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Quantification of fiber type differences in human gluteus medius muscle

Dissertation
zum Erwerb des Doktorgrades der Medizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
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aus Osnabrück
2020

Mit Genehmigung der Medizinischen Fakultät

der Universität München

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Tag der mündlichen Prüfung:	13.02.2020

"We absolutely must leave room for doubt or there is no progress and there is no learning. There is no learning without having to pose a question. And a question requires doubt."

Richard P. Feynman

Abstract

The survival of any human being is only possible with a highly specialized musculoskeletal system that provides functional independence in terms of day-to-day activities. The importance is underlined by the observation of declining functional abilities through loss of muscle mass and strength in aging people. Physical impairment, and thus declining independence, are major risk factors for adverse health outcomes with higher rates of institutionalization and mortality. Postural instability and falls are feared complications of old people, which are results of low skeletal muscle strength and function, also known as sarcopenia. Changes of skeletal muscle during ageing have already been investigated, nevertheless it is yet unclear, if skeletal muscle differs between sarcopenic and non-sarcopenic patients.

To address this question, we took biopsies of the gluteus medius muscle from 20 patients, who underwent surgical intervention due to traumatic proximal femur fractures. We employed immunohistochemical staining methods to reveal specifics of skeletal muscle morphology. Additionally, we estimated physical performance with a questionnaire containing questions regarding activities of the daily living, measured handgrip strength and blood levels of 25-hydroxyvitamin D, thyroid-stimulating hormone, total protein and calcium. We used the definition provided by the European Working Group on Sarcopenia in Older People to divide the study cohort into sarcopenic and non-sarcopenic patients. Results revealed that with increasing age, the cross sectional area of fast-twitching fibers declined, whereas the slow-twitching fibers seemed to be unaffected. Also, when compared to relative muscle mass, we could show that lower skeletal muscle mass did not correlate with the skeletal muscle fiber cross-sectional area. The proportion of type I fibers was generally the highest throughout all patients, followed by type IIa fibers and then type IIx fibers. Nevertheless, we could find no age-dependent

trend in the proportion of fiber types. Additionally, we analyzed fiber size and proportion between sarcopenic and non-sarcopenic patients, but we could not find any differences.

In sum, we found that aging skeletal muscle of our study cohort depicted morphologic characteristics concordant to existing literature of different skeletal muscles (e.g. vastus lateralis muscle), but skeletal muscle fibers from gluteus medius muscle of sarcopenic patients were not different in size nor proportion compared to those of non-sarcopenic people. We therefore concluded that sarcopenia was likely not caused by a single mechanism in our study and thus, causes that lead to primary sarcopenia have yet to be elucidated.

Zusammenfassung

Das Überleben eines jeden Menschen ist nur mit Hilfe eines hoch spezialisierten muskuloskelettalen Systems möglich, welches funktionale Unabhängigkeit im alltäglichen Leben ermöglicht. Die Bedeutung wird durch die Beobachtung unterstrichen, dass die funktionellen Fähigkeiten durch den Verlust von Muskelmasse und -kraft im Alter deutlich abnehmen. Körperliche Einschränkungen und damit die schwindende Unabhängigkeit stellen große Risiken für die Gesundheit dar und erhöhen sowohl die Rate an Krankenhausaufenthalten als auch die Mortalität. Posturale Instabilität und Stürze sind gefürchtete Komplikationen des Alterns, welche durch den Verlust von Muskelkraft, auch Sarkopenie genannt, bedingt sind. Die Veränderungen von Skelettmuskel im Alter sind bisher in vielen Belangen untersucht worden, dennoch ist bisher wenig darüber bekannt, welche Unterschiede Skelettmuskel sarkopener Patienten im Vergleich mit Skelettmuskel nicht sarkopener Patienten aufweist.

Um dieser Frage nachzugehen, sammelten wir Biopsien aus dem Musculus gluteus medius von 20 Patienten, die sich zu dieser Zeit in der Behandlung wegen einer proximalen Femurfraktur befanden. Wir wandten immunhistochemische Färbungen an, um die Morphologie der Skelettmuskulatur zu untersuchen. Zusätzlich bewerteten wir die körperliche Leistungsfähigkeit mittels eines Fragebogens mit Fragen, die auf Aktivitäten des Alltagslebens gerichtet waren, maßen die Handkraft und die Blutwerte von Vitamin D, Thyroid-stimulierendem Hormon, Gesamtprotein und Calcium. Wir benutzten die Definition der European Working Group on Sarcopenia in Older People um die Studienkohorte in sarkopene und nicht sarkopene Patienten aufzuteilen. Unsere Ergebnisse zeigten, dass mit steigendem Alter die schnellen Muskelfasern in ihrer Gesamtgröße abnehmen, wobei die langsamen Muskelfasern weniger beeinflusst zu sein scheinen. Im Vergleich zur Muskelmasse der Probanden zeigte sich allerdings, dass die Größe

der Muskelfasern nicht mit der Muskelmasse im Zusammenhang steht. Der Anteil an Typ I Fasern an der Gesamtzahl war in allen Biopsien am größten, gefolgt von Typ IIa und danach Typ IIx Fasern. Dennoch konnten wir keinen Zusammenhang zwischen dem Alter und dem Anteil an Muskelfasern erkennen. Zusätzlich untersuchten wir die Größe der Muskelfasern und den Anteil an der Gesamtzahl zwischen sarkopenen und nicht sarkopenen Patienten. Wir konnten allerdings keinen signifikanten Unterschied zwischen den beiden Gruppen feststellen.

Schlussendlich konnten wir herausfinden, dass die Größe der schnellen Typ IIa und IIx Muskelfasern mit dem steigenden Alter abnimmt, was im Einklang mit der schon existierenden Literatur bzgl. anderer Muskeltypen wie Musculus vastus lateralis steht. Allerdings konnten wir keinen Unterschied zwischen sarkopenen und nicht sarkopenen Patienten darstellen. Wir schließen daraus, dass die Gründe für Sarkopenie in unserer Kohorte nicht auf eine einzelne Ursache zurückzuführen sind und das die Ursachen für eine solche primäre Sarkopenie weiter untersucht werden müssen.

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Chapter 1

Introduction

1.1 The importance of locomotion

The survival of a species is only possible by consuming food as an energy source, by repelling natural predators and by reproduction. All these aspects heavily rely on the locomotion of the organism. Depending on the animal, the movements to suit these specific tasks are more or less complex. Functional independence in terms of breathing, generating heat and maintaining health can only be sustained by a complex system of muscles in most animals. Especially in higher organisms such as humans, social participation plays a key role in day-to-day living, which also requires distinct sets of muscles.

In humans, locomotion involves diverse kinds of movement and different parts of the body, including limbs for walking, jumping and gripping, the neck to move the head, the jaw and pharynx to chew and swallow food and facial muscles to make expressions. In addition, not only does the execution of dynamic movements require muscles, but also static tension to keep posture.

The importance of skeletal muscle can be seen by the proportion of muscle tissue in the whole body. On average, around 40% of the body weight is made up of skeletal muscle mass, containing 50 – 75% of all proteins and being responsible for 30 to 50% of all protein turnover (Spargo et al., 1979; Frontera and Ochala, 2015). For maintaining the integrity of the body, muscles demand a large amount of energy, which is stored inside the muscle cells in form of glycogen. This storage is not only used by muscles, but is also used to maintain a constant level of glucose in the blood, which is specifically

needed by the neuronal system (Stump et al., 2006). These facts show that muscle tissue is not only important for movement, but also plays an important role in the supply of metabolic substrates for other organs (Wolfe, 2006).
By summarizing the diverse functions of skeletal muscles, it is easily imaginable that loosing muscle mass and/or function poses a pivotal problem for the organism. Muscular diseases, as well as normal aging, cause complications to different extents. Patients with muscular dystrophies, such as Duchenne or Becker, show a significantly reduced life span, due to an early onset of advancing atrophy of skeletal muscles and ultimately the failure of respiratory muscles. Additionally, loss of muscle mass through aging entails a health risk, as low skeletal muscle mass is a major risk factor for higher mortality (Srikanthan and Karlamangla, 2014).

1.2 Architecture of skeletal muscle tissue

1.2.1 Macroanatomy

Skeletal muscle is a well-defined tissue, consisting mainly of muscle fibers and little surrounding connective tissue, in which a densely distinctive vascular network, innervating axons and muscle-specific fibroblasts are embedded. Tendons connect muscles and bones through myotendinous junctions and entheses, respectively, to transfer mechanical force over joints and thus generate locomotion. Most muscles are surrounded by a firm layer of fascia. Underneath the fascia, the fiber is surrounded by the epimysium, a layer of loose connective tissue, which also divides the muscle into fascicles (Figure 1.1). The fascicles are each subdivided into muscle fibers by the endomysium. Within this connective tissue, blood vessels form a densely packed capillary network and nerves branch to innervate each individual muscle fiber and spindle. The muscle fibers represent the majority of skeletal muscle cells that composed of a single cell membrane, surrounded by a basal lamina. Muscle fibers contain myofibrils, the sarcoplasmic reticulum, a network of mitochondria and multiple nuclei (Dahl et al., 2015; Picard et al., 2013). The size of a healthy and young muscle depends mainly on the number and the size of the muscle fibers and to a lesser degree on the amount of fat and connective tissue between the fibers. However, with advanced age or in presence

of myotendinous diseases, the amount of fat and connective tissue is decisively higher (Emery, 2002; Brown and Hasser, 1996).

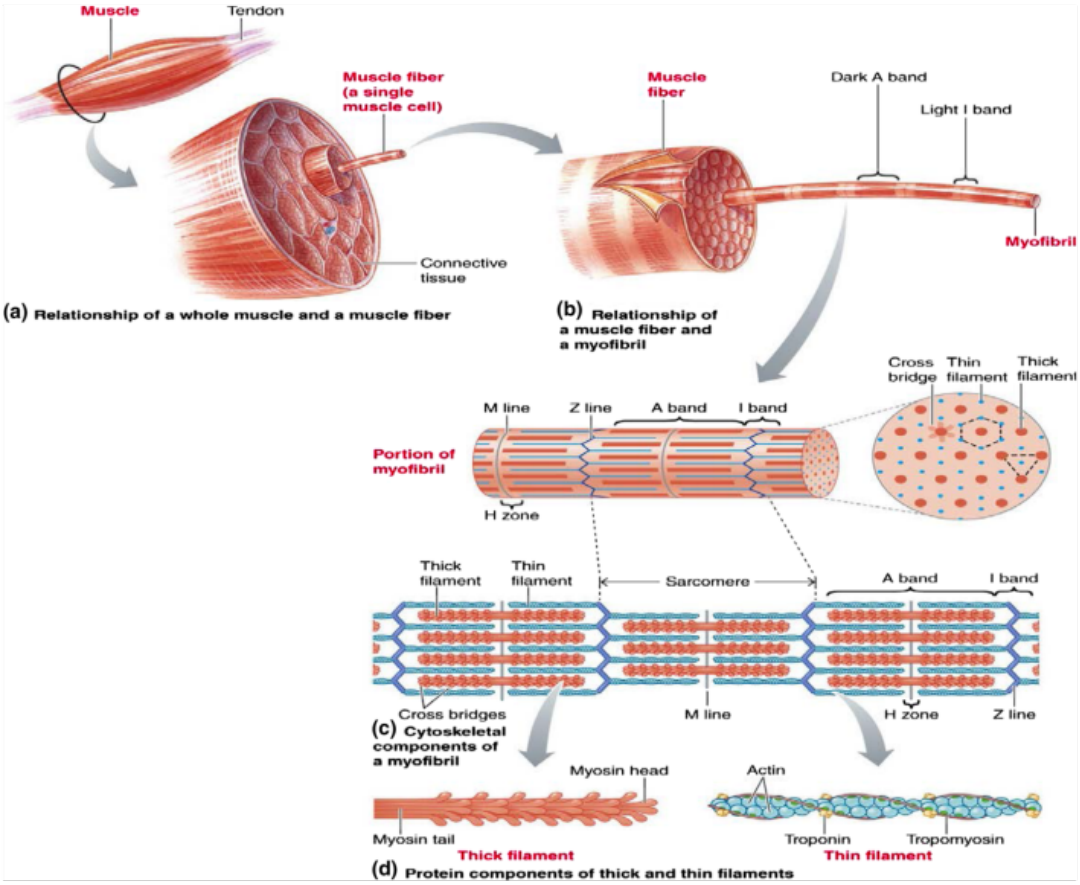

Figure 1.1: Structure of skeletal muscle.

(**A**) The whole muscle is surrounded by a layer of fascia and a layer of epimysium, which divides the muscle into fascicles. (**B-C**) Each fascicle is subdivided into muscle fibers that contain the contractile myofibrils. Myofibrils consist of overlaying thick myosin and thin actin filaments. (**D**) Thick filaments hold several myosin heads that build cross-bridges with the actin filament. Thin filaments are surrounded by troponin and tropomyosin (Sherwood, 2014).

1.2.2 Microanatomy

Within muscle fibers, myofibrils are densely packed and surrounded by sarcoplasmic reticulum, which itself has connections to transverse tubules. The tubules are inversions of the cell membrane, which create a large surface between the extracellular and intracellular compartments and a net of mitochondria. Two tubules of the sarcoplasmic

reticulum are accompanied by one transverse tubule, forming a triad orthogonal to the myofibrils. The mitochondria are located underneath the cell membrane and between the myofibrils (Figure 1.2).
As every muscle fiber is connected to only one neuromuscular junction, the large surface is important for the fast and even transduction of the excitation potential provoked by motor neurons (Slater, 2017). The myofibrils are long repetitive constructs consisting of sarcomeres, which are myosin filaments overlapping with actin filaments. These are the basic functional units of the contractile apparatus. The myosin filaments are able to build cross-bridges with the actin filaments and move their heads by the use of ATP and calcium (Figure 1.1, **C-D**). This generates a sliding movement against each other and thus the contraction of the muscle (Kuo and Ehrlich, 2015).

1.2.3 Muscle physiology

Muscle cells normally do not act independently, but require an external stimulus to initiate their contraction, which is necessary for a reliable and very narrow coordination between individual muscles and between muscle groups. Otherwise, a human body could not perform any tasks that require coordinated movement in any kind.
Directly responsible for the stimulus are somatic motor neurons in the ventral horn of the spinal cord. Each motor neuron has one axon that projects from the spinal cord via distinct nerve bundles to the target skeletal muscles, where it branches and innervates several muscle fibers. The amount of muscle fibers innervated by one motor neuron depends on the muscle and can vary from less than hundred fibers per neuron in extraocular and facial muscles to several hundreds of muscle fibers in soleus and quadriceps femoris muscle (Torre, 1953; Christensen, 1959; Burke et al., 1974). A neuron and its corresponding muscle fibers are known as motor units and represent the most basic functional unit within a muscle, because action potentials of one motor neuron trigger every innervated fiber simultaneously (Figure 1.3, **A**) (Fulton, 1931).

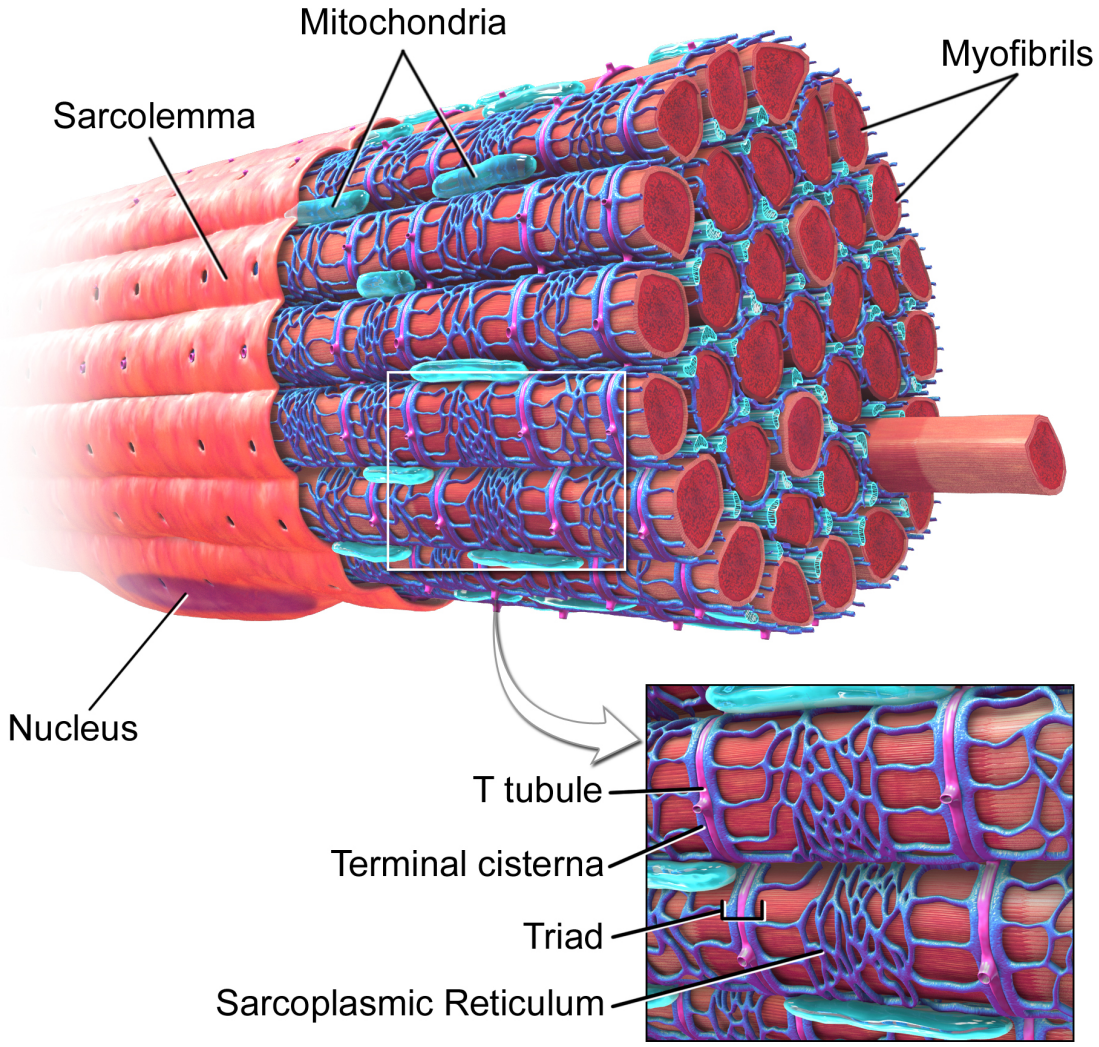

Figure 1.2: Three dimensional representation of the tubule system inside a skeletal muscle fiber.

The schematic representation of a skeletal muscle fiber depicts the three dimensional tubule system within the fiber. The transversal tubules, which are inversions of the cell membrane lie adjacent to two tubules of the sarcoplasmic reticulum, forming a triad. This network reaches through the whole muscle fiber. Additionally, rich deposits of mitochondria are scattered between the myofibrils (Staff, 2014).

