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## **Sarcopenia; Aging-related loss of muscle mass and function**

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Running title: Sarcopenia

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## **ABSTRACT**

Sarcopenia is a loss of muscle mass and function in the elderly that reduces mobility; diminishes quality of life, and can lead to fall-related injuries, which require costly hospitalization and extended rehabilitation. This review focuses on the aging-related structural changes and mechanisms at cellular and subcellular levels underlying changes in the individual motor unit: specifically, the perikaryon of  $\alpha$ -motoneurone; its neuromuscular junction(s), and the muscle fibers that it innervates. Loss of muscle mass with aging, which is largely due to the progressive loss of motoneurons, is associated with reduced muscle fiber number and size. Muscle function progressively declines because motoneurone loss is not adequately compensated by re-innervation of muscle fibers by the remaining motoneurons. At the intracellular level, key factors are qualitative changes in post-translational modifications of muscle proteins and the loss of coordinated control between contractile, mitochondrial, and sarcoplasmic reticulum protein expression. Quantitative and qualitative changes in skeletal muscle during the process of aging also have been implicated in the pathogenesis of acquired and hereditary neuromuscular disorders. In experimental models, specific intervention strategies have shown encouraging results on limiting deterioration of motor unit structure and function under conditions of impaired innervation. Translated to the clinic, if these or similar interventions, by saving muscle and improving mobility, could help alleviate sarcopenia in the elderly, there would be both great humanitarian benefits and large cost savings for health care systems.

## 1. INTRODUCTION

A progressive loss of function, decreasing fertility and increasing mortality represent fundamental biological aging processes intrinsic to most cellular systems. The aging-related muscle atrophy is the most common type of muscle atrophy in man and is associated with significant impairment of function, such as slowing of movement and muscle weakness. The importance of aging-related changes in muscle function is increasing dramatically due to socioeconomic factors related to the demographic development in modern society with a growing proportion of old individuals due to improved living conditions, health care, etc. The process of random mortality caused by intercurrent disease (e.g., infectious disease) has been replaced by mortality and morbidity caused by aging-associated injuries and diseases (e.g., falls and fall-related injuries). There is accordingly significant need for an increased understanding of the mechanisms underlying the changes in skeletal muscle structure and function during aging as well as the design of specific intervention strategies for multiple reasons. First, the demographic evolution with an increasing proportion of elderly citizens with problems related to the aging-related motor handicap and dependency with subsequent dramatic social and economic consequences has resulted in an increased awareness of the importance of research efforts aiming at improving the quality of life in the aged. Second, it cannot be excluded that aging plays an important etiological role in the progression of specific neuromuscular disorders, such as amyotrophic lateral sclerosis, muscular dystrophies, critical illness myopathy, ventilator induced diaphragm muscle dysfunction, and the post-polio syndrome. Third, elderly fragile men and women need to use their maximum muscle power to rise from a chair and an additional small impairment may accordingly change their life from an independent to a dependent one (288). Fourth, falls and fall-related injuries are common among older individuals and women are at higher risk than men (385, 857). The cause of falls and fall-related injuries in old age are complex and involve impairments in the neuromuscular, peripheral, and central nervous system (664). The fall may be separated into two components, the initiation of the fall and the ability to restore disturbed balance. First, aging-related changes in somatosensation, vision, and vestibular function have a negative impact on maintaining postural balance, resulting in an increased risk of initiating a fall. Second, the ability to recover from a threatening fall is also impaired in old age, especially in old women, but is not related to an altered somatosensory process or the initiation of muscle contraction by the central nervous system (809). This process, on the other hand, lies primarily in events after depolarization of the muscle membrane, *i.e.*, in force generation and contractile speed (809).

The aging-related loss of muscle function involves quantitative and qualitative changes in skeletal muscle structure and function. This process is typically slow and the functional loss varies significantly among individuals, but is observed in all humans, *i.e.*, also in healthy, well-nourished, and physically active individuals (70). The decline in muscle mass and function probably represents the most dramatic and significant of all changes during the aging process (117), a process referred to as *sarcopenia* (117). Although the fundamental biological processes underlying aging remain obscure, it is clinically of great importance to have detailed knowledge about muscle tissue-specific mechanisms of aging. These efforts need to include both state-of-the-art basic research and clinical investigations. The study of sarcopenia is however complicated by several factors, such as muscle-specific differences in the response to aging, differences among species, and methodological errors related to scientific approach. This is probably, at least in part, why so many different and sometimes conflicting results have been reported in the literature.

This review focuses on current knowledge about how aging influences the different components of the motor unit, underlying mechanisms, intervention strategies, and the association between specific neuromuscular disorders and old age. We will refrain from lengthy discussions related to the numerous divergent results in the literature regarding sarcopenia and underlying mechanisms. Although there are species differences in response to aging, all mammals are characterized by sarcopenia and conflicting results are frequently secondary to study designs with their different inherent methodological limitations. Throughout this review, changes related to the aging process will be referred to as “aging-related”, *i.e.*, a process which starts at the age of maturity and where old age is the age after an individual has passed the 50% survival age in the population (1000). On the other hand, “age-related” will be used to describe changes associated with the whole life-span, *i.e.*, including development and maturation in addition to aging. Further, sarcopenia will be referred to as its original definition (770), *i.e.*, the aging-related changes in skeletal muscle structure and function affecting mammals, including humans. Therefore, this review will pay specific attention to aging-related changes in skeletal muscle observed in both experimental models as well as in humans. Divergent or conflicting results observed in humans compared with experimental models may be related to species differences, but inherent errors in the design of the experimental model and limitations introduced in the study of sarcopenia in mammals with a long lifespan, such as humans, are more common.

## **2. EXPERIMENTAL MODELS**

### **a. Methodological problems in the study of aging skeletal muscle**

The study of sarcopenia in humans is complicated by the long duration of the aging process, large variability among individuals, and multiple factors affecting muscle that are not primarily related to aging *per se*. Studies of aging can be conducted using either a cross-sectional or longitudinal design, but neither are free from sampling errors (105, 514, 820). Many of the factors affecting skeletal muscle have changed dramatically in the past century, such as social-, nutritional-, and physical activity-related factors, adding a significant challenge in the interpretation of results in long-lived mammals such as humans. Cross-sectional studies are particularly vulnerable to these confounding factors and an additional weakness with the cross-sectional design is that very old subjects represent a specific cohort with a different rate of aging, resulting in the so called “reversible aging phenomenon”, a phenomenon also observed in aging rodents (805, 834). Longitudinal studies have significant advantages, but are difficult to conduct in mammals with a long life-span, such as in humans as well as in rodent studies, where a large proportion of the total muscle mass needs to be analyzed at each timepoint. Premature aging offers a significant advantage logistically in studies on aging in humans, although aging represents a very complex biological system that is not perfectly mimicked in premature aging models. The problem with tissue sampling may, at least in part, be overcome in rodent studies by using genetically identical recombinant inbred mice strains sacrificed at different time points, offering a “longitudinal” study with a cross-sectional design (572-574). In addition, longitudinal studies in humans are not immune to the dramatic changes that have taken place during the past decades in nutrition and other environmental influences. Rodent models have frequently been used in the study of sarcopenia, offering a cost-efficient alternative. Mouse models have the advantage of efficient genetic engineering, but it is becoming increasingly evident that the rat

is a superior rodent model for studies of both muscle disease and aging (416). This may in part be due to the fact that mice strains were originally selected for cancer research, while such selection has not occurred in rat strains primarily used for toxicological and physiological research (999). Further, the CRISPR interference technique has the potential to introduce cost-effective genetic manipulations of interest in aging research in rats. Finally, it is important to emphasize that many aging-related phenomena are closely related and impact on sarcopenia, adding to the complexity when interpreting results, such as endocrine, central and peripheral nervous system, social, nutritional, and central and peripheral circulatory influences and their impact on skeletal muscle (514).

#### **b. Muscle-specific differences**

Structural and electrophysiological changes in skeletal muscle are frequently reported first after the sixth decade in humans and followed by a progressive decline with increasing age (679, 847). However, aging-related changes in skeletal muscle power begin earlier according to observations in track and field Masters Athletes (321), *i.e.*, muscle power (world records) begins to decline after the age of 30 and continues to decline linearly with advancing age (321). Although Masters Athletes represent a unique population, they have the advantage that they are both highly motivated and are used to performing at maximum or near maximum muscle power, separating them from the general population, where measurements of muscle function may be less reliable. Maximum power is of less importance for daily life and quality of life in old age, but it reflects aging-related changes in skeletal muscle function. Thus, aging-related changes in muscle power appear to precede detectable changes in various morphological and electrophysiological changes. This may be due to lack of precision or sensitivity of the methods used, or more likely that qualitative changes in regulation of muscle contraction go undetected by morphological and electrophysiological methods, which primarily quantify muscle mass and motor unit size/organization. However, the application of more sophisticated techniques will allow for the detection of progressive aging-related changes in skeletal muscle at a much earlier time and sarcopenia may be considered an epiphenomenon of cellular and molecular changes that start early in life.

In quadruped and biped mammals, the forelimb (arm) and hind limb (leg) appear to be affected differently by aging, with hind limb (leg) muscles being more severely affected than forelimb (arm) muscles (311, 375, 623, 679, 847). This is of significant importance since lower extremity function has a stronger impact on quality of life and independence in old age than upper limb function in humans. Furthermore, the cause of falls and fall-related injuries in old age has been reported to lie primarily in events after depolarization of skeletal muscle, *i.e.*, in force-generation and contractile speed (809). In support of this, the decline in mobility and lower extremity disability have been reported most influential in predicting falls in the elderly (76, 596, 597, 761, 939), and the predictive value of the muscle weakness is further increased when muscle force is measured at a speed of movement resembling more functional limb velocities (939). Grip strength measurements have frequently been performed in the study of aging-related changes in skeletal muscle function, primarily due to the simple grip strength measurement procedure in both humans and rodents. However, it may be argued how well grip strength reflects the aging-related motor handicap and decreased quality of life in old age related to impaired mobility and risk of falls and fall-related injuries. Due to the strong impact of lower limb muscle function on falls and fall-related injuries and

the more pronounced changes in lower (hind) versus upper (fore) limbs, this review will focus on aging-related changes in lower (hind) limb muscles.

### 3. AGING-RELATED CHANGES IN THE MOTOR UNIT

#### a. Aging-related changes in motor unit organization

The concept of the motor unit introduced by Lidell and Sherrington (568) and defined as the single alpha motoneurone and the muscle fibers it innervates was emphasized as the final functional unit in the motor system (250). Numerous attempts have been made to classify motor units into different types according to physiological characteristics, metabolic properties, or myosin heavy chain (MyHC) isoform expression in the motor unit fibers, *i.e.*, the constituent muscle fibers within a single motor unit (645). Historically, the spatial organization of motor unit fibers was the subject of discussion for a long period and the scattered organization of motor unit fibers within the motor unit territory and the spatial overlap between different territories was not resolved until the single fiber EMG (852) and single motor unit glycogen depletion techniques were introduced. The glycogen depletion technique was originally proposed by Krnjevic' and Miledi (484) and successfully first reported by Edström and Kugelberg (257), Doyle and Mayer (240), Branstater and Lambert (98, 99), and Burke et al. (125). Originally, the spatial organization of motor unit fibers was considered to be random within the motor unit territory and metabolic properties to be uniform within single motor units, based on visual evaluation of glycogen-depleted motor unit fibers (124, 257, 662, 663). Albeit significantly smaller variation than among different motor units, microphotometric measurements of metabolic properties and computer-assisted measurements of spatial organization within the motor unit territory demonstrate heterogeneity in enzyme-activities and non-random organization of motor unit fibers (26, 79, 258, 358, 488, 516, 609, 610). In the large fast-twitch motor units in the rat tibialis anterior muscle, a gradient of the mitochondrial enzyme activity succinic dehydrogenase was observed along the superficial-deep axis of the muscle, indicating a biological phenomenon rather than a methodological error (516). The tibialis anterior muscle has a complex orientation of muscle fibers and the mechanical strain on muscle fibers both in the relaxed and activated strain may vary along to the superficial deep axis. This is of specific interest since mitochondria are mechanosensitive and respond to passive mechanical load (442, 443). In old age, a significant reorganization of motor unit fibers is observed, which precedes the fiber type grouping and grouped atrophy reported at an advanced age (24, 26, 258). The motor unit fiber reorganization is paralleled by an increased number of muscle fibers innervated by the  $\alpha$ -motoneurone, a decreased total muscle fiber number, and a decreased number of large myelinated neurons in peripheral nerves and ventral roots, suggesting denervation due to loss of  $\alpha$ -motoneurons and incomplete reinnervation of previously denervated muscle fibers. In the tibialis anterior muscle, motor units are organized according to the spatial organization along the superficial deep axis. That is, the largest motor units with the largest muscle fibers expressing the type IIb MyHC are located in the superficial region of the muscle and the small motor units expressing the type IIa MyHC isoform are located in the deep region of the muscle. In between these two motor unit types, motor units are localized having an intermediate size and fibers expressing the IIx MyHC isoform (**Fig. 1**, (524)). During aging, there is a fast to slow myosin transition, with a decreased amount of IIb MyHC fibers (522, 535) and motor unit fibers expressing the type IIx MyHC isoform are expressed in regions restricted to type IIb MyHC motor

unit fibers in young animals (521). Further, in old age, motor units have been identified including muscle fibers expressing different MyHC isoforms in contrast to motor units from young animals where motor unit muscle fibers are homogenous with regard to MyHC isoform expression (521) (**Fig. 2**). Such inhomogeneity has not been reported in motor units from in young animals and it may be speculated that this is secondary to an aging-related reinnervation but incomplete MyHC transition within the motor unit. However, muscle fibers co-expressing different MyHC isoforms are observed in both young and old animals and may increase in number in old age.

### **b. Motoneurone**

During the aging process, the neuromuscular system is believed to undergo a continual reorganization of motor units due to a loss of motoneurons and reinnervation of denervated muscle fibers via collateral sprouting (265). This is supported by an aging-related decline in myelinated neurons in sensory and mixed peripheral nerves, in the mouse (126, 792), the rat (24, 265, 350, 376, 865, 916), the horse (938) and in humans (422, 858, 864, 882, 885), as well as in ventral roots from rats (24, 248) and humans (174, 319, 641, 753) (**Fig 3**). The loss of large anterior horn cells in the mouse (964); rat (376, 420) and humans (455, 456, 885) lend further support to an aging-related loss of motoneurons and the large  $\alpha$ -motoneurons appear to be more affected by old age than the smaller  $\gamma$ -motoneurons (24, 374, 376). However, this is a slow gradual process including axonal, demyelinating and Schwann cell pathology, and the motoneurone loss is preceded by decreased metabolic capacity and structural changes at the motoneurone level, such as Wallerian degeneration, myelin sheet irregularities, myelin ovoids, infolded loops of myelin, macrophages overloaded with myelin debris, and myelin reduplication (439, 520). This condition is referred to as “sick” motoneurons which has significant consequences for the functional capacity of the motoneurone (265, 621). In parallel with the structural changes, there is a decreased rate of axoplasmic transport, speed of nerve regeneration and axonal conduction velocity (118, 444, 626, 705). The morphological changes indicating axonal degeneration have been reported more prominent in peripheral nerves than in ventral roots (24). This is suggested to be secondary to decreased protein synthesis in the neuronal cell body and/or the decreased rate of axonal transport in old age rendering the distal part of the neuron more vulnerable to these effects of aging (470, 475, 626). Axonal transport is highly dependent on intact microtubules (827) and the decreased axonal transport in experimental diabetes coincides with the glycation of tubulin (188). Reducing sugars target lysine residues during non-enzymatic glycosylation (glycation) and reactive lysine residues are essential for tubulin assembly into microtubules (188, 632). This offers a plausible mechanism underlying the slowing of axoplasmic flow in old age, since post-translational modifications of proteins and nucleic acids by non-enzymatic glycation are considered an important pathophysiological aging-related mechanism (115, 123). Ultimately, this process leads to a compromised ability of the old motoneurone to reinnervate via collateral sprouting, preceding the final aging-related motoneurone loss (265). Furthermore, the largest and fastest conducting neurons appear to be preferentially lost during aging with consequences for reinnervation capacity and muscle physiology (176, 641, 674, 677, 882). Thus, the loss of the largest motoneurons included in fast-twitch motor units, the impaired reinnervation capacity by “sick” motoneurons will contribute significantly to quantitative and qualitative changes of skeletal muscle structure and function in old age, such as muscle fiber loss and fiber type transitions (see(520)).



Mechanisms underlying the progressive loss of  $\alpha$ -motoneurons during aging are highly complex and involve multiple factors. DNA damage and modifications accumulating throughout life eventually leading functional deterioration and cell death have been suggested to play an important role for the motoneuron loss in old age (197). This is supported by experimental studies in progeroid mice with a deficient DNA repair mechanism resulting in accumulation of DNA damage and a phenotype resembling the neuromuscular system observed in old age, *i.e.*, degeneration of motoneurons and progressive disruption of the neuromuscular connectivity (197). Thus, accumulation of DNA damage and genotoxic stress have therefore been forwarded as a plausible mechanism contributing to the aging-related motoneuron loss (89, 197). Reactive oxygen species, malondialdehyde as well as non-enzymatic glycosylation may contribute to this process. Spinal motoneurons show selective vulnerability to intermediate products of glycation with neurotoxic effects, leading to apoptotic cell death (822). An inefficient glutathione defense system in old age will contribute to increased levels of glycated proteins and a progressive loss of spinal motoneurons, a pathophysiological mechanism also suggested to underlie the progressive loss of spinal motoneurons in patients with amyotrophic lateral sclerosis (ALS) (822). As discussed below, aging-related changes in the microcirculation of the spinal cord may contribute to the motoneuron loss in old age (see below).

### **c. Neuromuscular junction**

There are clearly a bewildering array of deficiencies reported in the physiology, biochemistry and morphology of muscle fibers in aging animals. These have been covered extensively in numerous, excellent reviews of this subject (cf. (37, 107, 228, 421, 426, 763, 880, 897, 959)) and are covered elsewhere in this review. Given the close, reciprocal influence of nerve on muscle (for example, the activity patterns conveyed to the muscle by their nerves influence the size and contractile properties of the muscle fibers (cf. (34, 339)) and muscle and its activity on nerve (for example, the activity pattern of the nerve and muscle can influence the size and detailed morphology of the synaptic contact itself (749)s and disconnecting the two serves as a major stimulant for nerve growth (cf. (113))), it is not surprising that there are corresponding aging changes in the physiology, biochemistry, and morphology of the neuromuscular junction (NMJ) itself. There is also an equivalently bewildering array of manipulations to the neuromuscular system that can alter these properties both of the muscle and the NMJ that produce effects similar to those seen in aging; some of these are discussed below. It is undoubtedly the case that, given these complex interdependencies of the neuromuscular system and the number of manipulations that can cause alterations, that the mechanisms of aging are quite complex. Nonetheless, understanding what happens to NMJs during aging is important not only for the possible interventions that could be made to improve the quality of life but also for understanding the basic science of these synapses as well many diseases that impact the NMJ.

### ***Developments allowing for vital imaging***

One approach to understanding how NMJs age is to directly observe the changes as they occur. Such an approach was made possible by two developments. Firstly, the application of fluorescent stains that vitally label the synaptic components made it possible to expose a muscle through simple surgery, briefly image the synapse through a dipping microscope objective, allow the animal to recover, and then return at a later time to examine the same synapse. Much of the early efforts at

vital imaging were made by the use of a mitochondrial dye (4-Di-2-Asp) (595) combined with a fluorescent conjugate of a snake toxin alpha bungarotoxin (BTX, that binds irreversibly to the postsynaptic acetylcholine receptors (19). A second development, the advent of genetically encoded fluorescent proteins (cf.(283)), considerably expanded the effectiveness of labeling as well as the number of components that could be labeled at the same NMJ.

Initial efforts at imaging concentrated on use of the sternomastoid muscle of the mouse (567), an easily accessible muscle located just beneath the salivary glands in the neck, but these efforts have since expanded to additional muscles. One of the chief concerns about imaging like this is its vital nature. Neither the bungarotoxin applied nor the fluorescent proteins expressed should perturb synaptic transmission; any perturbation here will itself alter the NMJ. BTX is a competitive antagonist of acetylcholine at acetylcholine receptor. It binds essentially irreversibly and, while it blocks the receptor to which it is bound and would paralyze the muscle, BTX can be used at a dosage that blocks only a fraction of the receptors. Because NMJs operate at a huge safety factor (i.e. they release enough quanta of acetylcholine to produce an excess of the depolarization necessary to bring the muscle fiber to threshold), it is estimated that up to 70% of the receptors can be blocked without rendering transmission across the synapse ineffective (571).

Moreover, the BTX will persist in its bound state until the receptors to which it is bound turns over. As long as transmission remains intact, the turnover of these receptors proceeds normally (9). Hence, it is the case that the BTX can be applied once at low concentrations and the labeling of a fraction of receptors persists for some weeks (567). As which receptors are labeled is random, an image of the entirety of the postsynaptic acetylcholine receptor area (in the endplate) is visible. Although the turnover of receptors and the structure of the synapse is altered in the presence of a block of neurotransmission and action potential activity in the muscle (9), this does not occur if the number of BTX-bound receptors is insufficient to produce a block (45). After an initial imaging session, the incision can be closed and the animal recovered from anesthesia. At a subsequent imaging session, the receptor labeling can be repeated with another application of BTX, even one conjugated to a different fluorochrome, again with the caveat of not producing a block of transmission. This allows the visualization of changes that have occurred in the distribution of receptors since the last imaging session and in particular the identification of any new sites on the muscle fiber at which newly synthesized receptors have been added. Of course, it is essential that one be able to identify the same synapses at the different imaging sessions. This can be easily achieved in most cases because the junctions each have a unique pretzel-like morphology created by the aggregation of receptors underneath the branches of the nerve terminals at the sites in the muscle fiber where they come into contact. These receptors usually occur within depressions in the muscle fiber surface occupied by the nerve terminal called synaptic gutters. Each pretzel is said to be unique owing to the unique pattern of the nerve branches on the surface of the muscle fiber, making each junction uniquely identifiable. The nerve branches control the localization of the underlying receptors by inducing and stabilizing their presence. In addition to the pretzel shape of the junction of interest, each of the neighboring NMJs on adjoining muscle fibers within the so-called "endplate band" has its own unique pretzel shape and this can aid considerably in localization. Moreover, the presence of nerve branches and blood vessels and the rough position within the muscle help in re-identifying the junction originally imaged in a subsequent imaging session. Of course, this ability depends on how constant these structures remain over time, and the

observations to date presented briefly below show an amazing constancy except in certain circumstances where the usually stable junctions undergo morphological change ((43). However, even in these cases it is usually possible to reliably re-identify the same junction, especially as the pattern of the endplate band does not usually change and moreover the surrounding junctions have sometimes not changed dramatically.

Soon after their initial reports on how the nerve terminals at NMJs did not appear to change over time, Lichtman and colleagues were able to combine labeling of the terminals with labeling of the postsynaptic acetylcholine receptors with BTX, allowing the use of visualization through fluorescence of both pre- and postsynaptic components of the NMJs. In the initial years of their studies of NMJs, they relied heavily on the use of the 4-Di-2-Asp dye together with BTX (cf. (755). However, with the discovery of fluorescent proteins like GFP, the isolation of the genes encoding these fluorescent proteins, the discovery of promoters that could be used to drive gene expression in a restricted set of cells, and the developments in mouse transgenesis, investigators achieved the ability to vitally label individual cells in organisms. In particular, the so-called thy1 promoter was found to drive expression in neurons of the central nervous system (457) and could be utilized with the spectral variants of GFP to produce vital labeling of motoneurons, their axons within muscles and their nerve terminals present at the NMJ (283). Moreover, it was found that the same transgene, depending on its integration site in the mouse genome during the process of transgenesis, could produce lines of mice in which “subsets” of motoneurons (rather than whole motoneurone pools) were consistently labeled within each individual line (283, 910). These subset lines have been particularly useful in the same way Golgi stains were useful in the early stages of neuroanatomy: staining every cell or structure of one type makes it impossible to visualize individual cells; labeling of only a few makes individual components identifiable. This has been useful in the vital imaging of development of muscle innervation (cf. (75, 899, 900, 910)) and in investigating changes in innervation during pathologies (cf. (426, 803)). Since the development of the thy1-fluorescent protein (FP) mice, a number of other transgenics have been created, enabling, for example, the vital labeling of other structures at the NMJ, including glial cells (996, 998), the acetylcholine receptors in the postsynaptic muscle fiber (324), the cytoskeleton of the synapse and reactive glia (446) and resident macrophage (998). Essentially, the only limitation is the discovery of selective promoters (or alternately, the production of fusion proteins as knock-ins) and the cost and time involved in producing and maintaining lines of mice. While following up on successes in the imaging of neuromuscular synapses, the techniques have been adapted for imaging neuron-neuron synapses within the peripheral and central nervous system and these studies have added to our understanding of neural connectivity and plasticity.

