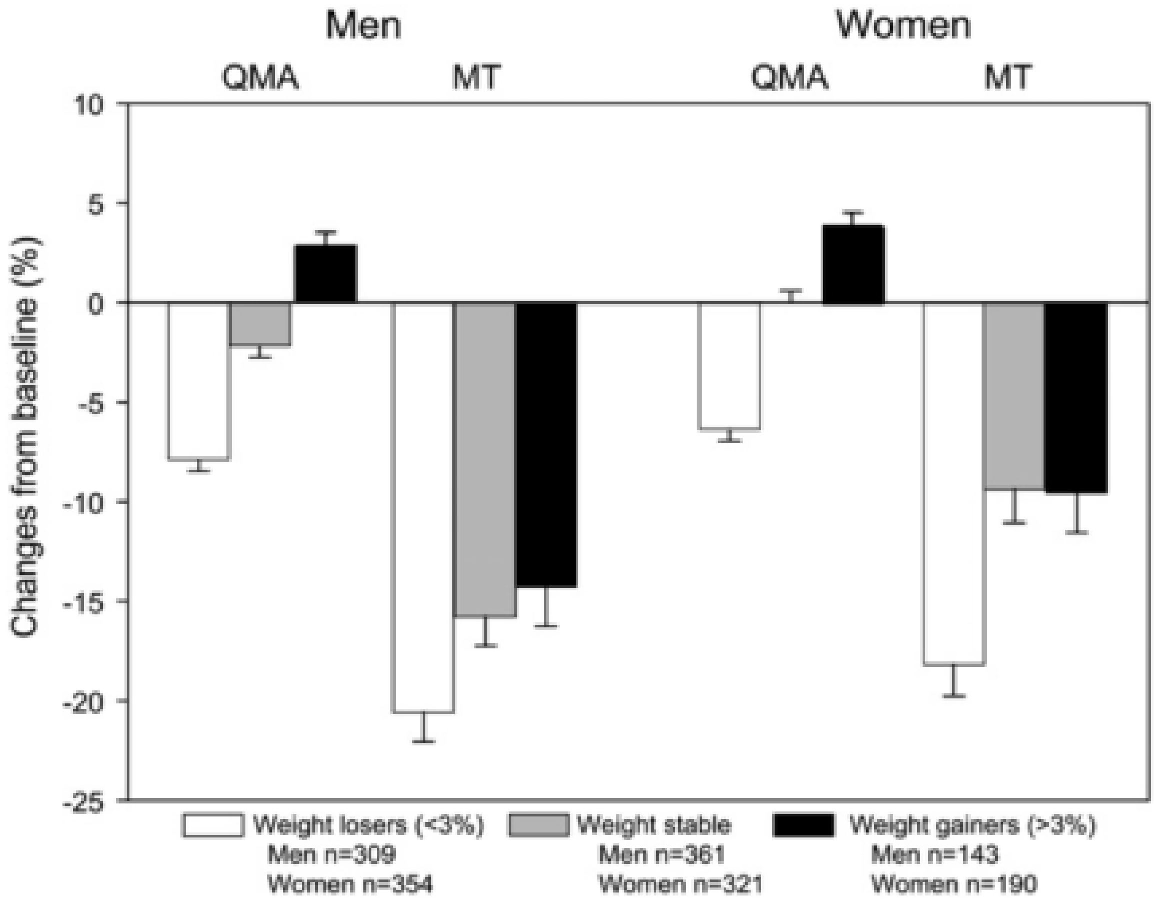


Figure 2: Percentage change in leg lean mass and muscle strength after 5- years within different weight change groups for men and women. QMA; Quadriceps muscle area, MT; Muscle torque (Delmonico et al., 2009).

Muscle architecture and its alteration during ageing might contribute to the loss of muscle strength (Narici et al., 2003). The decrease in fascicle length and pennation angle were seen to be highly related to the loss of muscle strength and shortening velocity (Narici, Franchi, & Maganaris, 2016). A reduction of 10 to 13% in muscle fibre pennation angle of the plantar flexor in old towards young individuals has been demonstrated by Narici et al. (2003) and also Kubo et al. (2003) who observed a decrease in pennation angle in the m. vastus lateralis in older individuals. These findings lead to the assumption that to some part the decrease in muscle function during the ageing process could potentially be related to changes in muscle architecture.

The alteration in MU characteristics is assumed to occur in the sixth decade of life caused by the loss of motoneurons (MNs) as well as a decreased discharge rate (Lexell et al., 1983). The consequences are the atrophy of former innervated muscle fibres while remaining fibers tend to have slow-twitch rather than fast-twitch properties (Hanson et al., 2013; Lexell et al., 1983). Furthermore, it is suggested that a decreased discharge rate of MNs might inhibit the nervous system to fully drive the remaining MU. Thus, older individuals might not be able to activate the muscle completely to generate the highest force (Karmen & Knight, 2004).

Ageing muscle fibres also lose their contractile properties which leads to inhibited muscle contraction (Porter, Vandervoort, & Lexell, 1995). The decreased proportion of fast-twitch fibers participating during the contraction is known as contributing factor and would partly explain lowered muscle strength at higher age (Frontera et al., 2000). Further findings by Ochala et al. (2007) showed a lowered specific force and velocity in type-I and type-IIa fibres, manifesting the previous findings that the ability to generate force might be age-related independently of changes in fibre size.

Fat infiltration within the muscle, was demonstrated to be greater in older individuals compared to younger individuals having the same body mass index (BMI). Furthermore, increased fat accumulation within the thigh muscle CSA of older men and women has been demonstrated to be associated with the loss of muscle strength and functional capacity, resulting in the reduction of MQ (Delmonico et al., 2009; Goodpaster et al., 2001; Reinders et al., 2016). An additional determining aspect of increased fat accumulation within the muscle, such as intermuscular adipose tissue (IMAT), is its association with insulin resistance observed in obese individuals. This is inasmuch problematic as one of the consequences of an impaired insulin sensitivity combined with inactivity is the development of type-2 diabetes (Goodpaster, Leland Thaete, Simoneau, & Kelley, 1997).

2.4 Annual changes in muscle strength and muscle mass at higher age

Both muscle mass and muscle strength are strongly related to each other and determine the quality of a muscle, although it has been reported that strength loss occurs independently of changes in lean mass (Delmonico et al., 2009; Goodpaster et al., 2006). This topic is still controversially debated since it is considered that the decline in muscle mass is the main contributor for the loss of muscle strength during ageing (Janssen et al., 2000). Therefore, the following chapters should provide insight into the linkage of these two determinants during the ageing process.

In a cross sectional study with an even distribution of 100 healthy men and women, Skelton, Greig, Davies, & Young (1994) observed a loss in isometric strength in the m. quadriceps of 1 to 2% per annum between the age of 65 to 89 years. A longitudinal study over 12-years with a starting mean age of 64 years displayed a yearly decline of 2% to 2.5% in the isokinetic knee extensor strength of 9 subjects and a ~1% in the thigh muscle CSA of 7 subjects (Frontera et al., 2000). As previously mentioned, Delmonico et al. (2009)

demonstrated astonishing results from the Health ABC study after 5-years of investigations concerning the relation between quadriceps muscle area and muscle strength. In this study analyses presented an annual loss in muscle strength of 2.9% despite a simultaneous gain in lean mass of 0.6%. All three studies demonstrated similar results in the percentage of annual strength loss. Furthermore, it was seen that annual decrease in muscle strength occurred more rapidly than the change in lean muscle mass.

As part of the Health ABC study the mortality risk under 2,292 participants aged 70–79 years was investigated as well. The outcomes suggested that muscle strength, but not muscle mass, was associated with higher mortality risk after 6-years of observation. Reduced muscle strength in the extensor and hand grip were seen to be predicting factors of mortality in elderly individuals while lower lean mass was not associated with mortality (Newman et al., 2006).

2.5 The effect of resistance training on muscle strength in elderly individuals

The main goal of a resistance training programme is to increase muscle strength. In order to effectively reach this goal, the principle of progressive overload is applied which implicates that muscles work against resistance close to its force generating capacity (Brooks, Fahey, & Baldwin, 2005). Although the effects of resistance training strongly depend on duration, frequency or intensity, a general protocol for interventions has been established during the last decades (Macaluso & De Vito, 2004). In clinical research, mostly two to three units, with an intensity of 80% of 1RM, for a period of 8 to 12 weeks were suggested as sufficient to show reasonable impact of resistance training (Frontera, Meredith, O'Reilly, Knuttgen, & Evans, 1988; Harridge, Kryger, & Stensgaard, 1999; Hvid et al., 2016; Macaluso & De Vito, 2004). Thus, the learning effects and neuronal adaptions were meant to be in focus until week six resulting in strength gain accompanied with less effect on muscle size. Nevertheless, greater adaptions in muscle size were noticed after six weeks (Macaluso & De Vito, 2004).

In the late 80s Frontera et al. (1988) presented one of the first studies investigating the outcomes of a standardised 12-weeks resistance training programme on muscle size in sedentary elderly volunteers aged between 60 to 72 years. As a result of the intervention a 10.2% increase in the knee extensor muscle CSA and a 112% increase in the 1RM of knee extensor strength were measured. Interestingly, the gain in isometric strength was ~10 times lower in comparison to the results in 1RM. The authors suggested that neural adaption specifics as possible reasoning for that matter. In a study of 11 very old individuals aged 86 to 97 years, a 12-weeks intervention of progressive resistance training for the knee extensor displayed an increase in both m. quadriceps strength (Figure 3 A) from 7.7 to 15.5kg (+ 134%) and m. quadriceps CSA of 9.8% (Figure 3 B) (Harridge et al., 1999).

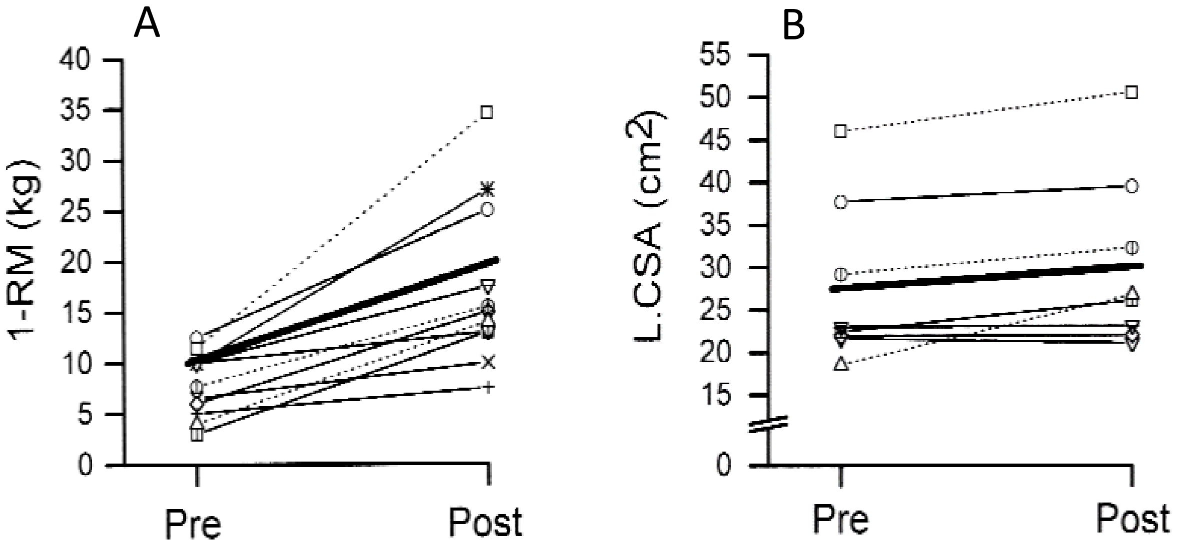


Figure 3: Individual changes from pre- to post intervention (N=11). 1RM; One repetition maximum, LCSA; Muscle cross-sectional area. Each symbol corresponds to a given individual. The solid lines represent women and the dotted lines men. The solid bold line without symbol represents the mean. A) Individual changes in 1RM. B) Individual changes LCSA (Harridge et al., 1999).

In order to show that not only resistance training by itself leads to positive muscular adaptions, Goodpaster et al. (2008) demonstrated reasonable effects when conducting a 1-year moderate training intervention (strength, aerobic, balance, flexibility) in about 77-year old subjects (Figure 4 A & B). Results displayed a 10% loss of knee extensor strength in the control group, whereas the loss within the physically active group was only 1.5%. In addition, a smaller loss in m. quadriceps CSA was seen in the physically active group (-1 ± 1%) versus the control group (-3 ± 1%) but with no significant differences between the groups.

Thus, findings demonstrated that moderate physical activity has preventive effects on the loss of muscle strength, muscle size and functional performance but not to the same extent as high intensity resistance training (Janssen et al., 2000; Suetta et al., 2004).

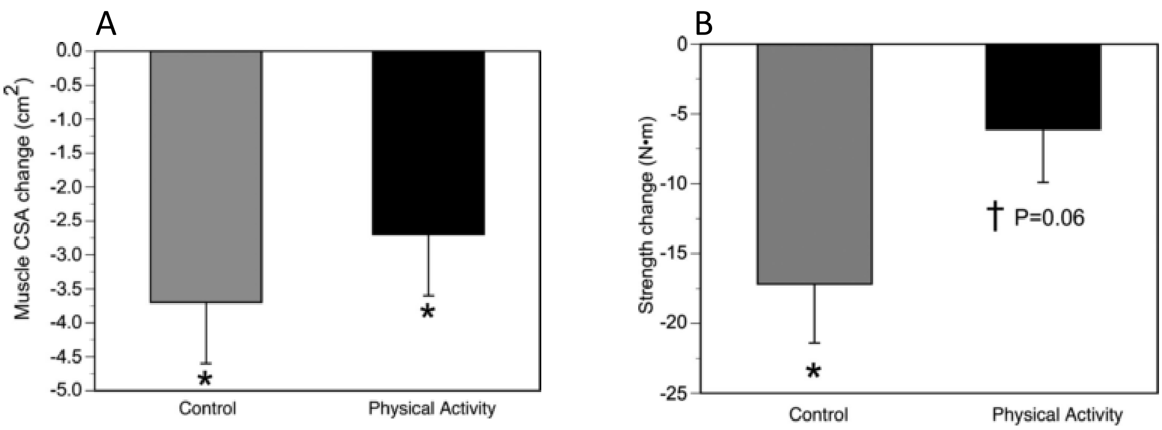


*Figure 4: A) Changes in mid-thigh muscle cross-sectional area (CSA) and B) Changes in absolute knee extensor strength in the physical activity and control groups after a 1-year intervention. N = 42, Age 77.4 ± 1.0 years, BMI 30.4 ± 1.3 kg/m², *: within group changes, P<0.05, †: between-group change, P=0.06 (Goodpaster et al., 2008).*

2.6 Protein intake during a strength training period

Protein supplementation is known to stimulate muscle protein synthesis to a degree where net protein balance (muscle protein synthesis minus muscle protein breakdown) becomes positive which is suggested to be essential to ensure a sufficient amino acid supply to the muscles (Phillips, Hartman, & Wilkinson, 2005). This is claimed to support the recovery and hypertrophy during a strength training period while a lack of nutrient intake would generally result in a negative net protein balance (René Koopman & van Loon, 2009).

A combination of resistance training and supplementation even enhances the stimulation of muscle protein synthesis resulting in a greater shift to a more positive net protein balance than with feeding only (Børsheim et al., 2008; Phillips et al., 2005). This further increases muscle fiber hypertrophy (Breen & Phillips, 2011). Muscle protein synthesis responded to protein intake and resistance type exercise in elderly individuals (75 ± 1 years, N=8), but to a lower extent than in younger individuals (20 ± 1 years, N=8) (Koopman et al., 2006). This age-related lowered response to anabolic stimuli of amino acids and exercise is summarized under the term *"anabolic resistance"* (Cuthbertson et al., 2005).

Taken together, a minimum of 20g of protein per meal (Breen & Phillips, 2011) or a dietary protein allowance of 0.85 $g \cdot kg^{-1} \cdot day^{-1}$ respectively (Campbell, Johnson, Mccabe, & Carnell, 2008) is suggested to be adequate to observe exercise-induced muscle fiber hypertrophy at advanced age.

2.7 Voluntary muscle activation

The recruitment of MU and their fire rate respectively, are two mechanisms available to the nervous system to coordinate the muscle contraction (Broman, De Luca, & Mambrito, 1985). Together, a MN and skeletal muscle fibers form a MU, innervated by the motor cortex. Within a MU all muscle fibres are of the same fibre type and they have the ability to contract the muscle maximally if innervated optimally (B. Clark & Taylor, 2011; Merton, 1954). In relation to the type of voluntary muscle activation smaller MU were seen to be recruited first before larger MU (e.g. only small MU were activated during submaximal voluntary muscle activation) (Paillard, Noé, Passelergue, & Dupui, 2005). Thus, numerous determinants such as changes in MN properties or excitatory and inhibitory sensory feedback were suggested to be influencing factors for voluntary contraction; whereas the descending motor pathway outgoing from the motor cortex seems to have the greatest impact on voluntary muscle activation (Clark & Taylor, 2011).

In conclusion, maximum voluntary activation is complete if the recruitment of all MU is achieved together with the unit’s discharge at their maximum frequency (Klass, Baudry, & Duchateau, 2007).

2.8 Age-related loss of muscle strength associated with the neuromuscular system

The decrease in muscle strength is associated with various changes in skeletal muscle and the neuromuscular system. These changes are amongst others, a loss of muscle mass, a type-II fibre atrophy, a lowered tendon stiffness (Narici & Maganaris, 2006) and a reduction in MU number beginning at the sixth decade of age (Lexell et al., 1983; Neil, Doherty, Stashuk, & Rice, 2005). Conversely the MU size is suggested to be increased in older individuals compared to younger ones explained by the reinnervation of denervated muscle fibres through collateral sprouting of nearby survived MNs (Aagaard, Suetta, Caserotti, Magnusson, & Kjær, 2010; Fling, Knight, & Kamen, 2009; Roos, Rice, & Vandervoort, 1997). However, it is suggested that the reinnervation occurs more likely in type-I and is less targeted onto type-II fibres. The reasons for the disparity of muscle fibers degeneration and reinnervation is still unclear but the disuse of fast-twitch fibres presents at least in part a contributing factor (Aagaard, Magnusson, Larsson, Kjær, & Krustrup, 2007; Macaluso & De Vito, 2004; Newman et al., 2006).

Another process appearing at higher age is a higher antagonist coactivation which contributes to reduced performance in the agonist (Klass et al., 2007). During the process of ageing, increased coactivation of the antagonist was seen to occur especially in bigger muscle groups like the m. quadriceps (Macaluso et al., 2002). Although it was demonstrated that a small level of coactivation is considered to be useful for the joint stabilization (Baratta et al., 1988) it simultaneously impairs the expression of muscle force generation in the agonist. In this regards, a negative association between the level of coactivation and development of submaximal and maximal torque production was reported (Simoneau, Martin, & Van Hoecke, 2005; Tracy & Enoka, 2002), manifesting a decreased force generation in the agonist muscle (Klass et al., 2007).