The motor neuron-derived action potentials reach the neuromuscular junction, a specific synapse between neuronal and muscular cells (Figure 1.4). Vesicles in the presynaptic terminal of the neuron fuse with the membrane and release acetylcholine into the synaptic cleft, which binds to the postsynaptic ionotropic nicotinic acetylcholine receptors (AChR). These ligand-gated ion channels then let cations pass through and thus cause a cascade of opening voltage-dependent calcium channels throughout the

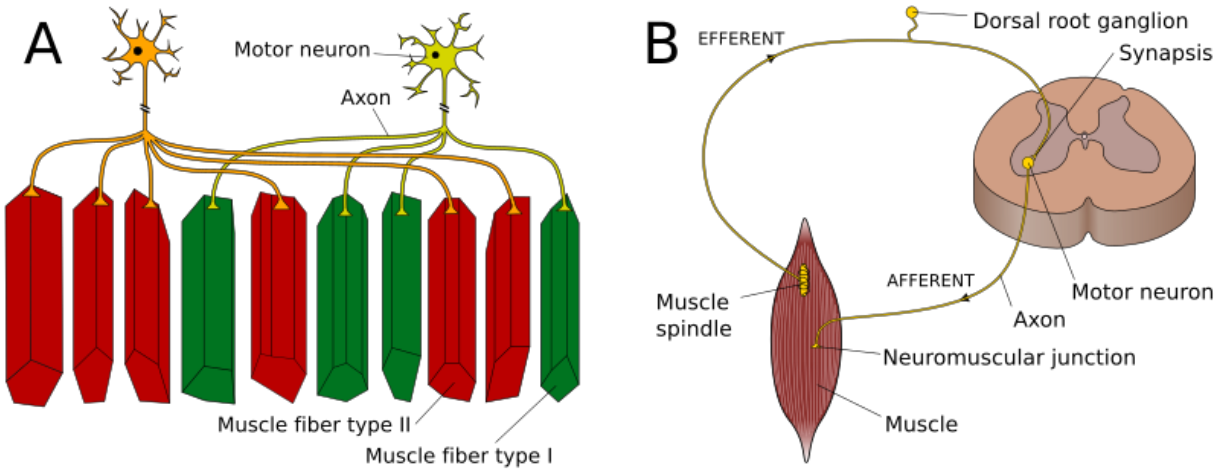

Figure 1.3: Schematic concept of two motor units.

Panel (**A**) shows two motor units in a skeletal muscle. One motor neuron innervates several skeletal muscle fibers of the same fiber type. Each skeletal muscle fiber is only innervated by one motor neuron. Muscle fibers of one motor unit do not necessarily lie adjacent to each other, but can intersect, which is responsible for the checkerboard-like distribution of fiber types within a muscle cross-section. (**B**) A motor neuron in the ventral horn of the spinal cord innervates skeletal muscle via an afferent axon and receives information about tension of muscle spindles via dorsal root ganglia. This creates a monosynaptic circuit, which can quickly and efficiently propagate information from the muscle spindle to the muscle.

transverse tubules and thereby depolarizing the muscle fiber. Dihydropyridine receptors (DHPRs), specific calcium channels in the cell membrane, physically interact with ryanodine receptors (RyRs) in the membrane of the sarcoplasmic reticulum directly adjacent to the transverse tubules. DHPRs then open due to the depolarization of the sarcolemma and open the RyRs themselves, which then release calcium stored within the sarcoplasmic reticulum (Kuo and Ehrlich, 2015).

When calcium is released from the sarcoplasmic reticulum, calcium binds to troponin, a protein on the actin filaments. Following the binding, troponin exposes the myosin binding site and allows myosin to establish a cross-bridge to the actin filament. This connection dissociates when adenosine triphosphate (ATP) binds to the myosin adenosinetriphosphatase (ATPase). ATP is then hydrolyzed into adenosine diphosphate (ADP) and phosphate (P_i), which generates a conformation change of the myosin where the head swings to the next actin molecule of the filament and binds again. Phosphate is then released and the head performs a “power stroke” releasing the ADP. At this point, new ATP must be bound to release the myosin heads from the actin filaments, be-

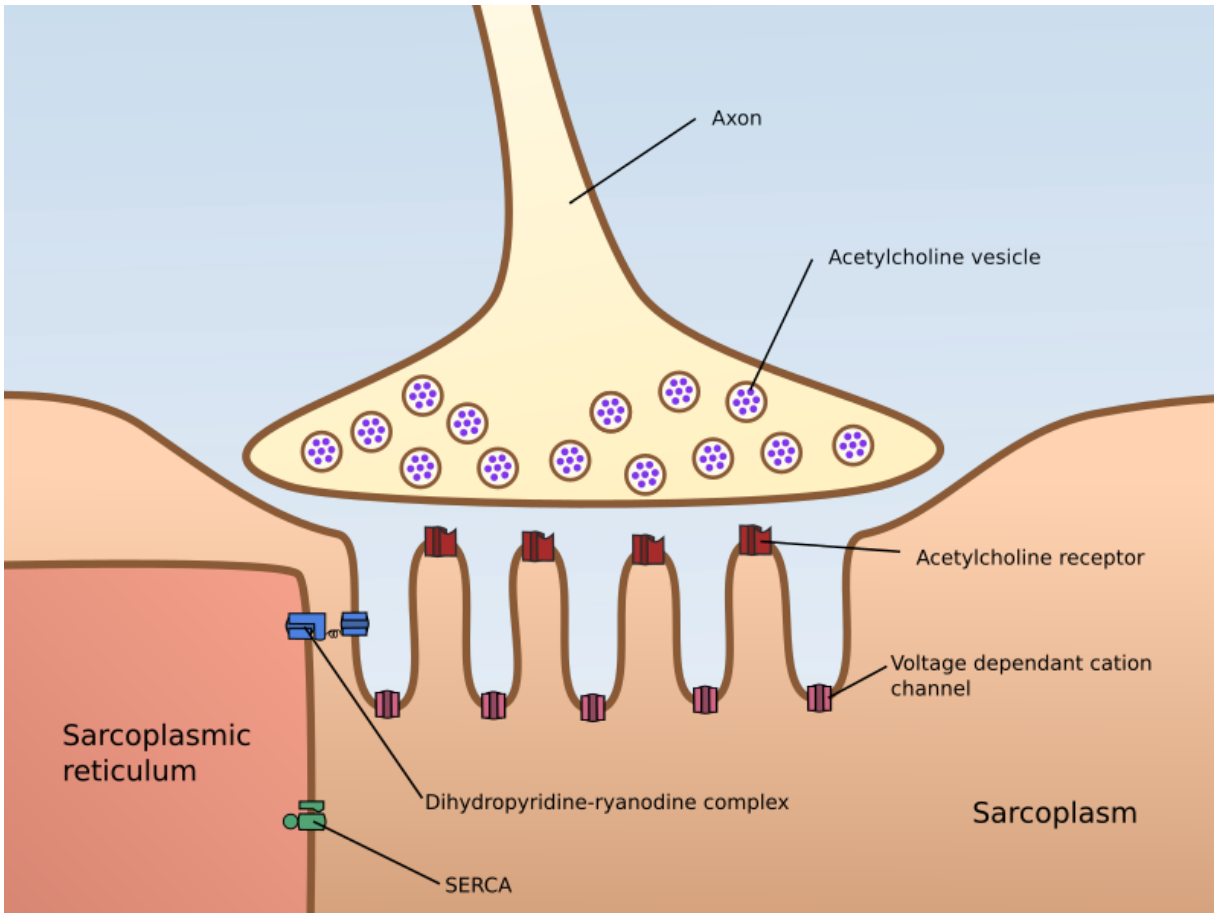

Figure 1.4: Illustration of a neuromuscular junction.

The figure shows a schematic display of a neuromuscular junction. Acetylcholine, which is stored in presynaptic vesicles, is released into the synaptic cleft upon depolarization by an action potential. Acetylcholine is then bound by the postsynaptic acetylcholine receptor (AChR) and thus induces a cascadic depolarization of the sarcolemma. Voltage dependent dihydropyridine receptors (DHPR) open sarcoplasmic ryanodine receptors (RyR), which then release calcium into the sarcoplasm.

ginning a new cycle of the contraction mechanism (Figure 1.5). Afterwards, the calcium gradient is reestablished by sarco/endoplasmatic reticulum calcium-ATPase (SERCA) transporters.

The central nervous system does not only activate muscles, but also receives information about the length and tension by the use of muscle spindles and Golgi tendon organs. While muscle spindles are specific stretch receptors, which are located within the muscle between the muscle fibers, Golgi tendon organs are stretch receptors within the tendons. Both organs combined give information via afferent fibers of a nerve to the

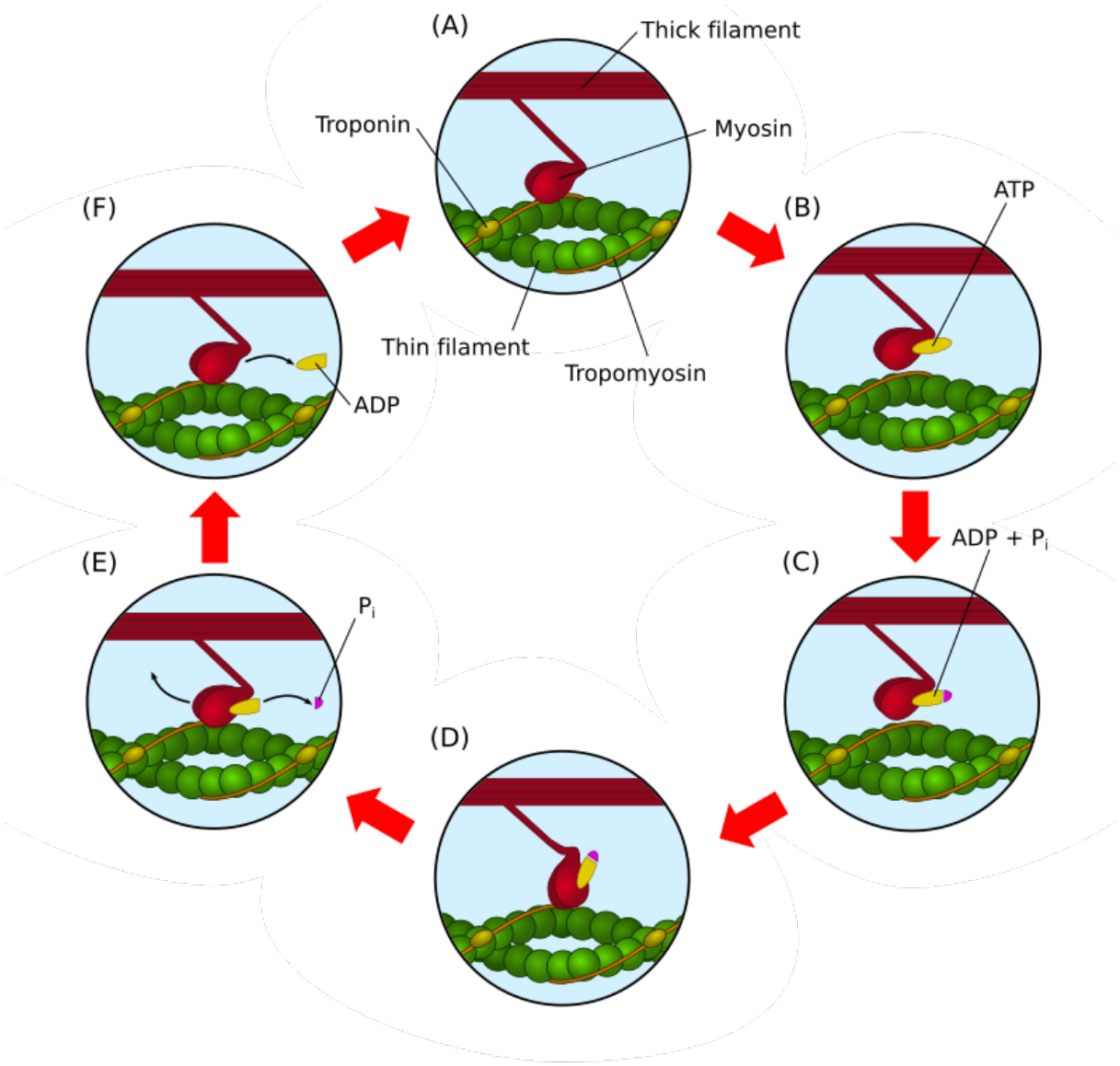

Figure 1.5: Excitation-contraction cycle sequence.

The figure shows the sequence of actions within skeletal muscle in the presence of stimulation by a motor neuron. (**A**) The myosin head of the thick filament is tightly bound to the actin of the thin filament. (**B**) This binding dissociates when the myosin head bind ATP. (**C-D**) ATP is then hydrolyzed into ADP and P_i by the myosin ATPase. This leads to a confirmation change in the myosin head, which then swings to another actin molecule. (**E-F**) Dissociation of ADP and P_i lead to a change of confirmation and initializes a 'power stroke'. Afterwards, myosin is again tightly bound to actin. Adapted and modified form (Frontera and Ochala, 2015)

central nervous system about the position and contraction of muscles. Muscle spindles and the Golgi tendon organs are innervated by sensory neurons situated in the dorsal root ganglia, which send their axons through the dorsal horn of the spinal cord, where they connect to the motor neurons of the corresponding muscle. This is a monosynaptic

connection from the muscle spindle, respectively the Golgi tendon organ, through the spinal root ganglion and the motor neuron to the same muscle (Figure 1.3, **B**) (Kröger, 2018; Sherrington, 1894). Both neurons are excitatory neurons and together have the function of triggering a contraction when the muscle is stretched, an indispensable feature to prevent muscles, tendons and joints from injury.
This is the most basic functional unit of movement regulation. It is observable when provoking stretch reflexes such as the patellar reflex or the ankle jerk reflex. In addition, each neuron branches out to various other neurons and interneurons and receive axons from many other neurons, making the whole mechanism vastly more complex and allowing simple monosynaptic reflexes to modulate muscle tension and contraction of different muscle groups, lowering the tension in antagonizing muscles and even bring up “primitive” reflexes such as walking or swimming.

1.2.4 Molecular biology

Myofibrils are connected to the cell membrane and proteins of the extracellular matrix via a dystrophin-associated glycoprotein complex (DGC). These complex structures contain a variety of proteins, such as dystrophin, dystroglycan and sarcoglycan. Dystrophin, a major protein of the DGC, and utrophin are directly connected to the actin filaments of the myofibrils and are themselves anchored in the cell membrane through sarcoglycan and dystroglycan, amongst others (Bhat et al., 2018). On the extracellular side of the cell membrane, the DGC is connected to the lamina densa of the basement membrane via laminin 2 (Figure 1.6). This connection allows the force generated by the contractile organelles of the cell to be conducted through the DGCs, the myotendinous junctions and the tendons finally to the bones (Townsend, 2014). The importance of the DGCs can be observed in people whose proteins of the DGCs are genetically compromised and are not fully functional as in various myopathies, such as Duchenne or Becker muscular dystrophy (Hoffman et al., 1987).

The basal lamina of each muscle fiber also surrounds several satellite cells, which are pre-mitotic stem cells of the muscle tissue, providing the cellular source of physiological muscle hypertrophy and regeneration (Bareja et al., 2014). The myonuclei are

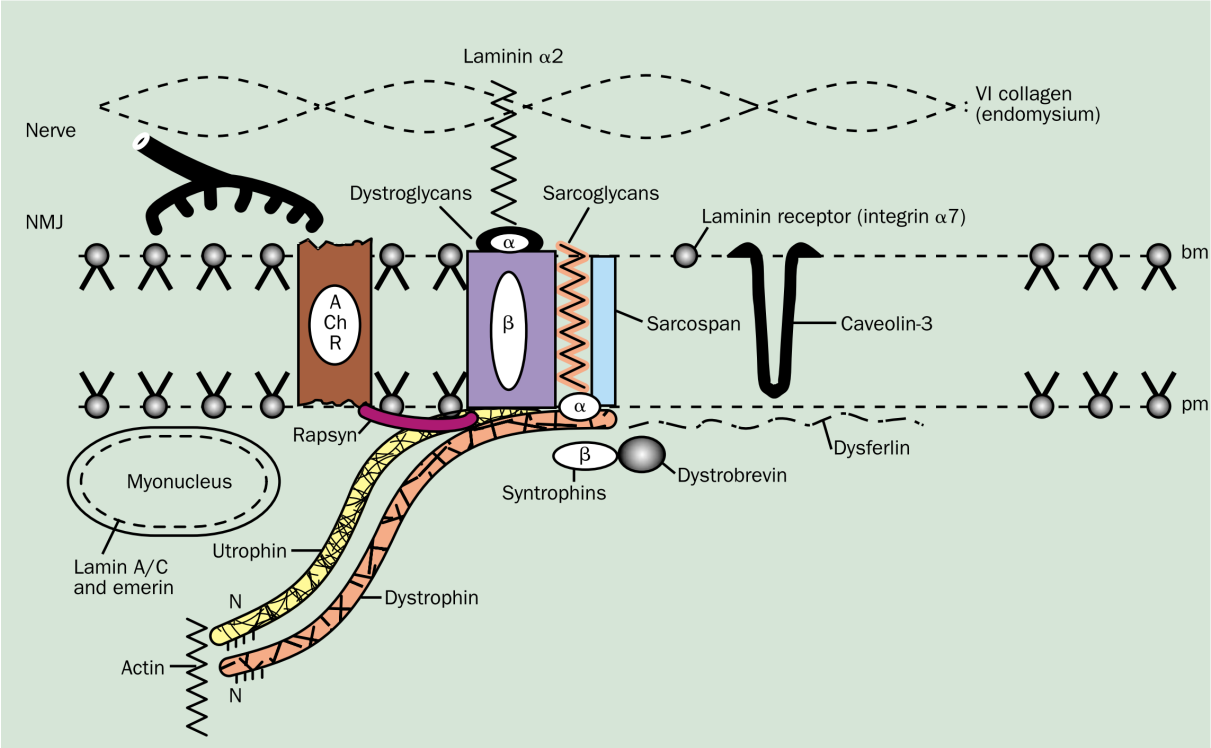

Figure 1.6: Dystrophin-associated glycoprotein complex (DGC) in muscle fibers.

The Dystrophin-associated glycoprotein complex is a complex of several proteins within the membrane of a skeletal muscle fiber, which interacts with various intra- and extracellular proteins. Actin filaments are connected via utrophin and dystrophin with various proteins in the sarcolemma. On the outside of the skeletal muscle fiber the DGC is linked via laminin to extracellular collagen. This connection holds the sarcomeres in place and conducts the contracting force to the whole muscle (Emery, 2002).

typically located at the periphery of the muscle cell below the cell membrane, unlike in many other cells, as the vast amount of sarcomeric and DGC proteins within the fibers push the nuclei below the outer cell membrane. This process is mediated by a complex interaction of many proteins such as fibronectin, α-integrin, FAK and Src during the maturing of the myoblasts (Roman et al., 2018) (Figure 1.7). Each nucleus within the fiber expresses proteins to form the myofibrils, but only in a closely defined area, which is consequently called nuclear domain (Ralston and Hall, 1989). These domains are highly coordinated throughout the muscle and thus, all nuclei normally express the same isoforms of MHC. However, muscle fibers in healthy people can also express multiple isoforms of myosin heavy chains (Pette and Staron, 2000).

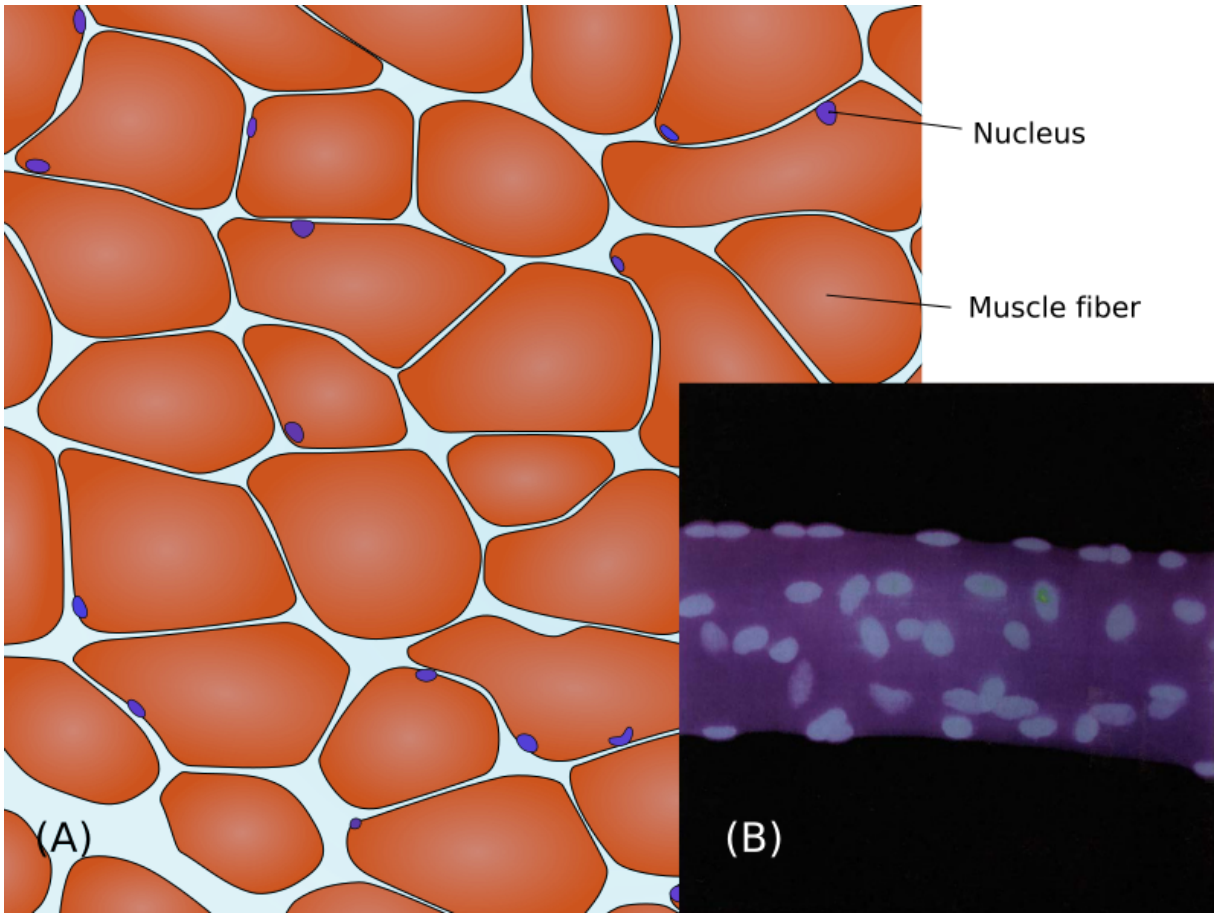

Figure 1.7: Localization of multiple nuclei in skeletal muscle fibers.