Of course, there are limitations of what one can stain vitally within the muscle with the dyes available and there are cases where fluorescent transgenes are not readily available. In these cases, it is possible to vitally image the morphology of the synapse with the vital reagents one has available and then examine the same synapses *ex vivo* with reagents such as antibodies by the normal staining procedures (187). Once again, the constancy and uniqueness of the pretzel pattern of the receptors can serve to re-identify the synapse(s) studied *in vivo in vitro*. This has expanded the usefulness of vital imaging, although the investigator is limited to examining the status of the synapse at the endpoint of a series of vital images.

### ***How constant is the morphology of the NMJ?***

The question of how NMJs change during normal life is fundamental for how one interprets the results of aging and, for that matter, of disease as well. Purves, Lichtman, and colleagues used vital imaging of NMJs in the sternomastoid muscle of mice to examine whether these synapses are plastic and undergo morphological alterations during the normal life of mice, but did not look at the events of late aging. At the time of their studies there had been numerous studies involving static imaging of NMJs in mammals (cf. (53, 135, 136, 177, 275)) and particularly in frogs (cf. (27, 178, 187, 192, 471, 935)). It was clear that frog NMJs underwent seasonal adjustments in size by retraction and extension of nerve processes across the surface of individual muscle fibers. Furthermore, static observations in mammalian muscles (cats and rabbits) led to the conclusion that there was a significant amount of nerve sprouting that led to continual turnover of NMJs even in normal muscles (53). Therefore, it was surprising when the vital imaging results from mice suggested that their NMJs were mostly stable. There were occasional small deletions or additions to the pretzel and the pretzels grew in size as the muscle fibers themselves expanded in diameter as the animal itself grew. However, the overall message was one of stability. The growth in size of NMJs was described as intercalary (608), where the size of the synaptic contact changes but the overall morphology does not, a quite different picture from the malleability of the NMJs that then was prevalent. When androgen-sensitive, sexually dimorphic muscles in the rodent were caused to atrophy or hypertrophy by use of castration and/or administration of androgens, the muscle fiber diameters changed but the NMJs expanded or shrank without dramatic changes in their overall morphology (42). Similar imaging efforts by Wigston (944) chose to examine the morphology of the slow soleus muscle in the mouse (in contrast to the fast contracting sternomastoid utilized previously). Once again, the overall picture was one of constancy in morphology. But in contrast to sternomastoid, a significant fraction of the NMJs examined over time periods of months changed significantly in their morphology. Even most of those that changed still had significant remnants of the old, initially viewed morphology, having added or lost small portions of their synaptic contact. When these same experiments were repeated using the lateral gastrocnemius (945) (a muscle composed of fast contracting fibers like the sternomastoid), the observed number of changes were more in line with those observed in sternomastoid. There were a small number of instances where NMJs did undergo a substantial loss of receptor area. In these cases there was a loss of the originally observed receptor area where the nerve enters the junction (43). Along with the loss of receptor area, the portion of the nerve above the lost receptors had apparently become myelinated, suggesting that one of the glial (Schwann) cells of the junction had changed its phenotype from non-myelinating to myelinating and perhaps intruded between the nerve and the muscle at this site, disconnecting the two. Since studies of the arrangement of Schwann cells above the junction and the preterminal axon have shown lack of overlap of the cells above the junction and the myelinating cells along the axon (and even a tiling of the coverage of the terminal by individual Schwann cells of the junction) (101), such a change could easily occur without a total rearrangement of the glia at the synapse.

Secondly, it is also clear that whenever there is an interruption of the motor axon innervating a NMJ (experimentally by nerve section or crush, or even laser ablation) there can be a dramatic change in

the configuration of the synapse when the axon regenerates to reoccupy the old endplate site (754). This was beautifully demonstrated by Gutmann and Young in 1944 (361). Usually if the axon returns rapidly (which is the case after simple crush injuries since the axon can quickly regenerate down the old endoneurial tube filled with Schwann cells), the old sites containing high concentrations of acetylcholine receptors that remain even when the nerve is disconnected, are accurately reoccupied, usually by the very same axon that previously made contact (445, 667). However, if the nerve damage is more severe and creates a discontinuity in the old nerve sheaths that contained the motor innervation, reinnervation is much slower and it is unlikely that the nerve returns to each and every location on the fiber containing a high acetylcholine receptor density. Some of these unoccupied original sites turnover and some new sites are generated, likely due to the action of agrin secreted from sprouting nerve fibers (445, 667, 755).

An explanation of this receptor turnover during reinnervation has been provided by Balice-Gordon and Lichtman (45): they have shown that portions of nerve terminals that are rendered ineffective in synaptic transmission (by localized application of BTX to block the receptors underneath this portion of the nerve terminal) can lead to loss of the blocked receptors and withdrawal of that portion of the nerve terminal. However, such a loss occurs only if enough receptors are left unblocked in the synapse to allow effective transmission of excitation to the muscle fiber. This kind of loss does not occur if the entire synaptic site is blocked with BTX. Further, loss of receptors at the endplate does not occur if the entire junction is denervated and remains so (11). The implications of these experiments are profound. If some receptor sites are present that are not continually part of the excitation of the muscle fiber, these sites are lost. Thus, it is easy to see why asynchronous reinnervation of the synaptic sites on a muscle fiber could lead to remodeling of the synaptic site as portions would become active in advance of others, perhaps even before these other sites are reinnervated. Moreover, such a mechanism points to how important it is to maintain transmitter release across the synaptic site (even though the release of neurotransmitter from each active zone in the terminal is quite variable). Any long-term inactivity of a portion of the synapse would lead to remodeling, as would any unintentional damage during imaging. It is also easy to understand those cases where the preterminal axon becomes myelinated (43): the portion of the synapse whose apposing nerve becomes myelinated would be rendered effectively non-functional in transmission. It is understandable as well why prolonged denervation prior to reinnervation leads to increased asynchrony in reestablishment of nerve contacts at the old synaptic sites and therefore more profound remodeling of this site.

A third example is the case of sprouting. Sprouting (the growth of new nerve processes from the terminal or axon) is a common phenomenon in muscle during reinnervation (840) and particularly during reinnervation following partial denervation (839). In partial denervation, when some of the axons innervating a muscle are damaged leaving other axons intact in their innervation, there is considerable terminal and nodal sprouting supposedly stimulated by the presence of the denervated muscle fibers. These sprouts can grow along glial cells to reinnervate the endplates on nearby muscle fibers (839, 840). In these cases, the morphology of the synapses formed can change. Some of this change has been attributed to polyneuronal reinnervation of some junctions with subsequent competition for the endplate site, displacement of one of the innervating axons and the

loss of receptors underneath the losing axon (755). However, from the arguments above and other studies, such remodeling can occur even in the absence of polyneuronal innervation (445).

Other investigators have used other methods of marking nerve terminals for vital imaging and they have come to somewhat different conclusions than those just presented. Robbins and collaborators labeled nerve terminals with fluorescently conjugated tetanus toxin C fragment (393, 394), a component of tetanus toxin that enters motor nerve terminals and is retrogradely transported but is otherwise non-toxic. Imaging the pectineus muscle in the mouse, they observed a remodeling of NMJs during their growth that was created by extension and withdrawal of nerve terminal processes with corresponding alterations in the receptor pretzel. The lack of stability of the NMJs in this study and ones previously discussed could be due to a number of factors: the different reagents for labeling nerves, the steps taken to limit phototoxic damage from imaging, the character or type of the different muscles investigated, the ability to approach the muscle without a reaction to the skin wound necessary to provide access to the muscle, and the time period in the life of the animal over which observations were made. However, it is important to recognize that the nerve terminal processes observed to be extended (and retracted) in these experiments were generally very small and many of them were resolved without any change in the postsynaptic acetylcholine receptor distribution. While some junctions were observed to change substantially over months, such changes have been observed even in the studies to be discussed below and have been attributed to the damage to muscle fibers that can occur during their normal use in contractions.

Despite the arguments from vital imaging made above that remodeling of the NMJ during most of the life of the animal is minimal, there have been very few muscles examined in this way and there likely are muscle-to-muscle differences based on the patterns of their usage. Similarly, few species have been carefully examined, and there could be differences here. While conclusions based upon static imaging are important (especially when the changes are profound), it seems most convincing if the examinations are made so that changes in individual synapses can be monitored over time. It is also clear that any situation where even temporary changes in synaptic transmission occur, the NMJ can be remodeled accordingly. So, any claim as to the plasticity present must carefully account for possible confounding changes that occur during the observations themselves.

### **Morphological changes to the NMJ during aging observed through static imaging**

Whether or not NMJs are generally stable through most of life as argued above, something dramatic happens during advanced aging, although exactly what is happening and its consequences for synaptic function are less than clear and still controversial. Gutmann and colleagues reported on aged muscles of rats in the 1960's. They examined NMJs in 24 mo. old rats by electron microscopy, cholinesterase staining, and electrophysiology (359, 360). Despite atrophy of the muscles, and slowing of their contractile properties they reported no signs of obvious denervation. However, NMJs revealed by cholinesterase staining and some electron microscopy were reported as significantly changed. The cholinesterase staining was more "fragmented" and in electron micrographs there was some evidence of invasion of Schwann cells to surround some nerve terminals. Moreover, the frequency of miniature endplate potentials (mepps) was reported to be reduced (but see below). Histology of muscles and peripheral nerves were said to show a loss of

about a third of the muscle fibers in soleus but no obvious loss of motor axons (see 3b above and below for contrasting results). In general, these reports laid the groundwork for a host of subsequent studies on NMJs in old rodents. Cardasis and colleagues likewise reported in several papers an examination of NMJs, primarily in the soleus muscle of aged rats using cholinesterase staining in whole mounts and electron microscopy (134-136). The light micrographs showed enlarged but fragmented contacts on the muscle surface whose frequency increased with age. Many of these contacts were only partially occupied by nerve terminals. Electron micrographs of synaptic sites revealed large stretches of muscle fiber membrane where prominent junctional folds were present but that were unoccupied by nerve terminals or the nerve terminals were wrapped by Schwann cells. These observations led these investigators to propose that aging was a continuous process of withdrawal of nerve terminals from synaptic contact resulting in focal denervation followed by regrowth of nerves/sprouting and partial reinnervation. Robbins and colleagues (50, 275, 763) made similar findings in studying aging mice, employing zinc-iodide-osmium and silver/cholinesterase staining together with light microscopy and electron microscopy. Their findings were similar to those of investigators cited above (although they performed a much more thorough analysis of synaptic physiology), reporting as others have subsequently, a remarkable preservation of transmission (see below). Their observations of morphology showed a number of pronounced changes (expansion of the perimeter of the junction along the fibers, increased and more complex branching of the nerve terminal within the synaptic site, fragmentation of the nerve contacts into small islets contained within the enlarged perimeter of contact) but no obvious complete denervation of individual fibers and no evidence of obvious collateral sprouting. Their assays of radioiodide-BTX binding suggested there was little change in the number of acetylcholine receptors. Other investigators have made similar observations with some exceptions in the details of the particular muscles affected, the particular fiber types affected, the species examined, and the methods for examining morphology (177, 678, 705, 772). However, the general picture emerging from all these studies is that there is a loss of innervation on some fibers, followed by a compensatory reinnervation/sprouting that replaces the loss in innervation. This reinnervation changes the synaptic contact in such a manner that the perimeter of NMJs on individual fibers is enlarged but the contacts of the individual nerve terminal boutons form smaller islands on the fiber surface. It is commonly suggested that these changes occur as a continuation of remodeling believed to occur constantly but that becomes enhanced/exaggerated during aging. Most investigators believe the morphological changes are an attempt to compensate for a gradual loss of motoneurons during aging by supplying new innervation to fibers that have lost it.

### ***Vital imaging of changes in innervation during aging.***

It seems that a clearer understanding of changes in NMJs during aging could be by repeatedly imaging the same synapse over time in an aging animal. The first effort at such vital imaging of aging NMJs was made by Balice-Gordon (41). The imaging was carried out by the protocols described above in the sternomastoid muscle of the mouse using 4-Di-2-Asp to label motor nerve terminals and BTX to label the acetylcholine receptors. By following individual junctions over time, Balice-Gordon demonstrated that the NMJs, in contrast to the junctions viewed repetitively in younger animals, were profoundly altered just as would have been predicted by the static imaging that had been performed previously. Unfortunately, most of the images were collected at intervals of

a month or longer, and, although the changes appeared to become more exaggerated with time, no new conclusions as to how the changes occurred could be made. By the time the next attempts at vital imaging were made, the technology had improved with the advent of fluorescent-protein-labeled motoneuron axons. Using transgenic mice in which motor axons were labeled blue with cyan fluorescent protein (CFP) and receptors could be labeled red at the time of the experiment with rhodamine-BTX, imaging of both structures was made possible by changing filter sets on a fluorescence microscope (see **Fig. 4**). Because of the spectral overlap between rhodamine-BTX and 4-Di-2-Asp and the relative intensity of their labeling, previous experiments had required separate labeling of each fluorophore; with fluorescent protein mice, this was no longer necessary. Li et al. (564) collected images from mice aged 19-26 months, taking images at much more frequent intervals than in the study of Balice-Gordon. They encountered two types of NMJs in the aged sternomastoid: those that had the pretzel-like appearance of “young” NMJs (**Fig. 4**) and junctions that had acetylcholine receptors that were fragmented into small islands (**Fig. 5**). The nerve terminals above the receptors had varicosities along their branches; these varicosities were apposed to the receptor islands. Reassuringly, the same two types of junctions were encountered in statically imaged junctions from these mice and the frequency of the aged phenotype increased with age. Li et al. had expected to observe junctions that underwent a slow transformation from young to fragmented and fragmented to more fragmented (564, 565). Surprisingly, this was not what they found. They saw that most of the small sample of NMJs they had previously imaged underwent almost no changes between imaging sessions. To insure that the imaging procedure itself did not change junctions, they had to limit their observations in each animal to a few junctions. Junctions that had a “young” appearance at the time of the first imaging session mostly remained young at subsequent imaging sessions. The same was true for fragmented junctions: they remained fragmented as in the first image acquired. However, a fraction of the junctions (something like 8%) underwent a remarkable transformation over as short a time as a few days. Further evidence that these junctions were undergoing a major transformation was from the labeling of the acetylcholine receptors. Normally, one application of a non-blocking dose of BTX labels a small fraction of the receptors at the NMJ. Typically in young muscles, some of the labeling achieved at the first imaging session remains when a second image is acquired of the same junction a week or so later because the binding is so avid and the receptors turn over slowly. Gradually over time, the labeling does disappear as the labeled receptors are replaced with new, unlabeled ones (9). While most of the junctions visualized in the aging muscles were like this, the junctions undergoing the transformation were not: they underwent a dramatic loss of the original label so it could barely be perceived (**Fig. 5**). Application of additional BTX in these cases labeled a set of receptors many of which were not in the original pattern of the previous image taken of this junction (**Fig. 5**). That these junctions were the same ones as previously imaged was obvious from the identification of neighboring junctions. Further, the site where the nerve entered the junction and some of the major terminal branches were the same. Li et al. concluded that these transformed junctions had undergone a major change in their synaptic structure: the original receptors present prior to the transformation event had been lost and been replaced by a new set of receptors that appeared fragmented. The nerve terminal had changed in its branching pattern and become more varicose. The Schwann cells at the junction had undergone changes as well (9). Two types of transformation were noted: young-appearing junctions with pretzel-like structure changed into the fragmented, varicose phenotype. Moreover, junctions that were already fragmented underwent a further transformation into an even more



fragmented phenotype. Li et al. concluded that the junctions in the aging muscle underwent an apparently stochastic transformation from the young to the old phenotype. To confirm their results without vital imaging they made use of the change in BTX labeling. They labeled the sternomastoid muscle in a set of aging mice under brief anesthesia with one fluorescent color of BTX. They then sacrificed the mice at an interval following this labeling, dissected the sternomastoid and labeled it with a second fluorescent color of BTX. They found junctions that were strongly labeled with the second color that were weakly labeled with the first color and these junctions were found in a state of fragmentation.

What causes this fragmentation of junctions? A possible clue comes from the location of nuclei in muscle fibers. Muscle fibers are multinucleate cells and the nuclei in a mature fiber are normally located peripherally just beneath the sarcolemma. However, in aging muscle many fibers have been reported to have strings of nuclei located not at the periphery but near the center of the fiber. These “central nuclei” were noted in some of the earliest studies of aging (446, 886). They are believed to be signs of “segmental necrosis” (457) in muscles, where a damaged muscle fiber degenerates, is phagocytosed by invading immune cells. Satellite (stem) cells of the muscle become activated, undergo mitosis, and differentiate into myoblasts that then fuse together to regenerate the original muscle fiber inside its basal lamina sheath. As in developing muscle fibers, these nuclei in regenerated segments are located, at least initially, centrally. In some cases it was possible for Li et al. to perform static imaging after observing such fragmentation of a NMJ *in vivo*; they then imaged the same fiber *in vitro* after staining with a nuclear dye (DAPI). Strings of central nuclei were found in some cases, but such examinations are typically difficult. A massive addition of nuclei occurs in the vicinity of the NMJ in aged animals and this obscures the location of the nuclei in the fiber; it is often difficult to positively determine whether any observed string of nuclei are in the fiber on which the junction of interest is located. One would predict that *in vitro* observations of fibers on which fragmented NMJs were located would all contain such central nuclei. However, this is not the case in static images of these fibers, suggesting several possibilities: the presence of central nuclei is unrelated to junctional fragmentation, the presence of central nuclei is transient and the nuclei in such fibers eventually move to the periphery (the junctions encountered in any muscle would vary as to when in the past fragmentation occurred), central nuclei are present somewhere along the fiber but do not extend through the region containing the junction. Whatever the case, this study shows that the morphological change in NMJs during aging is a sudden event rather than a slow, gradual process.

### ***Damage to muscle fibers causes fragmentation of NMJs***

A variety of experimental approaches that damage muscle fibers produce an aging-like phenotype in their NMJs. As aging muscles are more prone to contraction-induced damage and experience such damage (cf. (109, 110, 277)), it is relevant to examine how muscle damage produces neuromuscular changes.

Deliberate damage to muscle fibers can produce changes in NMJs that mimic those in aging. Rich and Lichtman (754) conducted a study in which they deliberately damaged muscles in the sternomastoid muscle of mice by mechanical means and vitally imaged the effects on the NMJs

using 4-Di-2-Asp and BTX. They observed the degeneration of the underlying muscle fiber, the loss of most of the acetylcholine receptors, and the withdrawal of the nerve terminal from the muscle fiber. The nerve quickly returned and within 3 days, the junction had been dramatically transformed to have a new set of acetylcholine receptors that were in a fragmented configuration on the previously damaged fiber. Central nuclei were present, suggesting fiber regeneration. Rich and Lichtman (754) commented that the junctions had the appearance of aged junctions and concluded that the muscle fiber was necessary moment-to-moment to provide factors to maintain the nerve terminal. These results have been subsequently followed up by examining the consequences of postsynaptic injection of protein synthesis inhibitors (35, 620), showing that this manipulation can result in retraction of the nerve terminal. As another method of fiber damage, Van Mier and Lichtman (913) used a laser microbeam to ablate individual fibers by interrupting the sarcolemmal membrane outside of the NMJ. The result was the same as that in the case of Rich and Lichtman, only here by confining the damage to individual muscle fibers, they were able to detect a directional sprouting response from nearby undamaged NMJs.

Somewhat different results were obtained when Li and Thompson (565) utilized the Van Mier protocol to vitally image the consequences of ablating single muscle fibers in sternomastoid muscles of young mice with a laser microbeam. The laser microbeam was aimed *in vivo* at individual muscle fibers on which the NMJs were identified by labeling receptors with a non-blocking dose of rhodamine BTX. The microbeam was applied to the fiber on each side of the junction some 100-150 microns distant. The muscle fiber segment underneath the NMJ underwent necrosis. Indeed, within 1 day the site of the old muscle fiber was filled with phagocytic cells. The acetylcholine receptors originally labeled in the muscle fiber membrane became very dim within hours, suggesting these receptors had been mostly turned over during this interval, not surprising since the fiber itself was degenerating and being phagocytosed. However, beginning at least 3 days later new receptors could be identified by another application of BTX and a regenerating muscle fiber with sarcomeres could be identified underneath the old synaptic site. However, the nerve terminal during the period prior to the regeneration of the muscle fiber remained with all its branches, together with the associated Schwann cells and their processes (**Fig. 5**). These nerve terminals, likely attached to the basal lamina of the now absent muscle fiber, showed no signs of sprouting or terminal withdrawal, although the terminal area appeared to shrink, likely as a consequence of the collapse of portions of the basal lamina. When receptors began to reappear in the regenerating muscle fiber they were in a fragmented state and apposed by varicosities on old and new branches of the nerve terminal. When followed over months, the fragmented state remained and there was no sign the junction reverted to the pretzel configuration. These findings suggest that the damage produces an irreversible change in the configuration of the synapse. Moreover, the stimulus for new growth of the nerve terminal arises from regeneration of the muscle fiber, a conclusion also suggested by Van Meir and Lichtman, although in their case the growth was the formation of sprouts from adjoining muscle fibers. It is not clear what explains the difference between the findings of Van Mier and Lichtman and of Rich and Lichtman in the withdrawal of nerve terminals vs. their maintenance. One explanation might be the use of a mitochondrial dye rather than a fluorescent protein. It is possible the soluble fluorescent protein expressed transgenically in mice provides a more persistent labeling than the 4-Di-2-Asp or that the mitochondria labeled by this dye become redistributed or stain differently after loss of the muscle fiber. In this regard, it is interesting to make a comparison to the

frog. Here muscle fibers in the thin, flat cutaneous pectoris muscle can be removed without damaging the nerve terminals: the fibers are cut on each side of the endplate band and regeneration of these fibers prevented by irradiation (43, 797). Although a few of the terminals withdraw, most remain intact in contact with the basal lamina and do not sprout (27, 37, 247, 978). They even continue to release and recycle synaptic vesicles (19, 247) upon nerve stimulation, even at long periods after removal of muscle fibers. One change that is reported is that the terminals become enwrapped by the Schwann cells (37, 978).

Similar changes are observed in rodent muscle fibers in which muscle fibers are caused to degenerate by administration of myotoxic drugs (41, 42, 44, 178, 243, 431). Commonly these experiments use fractions of cobra venoms (“cardiotoxin”) that are purported to have rather selective actions on muscle fibers, causing their necrosis, but do no damage to nerves, nerve terminals or blood vessels. These toxins produce rapid loss of the muscle fiber (and loss of most acetylcholine receptors) but are reported to leave the nerve terminal intact. New acetylcholine receptors appear on the surface of the muscle fiber as it regenerates from satellite cells within the old basal lamina sheath. The nerve terminal contacts this fiber. The nerve contacts are formed by varicosities in close vicinity to where the synaptic site was originally located. The acetylcholine receptors on the regenerating fiber appear in a fragmented pattern (like that seen in **Fig. 6, 7** after laser damage to individual fibers). Central nuclei are present within the regenerating fibers (like that seen in laser damage in **Fig. 8**), indicating that the damaged myofiber has been replaced. Thus, the events that follow the administration of myotoxins appear to mirror rather exactly the events following mechanical or laser damage to muscle fibers (cf. (42, 178)).

One of the well-known ways to damage muscle fibers either *in vitro* or *in vivo* is to cause them to undergo so-called “eccentric” contractions (30, 108, 724). In these cases, the muscle, instead of shortening during contraction, becomes lengthened (stretched) due to the activity of antagonist muscles. Use of membrane impermeant dyes or proteins normally enriched in the extracellular space show penetration of these components into fibers damaged by eccentric contraction, likely due to tears in the sarcolemma as the forces exerted on the molecules linking the cytoskeleton of the muscle and its cell membrane to the extracellular matrix in the basal lamina of the fiber itself exceed the ability to resist the strain (278, 303, 851). These tears can result in segmental necrosis. Interestingly, a muscle’s susceptibility to damage from these types of contractions increases over the lifetime of the animal (30, 108, 110) and during certain disease states (see below). In both cases, it appears that the structure of the basal lamina and its linkage to the cytoskeleton of the muscle fiber become weakened. Some fibers unable to resist such eccentric damage even in young, normal animals consequently undergo fragmentation of receptors and the generation of central nuclei (564).