2.9 Measurement of voluntary muscle activation

For the understanding of human muscle function, the measurement of muscle activation displays an essential component in quantifying neuromuscular mechanisms. Unfortunately, no gold standard has been established so far. Nevertheless, methods assessed extensively in this regard are the interpolated twitch technique (ITT) (Folland & Williams, 2007) and a derivative of this method as the central activation ratio (CAR; see Kent-Braun & Blanc, 1996; Stackhouse et al., 2001) which differentiates only between complete and incomplete activation. The principle of both methods is to electrically stimulate the muscle on the belly or the motor nerve of the muscle while performing a maximal voluntary contraction attempt.

When applying the ITT to measure voluntary muscle activation it should be noticed that the force which can be produced by a muscle is measured, rather than the descending drive to the MN. Any evoked increase in force indicates a submaximal voluntary activation of the muscle (Taylor et al., 2009). However, when applying the ITT method a voluntary activation of 100% is generally not achievable even in healthy adults (Shield & Zhou, 2004). Therefore, the *“cut point”* for voluntary activation was defined at 90%, where every activation above was determined as fully voluntary activated muscle (Shield & Zhou, 2004; Suetta et al., 2009).

Unfortunately, no standard technique assessing voluntary muscle activation has been established yet which makes it difficult to compare outcomes, since they might vary strongly (Klass et al., 2007). The following chapter provides a few explanations why the ITT with adjusted equation might be preferred when investing voluntary muscle activation.

2.10 Twitch interpolation technique – adjusted equation

The ITT is a widely used non-invasive method in order to investigate voluntary muscle activation introduced by Merton (1954). It uses a superimposed stimulus (electrical stimulus applied during voluntary muscle activation) to recruit MU and to evoke a contraction of muscle fibres that are not already activated. Furthermore, the superimposed technique recruits lager MU before smaller ones, which is in contrary to the voluntary muscle activation. This helps to reach a bigger spectrum of MNs and fully activate the muscle (Paillard et al., 2005).

The quantity of applied stimuli to evoke a contraction during the muscle activation is also subject to extended discussions. While Merton (1954) originally used a singlet (one single stimulus), there is evidence that doublets (two stimuli within 10ms) as well as multi stimuli may enlarge the evoked force increments, reduce variation and thus become more reliable (Shield & Zhou, 2004; Suter & Herzog, 2001). Apart from this, both singlets and doublets were suggested to be easily tolerated (less painful) by the elderly population and subjects who are not familiar to electrical stimulation (Klass et al., 2007).

Taking a look at the ITT with superimposed technique, it can be seen that it has its limitations when the standard equation (classic equation) is applied. As Folland & Williams (2007) reported in their methodological review, it is very difficult to evoke the stimulus at the time point when greatest torque level is voluntary reached. However, this is necessary as otherwise the calculations would result in an underestimation of the achieved maximum voluntary activation. In this regard it is recommended to apply the adjusted equation (Strojnik & Komi, 1998); it includes the variation of the torque and the timepoint when the stimulus is given and therefore delivers more accurate results (Mau-Moeller, Behrens, Lindner, Bader, & Bruhn, 2013). The two formulas are illustrated below while a more detailed description of the test protocol is presented in chapter 4.7.3 “Maximum voluntary muscle activation”.

Classic Equation: $$Activation\,(\%) = 100 - \left(\left(\frac{D}{TStimRest}\right) \cdot 100\right)$$

Adjusted Equation: $$Activation\,(\%) = 100 - \left(\left(D \cdot \frac{\frac{TStim}{TMVC}}{TStimRest}\right) \cdot 100\right)$$

Figure 5 demonstrates the assessment of the twitch interpolation technique where TMVC is torque at MVC, D the difference between torque at stimulus (TStim) and total torque (voluntary + electrically generated torque response), and TStimRest the torque attained due to stimulation of the relaxed muscle (Hvid et al., 2016).

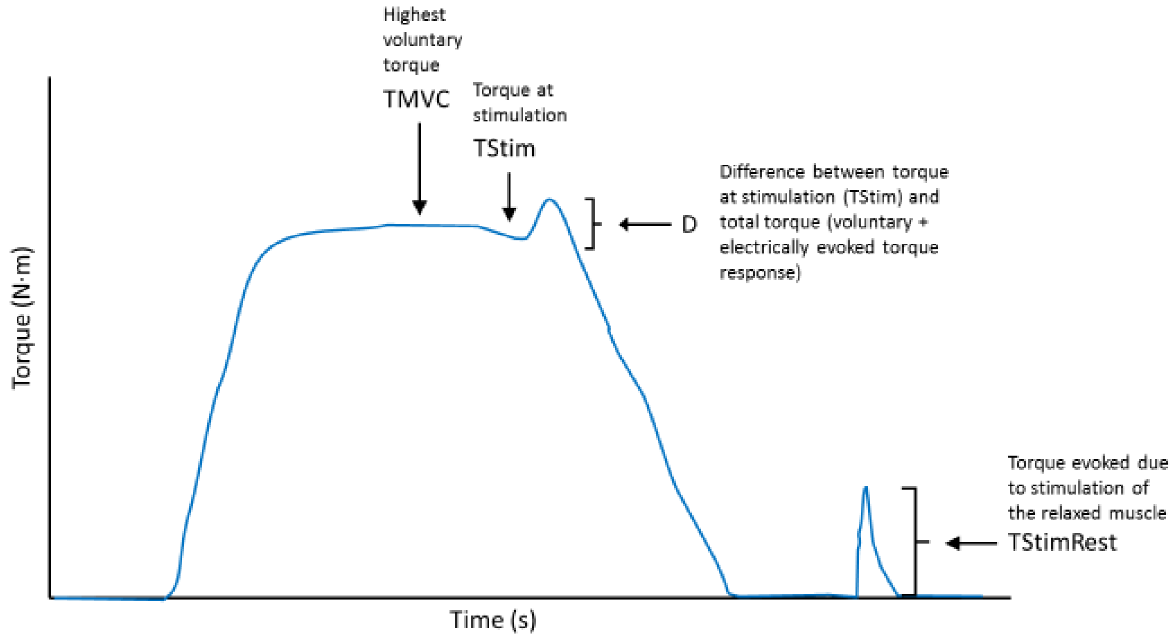


Figure 5: Example of the twitch interpolation technique during maximal isometric voluntary contraction. TMVC, Torque at maximal voluntary contraction; TStim, Torque at stimulus; TStimRest, Torque at stimulation in relaxed muscle; D, Difference between TStim and total torque.

2.11 Level of maximal voluntary muscle activation in elderly individuals

For the estimation of maximal voluntary muscle activation in elderly individuals, primary the isometric form of voluntary contraction is conducted due to methodological difficulties under dynamic contraction (e.g. changes in force-velocity and length-velocity relationship during applied muscle force) (Gabriel, Kamen, & Frost, 2006). Furthermore, reaching an activation level above 90% in the knee extensor is proposed as fully voluntary activated muscle, since this *"cut-point"* is commonly achieved in young active individuals (Hvid et al., 2016; Shield & Zhou, 2004; Suetta et al., 2009). An overview of activation levels is presented in table 1, it shows various studies utilising isometric contraction during the past decades in higher aged individuals.

Interestingly, except the study by Cannon, Kay, Tarpenning, & Marino (2007), all other studies displayed submaximal activation level, regardless of the participants' physical condition. In the survey of Suetta et al. (2009) even moderately active 61 to 74 years old subjects showed an activation level below 90%, indicating that incomplete voluntary knee extensor activation in older individuals may be explained to a greater extent by age-related neuronal deterioration rather than by inactivity (Aagaard et al., 2010; Hvid et al., 2014; Tracy & Enoka, 2002). Nevertheless, non-active individuals showed a constantly lower activation level as compared to active individuals. In this regard, the study by Venturelli et al. (2015)

demonstrated a 7.1% higher level of muscle activation in mobile vs. immobile subjects emphasising hereby a disuse-related reduction in voluntary muscle activation.

The ability to produce muscle force was seen to depend on the contraction form. While Tracy & Enoka (2002) observed reduced force production in the m quadriceps during isometric contraction compared to dynamic contraction, results reported by Babault, Pousson, Ballay, & Van Hoecke (2001) showed highest force production during isometric (95.2%) compared to concentric (89.7%) and eccentric (88.3%) contractions.

However, the data should be studied with care and evaluated in the light of the different methods, stimulus quantity, as well as type of contraction.

Table 1: Overview of voluntary muscle activation level in elderly individuals.

Study	Age (years)	N	Condition	Activation level (%)	Method	Stimulus	Contraction
Hvid et al. (2016)	76 – 93	16	Mobility-limited	78.9 ± 3.5	ITT (adj. equ.)	Doublet	Isometric
Harridge et al. (1999)	85 – 97	11	Frail	81.0 ± 7.0	ITT	Singlet	Isometric
Venturelli et al. (2015)	86 – 92	8	Immobile	78.2 ± 3.0	ITT	Singlet	Isometric
Venturelli et al. (2015)	85 – 92	8	Mobile	85.3 ± 2.2	ITT	Singlet	Isometric
Cannon et al. (2007)	64 – 78	8	Moderate active	96.2 ± 2.0	ITT	Doublet	Isometric
Suetta et al. (2009)	61 – 74	9	Moderate active	88.6 ± 1.6	ITT (adj. equ.)	Doublet	Isometric

N, Number of participates; ITT, Interpolated twitch technique; Adj. equ., adjusted equation.

2.12 Effects of strength training on maximal voluntary muscle activation and functional benefits in advanced age

The negative effects of disuse on the level of maximal voluntary muscle activation in higher aged individuals have been stated above. With respect to the impact of resistance training on older adults, increases in maximum MU firing rate were achieved in the m. vastus lateralis (Karmen & Knight, 2004) evidencing that increases in voluntary muscle activation might be possible. However, only a few studies investigated the neuromuscular changes of activation level in higher aged adults using the ITT.

A study worth mentioning was conducted by the research group led by Suetta et al. (2009), in which the change of muscle activation following a unilateral immobilisation period was investigated in young (21 to 27 years) and older (61 to 74 years) moderately active men. One leg of each of the eight subjects was immobilised using a lightweight fiber cast for two weeks before starting a 4-weeks strength training intervention, three times a week. Two weeks of immobilisation resulted in significant reduced voluntary muscle activation in older, but not in younger men as shown in table 2. In the group of the older men the activation level in the control leg remained significantly unchanged while the immobilised leg showed a significant improvement after re-training. The authors suggest that aged individuals could be more affected by neuronal changes in response to short-term immobilisation.

Table 2: Effect of immobilisation and re-training on voluntary muscle activation in young and older moderate active individuals (Suetta et al., 2009).

	Young men (N = 11)		Older men (N = 9)	
	Immobilised	Control	Immobilised	Control
Baseline	91.6 ± 1.6	92.3 ± 1.5	88.6 ± 1.6	86.9 ± 3.2
2-weeks immobilisation	90.6 ± 2.8	89.6 ± 2.9	80.2 ± 2.8†	83.2 ± 2.5
4-weeks re-training	95.2 ± 1.5*†	92.4 ± 1.7	90.6 ± 2.8‡	90.8 ± 2.8

Values are mean ± SE. *: significant different from 2-wk immobilisation, †: Significant different from Pre, ‡: old men significant different from young men $P<0.05$.

Furthermore, two studies investigated the effects on voluntary muscle activation by a 12-weeks progressive resistance training. The first study was conducted by Harridge et al. (1999), in which the subjects were frail older adults (85 to 97 years, N=11) and recruited from a geriatric day hospital. The resistance training for knee extensor and flexor muscles was conducted three times per week but demonstrated no significant impact on m. quadriceps activation level (81% to 85%).

The second study performed by Hvid et al. (2016) targeted on changes in gait speed in older adults with mobility limitations (82.3 ± 1.3 years, N=16). A significant 6% (78.9% to 84.9%) improvement in voluntary muscle activation in m. quadriceps was detected and positively associated with increased gait speed. Greater improvements were discovered in subjects having an incomplete activation level (Figure 6).

A third but slightly shorter research in moderate active older women (64 to 78 years, N=8) comprised 10-weeks of resistance training for knee extensor and flexor with three training sessions per week. But it failed to demonstrate statistically significant changes in knee extenstor activation level (96.2% to 98.3%) (Cannon et al., 2007).

To summarise, the duration of 10 to 12-weeks regular resistance training is generally suggested as sufficient to demonstrate neuromuscular adaptions (Macaluso & De Vito, 2004), whereas improvements in voluntary muscle activation were seen to be difficult to achieve during this period (Cannon et al., 2007; Harridge et al., 1999; Lars G. Hvid et al., 2016).

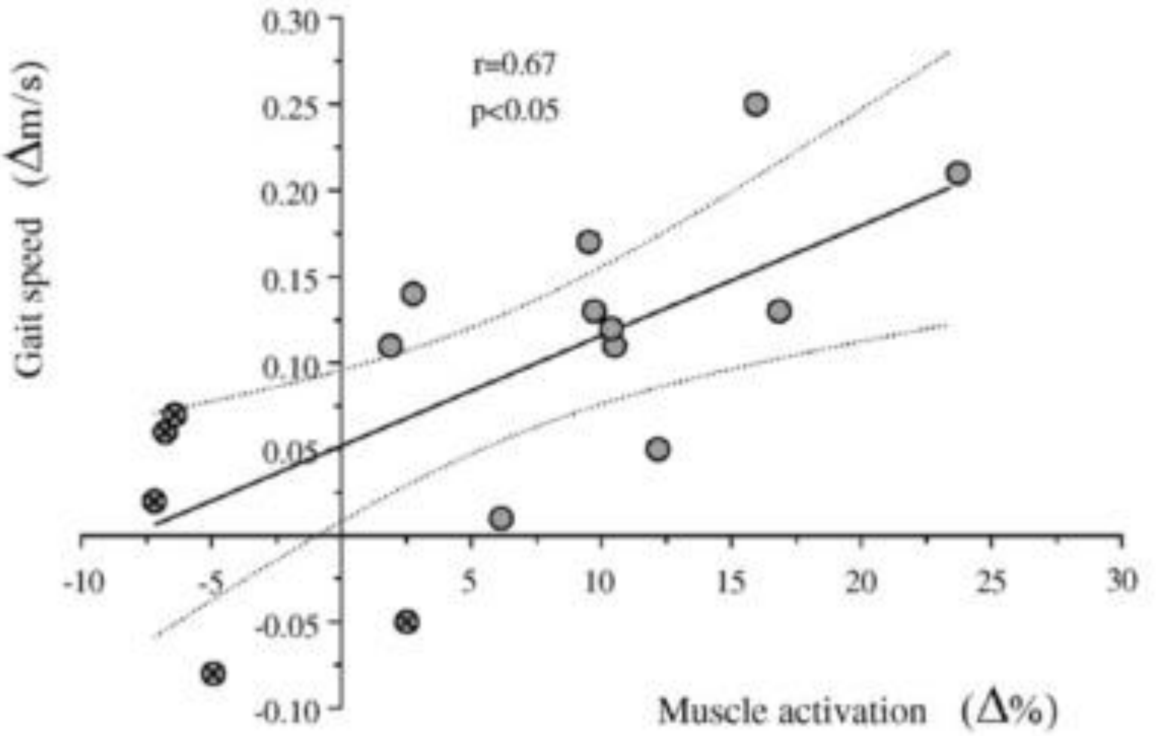


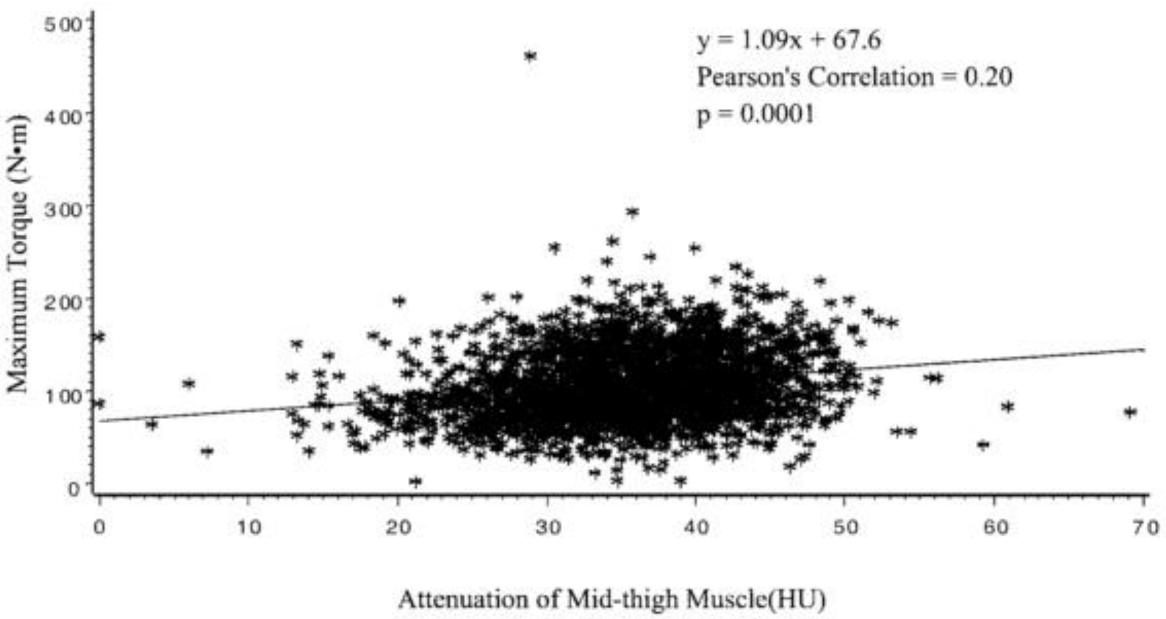
Figure 6: Association between training-induced changes in knee extensor voluntary muscle activation and two minutes walking test maximal gait speed. N=16, age 76 – 93 years. Symbols marked with an X determine participants having an activation level over 90% (Hvid et al., 2016).

2.13 Myosteatosis

Within the skeletal muscle two fat depots can be discriminated: a) fat infiltration within the muscle, known as intramuscular adipose tissue (Intramuscular AT) which can further be divided into intra- and extramyocellular lipid (IMCL and EMCL, respectively) and b) fat within the muscle fascia and between muscles bundles defined as intermuscular adipose tissue (IMAT). Both, intramuscular AT and IMAT are known as myosteatosis and associated with impaired skeletal muscle function when increased. (Goodpaster et al., 2001; Miljkovic & Zmuda, 2010).

Age-related loss of muscle strength associated with fat infiltration

Ageing is associated with a continuous annual loss of muscle strength (Delmonico et al., 2009; Frontera et al., 2000; Skelton et al., 1994) by a simultaneous increase in fat accumulation in the muscle (Marcus, Addison, Kidde, Dibble, & Lastayo, 2010; Reinders et
2.14 al., 2016). Further factors such as sarcopenia (Janssen et al., 2000), MU degeneration (Lexell et al., 1983) or alterations in muscle architecture (Narici et al., 2003) are well known as strong contributors regarding the decline in muscle strength. However less is known about the impact of muscle fat infiltration on muscle strength at higher age. During the Health ABC study Goodpaster et al. (2001) investigated the relationship between fat-infiltrated lean tissue and maximum torque in 2,627 individuals aged 70 to 80 years. Results showed a decreased attenuation value (which is associated with higher muscle fat infiltration) with increasing age. Furthermore, isometric knee extensor strength was positively related to higher mid-thigh attenuation independent of muscle mass (Figure 7). Thus, these findings demonstrated that lower muscle strength is associated with higher fat accumulating within the thigh muscle CSA.