(**A**) The schematic depiction of a skeletal muscle cross-section shows the form of skeletal muscle fibers and the localization of myonuclei within the cells. (**B**) The picture shows the position of multiple myonuclei under the sarcolemma on the outside of one single muscle fiber (Frontera and Ochala, 2015; Allen et al., 1995).

1.2.5 Diversity of skeletal muscle fiber types

Skeletal muscle tissue has to satisfy many different demands, enabling the human body to successfully navigate within their environment. These tasks include generating static force for long periods, for example in trunk muscles, which straighten and stabilize the spine, while standing upright. On the other hand, leg muscles have to be able to act dynamically, as sprinting and jumping demand a high magnitude of force at once but only for a very brief time. This means that muscle tissue has to conserve its energy resources for long-lasting, low-intensity activity, but must also be able to increase the energy output by a high margin almost instantaneously to successfully execute explosive force needed in fight-and-flight situations. To better suit these diverse demands,

evolution developed different muscle fiber types, which are adapted to exhibiting either more static or dynamic activity.
Fiber types can be characterized by their biochemical attributes in terms of fatigue-resistance, contraction time, mitochondria content, calcium release and reuptake by the sarcoplasmic reticulum, predominance of metabolic pathways, ATPase activity and extracellular properties such as the pericellular capillary density (Peter et al., 1972; Lowry et al., 1978). These differences are not only attributes for the identification of the fiber types, but reflect the adaptation during the evolutionary process (Buchthal and Schmalbruch, 1980). In human skeletal muscles, there are three dominant fiber types present, slow-twitching and fatigue resistant type I fibers, fast-twitching and moderately fatigue resistant type IIa fibers and fast-twitching and fatigable type IIx fibers (Schiaffino and Reggiani, 2011). Each muscle in the human body has slightly different tasks, as muscles close to the trunk and those extending joints, especially in the lower limbs, are particularly needed to maintain posture. Muscles more distant to the trunk, such as forearm muscles, are generally rather utilized for fine motor skills, and thus rely to a lesser extent on fatigue resistant fibers. It is therefore comprehensible that the proportion of the different fiber types varies between muscles with almost exclusively postural function and those used for precise and dynamic movement (Johnson et al., 1973).
Although muscle fibers can be characterized by the attributes mentioned above, the identification is based on the different isoforms of myosin. In general, each muscle fiber expresses only one specific isoform of myosin (Westerblad et al., 2010; Weiss et al., 1999). Fiber types and their corresponding myosin heavy chains are listed in table 1.1. Other histological and biochemical attributes, such as stronger capillarization, glycogen content or the balance between oxidative and glycolytic metabolization are substantiating factors to the function given by the myosin isoform (Pette and Staron, 2000).

Cross-sections of muscles reveal that the fibers of each type do not necessarily lie directly next to each other but are distributed throughout the muscle in a checkerboard like fashion (Figure 4.4) (Edström and Kugelberg, 1968). As motor neurons innervate several ten to hundreds of muscle fibers and each of them are of the same fiber type, it is obvious, that the territory taken up by the muscle fibers of one motor neuron overlaps with the territory of others (Burke et al., 1974).

Table 1.1: Overview of different fiber types and myosin isoforms.

	Type I fiber	**Type IIa fiber**	**Type IIx fiber**
Myosin isoform	MHC Iβ	MHC IIa	MHC IIx
Twitch speed	slow	fast	fast
Twitch force	low	medium	high
Fatigue resistance	high	high	low
Glycogen content	low	high	high
Capillary supply	rich	rich	poor
Myoglobin	high	high	low
Mitochondrial density	high	high	low
Alkaline ATPase activity	low	high	high
Acidic ATPase activity	high	high	low

Adapted and modified from (Peter et al., 1972; Pette and Staron, 2000).

Muscle fibers exhibit a limited potential of changing their biochemical properties, particularly the expression of different myosin isoforms, which renders the trans-differentiation from one fiber type into another a high-energy process. This might occur under physiological influences, such as a different pattern of excitation and by demanding a more dynamic response, which can trigger a shift from slow- to fast-twitch fibers or a more static response. Changes due to these influences can be observed in marathon runners, who exhibit a larger amount of type I fibers or high jumpers, who exhibit many type II fibers, respectively (Goldspink, 1998; Gutmann et al., 1972). Furthermore, hormone blood levels, especially thyroid-stimulating hormone (TSH) (Ianuzzo et al., 1977), as well as aging (Miller and Toth, 2013), contribute to an accentuated decrease in type II fibers and to overall muscle atrophy. There is also evidence that hybrid fibers, expressing two or more different isoforms of myosin, occur more commonly in aging patients than in younger adults (Andersen, 2003).

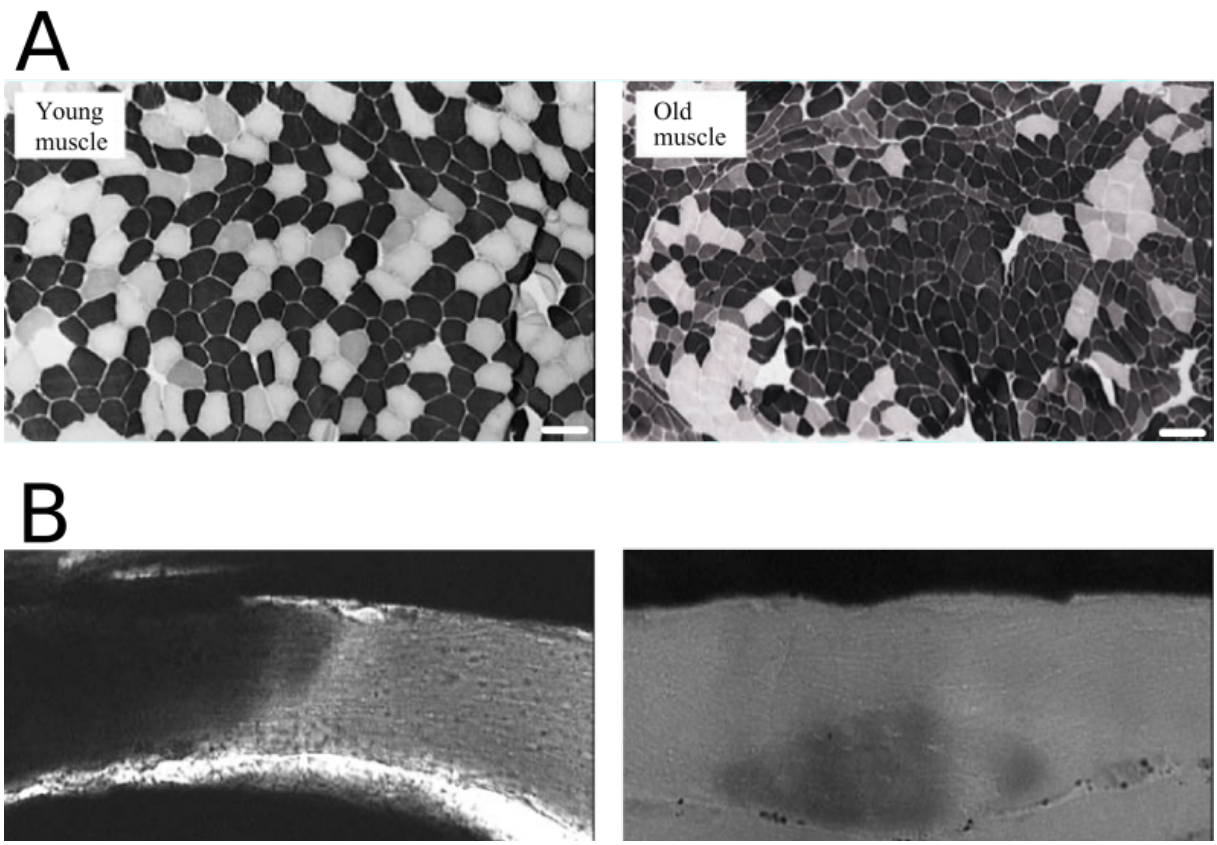

Figure 1.8: Different fiber types and their distribution within muscle tissue.

The figure illustrates enzymatic activity of adenosinetriphosphatase (ATPase) in skeletal muscle of humans. (**A**) Cross-sections of skeletal muscle of a young (left) and an old (right) person. The young muscle depicts a checkerboard like distribution of fiber types whereas the old muscle shows a distinct bundling of muscle fibers. ATPase staining (pH 4.6). (**B**) Both pictures show ATPase stained longitudinal sections of a single skeletal muscle fiber of a person older than 85 years. In both cases a single muscle fiber exhibits more than one MHC type. The shift of muscle fiber type can appear along the longitudinal axis (left) or originating from the domain of a myonucleus (right). ATPase staining (pH 4.6). Dark fibers, type I fibers; white fibers, type IIA fibers; grey fibers, type IIx (or I/IIa) fibers; scale bar = 100μm. Adapted and modified from (Andersen, 2003).

1.3 Adaptation of skeletal muscle in aging

Aging is a complex process to which all organisms succumb, which affects many organs including the musculoskeletal system. While skeletal muscle comprises up to 40% of the body mass, changes in skeletal muscle are most likely to affect the mobility, especially of elderly people, to a great extent. In fact, various studies describe the loss of skeletal muscle mass starting at the age of 40-50 years, with an increased loss from 60 years onwards and a loss of up to 50% of total skeletal muscle mass by the age

of 80 years (Lexell et al., 1988; Booth et al., 1994; Lexell et al., 1986). Although the loss of muscle mass is observable in all aging people, some people show a more rapid progression of muscle loss. Additionally, the changes in muscle mass are shown to be more obvious in males than in females (Yamada et al., 2014) (Figure 1.9).

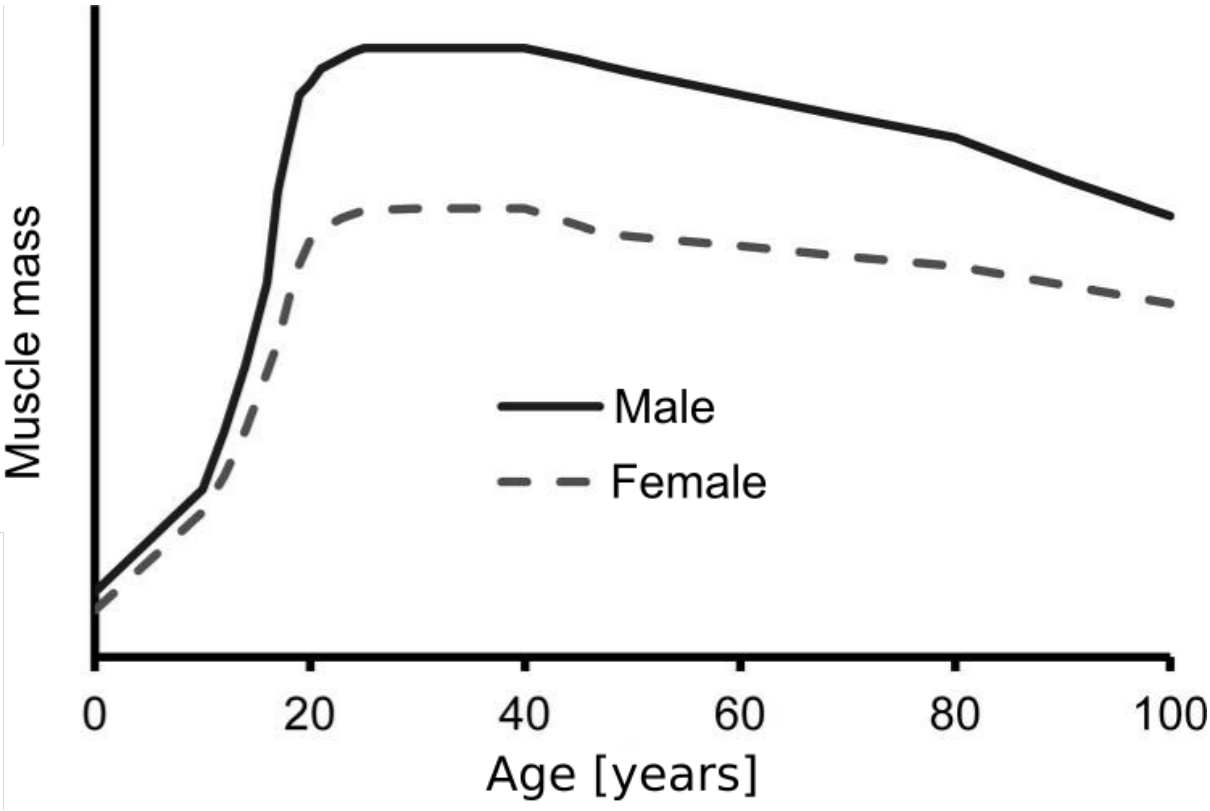

Figure 1.9: Age-dependent loss of skeletal muscle mass.

The figure displays the relation of skeletal muscle mass of male and female humans to age. Throughout the whole life females have less skeletal muscle mass than men, especially after puberty the increase in muscle mass in males is accelerated. Loss of skeletal muscle mass begins in midlife and is enhanced by the sedentary lifestyle of old people. Although females generally depict less muscle mass, the loss during aging is less distinct in females compared to males. Adapted by Smith and Mittendorfer (Smith and Mittendorfer, 2016).

This loss of muscle mass and function is also associated with lower mobility, resulting in a higher overall mortality (Kalyani et al., 2014; Dodds et al., 2015; Srikanthan and Karlamangla, 2014) and is discussed to be a product of frailty, a syndrome characterized by the reduction of musculoskeletal function, aerobic capacity, cognitive and integrative function and nutritional reserves (Campbell and Buchner, 1997). Hence, an overall reduced capability in life-sustaining body functions, which leads to higher risks

of chronic diseases, complicated courses of disease and a hindered ability to overcome traumas and hospitalization (Muscedere et al., 2017). The interaction between skeletal muscle loss, physical impairment, chronic disease and frailty has already been hypothesized as a cycle with a reciprocal sustentation ultimately leading to death (Aguirre and Villareal, 2015). The causes for this cycle however have not yet been fully uncovered and might considerably differ between individuals.

During aging, skeletal muscle tissue also depicts very distinct changes. Although the loss of muscle mass is generally accelerated, lower limb muscles show an accentuated weight loss compared to upper limbs muscles (Lynch et al., 1999), which demonstrates a non-uniform muscle adaptation throughout the whole body. The reasons for the loss of muscle mass and a decrease in physical performance include the atrophy of muscle fibers and the increase of connective and fat tissue between the fibers (Fielding et al., 2011). In addition, muscle fibers are shown to be affected by atrophy depending on their fiber type. In vastus lateralis and gastrocnemius muscle, the atrophy and eventual loss of fast-twitching type II muscle fibers was shown to be more prevalent than in the slower acting type I fibers (Tellis et al., 2011; Coggan et al., 1992). This leads to a higher proportion of fatigue resistant type I fibers within the skeletal muscle and thus to a better conservation of energy, which might be beneficial for older people, who exhibit lower reserve capacities than younger adults. The reduced number of motor neurons innervating skeletal muscles contributes to the transition from fast-twitching skeletal muscle fibers to slow-twitching fibers. Motor neurons in the spinal cord degenerate with aging and thus decline in numbers, resulting in an accumulation of denervated muscle fibers (Aare et al., 2016; Rowan et al., 2012). The residing motor neurons adapt to this atrophy by re-innervating denervated muscle fibers to a limited extend, potentially forcing the reinnervated fibers to change their fiber type, developing large groups of muscle fibers of the same fiber type (Doherty et al., 1993; Kanda and Hashizume, 1989; Tudoraşcu et al., 2014).

In addition, within skeletal muscles, the number of satellite cells reduces during aging, especially around type II fibers, which might contribute to the ongoing loss of such muscle fibers (Verdijk et al., 2007). The performance of single muscle fibers themselves is also impaired, as it is shown that cell organelles and contractile proteins degenerate over time. Muscle fibers of old humans contain significantly less actin and myosin compared to muscle fibers of younger patients (Miller and Toth, 2013). Thus, the force

generated and the elasticity of a single muscle fibers is lower in elderly than in young adults (Ochala et al., 2007). This relation of force generation and elasticity is thought to be an adaptive mechanism to reduce the energy needed for efficient day-to-day activities. Increasing stiffness could thereby compensate less efficient muscle contraction and sustain motoric stability. The sarcoplasmic reticulum is noticeably reduced in its capacity to hold calcium homeostasis due to an increasing number of segregated compartments, which hold calcium that cannot be mobilized and is thus separated from the excitation-contraction mechanism (Weisleder et al., 2006). Beside the changes of calcium homeostasis within the sarcoplasmic reticulum, also mitochondrial function is affected during aging. Although it is not yet clear if the loss of fibers is due to a decreased number of mitochondria or vice versa, it has been shown that age alone is not solely responsible for a lesser density of mitochondria. Elderly people often tend to a more sedentary lifestyle, but are generally able to regain mitochondrial function with sufficient exercise (Broskey et al., 2014; Cadore and Izquierdo, 2013). However, mitochondrial function might also be compromised by accumulated mutations in mitochondrial DNA, when mutated mitochondria reach a critical threshold in muscle fibers (Wang et al., 2001). This observation is underlined by the fact that induced mutations in mitochondrial DNA lead to a significantly increased loss of muscle fibers (Herbst et al., 2016). Furthermore, mitochondria produce reactive oxygen species (ROS) that can harm the content of the cell by uncontrolled oxidation of cell structures. The generation of ROS is increased with aging and the oxidative stress affects proteins of the contractile apparatus. Especially for the binding sites of actin and myosin it is shown, that oxidative stress can impair muscle function by decreasing the gene expression and therein lowers the content of contractile protein and by modifying amino acid side chains, which leads to an impaired ability to produce force (Moen et al., 2014).

This underlines that not only loss of muscle mass itself leads to the impaired physical ability of the elderly human, but also a wide range of effects, caused by aging, result in the functional loss of skeletal muscle, although their relative contribution is not yet fully resolved.

1.4 Sarcopenia: a pathologic form of aging?

For many decades, aging has been known to be associated with a generalized atrophy of several organ systems. Especially the loss of appendicular lean body mass, and therewith fading strength, are perceptible changes that occur in all humans. The increasing loss of skeletal muscle mass in aging adults has long had no specific name, although it has been of interest at least since the early twentieth century. With intensified focus on the aging society the term sarcopenia first occurred in an article by Irwin Rosenberg in 1987 (Rosenberg, 1989). The term sarcopenia was derived from the greek word 'sarx' for flesh and 'penia' for loss and was used to describe changes in body composition and their related functions in the context of aging. The subsequently defined syndrome sarcopenia featured the age-related loss of muscle mass, which was believed to be a predictor of impaired physical performance and therefore waning independence of people in old age. Looking solely on muscle mass, at what point does a human experience constraint in fitness?