The primary example of purported eccentric damage to muscle fibers occurs in Duchenne muscular dystrophy (DMD). This disease results from mutations in the gene for the protein dystrophin, a large protein localized to the sarcolemma that links the actin cytoskeleton of the muscle fiber through the sarcolemmal membrane protein dystroglycan to proteins of the extracellular matrix. In addition, the dystrophin protein has several motifs that serve to localize important signaling molecules to the muscle membrane (e.g. nitric oxide synthetase) whose localization is defective in mutant fibers. This localization defect disturbs circulatory homeostatic reaction to active fibers, as synthesis and release

of nitric oxide normally stimulates the local dilation of blood vessels. Dystrophin-lacking myofibers are clearly more susceptible to damage from eccentric contractions. There are a number of other mutations to components of this “dystrophin glycoprotein complex” that increase the susceptibility of muscle fibers to contraction-induced damage (192). Taken together, all these cases of fiber injury point to the crucial role of linkages between the extracellular matrix and the muscle cytoskeleton in resisting mechanical damage to a muscle undergoing active contraction. Without these linkages, contractions generate increased strain on the sarcolemma (471, 744).

### ***Molecular mechanisms underlying changes to aging NMJs***

The precise molecular mechanisms that produce aging-associated fragmentation of NMJs remain poorly understood. One indication of the likely complexity of mechanisms here are experiments that show that the course of fragmentation can be profoundly influenced by exercise and by placing animals on a calorically restricted diet (cf. (908)). Moreover, a number of genetic manipulations result in structural changes like those in aging but occur without any evidence of overt fiber damage and regeneration. These results suggest the existence of additional mechanisms for aging of this structure. Several molecules involved in the formation and the maintenance of the NMJ, when absent, appear to produce pre- and/or post-synaptic alterations observed in aged muscles. Genetic deletion in adults by conditional knockout of the nerve supplied synaptogenic molecule agrin (114, 793) or its muscle receptor, Lrp4 (45, 52), results in dismantling of mature NMJs and features of the aged synapse. These include fragmentation of postsynaptic acetylcholine receptors, varicose motor axon terminals and in some cases partial or complete denervation of endplates. A similar change occurs after knockout of MuSK in the adult (391, 476). Further evidence of the importance of this pathway comes from results from the Burden laboratory (132), examining NMJs and synaptic transmission in mice overexpressing a mutant human superoxide dismutase transgene as a model of amyotrophic lateral sclerosis (ALS). In this model of ALS, components of the agrin signaling pathway were manipulated. Neural agrin binds Lrp4 which in turn activates a kinase in the muscle (MuSK) that has profound effects on synaptic differentiation. NMJs in this ALS model undergo denervation and reinnervation, both reported to occur in aging (145, 907). Burden et al. report that administration of an antibody engineered to activate the kinase MuSK, can ameliorate muscle denervation and improve synaptic transmission even after the advent of symptoms but has minimal effects on the life expectancy of the affected mice. Lastly, mice lacking ERK1/2 (134, 814) or having reduced trkB signaling (337, 495) generate NMJ fragmentation in the absence of central myonuclei, the usual criterion of muscle fiber damage and regeneration.

Precocious accumulation of aging -like NMJ morphologies are also seen in absence of synaptic basal lamina components laminin  $\alpha 4$  (114, 793) and type IV collagen  $\alpha 5$  (53, 300). These are required for expression of both synaptic collagen IV trimers,  $(\alpha 3)(\alpha 4)(\alpha 5)$  and  $(\alpha 5)_2(\alpha 6)$  – where the initial formation and maturation occur normally. The absence of synaptic collagen IV trimers did not lead to a general disruption of muscle basal lamina or muscle fiber integrity as central myonuclei were rarely observed (53, 300). The muscle basal lamina and muscle fiber likely remain intact also for deletions of laminin  $\alpha 4$ , but this is presently unconfirmed.

Skeletal muscle fibers possess special mechanisms to ensure that proteins required to form and maintain a functioning synapse are synthesized by the ribosomes and nuclei located at the site of the synaptic contact. There are several muscle nuclei localized just postsynaptically beneath the synapse (the so-called sole plate nuclei) that aggregate here and carry out the transcription of so-called “synapse specific” genes (cf. (796, 828)). Among these genes selectively transcribed are those for acetylcholine receptors and the scaffolding proteins that allow the aggregation of these receptors at high density in the postsynaptic membrane. The molecular signaling allowing for this selective gene expression are largely known (390, 880) as are some of the mechanisms for the nuclear aggregation (267, 346). Genetic inactivation of Erm, a transcription factor that promotes gene expression by sole plate nuclei, also produces the fragmented postsynaptic appearance (395) and further implicates modulated expression of genes involved in NMJ formation in its aging-associated remodeling. An interesting recent paper by Liu et al. (578) reports a failure of this nuclear aggregation at the NMJ in aging muscles. The number of sole plate nuclei is drastically reduced and this reduction is mirrored by a decrease in the density of postsynaptic acetylcholine receptors, suggesting that aging muscles have a reduced ability to maintain the specialized synaptic contact. Several questions remain unanswered. Are the sole plate nuclei at aged synapses lost or is there some kind of failure in their maintained localization? Is the nuclear loss a general one occurring throughout the muscle fiber, or is it selective to the synapse? Aging adult muscle fibers following rounds of degeneration and reinnervation appear to be hypernucleated (Ryan Massopust, personal communication). However, Liu et al. (578) present evidence of some failure of generation of muscle stem cells in aging muscle to generate cells to renew or regenerate lost synaptic nuclei. Because the sole plate nuclei located underneath the synapse are specialized transcriptionally for the production of synaptic proteins, such a reduction in nuclei could produce alterations in synaptic proteins necessary for the maintenance of aged synapses. However, what causes the loss of the sole plate nuclei in these cases is unclear, as is also the role of muscle fiber damage. The purported failure of satellite cell generation is a controversial topic in aging and dystrophic muscle (7, 77, 169, 198, 327, 480, 598, 680, 810, 926).

Recent study also implicates glial involvement in producing aging-associated morphology. A transgenic increase in neuregulin1 expression by motor axons resulted in fragmentation of acetylcholine receptor aggregates and varicose appearance of presynaptic nerve terminal branches in every muscle in the animal (110, 549). This phenotype was a consequence of enhanced activity of terminal Schwann cells and occurred in absence of any muscle fiber damage. Moreover, recent evidence suggests that, in addition to neuregulin1, a set of molecules collectively known as “damage-associated molecular patterns” (DAMPs) or “alarmins” – released as a result of cellular damage or disease (50, 109, 155, 602, 878), can activate SCs (75, 136, 331, 902). Terminal SCs, therefore, may be recruited under various conditions (disease, muscle fiber damage, denervation, inflammation, etc) to produce synaptic fragmentation.

Absence of yet another synaptic basal lamina component, ColQ, leads to a fragmented postsynaptic morphology (282). However, unlike the molecules above, lack of ColQ may lead to synaptic remodeling as a consequence of muscle fiber necrosis (and regeneration) specifically at synaptic segments. This local muscle fiber damage likely results from the lack of acetylcholinesterase (282), whose acute inhibition results in such local muscle fiber degeneration (541).

Still another promising approach to understand the changes that occur at the NMJ in aging has been undertaken by Nishimune et al. (669). They have employed high resolution fluorescence microscopy of NMJs following immunolabeling of the molecule bassoon, a known component of the active zones where neurotransmitter is released from nerve terminals. Examination of the nerve terminals in mice has revealed a distribution of puncta of bassoon immunoreactivity in the synaptic membranes of nerve terminals that likely reflects the distribution of active zones in these terminals. They report a decrease in the density of these puncta in aging animals, suggesting a mechanism that could explain any diminution in neurotransmission that occurs at aging junctions.

### ***Physiological changes in synaptic transmission at aging junctions***

The physiological consequences of fragmentation are far more ambiguous. Careful studies of neuromuscular transmission at fragmented junctions have shown that, at least in the muscles studied, there is no change in the size of the mepps or in the quantal content of the endplate potentials, even when junctions known to be fragmented are studied physiologically, whether these junctions become fragmented in aging or in muscular dystrophy (243, 593, 947). There are several limitations to the interpretations possible from these studies, including the particular muscle(s) studied, the animal that was the source of the muscle, and the age of the animal. Most studies claim that the fragmentation in mice becomes most dramatic after around 20 months of age, even though some fragmented junctions can be found even in very young mice (9, 198). Most studies that claim to see a physiological difference have been conducted at advanced age. Another problem with these studies is that, in many cases, the evoked responses are studied only in response to single stimuli rather than the spike frequencies that occur *in situ*. That is, it is possible that there is a problem in transmission at fragmented junctions that occurs only upon repetitive stimulation, e.g. in the fatigue in transmission. Nonetheless, one can also point to a simple disease model in mice (mdx-the mouse model of Duchenne) where there is quite profound fragmentation of the receptors at NMJs without an obvious deficiency in synaptic transmission (593). Indeed, the mdx mouse is renowned for the lack of obvious behavioral deficits despite the rather extreme fragmentation of junctions that occurs early in the life of the animal (for example, after ca. 9 weeks of age) as a consequence of its muscle pathology.

So, is it possible to reconcile the obvious defects in muscle mass and contractile performance during advanced aging with the apparent effectiveness of NMJs when they are evaluated physiologically? Something is clearly hidden in the studies discussed to this point. One possibility is that atrophy and disappearance/necrosis of denervated fibers is rapid and they are not sampled in the various physiological assays. Another obvious possibility is that the defects in neuromuscular performance occur not at the level of individual synapses, but in the contractile performance of the fibers or alternatively in the way the synapses are utilized within the muscle. Compared to evaluating individual junctions, the evaluation of the ensemble of synapses made by individual motoneurons (i.e. motor units) is much more difficult. By various techniques, most commonly teasing filaments from ventral roots after laminectomy cf. (282, 487) it is possible to isolate bundles of axons that, if teased sufficiently small, contain among the several axons present, just a single axon innervating the muscle of interest. It is then possible to evaluate the contractile performance of that single motor

unit by monitoring the contractions of its unit fibers by tension recording. Moreover, it is possible to employ techniques that mark the utilization of metabolic energy by the activated fibers. Most prominent among these methods is the technique of glycogen depletion: activation of the fibers in the unit by stimulation of the motor axon can, under repeated stimulation in which efforts are made to ensure that neuromuscular failure does not occur, forces the active fibers to utilize their stores of glycogen. After staining muscle sections for glycogen, fibers activated during this procedure can be identified. What makes this procedure very tedious is that the depletion is usually conducted *in vivo* after extensive surgery and attendant difficulties of maintaining a living animal, there is just one unit identified in each experiment, and in slow-twitch motor units good glycogen depletion usually requires the imposition of anoxia during the stimulation. Despite these problems, the results obtained define some new major issues that would be missed by examining either the physiology of individual motor units or the morphology of individual junctions, even if these experiments are carried out with the most sensitive vital imaging techniques. Clearly, with the new technologies now available, including use of activity sensitive dyes (316, 390), optogenetics (303, 888), retrograde labeling of innervating axons with lipid soluble dyes injected at junctional sites (283, 291, 316, 317), the labeling of subsets of axons by transgenic expression of fluorescent proteins (283), and the tracing of individual labeled axons as they extend through the muscle by use of high resolution microscopy (299, 300, 396, 590) this situation will certainly change in the near future.

Glycogen depletion experiments have been performed on single motor units in individual muscles and the results compared between young and old animals. By far the most extensive of these experiments are those conducted in rats by Edstrom, Larsson, and colleagues (246, 247, 259, 520, 523, 524). Several profound conclusions can be made collectively from these experiments. First, there is clear evidence for a rearrangement of the distribution of fibers within motor units. The unit fibers, especially in the soleus muscle, that are normally spread in a random, scattered distribution in the muscle occupy an enlarged portion of muscle section, although very seldomly are fibers in a single unit adjacent to each other. This shows that there is a remodeling of the muscle innervation, but this remodeling differs from that seen after nerve damage leading to wholesale muscle denervation (or partial denervation) followed by reinnervation, in which case large, contiguous areas composed of adjacent fibers in the muscle can be innervated by single units (275, 487). The situation in old age could be explained by more moderate, gradual loss of innervation of individual fibers in a unit followed by sprouting of the axon losing the synapse or by neighboring motor axons (cf.(839). The conduits of the nerve basal lamina sheaths remaining behind would encourage such sprouting within the intramuscular nerves or even by terminal sprouts that enter these conduits and grow retrogradely along the pathway of the lost axon (Kang, Tian, and Thompson, unpublished observations). Such axons entering the previous endplate site might be indistinguishable from the normal innervation pattern (i.e. absent the sprouts from adjacent junctions in the muscle) or certainly the reformation of synapses upon regenerating fibers as argued above. Such events might explain the apparent absence of major sprouting in aging muscle. One might not expect to encounter such a change in the identity of individual motoneurons innervating individual fibers in aging muscle in vital imaging experiments in which the frequency of such events is low and the speed with which reinnervation of a fiber lacking innervation is rapid.

A second revelation of the glycogen depletion experiments is that the number of motor units is decreased (in some rats by as much as 25% at 20-25 months of age). Although there is a loss of muscle fibers, the average size of the remaining units is increased. This suggests there is a slow, gradual loss of motoneurons to the muscle, an observation supported by several other observations, including counts of axons in ventral roots and motoneurons in the ventral horn. The cause of this loss is not presently clear, but one idea that has received attention is that motoneurons, in face of continued, gradual loss of synapses are induced to sprout and this sprouting expands motor units and places additional stress on them for maintenance of an enlarged peripheral arbor. Indeed, it is very clear that even young motoneurons have a limit in their ability to expand their peripheral arbors (877) and that this ability is reduced in aged animals (705, 771). If the number of motoneurons is being reduced and the arbors of remaining motoneurons are expanding, any deficit in the ability of motoneurons to reinnervate the denervated fibers could lead to the atrophy of these fibers and their loss from the muscle (see above for further information)

A third revelation of the glycogen depletion experiments is that the motor unit fibers undergo a transformation in the differentiation of the contractile properties of their fibers. Evidence suggests that this transformation occurs unit by unit, likely due to changes in the pattern of activation of the fibers in the unit. As a motor axon comes to innervate additional fibers (or experiences a change in its pattern of activation), it is very clear that this axon can change the differentiation of the fibers that it innervates. This transformation is particularly prominent in muscles where the muscle fibers in individual muscle units shift away from fast a slower type (259, 521).

Taken together, these observations suggest that even if individual synapses apparently function well, the ability of the muscle to carry out different motor tasks is altered by a loss of motor units (which would be expected to decrease the fineness of motor control), a loss of muscle fibers (which would decrease the total strength of the muscle), and a change in fiber type within muscles (which would change their contraction kinetics and their ability to resist fatigue). What is really needed is a careful evaluation of how an individual muscle changes its performance and whether these changes can be accounted for by the changes that occur throughout the complement of units within the muscle.

## **Muscle Cell**

Pathological changes at the motoneurone level preferentially affecting the largest motoneurons included in fast-twitch motor units, resulting in peripheral denervation and incomplete reinnervation by motoneurons included in slow-twitch motor units have been forwarded as the dominant mechanisms underlying the muscle wasting and loss of muscle function in old age (137, 265, 376, 420, 444, 709). A fast-to-slow myosin isoform (fiber type) switching was originally reported four decades ago in sedentary men ranging in age between 22 and 65 years (539) and repeatedly documented within the a same age-range as well as among older individuals (377, 415, 423, 644, 801, 825). This is in accordance with the aging-related decreases in fast MyHC mRNA levels in human limb muscles, while the slow MyHC mRNA levels were unaffected by aging (40, 102, 825, 928). Others, have not confirmed this fast-to-slow conversion during aging in humans (190, 423, 469, 644). This discrepancy may be secondary to the influence of differences in physical activity levels interfering with aging-related effects as previously discussed (130, 514, 515, 520). It is



therefore of specific interest to note that in the sternocleidomastoid muscle, a muscle which is considered to be less affected by aging-related changes in physical activity, a similar aging-related fast-to-slow MyHC isoform transition was recently reported as in human limb muscles (584). In experimental animal models, where secondary influences from environmental factors not related to the aging-process *per se* are more easily controlled, an aging-related fast-to-slow myosin isoform transition is more widely accepted (23, 25, 56, 85, 126, 258, 265, 444, 463, 482, 519, 521-523, 540, 586, 709, 855, 865). The fast-to-slow fiber type transition during aging may contribute to the slowing of movement as well as decline in maximum force since fast muscle fibers are considered to have a higher force generation capacity than slow fibers. However, it is important to emphasize that the relative changes in MyHC/fiber type proportions during aging in humans are relatively small (see (520)). Besides the aging-related motor unit loss, fast-to-slow muscle fiber shift, and fiber atrophy that accompany the progressive motoneurone loss, other intrinsic qualitative changes at the muscle fiber level as well as the loss of the coordinated expression of muscle proteins in old age may have a significant impact on overall muscle function. For instance, the coordinated expression of contractile vs. SR proteins, contractile vs. mitochondrial proteins and myosin heavy vs. light chain isoform expression observed at the single muscle fiber level in humans and in experimental animal models are at least partly lost in old age (520, 563, 791) and these changes have most probably far more reaching consequences for regulation of muscle contraction and muscle function in old age than aging-related small perturbations in fiber type/MyHC isoform expression.

### ***Nuclear organization***

Skeletal muscle fibers are the largest cells in the body. They may be several centimeters long and have a large cytoplasm. In order to support this large cytoplasm, muscle fibers are truly multinucleated cells and contain hundreds to thousands of nuclei (myonuclei). Myonuclei are post-mitotically fixed and typically located in the periphery of the muscle fiber, just beneath the plasmalemma, but central localization may be observed during transitional or pathological conditions. Myonuclei are evenly dispersed along the length of the fiber except for the neuromuscular junction and myotendinous ends, where nuclei are clustered and have specialized functions (185, 648). The even distribution of myonuclei along the major part of the fiber has been speculated to be the result of myonuclei repelling each other to minimize transport distances (120, 121) or the attractive interactions between microvasculature surrounding muscle fibers (especially in slow-twitch fibers) together with the repelling interactions between myonuclei and the intermediate filament desmin (739). Each myonucleus regulates the gene products in a defined cytoplasmic volume known as the myonuclear domain (MND) or DNA unit (12, 13, 150, 364, 507, 699). Myonuclei are not transcriptionally active simultaneously and regulation of transcription occurs in a pulsatile manner (665), but it remains elusive how transcriptional activities are coordinated between individual myonuclei.

The MND size is critical for the function and adaptability of the muscle cell. MND size may change in response to muscle hypertrophy/atrophy and aging (120, 121), and it is affected by various factors such as muscle fiber type (MyHC isoform) (12, 450, 778, 895), mitochondrial activity (576), and species (body size) (576). Slow-twitch oxidative muscle fibers have smaller MNDs than glycolytic fibers coupled to their higher protein synthesis and degradation rates than fast-twitch fibers (253, 553, 675, 917). Further, there is a tight coupling between muscle hypertrophy and the addition of

new myonuclei via satellite cell activation to maintain a similar MND size during muscle hypertrophy (119-121, 778). However, satellite cell activation and myonuclear addition may lag behind the initial phase of hypertrophy coupled to an increased transcriptional and translational activity (153). Single muscle fiber *in vivo* analyses of myonuclear organization show that myonuclei are not lost in proportion to atrophy after denervation, resulting in smaller MND size in response to atrophy which may serve as a “muscle memory” when later exposed to e.g. a hypertrophic stimulus (119). Further, there are significant differences in MND size between different mammalian species and a significant difference in MND size has been shown in single muscle fibers in six different mammalian species with a 100,000-fold difference in body mass (**Fig. 9**), presumably reflecting the lower metabolic demand and lower protein turnover rate in large vs. small mammals (576).

Conflicting results have been presented in the literature regarding the effects of how different muscle atrophy conditions, including sarcopenia, impact on myonuclei organization and MND size (119-121, 912). Aging has been reported to be associated with an increased (106, 441, 912) or unchanged (314, 605, 730, 856) MND size in both rats and humans. These differences may, at least in part, be related to methodological differences and inherent methodological limitations. Estimates of MND size during aging have frequently been based on measurements from single muscle cross-sections, *i.e.*, counting the number of nuclei surrounding each fiber and assuming a constant shape and size of myonuclei as well as the exclusion of non-muscle fiber nuclei. However, myonuclei have different shapes and in human muscle the shape of myonuclei is affected by aging, *i.e.*, myonuclei frequently present deviations from the rounded or elliptical appearances observed among young individuals with elongations and indentations of the nuclear envelope in old age adding a systematic error when calculating aging-related changes MND size based on measurements from muscle cross-sections assuming constant myonuclei size and length (180). Further, the distinction between myonuclei and the numerous other nuclei located between muscle fibers is not trivial (95, 182, 576). To overcome these limitations, myonuclear organization has been investigated in 3D along the length of individual muscle fibers and this review will focus on 3D measurements of MND size and how it is affected by aging. Bruusgaard and co-workers (120) introduced a novel *in vivo* method to visualize myonuclei in intact muscle fibers from mice by microinjection of labeled DNA into single muscle fibers allowing longitudinal studies of myonuclear organization of myonuclei in single living cells and excluding satellite and non-muscle myonuclei. By using this model in young (2 mo.), middle-aged (14 mo.) and old (23 mo.) mice, the effects of aging on myonuclear organization was studied in muscle fibers from the slow-twitch soleus and the fast-twitch extensor digitorum longus (EDL) muscles (121). Significant aging-related changes were observed, such as an increased number of fragmented myonuclei, a less regular myonuclear shape, a decreased number of myonuclei in parallel with the preferential atrophy of fast-twitch muscle fibers as well as an impaired orderly distribution of nuclei in old age (121). The altered spatial organization of myonuclei in old age was suggested to be secondary to parallel changes in the microtubule network (121). Thus, these 3D *in vivo* analyses add significant novel information on aging-related changes in myonuclear morphology and organization which may have a significant impact on skeletal muscle function and adaptability. However, as discussed above, the mouse is not an ideal species for the study of human aging. In order to bypass experimental models, a 3D method combining confocal imaging and specific digital algorithms was introduced to determine the morphology and spatial organization of myonuclei along the length of individual single human membrane permeabilized muscle fiber

segments characterized according to muscle fiber size and MyHC isoform expression (180). In short, 3D confocal reconstructions of single human membrane permeabilized muscle fiber segments were performed after attaching both ends to 3-way positioners and setting optimal sarcomere length for force production. The spatial distribution of nuclei and  $x,y,z$  coordinates were determined and a custom made software calculated the cytoplasmic volume and size of MNDs corresponding to each myonucleus (**Fig. 10**) (180). In contrast to previous observations in rodents (120), myonuclear shape was not dependent on muscle fiber type, *i.e.*, round or elliptical nuclei were observed in the same fiber independent on fiber type, age and gender. In old age, deviations from the rounded or elliptical shapes were frequently observed with indentations of the nuclear envelope as well as myonuclei aggregated in groove-like structures along the length of the fiber irrespective gender and fiber type. Analyses of 3D nearest neighbor distances in ~15,000 myonuclei using a custom made software confirmed aging-related aggregation of myonuclei (180) in accordance with previous observations in humans (95) and mice (121). This is consistent with a dynamic positioning of myonuclei in adult muscle (739). Aggregation of myonuclei has been reported in response to denervation (946) and the well-established long-term denervation reinnervation process during aging in both rodents and humans (520) may accordingly be an important factor triggering myonuclear aggregation in old age (180).

In muscle fibers expressing the IIa MyHC isoform, a small but significant decline in MND size was observed in old age. On the other hand, average MND size was not significantly affected by age in muscle fibers expressing the type I MyHC isoform, but there was an increased variation in MND size in old age independent of gender (**Fig. 11, 12**). The lower MND size in muscle fibers expressing the IIa MyHC isoform may represent a physiological adaptation to the preferential type II muscle fiber atrophy observed in old age (539, 801, 886), but it is unlikely that the increased variability in MND size in type I fibers was secondary to a denervation-reinnervation process since this process is not restricted to slow twitch motor units (520). The increased MND size variability in old age due to an altered spatial arrangement and aggregation of myonuclei may represent an important aging-related biological process (180) and it is consistent with an increased MND size variability in old mice (95). In old mouse muscle, the increased MND size variability appeared to be related to a loss of satellite cells and hindering of efficient nuclear replacement (95). The increased complexity of the spatial organization of myonuclei organization may impact on the efficiency of transcriptional activity with consequences for local protein synthesis and degradation along the length of the muscle fiber. In this context, it is of interest to note that accumulation of dysfunctional mitochondria and oxidative stress occur locally along the length of the muscle fiber and not homogeneously in the muscle fiber (682, 918). Regions along the length of the muscle fiber characterized by large MND size may accordingly have a deficient protein turnover resulting in a lower concentration of contractile proteins with a negative effect on force generation capacity (183, 190, 529, 875) and/or post-translational modifications of proteins with a slow turnover such as contractile proteins in muscle fibers expressing the type I MyHC isoform (405, 529, 563, 740). Further, there appears to be a critical volume individual myonuclei can support to efficiently maintain function (732) and local areas within individual muscle fibers from old individuals may have exceeded this volume with negative consequences for muscle function.