2.15 *Figure 7: Association between mid-thigh attenuation value and torque in men and women with a mean age of 73.6 years (N=2,627). HU; Hounsfield units (Goodpaster et al., 2001).*

In vivo measurement methods for muscular fat infiltration

Besides utilising invasive techniques such as muscle biopsies to quantify fat infiltration within the muscle, non-invasive methods such as computed tomography (CT), magnetic resonance spectroscopy (MRS) or magnetic resonance imaging (MRI) have demonstrated to be promising methods delivering accurate information about fat infiltration within the muscle CSA. Although non-invasive methods have limitations in measuring fat infiltration within the muscle cell directly or in differentiating between IMCL and EMCL (Goodpaster et al., 2000a; Larson-Meyer et al., 2006), they are able to obtain highly reliable information about the fat infiltration within a muscle CSA. Furthermore, the shorter processing time compared to invasive techniques makes CT suitable for clinical investigations (Goodpaster et al., 2000a; Larson-Meyer et al., 2006; Strandberg, Wretling, Wredmark, & Shalabi, 2010).

Muscle fat infiltration measured by computed tomography

The imaging technique CT records large series of two-dimensional images and creates a
2.16 three-dimensional image that allows to reconstruct volume structures of the inside of a body. With a mathematical algorithm it is possible to calculate the degree of attenuation and reconstruct a picture for each voxel (volume element) of the three-dimensional section. A voxel equals a pixel (picture element) in a two-dimensional image but includes the slice-thickness and is allocated to the same area as the pixel. Consequently, a given pixel represents the average tissue properties within a voxel (Goodpaster, Thaete, & Kelley, 2000b), Kalender, 2011).

> Each pixel in the two-dimensional CT image has a specific number that corresponds to a specific location within the patient. The typical matrix of pixels is 512 × 512, and the range of numbers is from −1000 to +3095 (4096 values). The numerical value of each pixel within the matrix corresponds to a specific level of gray within the image. These values are called CT numbers or Hounsfield Units, corresponding to the linear attenuation coefficient, which, in turn, depends on physical properties of tissue within the voxel (Goodpaster et al., 2000b).

Thus, the attenuation value, respectively the Hounsfield Unit (HU) represents the relative density and chemical composition of tissue. In that regard CT allows to distinctive distinguish between different types of tissues since fat displays a negative attenuation (darker areas) value while lean tissue has a positive attenuation (lighter areas) (Figure 8). In CT analyses of the muscle CSA, it is further possible to characterise the composition of lean tissue based on its attenuation which is inversely related to the fat content. Therefore, the skeletal muscle is commonly divided into areas consisting of normal-density lean tissue

(NDM) and areas consisting of low-density lean tissue (LDM). In this regard, lower attenuation values are associated with a higher lipid accumulation in lean tissue and vice versa (Figure 9) (Aubrey et al., 2014; Correa-de-Araujo et al., 2017; Goodpaster et al., 2000b; Larson-Meyer et al., 2006).

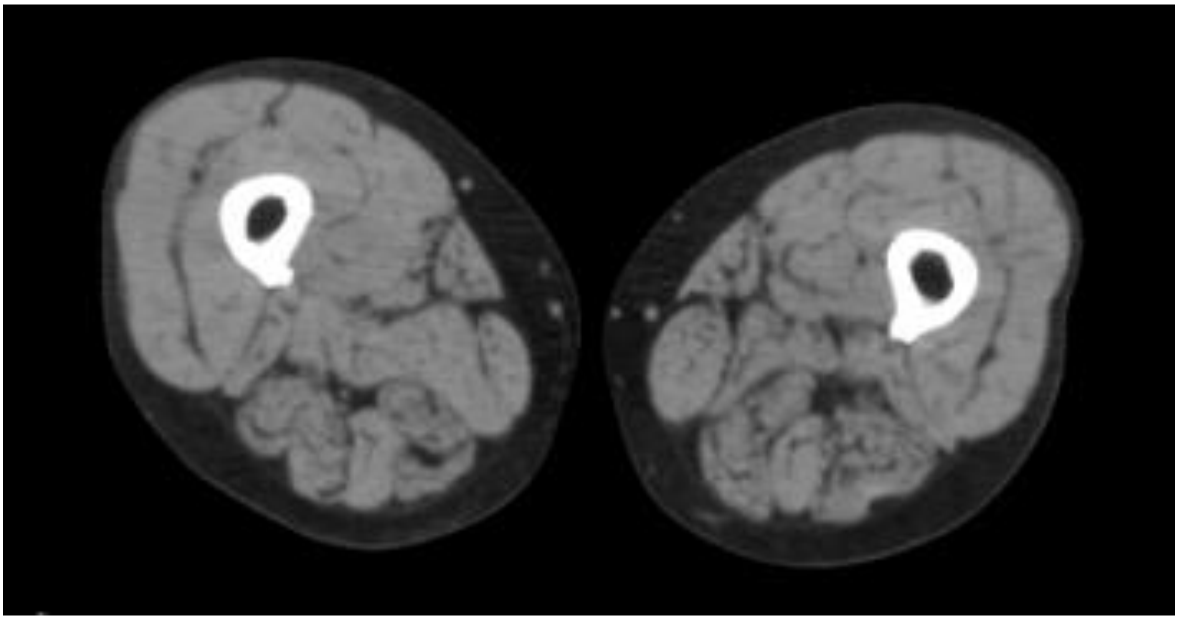


Figure 8: Mid-thigh CT scan. Male 72 years of age demonstrating adipose tissue (black), muscle (grey) and bone (white).

Concerning the quantification of the attenuation value, the HU scale uses the original linear attenuation coefficient of the radiodensity which utilises water at standard pressure and temperature (defined as 0 HU) and air at standard pressure and temperature (defined as -1,000 HU) as reference (Goodpaster et al., 2000a; Goodpaster, Kelley, Wing, Meier, & Thaete, 1999).

$$HU = CT\ Number = \left(\frac{\mu X - \mu Water}{\mu Water - \mu Air}\right) \cdot 1000$$

The equation (Kalender, 2011) used to obtain HU (also known as CT number) defines µ X as the linear attenuation coefficient of tissue, while µ Water and µ Air represent the linear attenuation coefficient of water respectively air.

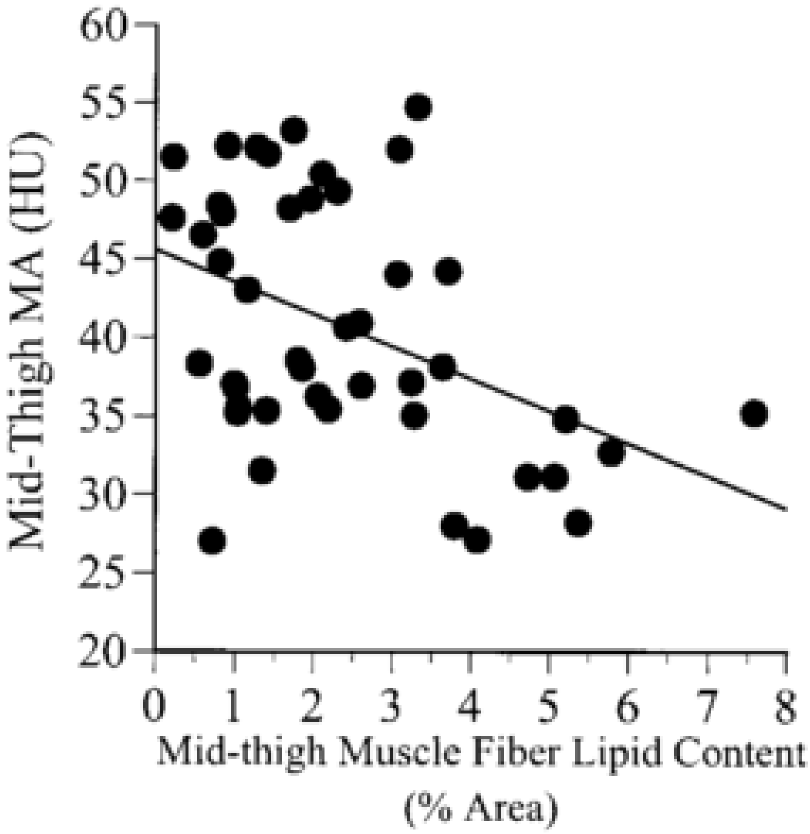


Figure 9: Association between mid-thigh muscle attenuation and muscle lipid content. Histologically determined with oil red O staining within muscle fibers. R = -0.43, P < 0.01, (N=45) (Goodpaster et al., 1999).

2.17 Attenuation and Hounsfield Units

When taking a closer look at the HU, different ranges were introduced in the past few years to discriminate between muscle and adipose tissue based on is density. In this regard, Kelley, Slasky & Janosky (1991) used a range of -200 to -1 HU to identify AT. Furthermore, a concept of classification within muscle density has been established. Lean tissue was determined between 0 to 100 HU, whereby regions with 1 to 34 HU were defined as low-density lean tissue and values regions between 35 to 100 HU as normal-density muscle. In contrast, Chowdhury et al. (1994) did not differentiate within lean tissue but used ranges from -190 to -30 HU for AT and -29 to 152 HU for lean tissue.

A few years later Goodpaster et al. (1999) established the most frequently applied cut-offs reflecting muscle density by defining the range of LDM between 0 to 30 HU and NDM between 31 to 100 HU. The cut-offs for AT were set between a range of -190 to -30 HU. However, the cut-offs were not standardised so far which limits the possibility to compare between various investigations (Aubrey et al., 2014). The histograms in figure 10 demonstrate the range of muscle attenuation within a lean and an obese individual where lower attenuation values indicate an increased lipid content within the muscle (Goodpaster 200b).

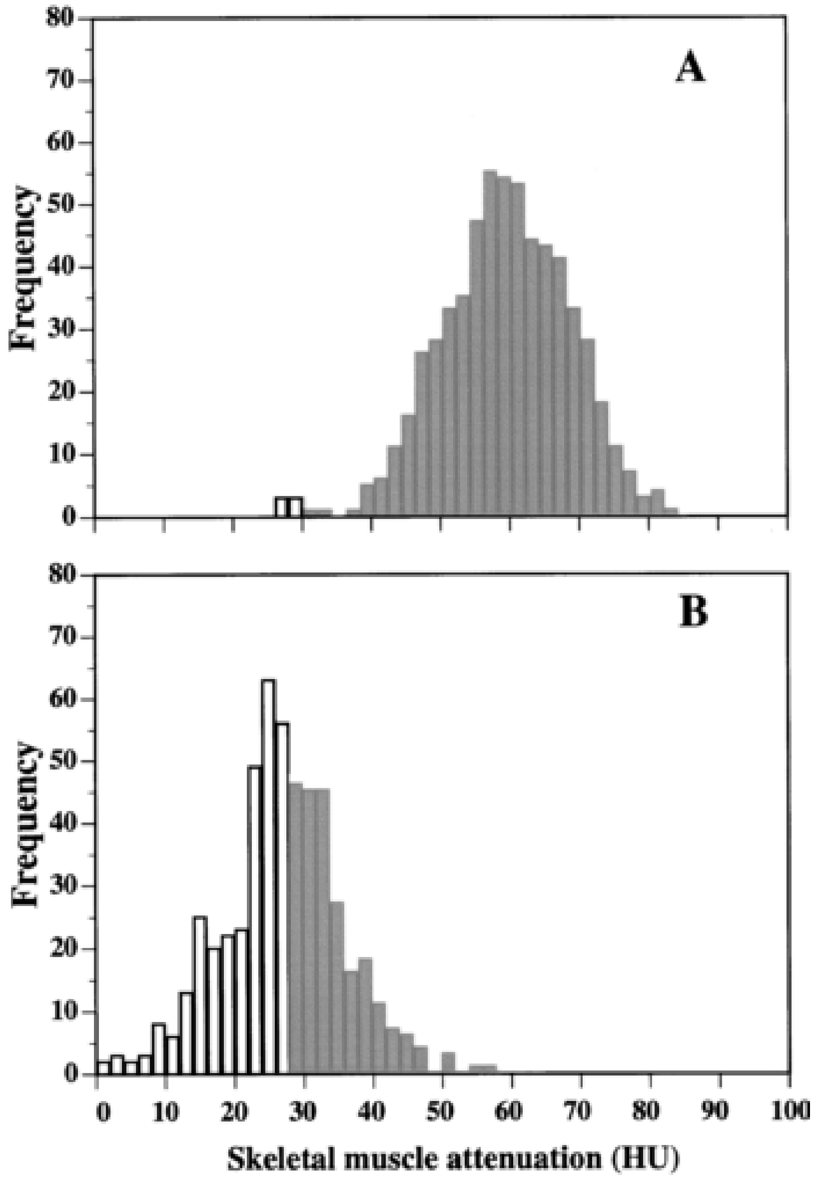


Figure 10: Muscle attenuation characteristics determined by CT in two exemplary histograms of (A) a lean and (B) an obese individual. The frequency of pixels from 0 to 29 HU (LDM) is shown in open bars while the frequency of pixels from 30–100 HU (NDM) is shown in filled bars. The lean person presents very few open bars
2.18 *in the LDM range, whereas a lot more pixels are presented in the obese individual by LDM (Goodpaster et al., 2000b).*

Age-related fat accumulation within the muscle CSA

Myosteatosis, the accumulation of IMAT and intramuscular AT within the muscle CSA, was obsered to increase during the process of ageing (Miljkovic & Zmuda, 2010; Reinders et al., 2016). Similar outcomes were discovered by Marcus et al. (2010) in ambulatory individuals aged 18 to 87 years as higher age was associated with greater IMAT accumulation in the thigh muscle (Figure 11).

Figure 11: Association between age and intermuscular adipose tissue (IMAT) in the thigh in ambulatory individuals. (N=88, Age 18 to 87 years) (Marcus et al., 2010).

In the Health ABC study, older healthy men (N = 813, 73.6 years) and women (N = 865, 73.2 years) showed increased IMAT content of 3.1% and 1.7% respectively, within an observation period of 5 years. This increase in IMAT was observed independently from body weight changes or changes in subcutaneous AT (Figure 12). The findings suggest, that IMAT accumulation might be a constant process in ageing since it was even seen in individuals who were losing weight and reducing subcutaneous AT (Delmonico et al., 2009).

It is suggested that type-I muscle fibers have a greater capacity for fatty acid uptake and increased triglyceride stores (Dyck et al., 1997). Additionally, ageing is associated with an increase in the relative proportion of type-I muscle fibers (Askanas & Engel 1975). Linking these findings together, this may explain the decline in muscle attenuation in elderly individuals (Goodpaster et al., 2001). Together with the aforementioned degenerative processes during aging this could lead to the replacement of functional muscle tissue with IMAT (Vettor et al., 2009).

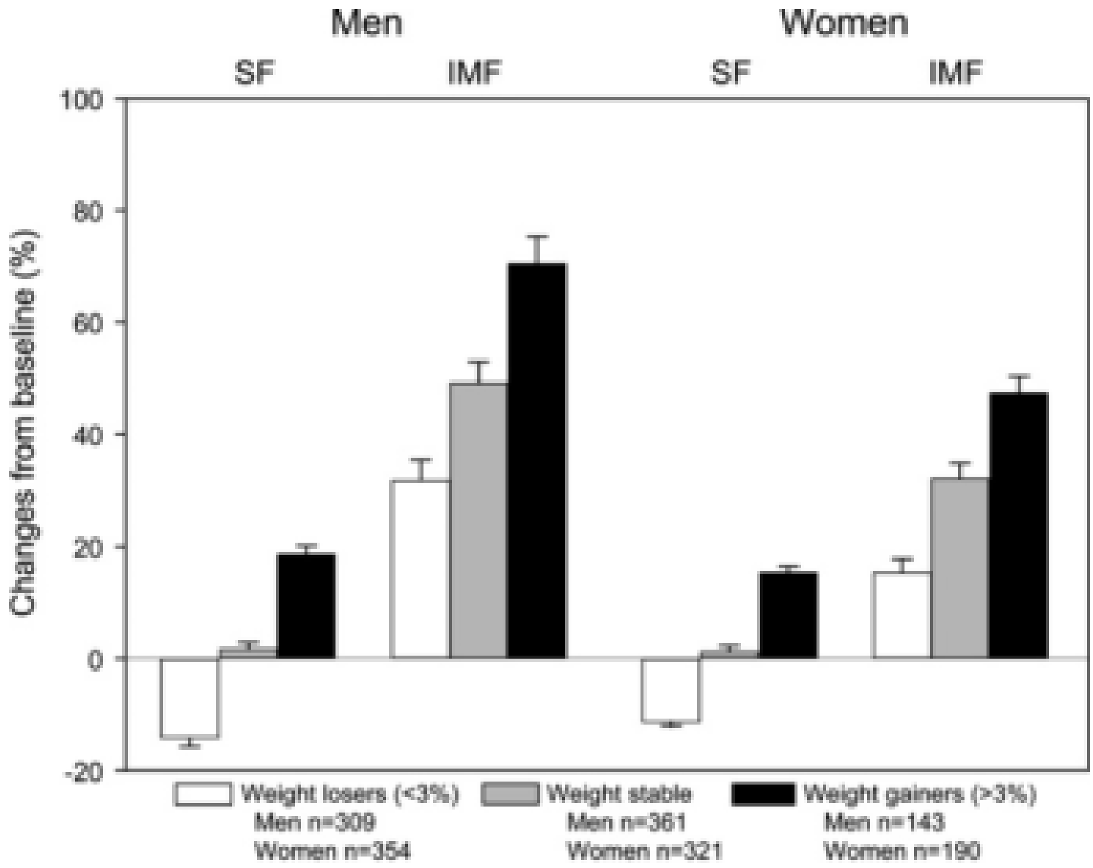


Figure 12: Percentage change in in fat accumulation after 5- years within different weight change groups for men and women. SF; Subcutaneous adipose tissue, IMF; Intermuscular adipose tissue (Delmonico et al., 2009).

2.18.1 Pathophysiological implications of IMAT accumulation

The pathophysiological accumulation of IMAT demonstrated by a lower mid-thigh muscle attenuation is associated with a higher incidence of mobility limitation (Visser et al., 2005). It is further considered to have a strong relationship to the total amount of body adipose tissue (Gallagher et al., 2005). Since IMAT content was greater in patients with type-2 diabetes mellitus (BMI 31.6kg/m²) as compared to a healthy control group (BMI 29.6kg/m²), a relationship with diabetes mellitus type-2 is assumed (Gallagher et al., 2009). This assumption is strengthened due to an IMAT-attributed increase in insulin resistance (Goodpaster et al., 2000a). Nevertheless, not only does the gain of non-contractile tissue support IMAT accumulation, the loss of muscle mass was also demonstrated to be tightly related to increased IMAT (Vettor et al., 2009). Furthermore, central muscle activation was seen to be decreased in older individuals (N=15, mean age of 75 years) with higher IMAT, while those with low levels of IMAT displayed normal central muscle activation. Findings suggest that the MU array might be inhibited in elderly individuals through fat accumulation within the thigh (Yoshida, Marcus, & Lastayo, 2012).