By looking at world-class athletes in various fields, it is peculiar how each individual adapts to different physical tasks and excels peak performances. It is therefore deducible that strength, and hence physical performance, does not necessarily depend solely on sheer skeletal muscle mass. Indeed, it has been shown that not muscle mass but muscle strength is independently associated with physical performance (Visser et al., 2000). Consequently, if in young humans, as well as aging humans, skeletal muscle mass is not the only crucial factor that determines fitness, skeletal muscle strength could be a relevant determinant of independence (Dodds et al., 2015). In fact, muscle strength and physical function are directly associated with higher rates of disability and mortality (Cesari et al., 2009). This was demonstrated by the fact that neither muscle mass in calf muscles nor fat mass are significantly relevant risk factors for mortality, but that strength measured in quadriceps as well as grip strength provide equally sensitive markers to determine the mortality risk (Manini et al., 2007; Newman et al., 2006). These findings are crucial information to broaden the picture of sarcopenia, and thus, a revised definition was created by the European Working Group on Sarcopenia (EWGSOP) in 2011 that describes sarcopenia as a syndrome consisting of low skeletal muscle mass and additionally either low muscle strength, physical performance or both (Cruz-Jentoft et al., 2010). The definition of sarcopenia by the EWGSOP has been

proven as a valid predictor of poor health outcomes (Landi et al., 2012b). As the body composition changes and muscle strength declines with age, although individually different, it is an inevitable process found throughout all humans. This raises the question, if sarcopenia can be considered a form of physiological aging, a disability or a disease. To address this question properly, it is important to elucidate the causes of sarcopenia. Sarcopenia is a medical state, mediated by a complex multifactorial pathophysiology with various differently weighted factors contributing to the loss of muscle mass and strength. There are major mechanisms considered to be involved in the genesis, which include the physical activity, neuromuscular integrity, protein metabolism, regulation of gene expression, endocrine milieu, circulating pro-inflammatory mediators and apoptosis. A sedentary lifestyle is the main factor that contributes to muscle weakness and this again promotes a further emphasized loss of strength itself (Aguirre and Villareal, 2015; Groessl et al., 2019). In contrast, physical exercise represents an important countermeasure to prevent weakness (Marzetti et al., 2017). Resistance training influences muscle mass and strength, mainly by the increase of the size and number of myofibrils, whereas type II muscle fibers are predominantly involved (Suetta et al., 2008). While this effect is observable in exercising muscles and represents a major benefit for older people, it is obvious how the absence of physical exercise can negatively affect skeletal muscle function and can therefore be considered a primary risk factor for the development of physical disabilities (Steffl et al., 2017).

Furthermore, skeletal muscle protein translation is also dependent on the protein metabolism. Imbalances in the synthesis and degradation in favor of degradation tends to weaken the contractile elements of skeletal muscle fibers. There are various causes for imbalances in protein turnover, with a driving reason being non-sufficient intake of metabolites, especially amino acids (Pasini et al., 2018). It has been demonstrated that in addition to resistance training, a supplementation of amino acids, mainly leucine, represent important anabolic stimuli in skeletal muscle protein synthesis (Makanae and Fujita, 2015). Both a leucine enriched nutrition supplementation and resistance training induce protein synthesis by activating mammalian target of rapamycin (mTOR) and ribosomal protein S6 kinase (S6K1) pathways (Anthony et al., 2001; Ogasawara et al., 2014). A lack of resistance exercise or appropriate nutrient intake negatively affects muscle hypertrophy and thus strength. Indeed, meta-analyses that evaluated the association of low protein intake with physical function could show that a high protein intake of >1.0 g/kg/d was

correlated to higher lower limb strength (Coelho-Júnior et al., 2018).
As skeletal muscle fibers do not act independently, it is evident that the motor neurons in the spinal cord must play a key role in the physiological function and maintenance of skeletal muscle. Analogous to known neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), the loss of motor neurons in both ALS and aging is a further risk factor in the development of weakness and disability. The atrophy of neurons responsible for the activation of muscle fibers leads to weakness and further atrophy of the given muscle. Pathophysiological causes are theorized to be “dying-back” and “transsynaptic degeneration” mechanisms, pathological changes in axons prior to neuron degeneration, which are also found in humans depicting sarcopenia, but to a lesser extent respectively (Fischer et al., 2004; Tintignac et al., 2015). Although the decline in motor neuron number with increasing age does not have the same impact as neurodegenerative diseases, it has nevertheless been shown that it is significantly associated with the occurrence of sarcopenia and could help as a role model in studying the influence of neuromuscular integrity in sarcopenic patients (Drey et al., 2014; Rowan et al., 2012; Pannérec et al., 2016).
Skeletal muscle structure, metabolism and neuromuscular function as well do all stand, to some extent, in the context of the endocrine milieu and circulating inflammatory mediators. Both skeletal muscle mass and strength are known to be negatively influenced by chronic diseases and inflammation (Bano et al., 2017). In addition to that, several studies have demonstrated that imbalances in the endocrine milieu as they occur in several diseases, have a negative effect on skeletal muscle. Glucocorticoids feature metabolic effects, among other things shifting protein turnover towards catabolic states. Significantly higher excretion of glucocorticoids in patients with Cushing’s syndrome therefore lead to higher protein degradation and consequently to significantly less muscle strength (Berr et al., 2017). Vitamin D has been demonstrated to exerpt a positive effect on the growth of skeletal muscle (Wagatsuma and Sakuma, 2014). Although the exact molecular effects have not yet been fully understood, it is possible to assume, that a lack of vitamin D might be one influential factor on sarcopenia. Likewise, it could be demonstrated, that a supplementation in vitamin D has a positive effect in the treatment of sarcopenic patients (Bauer et al., 2015; Tessier and Chevalier, 2018). Furthermore, chronic inflammation affects skeletal muscle tissue negatively. Increased expression of tumor necrosis factor α (TNF-α) stimulates ubiquitin-dependent ligases E1, E2 and E3,

which increase protein degradation (Jo et al., 2012; Patel and Patel, 2017).
Although sarcopenia as a syndrome is contoured clearly, the complexity of the multifactorial cause underlines the quandary of clinical practice. Identifying patients with a clear cause might prove to be difficult if not impossible, given the fact that the various factors determining loss of muscle mass and strength weighing differently from individual to individual or might even be absent at all. It is therefore important to further investigate causes for sarcopenia that are generally present and to differentiate them from those which are specific to certain diseases that promote sarcopenia themselves.

1.5 Prevalence and socio-economic significance

The question whether sarcopenia is a form of aging, a disability or a disease has not yet been fully elucidated, yet the consequences of weakness in old age, with no doubt, cannot be denied. Beside the difficulties in clinical practice, such as defining and diagnosing sarcopenia efficiently, it is important to understand the impact of sarcopenia on the society. Clearly defining a syndrome and attribute diagnostic parameters for comparability are needed to consistently survey large cohorts in a society.
Using the definition provided by the EWGSOP, several studies have estimated numbers of people deemed to be sarcopenic. A systematic review by Cruz-Jentoft et al. examined multiple prevalence studies on sarcopenia in western countries and revealed a share of 1 to 29% of community-dwelling people, 14 to 33% of elderly in long-term housing and 10% of patients in acute hospital-care to be sarcopenic (Cruz-Jentoft et al., 2014). The German FORMoSA study found 4.5% of 1,325 community-dwelling female participants aged 70 years and above to be sarcopenic (Kemmler et al., 2015), a relatively low amount. However, participants in the study with osteoarthritis were found to be at a higher risk for sarcopenia. Prevalence in Japan has been shown to be 21.8% for men and 22.1% for women in community-dwelling elderly aged 65 to 89 years (Yamada et al., 2013). Different studies also focused of special cohorts, which are thought to exhibit risk factors for poor health outcomes. In the group of people aged 80 years and above, the BEFRAIL study, conducted in Belgium, classified 12.5% of the 567 participants as sarcopenic (Legrand et al., 2013). The Newcastle 85+ study investigated the prevalence of people aged 85 and above in the UK and came to a share of 21% of

the 845 participants (Dodds et al., 2017). Additionally, 32.8% of elderly over 70 years living in nursing homes were sarcopenic (Landi et al., 2012a). Furthermore, multivariate analysis depicted a higher risk for sarcopenia in male nursing home residents and in those with cerebrovascular disease and/or osteoarthritis. Sarcopenia was less likely in those residents involved in regular physical activity, underlining the importance of exercise, even in old age. These studies also suggest that the prevalence of sarcopenia increases with increasing age.

The importance of sarcopenia cannot be deduced by the high prevalence in aging people alone. In fact, sarcopenia has been shown to be associated with significantly poorer health outcomes compared to non-sarcopenic people. A meta-analysis to review short-, middle- and long-term outcomes of sarcopenia came to the result that sarcopenia significantly affects mortality negatively (Beaudart et al., 2017). In addition, a higher fall rate and a higher incidence of hospitalization were reported. In nursing homes, residents with sarcopenia were 2.34 times more likely to die (Landi et al., 2012b). Additionally, in South Korea the risk for death in sarcopenic people was assessed to be between 2.99 and 3.22 times higher than in non-sarcopenic people (Kim et al., 2014).

The prevalence of sarcopenia in people of old age is relatively high and with sarcopenia being a major risk factor, the economic burden is conspicuous. Furthermore, the share of people aged 80 yeas and higher is the fastest growing group worldwide (United Nations and Social Affairs, 2017) This means, that not only the aging of the population itself, but also the associated risks for poor health represent a major economic burden that will continue to grow in the future (Bloom et al., 2015).

Chapter 2

Aim of the study

It has been shown that with increasing age, humans lose muscle mass and are increasingly impaired in physical performance, which diminishes their ability to participate in activities of day-to-day living. With reduced fitness health risks such as falls, hospitalization rates are also increasing. Furthermore, sarcopenia represents a health issue of increasing economic burden, which will further grow with the demographic change of the population. It is therefore obvious that musculoskeletal function is of high interest and containing strength is necessary for managing health demands of elderly patients. Current recommendations for treatment of sarcopenia contain supplementation of vitamin D and proteins (Bauer et al., 2015; Malafarina et al., 2013), as well as strength training and conditioning (Cadore and Izquierdo, 2013; Theou et al., 2011). However, especially training is often difficult for old adults, which makes a specific pharmacological therapy highly valuable.

For the challenge of finding new possible treatments for sarcopenia, we must clarify the impact of the different causes that lead to sarcopenia. Physiological, as well as pathological influences, such as weight-load, innervation and hormone levels can change the relative proportion of fiber types in the skeletal muscle (Izumo et al., 1986; Pette et al., 2002). To this date, it has been investigated that human skeletal muscle undergoes change during aging. However, morphological differences between skeletal muscle of sarcopenic and non-sarcopenic humans have not been described. For this purpose, we took samples of gluteus medius muscle from patients with proximal hip fractures, who underwent surgery at the Department of General, Trauma and Reconstructive Surgery at the University Hospital of Munich. We assessed skeletal muscle mass via bioelectri-

cal impedance analysis (BIA), measured handgrip strength with a hand dynamometer and analyzed blood levels of thyroid-stimulating hormone (TSH), 25-hydroxyvitamin D (25(OH)D), total protein and calcium. Furthermore, we hypothesized that patients with a higher skeletal muscle index depict generally bigger muscle fibers, especially of type IIa and IIx fibers and a higher proportion of fast-twitching fibers compared to patients with lower skeletal muscle index.

Chapter 3

Materials & Methods

3.1 Ethics statement and sample acquisition

This study was approved by the ethics committee of the Ludwig-Maximilians-University Munich (265-16) and declares itself to adhere to ethical standards and the generally accepted rules of medicine. Between 08/2016 and 03/2017, 20 patients with proximal femoral fractures were included in this study after signing an informed consent. Patients with acute or chronic infections, psychiatric or neurologic diseases, cancer, pregnancy, infections with human immunodeficiency virus (HIV), hepatitis B (HBV) and C (HCV) were excluded. All biopsies were collected by a trained surgeon under adherence to all current surgical standards during operation.

3.2 Clinical assessment and bioelectrical impedance analysis

The patients' mobility and activities of daily living prior to the fracture were assessed with a questionnaire including the Barthel index (Mahoney and Barthel, 1965), the Parker mobility score (Parker and Palmer, 1993) and the SARC-F score (Malmstrom et al., 2016). Each of those contain questions about the ability to conduct daily activities independently, such as walking, climbing stairs, getting up from chairs, dressing, using the toilet, etc. (for detailed view, the questionnaires used in this study are appended

in the supplementary data). The maximum handgrip strength was determined by a dynamometer (DynExTM, Akern, Italy). Three measurements on each side were performed and the highest value was selected. Additionally, the skeletal muscle mass was approximated using a bioelectrical impedance analysis (BIA). Patients had to be lying straight for at least five minutes before the examination was performed with a BIA device (BIA 101 Anniversary, SMT medical GmbH & CO. KG, Germany). The BIA was conducted bipolar with two electrodes on either the left or right hand and foot, regarding the absence of implants on that side of the body, avoiding contact between the extremities. The resistance value was then used to determine the total skeletal muscle mass and the skeletal muscle index (SMI) using the Janssen equation

$$SMM = \left(\frac{Ht^2 * 0.401}{R}\right) + (gender * 3.825) + (age * -0.071) + 5.102$$

where SMM = skeletal muscle mass [kg], Ht = height [cm], R = resistance [Ω], gender = 0 for female or 1 for male and age = patient's age [years] (Janssen et al., 2000). The SMI was then calculated by dividing the total skeletal muscle mass by the height in meters squared.

3.3 Collection and processing of skeletal muscle biopsies

Biopsies of the gluteus medius muscle were obtained during surgical treatment of the proximal femur fractures either by cephalomedullary nailing or hip arthroplasty. No longer than ten minutes after acquisition the collected samples were immersed in TissueTek O.C.T. (Sakura Finetek, Japan) and immediately frozen in liquid nitrogen cooled isopentane (Sigma-Aldrich, USA). Biopsies were then cut into 10 μm thick sections with a cryotome (ThermoFisher Scientific, USA) and stored at -80°C until further usage.

3.4 Immunohistochemistry

Sections were thawed at room temperature, fixed in acetone (Sigma-Aldrich, USA) for 10 minutes, rehydrated with PBS-T (phosphate-buffered saline with 0.1% Triton) (Sigma Aldrich, USA) and blocked in heat-inactivated horse serum (10% in PBS, ThermoFisher Scientific, USA) at room temperature for one hour. Afterwards, sections were incubated at 4°C overnight with primary antibodies against myosin heavy chain (MHC) type 7 (BA-F8, 1:25), MHC type 1 (6H1, 1:25, all MHC antibodies were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242) and Laminin 1 (ab11575, 1:200, Abcam, UK). The sections were washed in PBS-T three times for 5 minutes at room temperature and incubated at room temperature for one hour with appropriate fluorochrome-conjugated secondary antibodies (1:250, ThermoFisher Scientific, USA). Finally, sections were washed in PBS, counterstained with 3,3' diaminobenzidine (DAPI), rinsed in PBS and mounted with Fluoromount (Sigma-Aldrich, USA).

3.5 Cytochrome C oxidase/succinate dehydrogenase (COX/SDH) histochemistry

The double-labeling method reveals both cytochrome c oxidase and succinate dehydrogenase function. Cells with functioning cytochrome c oxidase enrich brown indamine polymer product, while cells with defect mtDNA are not saturated by indamine polymer but succinate dehydrogenase reduces nitro blue tetrazolium (NBT) to blue formazan. Thus, COX/SDH staining reveals cells without defects in mtDNA, or negative cells, as brown and cells with altered mtDNA, or positive cells, as blue.

Consecutive slides of the immunohistochemical staining were thawed at room temperature and placed in an incubation medium containing 3,3'-diaminobenzidine, bovine catalase, cytochrome c oxidase, sucrose (all from Sigma-Aldrich, USA) in PBS at room temperature for two hours. Afterwards, the sections were rinsed in distilled water and placed in a counterstaining medium containing NBT (Sigma-Aldrich, USA) and sodium

succinate (Sigma-Aldrich, USA) in a sodium dihydrogen phosphate and disodium hydrogen phosphate solution (Sigma-Aldrich, USA) at 37°C for two hours. The slides were then rinsed in water, dehydrated ethanol, cleared in xylene and then mounted using Roti-Mount (Carl Roth GmbH + Co. KG, Germany).

3.6 Image analysis

All pictures of the whole biopsy cross sections were taken via fluorescence microscopy (AxioObserver Z1, Zeiss). Fibers were counted and their area measured using Fiji (ImageJ, National Institute of Health, USA).

3.7 Statistical analysis

The collected data was evaluated for statistical significance with GraphPad Prism (USA). For statistical analysis a t-test for parametric data was performed, respectively a Mann-Whitney-U-test for non-parametric data. A p-value ≤ 0.05 was considered significant. For correlation analysis, the Spearman's rank correlation coefficient was calculated. Group data are represented by the mean and the standard deviation (SD). Correlation are represented by mean and confidence interval (CI) of 95%.

Table 3.1: Instruments & chemicals.

Instruments	
DynExTM dynamometer	Akern, Italy
BIA 101 Anniversary	SMT medical GmbH & CO. KG, Germany
CryoStar^{TM} NX50 Cryostat	Thermo Fisher Scientific Inc., USA
AxioObserver Z1	Zeiss, Germany
Antibodies & Chemicals	
TissueTek O.C.T.	Sakura Finetek, Japan
BA-F8, against MHC type 7	DSHB, USA
6H1, against MHC type 1	DSHB, USA
ab11575, against laminin 1	Abcam, UK
DAPI (4',6-diamidino-2-phenylindole)	Thermo Fisher Scientific Inc., USA
Fluoromount^{TM}	Sigma-Aldrich, USA
DAB (3,3'-diaminobenzidine)	Sigma-Aldrich, USA
bovine catalase	Sigma-Aldrich, USA
cytochrome c oxidase	Sigma-Aldrich, USA
sucrose	Sigma-Aldrich, USA
NBT (nitro blue tetrazolium)	Sigma-Aldrich, USA
sodium succinate	Sigma-Aldrich, USA
sodium dihydrogen phosphate	Sigma-Aldrich, USA
disodium hydrogen phosphate	Sigma-Aldrich, USA
Roti-Mount^{TM}	Carl Roth GmbH & Co. KG, Germany

Chapter 4

Results

4.1 Patient collective

We included a total of 20 patients from the Department of General, Trauma and Reconstructive Surgery at the Munich University Hospital, LMU into our study. Of those, 15 were female and 5 were male. The average age of all individuals was 79.6 $\pm$ 10.4 years, with a mean age of female patients of 82.7 $\pm$ 7.1 years and a mean age of male patients of 70.2 $\pm$ 12.7 years.
All individuals were measured for their skeletal muscle mass (SMM), their body mass index (BMI) and their skeletal muscle index (SMI). The average SMM in the study group was 23.3 $\pm$ 6.2 kg, the average BMI was 23.2 $\pm$ 4.1 kg/m^2 and the average SMI was 8.2 $\pm$ 1.5 kg/m^2. The female patients' SMM was 20.5 $\pm$ 4.5 kg, the BMI was 23.2 $\pm$ 3.9 kg/m^2 and the SMI was 7.7 $\pm$ 1.4 kg/m^2, whereas the males' SMM was 31.4 $\pm$ 2.6 kg, the BMI was 23.3 $\pm$ 4.5 kg/m^2 and the SMI was 9.6 $\pm$ 0.6 kg/m^2.
We also compared the SMM, BMI and SMI of the female patients with the male patients and could show that both the SMM and the SMI of males were significantly higher than in women (Figure 4.1, **A** $p \leq 0.01$; **C** $p \leq 0.01$), but the BMI did not differ between the two groups (Figure 4.1, **C** $p = 0.96$), hence showing that the men had a higher proportion of skeletal muscle than the women (Table 7.1).