### ***Mitochondrial function, oxidative stress, heat shock proteins, post-translational protein modifications***

Mitochondria are small intracellular organelles that play a crucial role in the life and death of cells. They produce most of cellular ATP through oxidative phosphorylation (OXPHOS), but are also essential for thermogenesis, for the control of the homeostasis of calcium, one of the main intracellular second messengers, and are the key effectors of the intrinsic pathway of apoptosis, the cellular pathway that leads to programmed cell death. All these functions are interconnected and are tightly regulated (129). Moreover, mitochondria host several other biochemical pathways such as the beta oxidation of lipids, portions of the urea cycle and several enzymes involved in amino acid metabolism.

Mitochondria derive from ancient Gram-negative bacteria, similar to modern-day Rickettsiae, which started an endosymbiotic process with the progenitor of the eukaryotic cells about two billion years ago (20). Mitochondria still retain many features of their bacterial progenitor. They are enveloped by two distinct membranes: a permeable outer membrane and a highly impermeable inner membrane (MIM). The MIM presents several inflexions named cristae, which expand the total area of the MIM, thereby enhancing its ability to produce ATP (266).

### Mitochondrial genetics

Mitochondria have their own genome (mitochondrial DNA – mtDNA), which is distinct from the nuclear genome (nDNA) and is similar to bacterial chromosomes. In fact, mtDNA is circular, it is not organized into chromatin, genes are not interrupted by introns (and non-coding sequences comprise less than 5% of the genome) and is present in multiple copies per mitochondrion. The mitochondrial genetic code is also different from the universal code, therefore mitochondria have their own translation apparatus. These peculiar characteristics of mtDNA account for the unique laws of mitochondrial genetics, which are different from those of classical mendelian genetics and resemble more closely the laws of population genetics (234). First, mtDNA is transmitted only from the mother (paternal DNA is actively eliminated from the Zygote). Second, the presence of multiple genomes per mitochondria (a single muscle fiber can contain several hundreds of thousands copies of mtDNA) implies that whenever a mutation is present, wild type and mutant mtDNA may coexist at variable proportions, within individual mitochondria in a given cell, in distinct cells within a tissue, or in different tissues within the same patient. This phenomenon is called heteroplasmy. Strictly linked to heteroplasmy is the threshold effect. In fact, mtDNA mutations must affect a critical proportion of the mtDNA molecules within a cell in order to cause a biochemical phenotype. This threshold is in the range of 70-95%. In other words, the majority of mtDNA molecules in a cell must carry the mutation for the biochemical defect to become evident.

Last, the proportion of mutant versus wild type mtDNA may drift with cell divisions due to the random segregation of affected genomes at mitosis. In post-zygotic cells, selective replication of individual mitochondria with high mutational load may also change the proportion of mutant to wild type genomes, favoring the accumulation of defective mtDNA in individual cells (see below).

### The respiratory chain

mtDNA encodes a total of 37 genes: the 2 ribosomal RNAs and 22 tRNA required for mitochondrial protein synthesis, and 13 structural genes, all of which are subunits of the respiratory chain. The respiratory chain (RC) is comprised of five multisubunit enzymatic complexes (C.I-C.V) and of two

electron carriers, coenzyme Q (CoQ) and cytochrome *c*. All complexes (except for C.II which contains only nDNA-encoded subunits) include both mtDNA and nDNA-encoded subunits. Moreover, nDNA provides the genes required for mtDNA maintenance and replication, the proteins involved in mitochondrial transcription and translation, and those required for the assembly of RC complexes and CoQ biosynthesis. Biogenesis of the RC requires a tightly regulated interplay between the two genomes and OXPHOS defects may derive from dysfunction of any of the two.

The first four complexes of the RC (C.I-C.IV) catalyse the transfer of electrons from the high energy compounds (NADH, FADH<sub>2</sub>) generated by the reactions of the Kerbs cycle to oxygen. The energy liberated is used to translocate protons across the MIM generating the electrochemical gradient which is used by C.V to synthesise ATP (266).

RC complexes are not isolated structures, but are physically associated to form huge macromolecular structures termed “supercomplexes”. Supercomplexes continuously form and disassemble and are required for the optimal respiratory efficiency of the cells (4).

#### RC dysfunction, mtDNA mutations, ROS, and aging

Because their central role in cellular metabolism, especially in skeletal muscle, mitochondria have been considered key players in the aging process for more than four decades (372). In fact the RC is one of the main sources of ROS in cells and mtDNA is particularly susceptible to ROS damage due to the lack of histones and the relatively simple DNA repair system. The traditional mitochondrial free radical theory of aging postulates that ROS produced by the RC cause damage to mtDNA, resulting in mutations which progressively impair OXPHOS. This creates a vicious cycle, with more ROS produced by the dysfunctional RC, which result in further mtDNA damage (**Fig. 13**) (49).

However, in the last fifteen years it has become evident that the situation is far more complex and the relationships between ROS, mitochondrial dysfunction, aging, and sarcopenia are not as simple as previously thought (921). Furthermore, a key issue regarding the role of mitochondria in aging and sarcopenia is whether the observed mitochondrial alterations are primary events or are secondary to other extramitochondrial abnormalities.

A large series of studies have evaluated the changes in mitochondrial bioenergetics during aging in different models. Several approaches have been used from the (relatively) simple spectrophotometric assessment of RC enzymatic activities on muscle homogenates (54, 56, 224, 362, 408, 556, 592, 689), or polarographic studies on freshly isolated mitochondria (81, 133, 144, 342, 893), to the more complex *in vivo* spectroscopic measurements (170, 458, 509, 622). Results however are conflicting. Several factors have been invoked to explain the discrepancies in the different studies: technical issues (715, 846), confounding factors, like exercise or immobility, or, for example, altered blood flow in the case of *in vivo* spectroscopic studies (722), the parameters used for normalization of data (386), and species-specific features that make the situation in rodents different from that of humans (344). Even the type of muscle studied (fast vs. slow twitch) appear to be differently affected and the specific mitochondrial subpopulations (*i.e.*, the subsarcolemmal and intermyofibrillar mitochondria) may play different roles in the processes triggering muscle atrophy (544), adding more confusion to an already complex issue.

A good example of how methodological details may profoundly affect results is provided by the observation that the mitochondrial isolation procedure may exaggerate the impact of aging. When confronting mechanically separated mitochondria and saponin-permeabilized myofibers, Picard and coworkers showed that several parameters (maximal respiratory capacity, ROS production, RC activities) were significantly altered when determined in isolated mitochondria, but were normal or nearly normal in permeabilized fibers. The authors suggested that this may reflect the fact that aged mitochondria are more rigid, and reseal less efficiently during mechanical isolation, resulting in loss of mitochondrial matrix constituents, in contamination with cytoplasmic material, and in altered coupling (714). Moreover, some of the protocols used in the past to assay RC complexes activities, especially C.I and C.III, are questionable (846), and there is still no widely accepted method to reliably assay C.V spectrophotometrically (55).

Several studies have investigated the mtDNA alterations associated with aging. It should be noted that, rather than point mutations, skeletal muscle mtDNA accumulates large-scale rearrangements (deletions (451), but also duplications and more complex alterations (869) of the mitochondrial genome) during aging. The exact mechanisms generating these rearrangements are not completely understood but they involve polymerase slippage (59), homologous recombination or slip replication, as it has been hypothesized for the common 4.9 kb deletion which is mediated by a 13 base-pair direct repeat (808, 823), double strand breaks or inefficient repair mechanisms (122, 631). The abnormal genomes then begin to accumulate in mitochondria by a process termed clonal expansion and the affected fibers become filled with dysfunctional mitochondria. These fibers appear as ragged-red fibers (RRF) on Gomori trichrome stain or ragged blue fibers on succinate dehydrogenase stain (SDH) (811). During aging, muscle becomes a mosaic of normal fibers, and of respiratory deficient fibers harboring very high levels of mutant mitochondrial genomes (>90% of total) (656). However only a minority of fibers appear to be respiratory deficient; this proportion varies in different studies from 5% to 14% (in most severely affected muscles) (100, 122, 785), hence the real impact of these alterations on muscle function is questionable. Moreover, these fibers do not seem to be smaller than those with normal mitochondria (775), therefore their contribution to atrophy is questionable. Finally, mtDNA deletions would cause reduced production of otherwise normal mtDNA-encoded RC subunits that would still function properly.

The issue of mtDNA point mutations is even more controversial. Disease-causing mtDNA mutations have been reported in aging skeletal muscle (440, 579, 654, 990), but the validity of these findings has been questioned (655, 690). Moreover, these mutations were mostly found at low levels (less than 2%), far below their pathogenic threshold. Although some point mutations may behave in a dominant fashion, where as little as 5% of mutant mtDNA in a cell is sufficient to impair OXPHOS (786), they appear to be rare, and have never been reported in aging muscle.

A mouse model harboring a mitochondrial DNA polymerase with a faulty proofreading activity (the “mutator” mouse) displays a marked increase in the rate of mtDNA point mutations with premature aging and muscle atrophy (892). The muscle phenotype of these mice is however different from that of normal aged mice. In fact mutator mice display reduced RC enzyme activities, but a negligible increase in oxidative stress (494, 891), and increased mitochondrial fission and autophagy, whereas normal aged mice have normal RC enzyme activities, increased mitochondrial fusion, and decreased autophagy (640).

Moreover, patients with somatic mutations affecting the MT-CYB gene (encoding a component of RC complex III) in skeletal muscle display markedly reduced complex III activity, exercise intolerance, and myoglobinuria, with no sign of atrophy or sarcopenia (21).

More recently, attention has shifted from mtDNA point mutations to mtDNA copy number. An elegant study has evaluated mtDNA integrity and function in three generations of women (grandmothers, mothers and daughters) from 18 families. No significant increase in mtDNA mutational load in the coding region was detected, but rather a progressive decline in mtDNA copy number which paralleled the decrease in mitochondrial function (382). The decline in muscle mtDNA copy number associated with aging has been reported by several groups in humans (510, 764, 824, 903), and in an equine model (557). Two studies also detected accumulation of mutations in the non-coding control region of mtDNA (382, 903) especially at position 408; however, there is no conclusive proof that these changes are affecting mtDNA replication. Increased oxidative damage has also been invoked to explain the reduction of mtDNA copy number (824), however this explanation seems unlikely since there was a reduction also of nDNA encoded enzymes such as citrate synthase, which is usually elevated in case of mtDNA depletion syndrome (MDS) (603).

The concept of threshold applies also to mtDNA depletion, even though much less data are available than what is known about point mutations. Therefore copy number must fall below a certain level for the pathological phenotype to develop. This issue is critical to determine whether the reduction in mtDNA copy number is a primary event or if it reflects a more general impairment of mitochondrial biogenesis (see below). A study analyzing single muscle fibers of patients with the myopathic form of MDS, showed that fibers develop COX deficiency when their mtDNA content is less than 10% of that of controls (249). Moreover patients treated with antiretrovirals often display reduction in mtDNA copy number in muscle, sometimes with a partial reduction also of complex IV, but are clinically asymptomatic, and have normal oxygen consumption (584). Finally, conditions of severe neurogenic atrophy, as seen in patients with spinal muscular atrophy, may provoke a profound reduction in mtDNA copy number in muscle (70).

Another component of the RC, ubiquinone (coenzyme Q –CoQ) deserves a special mention. It is an electron shuttle from RC complexes I and II, to complex III, it is one of the principal cellular antioxidants, and is a modulator of the mitochondrial permeability transition pore, therefore controlling apoptosis (5). CoQ is also a cofactor of several other mitochondrial dehydrogenases, among which di-hydroorotate dehydrogenase (DHODH) and electron-transfer flavoprotein dehydrogenase (ETFHDH), and of uncoupling proteins. CoQ deficiency is associated with decreased ATP production, increased ROS, abnormal lipid metabolism, and altered pyrimidine production (227, 583, 736, 890) (**Fig. 15**). Given the central role of CoQ in mitochondrial metabolism and homeostasis and the observation that muscle CoQ levels tend to decrease with aging (542), the impairment of CoQ biosynthesis has been considered an important player in the aging process. Consequently, CoQ administration has been proposed as a simple measure to counteract the aging process. Recent data however questioned this view and animal models (from nematodes to mice) with partial defects of CoQ biosynthesis actually display a longer lifespan and reduced markers of aging despite an increase in ROS and a decrease of ATP production (921). Mice in which the *Coq7* gene (encoding a hydroxylase essential for CoQ biosynthesis) was conditionally ablated at 2 months of age rapidly develop a multisystem disorder leading to death at age 9 months. However, if these

animals are treated immediately before death with 2,4-dihydroxybenzoate, which can bypass the *Coq7* defect and restore CoQ biosynthesis (972), they recover virtually all symptoms and their lifespan is normal (922).

Patients with mutations in genes involved in CoQ biosynthesis and low CoQ levels in skeletal muscle do not develop atrophy or sarcopenia (unless as a consequence of the severe encephalopathy which can be present in these cases) (226, 890). This holds true also for most other forms of mitochondrial encephalomyopathies.

A further proof of the controversial role of ROS comes from mice lacking one copy of *Sod2*, a mitochondrial matrix superoxide dismutase which is essential for protection against ROS. These animals display increased oxidative stress, decreased RC enzyme activities, and increased oxidative damage to both mtDNA and nDNA, but appear phenotypically normal and do not show premature aging (915).

Overall, these observations question the classical mitochondrial theory of aging and their role of ROS as the primary determinants of the aging process in muscle. Nevertheless, ROS may still play an important role in the process, and even if there is no hard evidence showing increased ROS emission with age, reduced endogenous anti-oxidant buffers and increased abdominal fat in older people can cause oxidative stress.

#### Mitochondrial dynamics and mitophagy

During the past 15 years other aspects of mitochondrial function have emerged that are not directly involved in OXPHOS, but have a crucial role in the functionality of these organelles.

Mitochondria continuously undergo a reshaping process with fusion and fission events which are controlled by a specific set of mitochondria-shaping proteins among which are OPA1, Mitofusin 1 and 2 (which promote fusion), DRP1 and FIS1 (promoting fission) (813) (**Fig. 15**).

All these aspects are tightly connected; in fact, cristae morphology modulates the organization and function of the OXPHOS system, with a direct impact on cellular metabolism (166). OPA1 controls RC supercomplex assembly, increasing the efficiency of respiration (167). This property has been exploited to ameliorate the bioenergetics defect of mouse models with C.IV deficiency (160). Cristae shape is also crucial for controlling apoptosis (159, 302).

Interestingly OPA1 is essential also for maintenance of mtDNA. Yeast lacking the OPA1 orthologue *MGM1* lose their mitochondrial genome (432), and patients harboring dominant-negative OPA1 mutations accumulate deleted mtDNA genomes in skeletal muscle (18).

Directly linked to mitochondrial dynamics is another process called mitophagy. Mitophagy is a specialized form of autophagy aimed at removing dysfunctional mitochondria. Small, fragmented, depolarized mitochondria are encapsulated by a double membrane and form the autophagosome, which is then fused to a lysosome (462) (**Fig. 15**). Mitophagy is designed to recycle whole mitochondria (785), but there is also a set of proteases which selectively degrade misfolded or damaged mitochondrial proteins. These mitoproteases are located in both the matrix compartment (Lon, ClpP, and m-AAA), and the intermembrane spaces (i-AAA, Yme1L1, HtrA2/Omi, OMA1, and



PARL) (767). They are also involved in controlling dynamics (by activating OPA1)(159, 381) and mitophagy (PARL modulates mitophagy by degrading PINK1)(430). The cytosolic ubiquitin-proteasome pathway is also involved in the degradation of several outer membrane and intermembrane space proteins. Recently, an additional system was discovered which is involved in removing localized portions of mitochondria in response to oxidative stress. Mitochondria-derived vesicles (MDV) bud off from mitochondria in response to oxidative stress, are enriched in oxidized proteins, and are directed to lysosomes for degradation (843, 844). MDV are induced by the Pink-Parkin system but are independent from the fission machinery and from the classical autophagy proteins (767).

Overall, these processes are indispensable for mitochondrial quality control and prevention of atrophy. In fact mitophagy is essential for maintaining muscle mass. In mice, muscle specific knockout of an essential mitophagy gene, *Atg7*, resulted in muscle atrophy and aging-dependent decrease in force, and precipitated muscle loss during denervation and fasting. Affected muscles displayed accumulation of abnormal mitochondria, distension of sarcoplasmic reticulum, disorganization of sarcomere, and formation of aberrant concentric membranous structures (616). Accumulation of dysfunctional mitochondria may also contribute to atrophy by increasing pro-apoptotic signaling. Aged muscles, in fact, display an increased sensitivity to permeability transition (343) and apoptosis (615).

Ablation of Pink and Parkin reduces mitophagy and causes mitochondrial dysfunction, increased sensitivity to oxidative stress and muscle degeneration (74, 161, 694). On the other hand, excessive autophagy can become detrimental to muscle homeostasis. Overexpression of FoxO3, Muf1, Bnip3, or Nix triggers autophagy and induces muscle atrophy (582, 601, 766).

Defects of the proteolytic pathways have been reported in different tissues during aging, but data in muscle are scarce and only the Lon system was unambiguously shown to be altered (92), causing accumulation of carbonylated proteins in muscle mitochondria (93). Curiously, autosomal recessive mutations of the *LONP1* gene cause CODAS syndrome (850), characterized by developmental delay, craniofacial anomalies, cataracts, delayed tooth eruption, hearing loss, short stature with delayed ossification, but without prominent muscular symptoms.

Analysis of carbonylated proteins in normal, aged, and sarcopenic human subjects, showed that aging was associated with increased mitochondrial (but not myofibrillar or sarcoplasmic) protein carbonyl adducts, independently of sarcopenia. Mitochondrial protein carbonyl abundance negatively correlated with muscle strength, but not muscle mass. Levels of cytoprotective proteins, (HSP 27, 70 and CRYAB), were unaffected (67).

A well-regulated balance between pro-fusion and pro-fission proteins is also essential to maintain muscle mass. Electron microscopy analyses of murine aged muscles showed that sarcopenia is associated with larger and less circular subsarcolemmal mitochondria while intermyofibrillar mitochondria appeared longer and more branched. While total levels of shaping proteins were not changed, the Mfn2/Drp1 was altered, indicating increased fusion and/or decreased fission (545). Excessive fission (observed in mice overexpressing *Drp1*) also results in reduced muscle mass, compromised exercise performance, and severe remodeling of the mitochondrial network (887). In

mice, aging is associated with a progressive reduction in *Mfn2* levels, while conditional ablation of *Mfn2* in muscle results in sarcopenia, impaired autophagy and accelerated aging (816).

However, among mitochondrial shaping proteins the role of OPA1 is probably the most important. In fact, its mild overexpression protects mice from muscle loss secondary to denervation (924). Denervation appears to be a key player in the development of muscle atrophy in old rats (776) and of octuagenarians (845) (see above). A recent work has further stressed the protective role of OPA1. A decline of *MFN1/2*, *OPA1* and *DRP1* transcripts was found in muscle biopsies of old sedentary individuals, while expression was maintained in muscle of senior sportsmen. Most importantly, reduction in muscle mass and force correlated with the decrease in OPA1, but not with other shaping proteins. Data in aged mice recapitulated the human results; interestingly one week of exercise training could restore normal *Opa1* expression in aged mice. Moreover, inducible *Opa1* ablation in muscle of adult mice caused muscle loss and weakness, but also a systemic phenotype (white hair, liver steatosis, hypoglycemia, and denervation) which was mediated by Fgf21 (870) (Fig. 15).

### Mitochondrial biogenesis

Biogenesis of mitochondria requires a complex interplay between mtDNA and nDNA to ensure coordinated expression of proteins encoded by each genome (266). A specific transcription factor (peroxisome proliferator-activated receptor  $\gamma$ , coactivator 1 -PGC1 $\alpha$ ) appears to be the master regulator of the process (569). Specific metabolic sensors such as sirtuin 1 (SIRT1), mitogen-associated protein kinase (p38MAPK), and AMP-activated protein kinase (AMPK), activate PGC-1 $\alpha$  in the cytoplasm inducing translation of PGC-1 $\alpha$  to both the nucleus and mitochondria where it regulates the transcription of nDNA and mtDNA-encoded genes, as well as mtDNA replication (787).

SIRT1 is a member of the sirtuin family, a highly conserved group of NAD<sup>+</sup>-dependent protein deacetylases. It is located predominantly in the nucleus but it is also found in the cytosol, and it can shuttle between these two compartments (570). Increases in NAD<sup>+</sup> intracellular levels stimulate its activity allowing it to deacetylate (among its targets) PGC-1 $\alpha$  (765). Phosphorylation of PGC-1 $\alpha$  by AMPK (which in turn is activated by high AMP/ATP ratio (647) is a prerequisite for its deacetylation (131). SIRT1 is also involved in the deacetylation of FOXO3A in response to oxidative stress, promoting the transcription oxidative stress resistance genes among which SOD2 and catalase (117). SIRT1 is also involved in the mitochondrial quality-control machinery. Overexpression of SIRT1, increases the protein levels of Hsp60 and of the CLpP protease which are involved in the mitochondrial unfolded protein response (651). Finally, SIRT1 appears to be a modulator of the permeability transition pore (562). Other sirtuins also have important roles in mitochondrial metabolism by controlling antioxidant defenses, mitochondrial dynamics, autophagy, and mitochondrial stress responses (570).

Activated PGC-1 $\alpha$  induces expression of nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) but it also directly interacts and coactivates the transcriptional function of NRF-1 on the promoter of mitochondrial transcription factor A (*TFAM*) (965). TFAM promotes transcription of mtDNA, but it is also important for mtDNA replication and packaging (447). Mutations in TFAM cause a severe form of hepatocerebral MDS (849) which affects also muscle mtDNA copy number.

Overexpression of PGC-1 $\alpha$  causes an increase of mitochondrial mass (965), and its effect is not limited to proteins related to energy metabolism, but affects also mitochondrial proteins unrelated to energy metabolism (237). However, PGC-1 $\alpha$  is not the only regulator of the process since exercise-induced mitochondrial biogenesis is still observed in mice with skeletal muscle-specific knock-out of PGC-1 $\alpha$  (777). A closely related protein, PGC-1 $\beta$ , can also activate mitochondrial biogenesis, and deletion of both genes in skeletal muscle has a synergistic effect, causing profound impairment of mitochondrial function (29, 989).

Several studies have addressed PGC-1 $\alpha$  levels during aging, but again results are conflicting (428). Because PGC-1 $\alpha$  levels are reduced also in sedentary individuals, and physical training can increase its expression, exercise could represent a simple measure to counteract/reduce the effects of aging on mitochondrial biogenesis. In fact, another recent report confirms that aging-related muscle mitochondrial dysfunction can almost completely be rescued by aerobic exercise and the functional improvements are related to the reversal of muscle mitochondrial proteome alteration (764).

Besides its role in boosting bioenergetics, PGC-1 $\alpha$  seems to be involved also in the maintenance of the neuromuscular junction (NMJ).-It modulates denervation-induced mitophagy in skeletal muscle (906) and together with WldS it has a protective effect on neurons after axonal injury (672). Moreover, pyrroloquinoline quinone, a naturally occurring antioxidant with multiple functions including mitochondrial modulation, prevents denervation-induced muscle atrophy by increasing PGC-1 $\alpha$  levels (498).

Other pharmacological stimulators of mitochondria biogenesis have been successfully used in murine models of mitochondrial myopathy. Nicotinamide riboside (which stimulates the SIRT1 cascade by increasing intracellular NAD<sup>+</sup> levels) corrected the myopathic phenotype of mice with mutations in Sco2 (142) and in Twinkle (459), while treatment with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an activator of AMPK, resulted in partial correction of the COX deficiency of Surf1 and Cox15 knockout mice (954). Another compound, bezafibrate, was shown to induce expression of mtDNA-encoded subunits in both normal and SCO2 patient cells, with an improvement of COX activity and of the bioenergetics status in patient cells (141). These compounds represent interesting candidates to improve mitochondrial biogenesis in aged individuals.