2.18.2 Pathophysiological implications of low muscle density

Investigations of Goodpaster et al. (2000a) detected an association between skeletal muscle attenuation and muscle lipid content in the mid-thigh. Results demonstrated that subjects (aged 25 to 49 years, BMI 18.5 – 35.9 kg/m^2) with greater lipid content within the muscle had lower muscle attenuation. Additionally, Goodpaster & Kelley (2002) showed an association between increased triglyceride accumulation in the muscle and reduced insulin sensitivity. A few years later observations over 4.7 ± 0.8 years of follow up in older adults aged 70 to 80 years (N=1,678) indicated that low muscle strength and low muscle density (but not muscle size or lean mass) were associated with increased risk of hospitalisation (Cawthon et al., 2009). In summary, all these findings suggest LDM as a potential marker for poor muscle function, disease and higher risk of developing insulin resistance. In return a higher value of muscle density was seen as contributor to greater absolute muscle strength in older adults (Goodpaster et al., 2001).

When investigating fluid shift from standing to acute supine rest on mid-thigh muscle CSA, a significant decrease in NDM by 1.6% was observed between 5 and 15 minutes of rest compared to non-significant changes in LDM. The exact mechanism for the evident differences between NDM and LDM in response to spine rest are not clarified. The authors attributed the results to the relatively anhydrous characteristics of fat tissue compared with muscle tissue. Findings would therefore underline the association of an increased lipid content within LDM (Cerniglia, Delmonico, Lindle, Hurley, & Rogers, 2007).

2.19 Effects of strength training on fat infiltration in higher age

The beneficial effects of physical activity were shown by reduced IMAT after 1-year of moderate exercise (Figure 13) (Goodpaster et al., 2008). Furthermore, fat accumulation within the muscle was observed to be inversely associated to the level of physical activity and it is suggesting that doubling the level of physical activity potentially halves the amount of intramuscular AT (Narici & Maffulli, 2010).

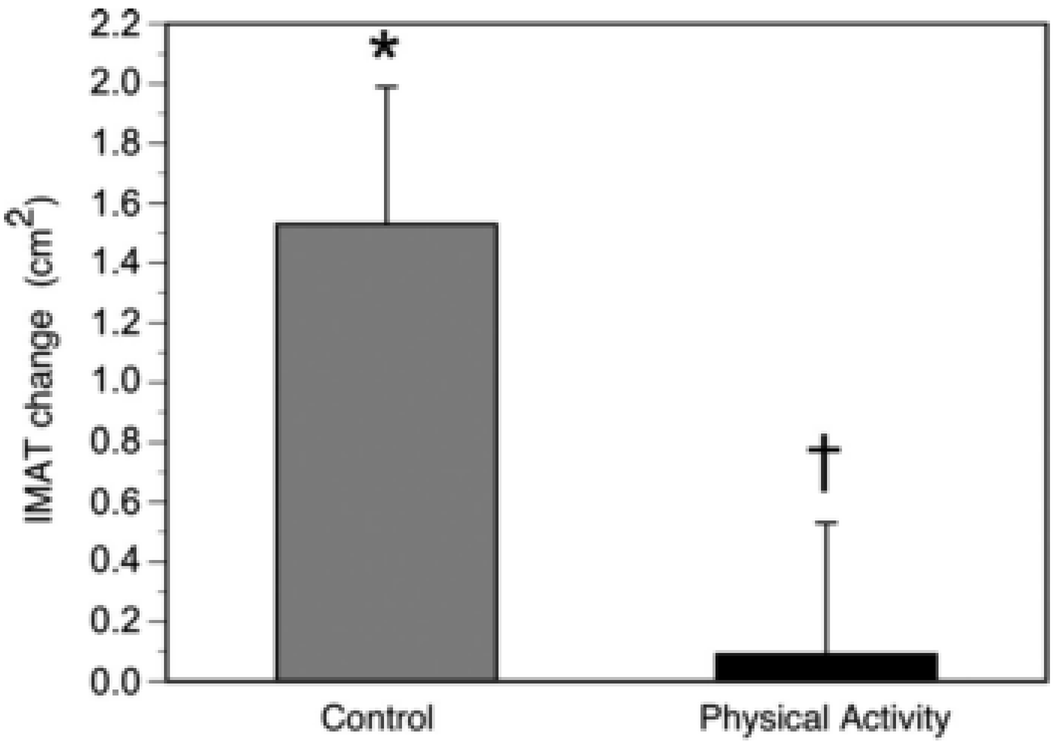


*Figure 13: Changes in mid-thigh intermuscular adipose tissue (IMAT) in the physical activity and control groups. *: within group change, P < 0.05, †: between group change, P < 0.05 (Goodpaster et al., 2008).*

Marcus et al. (2010) investigated the impact of a 12-weeks high-force resistance training on the knee extensor in ambulatory subjects over 55 years of age (N=32). Results displayed a decrease of 11% in thigh IMAT and of 7% in thigh lean tissue. However, this study had its limitations as only the percentage change was presented and not absolute numbers. A few years later Marcus, Addison, & Lastayo (2013) reported a significant increase in thigh-MQ from 2.7 to 3.0 N/cm^2 in limited elderly (BMI 28.9 ± 5.5 kg/m^2) after a 12-week multicomponent training in a low intramuscular AT group, whereas no changes were found in a high intramuscular AT group. This indicates that greater intramuscular AT potentially inhibited the increase in thigh-MQ while lower intramuscular AT did not show any negative effect.

Taaffe et al. (2009) presented a highly interesting study, investigating the change in HU after a 60-weeks intervention composed of resistance training (24-weeks), detraining (24-weeks) and retraining (12-weeks) in healthy elderly individuals (N=13, 65 to 83 years of age, BMI 27 kg/m^2). Detraining resulted in a 7.7% decrease in HU in m. quadriceps. Subsequent retraining caused an increase of 5.4% (for unnamed reasons no data of the initial 24-weeks training were presented in the study) (Figure 14). Furthermore, m. quadriceps volume changed by 3% following retraining (122cm^3 to 126cm^3) while IMAT investigated for the whole thigh did not significant change in volume neither after detraining nor retraining. Results in knee extensor strength (1RM) displayed changes after baseline (35.5kg), pre-detraining (59.8kg), post-detraining (46.9kg) and retraining (57.3kg). Thus, a positive impact on MQ with increased strength and greater muscle density after a 12-weeks resistance training could be observed.

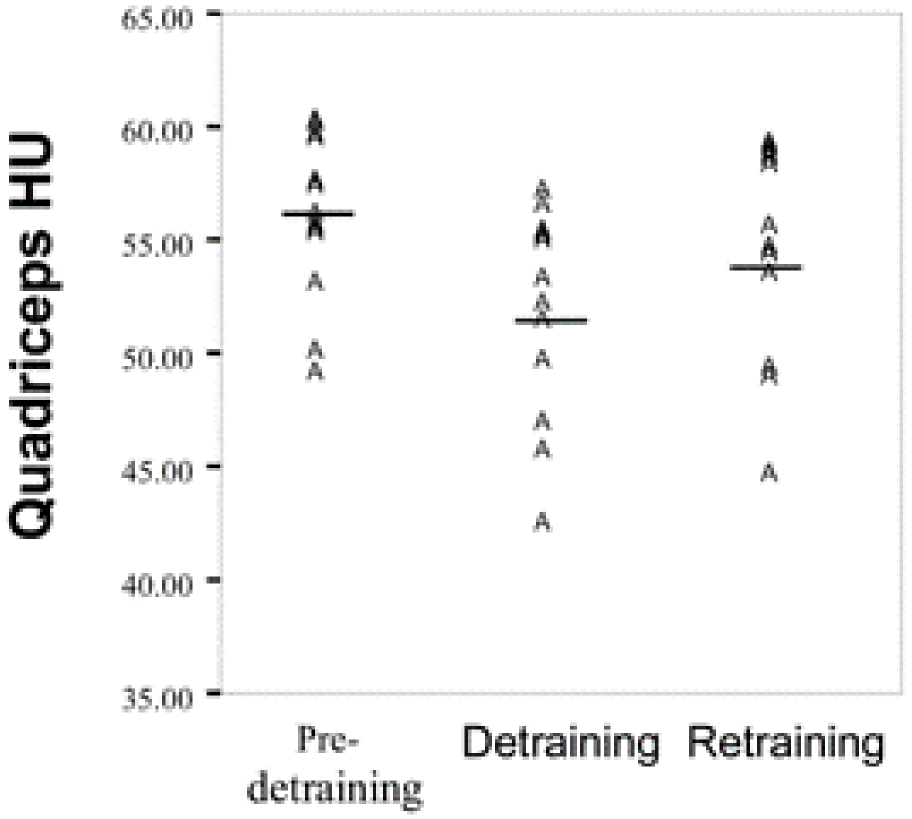


Figure 14: Changes in muscle attenuation in the knee extensor expressed in Hounsfield Units (HU) following detraining (24-weeks) and retraining (12-weeks) in elderly men and women (N=13) (Taaffe et al., 2009).

3 Aims

Knowing that fat infiltration and neuronal deterioration are two considerable contributors to the reduction of muscle strength during ageing, this thesis aims to demonstrate the influence of both factors on determining physical performance in frail elderly individuals.

The main focus of this thesis is to investigate the relations between muscle size and density measured with CT and force-generating capacity in the knee extensor. A secondary aim examines the level of deterioration of MU activation capacity in the same cohort.

Furthermore, it is hypothesised that a 10-weeks resistance training intervention leads to a reduction in IMAT by a simultaneous increase in density within the m. quadriceps. It is also hypothesised that the same training intervention results in an improvement in the level of voluntary muscle activation.

4 Materials and Methods

4.1 Study design

The study was designed as a randomised controlled trail, and subjects were randomised by a computer program using a 1:1 allocation ratio into a training group (TG) and a control (CG).

The randomisation ranking was stratified by strength in knee extension and gait speed and separately performed for participants living in nursing homes and participants not living in nursing homes to ensure that living environment did not comprise allocation.

4.2 Study flow

To determine whether a strength training intervention impacts muscle fat infiltration and MQ, tests were done on different days. An overview about study flow is given in figure 15, whereas a more detailed description of the single steps is provided in the following paragraphs. Except computed tomography (Aleris Røntgen, Oslo) and functional tests all pre- and post-intervention tests were performed at the Norwegian School of Sport Sciences (NIH).

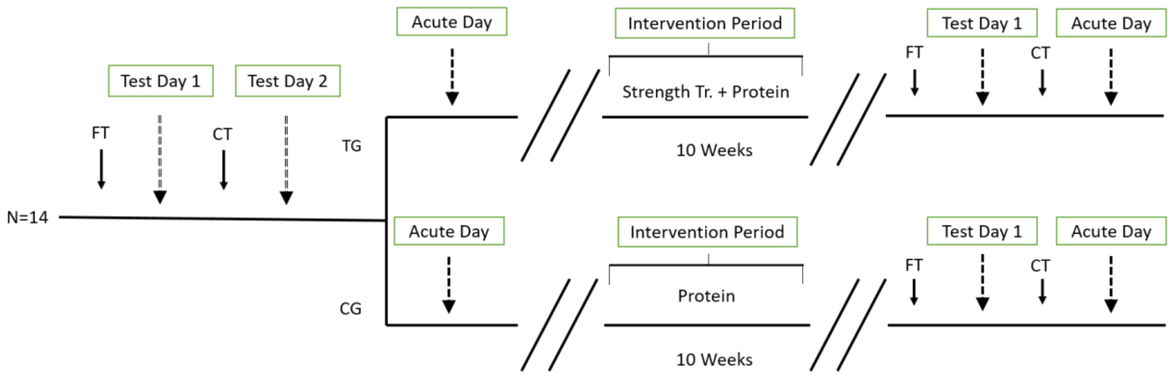


Figure 15: Overview of the study flow. TG, Training group; CG, Control group; CT, Computed tomography; FT, functional tests.

4.3 Recruitment

4.3.1 Subjects

The STAS-Study (Strength Training, Aging and Sarcopenia) included frail women and men aged ≥65 years. Subjects were recruited from nursing homes and day care centres in Oslo, Norway. The study was approved by the South-East Regional Ethical Committee of Norway

(2016/895/REK Sør-Øst) and carried out in agreement with the Declaration of Helsinki Committee.

During the first recruitment phase nursing homes and day care centre were contacted. General project aims and assignments were explained. Interested individuals were invited to preliminary examinations to check if inclusion criteria were fulfilled.

4.3.2 Inclusion and exclusion criteria

To determine whether subjects were eligible for participation, inclusion- and exclusion criteria summarized in table 3 had to be met.

Table 3: Inclusion- and exclusion criteria for STAS-Study.

Inclusion criteria	Exclusion criteria
Individuals ≥ 65 years	Musculoskeletal injury or health condition (e.g. heart attack within last six months)
SPPB ≤ 8	Uncontrolled hypertension (high blood pressure)
MMSE ≥ 22	Allergies to local anaesthesia
FFC ≥ 2	Lactose intolerance/Milk allergies

SPPB, Short physical performance battery (Guralnik et al., 1995); MMSE, Mini mental status examination (Strobel & Engedal, 2014); FFC, Fried frailty criteria (Fried et al., 2001); SF-36 Short form health survey (Ware & Sherbourne, 1992).

For this thesis, 17 participants were assessed for eligibility and allocated to the two groups as shown in the participants flow (Figure 16). Due to an unexpected high dropout rate in the in TG (6 participants in total), four more participants were recruited for this group (explained in more detail in the chapter 6.6 “Methodological considerations”). It should also be clarified that not all participants were able to perform every test due to health risks or acute pain on the day of the test. Participants fitted with a pacemaker were unable to perform the muscle activation test. Some participants were fitted with a hip prosthesis that caused problems in the CT image reproduction.

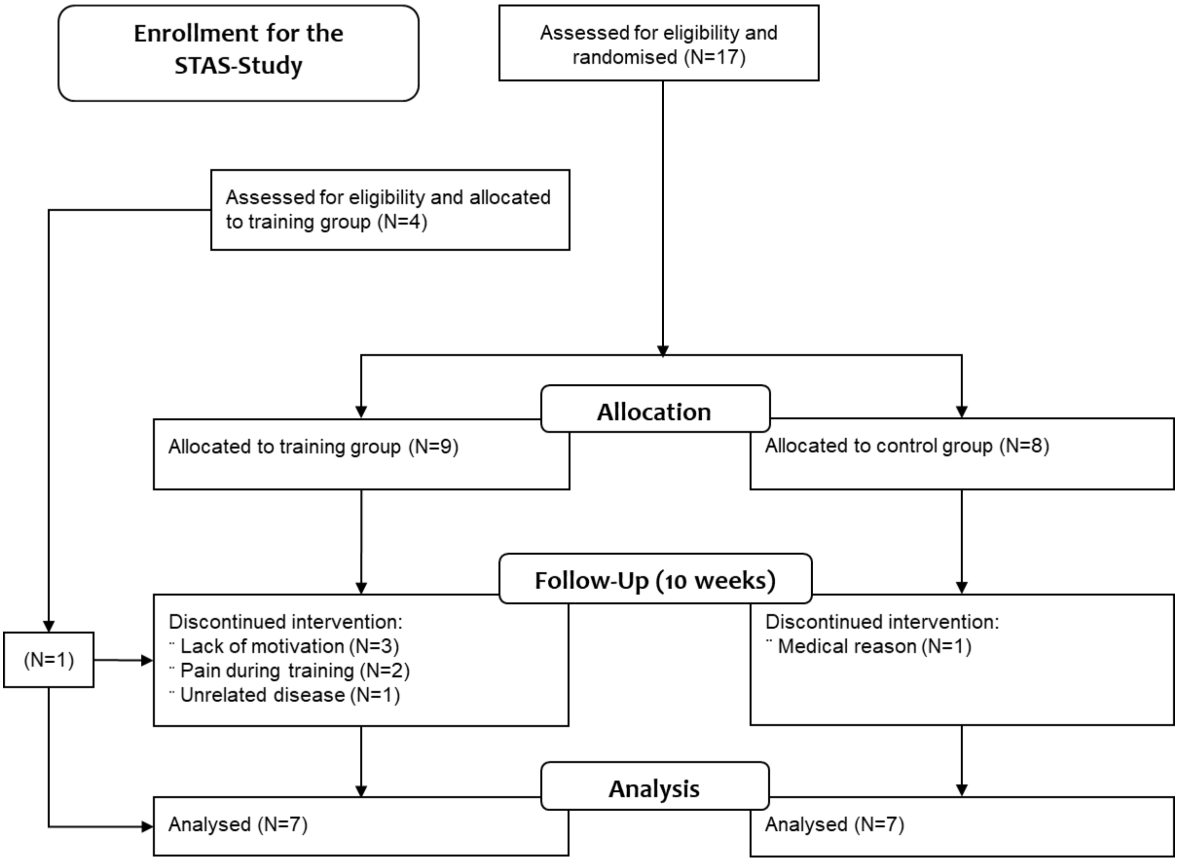


Figure 16: Participants flow

4.3.3 Fried frailty criteria

Since frailty was a substantial inclusion criteria for this study, Fried et al. (2001) frailty criteria was used as protocol to determine whether subjects were considered eligible for inclusion. It comprises five different aspects (weight loss, weakness, poor endurance, slowness and low activity), giving a possible score from 0-5 points. An older person (65+ years) is considered frail if three or more of these criteria are assessed as positive. Subjects characterized as frail were eligible for participation in this study. Furthermore, individuals determined with one or two positive criteria were characterized to be pre-frail and were included as well, given that they achieve at least two of the following criteria: weakness,
4.3.4 slowness and low physical activity. More detailed information about tests/cut-offs is shown in the appendix.

Short physical performance battery

A short physical performance battery (SPPB) related to the protocol of Guralnik et al. (1995) was executed in addition to the Fried frailty criteria to detect whether participants fulfil inclusion criteria. Subjects with a score of 8 or lower on the SPPB were included regardless of characterization based on the Fried frailty criteria. The SPPB comprised three tests:

1) To perform the standing-balance test, individuals were instructed to attempt to place their feet in the side-by-side, semi-tandem (heel of one foot beside the big toe of the other foot) and tandem position (heel of one foot in front of the other foot) for 10 seconds each. The score used for additional analyses was dependent on the time participants could hold their position.

2) To perform the sit-to-stand test, a chair with armrest was used. Subjects started in seating position, leaning against the backrest and arms crossed in front of the body. A repetition was completed correctly if the participant got up to fully extended hip and knee and set down again touching the backrest with his back. Time for five correctly performed repetitions was taken by the test supervisor. The participant had two attempts with a two-minute rest in between. Participants that were not able to stand up without using their arms received no points. The highest score was used for additional analyses.

3) To perform the modified 4m-gait test, referred to Guralnik et al. (2000) with a static start a marked distance of 4 meters was to be completed. Therefore, light beams were placed on the start and 4 meters. Participants were instructed to accomplish the distance two times in their habitual gait speed. The fastest attempt was used for additional analyses.