As one criterion for diagnosing sarcopenia is muscle function, we had to utilize a surrogate test to evaluate the fitness of our participants prior to the hip fracture. For this, the physical performance was estimated by the utilization of the clinical scores:

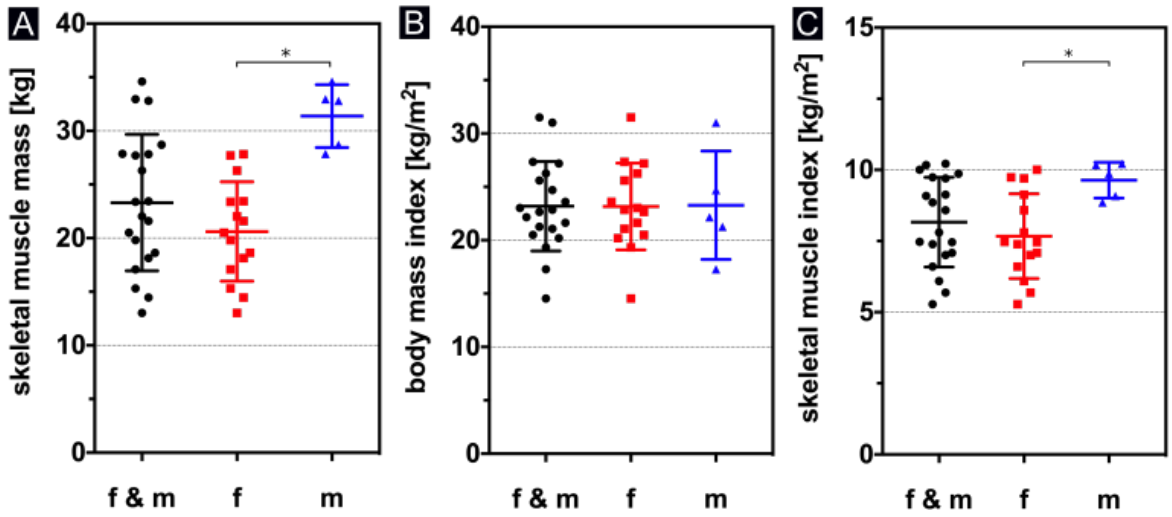

Figure 4.1: Comparison of skeletal muscle mass, body mass index and skeletal muscle index in the patient collective.

The graphs show the individual data of the overall study group and divided into female and male patients, plotted for skeletal muscle mass, body mass index and skeletal muscle index. The skeletal muscle mass of men was significantly higher compared to women (**A** $p \leq 0.01$). In contrast, there was no difference of the body mass index between men and women (**B** $p = 0.96$), with the skeletal muscle index of men also being significantly higher than the skeletal muscle index of women (**C** $p \leq 0.01$). f: female, m: male. Data points represent individual donors. Error bars represent the mean $\pm$ SD. * equals $p \leq 0.05$.

Barthel index, parker mobility score and SARC-F score. In addition, handgrip strength was measured in all patients using a handgrip dynamometer.

On average, the study group conducted 20.9 $\pm$ 10.6 kg weight force with their dominant hand. Additionally, they scored 94.4 $\pm$ 7.2 points in the Barthel index, 7.1 $\pm$ 1.7 points in the parker mobility score and 3.4 $\pm$ 2.4 points in the SARC-F score. The female patients conducted 16.7 $\pm$ 5.6 kg weight force and scored 94.3 $\pm$ 7.2 points in the Barthel index, 6.9 $\pm$ 1.7 points in the parker mobility score and 3.6 $\pm$ 2.2 points in the SARC-F score, whereas the male patients conducted 33.7 $\pm$ 11.6 kg weight force and scored 95.0 $\pm$ 7.1 points in the Barthel index, 8.0 $\pm$ 1.4 points in the parker mobility score and 2.3 $\pm$ 2.6 points in the SARC-F score. By comparing the female and male participants in regard to the values of the physical performance questionnaires, we could show no significant difference between men and women (Figure 4.2, **A** $p = 0.73$; **B** $p = 0.38$; **C** $p = 0.57$). Thus, we could say that men in our study group did not generally state higher values in the physical performance questionnaires, despite higher skeletal muscle mass and handgrip strength.

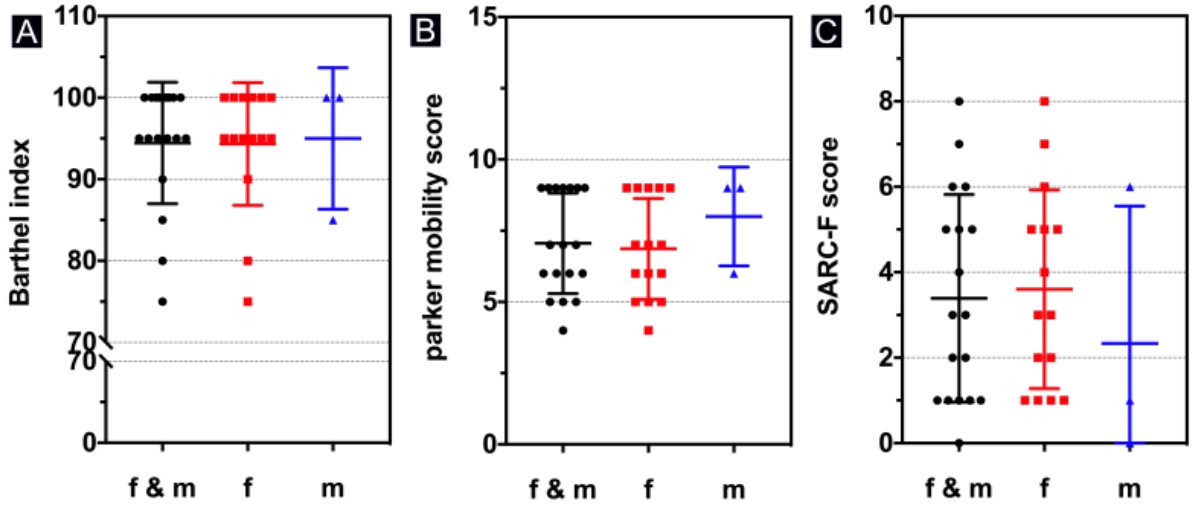

Figure 4.2: Physical performance values of the patient collective.

The graphs show the individual data of the overall study group and divided into female and male patients, plotted for Barthel index, parker mobility score and SARC F score. Between men and women we could not find any significant differences in regard to physical impairment, as the Barthel index scores, the parker mobility score values and SARC F score values do not significantly differ(**A** p = 0.73; **B** p = 0.38; **C** p = 0.57). f: female, m: male. Data points represent individual donors. Error bars represent the mean ± SD.

In sarcopenia, endocrine function and osteoporosis are known confounding variables that might influence the onset of muscle loss. Hence, we took blood samples from the study participants and quantified serum levels of 25(OH)D, TSH, total protein and calcium to evaluate disrupting factors, as particularly low thyroid function, osteoporosis and malnutrition might not yet have been diagnosed in our patients. In all individuals combined, the average level of 25(OH)D was 16.0 ± 11.5 ng/ml, TSH was 1.35 ± 0.69 μU/ml, total protein was 5.4 ± 0.5 g/dl and of calcium was 2.05 ± 0.1 mmol/l. The average serum level measured in all female participants of 25(OH)D was 17.3 ± 12.8 ng/ml, TSH was 1.39 ± 0.73 μU/ml, total protein was 5.3 ± 0.5 g/dl and calcium was 2.05 ± 0.11 mmol/l, whereas for males the mean serum level of 25(OH)D was 11.6 ± 2.9 ng/ml, TSH was 1.24 ± 0.57 μU/ml, total protein was 5.7 ± 0.3 g/dl and calcium was 2.05 ± 0.05 mmol/l. Furthermore, we compared the blood levels of all the parameters mentioned above in the female participants with the blood levels of the male participants and could find no significant difference between these two groups (Figure 4.3, **A** p = 0.18; **B** p = 0.69; **C** p = 0.09; **D** p = 0.99). Although female and male adults did

not show differing blood levels of 25(OH)D, a striking fact is that aside from one female patient, all other study participants did show a lack of 25(OH)D.

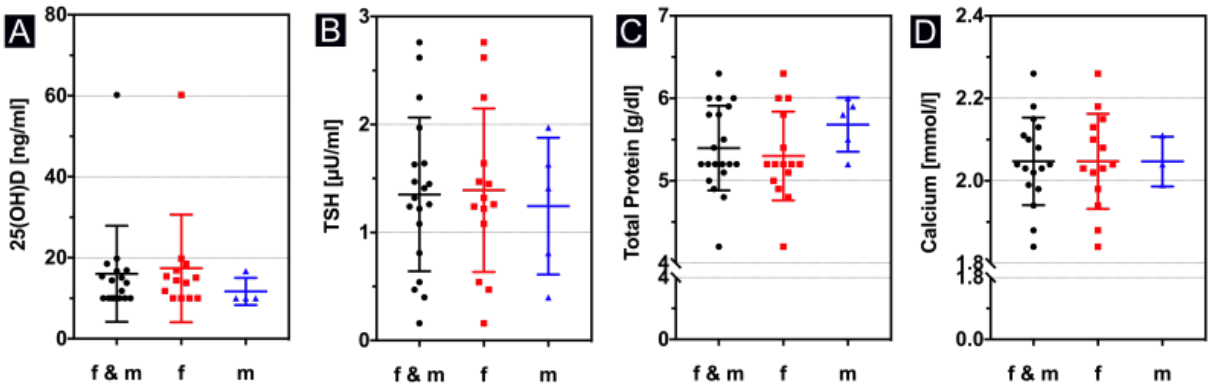

Figure 4.3: Blood levels of 25(OH)D, TSH, total protein and calcium in the patient collective.

The graphs show the individual data of the overall study group and divided into female and male patients, plotted for 25(OH)D, TSH, total protein and calcium. The comparison of the blood levels showed no significant difference in neither 25(OH)D (**A** p = 0.18), TSH (**B** p = 0.69), total protein (**C** p = 0.09) nor calcium (**D** p = 0.99) between men and women. f: female, m: male. Data points represent individual donors. Error bars represent the mean ± SD.

4.2 Morphological analysis of cross-sections from gluteus medius muscle

From each patient in our study, we collected a muscle biopsy of the gluteus medius muscle during the operation of their proximal femoral fracture. We cut the biopsies into cross-sections and stained them according to our protocol for slow twitching type I fibers and fast twitching type IIx fibers, leaving the type IIa fibers unstained and thus appearing black in fluorescence microscopy. Hence, we were able to count all fibers of each fiber type present in all biopsies and measure their size.

On average, each biopsy contained 3,040 ± 1,779 fibers, which was vastly dependent on the overall size of the biopsy. Each fiber had an average size of 3,385 ± 952 μm^2. The mean fiber area of each fiber type was measured individually, which revealed, that type I fibers had an average fiber size of 5,885 ± 2,363 μm^2, while type IIa fibers had an average fiber size of 2,996 ± 1,523 μm^2 and type IIx fibers of 2,232 ± 1,041 μm^2. This

showed, that type I fibers were significantly bigger than type IIa ($p \leq 0.01$) and type IIx fibers ($p \leq 0.01$). The average size of muscle fibers in females was 5,832 $\pm$ 2,438 μm^2 for type I, 2,282 $\pm$ 1,375 μm^2 type IIa and 1,522 $\pm$ 879 μm^2 for type IIx respectively. In male participants, muscle fibers were 4,803 $\pm$ 1,928 μm^2 for type I, 2,980 $\pm$ 1,707 μm^2 for type IIa and 2,020 $\pm$ 1,474 μm^2 for type IIx. Additionally, we analyzed the fiber type proportion within the biopsies and could show that type I fibers were significantly dominant in all biopsies with a proportion of 79.14 $\pm$ 16.50% ($p \leq 0.01$). In contrast, the proportion of type IIa fibers was 11.84 $\pm$ 9.64% and the share of type IIx fibers was 9.02 $\pm$ 8.98%. In females the share of type I fibers with 81.41 $\pm$ 15.47% was higher than in men with 72.24 $\pm$ 17.5%, whereas type IIa and type IIx fibers were less dominant. Type IIa fibers held a share of 11.06 $\pm$ 9.3% in female and 14.36 $\pm$ 10.06% in male. Type IIx fibers were less common with 7.5 $\pm$ 7.9% in females and 13.4 $\pm$ 10.4% in males. Noticeably, while analyzing the cross-sections, we discovered that 10 out of 20 biopsies depicted fiber bundling of type I fibers (Figure 4.4). In all these cases several fiber bundles contained only type I fibers. This ranged from several bundles without type IIa and IIx fibers to biopsies completely without type IIx fibers and almost no type IIa fibers, which was the case for four biopsies. We could see in one biopsy the lack of both type IIa and IIx fibers.

4.3 Handgrip strength declines with age

We analyzed handgrip strength of the dominant hand with a dynamometer in all patients and compared the achieved weight force to age and SMI. We found that handgrip strength declines with aging ($p \leq 0.05$, $R^2 = 0.57$). Also, by comparing handgrip strength to SMI, we found that strength correlated to skeletal muscle mass ($p \leq 0.05$, $R^2 = 0.51$). These results were expected and are concordant to existing literature (Visser et al., 2000). The corresponding figures are appended in the supplementary data (Figure 7.1).

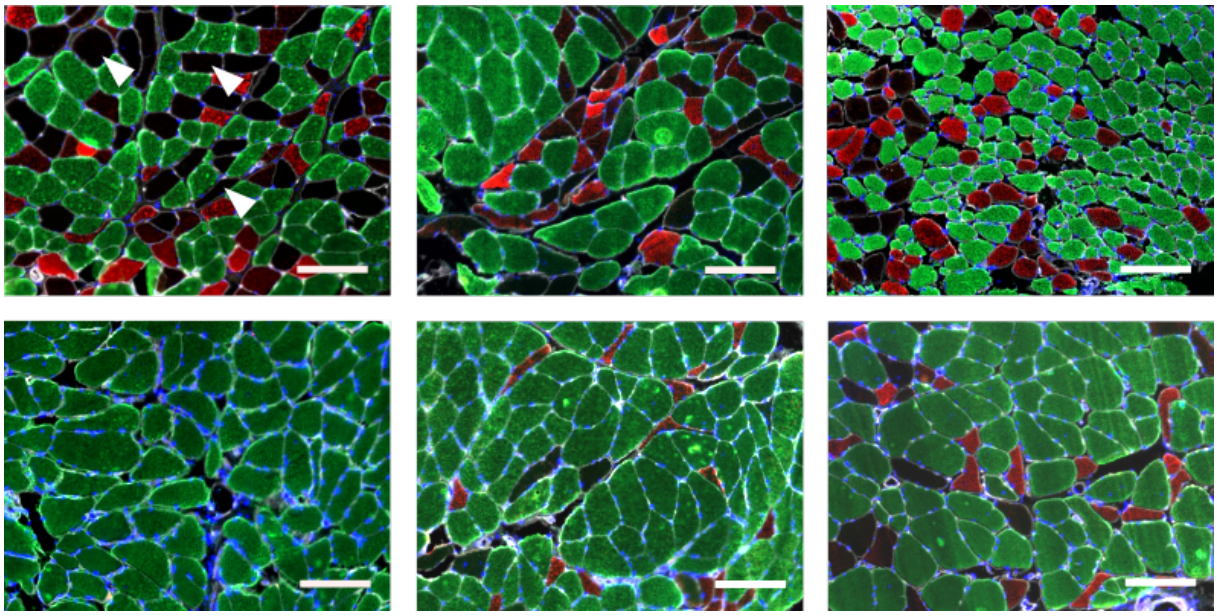

Figure 4.4: Skeletal muscle biopsy cross-sections of six individuals.

The figure depicts the immunohistochemical staining of skeletal muscle cross-sections of six individual participants in the study. MHC7 (type I; green), MHC1 (type IIx; red), laminin 1 (white), DAPI (blue). Type IIa fibers are not stained and appear black (white arrow head). Type I fibers are dominant in all six participants, whereas type IIa and type IIx fibers are less common. Additionally, the proportion of fast twitching fibers was highly variant. Fast-twitching fibers were generally smaller and appeared "flattened" more often compared to type I fibers. Several cross-sections depicted pronounced bundling of type I fibers with few to no type II fibers in fiber bundles. Scale bar: 200 μm.

4.4 Prevalence of sarcopenia does not correlate to mitochondrial dysfunction

In addition to immunohistochemical staining, we also stained gluteus medius cross-sections for mitochondrial dysfunction with cytochrome c oxidase/succinate dehydrogenase histochemistry (Ross, 2011). We analyzed gluteus medius cross-sections of all participants via COX/SDH staining and found out, that the amount of blue positive fibers was extremely low. In fact, there were few to none positive cells in each of the participants (Figure 7.2).

4.5 The mean fiber area of fast-twitching fibers declines with aging

As muscle mass in aging adults decreases from about 30 years onwards with an accelerated loss from the age of 60 onwards (Lexell et al., 1986), we correlated the loss of muscle mass to either the loss of muscle fibers in number and to the loss in size of each individual fiber. We compared the mean fiber area of each fiber type to the age of the corresponding individual. The analysis revealed that the mean fiber area of type I fibers does not correlate to age (**A** p = 0.96, $R^2 \leq 0.01$). However, type IIa and type IIx fiber size decreases with aging (**B** $p \leq 0.01$, R^2 = 0.38, respectively **C** $p \leq 0.01$, R^2 = 0.65). A noticeable feature is the variance of the mean fiber area between the individuals, most obvious in type I fibers, but also in type IIa and type IIx fibers (Figure 4.5, **A-C**). Furthermore, the percentage of fiber types in the biopsies varies strongly from individual to individual, as the share of type I fibers ranges from 50% to 100% (on average 79.1% $\pm$ 16.5%). Type IIa fiber proportion ranges from 0% to 30% (on average 11.9% $\pm$ 9.6%) and type IIx fiber share ranges from 0% to 30% (on average 9.0% $\pm$ 9.0%). With higher age, the distribution does not change to the favor of one fiber type but varies randomly depending on each individual (Figure 4.5, **D** type I: p = 0.96, $R^2 \leq$ 0.01; type IIa: p = 0.87, $R^2 \leq 0.01$; type IIx: p = 0.78, $R^2 \leq 0.01$).