The role of denervation in causing mitochondrial dysfunction in aged humans was also investigated confronting cohorts of young or active aged individuals, with old, inactive subjects, and with a rodent model of sporadic denervation. Interestingly the main effect of denervation on mitochondria was shown to be increased sensitivity to mitochondrial permeability transition (845).

Despite all these advances, the precise role of mitochondria in aging and sarcopenia remains controversial. The classical mitochondrial theory of aging has been largely abandoned, but it has not been replaced by a novel formal set of hypotheses. It is still not clear whether the observed mitochondrial alterations are primary or secondary events and what is the main trigger that determines these changes. Paradoxically, even the exact nature of these alterations is still not completely clear. Apart from the methodological issues that we have discussed, some of the conflicting evidence could also be related to the animal models employed in many studies. In fact, overexpression or tissue-specific knockout of individual genes are situations which are not observed

in human mitochondrial diseases (most human genetic defects are hypomorphic and are not restricted to muscle), let alone during aging. Although these “extreme” situations may provide very useful information, they could also overemphasize some aspects that have marginal importance in the physiology of aging.

Nevertheless, mitochondria remain one of the main focus areas of aging research and altered mitochondrial homeostasis is likely to be one of the main players in the process. Given the tightly interconnected nature of the main mitochondrial functions (biogenesis, dynamics, mitophagy, bioenergetics, apoptosis), it is reasonable to think that they all participate to some extent to the physiological aging process, and that they could represent potential therapeutic targets.

### ***Microvasculature***

Sarcopenia is the result of an ever so slight, but prolonged, negative protein balance. Consequently, many investigators have focused their attention on signaling pathways and factors that may underlie the reduced rate of protein synthesis and increased rate of protein breakdown during aging (see below). Another factor that may contribute to sarcopenia is the gradual accumulation of incompletely repaired contraction-induced micro damage (277). Such repair requires activation of satellite cells (380) and it has been suggested that an aging-related decrease in their number, and ability to proliferate and differentiate, also contribute to sarcopenia (202). While all those factors undoubtedly play a causal role, little is known about a potential contribution of capillary rarefaction or capillary hypo perfusion to sarcopenia. This is surprising, as an adequate microvascular network is not only required for oxygen supply and removal of heat and metabolites, but also for the delivery of nutrients and (anabolic) hormones (966). The importance of an adequate blood supply for muscle size and function is readily seen in the ensuing atrophy during prolonged ischemia (230) and a dramatically hastened onset of muscle fatigue during a series of intermittent isometric contractions during acute ischemia (971), respectively. The impact of capillary rarefaction *per se* on skeletal muscle structure and function has hitherto not directly been investigated. However, in the heart a 10% arteriolar occlusion results in a large reduction in cardiac function and increase in size of hypoxic regions (379). This highlights the importance of local capillary supply for adequate muscle function and maintenance of muscle mass. Such and other observations have led some investigators to hypothesize that hypoxia contributes to sarcopenia (231). Given the above observations and the importance of the capillary bed for tissue oxygenation, removal of heat and metabolites, and delivery of nutrients and (anabolic) hormones, we will below explore how the capillary bed in the muscle is affected during aging and to what extent capillary rarefaction may or may not contribute to sarcopenia and skeletal muscle dysfunction in old age.

### ***Relationship between capillary supply and size of a muscle fiber***

Capillaries are often seen as the source of oxygen for the mitochondria in the surrounding tissue cells. In line with this, it has been found that muscles with a high oxidative capacity have a denser capillary network than those with low oxidative capacity, and even within a given muscle, regions with a higher oxidative capacity have a denser capillary bed (205, 210, 215-217, 347, 411). This relationship between capillary supply and oxidative capacity seems also to hold for individual fibers (65, 618). These latter comparisons, however, did not consider the influence of fiber size on this relationship.

The method of capillary domains (**Fig. 16**) calculates capillary domains (estimates of their oxygen supply area), defined as the area surrounding a capillary delineated by equidistant boundaries from adjacent capillaries. This method allows one to calculate the capillary supply to individual fibers and in relation to their oxidative capacity and size, and to estimate the maximal oxygen demand supported by a capillary (91). Using this method, it has been shown that the capillary supply to a fiber depends more on the size (8, 216, 969, 970) than on the oxidative capacity (969, 970), or type (8) of the fiber (91), observations confirmed also with other approaches (424, 448). The importance of fiber size rather than oxygen demand is emphasized by the observation that the relationships between fiber size and capillarization during maturation are similar under conditions of reduced oxygen supply (maturation in hypoxia) or increased oxygen demand (treatment with thyroxin) to that seen during normal maturational growth (837). The intricate interrelationship between the size and the capillary supply to a fiber is also shown by the proportional increase in capillary number and fiber size in the quadriceps after 12 weeks resistance training in young men (349). Finally, the similar time course of muscle fiber hypertrophy and angiogenesis during overload (263, 719) even more forcefully suggests that fiber size and fiber capillary supply are tightly linked and controlled by common factors. This tight link between fiber size and capillary supply to a fiber lends further support to the idea that a potentially slow, but gradual capillary rarefaction during aging contributes to sarcopenia.

#### *Aging-related changes in muscle capillarization*

The capillary supply to a muscle is often described in terms of capillary density (CD in number of capillaries per mm<sup>2</sup>) and capillary to fiber ratio (C:F). A reduction in C:F indicates capillary rarefaction, while an increase in C:F reflects capillary proliferation, assuming the fiber number is the same. Table 1 gives an overview of the observed aging-related differences in CD, C:F and fiber cross-sectional area (FCSA) in rodents, the Beagle and human.

#### *Rodent studies*

Most rodent studies do not report an aging-related difference in C:F or FCSA, suggesting there is neither capillary rarefaction nor atrophy during aging. Even an aging-related increase, rather than decrease, in FCSA has been reported in the soleus, plantaris and red, but not white, rat gastrocnemius muscle (389, 448). It should be noted, however, that in the majority of cases animals of less than 1 year old were compared to animals of 24 months or older. Such comparisons can be marred by maturational increases in FCSA, fiber type transitions and changes in function up to the age of around 9 months (204, 214, 591, 600) that are accompanied with capillary proliferation and increases in C:F (206, 757, 836). Too young animals in the young group may not have reached the largest FCSA and highest C:F, while those in the oldest group may not yet show signs of muscle aging (47, 204, 591). Consequently, any aging-related reduction in FCSA and C:F may be missed as illustrated in **Figure 17**. In support of this, it has been found that the FCSA and C:F were higher in the plantaris muscle of 13- than 5- and 25-month-old rats (215, 216) and that C:F in the soleus and EDL of rats decreased between 103 and 121 week of age (85). Other studies showing muscle fiber atrophy report, however, no change in C:F (111, 112, 274, 389), even when muscles from 12- and 24- were compared with those of 35-month-old rats (619). The latter suggests that aging-related muscle atrophy can occur without capillary rarefaction. This conclusion must, however, be taken with some caution as in theory capillary rarefaction can result in a maintained C:F when accompanied with

loss of fibers. For instance, in one study the C:F was higher in muscles of 28- than 6-month-old mice (193) which at first glance suggests capillary proliferation. It is more likely, however, that this increase in C:F is due to fiber loss, as reflected by the lower fiber count in the muscles of the old than the young mice (193). It is thus difficult to decide whether capillary rarefaction plays an important role in rodent skeletal muscle aging. It is recommended that future studies dealing with muscle fiber atrophy and capillary rarefaction in old age in rodents have 1) more than two age groups, 2) choose fully matured animals in the youngest and animals at >90% of average life span in the oldest group (47), and if possible 3) get an idea of the number of fibers in the muscle. Nevertheless, the current data suggest that capillary rarefaction does not play an important role in the aging-related muscle wasting in rodents.

While there does not appear to be a clear link between aging-related changes in capillarization and muscle fiber size in rodents, it is possible that the capillarization follows, or determines the aging-related changes in muscle oxidative capacity. As discussed above, oxidative muscles, muscle regions or muscle fibers have in general a denser capillary supply than non-oxidative muscle (fibers). The lower aerobic power in old (28-30 months) than young (8 months) rats with artificially matched convective oxygen delivery (387) could arise from a lower muscle capillarization. However, no significant aging-related reduction in C:F or CD was found, but rather a decreased oxidative capacity (389), suggesting that the old muscles had an excess capillary supply. Also in the soleus and EDL of 35- vs 12- and 24-month-old rats the decrease in mitochondrial volume was proportional to the decrease in FCSA, without any concomitant change the C:F ratio, thus resulting in a higher CD in old muscles (186, 619). This superfluous capillarization may serve to maintain an adequate oxygen supply during exercise in the face of reduced exercise-induced vasodilation and hence blood flow in the skeletal muscle of old rodents.

The superfluous capillarization in old rodent muscles may be the result of an accelerated rate of atrophy as a consequence of an accelerated denervation of fibers at advanced age and incomplete reinnervation due to motoneurone loss (209). Indeed, during the early stages of denervation, rodent (208, 729) and human (139) muscle appear to have a superfluous capillary supply. Interestingly, the regression of the capillary bed during denervation was slower in old than young-adult rat soleus, resulting in an increased CD in old denervated muscles only (208). The loss of muscle fibers that may ensue after motorneurone loss in the absence of capillary rarefaction will accentuate the superfluous muscle capillary supply, as observed in old mouse muscle (193).

### Human studies

In Table 1 it can be seen that where in most rodent studies no aging-related fiber atrophy or reductions in C:F are observed, in human muscle often both atrophy and a reduction in C:F are seen. In most of these studies the loss of capillaries and reduction in fiber size are proportional, resulting in a similar CD in muscles from young and old people. The reduction in C:F can even occur without a reduction in FCSA (306, 784). In none of the studies an increase in CD was observed, suggesting that in contrast to what can occur in old rodent muscle there is no superfluous capillary supply in old human muscle and the coupling between fiber size and oxidative capacity with fiber capillary supply is maintained in old age (817).

It has been observed that the lower CD in old than young muscles was associated with a reduced oxidative capacity (164). A lower CD may partly be due to low activity levels as master athletes and young people matched for the 10-km run performance of the master athletes had a similar CD in the gastrocnemius muscles, even though the masters had a higher oxidative capacity (165). The higher C:F in the athletes than in the performance-matched young athletes was associated with larger fibers in the master athletes (165), supporting the concept that FCSA rather than oxidative capacity determines also in muscles of old people the capillary supply to a fiber.

Another factor that may contribute to a reduced capillarization of old muscle is insulin resistance, as it were particularly older (70 years) men with diabetes who had a lower C:F than young (24 years) men (356). In the diabetic older men, but not the healthy older men, also the muscle fiber atrophy was more pronounced and the CD less than in the young men (356). This is significant as insulin activates endothelial nitric oxide synthase (eNOS) and leads to vasodilation, where insulin resistance may result in an impaired post-prandial insulin-induced vasodilation (905). The consequent reduction in perfusion of the capillary bed may result in reduced shear stress and together with a reduced nitric oxide (NO) production leading to a lower vascular endothelial growth factor (VEGF) expression and impaired maintenance of the capillary bed.

In a 12-year longitudinal study a reduction in the C:F was found without a significant decrease in FCSA, even though the muscle size had decreased (306). The latter suggests fiber loss, considered an important factor contributing to the aging-related muscle wasting (555, 965) and implies, if fiber loss had occurred, an even larger capillary rarefaction in the 12-year period than suggested by the decrease in C:F. It is possible that the lower capillary supply to the remaining fibers, indicated by the lower C:F, creates a less than optimal environment for these fibers and precedes subsequent atrophy of the remaining fibers. In fact, it has been observed that in rats with heart failure endothelial apoptosis preceded muscle fiber apoptosis and atrophy (937). The authors suggested that the relative ischemia consequent to endothelial apoptosis causes an imbalance in fiber nutrition that in turn triggers fiber atrophy. Also during aging this may occur, as it has been observed in mouse gastrocnemius muscle, that the aging-related increase in apoptotic endothelial cell nuclei exceeds the apoptosis in satellite cells and myofibre nuclei by at least one order of magnitude (382). It is thus possible that the aging-related capillary rarefaction and hypoperfusion do contribute, and may even precede, sarcopenia.

#### *Aging-related changes in the heterogeneity of capillary spacing*

While CD and C:F give an idea of the quantitative capillary supply they do not tell us how the capillaries are distributed within a muscle. This is potentially important as an increased heterogeneity in capillary spacing may affect muscle oxygenation negatively (206, 212, 213, 264, 716, 898). It has been suggested, for instance, that heterogeneous stoppage of capillaries plays an important role in skeletal muscle pathology during sepsis (332). Yet, little is known about possible aging-related changes in the heterogeneity of capillary spacing, but there are some indications that it is elevated in old age. It has been reported that in the rat plantaris muscle the heterogeneity of capillary spacing was higher in 25- than 5-month-old rats, which was related to the increased heterogeneity in fiber size (211), which may cause an increased heterogeneity of muscle oxygenation (575).

#### *Aging-related structural changes in the microcirculation*

In addition to changes in C:F and CD during aging, other structural changes in the microcirculatory bed may affect delivery of oxygen and substrates, and removal of heat and waste products, and thereby have a negative impact on muscle function. Although a reduction in luminal diameter, as observed in rats after hind limb suspension (449), could be such a factor, no differences in capillary (448) or arteriolar (171) luminal diameter have been observed in aging rodents. Another factor is a decreased tortuosity and branching of capillaries that has been observed in the soleus but not EDL of 35- vs 12- and 24-month-old male FBN rats (619). This change is unlikely, however, to have a significant impact, as it did not result in a decreased capillary surface to fiber surface area or capillary length per fiber volume (619). Even though the capillary – fiber exchange area may stay the same, the aging-related increase in the periodic acid-Schiff (PAS) reactivity of the extracellular matrix and basement membrane of capillaries, arterioles and venules, may be reflective of a thickening and enhanced advanced glycation end-product formation in the basement membrane (838). Indeed, an aging-related thickening of the capillary basement membrane has been observed between the age of 28 and 63 years in men, that was, however, unrelated to glucose tolerance and readily reversible by endurance training (949). How such thickening of the basement membrane affects capillary function is unknown, but it is likely to increase the resistance to diffusion over the capillary membrane and to contribute to the impaired dilatory function of arterioles (701). In addition to these biochemical changes in the capillary and arteriolar wall, there may also be an increase in the segment length of arterioles as observed between 12 and 24 months in the cremaster muscle of male rats, implying reduced branching (171). Finally, in aging (25-month-old) BUFMna rats that suffer from muscle weakness and atrophy, discontinuous capillary segments with fenestrations may contribute to atrophy as they were particularly associated with bundles with small muscle fibers (225).

#### *Aging-related changes in microcirculatory flow*

The impaired vasodilatory response to adenosine (171), acetylcholine (829) and diazoxide may explain the reduced blood flow and fatigue resistance in old male Fischer344 rats (419) and indicates that the reduced dilatory response of arteries is most likely attributable to impaired endothelial function (229, 233). Endothelial dysfunction may underlie the lower exercise-induced blood flow and vascular conductance in older (55-68 years) men. Furthermore, the absolute leg blood flow and vascular conductance during exercise are less than in young (22-30 years) men working at the same absolute or relative workload (238, 726), while the venous oxygen pressure was lower. Not only the blood flow during submaximal exercise is reduced, but also the maximal mass-specific blood flow decreases with increasing age (478). The lower venous oxygen pressure suggests that the lower oxygen delivery due to the diminished exercise-induced blood flow is compensated, at least partly, by a larger oxygen extraction from the blood. A side effect is that average oxygen tension in the muscle tissue would be lower with muscle of the older person working in a somewhat locally hypoxic environment.

Endothelial dysfunction is also reflected by a reduced production of nitric oxide (NO) in aortic rings and impaired acetylcholine-induced vasodilation in old rabbits (759). The reduced availability of NO is not only due to a lower expression of eNOS, but also to oxidative stress and low-grade systemic inflammation where superoxide may capture NO and form peroxynitrite (233, 812, 904). To make matters worse during oxidative stress eNOS itself starts to produce more superoxide, making the bioavailability of NO even less (812). Together, the increased oxidative stress and lower expression



of VEGF, that has anti-apoptotic properties, will result in endothelial cell apoptosis (812), which has been suggested to precede muscle fiber apoptosis in heart failure (937) and aging (936). Lower flow, and hence shear stress, which is an important stimulus for angiogenesis and maintenance of the capillary bed (410) may thus underlie the capillary rarefaction and subsequent muscle wasting during aging.

Impaired vascular function is not limited to an impaired dilation, but also the arteriolar myogenic constriction (vasoconstriction in response to elevated blood pressure) has been reported to be impaired in old age (326). Where the vascular dysfunction in oxidative muscles is primarily related to changes in NO signaling, in glycolytic muscles it is a combination of impaired NO and prostanoid signaling (653). Such differential disturbances in vascular function may well cause the observed redistribution of blood flow from low-oxidative to highly glycolytic muscles during submaximal exercise in old compared with young rats (653, 658). This redistribution of blood flow in turn may result in a disturbed matching of oxygen demand and supply during exercise and contribute to the aging-related decrease in maximal oxygen uptake and exercise tolerance (199, 295, 363, 653).

#### *Aging -related changes in angiogenesis and maintenance of the capillary bed*

While capillary rarefaction does appear to occur during aging, this may be at least partly attributable to the aging-related decline in physical activity, a widespread phenomenon in aging organisms including humans (418). Disuse indeed does not only result in muscle atrophy, but also induces capillary rarefaction (437, 449, 901). In support of a role for reduced physical activity levels, the CD and C:F were higher in both young (21-30 years) and old (51-62 years) fit than sedentary people (727). Similarly, the gastrocnemius muscle of master athletes (63 years) had a higher C:F, muscle oxidative capacity and larger type I fibers, but similar CD as young runners (26 years) matched for 10-k run performance, while top 10% young runners had even higher C:F and CD than the master athletes (165). Although these observations do indeed suggest that part of the capillary rarefaction in older people is related to disuse, these were not training studies and therefore no conclusion can be drawn as to a similar or reduced angiogenic response in old age. What is known, however, is that in the vastus lateralis muscle of 65-74-year old men both endurance and resistance training can induce (330, 388, 933), although not always (835), angiogenesis that in the case of resistance training is proportional to the increase in fiber size (388). However, the more pronounced capillary proliferation in young (20 years) than old (62 years) endurance trained men suggests an attenuated angiogenic response in old age that may even be associated with muscle fiber atrophy (221). Also in mice, the blunted hypertrophic response in old age was associated with impaired capillary proliferation (47). Further, evidence for an attenuated angiogenic response has been observed in ischemic hind limbs of old rabbits and mice (274, 759).

The significance of the capillary bed for subsequent resistance exercise-induced hypertrophy is further reflected by the observation that older men with a poorer capillary supply to type II fibers than those with a higher capillary supply to type II fibers develop less hypertrophy in response to resistance training (835). Interestingly, those individuals with a lower capillary supply to type II fibers not only showed little to no hypertrophy, but also did not exhibit satellite cell proliferation (835) reinforcing the ideas that 1) there is a cross-talk between endothelial cells and satellite cells and 2) capillary rarefaction may precede the aging-related muscle wasting.

### Potential causes of capillary rarefaction and attenuated angiogenesis in old age

The question arises as to what may cause capillary rarefaction and the impaired angiogenesis in old age. Vascular endothelial growth factor (VEGF) is a potent endothelial mitogenic factor (284) that is increased in expression under conditions with elevated flow (636) and hypoxia (407), where blocking VEGF abolishes skeletal muscle angiogenesis induced by overload or shear stress (948). VEGF is also important for the maintenance of the capillary bed, as supported by the positive correlation between pre-exercise interstitial VEGF protein and capillary contacts per fiber (322). Indeed, targeted inhibition of VEGF expression in mice led to capillary rarefaction and apoptosis in skeletal muscle (866). It is thus possible that an aging-related reduction in expression, ability to increase VEG, and/or impaired response to VEGF expression underlie an impaired maintenance of the vascular bed and/or angiogenesis in old age.

Both lower basal levels of vastus lateralis muscle VEGF protein (184, 222, 784) and an attenuated acute exercise-induced increase in interstitial VEGF protein have been observed in old (65-72 vs 21-32 years) men and women (184, 322). Yet, the increases in VEGF and angiogenesis were similar in young (25 years) and old (64 years) men after 8 weeks endurance training (322). It is likely, however, that the endurance training-induced angiogenesis occur

ed somewhat slower in old age because of the lower basal levels of VEGF and attenuated exercise-induced increase in VEGF expression. In old rabbits and mice, a lower induction of VEGF and impaired endothelial function contributed to the impaired angiogenesis in the ischemic hind limbs, where the angiogenic response was rescued to some extent by administration of recombinant VEGF (759, 821).

In cell cultures of aortic smooth muscle cells isolated from young and old rabbits it was subsequently found that the hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) DNA binding, important for transcription of VEGF, is less in old than young cells, suggesting that the impaired angiogenesis in old age is related to impaired HIF-1 $\alpha$  activity (758). This reduced activity may be due to an aging-related reduction in HIF-1 $\alpha$  expression and a concomitant increase in the oxygen-dependent prolyl-4 hydroxylase domain proteins that play a role in the breakdown of HIF-1 $\alpha$  (452). In addition to the reduction in HIF-1 $\alpha$  activity, an aging-related reduction in eNOS has been shown to underlie the impaired angiogenic response in old ischemic mouse muscles (274). Finally, an impaired angiogenesis, and consequent reduced maintenance of the capillary bed, may also be due to reduced secretion and sensitivity to growth factors and an impaired ability to break down the extracellular matrix by matrix metalloproteinases that is crucial for angiogenesis (812).

Impaired angiogenesis may not only be caused by a reduction in the ability to increase VEGF expression, but also to an impaired angiogenic response to VEGF. Indeed, it has been found that the angiogenic capacity of VEGF was impaired in mice with an endothelium-specific SIRT1 knock-out. That such a situation may occur in old age was reflected by the fact that the capillarisation in old mice treated with nicotinamide mononucleotide, a precursor of NAD<sup>+</sup>, that activates SIRT1, restored the capillarisation and endurance and hence rejuvenated the muscle (903).

### Mechanisms controlling both capillarization and muscle fiber size

Above we have discussed the close link between muscle capillarization and fiber size and that part of the muscle atrophy during aging is associated with capillary rarefaction, at least in humans. Here we will discuss the potential mechanisms controlling both capillarization and fiber size. One common factor is that not only muscle fibers and satellite cells (298), but also endothelial cells act as mechanotransducers not only of high shear (e.g. hyperaemia), but also strain (e.g. muscle stretch) (262). The association between angiogenesis and fiber growth is reinforced by the increased capillary density, myogenic cell hyperplasia and transient rise in VEGF protein during 25% fiber hypertrophy after voluntary treadmill running in 18-month-old mice treated with an NO donor, but not when exercising only (551). This suggests that NO is important for the induction of VEGF, angiogenesis and fiber hypertrophy (551).

Satellite cells are in close proximity of capillaries (661) and it appears that activated satellite cells stimulate angiogenesis and *vice versa* that endothelial cells stimulate myogenesis (158). In co-cultures where satellite cells and endothelial cells were in separate layers without physical contact, the stimulation of satellite cell proliferation must have been due to factors released from the endothelial cells (158). Among these factors was VEGF. Neutralizing VEGF by VEGF antibodies reduced the satellite cell proliferation induced by endothelial cells by at least 41%, and even by 90% if a host of antibodies against factors released by the endothelial cells was used, while recombinant VEGF administration stimulated satellite cell proliferation (2, 158). VEGF probably acts via the VEGF receptors Flk-1 and Flt-1 on satellite cells where it stimulates satellite cell migration and inhibits satellite cell apoptosis (325). These observations indicate that VEGF has a direct effect on satellite cell proliferation, migration and survival. In turn, satellite cell conditioned growth medium stimulated the formation of connected endothelial cells *in vitro*, indicating a reciprocal interaction between endothelial and satellite cells (158). The close proximity of capillaries and satellite cells in the muscle facilitates the coordinated angio-myogenesis. Loss of capillaries may thus be associated with a loss of niches for satellite cells. In old human muscle the distance between capillaries and satellite cells in particularly type II fibers are greater than that in muscles from young people (661). The resulting impaired diffusion-based interaction between satellite cells and capillaries may underlie the impaired regenerative capacity and plasticity of old muscle. In line with this, conditions with capillary loss, such as amyopathic dermatomyositis, are associated with a proportional loss of satellite cells, and *vice versa* the increase in capillaries in resistance-trained individuals was associated with an increase in satellite cells (158).