The total performance score was calculated by summation of the scores for the tests of standing balance, sit-to-stand five times and gait speed. More detailed information about SPPB is presented in appendix 2.

4.3.5 Mini-mental state examination

To evaluate mental status and inclusion eligibility of participants a mini mental state examination MMSE-Nr2 (Strobel & Engedal, 2014) was performed. Only long-term nursing home residents had to complete this test and were entitled to take part in the study with a score equal 22 of 30 or higher.

4.4 Functional tests

Two functional tests were conducted before and after the intervention to see if the resistance training leaded to performance changes. Before the intervention tests were executed together with the SPPB and after the intervention tests were completed a few days before test day 1. The tests were a sit-to-stand and a gait test. The sit-to-stand test was the same as in the SPPB and the highest score was used for additional analyses. For the gait test a ten meters walk was completed. Therefore, light beams were placed on 4 and 8 meters. Participants were instructed to accomplish the distance three times in their habitual gait speed. Subsequent a two-minute rest, participants completed a second series of three attempts, now instructed to go as fast as possible. The fastest attempts of both series were used for additional analyses.

4.5 Test days

Before starting the intervention period, all subjects signed an informed consent form and underwent three days of testing (test day 1, test day 2 and acute test day). Test day 1 and test day 2 were separately implemented and by at least three days (same strength tests on both days). Further, the acute test day was held at least three days after test day 2. However, after the invention period only test day 1 and the acute test day were performed. All tests were performed at the NIH except for the CT-scans.

4.5.1 Test day 1

On test day 1 the participants' blood pressure were measured before a subsequent DXA scan to determine body composition. Furthermore, m. vastus lateralis was examined by ultrasound, before the strength tests began. All tests are explained in more details on the following pages.

4.5.2 Test day 2

Since most of the participants had no former experience in heavy strength training and testing, a second test day (test day 2) for the strength test was implemented 7 or 8 days after test day 1. The additional test day was conducted to ensure proper testing performance.

4.5.3 Acute test day

Although results from the acute test day are not relevant in this thesis, a brief overview is given for a better understanding of the whole testing procedure. The day started in the morning with a blood profile (cholesterol, glucose, triglycerides) followed by a standardized breakfast.

One hour after breakfast a muscle biopsy of the mid-section of the m. vastus lateralis was taken, applying the Bergström technique (Tarnopolsky, Pearce, Smith, & Lach, 2011). Fifteen minutes post biopsy both groups underwent a 5-min warm up on a stationary bike (Monark Ergomedic 828 E, Quebec, Canada) before testing MVC. After the MVC test a training session (for the TG) was performed in combination with protein supplementation (for both groups) followed by a second MVC test. Approximately 2.5 hours after the training session a second muscle sample was taken.

4.6 Intervention groups

The study was designed as a randomized controlled trail, and subjects were randomized by a computer program using a 1:1 allocation ratio. The eligible participants were randomly, but stratified by strength in knee extension and gait speed assigned and allocated to either the TG or CG. The randomisation was separately performed for participants living in nursing homes and participants not living in nursing homes to ensure that living environment did not comprise allocation.

4.6.1 Protein supplementation

Throughout the intervention period both groups (TG and CG) received a daily protein supplementation Tine Styrk 330 ml (Tine, Oslo, Norway, 330ml contain: 0.7g fat, 18.5g carbohydrates, 16.8g protein). The servings were done once (in the evening). Further a control form was handed out for documenting the intake.

4.6.2 Training intervention

The training period for the TG started once the test days and randomization was completed. Training was performed for ten weeks with two sessions per week. Protein supplementation was given once a day throughout the intervention period. Training was conducted in groups of one to three subjects maximum. The training program comprised two lower body exercises: leg press and knee extension (Table 4). Load was increased gradually based on individual progress. Each session lasted for 20-40 minutes. One or two instructors supported by a caregiver supervised all training sessions. To hold the costs for the participants to a minimum the training session were executed on different locations nearby the participant’s homes or day care centres.

Table 4: Training program.

Week	Exercise	Session 1			Session 2		
		Series	Reps	Load	Series	Reps	Load
1	Leg Press	3	12	<RM	3	12	<RM
	Knee Extension	3	12	<RM	3	12	<RM
2 -4	Leg Press	3	12	RM	3	10	RM
	Knee Extension	3	12	RM	3	10	RM
5 -7	Leg Press	3	10	RM	3	8	RM
	Knee Extension	4	10	RM	4	8	RM
8 -10	Leg Press	3	8	RM	4	6	RM
	Knee Extension	4	8	RM	4	6	RM

RM, repetition maximum.

4.6.3 Control group

Participants randomized to the CG consumed only protein supplementation once a day for 10-weeks and were encouraged to maintain their current habitual life style. After post-tests patients in the control group were offered supervised strength training twice a week for 10-weeks.

4.7 Assessments

4.7.1 Body Composition

All DXA analyses (DELPHI, QDR 4500 series, or QDR 1000, Hologic, Massachusetts, USA) were performed ahead the testing on test day 1. Body Composition included total and regional measurements. Analyses covered spine status (L2-T12) as well as thighs and full body composition scans. A pad as headrest was provided to prevent vertigo while lying flat on the bench.

4.7.2 One repetition maximum

For testing 1RM strength in knee extension, a leg extension machine (Gym 2000 Gym Equipment, Vikersund, Norway) was used (Figure 17). After a 5-min warm-up on a stationary bike (Monark Ergomedic 828 E, Quebec, Canada), participants were seated in upright position in the Gym 2000, fastened with a seatbelt to prevent movement of the hip, and instructed to cross their arms in front of their chest.

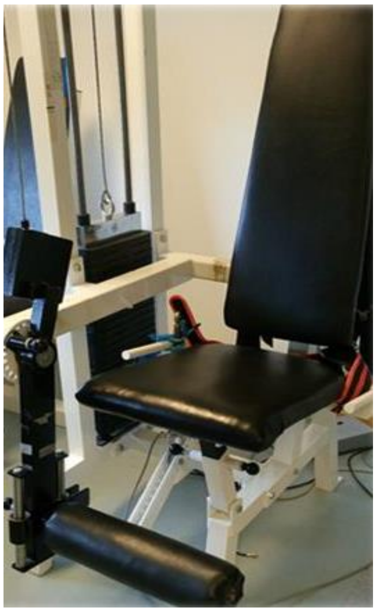


Figure 17. Gym 2000 Gym Equipment, Vikersund, Norway

The start angle of the knee joint was at 90° and for a valid trail participant had to reach an angle of 0° (full extension). After settings for backrest, knee angle and ankle joint position were adjusted, standardized and protocolled, warm up sets with both legs were completed. Since determining the proper starting weight in the older population is challenging, the starting weight was set on a low level and gradually increased. Based on expertise and observations of the test conductor 10, 6 and 3 repetitions (rep) at estimated 50%, 65% and 80% of the 1RM were performed.

In subsequence, one attempt at estimated 90 % of 1RM of one leg was performed before 1RM testing. 1RM was tested for both legs separately. An effort was made to reach 1RM within three attempts. The rest period between the trials was accounted for 1-min.

4.7.3 Maximum voluntary muscle activation

For this test participants with a pacemaker were excluded due to health risks.

Maximum isometric voluntary contraction was measured unilaterally using an isokinetic dynamometer (Humac Norm Model 502140, CSMi Medical Solution, Massachusetts, USA). The device was coupled to a generator (Digitimer DG2A Train/Delay Generator, Digitimer Ltd, Hertfordshire, United Kingdom) to assess voluntary muscle activation as well. The signal was sent twice (ITT) at 100Hz with a duration of 10ms each. Therefore, related to the test protocol used by Hvid et al. (2016), two surface stimulation electrodes were utilised (Veinoplus, neurostimulation electrodes, 8x13cm, Paris, France). One electrode was positioned on the distal (approximately 10 cm above the patella) and one on the proximal (approximately 15 cm below spina iliaca anterior superior) part of m. quadriceps femoris.

The exact distance was not measured. Singlets (single stimuli) were applied with gradually incremented amplitude, until no increase in force was observed. For the rest of the protocol, an amplitude of 10% above this amplitude was used.

For the test series participants were seated upright in the Humac apparatus, fastened with a seatbelt at the hip and instructed to cross their arms in front of their body to reduce coactivation through upper body movements. In addition, a knee joint angle of 70° in resting position was determined. After recording all adjustments, electrodes were attached. However, before testing maximum voluntary muscle activation, subjects were familiarized with the stimulation protocol followed by a knee extensor warm-up on the apparatus.

The warm-up included 8-10 isokinetic knee extensions and -flexions. All participants were advised to increase force production gradually (submaximal, 20%-70%). The warm up was followed by three isometric contractions at 70° with approximately 30%, 50% and 70% of the subjects MVC.

Subsequent a 2-min break, four singlets were applied at rest at 70° with approximately 5-sec between each singlet and followed by three MVC trails without stimulation. The contraction was maintained for approximately 3-sec or until the maximal force declined. A 1-min resting period between trials was given.

Following a 3-min break, two trails of MVC with stimulation were conducted. The protocol was the same as before, except when maximal force was achieved, a doublet (paired stimuli) was carried out. The subjects were then instructed to relax, before another doublet was given 2-sec later. A 2-min break between the two trials was given. The best of both results was used for further analyses.

The P MVC was calculated of the data received from the MVC and maximal voluntary muscle activation.

4.7.4 Twitch Interpolation technique

For this study, the interpolated twitch technique was used applying stimuli onto the muscle belly. On the purpose to recruit muscle fibres which were not activated by voluntary exertion, singlets and doublets were manually delivered at the plateau of MVC and two seconds after in the rested muscle. The extent of voluntary activation was quantified by the ratio of the superimposed torque during the MVC to the evoked torque measured at rest after the MVC (Figure 18).

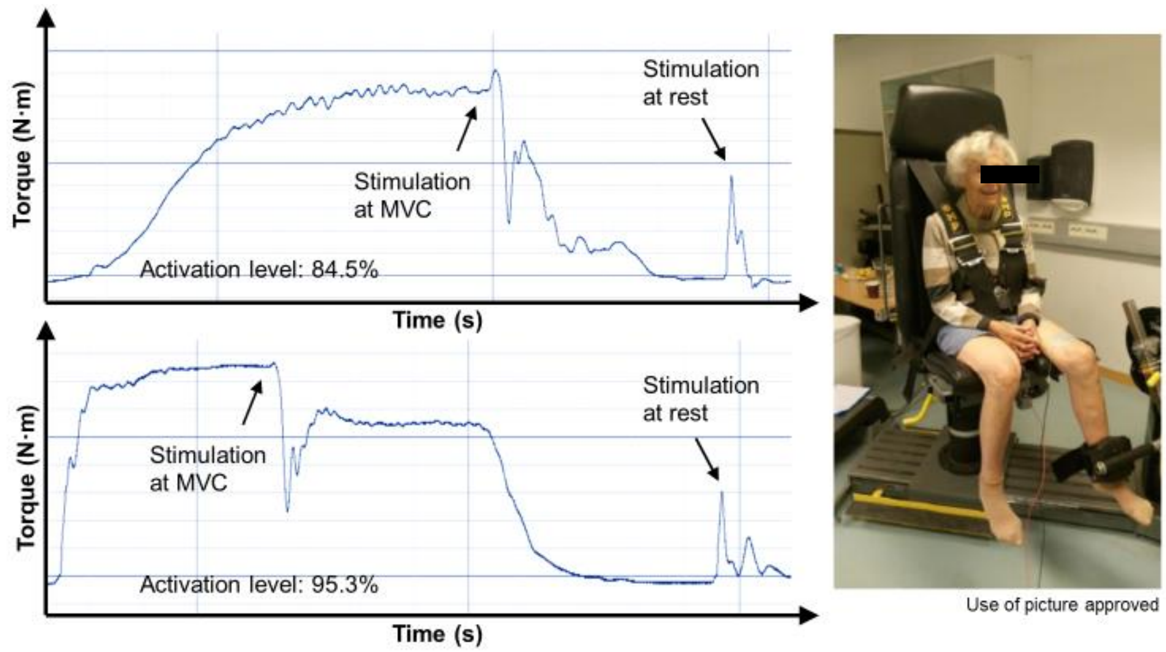


Figure 18: Twitch interpolation technique. MVC, Maximum voluntary isometric muscle contraction. Subsequent MVC an electrical stimulus evokes an additional torque, the muscle is then considered to be fully activated, whereas voluntary activation is considered to be sub-maximal.

4.7.5 Computed Tomography

Axial computed tomography (Toshiba Aquilion Prime, Canon Medical Systems Gmbh, California, USA) of the thighs, performed both before and after the intervention period, was providing information on muscle CSA and level of non-contractile tissue. Thus, three 8-mm-thick sectional scans (120 kVp, 300 mA) of both thighs were obtained for each subject and saved as DICOM images for further analysis. The sectional scans were obtained at the distal- (33%), proximal- (66%) and mid-thigh (50%) of the distance between the medial edge of the greater trochanter of the femur and intercondyloid fossa of the left leg (Figure 19).

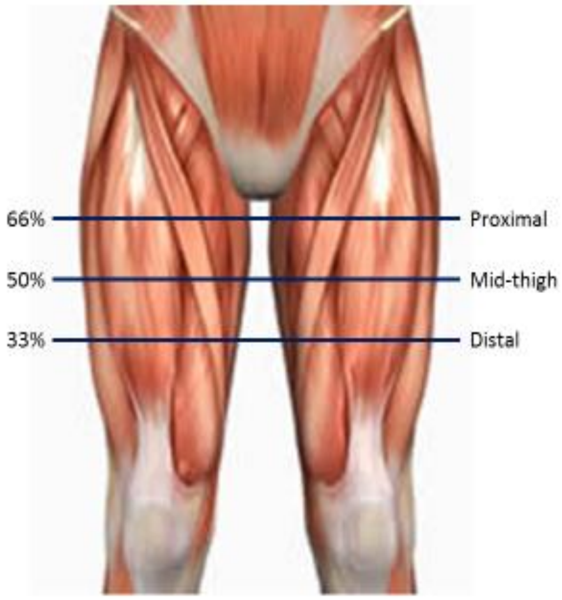


Figure 19. CSA scans of the thigh. (Myfit Fitness, 2017)

Average deviation for the ROI (region of interest) between pre- and post-scans was at 0.4 cm, ranging from 0.0 to 0.8 cm. If participants were not able to stretch their legs to full extent, a foam roll was applied under the knee to take the pressure.

4.7.6 Computed tomography analysis

For analysing CT-scans, Fiji a distribution of ImageJ2 (U.S. National Institutes of Health, Maryland, USA) was used to receive information about the composition of the muscle CSA of the mid-thigh. The software allows calculating muscle- and fat density based on its attenuation characteristics, expressed in HU. Adipose tissue and skeletal muscle area were estimated by the range of attenuation values of adipose tissue (-190 to -30 HU) and skeletal muscle (0 to 100) and presented in cm². The skeletal muscle was divided into NDM (31 to 100) and LDM (0 to 30) (Goodpaster et al., 1999). The area of the bone was manually excluded from the calculations. Values for muscle attenuation were gathered by estimation of all pixels between the ranges of 0 to 100 HU.

Figure 20 shows an example of the muscle CSA of the right mid-thigh and the performed steps before muscle attenuation was calculated. The IMAT was separated from the subcutaneous AT by manual drawing of a line along the deep fascial plane surrounding the thigh muscles (Figure 20 A & B). Bone was excluded and m. quadriceps was manually differentiated with care taken from hamstring- and adductor muscles (Figure 20 C & D).

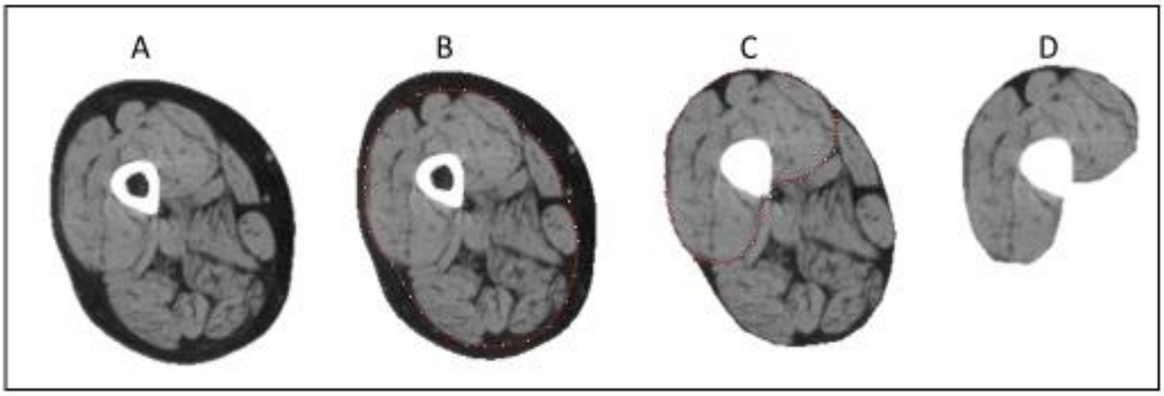


Figure 20: Right mid-thigh muscle CSA. Male, 83 years of age. CT scan total muscle CSA (A). Separation of subcutaneous AT (red line) (B). Separation of oss femoris and m. quadriceps (red line with white dots) (C). Separated m. quadriceps (D).

Figure 21 displays an example of the muscle CSA of the right mid-thigh and the performed steps after various ranges for muscle attenuation were set. In figure 21 I, the white area shows lean tissue while the black area demonstrates subcutaneous AT and IMAT, followed by a negative of the muscle area with excluded oss femoris (Figure 21 II). The next step was the separation of the m. quadriceps (Figure 21 III). In this image the range for calculations was set between 0 to 100 HU to receive information of the total muscle CSA followed by a set range between 31 to 100 HU for NDM (Figure 21 IV). The same procedure was performed to gather information of the IMAT, however with a set rage between -190 to -30 HU. LDM was calculated by subtracting NDM from total muscle CSA. For all analysing steps macros were applied to accelerate the data evaluation.

Figure 21: Right mid-thigh muscle CSA. Male, 83 years of age. Black: adipose tissue, white: muscle area (I). Black: muscle CSA and white: IMAT (II). M. quadriceps CSA, black: muscle, white: IMAT (III). M. quadriceps CSA, black: NDM, white: LDM and IMAT (IV).

4.8 Statistical Analyses

All analyses were performed using Graph Pad Prism 7.03 (GraphPad Software Inc, San Diego, CA, USA). Level of significance was set at $P < 0.05$ for all statistical analyses, P values between 0.05 and 0.1 were interpreted as trend. The results were presented in mean ± standard deviation (SD). Data were tested for normality using Shapiro-Wilk test. All results were received using parametric tests and covered by performing non-parametric tests as sample sizes were generally low. Group differences were analysed using chi-square analysis and T-Tests. Paired- and unpaired T-Test were conducted to measure within and between group differences following the intervention. A paired T-Test was used to assess differences in distinct images of the knee extensor CSA. The association between lean tissue, IMAT, attenuation value, muscle activation level and force generation capacities was determined using Pearson's correlation. For comparison of muscle activation, MVC and predicted MVC (P MVC) only values from one leg were used and compared with muscle CSA and -attenuation of the same leg. In contrary, for concentric knee extension mean values of both legs were compared to mean values of muscle CSA and -attenuation.