4.6 The mean fiber area does not correlate to skeletal muscle index

Sarcopenia is defined by the EWGSOP as a loss of muscle mass and additionally, a loss of muscle strength and/or physical performance (Cruz-Jentoft et al., 2010), where the muscle mass is determined through calculating the skeletal muscle index (SMI) of each individual with the Janssen equation (Janssen et al., 2000). Thus, we evaluated, if individuals with lower skeletal muscle mass showed any differences in fiber type size or distribution within their muscles. We therefore compared the mean fiber size to the SMI of any individual and we could show that the mean fiber area of each fiber type does not correlate to the measured SMI (Figure 4.6, **A** p = 0.54, $R^2 \leq 0.02$; **B** p = 0.5,

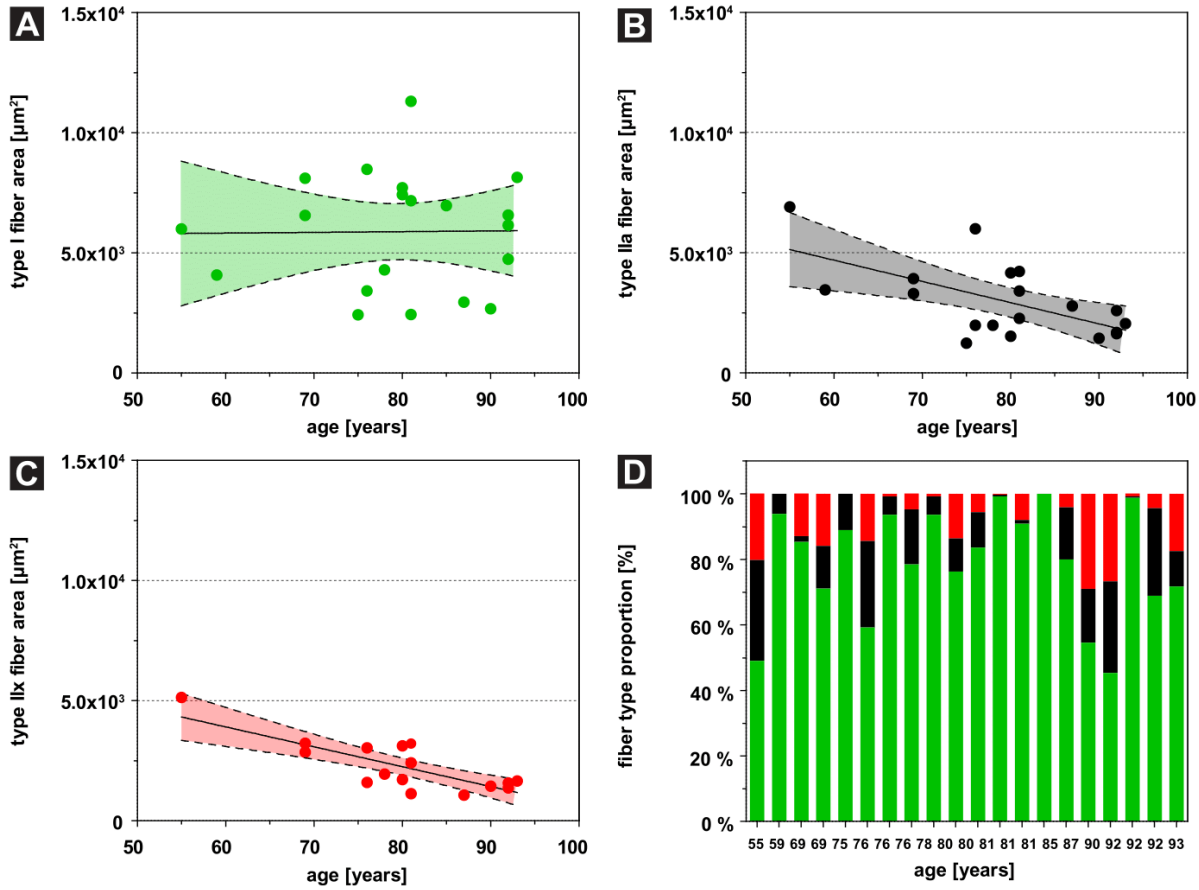

Figure 4.5: Change of mean fiber area during aging and fiber type proportion compared to age.

Fibers of each type were measured in size and the average was compared to the age of the given individual (**A-C**). Each patient's fiber type distribution is displayed as a bar of corresponding color (**D** fiber type I: green; type IIa: black; type IIx: red). While the mean fiber CSA of type I fibers shows no significant change during aging (**A** $p = 0.96$, $R^2 \leq 0.01$), the CSA of type IIa (**B**) and type IIx (**C**) fibers revealed a significant decline in aged patients (**B** $p \leq 0.01$, $R^2 = 0.38$; **C** $p \leq 0.01$, $R^2 = 0.65$). Quantification of the fiber type proportion of the gluteus medius muscle does not show any obvious correlation with aging (D type I: $p = 0.96$, $R^2 \leq 0.01$; type IIa: $p = 0.87$, $R^2 \leq 0.01$; type IIx: $p = 0.78$, $R^2 \leq 0.01$). Data points and bars represent individual donors. Regression lines indicate the mean $\pm$ CI (95%).

$R^2 \leq 0.03$; **C** $p = 0.11$, $R^2 = 0.16$). In addition, the fiber type distribution compared to the SMI shows no change in favor of one fiber type (Figure 4.6, **D** type I: $p = 0.11$, $R^2 = 0.14$; type IIa: $p = 0.11$, $R^2 = 0.14$; type IIx: $p = 0.22$, $R^2 = 0.09$).

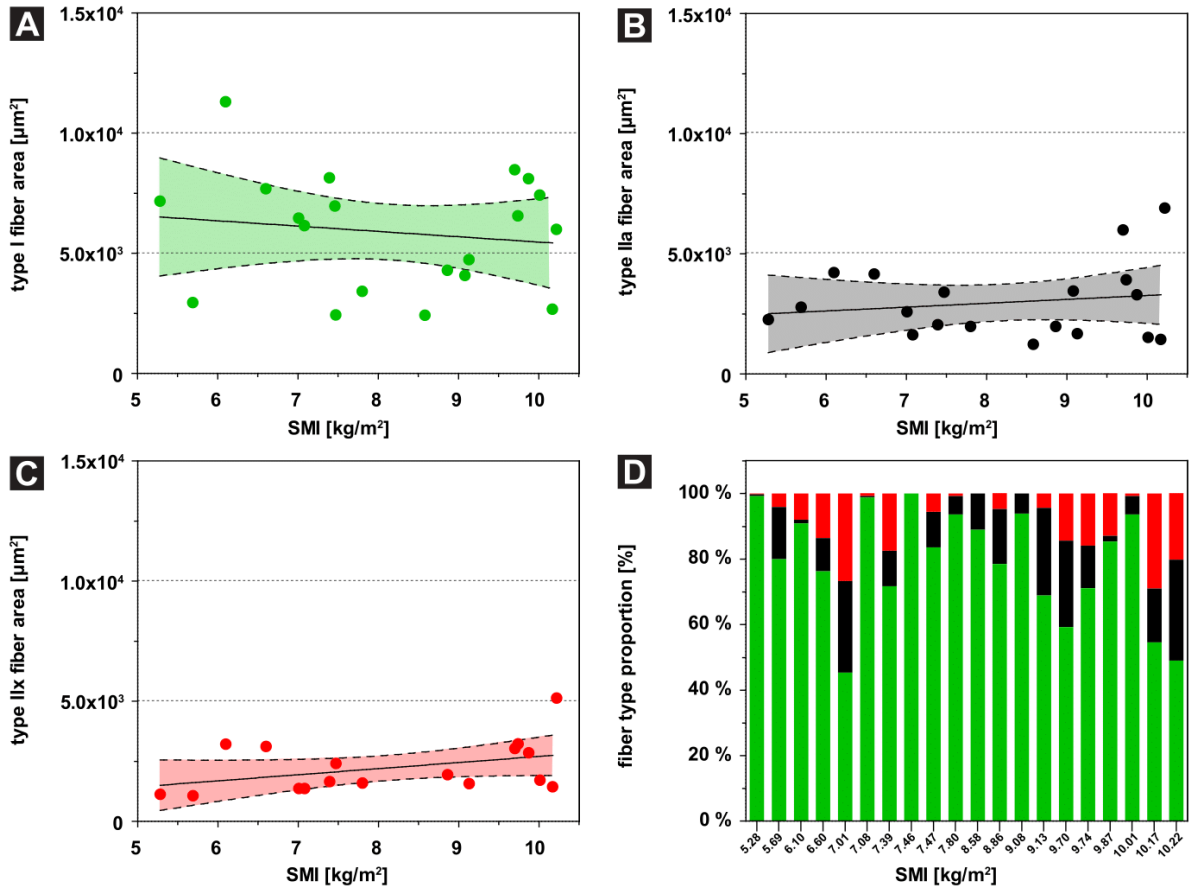

Figure 4.6: Change of mean fiber area during aging and fiber type proportion compared to SMI.

Fibers of each type were measured in size and the average was compared to the SMI of the given individual. Each patient's fiber type distribution is displayed as a bar of corresponding color (fiber type I: green; type IIa: black; type IIx: red). Compared to the skeletal muscle index no fiber type shows any correlation to its mean area (**A** p = 0.54, $R^2 \leq 0.02$; **B** p = 0.5, $R^2 \leq 0.03$; **C** p = 0.11, $R^2 = 0.16$). The fiber type proportion shows a heterogeneous distribution compared to SMI (**D**, fiber type I: p = 0.11, $R^2 = 0.14$; type IIa: p = 0.11, $R^2 = 0.14$; type IIx: p = 0.22, $R^2 = 0.09$). Data points and bars represent individual donors. Regression lines indicate the mean ± CI (95%).

4.7 Physical impairment increases with aging

To estimate physical performance of patients with proximal femoral fractures, we used three different clinically utilized questionnaires targeting the activities of the daily living. The score values determined for each individual was then compared to the age and the SMI. The goal was to detect possible changes of physical impairment with higher age or lower muscle mass.

The analysis showed, that neither the Barthel index, the parker mobility score nor the

SARC-F score correlate to the SMI (**A** p = 0.48, $R^2 \leq 0.05$; **B** p = 0.43, $R^2 \leq 0.05$; **C** p = 0.2, R^2 = 0.09) (Figure 4.7, **A-C**). However, while the Barthel index showed no correlation to the age of the corresponding individual, the parker mobility score and the SARC-F score values significantly decline with higher age (**D** p = 0.23, R^2 = 0.08; **E** p $\leq$ 0.05, R^2 = 0.21; **F** p $\leq$ 0.05, R^2 = 0.31). Thus, we could show that physical fitness declines with aging, especially in activities that involve the lower extremities (Figure 4.7, **D-F**).

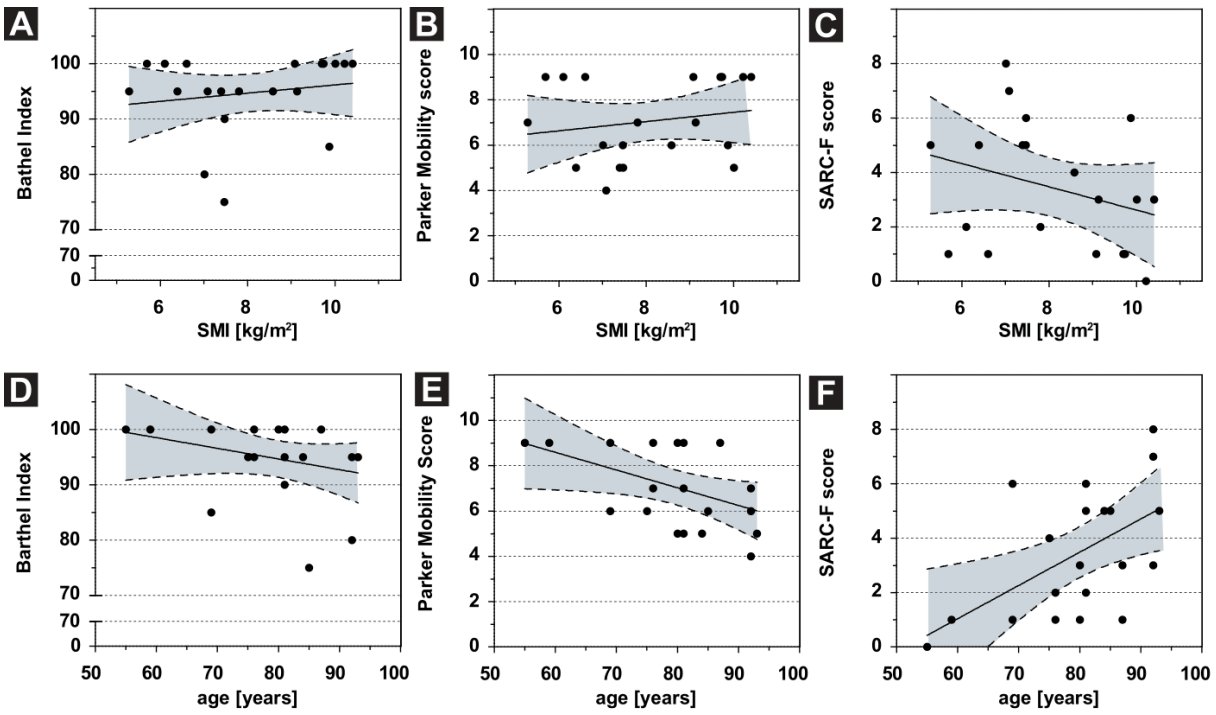

Figure 4.7: Correlation of mobility scores to SMI and to age.

The graphs show the comparison of the clinical scores we surveyed to either the SMI (**A-C**) or the age (**D-F**). Neither the Barthel index, the parker mobility score nor the SARC-F score values correlate to SMI (**A** p = 0.48, $R^2 \leq 0.05$; **B** p = 0.43, $R^2 \leq 0.05$; **C** p = 0.2, R^2 = 0.09; **D** p = 0.23, R^2 = 0.08). However, the parker mobility score values and SARC-F score values decline compared to higher age (**E** p $\leq$ 0.05, R^2 = 0.21; **F** p $\leq$ 0.05, R^2 = 0.31). Data points represent individual donors. Regression lines indicate the mean $\pm$ CI (95%).

4.8 Data of sarcopenic patients does not differ from data of non-sarcopenic patients

For comparison between sarcopenic and non-sarcopenic patients, we divided the study population into two groups depending on the measured SMI and low grip strength. As a reference, we took the definition contributed by the EWGSOP (Cruz-Jentoft et al., 2010) and thus, patients with a SMI lower than 8.87 kg/m^2 and grip strength lower than 30 kg for men and a SMI lower than 6.42 kg/m^2 and grip strength lower than 20 kg for women were deemed sarcopenic. Patients with higher SMI and grip strength were considered non-sarcopenic. Hence, per definition the SMI and the skeletal muscle mass between these two groups were significantly different ($p \leq 0.05$). Despite that, we could not find any difference in the body mass index ($p = 0.95$). This showed that patients with sarcopenia do not necessarily weigh less than non-sarcopenic patients but still exhibit a deficiency of muscle mass (Figure 4.8).

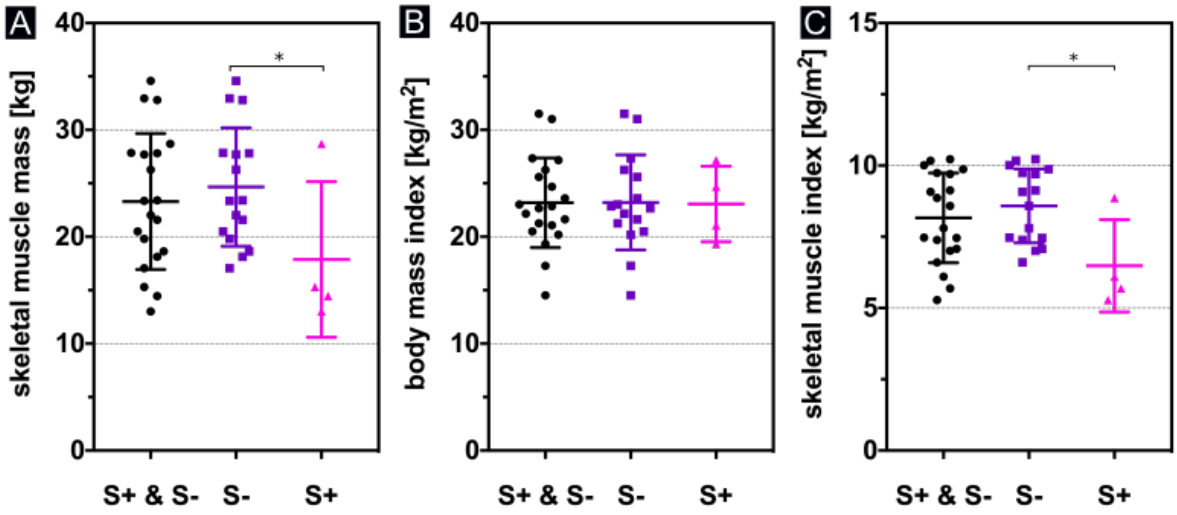

Figure 4.8: Comparison of skeletal muscle mass, body mass index and skeletal muscle index in non-sarcopenic and sarcopenic patients.

The graphs show the individual data of the overall study group divided into non-sarcopenic and sarcopenic patients, plotted for total skeletal muscle mass, body mass index and skeletal muscle index. By definition, the skeletal muscle mass and the SMI are significantly different, but we could not find any difference in the body mass index between these two groups (**C** $p = 0.95$). S-: non-sarcopenic, S+: sarcopenic. Data points represent individual donors. Error bars represent mean $\pm$ SD. * equals $p \leq 0.05$.

We analyzed the values in the physical performance questionnaires and found that sarcopenic patients did not score significantly worse in either of all three tests (Figure 4.9, **A** p = 0.45; **B** p = 0.13; **C** p = 0.57).

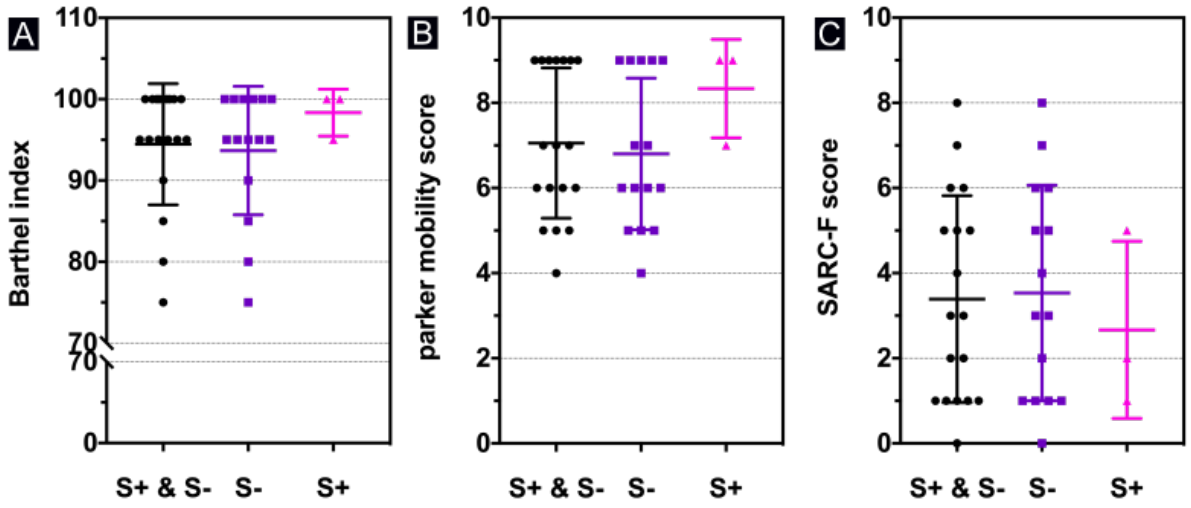

Figure 4.9: Physical performance scores in non-sarcopenic and sarcopenic patients.

The graphs show the individual data of the overall study group and the subdivision into non sarcopenic and sarcopenic patients, plotted for Barthel index, parker mobility score and SARC F score. We could find no difference between non sarcopenic and sarcopenic patients in regard to physical impairment (**A** p = 0.45; **B** p = 0.13; **C** p = 0.57). S-: non-sarcopenic, S+: sarcopenic. Data points represent individual donors. Lines represent mean ± SD.

Additionally, we investigated the fiber type proportion and fiber size of all study participants to comprehend, if any difference that might be correlated to the occurrence of sarcopenia exists. Looking at the mean fiber area, the striking difference in size between type I and type IIa and IIx fibers is also present in sarcopenic patients and thus does not differ from the overall population. Concordant to that, there is no difference between the mean fiber area of non-sarcopenic and sarcopenic patients (Figure 4.10, **A** type I: p = 0.64; type IIa: p = 0.75; type IIx: p = 0.93). By comparing the fiber type proportion in the two groups, we could not find any significant difference for neither type I, IIa nor IIx fibers (Figure 4.10, **B** type I: p = 0.16; type IIa: p = 0.46; type IIx: p = 0.06). Although people with sarcopenia exhibit a lack of muscle mass, we could neither associate this deficiency with generally smaller fibers nor with a lack of one specific fiber type such as fast twitching type II fibers.