Angiogenesis and VEGF expression can be stimulated independent of HIF-1 $\alpha$  via Akt, both during fiber hypertrophy and in ischemic hind limbs (863). It is therefore possible that the attenuated hypertrophy in older people that was associated with reduced satellite cell proliferation (707) finds its origin in impaired angiogenesis. In support of a role of both satellite cells and angiogenesis in muscle plasticity, it has been found that the blunted hypertrophic response in old mice is associated with both a lower satellite cell number (48) and impaired angiogenesis (46) in their muscles. Finally, it has been suggested that part of sarcopenia is attributable to incomplete repair of accumulated contraction-induced damage (277) and the importance of the microcirculation for muscle plasticity, repair and regeneration may well be underestimated. In support of this, it has been found that muscles from myostatin knockout mice that also overexpress estrogen related receptor gamma have a better regenerative capacity than those from myostatin knockout mice even though they have fewer satellite cells, but indeed a denser capillary network (685). In this context it is significant to note that an

adequate vasculature is crucial for muscle maintenance and repair, as indicated by the slower recovery from muscle damage that also includes damage to the neurovascular system than recovery from damage not affecting the neurovascular system, particularly in older (22-month-old) female C57BL/6J mice (546).

Peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$  (PGC-1 $\alpha$ ) not only plays an important role in mitochondrial biogenesis, but also the release of VEGF from myocytes (28) and it is suggested that a defect in this pathway may underlie the ageing-related capillary rarefaction (903). This pathway may underlie the higher capillary density in oxidative than glycolytic regions of the muscle and reflects that the cross-talk between muscle cells and endothelial cells is not limited to regulating the match between fiber size and capillary supply to the fiber, but also matching to some extent the aerobic metabolism with the capillary supply of the fiber.

### Capillary supply in the nervous system

A significant part of the aging-related loss of muscle mass is considered to be due to loss of motoneurons, where reinnervation of the denervated muscle fibers is incomplete (209, 258, 279, 520, 521). As a consequence of the compensatory, though incomplete, reinnervation the size of the remaining motor units increases (209). The increase in motor unit size may reduce the efficacy by which contractile activity stimulates perfusion of the capillary bed in the muscle (310) and contribute to muscle dysfunction in old age apart from the muscle wasting induced by motoneuron loss. Little attention, however, is given to the cause of this loss of motoneurons, but here we suggest it is at least partly attributable to vascular disruptions in the spinal cord. Indeed, in a mouse model of amyotrophic lateral sclerosis (ALS) the loss of motoneurons appears to be preceded by a decrease in the capillary density (642). It is thus possible that the aging-related reduction in capillary density in the spinal cord (733) contributes to loss of motoneurons in old age.. Even in the absence of capillary rarefaction motoneuron loss may occur in the presence of reduced spinal cord perfusion (688). In the latter study, this was achieved by deletion of the HIF-binding site of the VEGF promotor, and was associated with weakness and signs of denervation-reinnervation in the muscle (688). Above we discussed that the aging-related impairment in the HIF-VEGF pathway may underlie the impaired angiogenic response during ischemia in skeletal muscle and similar changes at the spinal cord level may contribute to the aging-related loss of motoneurons.

Also peripheral nerves are sensitive to loss of capillaries, as reflected by the positive relationship between sciatic nerve degeneration and conduction failure with the degree of capillary embolization with microspheres in rats (461), similar to that observed in mice with deletion of the hypoxia-response element in the VEGF promotor (688). The latter is significant and similar to the cross-talk between satellite cells and endothelial cells, there is also such a cross-talk between Schwann cells that produce VEGF (652) and endothelial cells that produce brain-derived neurotrophic factor (BDNF), important for development, survival and repair of peripheral nerves (659). It is thus possible that the regression of myelinated fibers in old (21 months) compared to young-adult (7 months) rats is a consequence of the reduced diameters and branching of capillaries in the peripheral nerves (788). It is noticeable that there is not yet significant muscle atrophy in rats aged 21 months, and these observations thus suggest that the capillary rarefaction and morphological changes in peripheral nerves precede the aging-related loss of muscle mass.

Thus, there is considerable evidence, particularly in human studies, that sarcopenia is associated with capillary rarefaction. It is not yet clear, however, whether capillary rarefaction precedes or follows sarcopenia. Whatever the sequence muscle fiber capillary supply and fiber size appear to be closely linked and controlled by common factors. There is a considerable cross talk between satellite cells and endothelial cells, where in particular the endothelial mitogenic factor VEGF appears to play a key role in this cross talk. Finally, it is recommended to explore further the potential importance of aging-related changes in the microcirculation of the spinal cord and how this may contribute to the aging-related loss of motoneurons and sarcopenia.

### **Satellite cells and regenerative capacity**

Mitotically quiescent satellite cells are residing in a specialized niche between cell membrane and basal lamina. The satellite cell niche includes extracellular matrix proteins, growth factors, myofibers and muscle resident non-myogenic pro-fibrotic cells, macrophages and regulatory T cells (for refs see (14)). The satellite cells represent a muscle-specific adult stem cell population and muscle repair/regeneration processes are primarily mediated via the activation of satellite cells, resulting in satellite cell entrance into the cell cycle giving rise to proliferating myogenic precursor cells which differentiate and fuse with existing muscle fibers or form new muscle fibers (960, 981). The transcription factor paired box 7 (PAX7) is a marker of satellite cells and an important regulator of the repair process (357, 815, 961). Quiescent satellite cells express PAX7 but not MYOD or other markers associated with proliferation. Proliferating satellite cells maintain PAX7 expression and induced expression of MYOD. In a subset of these satellite cells, myogenin expression is induced and PAX7 down-regulated and these cells are committed to differentiation. Another subset of activated satellite cells self-renew by inhibiting MYOD to reinstate quiescence to replenish the quiescent satellite pool to ensure a sufficient number quiescent satellite cells which can be activated during future rounds of muscle repair. Complex transcriptional mechanisms regulating satellite cell quiescence involve approximately 500 uniquely expressed genes, elegantly reviewed by Almada and Wagers (14). The membrane-associated inhibitor of receptor Tyr kinase signaling, Sprouty 1 (SPRY1), expressed in slow cycling satellite cells appears to be required for the return to the quiescent state after muscle repair (146, 341). In addition, the forkhead box protein 3 (FOXO3) transcription factor is involved in the return to the quiescent satellite cell pool (338). Interestingly, FOXO3 appears to be directly linked to NOTCH signaling and suggested to play a key function in the maintenance of NOTCH receptor expression, essential for the maintenance of the quiescent state. MYOD and MYF5 have been forwarded as the master transcriptional factors orchestrating the activation of satellite cell genes and both these transcription factors are under both transcriptional and post-transcriptional control (for refs see (14)). It has been demonstrated that p38 $\alpha/\beta$  MAPK, activated by fibroblast growth factor receptor (FGFR) tyrosine kinase, induces MYOD by inhibiting a *Myod* mRNA-destabilizing protein (179, 232, 378, 433, 434, 453, 894). Further, miRNA-31 has been shown to post-transcriptionally repress *Myf5* mRNA in quiescent satellite cells, an inhibition lost during satellite cell activation (179). Recent results from zebrafish show that there are different populations of satellite precursor cells playing different roles in the initiation of fiber formation and fiber growth during muscle repair (717). Satellite cells expressing *pax7a* or *pax7b* gene reporters have different specific roles during muscle repair with *pax7a* preferentially initiate nascent muscle fibers while *pax7b* expressing cells more frequently fuse to repair and grow muscle fibers (717).

Adult stem cells have turnover rates and differentiation programs which are tissue-specific as well as common properties independent on tissue. However, independent of properties being intrinsic to the tissue or common to all stem cells, these traits are affected by aging and consequently impacting on tissue repair and regeneration capacity (96). In skeletal muscle, there are both quantitative and qualitative aging-related changes in satellite cells affecting muscle regenerative capacity, such as a ~65% decline in satellite cell number (95, 175), an increased DNA damage, a decline in antioxidant capacity and an altered gene expression (for refs. see (96)). The impaired skeletal muscle regeneration capacity in old age has been associated with intrinsic deficits in satellite cell function (141, 142, 175). However, no difference in muscle fiber size and number, muscle fiber specific force or myonuclear number were reported between control old and old genetically manipulated mice with an inducible depletion of satellite cells resulting in loss of satellite cells to the degree sufficient to impair regeneration from adult throughout their lifespan (131). Thus, in mice not challenged with a muscle injury during aging, satellite cells appear not to influence the loss of muscle mass and function, i.e., sarcopenia. On the other hand, the increased amount of muscle collagen and fibrosis associated with aging was significantly increased in mice depleted of satellite cells during aging, suggesting that the aging-associated loss of satellite cells contributes to the muscle fibrosis in old age (131). Syndecan-3 is a transmembrane proteoglycan which is expressed in satellite cells and is involved in signaling to maintain satellite cells quiescent. In mutant mice lacking syndecan-3, improvements in muscle regenerative capacity and reduced fibrosis were observed in old as well as in dystrophic mice (474). Manipulating syndecan-3 was accordingly forwarded as a promising target in the development of therapies enhancing muscle regeneration and reducing fibrosis in aged muscle (474).

Muscle satellite cells are sensitive to systemic soluble factors in serum, inflammation and local growth factors which all change during aging with an impact on satellite cell function (for refs see (14, 96)), resulting in an aging-related impairment or delay in the repair and regeneration process in response to a damaging insult to skeletal muscle (for refs see (14, 96)). It is of specific interest to note that neutralization of Wnt and TGF $\beta$  signaling pathways, both antagonizing Notch signaling critical for satellite cell activation, improved the muscle repair mechanism in old age (for refs see (96)). The increased TGF $\beta$  levels in old age has also been suggested to have a negative impact on satellite cell function via promoting pro-fibrotic muscle resident stem cell types known to cross-talk with satellite cells (96). The chronic low-grade “sterile” inflammation considered a hallmark of aging is associated with geriatric frailty, decreased muscle strength and incapacity to respond to stress (634). This chronic low-grade inflammation is closely associated with the senescence-associated secretory phenotype, comprising pro-inflammatory cytokines and chemokines (974). It has recently been shown by two independent groups that elevated levels of inflammatory cytokines regulate satellite cell function via the JAK-STAT3 pathway and inhibition of this pathway markedly improved satellite cell function, offering a promising therapeutic intervention for the treatment of sarcopenia (725, 879). In addition, the JAK-STAT pathway also plays an important role in cytokine production and JAK inhibitors decrease cytokine production in pre-adipocytes, aging-related adipose tissue accumulation, systemic inflammation as well as frailty (974). Thus, senescent cells contribute to the aging-related low-grade inflammation and inhibition of the JAK-STAT pathway may blunt the senescence-associated secretory phenotype as well as inhibit the direct negative effects of the pathway on satellite cell function (725, 879, 974). Further, elevated activity p38 $\alpha$ / $\beta$  MAPK signaling

pathway has been implicated in aging-related functional decline in satellite cell proliferative capacity and the inhibition of this pathway rescued the self-renewal capacity of satellite cells in old age (71, 175). The rescue of the deregulated FGFR- p38 $\alpha$ / $\beta$  MAPK pathway offers an interesting therapeutic intervention strategy in the treatment of sarcopenia (71, 175).

Although the aging-related loss of satellite cells may not be a prerequisite for sarcopenia, different intervention strategies aiming at improving or altering satellite cell function may ameliorate aging-related impairments in skeletal muscle regenerative capacity upon repeated injury and reduce the aging-related muscle fibrosis. As discussed above there is experimental evidence suggesting that this may be achieved by targeting (i) syndecan-3, (ii) Notch signaling, (iii) aging-related low-grade inflammation or the JAK/STAT pathway directly, or (iv) activity p38 $\alpha$ / $\beta$  MAPK signaling pathway. However, it needs to be emphasized that these interventions are primarily forwarded based on observations in genetically manipulated mice and extensive analyses of efficiency and side-effects need to be completed in both experimental and clinical studies prior to the translation to clinical research and practice.

### **Regulation of muscle protein synthesis/degradation and intracellular signaling pathways**

Muscle atrophy is secondary to the decrease in cell size involves the net loss of proteins, organelles and cytoplasm. The amount of organelles and proteins in the cell depend on the balance between biogenesis/biosynthesis and removal/destruction. This equilibrium defines the organelle and protein turnover and greatly affects the size and performance of cells including skeletal muscles. Growth of adult musculature occurs when enhancement of protein synthesis exceed rates of protein degradation. Few pathways have been shown to control protein synthesis and most of them converge to IGF1-PI3K-Akt/PKB-mTOR or TGF $\beta$ /myostatin/BMP signaling. Conversely, atrophy occurs when the main cellular degradation pathways, the ubiquitin-proteasome and the autophagy-lysosome systems, overcome protein synthesis. In sarcopenia, this dogma has been challenged by recent findings that put the quality more than the abundance of proteins and organelles as a critical factor for force generation and muscle function.

#### ***Protein synthesis***

##### *IGF1/AKT1/mTOR signaling*

The IGF1-PI3K-Akt/PKB-mTOR signaling pathway is considered the key controller of protein synthesis in muscle providing the means for potent control of S6, a ribosomal protein, and factor 4E binding protein 1 (4EBP1), an inhibitor of the ribosomal eukaryotic translation initiator factor 4E (eIF4E) (513). Simultaneously, this pathway suppresses protein breakdown because Akt negatively controls FoxO family of transcription factors that are sufficient and required for the expression of rate limiting enzymes of the ubiquitin-proteasome and autophagy-lysosome systems (635). Indeed, muscle specific insulin and IGF1 receptors double knockout mice display a tremendous muscle loss that is FoxO dependent (673). Therefore, this pathway has a unique function that is to enhance protein synthesis and simultaneously to reduce protein breakdown. The critical factor that controls protein synthesis is the mTOR kinase that however belongs to two different complexes named mTORC1 and mTORC2. While mTORC2 is related to glucose and lipids homeostasis (497, 505, 684), mTORC1 regulates several anabolic processes including protein synthesis, ribosome and

mitochondria biogenesis. Interestingly, inhibition of mTORC2 complex in mice reduces life span only in males but the mechanistic insight of this effect remains to be identified (504). The mTORC1 effect on translation relies on its activating action on S6K1 and inhibitory effect on 4EBP1. The mTORC1 complex is critical for muscle hypertrophy induced by exercise, feeding and growth factors and is the target of the drug rapamycin (806). Although inhibition of IGF1/Akt/mTOR pathway has been found in several catabolic conditions characterized by muscle wasting, its involvement in aging sarcopenia is contradictory. In the adult mammals, IGF-1 is synthesized predominately in the liver under the growth hormone action, and acts as a systemic growth factor. However, IGF-1 is also produced in extrahepatic tissues where it plays a predominantly autocrine/paracrine role (703). The muscle isoform but not the circulating one is critical for muscle mass maintenance and growth in adulthood (657). While in rodents the muscle-specific IGF1 expression declines during aging and its reactivation in old mice counteracts sarcopenia (657, 795), in humans this reduction was not found but, surprisingly, an upregulation of IGF1Eb isoform was detected in elderly subjects (795). Controversial data have been also reported for Akt1 activity in old mice while data on mTOR are more consistent. Indeed, most of the studies show the persistence or even an increase of mTOR signaling during aging in humans, rats and mice (320, 464, 607, 698, 795, 941). These findings argue against the idea that inhibition of protein synthesis is the cause of sarcopenia. However, growth-promoting stimuli such as exercise or nutrients do not efficiently activate mTOR pathway in elderly people and this anabolic resistance is believed to be an important factor in the reduced muscle recovery after an injury (312, 320, 696). Interestingly, acute and transient activation of Akt1/FoxO axis has been reported to be beneficial in mice and elderly people (10, 683).

### TGF $\beta$ Superfamily and signaling

The TGF $\beta$  superfamily comprises more than 30 secretable ligands with differing selectivity for specific receptor subtypes. The complexity of the TGF $\beta$  signaling network is related to the different biological actions executed within a cell, depending on the combination of ligands and receptors that are utilized. Downstream these ligands, two major signaling pathways are activated that impinge on two classes of transcription factors belonging to Smad family. Activins, some growth and differentiation factors (GDF) such as Myostatin/GDF8 and GDF11, and the TGF $\beta$  proteins promote activity of Smad2 and 3 and muscle loss. In contrast, other GDF ligands and the bone and morphogenetic proteins (BMP) rely on Smad 1, 5, and 8 to regulate target genes that control muscle growth. The manner in which the two subfamilies of ligands recruit different Smad proteins is a function of the different combination of type II and type I receptors with which ligands preferentially engage (798).

The Activin/Myostatin/TGF $\beta$  group bind plasma membrane associated activin type IIB and type IIA receptors (ActRIIB/IIA) and TGF $\beta$  receptors (TGF $\beta$ RII). Ligands binding with ActRIIB/IIA/TGF $\beta$ RII lead to recruitment and activation of activin receptor like kinase (ALK)-4, -7 and -5, and serine/threonine type I receptor kinase (617). ALK4/7 and ALK5 phosphorylate Smad2/3, to promote formation of a heterotrimeric complex with Smad4. Increased nuclear retention of an activated Smad2/3-Smad4 complex facilitates interaction with other transcription factors / co-activators / repressors, to modulate the transcription of target genes that either control atrophy or block hypertrophy (799, 889).



The other ligand group comprising BMP/GDF members preferentially binds to a combination of type II receptor that include BMP type II receptor (BMPRII), ActRIIA and ActRIIB, before promoting recruitment of type I receptors such as BMPRIA (ALK3), BMPRIIB (ALK6) and ActRIA (ALK2). These ligand/TypeII/TypeI receptor complexes promote phosphorylation and heterotrimerisation of Smad1/5/8 with Smad4 to effect transcriptional upregulation of still unidentified hypertrophy genes and suppression of atrophy-related genes such as MUSA1 (800, 950).

Myostatin is the most famous member of this superfamily in muscle field because of the profound hypermuscularity of Myostatin knockout mice (629, 630). Despite the observation that myostatin inhibitors are sufficient to promote muscle growth in mice and humans (63), Myostatin does not seem to be involved in the onset of sarcopenia. Indeed, several recent studies reported no changes or even decreased myostatin levels in serum of elderly people (260, 720, 745, 795, 804) (**Fig. 18**). Consistently, myostatin transcript and protein did not change in muscle of both old physical active and inactive people (795, 927, 930).

Another TGF $\beta$  family member that recently attracted the attention as a potential culprit of sarcopenia and frailty syndrome is GDF11. GDF11 and myostatin are very similar in aminoacid sequence and structure and can be confused by each other when antibodies are used to check their expression level (260, 804). Despite an initial observation suggesting that GDF11 expression declines with ageing in mice (581), recent data in humans and rats do not confirm this finding and actually show the opposite. Indeed, GDF11 blood levels have been reported to increase during aging and importantly, to correlate with frailty syndrome (260, 804) (**Fig. 18**).

Very few data are available on BMPs and therefore, the role of this pathway in sarcopenia is unknown. Recent data have shown a dominant anabolic function of BMPs over myostatin in mice (800, 950) and a transcriptomic study have found a suppression of BMP5 in elderly subjects (930) suggesting that this signaling might play a role in the control of muscle mass during aging. Interestingly, MUSA1, a BMP target that is negatively regulated, has been reported to increase during aging in rats (416) (**Fig. 18**). Further studies are required to identify the members of the TGF $\beta$  superfamily that are involved in sarcopenia and frailty.

### ***Protein breakdown***

The discovery of the atrophy-related genes was a major advance in the field of muscle wasting. The comparison of the gene expression profiling of muscles from tumor bearing mice with fasted, diabetic and uremic animals led to the identification of a common set of genes that were named atrogenes. Since all these diseases have muscle atrophy in common, these genes are thought to be important for protein loss. Among these atrophy-related genes there were several belonging to the major cellular degradation systems, the ubiquitin proteasome and autophagy-lysosome. The transcriptional-dependent induction of these atrogenes is required to replenish the enzymes/proteins that are lost during the enhanced protein breakdown. The ubiquitin ligase, the rate-limiting enzyme during the ubiquitination process, undergoes auto-ubiquitination (80) therefore, an increased ligase activity during catabolic conditions will inevitably amplify their auto ubiquitination and their proteasomal-dependent degradation. Therefore, the transcriptional upregulation is particularly important mostly to replenish the loss of the ubiquitin ligase proteins. Similarly, the autophagy-related proteins (LC3, Gabarap, p62, NBR1, Optineurin, PINK1, Parkin, BNIP3 and BNIP3/Nix),

which are critical for membrane commitment, cargo delivery and selective removal of damaged mitochondria, are entrapped into the autophagosome when the vesicle is formed and therefore are destroyed upon fusion of autophagosome with lysosome. This concept should be considered when protein expression does not match with transcript levels. Finally, recent findings suggest that the signalling pathways that control muscle loss vary between different muscles (103).

#### Ubiquitin-proteasome system

Proteasome activity was found decreased (36, 168, 285, 737), unchanged (90) or increased (16) in aged rats. However, since proteasome breaks ubiquitinated proteins, the *in vitro* measurement of proteasome activity does not mirror the level of protein breakdown inside muscles that instead is dependent on protein ubiquitination. In fact, in the ubiquitin-proteasome system, proteins are targeted for degradation by the 26S proteasome through covalent attachment of a chain of ubiquitin molecules. Different classes of enzymes, named E1, E2, and E3, are involved in protein ubiquitination. The E1 is the ubiquitin activating enzyme E1 and is encoded by only one gene. The ubiquitin ligase enzyme, or E3, binds the protein substrate and catalyzes the movement of the ubiquitin from the E2 enzyme to the substrate. This is the rate-limiting step of the ubiquitination process, which affects the subsequent proteasome-dependent degradation. Once the protein is ubiquitinated it is docked to the proteasome for degradation, unless the polyubiquitin chain is removed by the de-ubiquitinating enzymes. Different E2-E3 pairs function in the degradation of different proteins, and the specificity of the E3s for specific groups of proteins provides exquisite selectivity to this degradation process. The human genome contains around one hundred E2 and almost one thousand E3. This great heterogeneity is involved in the precise regulation of different cellular processes. The content of different E2s and E3s varies between tissues and with different physiological conditions, but is still not known which specific E2s and E3s normally work in muscle.

The two most induced atrogenes belong to the ubiquitin ligases superfamily and were named atrogen1/Mafbx and MuRF1. While these E3 enzymes are induced at the transcript level in many muscle wasting condition, they are not upregulated in muscles of aged rats or elderly people (795, 929, 943) and in some studies were found to be suppressed (223, 242, 256). Consistent with the concept that sarcopenia is caused by an impairment of the degradation systems more than an hyperactivation, atrogen-1 and MuRF1 knockout did not show any beneficial effect in terms of muscle mass and force during aging. Importantly, both these knockout are weaker than age matched controls (795) and life span of atrogen1 deficient mice is shortened due to the onset of hypertrophic cardiomyopathy in adulthood (987). Interestingly, MuRF1 knockout mice experience a higher decay in muscle strength during aging compared to controls despite the fact that muscle mass is preserved (413, 795). A novel ubiquitin ligase named MUSA1, which was recently shown to be necessary for muscle loss after denervation (800), is upregulated during aging in rats (416). Whether MUSA1 contributes to sarcopenia remains to be addressed in future studies. In conclusion, the literature suggests that the ubiquitin-proteasome system is not enhanced during aging but instead is reduced/impaired or unaffected. Therefore, there is no evidence that sarcopenia is caused by excessive protease-dependent degradation and data suggest the contrary.