5 Results

5.1 Baseline characteristics

Participant characteristics are presented in table 5. The population comprised 8 male participants (57%) and 6 females (43%) with a mean age of 86.6 ± 5.66 years. In general, no differences were detected between intervention groups at baseline, although fat percentage tended to be lower in the intervention group (35.1% vs. 40.8%, p=0.06). The BMI was considered as normal healthy (N=5) to slightly over-weight (N=9).

Table 5: Subject characteristics at baseline.

	TG (N=7)	CG (N=7)	Entire Cohort (N=14)	P Value
Sex M/F	5/2	3/4	8/6	0.26
Age (years)	86.2 (± 5.93)	87.0 (± 6.27)	86.6 (± 5.88)	0.81
Height (m)	1.7 (± 0.07)	1.6 (± 0.05)	1.6 (± 0.06)	0.66
Weight (kg)	69.5 (± 11.1)	70.6 (± 9.7)	70.1 (± 10.0)	0.85
BMI (kg/m^2)	25.6 (± 3.60)	26.3 (± 2.43)	26.0 (± 2.98)	0.66
Fat (%)	35.1 (± 5.8)	40.8 (± 4.3)	38.0 (± 5.7)	0.06
Fat mass (kg)	23.8 (± 5.91)	28.1 (± 4.81)	26.0 (± 6.55)	0.15
Lean mass (kg)	43.6 (± 7.12)	40.6 (± 5.05)	42.1 (± 6.12)	0.40
SPPB score	4.1 (± 2.4)	4.3 (± 2.4)	4.2 (± 2.3)	0.91
Gait speed (m/s)	0.74 (± 0.38)	0.73 (± 0.30)	0.74 (± 0.33)	0.94
Sit to stand x 5 (s)	28.4 (± 29.04)	25.2 (± 27.3)	26.8 (± 27.1)	0.83

Values are Means (± standard deviations). M, male; F, female; BMI, Body mass index; SPPB, Short physical performance battery; TG, Training group; CG, Control group. P values (P<0.05) for normal distribution between groups comparison.

5.1.1 Mid-thigh CSA composition

The mid-thigh CSA composition is presented in table 6. No differences between the groups were revealed. The total CSA of the m. quadriceps constituted 46.5% of the total mid-thigh CSA. The IMAT content in the m. quadriceps accounted for only 10.3% of the total mid-thigh IMAT content. IMAT content of the m. quadriceps related to the total CSA of the knee extensor was only 3.4% while the muscle area of the m. quadriceps showed a distribution of 75.9% in NDM and 20.6% in LDM. The muscle density expressed in HU was seen to be higher in m. quadriceps (42.3 HU) when separated from the total muscles (37.8 HU) which illustrates a higher quality in the isolated m. quadriceps compared to the remaining thigh muscles.

Table 6: Mid-thigh CSA composition at baseline.

	TG (N=7)	CG (N=7)	Entire Cohort (N=14)	P Value
Mid-thigh				
HU	38.2 (± 5.19)	37.4 (± 3.79)	37.8 (± 4.57)	0.75
Total CSA (cm²)	92.5 (± 18.1)	87.8 (± 17.8)	90.1 (± 18.1)	0.66
Muscle CSA (cm²)	80.7 (± 16.8)	73.8 (± 16.6)	77.2 (± 17.1)	0.48
NDM (cm²)	57.6 (± 16.6)	51.7 (± 15.0)	54.7 (± 16.1)	0.53
LDM (cm²)	23.1 (± 7.23)	22.0 (± 6.05)	22.6 (± 6.69)	0.78
Total Fat (cm²)	62.4 (± 20.6)	93.4 (± 44.9)	77.9 (± 38.2)	0.15
Subcutaneous AT (cm²)	50.7 (± 16.9)	97.3 (± 44.3)	65.0 (± 36.5)	0.16
IMAT (cm²)	11.7 (± 4.87)	14.0 (± 4.07)	12.9 (± 4.63)	0.40
M. Quadriceps				
HU	42.5 (± 5.54)	42.0 (± 2.86)	42.3 (± 4.42)	0.78
Total CSA (cm²)	43.4 (± 8.37)	40.5 (± 8.91)	41.9 (± 8.76)	0.57
Muscle CSA (cm²)	42.2 (± 8.54)	39.0 (±8.77)	40.6 (± 8.80)	0.53
NDM (cm²)	33.8 (± 9.26)	30.9 (± 8.27)	32.3 (± 8.90)	0.58
LDM (cm²)	8.4 (± 2.7)	8.1 (± 1.8)	8.5 (± 2.4)	0.79
IMAT (cm²)	1.2 (± 0.9)	1.5 (± 0.6)	1.4 (± 0.8)	0.48

Values are Means (± standard deviations). TG, Training group; CG, Control group; HU, Hounsfield units; CSA, Cross sectional area; NDM, Normal-density muscle; LMD, Low-density muscle; IMAT, Intermuscular adipose tissue; AT, Adipose tissue. P values ($P<0.05$) for normal distribution between groups comparison.

5.1.2 Muscle attenuation and IMAT in distinct slices of the m. quadriceps

The m. quadriceps CSA was divided by its attenuation characteristics and was investigated by its differences in three various locations (Figure 22, Table 7). In the scans from the proximal to the mid part only IMAT was decreased 0.38 ± 0.46cm² (P= 0.022) while muscle CSA 0.24 ± 2.72cm² (P=0.844) NDM 0.23 ± 3.00cm² (P=0.799) and LDM 0.45 ± 1.18cm² (P=0.241) remained unchanged.

Scans from the mid- to the distal part showed a significant decrease in muscle CSA of 10.87 ± 4.86cm² (P<0.001), NDM 10.96 ± 4.76cm² (P<0.001) (Figure 22 A and B) while IMAT 0.76 ± 0.95cm² (P=0.010) increased LDM (Figure C 22). No changes were seen in LDM 0.071 ± 1.47cm² (P=0.859) (Figure 22 D).

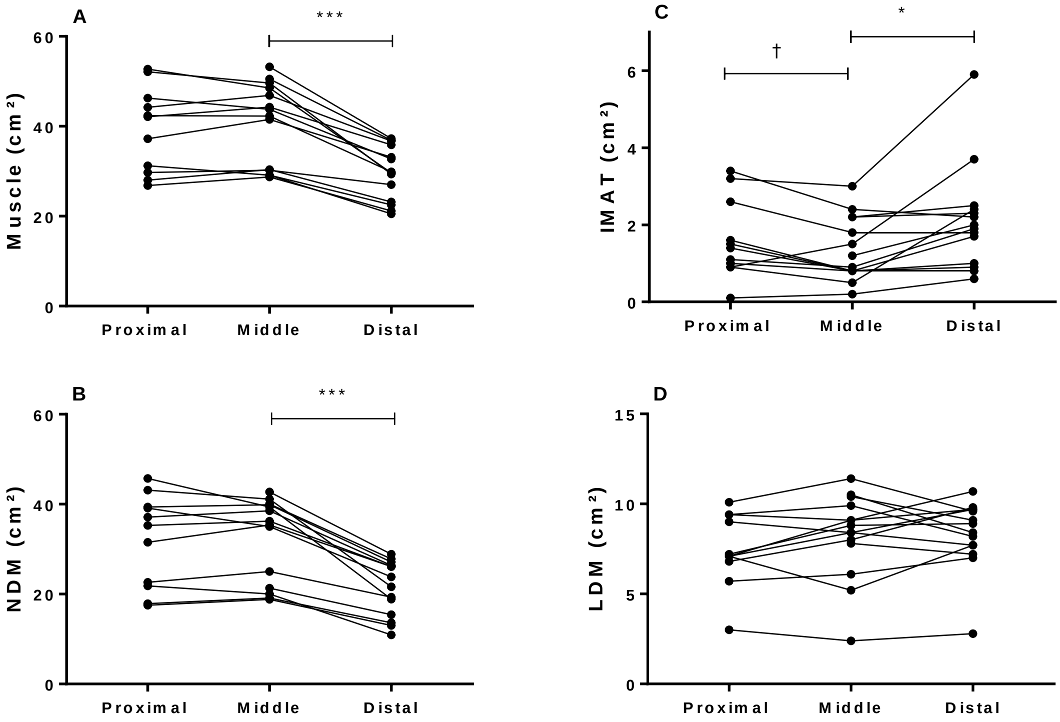


Figure 22: Individual baseline differences of both legs in proximal-, mid- and distal cross-sectional area (CSA) of the m quadriceps. The CSA of total muscle (A), normal-density muscle (NDM) (B), intermuscular adipose tissue (IMAT) (C) and low-density muscle (LDM) (D) are shown. Proximal- (N=11) mid- (N=14) and distal- (N=14) sections.

Table 7 presents the region-specific CSA of the m. quadriceps. The muscle CSA was at its biggest in the mid-scan while the highest amount of IMAT was located in the distal part of the knee extensor. The significant differences between various sections were already mentioned above.

Table 7. Region-specific m. quadriceps CSA divided by its attenuation characteristics at Baseline.

	Proximal-scan (N=11)	Mid-scan (=14)	Distal-scan (=14)
Total CSA (cm^2)	40.95 (± 8.87)	41.94 (± 9.09)	31.83 (± 6.00) ***
Muscle CSA (cm^2)	39.34 (± 9.38)	40.58 (± 9.14)	29.71 (± 6.05) ***
NDM (cm^2)	31.91 (± 10.27)	32.31 (± 9.22)	21.35 (± 6.16) ***
LDM (cm^2)	7.45 (± 2.02)	8.25 (± 2.36)	8.32 (± 1.93)
IMAT (cm^2)	1.61 (± 1.03)	1.36 (± 0.83) †	2.12 (± 1.36) *

Values are Means (± standard deviations). CSA, Cross sectional area; NDM, Normal-density muscle; LMD, Low-density muscle; IMAT, Intermuscular adipose tissue. Asterisks mark significant difference between mid- and distal-scan (* $P<0.05$, *** $P<0.001$). The cross marks significant difference between proximal- and mid-scan († $P<0.05$).

5.2 Force generation at baseline

Representative for the strength tests at baseline, only the correlation of the 1RM and m. quadriceps CSA is demonstrated in figure 23. Further correlations of strength parameters and m. quadriceps CSA are presented in table 8.

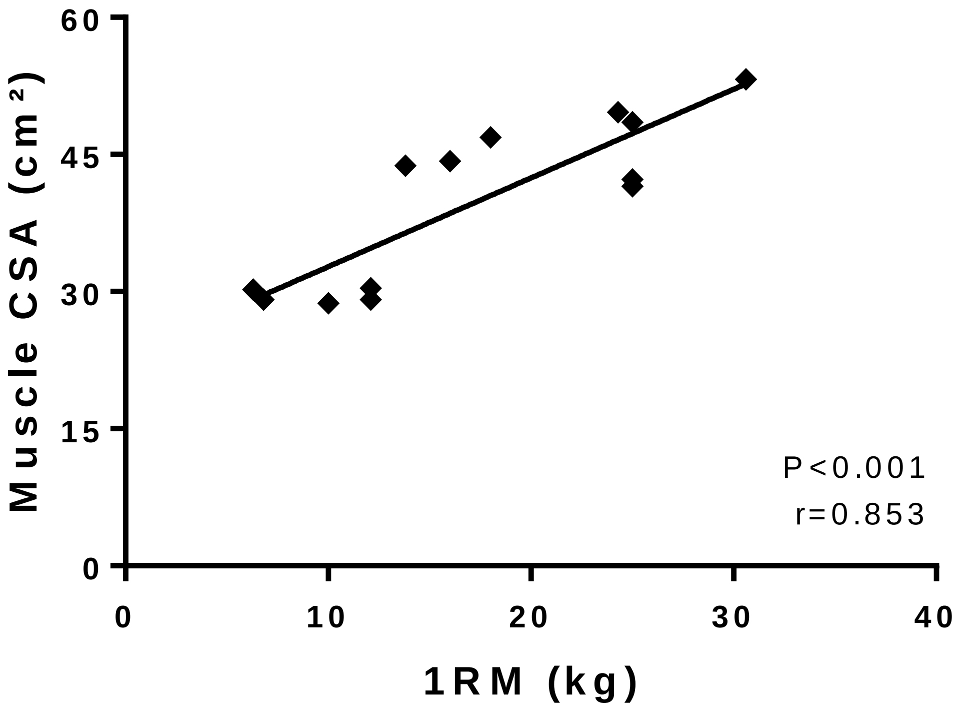


Figure 23: Association between m. quadriceps cross-sectional area (CSA) and one repetition maximum (1RM) in all subjects (n=13) at baseline.

Concentric knee extension strength (1RM) was significantly correlated with muscle CSA (r=0.853, P<0.001), NDM (r=0.857, P<0.001), total CSA (r=0.849 P<0.001) and HU (r=673, P=0.012). In contrast, IMAT (r=-0.185, P=0.544) and LDM (r=-0.119, P=0.697) were not associated with concentric knee extension 1RM. A trend to significance was seen when correlating MVC and m. quadriceps CSA including IMAT (r=0.549, P=0.099). Excluding IMAT from the quadriceps CSA did not alter the correlation substantially (r=0.572, P=0.084). However, when correlating MVC to NDM only, the correlation was slightly improved (r=0.625, P=0.053). In comparison a no correlations were observed between MVC and LDM (r =-0.322, P=0.874), MVC and IMAT (r=0.057, P=0.874) and MVC and HU (r=0.547, P=0.102).

Unlike MVC the P MVC and muscle CSA attenuation values were significantly associated. A strong correlation was found between P MVC and m. quadriceps CSA including IMAT (r=0.678, P=0.045). The exclusion of IMAT from the m. quadriceps CSA did not affect the correlation to a greater extent (r=0.703, P=0.035). However, when correlating MVC to NDM only, a small increase was observed (r=0.745, P=0.021). No significant correlations were observed between P MVC and LDM (r=-0.310, P=0.416) and P MVC and IMAT (r=0.114, P=0.770), while a trend to significance was observed between P MVC and HU (r=0.596, P=0.090). Furthermore, a strong negative correlation was found between voluntary muscle activation and IMAT (r=-0.881), P=0.002).

Table 8: Force generation. Correlations between various cross-sectional area attenuation values and strength parameters at baseline.

	MVC (N=10)	P MVC (N=9)	Concentric Knee Extension 1RM (N=13)	ACT (N=9)
Total CSA	0.55	0.68*	0.85***	-0.38
Muscle CSA	0.57	0.70*	0.85***	-0.31
NDM	0.63	0.75*	0.86***	-0.33
LDM	-0.32	-0.31	-0.12	0.11
IMAT	0.06	0.11	-0.19	-0.88**
HU	0.55	0.60	0.67*	-0.22

HU, Hounsfield units; CSA, Cross sectional area; NDM, Normal-density muscle; LMD, Low-density muscle; IMAT, Intermuscular adipose tissue; MVC, Maximum voluntary isometric contraction; P MVC, Predicted MVC; 1RM, One repletion maximum; ACT, Voluntary muscle activation. Values represent Pearson correlation coefficients, r. Asterisks mark significant correlations between CSA attenuation and strength parameters (*P<0.05, **P<0.005, ***P<0.001).

5.2.1 Relations between absolute and specific force generation and muscle attenuation

Baseline strength characteristics were not different between groups (Table 9). As expected, results of the P MVC were higher compared to MVC in absolute- and specific torque in both groups. Voluntary muscle activation at baseline was relatively high in this cohort (91.4% ± 4.51%). The activation level of all participants was above 80%, and six out of nine reached an activation level above 90%.

Table 9: Subject characteristics at baseline in absolute- and specific force generation.

	TG	CG	*Entire Cohort*	P Value
MVC	*(N=6)*	(N=4)	*(N=10)*	
Maximal torque (*N·m)*	88.4 (± 29.2)	89.2 (± 26.1)	88.8 (± 28.0)	0.97
Specific torque *(N·m/cm²)*	2.0 (± 0.58)	2.4 (± 0.14)	2.2 (± 0.49)	0.32
P MVC	*(N=5)*	*(N=4)*	*(N=9)*	

Maximal torque (*N·m)*	103.3 (± 27.0)	98.6 (± 29.4)	101.2 (± 28.2)	0.83
Specific torque *(N·m/cm²)*	2.3 (± 0.48)	2.6 (± 0.23)	2.5 (± 0.41)	0.22
1RM	*(N=7)*	*(N=6)*	*(N=13)*	
Maximal force (kg)	18.6 (± 7.8)	15.7 (± 7.2)	17.3 (± 7.6)	0.53
Specific force (kg/cm²)	0.4 (± 0.12)	0.4 (± 0.12)	0.4 (± 0.13)	0.53
Voluntary muscle activation	*(N= 5)*	*(N=4)*	*(N=9)*	
Activation Level (%)	91.7 (± 4.6)	90.9 (± 4.4)	91.34 (± 4.5)	0.82

Values represent mean (± standard deviation). MVC, maximum voluntary isometric contraction; P MVC, Predicted MVC; 1RM, one repetition maximum (concentric knee extension); Specific torque, torque per unit cross-sectional muscle area; TG, Training group; CG, Control group. P values for between groups comparison at baseline.

Looking at the MVC, no significant associations were detected between m. quadriceps attenuation and absolute torque (r=0.547, P=0.102) (Figure 24 A) respectively specific torque (r=0.205, P=0.570) (Figure 24 B).

The correlation between m. quadriceps attenuation and predicted absolute torque showed a trend to significance (r=0.596, P=0.090), whereas predicted specific torque (r=0.193, P=0.619) failed to reach statistical significance.

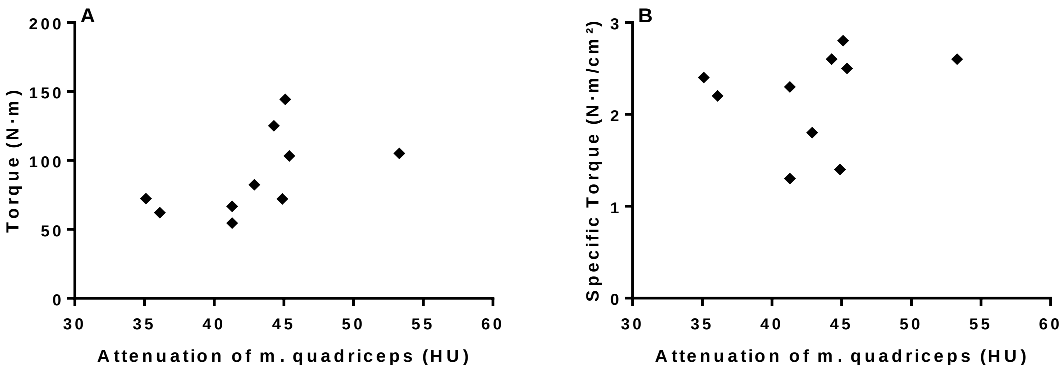


Figure 24: Associations between m. quadriceps attenuation values and maximal voluntary isometric contraction (MVC) in torque (A) and specific torque (B) in all subjects (N=10) at baseline. HU, Hounsfield units.