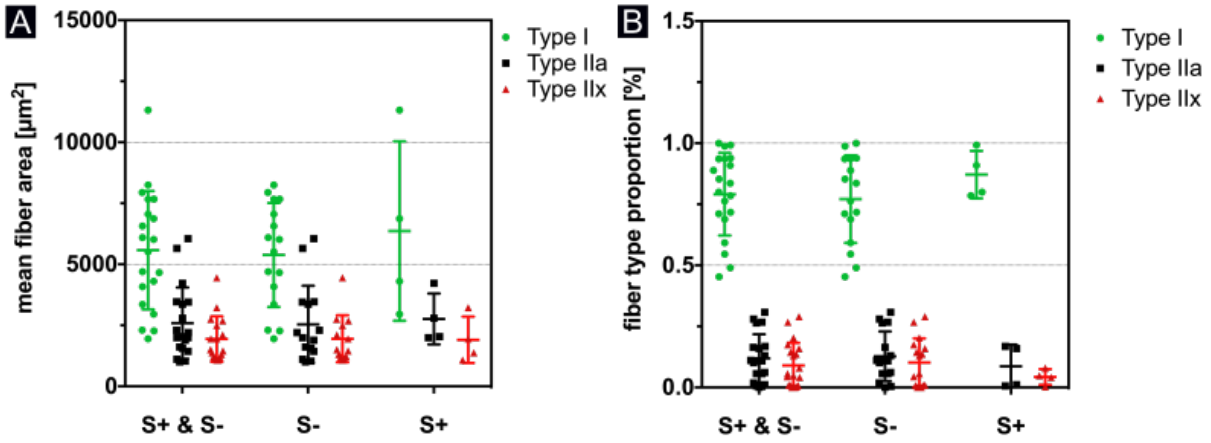

Figure 4.10: Comparison of mean fiber area and fiber type proportion of type I, IIa and IIx fibers in non-sarcopenic and sarcopenic patients.

The graphs show the individual data of the overall study group and divided into non sarcopenic and sarcopenic patients, plotted mean fiber area and fiber type proportion, each divided into type I (green), IIa (black) and IIx (red) fibers. In comparison, the mean fiber area of all three fiber types was not significantly different between the two groups (**A** fiber type I: p = 0.64; type IIa: p = 0.75; type IIx: p = 0.93). Additionally, fiber type proportion of all three fiber types was also not significantly different between non sarcopenic and sarcopenic patients (**B** fiber type I: p = 0.16; type IIa: p = 0.46; type IIx: p = 0.06). S-: non-sarcopenic, S+: sarcopenic. Data points represent individual donors. Error bars represent the mean ± SD.

Furthermore, we analyzed if the deficiency of muscle mass might be related to changes in blood levels of markers that are frequently used for diagnostics of muscle and bone diseases, such as 25(OH)D, TSH, total protein and calcium. The comparison between non-sarcopenic and sarcopenic patients revealed that blood levels of all the measured parameters did not differ from each other. (Figure 4.11, **A** p = 0.47; **B** p = 0.54; **C** p = 0.23; **D** p = 0.25). Altogether, the two groups of patients with sarcopenia and without did only differ, as per definition, in the SMI and the skeletal muscle mass.

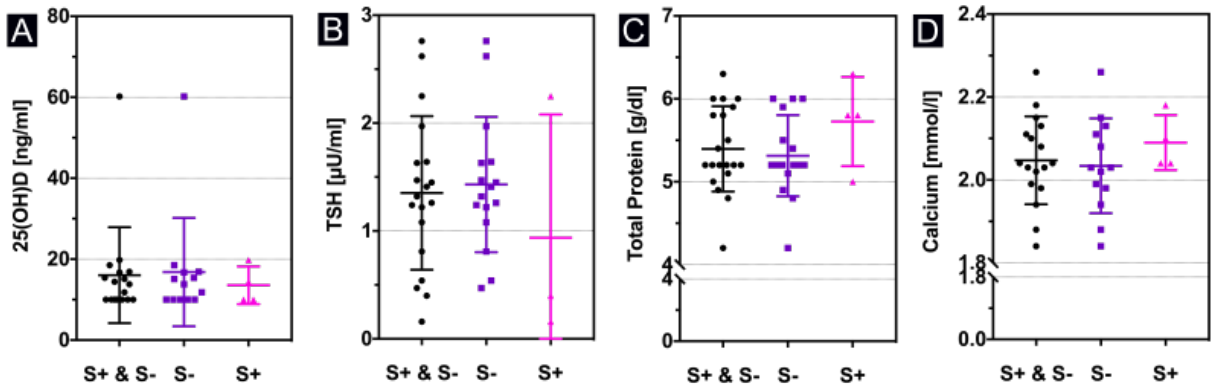

Figure 4.11: Blood levels of 25(OH)D, TSH, total protein and calcium in non-sarcopenic and sarcopenic patients.

The graphs show the individual data of the overall study group and divided into non sarcopenic and sarcopenic patients, plotted for 25(OH)D, TSH, total protein and calcium. Between non sarcopenic and sarcopenic patients, the comparison of the blood levels showed no significant difference in neither 25(OH)D (**A** p = 0.47), TSH (**B** p = 0.54), total protein (**C** p = 0.23) nor calcium (**D** p = 0.25). S-: non-sarcopenic, S+: sarcopenic. Data points represent individual donors. Error bars represent the mean ± SD.

Chapter 5

Discussion

To contribute to the still ongoing discussion regarding the causes and onset of sarcopenia with the aim of a characterization of this phenomenon, we investigated the role of muscle weakness in postural muscles, in particular in geriatric people with osteoporotic bone fractures. In this study, we systematically investigated the differences of fiber type proportion between sarcopenic and non-sarcopenic patients hospitalized for proximal femur fractures and compared them to clinical and biochemical markers. The impact of sarcopenia as an economical and health burden is of increasing interest and has been shown to be of even more importance in the future. To our best knowledge, there has not yet been any study investigating the morphological differences in gluteus medius muscle of both patient groups.

5.1 Skeletal muscle morphology in aging

Our analysis of gluteus medius muscle in the elderly showed that slow-twitching type I fibers were generally larger than fast-twitching type IIa and IIx fibers and type I fibers that were predominantly present within the muscle biopsies. As gluteus medius muscles are essentially used for leg and hip stability while being upright standing or walking, a high amount of type I fibers was expected. Nevertheless, the variance in the amount of type IIa ($11.84 \pm 9.64\%$) and IIx ($9.02 \pm 8.98\%$) fibers was high throughout all participants and thus we compared, in addition to the fiber type, the fiber size and proportion to age and SMI. We could show a negative correlation between the size of type IIa

fibers and IIx fibers and age, but not between type I fibers and age. These findings are consistent with current literature about other lower limb muscles in humans (Aniansson et al., 1986). We hypothesized that with progressed atrophy of fast-twitching fibers during aging, the share of the different fiber types within gluteus medius muscle would shift towards a higher proportion of type I fibers. However, unlike in fiber size, we could not show any differences in fiber type proportion compared to age. The great variance between each individual and the relatively low sample size made a comparison of skeletal muscle fiber morphology hardly possible. We therefore concluded that if atrophy of fast-twitching fibers would promote a proportional shift of fiber types, either more participants would have to be examined to even out the variance or participants would have to be reexamined in a longitudinal study for inter-individual comparison. Additionally, the muscle biopsies were very small compared to the whole muscle. It is possible, that the distribution of muscle fibers is not homogenous throughout the muscle and analyzing smaller sections of a muscle can contribute to a higher variance.
Nevertheless, the comparison of muscle fiber size versus age was already investigated, which showed that the cross-sectional area of skeletal muscle fibers shrinks with increasing age, and especially fast-twitching fibers are shown to be affected (Lexell et al., 1983; Lexell, 1995). As the significant loss of muscle mass is one of the main features that characterizes sarcopenia, we compared muscle fiber size to SMI. We hypothesized that participants with a higher SMI exhibit generally larger muscle fibers and, because fast-twitching fibers are shown to be more affected by atrophy in age, participants with a higher SMI also exhibit a higher proportion of type IIa and IIx fibers. This proved to be wrong, as neither type I, IIa nor IIx fiber size correlates to SMI and additionally, SMI does not correlate to the proportion of either fiber type. This led us to the conclusion, that while SMI does have a somewhat predictive value in clinical settings, because it correlates to poor health outcomes in patients with sarcopenia (Janssen et al., 2004), it does not give any information about the fiber type composition.
Furthermore, it has been shown that muscle fiber atrophy in aging muscles preferentially affects fast-twitching fibers (Lexell et al., 1988; Coggan et al., 1992). Causes for this phenomenon have yet to be elucidated, but it is theorized that both muscle fiber metabolism as well as the neuronal innervation play important roles in this process. Aare et al. discovered that repeated cycles of denervation and reinnervation in young (eight month) and very old (35 month) rats leads to fiber type grouping (Aare et al.,

2016). Additionally, very old rats showed a decreased ability to reinnervated muscle fibers, which results in further atrophied fibers. In our study cohort we observed that half of the participants depicted bundling of type I fibers to some extent, which is presumably either due to an increased atrophy of fast-twitching fibers or due to loss of innervation by the motor neuron and subsequent reinnervation by other motor neurons. Spatio-temporal analysis of ALS progression in superoxide dismutase 1 (SOD1) gene mutant mouse model, which is used to induce clinical weakness through the loss of motor neurons, suggests that this motor neuron disease begins at the distal end of the axons in a “dying-back” pattern (Fischer et al., 2004; Tintignac et al., 2015). Furthermore, the authors concluded that these distal pathological mechanisms in motor neurons are responsible for fiber type grouping. Our findings depicted bundling of muscle fibers, which underlines the involvement of motor neuron adaptation and potential axonal pathologies in the genesis of sarcopenia. These findings are emphasized by Piasecki et al., who found that the size of motor units (i.e. the number of muscle fibers innervated by one motor neuron) of older people is significantly lower than that of a young control group (Piasecki et al., 2016). They also hypothesized that changes in motor units prelude future impairment in physical fitness and might therefore be of interest in early diagnostics. Comparable results were found by Drey et al., who discovered that a significant amount of sarcopenic patients depicted pathologic motor unit number indices in hypothenar (Drey et al., 2014).

Fast-twitching muscle fibers need high energy resources, while conducting fast movements. With advanced age, resources and efficient energy yield are significantly less productive (Layec et al., 2015), which could possibly lead to alimentary difficulties regarding fast-twitching fibers. This has especially been demonstrated in the demand of ATP for fast dynamic contractions, which was significantly higher in older adults compared to young adults (Layec et al., 2018, 2016). Additionally, older people are likely to be more sedentary than younger people. As a sedentary lifestyle probably depends more on stable and slow than on fast movements, fast-twitching fibers are likely to be less stimulated than slow fibers. This lack of stimulus can also promote advanced degradation compared to activated fibers (Mosole et al., 2016). Both less productive energy output and sedentary lifestyle are possible causes for fiber type bundling.

In sum, these results underline that for complete understanding of muscle mass and strength loss, the neuromuscular fidelity should be investigated, contrary to only mus-

cle or neuronal tissue alone.

5.2 Morphological differences between sarcopenic and non-sarcopenic patients

To evaluate a potential sarcopenia-dependent effect onto skeletal muscle morphology, physical function and biochemical markers, we divided the study population into healthy and sarcopenic patients. While it was shown that muscle fiber size, especially of fast-twitching fibers generally decline with age, we hypothesized that fast-twitching fibers are even more affected in people with sarcopenia than in healthy patients. However, we could not show any correlation between sarcopenia and either fiber size or fiber proportion within the muscle biopsies for any of the three examined fiber types. The proportion of type IIa and type IIx fibers were generally low in all participants, which makes it even more difficult to detect possible changes in fiber area. Predominant changes in fast-twitching fibers would therefore be of lesser consequence. Additionally, the reason for the dominance in proportion of type I fibers is not known. Both more dominant atrophy of fast-twitching fibers and denervation due to atrophy of motor neurons with subsequent reinnervation pose possible explanations (Rowan et al., 2012). Each of those factors might represent independent mechanisms that lead to a shift of fiber types towards slow-twitching fibers. Additionally, we pooled all non-sarcopenic and sarcopenic patients in two groups but did not correct for age. This might also represent a confounding variable, as age itself is correlated to increased fiber atrophy and must therefore be considered when comparing these two groups.

5.3 Physical performance of the elderly

Along with the muscle fiber size, we assessed the physical function with questionnaires concerning independent abilities in day-to-day living and compared the obtained scores with age and SMI. We proposed that the study cohort would exhibit a negative correlation between physical function and age, as well as the muscle mass, which would affect strength and therefore lead to physical impairment. According with the already

published data that physical function declines with age (Frontera et al., 2008; Novak, 1972; Sathyaprabha, 2000; Tanaka et al., 2017), elderly patients of our cohort scored lower values compared to younger patients. Malmstrom et al. developed the five-item SARC-F questionnaire, which assesses the main features or consequences of sarcopenia (Malmstrom et al., 2016). They designed the test to reflect an approximation of the physical ability. In fact, the SARC-F score is correlated to physical performance and likelihood of hospitalization. Similarly, the Parker-mobility score is designed to provide an estimation of mortality after hip fractures and the Barthel index provides an estimate of physical impairment (Parker and Palmer, 1993; Mahoney and Barthel, 1965). However, the Parker-mobility score and the SARC-F score are age-dependent, whereas the Barthel index is not age-specifically validated. A possible explanation for this could be that the Parker-mobility score and the SARC-F score are devised to screen for disabilities in lower extremity strength, which affects walking and climbing stairs and the Barthel index is a questionnaire to assess whole body mobility in terms of self-care, which predominantly focuses on upper body mobility and continence (Mahoney and Barthel, 1965; Parker and Palmer, 1993; Malmstrom et al., 2016). For example, a person in a wheelchair with unrestricted mobility and strength in the upper body can score relatively high in the Barthel index compared to parker-mobility and SARC-F score. Loss of strength could therefore be initially noticeable in those questionnaires focusing on postural movements and stability. Nonetheless, we could not proof any relation between mobility scores and relative skeletal muscle mass, as neither Barthel index, parker-mobility score nor SARC-F score correlate to SMI.

We utilized all three tests to assess the physical function of the participants. However, we could not find any differences in the scored values of each questionnaires between sarcopenic and non-sarcopenic patients. Thus, we conclude that although the questionnaires are validated in predicting health outcomes, they do not represent a proper replacement for physical performance tests, due to their retrospective and subjective character.

5.4 Study design and methodology

In this study, we analyzed biopsies, clinical and biochemical data of 20 patients. Of those four were sarcopenic, with all but two patients, all were above 69 years old, which yields limitations in the possible interpretation of the results. The low number of participants can only give a rough estimate of sarcopenia prevalence in the population, which could otherwise evaluate the eventual robustness of the definition provided by the EWGSOP. In addition, the study cohort consists of only people with proximal femur fractures, hence, only people with traumatic injuries presumably due to weakness in postural skeletal muscle were included. As there was no control group without proximal femur fractures available, the differences between those two groups, and a possible impact of muscle morphology on postural instability, could not be further evaluated. Furthermore, with the average age of 79.6 $\pm$ 10.4 years, the cohort consisted mainly of elderly patients. This lack of young patients as a control group made a possible comparison of aging skeletal muscle to younger skeletal muscle particularly regarding normal, physiological morphological changes, in contrast to possible magnified changes by reason of sarcopenia, unfeasible.

Gluteus medius muscle inherits important functions in postural stability by stabilizing the femur and pelvis, which makes it interesting to investigate sarcopenia and age-related effects in regard to potential risks for falls. The amount of fast-twitching type IIa and IIx fibers is relatively low throughout all participants. Possible changes in size and number of type II fibers through age or sarcopenia, might have a mild influence on physical performance, as the relative proportion of those fast-switching fibers is low. Therefore, the effect of sarcopenia on fiber type composition could be more severe than expected, either higher or lower. In addition, we could show that the variance in proportion of fiber types within the muscle biopsy and throughout the individuals is distinctively exalted. Comparing biopsies yields a highly heterogeneous picture of gluteus medius muscle, which complicates synthesis of a general statement about the influence of age and sarcopenia on skeletal muscle fibers.

Grip strength is a widely accepted measurement of skeletal muscle strength, especially in the context of sarcopenia, as it correlates to general muscle strength and is a good predictor of disability and mortality in older people (Newman et al., 2006; Roh et al., 2017). Nevertheless, even if grip strength is an established marker for muscle

strength, the direct correlation to morphological markers of gluteus medius biopsies must be proven. As all our participants were hospitalized for proximal femur fracture, we were unable to effectively measure gluteus medius strength. Therefore, we resorted to use grip strength as a criterion for the presence of sarcopenia. To estimate physical function, a questionnaire was used that consisted of the Barthel index, the parker mobility score and the SARC-F score. All of these are designed to screen for disabilities in activities of the daily living and to predict long-term mortality. However, they do not make a direct statement on the actual physical function of the respondent, in contrast to short physical performance battery or gait speed. Furthermore, a confounding variable is the non-objectivity of questionnaires. Patients asked about their ability to conduct activities of day-to-day living might falsely answer questions through wrongful self-perception (personal communication with Pascal Martin). However, it has been shown that especially the SARC-F score has been proven to successfully screen for sarcopenia (Malmstrom et al., 2016), but is arguably useful in replacing objective physical performance tests.

5.5 Sarcopenia: Validation of definition and diagnostics

Our goal with this study was to investigate structural characteristics, especially fiber size and proportion, in gluteus medius muscle of sarcopenic and non-sarcopenic patients. This should result in an appropriate answer to the question whether sarcopenia is a form of physiological aging, a disability or even a disease. Our study revealed that loss of strength and hence sarcopenia, is not associated with morphological changes in gluteus medius muscle. We could not find differences between the two groups in neither muscle fiber morphology, physical performance nor blood levels of TSH, 25(OH)D, total protein or calcium, which makes sarcopenic patients in our study indistinguishable from other patients in those terms. Nevertheless, it was proven that sarcopenia is associated to higher risk of poor health outcomes and higher mortality (Newman et al., 2006; Visser et al., 2000; Beaudart et al., 2017).

Furthermore, sarcopenia is not only a product of continuous denervation and fiber atro-

phy during aging, but also includes various other causes like nutrition intake (Volpi et al., 2003), missing physical exercise (Trappe et al., 2004), chronic inflammation (Bano et al., 2017) and endocrine influences (Ng et al., 2018). Examples of the multitude of influence factors on muscle wasting are diseases that depict these causes to a significant degree and are not exclusively observed in older people. Examples of such diseases are ALS and Cushing's disease, which both lead to a loss of muscle mass and strength, although through either the death of motor neurons or an increased catabolic metabolism due to increased levels of glucocorticoids, respectively. To this date, it is not fully clear what contributes to increased muscle wasting and if it is possible to find a single main cause for sarcopenia that affects each patient. Nevertheless, we excluded patients with severe comorbidities in this study and therefore eliminated patients with muscle wasting due to other diseases. We concluded that because of no apparent diseases of the study cohort and no obvious differences between sarcopenic and non-sarcopenic patients, the cause for muscle wasting must lie in the multitude of subtle factors that in sum negatively affect muscle mass and strength. The differentiation between individuals with no evident cause and those where a clear single cause can be isolated is likewise found in conditions such as osteoporosis, dementia or high blood pressure and is therefore nothing new to clinical medicine. As Crutz-Jentoft et al. already pointed out in their review on sarcopenia, it might be beneficial for clinical practice that sarcopenia is categorized into a primary and secondary form (Cruz-Jentoft et al., 2010). Primary sarcopenia includes all elderly patients with no direct cause and is therefore considered age-related and secondary sarcopenia includes all patients with one or more obvious evidences.
Cruz-Jentoft et al. defined a consensus, which diagnostic tools should be utilized for clinical diagnosis. Muscle mass can either be assessed by dual energy X-ray absorptiometry (DXA) or BIA, physical performance via short physical performance battery or gait speed and grip strength via hand dynamometer. The availability of the different tools makes diagnostic procedure easier, due to the availability of at least one of these tools. It has been shown that DXA and BIA do not necessarily yield the same results in analyzing skeletal muscle mass (Reiss et al., 2016). The authors implied, that an estimated one out of six patients are misdiagnosed with sarcopenia, when using BIA compared to classification using DXA. However, this does not imply that either BIA or DXA is a better approach for determining skeletal muscle mass, but that there is still no consistency throughout the applied technique. These facts are one of the reasons that

block the progression towards a common diagnosis.