#### Autophagy-lysosome system

Autophagy is an evolutionarily conserved catabolic process through which damaged organelles and macromolecules are degraded and recycled within the cell. There are three different forms of autophagy named microautophagy, chaperone mediated autophagy and macroautophagy. A detailed description of the different types of autophagy and their role in muscle has been recently reviewed (82). The role of microautophagy and chaperone mediated autophagy in muscle and in sarcopenia remains to be unraveled. Instead macroautophagy, hereafter named autophagy, has been shown to be critical for muscle maintenance. Moreover, an extensive literature from yeast to mammals has linked tissue aging with the decline of autophagy (512, 594). The process of autophagy proceeds through several mechanistically distinct steps that include: i) induction, ii) membrane commitment and elongation to form the autophagosome, iii) engulfment of cellular components, the cargo, iv) autophagosome–lysosome fusion, v) degradation of sequestered components and recycling of aminoacids, lipids and glucose, vi) lysosomal rejuvenation and biogenesis. Each of these steps involves a number of conserved autophagy-related genes (Atg) that are transcriptionally regulated, reviewed in (512). Some of these Atg especially the ones related to membrane commitment and elongation (LC3, Gabarap), cargo recognition (p62, Bnip3) for selective degradation and lysosomal enzyme (CathepsinL) belong to the atrogenes and are under the regulation of FoxO transcription factors (635). Although autophagy was initially considered a non-selective degradation pathway, the presence of selective forms of autophagy is becoming increasingly evident. Indeed, autophagy can trigger the selective removal of either specific organelles such as mitochondria or proteins including protein aggregates. The specificity is elicited by some adaptor proteins such as p62, Nbr1, Optineurin, Bnip3 that simultaneously bind autophagosomes via LC3 and the dysfunctional organelles/damaged proteins/protein aggregates that usually, but not exclusively, are labeled by a poly-ubiquitin chain. In muscle disease an impairment of the selective removal of dysfunctional mitochondria, named mitophagy, results in an enhancement of aspecific autophagy that contributes to muscle loss and weakness (599). Interestingly, autophagy related genes and especially mitophagy-related genes are downregulated in elderly frail women (242) suggesting an impairment of the selective mitochondrial removal. Importantly, muscle specific and inducible muscle specific autophagy deficient mice display a muscle phenotype that recapitulates all the features of sarcopenia such as increased oxidative stress, accumulation of dysfunctional mitochondria, neuromuscular junction fragmentation and myofiber denervation, muscle atrophy and severe weakness that lead to a premature death (138, 580, 616). In aged rats, LC3II, a marker of autophagosome, and p62, the autophagy adaptor that is consumed by autophagosome-lysosome fusion, have been reported to accumulate in muscle (789, 941, 958) suggesting that autophagosome clearance (autophagy flux) is reduced during aging. Other studies have found reduction of LC3II, accumulation or no changes of p62 and/or reduction in the regulatory proteins like beclin1 suggesting that autophagy induction is impaired (138, 436, 627, 783). The reduction of autophagy leads to accumulation of dysfunctional mitochondria that generate reactive oxygen species (ROS). The increased oxidative stress contributes to precocious senescence of muscle stem cells and weakness in adult myofibers (138, 141, 580). Importantly, despite the finding that oxidative stress does not affect muscle atrophy, ROS-dependent oxidation of myosin and actin greatly reduces force production and contributes to aging-dependent weakness (138, 561, 585). In humans, a decrease of Atg7, the rate limiting enzyme of autophagosome formation, and LC3II has been shown in muscle biopsies of sarcopenic people confirming the concept of an aging-dependent autophagy decline (138). However, exercise and caloric restriction are sufficient to reactivate this

system in muscles of elderly rodents and humans (138, 940, 958, 976). Finally, reactivation of autophagy in aged mice by expressing Atg7 in skeletal or cardiac muscle ameliorates muscle mass and cardiac function (73, 138). In conclusion, most of the studies agree with an aging-dependent suppression of autophagy and in particular mitophagy in muscles supporting a direct contribution of an autophagy defect to sarcopenia.

### **Exercise and caloric restriction in sarcopenia affects similar but not identical pathways to improve muscle function**

Exercise and caloric restriction have been repetitively shown to improve life span and attenuate aging-dependent disease onset and organ functional decline. Both these conditions impinge on the same molecular patterns that control glucose and lipid homeostasis, proteostasis, mitochondrial function/shape and energy balance. Molecularly, these life styles activate, in muscle, the energy stress sensor AMPK to improve glucose homeostasis, ameliorate mitochondrial function/biogenesis via transcriptional- and non transcriptional-dependent program, activate FoxO transcription factors to induce genes related to proteostasis (552, 977). However, despite these similarities it has been recently reported that exercise differs from caloric restriction. While both improved glucose uptake only exercise ameliorates mitochondrial content, mitochondrial electron transport, fatty acid oxidation (633) and supercomplex formation (351). In fact, chronic caloric restriction does not induce transcriptional-dependent changes and mitochondrial biogenesis but it minimizes oxidative damage to DNA and proteins by decreasing oxidant emission (511). Thus, exercise seems to be more effective than caloric restriction in ameliorating muscle function of sarcopenic muscles. Consistently, it has been recently found that while resistance exercise training improves muscle force of obese aged persons both at the single myofiber level and in whole muscle, caloric restriction does not enhance such improvements (923). The lack of an additive effect may be related to the behavioral changes induced by chronic caloric restriction that cause high burst of physical activity within short time. This high intensity physical activity may account for the beneficial effect of the diet in terms of muscle force/performance and would explain why caloric restriction does not improve the exercise-mediated effect (914). An alternative explanation considers the fact that chronic caloric restriction does not enhance the nutrient- and insulin/IGF1-dependent circadian rhythms, which has been found to be critical to counteract sarcopenia and aging (697). Time-restricted feeding is much more potent than chronic caloric restriction in enhancing insulin and nutrients oscillation and, consequently, FoxO and mTOR activity that, ultimately, modulates autophagy genes expression. This periodic fluctuation of genes and proteins better sustain autophagy in aged mice and adapt the organism to the daily metabolic changes by preventing aging-dependent energy imbalance, dyslipidemia and glucose intolerance (611). These findings suggest that exercise or intermittent fasting by acting as acute stress stimuli are more potent in preserving muscle function and counteracting sarcopenia. Several recent studies show that the beneficial effects of physical activity impinge on mitochondrial quality control system and mitochondrial energetics (219, 236, 988). In fact, there is a close relationship between muscle strength, walking performance and mitochondrial energetics (236, 988) so that mitochondrial dysfunction is considered a predictor of sarcopenia (219, 870). For instance, mitochondria ADP sensitivity is impaired during aging leading to an increased mitochondrial ROS emission and oxidative stress (401). Mitochondrial function is controlled by several mechanisms including mitochondrial dynamics (e.g. fusion and fission), mitophagy and mitochondrial proteostasis. In sarcopenic people the genes and proteins that control mitochondrial

fusion and fission are dramatically suppressed (219, 416, 870) as well as autophagy (138) leading to mitochondrial dysfunction and oxidative stress. Acute and muscle specific inhibition of the fusion protein OPA1 or autophagy is sufficient to induce a sarcopenia and premature tissue senescence in adult mice (138, 870). Exercise counteracts most of these aging-related mitochondrial quality control changes by reactivating the transcription and translation of fusion/fission and autophagy-related genes. Physical activity also improves ADP sensitivity and mitochondrial bioenergetics normalizing the fraction of electron leak to ROS and oxidative stress (401) and activate transcriptional-dependent programs to promote mitochondrial fusion and optimize mitochondrial respiration. The transcriptional dependent action are mediated by at least three different mechanisms which include: 1) the transcriptional co-activator PGC1 $\alpha$ , which is a physical activity sensor being upregulated by exercise and suppressed by sedentary life, 2) the PGC1 $\alpha$  downstream targets such as PPAR $\gamma$  and  $\delta$ , and 3) the transcription factor TFEB, which is activated by calcium-calcieneurin pathway and controls PGC1 $\alpha$  expression, glucose homeostasis and metabolic flexibility during training (604). The metabolic changes of these factors not only affect muscle, but they systemically reverberate modulating the function of multiple tissues. For instance, the expression of PGC1 $\alpha$  in muscle of aged mice is not only sufficient to improve mitochondrial function and muscle endurance but also to maintain balance and motor coordination (328). The beneficial effects of exercise are not only transcriptional dependent. In fact, exercise prevents also the activation of DNA-PK, a protein kinase that senses DNA double-stranded breaks. During aging DNA-PK is more active in muscle and inhibits AMPK function by blocking the chaperone HSP90 $\alpha$  (695). Inhibition of DNA-PK increases AMPK, prevents the decline of mitochondrial function and of physical activity in middle-age mice and protects against type 2 diabetes (695). However, the different modalities of exercise do not impinge on the same molecular patterns and therefore, they slightly diverge in terms of functional effects in elderly people. While aerobic (high Intensity aerobic interval), resistance or combined (aerobic and resistance) exercise induce transcriptional adaptations, enhance insulin sensitivity and lean mass in aged people, only aerobic or combined improved mitochondrial function and aerobic capacity while only resistance or combined increases muscle strength (710, 764). Interestingly, only aerobic exercise induced the biggest changes of gene expression regardless of age while both aerobic and resistance training enhanced proteins involved in the translational machinery irrespective of age. The effect on protein synthesis by resistance exercises are potentially mediated by a relocalization of mTOR which moves from lysosome to plasma membrane in regions close to the blood capillary endothelium where it interacts with eIF3f and Rheb, two positive modulators of the translation machinery (841). Since, exercise and nutrients affect the same signaling pathway, although via slightly different mechanisms, they potentiate each other because of their synergistic action. Protein supplementation in combination with resistance exercise reduce aging-related muscle loss probably because both activate mTOR and translation machinery (97, 268). In the future, it would be interesting to combine exercise with fasting-mimetics to determine whether they can synergize. For instance it has recently been shown that chronic administration of Nicotinamide monucleotide that enhances NAD $^{+}$  production, improves mitochondrial respiration and prevents aging-associated gene expression changes in muscle (637). Interestingly, NAD $^{+}$  level typically increases during energy stress conditions and is a potent activator of sirtuins (SIRT) and especially of SIRT1, a longevity-related factor that controls the acetylation and activation status of many stress-dependent genes such as PGC1 $\alpha$  and FoxOs (345, 986).

#### 4. REGULATION OF MUSCLE CONTRACTION AT THE MUSCLE, MOTOR UNIT, MUSCLE CELL AND MOTOR PROTEIN LEVELS

##### ***Muscle function***

Since Quetelet's (734) pioneering study almost two centuries ago, aging-related changes in human muscle function have for many years received only sporadic scientific interest. However, the demographic development in modern society, rapidly increasing health care costs secondary to aging-related impairment in motor function, and concomitant consequences for quality of life and aging-related morbidity have triggered an increased scientific attention related to aging in general and the effects of aging on the motor system in particular. It is of interest to note an improved maintenance in hand-grip and back muscle strength in more recent studies compared with earlier studies, presumably related to the improvement of general living conditions, accounting also for the increased life expectancy (for refs see (514)). However, as discussed above (Experimental models, Muscle specific differences), hand-grip strength measurements are of limited importance in predicting fall and fall-related injuries and a maintained hand-grip strength has been reported up to the age of 60 (for refs see (514)), while profound changes in body composition with an increase in body fat and decrease in muscle mass resulting in decreased muscle strength and mobility have been reported in association with advancing age (57, 116, 189, 289, 307, 525, 671, 982).

The loss of maximum force in old age is related to quantitative changes in contractile material, i.e., a total loss of muscle fibers related to motor unit loss and incomplete reinnervation as well as muscle fiber atrophy (see (520)). In humans, the loss becomes prominent first at the end of the fifth decade and lower body muscle mass is preferentially affected during aging (427, 525). At the muscle fiber level, an atrophy is observed in old age and muscle fibers expressing fast myosin isoforms show a more pronounced decline in cross-sectional area than slow muscle fibers as originally reported four decades ago in humans (539, 886) and confirmed in multiple later studies (102, 182, 529, 554). More recently, a gender-related difference was reported regarding the preferential atrophy in fibers expressing fast myosin isoforms, i.e., the preferential type II fiber atrophy was only observed in men and not in women despite a significant aging-related decline in maximum voluntary force independent of speed of movement and gender (985). Further, the preferential type II fiber atrophy in sedentary men was diminished after a 3-month physical exercise intervention improving muscle strength irrespective subject age, but there was a persistent aging-related decline in maximum voluntary force irrespective speed of movement (517). Thus, it is unlikely that the preferential atrophy of the fibers included in fast-twitch motor units with a high threshold for activation plays a dominant role in the aging-related loss in muscle force. Based on whole muscle function measurements in humans, it remains controversial whether skeletal muscle undergoes qualitative aging-related changes in force generation capacity, i.e., a decreased maximum force normalized to the cross-sectional area of the contractile material (specific tension) or not (for refs. see (116, 520, 603)). However, the more pronounced decline in muscle strength at fast compared with slow speeds of movement indicates that the loss in force is not only due to loss in muscle quantity (525, 985). Several attempts have been made to normalize *in vivo* muscle force to the average muscle cell cross-sectional area in muscle biopsy specimens or total muscle cross-sectional area determined

from limb circumference, ultrasound, CT- or MRI-scans (57, 116, 289, 307, 525, 671, 982). However, these estimates of contractile material cross-sectional area do not take into account the gradual replacement of contractile proteins by connective tissue reported in old age in rodents (15, 23, 311, 482, 483, 523, 643) and in humans (301, 417). Cross-sectional muscle areas and muscle weights will accordingly overestimate the amount of contractile material in old age (520). Further, analyzes of muscle cell cross-sectional areas do not take into account the relative contribution of different muscle cell types during maximum voluntary contractions and aging may affect specific types of muscles, motor units and muscle cells differently (for refs. see (514, 520)).

In addition to the muscle atrophy and decreased force, slowing of movement is also a hallmark of sarcopenia with significant negative consequences for fall and fall-related injuries in old age due to an impaired capacity to quickly correct for loss in balance. However, the slowing of movement may be secondary to multiple factors ranging from the initiation of contraction in the central nervous system to mechanical properties of joints and tendons. However, the difficulty in rapid recovery from an impending fall in old age is primarily due to inherent aging-related changes in muscle tissue itself (809). At the muscle level, a fast-to-slow myosin isoform (fiber type) switching have been reported in both humans and in experimental animal models (see above). The fast-to-slow fiber type transition during aging may contribute to the slowing of movement as well as decline in maximum force since fast muscle fibers are considered to have a higher force generation capacity than slow fibers. However, the relative changes in fiber type proportions or myosin isoform expression in aging human muscle are relatively small and it may be questioned whether these changes will have a major impact on overall motor performance.

### Motor unit function

Decreased speed of contraction and loss of force represent significant phenotypic characteristics of sarcopenia. At the single motor unit level, speed of contraction refers to the duration of the isometric twitch, i.e., the duration of contraction and half-relaxation times and both being highly dependent on motor unit type with fast-twitch units having shorter contraction and half-relaxation times than slow-twitch units irrespective age (258). During aging, there is a slowing of the isometric twitch in various mammalian species, including humans (23, 66, 126, 128, 194, 258, 360, 523, 735) and the reduced speed of contraction precedes the aging-related loss in muscle mass (23, 523). An aging-related loss of fast-twitch motor units or altered properties of the contractile machinery, or both, have been suggested to underlie the slowing of the isometric twitch in old age (23, 128, 258, 523, 666, 709, 735, 861). The slowing of the isometric twitch observed in both fast- and slow-twitch muscles may accordingly be related to an aging-related shift within fast-twitch motor units or from fast- to slow-twitch motor units. However, the slowing of the isometric twitch at the motor unit level, irrespective motor unit type, indicates significant aging-related changes in the function of the contractile apparatus and are forwarded as the dominant mechanism underlying the decreased speed of contraction (258, 521, 537). A number of different factors may influence the duration of the isometric twitch ranging from the transmission of the action potential, the  $Ca^{2+}$  handling by the sarcoplasmic reticulum (SR),  $Ca^{2+}$  binding to regulatory proteins and the initiation of contraction to the function of contractile proteins. The capacity of the SR for  $Ca^{2+}$  release and recapture and the expression and function of the contractile proteins are the two key factors determining contractile speed. In adult mammalian motor units, there is a close co-ordinated expression of SR and

contractile proteins (104, 245, 489, 791), but this close co-ordination may be lost under different experimental conditions (104, 294) as well as during aging (791). A strong relationship has been reported between the contraction time of the isometric twitch and the SR  $\text{Ca}^{2+}$  uptake activity (104, 383), suggesting that altered SR properties underlie the slowing of the isometric twitch in old age. This is supported by the impaired structure, function and biochemical properties of the SR in old age as well as the correlation between the prolonged isometric twitch in single motor units and the impaired SR  $\text{Ca}^{2+}$  uptake activity in single membrane permeabilized muscle fibers from the same muscle (195, 537, 952). In this context it is interesting to note that the decreased speed of isometric contraction observed in cardiomyocytes from old rats has been reported to be coupled to a diminished rate SR  $\text{Ca}^{2+}$  uptake (501).

In glycogen depleted single motor units characterized according to type and motor unit fiber morphology, maximum twitch and tetanus forces have been reported to be maintained or increased in moderately old rats (20-24-month) (258, 521), but decreased in very old 27-month old rats (444). The increased or maintained maximum force in moderately aged animals at the same time as total muscle fiber number and muscle force are lower than in young animals is suggested to be secondary to the increased number of motor unit fibers in individual motor units caused by the ongoing denervation-reinnervation process related to the aging-related  $\alpha$ -motoneurone and motor unit loss (26, 258, 520, 521, 524). The decline in single motor unit force in the very old (27-month old) rats coincides with ultrastructural evidence of myofibrillar loss and increased myofibrillar space (22) in 27-month old rats as well as the preferential loss of the molecular motor protein myosin observed in very old rats and humans (183, 875). Specific tension measurements in glycogen depleted motor units is complicated by the impact of pennation angle on the force recording, all muscle fibers in a motor unit can typically not be identified in one single muscle cross-section and additional aging-related changes in neuromuscular transmission may introduce incomplete glycogen depletion of all motor unit fibers in old age. Single muscle fiber preparations offer a superior alternative for specific force measurements, i.e., a preparation where fiber orientation, sarcomere length and temperature can be controlled during maximum force recordings.

### Single muscle cell contractility

The maximum velocity of unloaded shortening ( $V_0$ ) varies considerably between different muscle cells and  $V_0$  is often used to characterize and separate muscle cells, motor units and muscles into different types (163). Muscles develop their maximum power at a shortening velocity of approximately one-third of  $V_0$  and  $V_0$  is therefore considered to be one of the most important design parameters of skeletal muscle (see (392, 625, 768)). The ability to generate power optimally over a wide range of movements is important for avoiding falls in the elderly and myosin isoforms are the major determinants for the wide range of shortening velocities under which muscle cells are operating. Studies at the single muscle fiber as well as motor protein levels have shown that there is a close relationship between  $V_0$  and myosin heavy chain (MyHC) isoform composition (see (650)). A modulatory influence of essential myosin light chain (MyLC) isoforms on  $V_0$  have a been documented in single skinned muscle fibers from small mammals such as the rat and rabbit (94, 348, 563, 859) as well as in *in vitro* motility assays using avian myosins (588). In humans, on the other hand, the modulatory influence of MyLC isoforms on  $V_0$  has not been confirmed (526, 529, 530, 534), indicating a species difference in the regulation of muscle contraction.



Whole muscle or fiber bundle preparations are of limited usefulness when studying  $V_0$  since the behavior of individual muscle cells is obscured by several independent factors, such as: (a) differences in intramuscular fiber orientation, (b) differences in the mechanical leverage provided by the bony anatomy of the joint, (c) the elasticity of the muscle and the muscle tendons, (d) patterns of motor unit recruitment, and (e) activation of antagonist muscles. Even if these difficulties were not present, multicellular preparations have the disadvantage that the roles played by different muscle fiber types in overall contractility are difficult to evaluate (see (392, 397, 398)).

The confounding influences of protein heterogeneity between muscles fibers or intercellular connective tissue in multicellular preparations are overcome in single muscle fiber preparations, allowing detailed studies of regulation of muscle contraction in a muscle fiber with an intact myofilament lattice. In humans, the skinned fiber preparation offers the advantage that several muscle cells can be studied from a single biopsy under near physiological conditions (292, 518, 534). The ability to investigate muscle fibers of different type (MyHC isoforms) is of crucial importance in studies on aging-related changes in muscle contractility since aging appears to have a differential influence on different muscles and muscle cell types. Further, the skinned fiber preparation has been found very useful for the study of regulation of muscle contraction under controlled conditions in human muscle fiber segments in both normal (292, 518, 532-534, 538, 848) and pathological muscle tissue (305, 508, 528, 531, 536, 560, 670).

Aging-related changes in regulation of muscle contraction at the single muscle fiber level have been investigated in both humans (181, 190, 220, 477, 529, 985) and rodents (218, 252, 463, 540, 781, 794, 842, 874, 984) and a decline in  $V_0$  in muscle cells expressing the  $\beta$ /slow (type I) and fast (type II) MyHC isoforms have been observed in both humans (102, 181, 190, 477, 529, 985) and rodents (218, 463, 540, 874, 984). As discussed above, there is a close relationship between  $V_0$  and MyHC isoform composition at the single-fiber level and the composition of MyHC isoforms determines the myosin ATPase activity (see (650)) irrespective species and essential MyLC isoforms have a modulatory influence on  $V_0$  in muscle fibers expressing fast MyHC isoforms (94, 348, 563, 859), at least in small mammals. It cannot be completely ruled out that our knowledge of MyHC isoforms is incomplete and that novel MyHC isoforms are expressed in old age causing the decreased speed of shortening at the single muscle fiber level. This is supported by the identification of multiple  $\beta$ /slow (type I) MyHC isoforms in mammalian skeletal muscle by using S1 nuclease mappings and immunocytochemical and electrophoretic techniques (280, 315, 367, 412, 425) as well as the existence of additional MyHC isoforms in fibers so far considered homogeneous regarding their MyHC isoform composition (see (807)). To date, eight different MyHCs have been identified in mammalian striated muscles, *i.e.*,  $\beta$ /slow (I),  $\alpha$ -cardiac, embryonic, fetal, IIa, IIx (IIc), IIb and a super-fast extra ocular myosin isoform. A ninth slow MyHC isoform has been identified in mammalian extra ocular and intrafusal muscle fibers which is antigenically different from the  $\beta$ /slow or type I MyHC observed in limb and trunk muscles (see (399, 807)). Furthermore, the observation by Smerdu and co-workers (833) of the presence of additional fast MyHC components in adult human skeletal muscle cells is of specific interest in this context. The sarcomeric MyHCs are encoded by three highly conserved multigene families and the human MyHC gene family includes several genes that code distinct isoforms. Our knowledge of the MyHC genes may not be fully understood (see (708)) and there are indications of as many as 31 MyHC genes in the chicken (762). Although relative changes in the MyHC isoform expression has been reported during the aging process in

mammalian skeletal muscle, including humans, there is to our knowledge no results demonstrating an increased expression of specific MyHC isoform in old age not expressed in adult skeletal muscle.

Aging-related changes in the molecular motor protein myosin can affect contractile speed by different mechanisms: (a) The reduced rate of protein synthesis in old age will affect enzymatic activities negatively (746). In accordance with this, the decreased rate of MyHC synthesis in old age (38) may have a negative effect on the myosin ATPase activity resulting in a slowing of contractile speed. (b) In adult laboratory mammals, MyLC isoform composition has been shown to have a modulatory impact on the primary influence of MyHC isoform composition on  $V_0$  (94, 348, 563, 589, 650) and the aging-related slowing in muscle cells may accordingly be related to an altered modulatory influence of MyLC isoforms. (c) Finally, aging-related changes in other thick and thin filament proteins or in cytoskeletal elements cannot be completely ruled out as a mechanisms underlying the aging-related slowing in contractile speed.

Proteins undergo a number of posttranslational modifications during aging which influence enzymatic activity, stability and digestibility (see (646)). Aging-related myosin posttranslational modifications induced by e.g. reactive oxidative species may accordingly have significant impact on regulation of muscle contraction. Peroxynitrite ( $\text{ONOO}^-$ ), formed under conditions of simultaneous generation of superoxide and nitric oxide, represents a potentially harmful reactive oxygen species due to its high reactivity with lipids and proteins leading to oxidation, hydroxylation and to ortho-nitrotyrosine (eg. (728)). Thus, reactive oxygen species have been forwarded as important factors inducing muscle cell protein modifications which affect skeletal muscle function (472, 750). During aging, 3-4 fold higher amounts of nitrotyrosine have been reported on the Ca-ATPase of the sarcoplasmic reticulum in muscle cells expressing the  $\beta$ /slow MyHC isoform (952), confirming periodical exposure of skeletal muscle to peroxynitrite. Thus, the slow Ca-ATPase isoform appears to be more susceptible than the fast Ca-ATPase isoform to this modification, possibly related to a less efficient antioxidant defense system of aged organisms (953)

Non-enzymatic glycation of proteins has been regarded as one of the biochemical basis underlying the pathophysiology of diabetes and aging (115). Non-enzymatic glycosylation (glycation) of proteins occurs by a chemical reaction of reducing sugars with primary amino groups in proteins to form Schiff's base linkage (925). That is, the aldehyde groups of free, unbound sugars react with free amino groups of proteins, forming Schiff's bases, which further undergo various rearrangements to generate advanced glycation end products (502). This reaction of proteins is a common post-translational modification of proteins. Glycation of myosin has been sporadically reported by workers in different muscle preparations, viz., actomyosin (925) and myosin ATPase (862). The effects of myosin posttranslational modifications during aging and the effects on myosin function are discussed below.