In relation to muscle attenuation a significant correlation was observed in absolute 1RM of the knee extensor (r=0.669, P=0.012), (Figure 25 A). Also, specific 1RM displayed a significant correlation of (r=0.640, P=0.019) (Figure 25 B). In this regard, both 1RM and specific 1RM were associated with higher muscle attenuation.

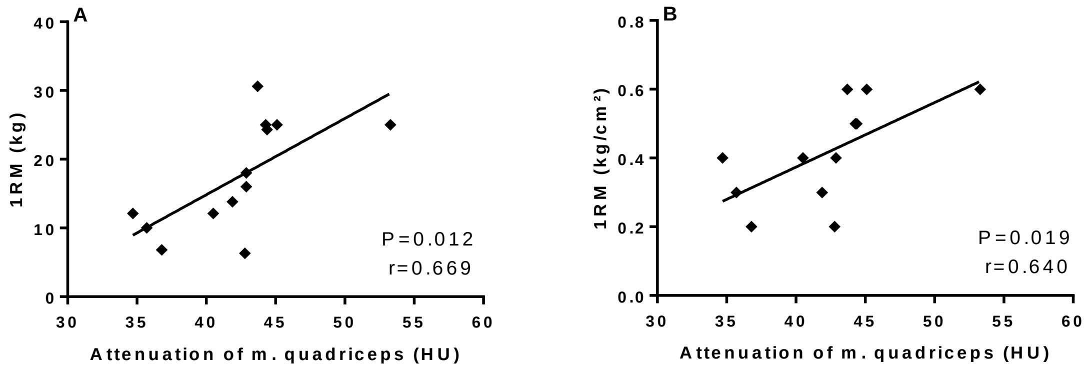


Figure 25. Associations between m. quadriceps attenuation values and 1RM (A) and specific 1RM (B) in all subjects (n ± 13) at baseline. HU, Hounsfield unit.

Influence of strength training on m. quadriceps CSA

5.3 Looking at the muscle attenuation, no significant changes from pre- to post intervention within both groups were observed. However, TG increased in muscle attenuation by 4.0% and demonstrated a tendency to significance (P=0.065) while the CG remained (-0.8%, P=0.517) unchanged. However, with respect to the attenuation changes from baseline a significant difference of 4.8% between the groups was observed (P=0.049) (Figure 26).

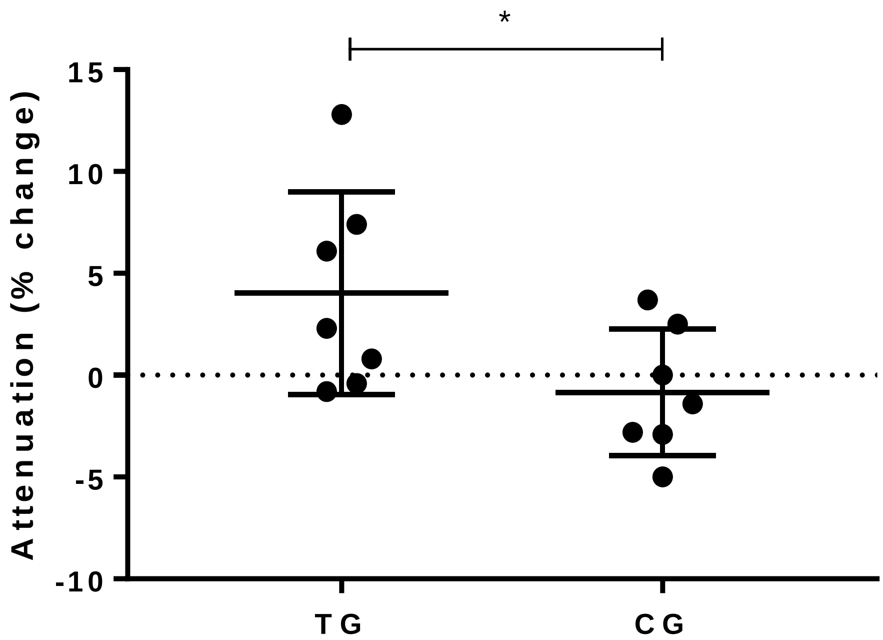


*Figure 26: Within group percentage changes in m. quadriceps attenuation from baseline; TG, Training group; CG, Control group. *: within group changes, P<0.05.*

Results from the total CSA (IMAT included) of the knee extensors showed a tendency to increase by 5.7% (P=0.084) in the TG, whereas no trend was detected in the CG (-0.9%, P=0.895) (Figure 27 A). The between groups comparison of the percentage change showed no significance (P=0.830).

When excluding IMAT, no changes in muscle CSA in the TG (P=0.100) and the CG could be detected (P=0.929). Furthermore, the percentage change was not different between groups (P=0.159). When looking at the NDM a significant 8.6% improvement from pre- to post intervention was observed in TG (P=0.040), while NDM was not altered in the CG (-0.9%, P=0.965) indicating that the strength training intervention led to an increase in NDM (Figure 27 B). The between groups comparison showed a tendency to significance (P=0.078). LDM remained unchanged in both groups, since no significant changes were observed either in TG (-0.1%, P=0.610) nor CG (-2.6%, P=0.970). Furthermore, the percentage change was not different between groups (P=0.767) (Figure 27 C). The IMAT content did not change in either of both groups, TG (0.16 cm², P=0.157), CG (-0.02 cm², P=0.745) and the percentage changes did not differ significantly (P=0.256).

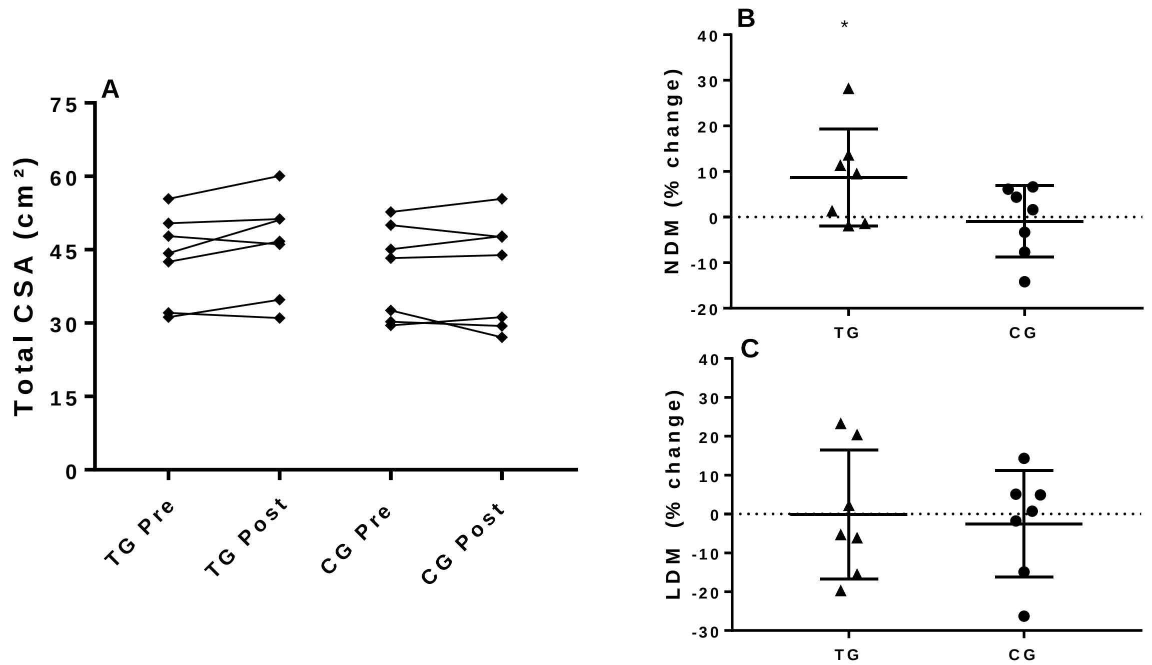


Figure 27: Changes in m. quadriceps total cross-sectional area (CSA) from baseline. NDM, Normal-density muscle; LDM, Low-density muscle; TG, Training group; CG, Control group. Within group change in total CSA (A), percentage within group change in NDM (B), percentage within group change in LDM (C).

5.4 Changes in force generation

To investigate the effect of a 10-week strength training on muscle CSA and muscle attenuation, 1RM changes were compared with muscle CSA in the TG.

The association between 1RM% change and m. quadriceps CSA% demonstrated a trend to significant (r=0.754, P=0.050) (Figure 28). Furthermore, no significant correlation (r=-0.161, P=0.730) was observed between 1RM% change and muscle attenuation percentage change.

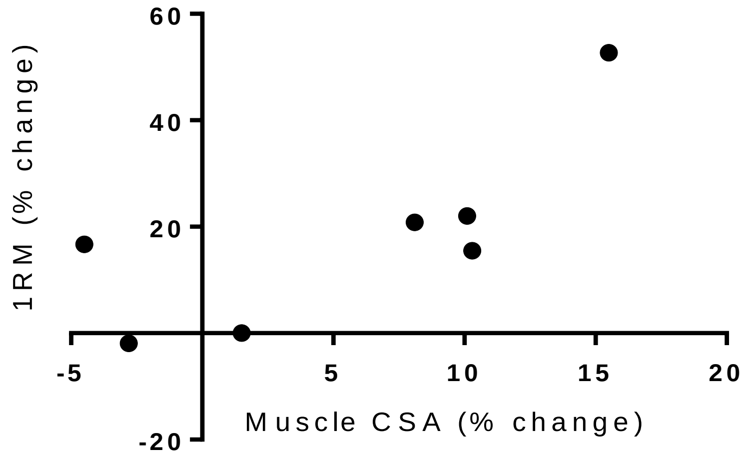


Figure 28: Association between 1RM (one repletion maximum) percentage change and muscle CSA (cross-sectional area) percentage change in the TG, Training group.

5.4.1 MVC

The MVC demonstrated no significant change (9.8%) in the TG (6.8 ± 11.2 N·m, P=0.200) following the intervention. However, a significant increase was detected in the CG by 9.3% (7.5 ± 4.4 N·m, P=0.042) was. No significant difference in percentage change was observed between groups (P=0.961) (Figure 29).

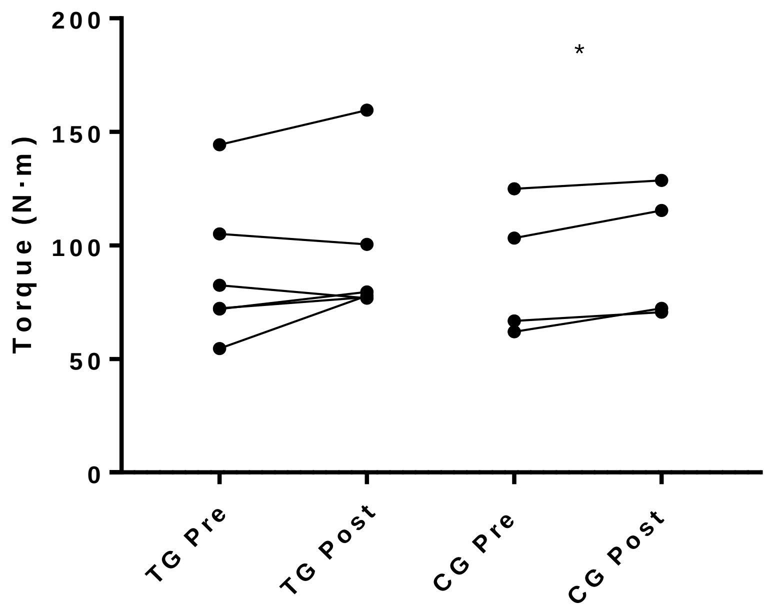


*Figure 29: Changes in maximum voluntary isometric contraction (MVC) from baseline in TG, Training group; CG, Control group. *: within group changes, P<0.05.*

5.4.2 Concentric knee extension

The concentric 1RM test for the knee extensor displayed significant changes from pre- to post intervention for both groups. Both TG and CG group increased their performance in 1RM by 18.0% (3.4 ± 3.02kg, P=0.025) and 8.9% (1.3 ± 1.18kg, P=0.047) respectively (Figure 30). The between comparison of the groups percentage change detected no significant difference (P=0.294).

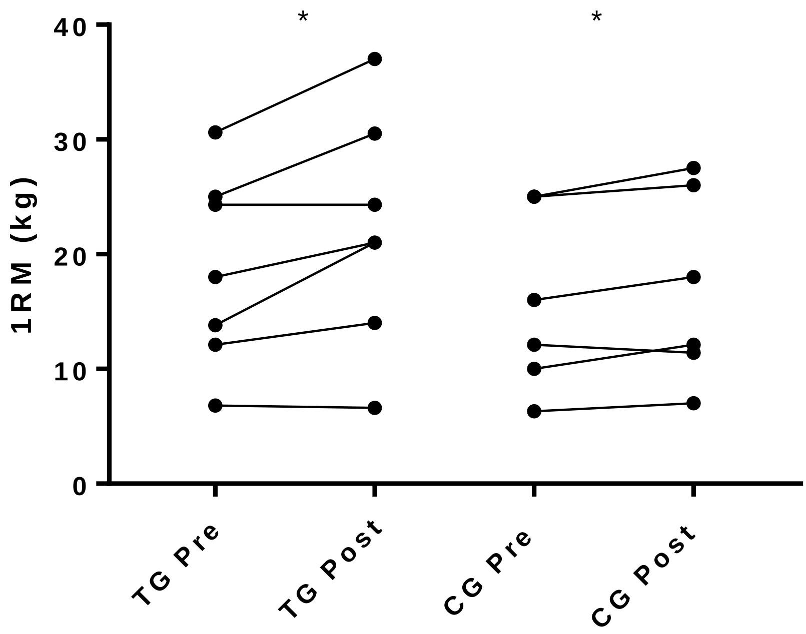


*Figure 30: Changes in one repetition maximum (1RM) from baseline in TG, Training group; CG, Control group. *: within group changes, P<0.05.*

5.4.3 Voluntary muscle activation

No significant differences either within groups change in TG (-2%, P=0.121) and CG (2%, P=0.528) nor between groups percentage change (P=0.166) were observed after a 10-weeks strength training intervention in voluntary muscle activation level (Figure 31).

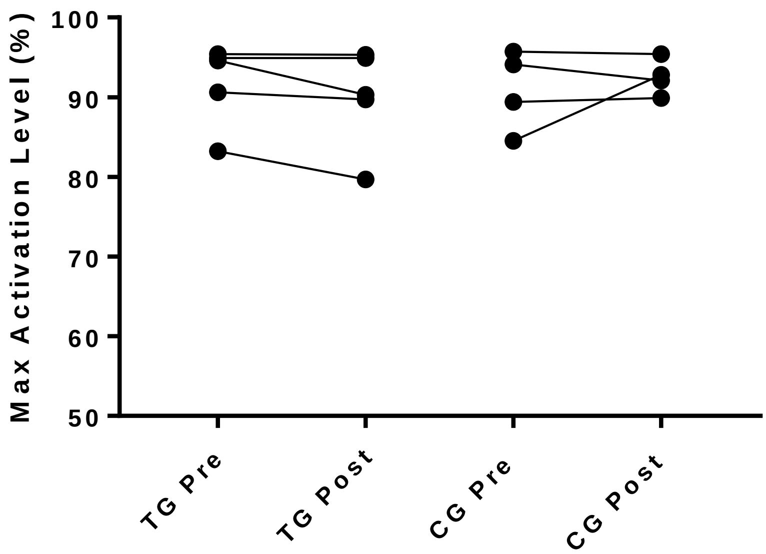


Figure 31. Percentage changes in voluntary muscle activation from baseline. TG, Training group; CG, Control group.

6 Discussion

This study was conducted to present a clearer picture of the relations between muscle size, fat infiltration and force generating capacity. The main finding of this study was that the degree of force production was determined by means of muscle attenuation. In this regard a greater attenuation value was associated with a greater force generation per unit muscle size. Moreover, the separation of lean tissue by its density illustrated a strong correlation between NDM and concentric knee extensor strength, while LDM was not affecting force production. Also, IMAT did not influence force production of the m. quadriceps. Interestingly, voluntary muscle activation was seen to be extraordinary high in this population compared to published research (Harridge et al., 1999; Hvid et al., 2016) but was not associated with muscle force production.

Outcomes from the intervention demonstrated that a 10-weeks resistance training regime can effectively increase NDM while LDM and IMAT remained unchanged. The performance in concentric 1RM was improved by 18% while the attenuation value within the knee extensor showed a trend (P=0.065) to increase. Against the hypothesis, no increase in activation level of the m. quadriceps was observed and thus contrary to previous findings applying the same method (Hvid et al., 2016).

6.1 Relations between muscle size, density and force generating capacity

Results from the baseline measurements showed that the degree of force production was determined by the level of muscle attenuation. This corresponds to recent research of Goodpaster et al. (2001), where low thigh muscle attenuation was associated with lower knee extensor strength. Another finding was that a greater attenuation value was associated with a better MQ defined by greater force generation per unit muscle size (Metter et al., 1999). Also, a significant relation between NDM and knee extensor strength was reported and consistent with previous findings (Goodpaster et al., 2001). In contrast to NDM, LDM was not affecting force generating capacity of the knee extensor. Weather greater fat accumulation in lean tissue directly impacts muscle fiber recruitment (nerve function), metabolism (energy utilisation) or contractility (cellular function) can only be hypothesised (Visser et al., 2002). However, greater fat infiltration in the muscle is not desirable since it is indirectly associated with glucose intolerance, diabetes mellitus type II (Goodpaster et al., 1999; Jacob et al., 1999) and might negatively affect lower extremity performance (Prior et al., 2007; Visser et al., 2002).

Interestingly, IMAT within the thigh muscle of frail older individuals amounted 12.9 ± 4.6cm^2 and was not higher as reported in other older individuals with limitations (11.0 ± 1.8cm^2) (Marcus et al., 2013). When further looking on the fat accumulation in the separated m. quadriceps it was seen that only 10.3% (1.4 ± 0.8cm^2) of IMAT were located in the knee extensor, calculated on the total amount within the thigh muscle. The association between IMAT in the thigh and deficits in strength and physical performance has already been demonstrated (Goodpaster et al., 2001; Marcus et al., 2012) however to the knowledge of the author this is the first study investigating the impact of m. quadriceps IMAT on force generation. In this regard, IMAT in the knee extensor did not influence force generation substantially which might be linked to the small amount within this muscle group.

The distribution in lean tissue was seen to be strongly differentiated within the thigh muscle. Nearly 60.1% NDM and 36.7% LDM calculated on the total amount within the thigh were located in the m. quadriceps. In that means myosteatosis in the other muscle groups of the thigh as e.g. the hamstrings might face a bigger issue maintaining strength and functional capacity in higher age (Macaluso et al., 2002). The attenuation value of the m. quadriceps CSA (42.5 HU) was also greater than the attenuation value for the whole thigh muscle CSA (38.2 HU). This observation is in accordance with previous findings (Goodpaster et al., 2001; Santanasto et al., 2011; Taaffe et al., 2009).