Furthermore, different studies implying slightly different thresholds. For muscle mass assessed by a BIA, there are two sets of cutoffs defined: Chien et al. defined low skeletal muscle mass as two standard deviations below the mean muscle mass of a young control group in Taiwanese subjects (Chien et al., 2008). Janssen et al. estimated skeletal muscle mass by a BIA in a cohort consisting of subjects being 60 years and above and defined cutoff values associated with the risk of physical disability (Janssen et al., 2004). Defining the skeletal muscle cutoff as two standard deviations below the mean of a young control group depicts the major drawback that by comparing aging people with a young group, eventually every patient would be deemed sarcopenic regardless of actual physical disabilities. In contrary, the approach by Janssen et al. appears to be much more plausible as patients are compared with a cohort of the same age. Nevertheless, this approach is based on a generally smaller group size as they firstly compared skeletal muscle mass to the likelihood of disabilities. This means that the thresholds are not as robust as the cutoff values by Chien et al. and therefore need further investigation. Additionally, Visser et al. compared both muscle mass and muscle strength with lower-extremity performance and could show that only leg extensor strength was independently associated with lower-extremity performance (Visser et al., 2000). Furthermore, it has been shown that lower extremity muscle and fat mass are no significant risk factors for disabilities and mortality (Cesari et al., 2009). Although this seems to contradict the findings of Janssen et al. that SMI is correlated to the likeliness of disabilities, it could be possible that the skeletal muscle mass might be associated with physical fitness, albeit probably dependently. Using skeletal muscle mass as the main criterion in the definition of sarcopenia seems therefore questionable, as the clinical relevance of this parameter is not out of question and strength and physical performance depict a more robust association with poor health outcomes.

In sum, Cruz-Jentoft et al. provide several methods per criterion and several threshold values based on different studies for each of all the three criteria, namely muscle mass, strength and physical performance, which implies that this clinical definition of sarcopenia can hardly be considered a consensus. We propose sarcopenia to be defined clinically by the loss of strength and/or physical performance, shifting the picture towards a disability, independent from the definite cause in a certain individual. Differentiating between primary and secondary sarcopenia might lead to different approaches

in clinical management.

Chapter 6

Conclusion & Outlook

We could not find morphological or biochemical differences between sarcopenic and non-sarcopenic patients with proximal femur fractures. We excluded any patient with severe comorbidities, and thus only included patients with primary sarcopenia. Concordant to the clinical definition of sarcopenia by the EWGSOP, we conclude that it is necessary to divide sarcopenia into primary and secondary sarcopenia, depending on the presence of distinguishable causes. Nevertheless, the definition provided by EWGSOP is not yet consistent in itself, as they present more than one threshold level for each criterion. For this purpose, we propose that the given thresholds should be reevaluated with the aim of generating a robust and universal definition of sarcopenia. Additionally, the focus of sarcopenia should be shifted towards muscle strength and physical performance as the driving parameters, as they are directly associated with poor health outcomes.

Future goals should include the derivation of a more robust definition of sarcopenia for clinical application, as well as the investigation of different influence factors on muscle wasting both in young and aging humans. For this purpose, strength and physical performance need to be measured throughout a large population cohort to estimate age-dependent threshold levels. This is a necessary step to compare the functional parameters with the population of the same age. As those parameters decline with age, comparison with young control groups would therefore inevitably lead to false positive diagnoses. Additionally, a new definition of sarcopenia should then be discussed, with the main criteria being strength and physical performance of the whole body and not

just individual muscles.

Sarcopenia is a geriatric syndrome due to a multitude of causes. Loss of muscle mass and strength in aging is not yet fully understood. Investigating the age – and disease-related effects leading to muscle wasting are therefore needed to understand the biochemical pathways that lead to sarcopenia. Eventually, biomarkers could be available to differentiate between different causes for muscle wasting and could help in the early diagnostics of sarcopenic patients. For this, various diseases associated with a significant loss of skeletal muscle can be used as model systems to better understand the various pathophysiological mechanisms. The economical and sanitary burden sarcopenia poses is conspicuous and is expected to aggravate within the next decades. To effectively address this problem, sarcopenia should be treated as early as possible. Therefore, it is necessary to devise a prevention plan to reach out for people and motivate them to obtain a more active lifestyle by exercising on a regular basis, as physical activity is the only treatment known to this date that effectively improves fitness and preserves mobility in age.

Chapter 7

Supplementary data

Table 7.1: Overview of collected data.

Donor	Height [cm]	Weight [kg]	Age [years]	Barthel index	Parker mobility score	SARC-F score	Handgrip [kg]
D01	169	90	76	100	9	1	22.9
D02	168	60	69	85	6	6	32.5
D04	170	42	81	90	5	6	9.9
D05	171	69	92	80	6	8	13.0
D08	160	70	92	95	4	7	11.3
D09	168	65	76	95	7	2	21.7
D10	153	48	80	100	5	3	20.4
D11	157	67	81	95	7	5	12.8
D13	168	57	80	100	9	1	17.0
D14	180	56	90	ND	ND	ND	34.7
D15	184	105	55	100	9	0	50.0
D17	160	58	92	95	7	3	15.4
D18	152	50	93	95	5	5	15.0
D19	175	70	75	95	6	4	20.7
D20	163	68	85	75	6	5	11.3
D21	154	50	81	100	9	2	13.2
D22	164	52	87	100	9	1	14.3
D23	169	75	69	100	9	1	31.3
D24	190	80	59	100	9	1	37.6
D25	180	80	78	ND	ND	ND	13.9

ND = not determined.

Donor	25(OH)D [ng/ml]	TSH [mU/l]	Total protein [g/l]	Calcium mmol/l	Resistance [Ω]	SMM [kg]	SMI [kg/m^2]	SMM proportion [%]
D01	10.0	1.45	6.0	2.03	409.1	27.70	9.70	30.78
D02	10.0	1.63	5.9	1.99	475.1	27.85	9.87	46.42
D04	13.8	1.47	5.2	2.03	521.1	21.59	7.47	51.41
D05	15.1	1.64	6.0	2.15	534.7	20.50	7.01	29.71
D08	16.9	2.76	5.2	2.26	524.7	18.13	7.08	25.91
D09	10.0	1.24	4.9	1.88	507.2	22.02	7.80	33.88
D10	15.4	1.26	5.2	1.94	391.1	23.42	10.01	48.80
D11	14.4	2.25	5.0	2.18	723.0	13.02	5.28	19.44
D13	18.5	1.32	5.4	2.02	589.3	18.63	6.60	32.68
D14	16.7	1.41	5.2	ND	427.2	32.95	10.17	58.84
D15	ND	1.97	5.5	ND	459.2	34.59	10.22	32.94
D17	ND	2.62	5.2	ND	413.9	23.37	9.13	40.30
D18	11.8	1.08	5.2	1.98	498.8	17.07	7.39	34.15
D19	60.2	0.47	4.8	2.13	463.3	26.28	8.58	37.55
D20	ND	0.54	5.1	2.08	513.6	19.81	7.46	29.13
D21	19.8	ND	5.8	2.04	629.5	14.46	6.10	28.92
D22	10.0	0.16	6.3	2.10	658.8	15.30	5.69	29.42
D23	10.0	1.22	4.2	1.84	414.7	27.82	9.74	37.09
D24	10.0	0.81	6.0	2.11	516.1	32.79	9.08	40.98
D25	10.0	0.40	5.8	2.04	513.4	28.70	8.86	35.87

ND = not determined.

	Fiber number				Fiber proportion			mean Fiber size [μm^2]		
Donor	**Type I**	**Type IIa**	**Type IIx**	**Sum**	**Type I %**	**Type IIa %**	**Type IIx %**	**type I area**	**type IIa area**	**type IIx area**
D01	646	288	157	1091	0.592	0.264	0.144	8244	5650	2681
D02	1736	36	264	2036	0.853	0.018	0.130	7663	2297	2492
D04	2946	382	196	3524	0.836	0.108	0.056	2299	3362	2108
D05	4061	2507	2388	8956	0.453	0.280	0.267	6093	2198	1199
D08	2219	8	20	2247	0.988	0.004	0.009	5508	1435	1074
D09	1964	117	17	2098	0.936	0.056	0.008	3357	1560	1306
D10	2309	117	33	2459	0.936	0.056	0.008	7053	1031	1149
D11	2306	13	5	2324	0.992	0.006	0.002	6866	2034	1375
D13	3041	402	544	3987	0.763	0.101	0.136	7668	3463	2746
D14	1026	309	545	1880	0.546	0.164	0.290	1946	1110	1213
D15	1349	847	558	2754	0.490	0.308	0.203	6020	6047	4450
D17	1946	754	124	2824	0.689	0.267	0.044	4658	1611	1452
D18	3059	459	748	4266	0.717	0.108	0.175	7940	1889	1504
D19	4642	578	0	5220	0.889	0.111	0.000	2271	1001	ND
D20	1417	0	0	1417	1.000	0.000	0.000	4698	ND	ND
D21	3225	39	283	3547	0.909	0.011	0.080	11312	4223	3221
D22	1568	312	80	1960	0.800	0.159	0.041	2958	2790	1074
D23	1249	228	279	1756	0.711	0.130	0.159	6562	1990	1947
D24	4721	305	0	5026	0.939	0.061	0.000	4083	3454	ND
D25	1114	238	68	1420	0.785	0.168	0.048	4304	1990	1947

ND = not determined.

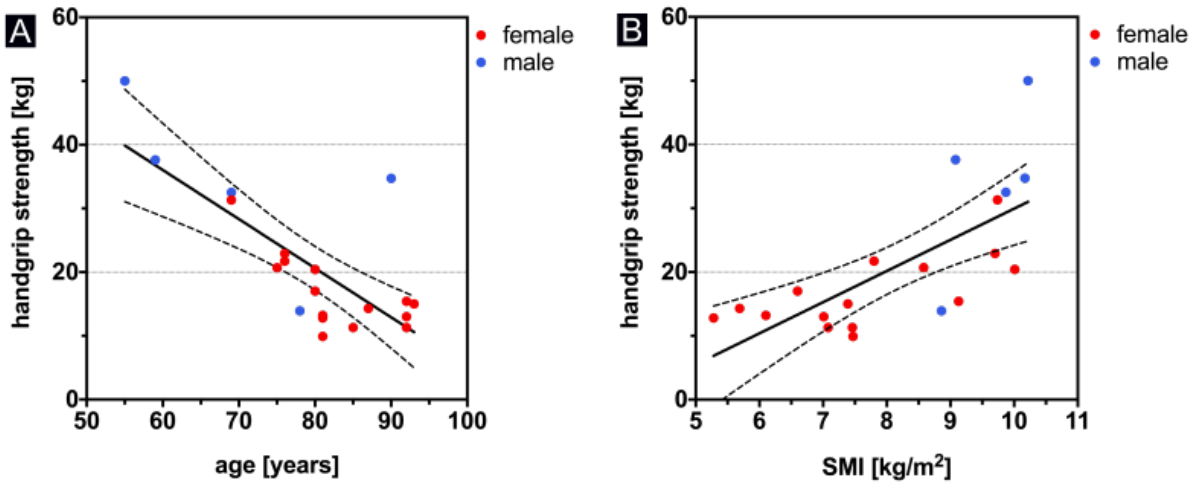

Figure 7.1: Measurement of handgrip strength.

We measured handgrip strength in all participants and compared them to both age and SMI. We found that with increasing age skeletal muscle strength significantly decreases (**A** $p \leq 0.05$, $R^2 = 0.57$). Additionally, handgrip strength positively correlated to relative muscle mass (**B** $p \leq 0.05$, $R^2 = 0.51$). This implies that muscle mass is at least indirectly correlated to muscle strength. Data points represent individual donors. Female: red, male: blue. Regression lines indicate mean $\pm$ CI (95%) of the whole study cohort.

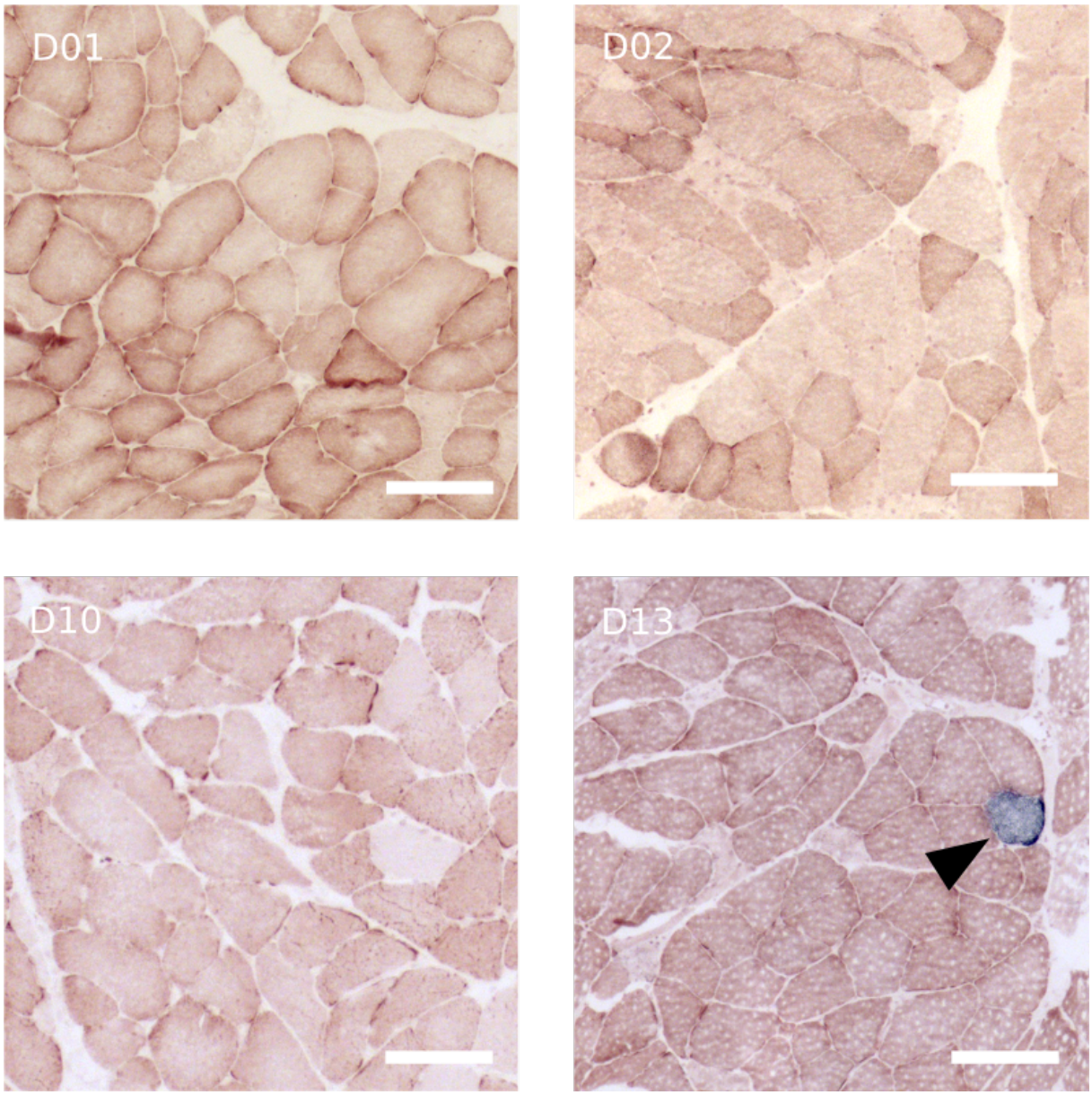

Figure 7.2: COX/SDH staining.

The figure shows COX/SDH stained cross-sections of 4 individuals representing the study cohort. We analyzed mitochondrial function in skeletal muscle biopsies of all participants. Most participants showed close to or only negative fibers (brown). Positive muscle fibers with mitochondrial dysfunction were very rare throughout all participants (blue, black arrow head). Scale bar = 200 μm.

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Patientenname

Interne Nummer

Etikett

Geburtsdatum

OP Datum

Größe/Gewicht

Vorerkrankungen

Scores

Barthel-Index	
Parker-Mobility-Score	
SARC-F Score	

BIA

Resistanz	
Reaktanz	
Phase	

Handkraftmessung Maximalwerte

Linke Hand	
Rechte Hand	

Laborparameter Datum:

25(OH)-Vitamin D		> 20 ng/ml
TSH		0,4 – 4 mU/l
fT_3		2,2 – 5,5 pg/ml
fT_4		0,6 – 1,8 ng/dl
Gesamteiweiß		66 – 83 g/l

Barthel-Index

Essen		[10]	[5]	[0]
Auf- und Umsetzen	[15]	[10]	[5]	[0]
Waschen			[5]	[0]
Toilettengang		[10]	[5]	[0]
Baden/Duschen			[5]	[0]
Aufsetzen und Gehen	[15]	[10]	[5]	[0]
Treppensteigen		[10]	[5]	[0]
An- und Entkleiden		[10]	[5]	[0]
Stuhlkontinenz		[10]	[5]	[0]
Harnkontinenz		[10]	[5]	[0]
Gesamt				

Parker-Mobility-Score

Mobilität im Haus	[3]	[2]	[1]	[0]
Mobilität außer Haus	[3]	[2]	[1]	[0]
Ohne Hilfe Einkaufen gehen	[3]	[2]	[1]	[0]
Gesamt				

SARC-F Score

Last tragen (5kg)		[2]	[1]	[0]
Gehen ohne Hilfe		[2]	[1]	[0]
Von Stuhl aufstehen		[2]	[1]	[0]
Treppensteigen		[2]	[1]	[0]
Stürze im letzten Jahr		[2]	[1]	[0]
Gesamt				

Handkraftmessung

Linke Hand	Rechte Hand
Versuch 1	Versuch 1
Versuch 2	Versuch 2
Versuch 3	Versuch 3

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Chapter 9

Appendix

Abbreviations

25(OH)D	25-hydroxyvitamin D
AChR	acetylcholine receptor
ADP	adenosine diphosphate
ALS	amyotrophic lateral sclerosis
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BIA	bioelectrical impedance analysis
BMI	body mass index
CI	confidence interval
CSA	cross-sectional area
COX/SDH	cytochrome c oxidase/succinate dehydrogenase
DAPI	4',6-diamidino-2-phenylindole
DGC	dystrophin-associated glycoprotein complex
DHPR	dihydropyridine receptor

DXA dual energy X-ray absorptiometry

etc. et cetera

EWGSOP European Working Group on Sarcopenia

MHC myosin heavy chain

mtDNA mitochondrial DNA

PBS phosphate buffered saline

PBS-T phosphate buffered saline with 0,1% Triton

Pi inorganic phosphate

ROS reactive oxygen species

RyR ryanodine receptor

SD standard deviation

SERCA sarco/endoplasmatic reticulum calcium-ATPase

SMI skeletal muscle index

SMM skeletal muscle mass

SOD1 superoxide dismutase 1

TSH thyroid stimulating hormone

Acknowledgments

Lastly, I want to deeply appreciate the following people, who contributed in various ways to the realization of this thesis:

Wolfgang Böcker and Christian Kammerlander, for accepting me in your group and offering me an interesting and challenging opportunity for my thesis.

Maximilian Saller, for constantly pushing this project forward. Without your help this project wouldn't have been what it is today. With the constant exchange of ideas I developed a thorough interest in research and am able to pursue my own way in science. The project with you was a stepping stone towards a new way in my life I would not have dared to take without making the experience of such a good supervisor like you are. Thank you very much!

Stefan Mehaffey, for offering me a position in this project and giving me the chance to finally work in a scientific environment. I want to thank you for the close collaboration during the time, which helped me develop our project. Your helpful advice and interest to push this project forward was always appreciated. Thank you!

Attila Aszodi, for providing good background in the lab and making my time there worthwhile.

Jelena Schwarz and Sara Taha, for constant exchange of thoughts, not only about the our projects but about all things we had in mind as well. You both always made the days more bright in the lab. Together we will keep the medium warm!

Sebastian Reiprich, for always encouraging me to go on. Sharing a working space with you was a more than pleasant thing throughout all the time in the lab. And remember: Heike is watching you!

Paolo Alberton, for being a heartwarming and awesome team mate. Thank you for the prosecco!

Maresa Demmel, for supporting our project by introducing me to your lab in Großhadern and making it possible to acquire muscle biopsies in Großhadern as well.

Zsuzsanna Farkas, Martina Burggraf and Heidrun Grondinger, for providing me a perfect background environment to complete my thesis focused and without any technical restrictions.

Finally, I also want to thank all other colleagues at ExperiMed and at the Department of General, Trauma and Reconstructive Surgery at the University Hospital of Munich, who I met during the last years.

Eidesstattliche Versicherung

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