In this study, two different types of contractions were applied to gather information about force generating capacities. First, the muscle CSA showed a significant correlation with dynamic concentric contraction ($r=0.853$, $P<0.001$) while secondly, only a trend to significance was observed during static isometric contractions ($r=0.572$, $P=0.084$). Referring to this, Macaluso & De Vito (2004) reported that the speed of movement can influence force production which might be an indication why force generation was only significant in dynamic concentric contraction.

6.2 NDM, LDM and IMAT distribution in distinct CSA of the m. quadriceps

The within-subject variance in muscle attenuation of only 1.3HU between three distinct slices of the thigh (midpoint, 40mm and 80mm inferior to the midpoint) was already demonstrated by Goodpaster et al. (2000a), but they did not describe the distribution of NDM, LDM or IMAT. In this regard, muscle CSA and NDM demonstrated no changes from the proximal- to the mid part of the thigh, whereas significantly lower values were observed in the distal part. IMAT was seen to be different in all three sections with its highest amount in the distal part. The intramuscular differences are in line with previous findings (Narici, Roi, Landoni, Minetti, & Cerretelli, 1989) where maximal anatomic quadriceps CSA was found to be located at 4/10 of the femur length and decreases distally towards the knee. Also Seynnes, de Boer, & Narici, (2006) detected similar intermuscular differences between the mid and distal CSA of the m. quadriceps, and demonstrated specificity in the hypertrophic response to 35-days of resistance training. In this relation a faster and greater increase in the mid- versus the distal part of the m. quadriceps was observed.

Therefore, awareness of the location where the muscle CSA is scanned has importance since it could make a difference in the results. Data gathered from a midpoint scan would be seen as a proper choice to increase inter-investigational reproducibility between studies (Narici et al., 1996; Seynnes et al., 2006).

6.3 Voluntary muscle activation

The present study illustrated that voluntary muscle activation had no major influence on force production. Indeed, it turned out that most of the participants had a close to complete voluntary muscle activation (Shield & Zhou, 2004; Suetta et al., 2009) whereas the average level of activation 91.4% (N=9) was unexpectedly high and controversial to recent research (Harridge et al., 1999; Hvid et al., 2016). In addition, all participants reached a higher activation level than 80% while six participants were even able to generate an activation level above 90%, which is considered as fully voluntary activated muscle (Shield & Zhou, 2004). These findings, however were surprising since age-related neuronal deterioration was assumed to be performance decreasing and seen as a limitation to fully drive MU and thus completely activate the muscle (Hvid et al., 2016; Shield & Zhou, 2004; Suetta et al., 2009). However, it was reported that voluntary muscle activation was known to be higher in static isometric compared to dynamic contractions (Babault et al., 2001). Despite that, no other study having an equivalent cohort with respect to a comparable physical performance (SPPB 4.2 ± 2.3), to the same method (ITT with adjusted equation), to the same contraction form (isometric) and to the same investigated muscle group (knee extensor) observed likewise high activation levels. In this regard Hvid et al. (2016) demonstrated an activation of 78.9% in mobility limited subjects (SPPB 8.75 ± 0.62), while Suetta et al. (2009) showed an activation level close to full activation (88.6%) but in moderately active participants.

The research group of Yoshida et al. (2012) detected an interesting link between IMAT and CAR, where the CAR was greater in older participants with lower IMAT and vice versa. Findings might indicate that voluntary muscle activation at higher age is not necessarily impaired when the IMAT level is low; this was the case in the present study (10.3%). Further arguments for the high level of voluntary muscle activation in the m. quadriceps could be either a coactivation of the synergist muscles which contributed to the total force production (Folland & Williams, 2007) or a reduced antagonist coactivation, which also allows more net force production of the agonist (Arnold & Bautmans, 2014; Häkkinen et al., 2003). However, the assumption of a reduced antagonist coactivation is divergent and contrasting with findings where antagonist coactivation was seen to be present (Aagaard et al., 2010) and further higher in older compared to younger individuals (Macaluso et al., 2002; Tracy & Enoka, 2002). To the knowledge of the author no study with a similar cohort has yet investigated the amount of coactivation while using the ITT to assess voluntary muscle activation. Therefore, tracing the electromyographic activity of the agonist, synergist and antagonist could be considered in further studies to clarify the level of coactivation in MVC in frail elderly individuals (Klass et al., 2007).

Another finding was a strong negative correlation of IMAT and voluntary muscle activation. This leads to the assumption that individuals with high IMAT have impaired muscle activation. Similar observations were reported by Yoshida et al. (2012) who applied the CAR method to measure central activation of the m. quadriceps. Lower CAR value was demonstrated in individuals showing high IMAT accumulation (>15.4%) in the thigh muscle.

6.4 Relations between muscle size, density and force capacity after intervention

The outcomes of the intervention demonstrated that 10-weeks of resistance training were not sufficient to significantly increase muscle attenuation (P=0.065). However, the change in muscle attenuation in TG was significantly different from the change in CG (4.8%). This was an important outcome since less is known about the impact of resistance training in very old individuals on the density of the m. quadriceps. Concerning these findings, only Taaffe et al. (2009) reported likewise changes (5.4%) after a 12-weeks retraining period. Results strengthen the relevance of a physically active life to positively counteract fat infiltration within the muscle (Goodpaster et al., 2008; Taaffe et al., 2009). This is of great importance since low muscle density is linked to disability and poor health conditions (Cawthon et al., 2009) while higher muscle density is associated with greater gait speed, quadriceps strength and physical activity (Khoja, Moore, Goodpaster, Delitto, & Piva, 2018).

Although concentric knee extensor strength increased by 18%, no correlation between the change in HU and the change in force generation was observed. These findings are in contrast to the results from the Health ABC study where an association between the change of HU and change of specific torque generation in healthy older adults (76.7 ± 1.0 years, SPPB 8.0 ± 0.2, N=20) was reported following an 1-year intervention (Goodpaster et al., 2008). Thus, it is conceivable that for frail older adults the relatively short period of 10-weeks might not be long enough to demonstrate a correlation between the change in HU and the change in force generation.

In contrast with previous investigations this study observed no changes in muscle CSA; this was unexpected since a general increase in muscle CSA was suggested to appear subsequently to a strength training intervention (Ferri et al., 2003; Häkkinen et al., 2003; Scanlon et al., 2014). However, changes were detected when separating the muscle CSA by its density; the area consigning of NDM increased by 8.6% (2.6 ± 2.7cm²) while LDM remained unchanged. Findings, therefore underline the importance of separating lean tissue by its fat infiltration to put muscle CSA and its association to strength in perspective. Indeed, from these observations it seems as if strength training for the knee extensor mainly affects lean tissue with higher attenuation indicating that strength training alone might be inadequate to change LDM or increase intramuscular lipid (Pruchnic et al., 2004; Ryan, Ivey, Prior, Li, & Hafer-macko, 2011). When combining strength training with a dietary intervention both an increase in NDM and a decrease in LDM were seen in obese older adults (66.0 ± 3.8 years, BMI kg/m² 31.6 ± 3.8) (Avila, Gutierres, Sheehy, Lofgren, & Delmonico, 2010).

The role of IMAT accumulation as a negative predictor for muscle metabolism and function has already been demonstrated (Addison, Marcus, Lastayo, & Ryan, 2014; Tuttle, Sinacore, & Mueller, 2012; Vettor et al., 2009). Nevertheless large surveys examining the effect of resistance training alone on lowering IMAT are limited (Ryan et al., 2011; Taaffe et al., 2009). In this study IMAT was seen to be unaffected by resistance training. The outcome was not unexpected since only changes of IMAT in the m. quadriceps were investigated and as mentioned previously merely 10.3% of total IMAT in the thigh were observed in the m. quadriceps. Since no further studies investigating the IMAT change in the knee extensor it can only be assumed why strength training alone showed no effects. First, the intervention might not have been long enough as a minimum duration of 12-weeks to 6-month is recommended to decrease IMAT level (Addison et al., 2014; Marcus et al., 2010). Second, strength training alone might not be effective in very older adults. Most of the studies that have detected a decline in IMAT have been studies on calorie restriction, aerobic exercise or a combination of both (Avila et al., 2010; Murphy et al., 2012; Prior et al., 2007; Santanasto et al., 2011). Third, the individuals might have been too old and frail and a strength training intervention might only be adequate as a preventive strategy to inhibit or stabilise further IMAT accumulation (Goodpaster et al., 2008).

In summary, the current findings demonstrated resistance training as eligible to increase NDM even in frail older adults. However but when the aim is an reduction in LDM and IMAT, interventions combining strength and endurance training (Marcus et al., 2008) or strength training and dietary interventions (Avila et al., 2010) seem to be more appropriate.

6.5 Voluntary muscle activation

It is suggested that a several weeks lasting strength training leads to an increase in voluntary muscle activation (Karmen & Knight, 2004; Suetta et al., 2004, 2009). In support of these findings, one aim of the present study was to investigate the neuronal changes induced by a strength training intervention. Unlike the hypothesis, after the 10-weeks intervention no significant improvement in the level of muscle activation was observed in this study, neither in the TG nor the CG. This is contrasting with investigations of neuronal adaptation by Hvid et al. (2016), but in line with the studies of Cannon et al. (2007) and Harridge et al. (1999) which detected no changes after a similar intervention. Findings are further in contrast to Macaluso & De Vito (2004) who described neuronal learning effects in healthy older adults occurring within the first six weeks. Probably the combination of frailty and high age inhibit neuronal adaption more than claimed (Aagaard et al., 2010) and in consequence, 10-weeks of resistance training are not sufficient for this population.

But to put findings in perspective, it should be considered that the baseline activation level of 90% was already very high and considered as fully activated muscle. Another study illustrated a baseline activation level below 90%, but nevertheless an improvement of 4% (Hvid et al., 2016). From that point of view results in this study would have been more surprisingly if changes were significant.

However, when comparing TG and CG the between groups difference of 4% illustrated that resistance training had an impact. Even if results were not seen to be significant it indicated that individuals in higher age, with low physical status and high baseline activation level are able to achieve neuronal adaption in the m. quadriceps by regular resistance training.

6.6 Methodological considerations

6.6.1 Limitations

The major limitation of this thesis was the low sample size, in particular for the intervention which impaired the explanatory power of the results.

In regard to the low sample size, six dropouts in the intervention group was substantial. It can be speculated about the reasons for the dropouts in the TG, but it seems that the training load at the beginning of the intervention was too much for unexperienced subjects.

To increase training adherence, an additional familiarization week with reduced training load might be helpful.

Since this study comprised adults of very high age in association with low physical function, moving from one place to another might be very exhausting and stressful, in particular when the training is performed two times a week. Although the training was conducted using training facilities in the nursing homes and fitness centers nearby, it was observed that even short ways of a few hundred meters were a challenge in this cohort. Therefore, when recruiting frail elderly participants, nursing homes with training facilities in the house should be preferred. This could lower the stress level and physical exhaustion before exercising and in return might increase training adherence as well.

During the first two weeks irregularities in protein intake were observed. After consultation with the participants it was reported that two servings of the protein drink (one in the morning and one in the afternoon) were too much for them. To avoid bias as undocumented omission of the supplementation, it was decided to reduce the standardized daily protein intake of two servings Tine Styrk 330 ml to one severing a day after the third week.

In advanced age individuals fitted with artificial joint quite common. However, hip or knee prosthesis might affect CT the measurements. Due to design and multiple materials of the prosthesis, complications in the image reproduction can occur. Besides ceramic prosthesis a lot of other implants consisting of metal are in use and might generate streak artifacts in the image, in particular when conducting a very proximal scan of the thigh. Thus, some materials as cobalt-chrome and stainless steel attenuate the x-ray beam and produce wrong calculations when analysing the composition of the muscle CSA (Roth, Maertz, Parr, Buckwalter, & Choplin, 2012).

To conclude, not all materials generate artifacts, participants with prosthesis can be scanned by CT. However, materials with a strong attenuation as cobalt-chrome and stainless steel should be considered as exclusion criteria when analysing the composition of the muscle CSA due to incorrect values.

6.6.2 Prospects

As our population ages muscle mass, neuromuscular function and physical performance decreases progressively regardless the individual activity level (Janssen et al., 2000). Nevertheless, the quality of ageing can be positively affected but requires effective interventions. In the last decades the level of muscle fat infiltration and IMAT has been recognised as important predictors of metabolic and muscle dysfunction. This study showed promising effects of resistance training on muscle attenuation in frail elderly individuals. However, the mechanism behind the regulation of IMAT and LDM is still not clear and needs more attention. Further research is required to find the most effective exercise prescription aiming at the reduction of IMAT and LDM in general but also more pronounced in areas with massive muscle fat infiltration as knee flexors and adductors.

Thus, work is necessary to determine the role of voluntary muscle activation in older adults. The primary aim should comprise the clarification of a standardised testing protocol for a better inter-investigational reproducibility, while a secondary aim might be targeted on potentially synergist or antagonist coactivation to provide a more accurate measure of the individuals activation level.

7 Conclusion

In this thesis findings demonstrated that in frail elderly individuals, muscle density as defined with CT scans is related to force generating capacity. A greater attenuation value was associated with a greater force per unit muscle size. The degree of force production was determined by the level of muscle density. Moreover, the segmentation of lean tissue into NDM and LDM illustrated that force production only increased lean tissue with low fat infiltration. Moreover, it was shown that 10-weeks of resistance training was sufficient time to elicit improvements in strength and NDM in frail elderly individuals. A surprise was the small amount of IMAT identified in the quadriceps, which had no negative influence on muscle force production. Voluntary muscle activation was detected as negatively related to IMAT but not seen as an inhibiting factor in muscle force production.

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Tables

Figures

Appendix

Appendix 1 Criteria to be used for assessment of frailty, based on the Fried Frailty Criteria (Fried et al., 2001).

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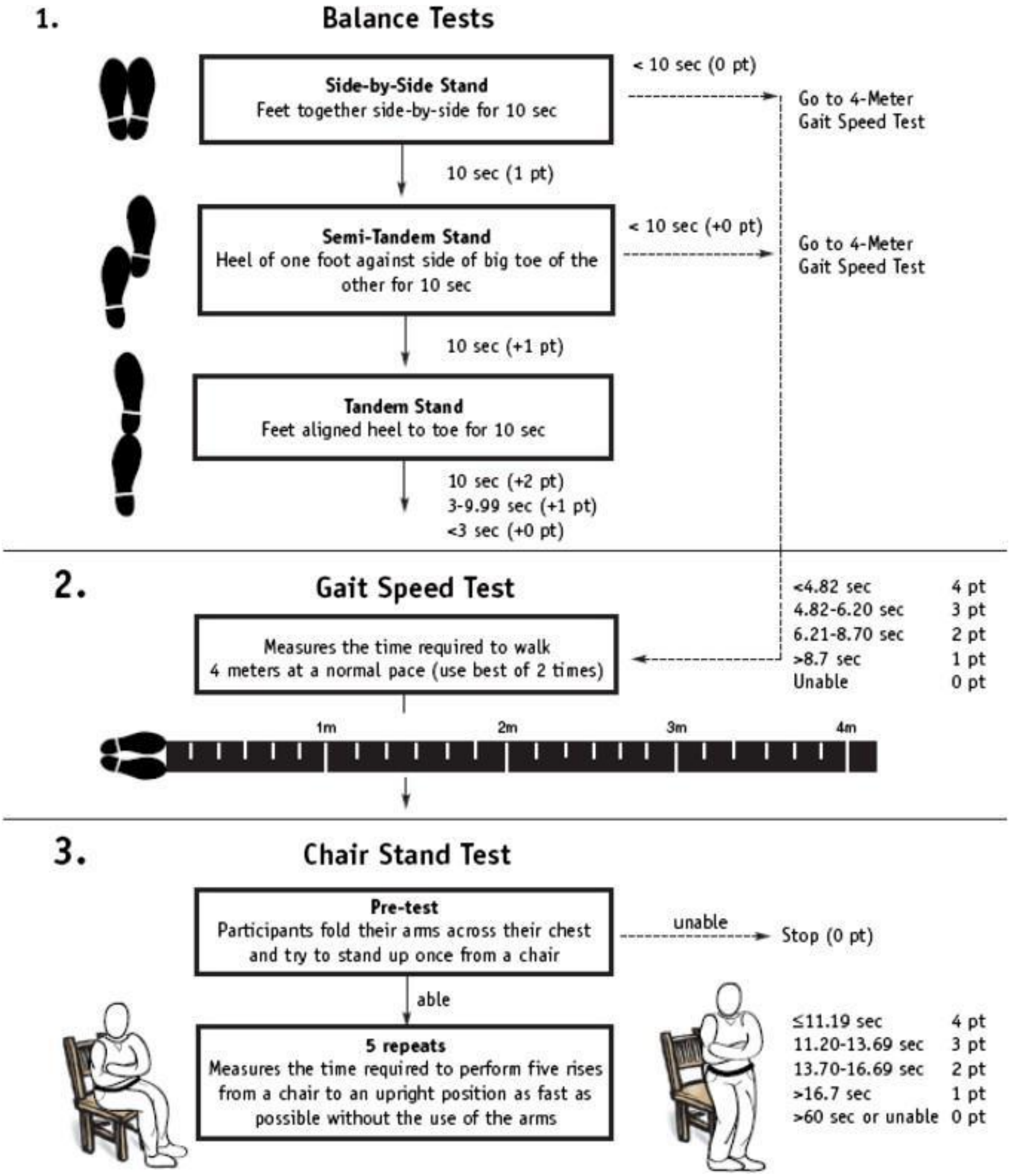
Shrinking, i.e. weight loss	Unintentional loss of at least 5% of the previous year's body weight.			
Weakness, i.e. low handgrip strength	Grip strength of the dominant hand (mean of three measurements)			
	BMI/male	Cut-off (kg)	BMI/female	Cut-off (kg)
	≤24	≤29	≤23	≤17
	24-26	≤30	23-26	≤17.3
	26-28	≤30	26-29	≤18
	>28	≤32	>29	≤21
Poor endurance, i.e. self- reported exhaustion	Evaluation of two statements of the CES-D scale: a) I felt that everything I did was an effort b) I could not get going Criteria fulfilled if at least one condition is present for 3 days or more during the last week.			
Slowness, i.e. low gait speed	Cut-off for time to walk 4 meters (static start)			
	Height/male (cm) ≤173	Cutoffs (s) ≥6.15 (0.65 m/s)	Height/female (cm) ≤159	Cutoffs (s) ≥6.15 (0.65 m/s)
	>173	≥5.25 (0.76 m/s)	>159	≥5.25 (0.76 m/s)
Low activity, i.e. reduced energy consumption	Physical activity will be assessed during an interview. Following the level of leisure physical activity performed daily during the last year, participants will be assigned to one of the following categories. 1) completely inactive or performing light-intensity physical activity (i.e., walking, light housework) less than 1 hour per week; 2) light physical activity: light-intensity physical activity 2–4 hours per week; 3) moderate–high physical activity: light physical activity at least 5 hours per week or moderate physical activity (i.e., gymnastics, playing soccer, gardening) at least 1–2 hours per week The low activity-criteria is fulfilled only for participants in category 1.			

Appendix 2 Criteria to be used for assessment of the short physical performance battery (Guralnik et al., 1995).

at



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