The association between the aging-related  $V_0$  decline and MyLC isoform shifts has been forwarded as a mechanism underlying the aging-related slowing within specific muscle fiber types (463, 681). This is confirmed by the parallel fast-to-slow transition of the regulatory MyLC and MyHC isoform expression (681) and the shift in essential MyLC isoform expression in single muscle fibers from old rats expressing fast MyHC isoforms (463). This is further supported by the attenuation of the aging-related slowing after recombinant adenovirus gene transfer of MyLC<sub>3f</sub> (463). However, the aging-

related essential MyLC shift and the attenuation of the decreased speed of contraction was mainly confined to muscle fibers expressing the type IIb MyHC isoform (463). This is an interesting finding, although muscle fibers expressing the IIb MyHC isoform are not expressed in normal skeletal muscle from larger mammals such as humans and the slowing in muscle fibers expressing the  $\beta$ /slow (type I) MyHC is not accompanied by a MyLC isoform switching (218). Further, the modulatory influence of the MyLC<sub>3f</sub> on  $V_0$  observed in young animals was not confirmed at the single muscle fiber level in old animals and the coordinated expression of MyHC and essential MyLC isoform expression observed in young animals were not observed in old age (563). At the filament level this may be secondary to the independent incorporation of MyHCs and MyLCs into the thick filament backbone and the differential turnover rates of MyHC and MyLC isoforms (442). This is of specific interest since a loss in the coordinated expression of MyHC isoforms and mitochondrial enzyme activities (521), and between MyHC isoforms and SR proteins (791), and between MyHC and MyLC isoform expression (563) have been reported in rodents and humans. Thus, the loss in the coordinated expression of contractile, SR and mitochondrial proteins may play a more important role for the overall aging-related impairment of muscle function than changes in individual proteins.

As discussed above, other factors than quantitative changes related to total muscle fiber number and muscle fiber size may play a significant role for the aging-related decline in muscle force. A significant dissociation between muscle mass and strength have been reported during aging, hormone treatment, exercise and inactivity (reviewed in (780)) and suggested to be secondary to central factors rather muscle specific changes (780). Central factors may play a significant role for aging-related changes in motor function, but as mentioned above factors distal to depolarization of the muscle membrane are the primary determinants underlying the impaired capacity to correct an impending fall in old age with consequences for falls and fall-related injuries (809). Furthermore, studies showing discrepancies between muscle mass and function during aging, hormone treatment, exercise and immobilization are frequently based on measurements of muscle mass using imaging methods such as computerized tomography, force measured during maximal voluntary contractions under isometric or isokinetic conditions and central activation estimated by measuring the amplitude of the surface EMG, i.e., methods which are all associated with significant methodological limitations. As an example, long-term immobilization by bedrest shows modest decline in muscle mass and a greater decline in muscle strength (69) together with concomitant altered surface EMG amplitudes, this may be interpreted as central factors playing a dominant role for the inactivity induced decline in muscle function (69). However, when the intrinsic properties at the single muscle fiber level were evaluated in the same individuals, a significant decline in muscle fiber force generation capacity (maximum force normalized to muscle fiber cross-sectional area, specific force) were observed which were closely associated a significant loss of contractile proteins which exceeded the decrease in muscle fiber size (527). Thus, the study of aging-related loss in muscle function needs to go far beyond the study of muscle mass and voluntary force and focus specifically on regulation of muscle contraction at the cell and subcellular levels.

Single muscle fiber results indicate intrinsic changes in regulation of muscle contraction with a decline in single muscle fiber specific force (maximum force normalized to muscle fiber cross-sectional area) in old age in both rodents and humans (102, 190, 309, 335, 529, 532, 586, 587, 874, 883, 991). The decreased specific force in old age has been attributed to both quantitative changes in the number of force generating cross-bridges (183, 190, 875) as well as the function of individual

cross-bridges (561, 586, 587, 991). In line with this, a preferential loss of the molecular motor protein myosin has been observed during aging in both men and women (183) as well as in rodents (874). However, other intracellular factors prior to activation of contractile proteins have been suggested to play a more important role for the aging-related decline in specific force than quantitative and qualitative changes in contractile proteins. Events prior to activation of the contractile proteins are affected by aging such as neuromuscular transmission (discussed above) and from muscle membrane depolarization to the  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR) also known as the excitation-contraction (EC) coupling (149). The events leading to SR  $\text{Ca}^{2+}$  release includes multiple steps involving the dihydropyridine receptor (DHPR) L-type  $\text{Ca}^{2+}$  channel and the SR ryanodine receptor (RR) for  $\text{Ca}^{2+}$  release (748). The depolarization of the muscle membrane and the propagation of the action potential along the muscle fiber and deep into the fiber via the transverse tubular system induces a conformational change in the DHPR acting as a voltage sensor, activation of the RR and  $\text{Ca}^{2+}$  release from the SR and activation of the contractile proteins. In addition to receiving a signal from the DHPR, the RR also generates retrograde signaling to the DHPR of importance for channel activity (51, 660). In old age, peak intracellular  $\text{Ca}^{2+}$  transients have been reported decreased (270, 336) due to an impaired SR  $\text{Ca}^{2+}$  release (429), resulting in decreased muscle fiber force generation capacity (270, 752). Changes in EC coupling in old age has been shown to be associated with the aging-related denervation process suggesting that the motoneurone loss may underlie the alterations in EC coupling in old age (700).

### Myosin and actin interaction

The ability of skeletal muscle to generate force and motion can be attributed to the mechanical interaction between the two contractile proteins, myosin and actin. Different *in vitro* methods allow direct measurements of myosin-actin interaction in the absence of secondary influence from structural and regulatory proteins (370, 485, 818, 819). Since the introduction in the 1980s, the *in vitro* motility assay has been continuously improved. In the early studies, the movement of single fluorescently labeled actin filaments on a myosin-coated glass surface was followed in a flow cell where myosin had been extracted from muscle homogenates and randomly adhered to nitrocellulose coverslips. Recent improvements include measurement of average force per cross-bridge by measuring the deflection an ultracompliant glass microneedle to which an actin filament was attached (468). In the technically challenging optical trap system, the interaction between a myosin molecule and a single actin filament can be measured allowing force and displacement measurement with pN and nm precision, respectively (290).

In the study of aging-related changes in myosin function, the single muscle fiber *in vitro* motility assay offers the opportunity to conduct detailed studies of the catalytic properties (motility speed) and force generation capacity of specific myosin isoforms or combinations of isoforms extracted from a single muscle fiber segment with a known myosin isoform expression (403, 404, 406, 558-561, 740, 741). In addition, myosin function can be investigated in detail in any mammalian species including humans (559). The single fiber *in vitro* motility assay has become a critical method for the study of myosin function, *i.e.*, catalytic properties (motility speed) and force generation capacity, during aging and the viscous drag produced by the solution in the experimental chamber when fluorescent-labeled actin filaments are driven by myosin is negligible compared with the force produced by the motor protein. Therefore, *in vitro* motility speed is, under most conditions, a good

analogue for the maximum velocity of unloaded shortening ( $V_0$ ) in single cells measured with the slack test (see (402)). The aging-related slowing of  $V_0$  at the single muscle fiber level (see above) has been confirmed at the motor protein level in both rodents and humans in muscle cells expressing the type I and IIa MyHC isoforms (404, 405, 526, 561). According to X-ray diffraction single muscle fiber analyses, allowing detection of structural alterations of contractile proteins with high temporal and spatial resolution (481), filament lattice spacing was not altered in muscle fibers from old humans but actin-myosin lattice ordering was altered leading to a disrupted actomyosin interaction during muscle contraction in old age (561). This observation was supported by mass spectrometry analyses demonstrating posttranslational modifications of the myosin rod domain in old age, i.e., a region essential for the packing of myosin molecules into myosin filaments (127, 561, 676). Oxidative stress related to accumulation of reactive oxygen and nitrogen species and the compromised ability to handle this stress in old age is the most probable cause underlying the increased amount of protein modifications in old age (149).

In response to oxidative stress, aldehyde groups of reducing sugars react preferentially with free lysine residues to form Schiff's base adducts, which may undergo further Amadori rearrangements and free-radical-mediated oxidation to form irreversible advanced glycation end products (AGEs) (502). Non-enzymatic glycation of proteins is a common post-translational modification affecting the structure, function and digestibility of extracellular proteins and has been regarded as one biochemical basis underlying the pathophysiology of diabetes and aging (115, 502, 925). The effects of glycation of long-lived extracellular proteins, such as collagen, have been described in detail during aging (896). However, intracellular muscle proteins are also affected by glycation (743). This is of specific interest, since myosin is a long-lived protein, it has a decreased turnover rate in old age, and it is lysine rich in critical regions of the motor protein, i.e., the actin binding site and in the catalytic domain (39). In line with this, glycation of actomyosin (925) and myosin ATPase (862) have been reported in aging muscle. Furthermore, exposure of myosin isoforms to a reducing sugar results in a significant impairment of myosin function linked to structural modifications of myosin at the catalytic domain (740) and the antioxidant glutathione protects myosin from the negative effects of a reducing sugar on myosin function (742).

Thus, significant qualitative and quantitative changes take place at the motor unit, muscle fiber and motor protein levels during aging significantly affecting skeletal muscle structure and function some of which may be affected by intervention strategies.

## 5. INTERVENTION STRATEGIES

Aging is accompanied by an imperceptibly slow progression of deterioration of tissue and organ function that ultimately results in death. Skeletal muscle is, however, highly adaptable and interventions that reverse or delay the progressive aging-related loss of muscle function will extend the number of years with a good quality of life.

### ***Caloric restriction***

Caloric restriction is an effective means to increase the life span in a wide variety of organisms ranging from yeast to primates and probably even humans (235, 384, 934). Caloric restriction in progeric mice even led to a staggering 3-fold increase in life expectancy (934). This is the consequence of redirecting resources to maintenance rather than growth and reproduction (934) to overcome periods of diminished food supply that severely reduces the chances of survival of offspring (467). Among the adaptations is a shift from glucose to fatty acid metabolism and a reduction in IGF-I and insulin (384, 934). As IGF-I is important for muscle growth (269) one may expect that a caloric restriction-induced reduction in IGF-I has a negative impact on muscle strength, but no such effect was seen in caloric restricted mice (934). Even so, any negative effects may be more than compensated for by the reduced mitochondrial leakage and ROS production (384) resulting in reduced oxidative modifications as indeed seen in caloric restricted mice with a mutation in a DNA-repair gene (934). As seen in isolated mitochondria, the diminished mitochondrial ROS generation during caloric restriction may be a consequence of a reduced mitochondrial membrane potential caused by a non-esterified fatty acid-induced increase in protonophoric activity of adenine nucleotide translocase (32).

The reduced oxidative stress may also explain the absence of an aging-related increase in expression of genes involved in the stress response, allowing resources to be channeled to growth, as reflected by the shift in gene expression toward increased protein turnover and decreased macromolecular damage (547) during caloric restriction. This increased protein turnover, decreased macromolecular damage and reduced ROS production may well explain why caloric restriction was associated with a decrease in Hsp27 and the DNA repair enzyme oxoguanine glycosylase 1 in the ventral horn of the spinal cord (157).

The caloric restriction-induced reduction in DNA damage in mice with deficiencies in DNA-repair genes not only tripled their life expectancy, but also improved maintenance of motoneurons and full motor function (934). Also in normal mice, life-long caloric restriction attenuated motoneuron loss and decreased the abundance of pre- and post-synaptic abnormalities in 24-month-old mice (907). Part of the attenuated loss of motoneurons can be ascribed to reduced caspase-dependent apoptosis (157). It may well be that the caloric restriction-induced attenuation of motoneuron loss underlies the prevention of muscle fiber loss during aging in rats that had been subject to 40% caloric restriction since the age of 4 months (624).

Caloric restriction not only exerts its beneficial effects on skeletal muscle mass by attenuating the aging-related decline in motoneuron numbers and alterations in the neuromuscular junction, but also via reducing the low-grade systemic inflammation observed in rats (712). The reduction in low-grade systemic inflammation diminished apoptosis, at least in the fast superficial vastus lateralis muscle in the rat (712).

There are some potential pitfalls in applying caloric restriction to humans, such as lowered blood pressure, slower wound healing and a danger of malnutrition (235). In addition, indications in older people with sarcopenic obesity that caloric restriction may lead to undesired loss of muscle mass has led to the suggestion that 'weight loss by caloric restriction in individuals with sarcopenic obesity should likely be avoided' (570). Overall, however, these observations bear great promise for dietary restriction to prevent and maybe even reverse some of the aging-related changes in skeletal muscle.

They also highlight the potential detrimental effects of present-day calorie-rich diets in the developed world that may accelerate skeletal muscle aging. Indeed, there are indications that the aging-related rate of muscle wasting is faster in obese and overweight people (647), and, in contrast to the above reservation, that caloric restriction normalised the inflammatory profile in the adipose tissue of obese people (162).

### **Activity/inactivity**

While life-long caloric restriction appears to be a potent intervention to extend the life expectancy by a staggering 30% (235), the benefits are likely to become less when started later in life. In addition, continuing caloric restriction demands a great deal of will power and is in practice rarely, if at all, achievable. It is therefore desirable to explore other routes that can reverse or attenuate the aging-related decrements in muscle mass and function. In one study, the investigators showed with time-lapse imaging that 1-month wheel-running exercise reversed, similar to - though to a lesser extent than - life-long caloric restriction, some of the aging-related changes in the morphology of the NMJ in 22-month-old mice (907).

A striking characteristic of skeletal muscle is its ability to adapt to altered functional demands. Examples are the hypertrophy and associated increase in muscle strength in response to a repeated overload stimulus, such as delivered during a resistance-training program, and the increased aerobic capacity and fatigue resistance of a muscle after an endurance-training program or chronic electrical stimulation (790). Likewise, a reduced use of skeletal muscle, such as occurs during hospitalization, paralysis, or bed rest, leads to muscle atrophy, weakness and a reduction in the oxidative capacity and fatigue resistance of the muscle (203).

The effects of reduced use on skeletal muscle resemble those seen during aging (203) and the lower levels of physical activity and increased sedentary behavior in old age (418, 628, 967) may thus well be the prime cause of the aging-related loss of muscle mass and strength. Given these observations it is no surprise that significant attention has been paid to the potential of exercise to improve the quality of life and independence, and a substantial number of studies have shown significant muscle hypertrophy and strength gains in elderly men and women (eg. (58, 271-273, 288, 308, 517)). The potential of maintaining high levels of physical activity for daily-life exercise capacity is exemplified by master athletes and a case of a 97-year-old man who cycled more than 5,000 km a year, even at that age (154).

It is well known that muscle activity increases the rate of myofibrillar protein synthesis, resulting in muscle hypertrophy (e.g.,(333)). Muscle hypertrophy *per se* is only valuable if it also leads to improvement in the ability to perform daily life activities, and it has been shown that progressive strength training does just that; it did improve gait velocity, stair-climbing power and spontaneous activity levels in elderly men and women (58). Proteomic analyses have indicated that this is associated with an increased expression of contractile and structural, metabolic and stress response proteins (651).

A rapid decrease in protein synthesis rate followed by an accelerated rate of protein degradation is typically observed in response to inactivity, resulting in weakness and muscle atrophy. Different models to simulate the unloading induced muscle atrophy during space flight have been introduced,

such as limb suspension and bed rest (see (83, 203), but the mechanisms underlying this loss of strength in response to unloading are not well understood (see (68, 244, 254)). While muscle atrophy is a significant cause of the force loss during unloading it cannot be the sole explanation, since the decrease in force is proportionally larger than that in fiber size or muscle cross-sectional area (e.g. (68, 244)). The extent to which the loss of voluntary force is related to neural factors or hormonal changes is a matter of controversy (244, 254), where some studies have shown that motor unit activation is not impaired in response to short-term unloading (68) and others observed a reduced ability to voluntarily activate the muscle (454).

There is evidence that at least part of the proportionally larger loss of force than muscle atrophy in response to unloading or reduced physical activity levels is attributable to a reduced specific tension of the muscle fibers. For instance, it has been observed in rat (751) that after hind limb suspension the specific tension in isolated muscle fibers was 12-15% lower. A more pronounced decline in specific tension was reported by Gardetto et al. (318), who found a 20% and 28% decrease in type I fibers from the gastrocnemius and soleus muscles, respectively, in response to 2 weeks of hindlimb suspension.

Specific tension is determined by the number of force generating cross-bridges per cross-sectional fiber area and the force generated per cross-bridge. The parallel decrease in specific tension and myofibrillar protein concentration in humans in response to 6 weeks bed rest (527) indicates that the reduction in specific tension is primarily related to a decreased number of force-generating cross-bridges. It has been shown in rats that the protein loss in response to hind limb suspension follows a triphasic time course, involving pre-translational, translational and post-translational control mechanisms: 1) an initial rapid reduction of protein synthesis, 2) a delayed increase in proteolysis, and finally 3) a decreased rate of myofibrillar protein degradation resulting in a new balance between rates of protein synthesis and degradation (see (84, 872)). The time-course of protein loss varies between sarcoplasmic, thick and thin filament proteins during hind limb suspension (84, 872).

Computed tomography (417) has not only shown an aging-related decrease in muscle cross-sectional area, but also a decreased density of lower limb muscles in humans, indicating both a loss of muscle mass and replacement of contractile material with fat and connective tissue, similar to the observations after hindlimb unloading in rats. This replacement of contractile material with fat may be a consequence of a decreased mixed muscle protein synthetic rate in the elderly (age>65 yr) compared to the young (age<35 yr) (931, 979, 980), where in particular the myosin synthesis rate was affected (38, 40). In fact, the aging-related decline in muscle strength per unit muscle mass correlated with the decreased rate of myosin synthesis. This suggests a compromised ability of skeletal muscle to maintain the quantity and quality of the molecular motor protein myosin in old age. The negative effect of inactivity on protein synthesis may accordingly add to the aging-related decline in myosin quantity and quality making the elderly more vulnerable to a period of immobilization.

The reduced myofibrillar protein synthesis rate makes the elderly not only more vulnerable to the detrimental effects of immobilization, but may also underlie the common clinical experience that the rehabilitation process is significantly slower in elderly than in young patients who have been immobilized for a long time because of various disabilities or diseases. This has a significant impact



on the quality of life and the health care system, where the older patient will need longer care during recovery and some even never fully recover from a period of immobilization. This effect of immobilization is at least partly related to the additive effects of inactivity and the normal aging-related impairments of neuromuscular function.

The reductions in muscle mass and strength induced by immobilization are paradoxically less in the old than in the young. For instance, both the absolute and the relative denervation-induced atrophy in rat soleus and gastrocnemius muscles was less in old than in young animals (17). A similar situation was seen after 2 weeks of unilateral leg casting, where the decline in muscle volume was less in old than young men (854). This was even reflected at the fiber level where in young men fibers of each type atrophied by 15-30%, while in the old men type IIa fibers only atrophied by just 13% (414). Yet, the rate of force development was decreased more in old than young men, and where in young men the muscles had fully recovered after 4 weeks retraining this was not so in the old men (414, 853). Even so, in older people post-operative resistance training resulted in a better recovery of muscle mass and strength and a shorter hospital stay after unilateral hip replacement (854).

It is intriguing that, as described above, the loss of muscle mass during immobilization is less in older, including humans, than in younger organisms. This observation has led to the idea that there may be a 'default' muscle mass (208) beyond which normally no further atrophy (can) occur(s). It could also be a reflection of a lower protein turnover in the old, where a reduced rate of protein synthesis is accompanied with an unaltered or even reduced rate of protein breakdown, as suggested by the lower specific activity of the ubiquitin proteasome pathway in muscles from old rats (285).

The slower rate of protein turnover is probably contributing to the accumulation of oxidized proteins in, and the lesser atrophy and slower recovery of, old muscles after a period of immobilization. This apparently negative characteristic of old muscle may, however, be turned into an advantage. If it results in a slower atrophy, as has been observed, one may expect that it also will result in a slower atrophy of muscles that had hypertrophied after a period of loading! That this is not just wild speculation was shown in an elegant study where indeed the loss of patagialis muscle mass after a period of loading was significantly less in old than in young quail (832). In fact, retraining may be even facilitated by a previous period of regular loading, due to the persistence of newly acquired myonuclei during the training regimen, as seen in temporarily overloaded mouse muscles (119). These observations may have significant clinical implications as, if this also applies to humans, pre-operative resistance exercise would then significantly shorten the duration of post-operative hospital stay, particularly when also followed by post-operative resistance exercise.

It has been observed that motor unit numbers are better preserved in muscles of the upper body than muscles of the lower body in both humans (313) and rodents (909). Given that both the activity and the mass of locomotor muscles decreases more than that of the arm during ageing(427), it may well be that maintained activity levels prevented motor unit loss in arm muscles. In support of a role of regular activity, it has been found that master athletes had a larger number of motor units than age-matched controls (723), with evidence of better reinnervation as reflected by larger motor units (713), fewer angulated fibres - indicative of denervation -, and a larger percentage of fibre type grouping (649).

This larger prevalence of fibre type grouping may, however, not be a reflection of better reinnervation, but rather a chance observation due to the larger proportion of type I fibres (our unpublished data), something also alluded to in a previous paper (649). In support of this, studies with larger numbers of participants observed an age-related reduction in the number of motor units (241). Importantly no evidence for preservation of motor units in master athletes was observed (713). Also in rodent muscles, there is no evidence that motor unit survival is enhanced by regular physical activity (1). There is thus little evidence that regular exercise prevents motor unit loss, or motor neuron death.

Overall, it appears that a significant part of the aging-related muscle wasting and weakness is attributable to reduced levels of physical activity. Even though somewhat attenuated compared to young adults, regular exercise has significant benefits for muscle strength and endurance in old age. This adaptability of the muscle may well be exploited in pre- and post-operative resistance exercise regimes that may prove to be a potent novel approach to reduce the length of hospital stay of the older patient. Surprisingly, this potential application of resistance exercise in a clinical setting has so far received little scientific attention.

### ***Hormone therapy***

While caloric restriction and exercise programs clearly have significant benefits for the older person and their muscles, they require considerable motivation and are not always possible because of frailty and co-morbidities (353). When considering other options it is important to recognize that many circulating anabolic and sex hormones show an aging-related reduction (503, 831). This does not need to be so when it comes to the concentrations of androgenic hormones in skeletal muscle. For instance, the muscle concentrations of dehydroepiandrosterone and androstenedione were similar while the estradiol and testosterone concentrations were even higher in post- than pre-menopausal women, the latter probably reflecting fat infiltration in the muscle (721).

Given the aging-related decrements in circulating sex hormones and their association with muscle wasting and weakness during aging, hormone replacement therapies or treatment with other anabolic factors has received considerable attention as a means to combat aging-related muscle wasting and as an alternative or enhancer of exercise-induced improvement in muscle mass and strength, particularly for the frail older person.

Anabolic steroids on their own may, however, not be enough to induce hypertrophy. For instance in old rats, nandrolone decanoate on its own did not induce hypertrophy (548) and the  $\beta_2$ -adrenergic agonist clenbuterol did not prevent atrophy during hind limb suspension (151, 152, 975). Only when nandrolone decanoate was combined with 7 days of functional overload did the soleus, but not the plantaris, muscle develop hypertrophy (548). Similarly, clenbuterol combined with intermittent weight bearing in young rats prevented the hind limb suspension-induced atrophy of the soleus, but not that of the extensor digitorum longus muscle in rats (975). The beneficial effect of nandrolone decanoate during an overload stimulus in old age has been shown to be associated with suppression of cytokine gene expression in the old, but less so in the young overloaded soleus muscle (876). These data indicate potential of combined overload and anabolic hormone treatment that appear, however, to be limited to slow fibers/muscles only.

Also in humans, hormone replacement therapy has benefits for skeletal muscle mass and can even be effective without concomitant exercise in hypogonadism. For instance, continuous testosterone replacement via a gel over the skin (50 mg/day) for 6 months preserved muscle thickness in intermediate-frail and frail hypogonadic elderly men (33) and a meta analysis showed improvements in muscle mass in hypogonadic elderly men (409). In addition to these benefits on muscle mass, testosterone treatment also improved insulin resistance in hypogonadal frail elderly men, all stimulating further study on the effectiveness of testosterone treatment to improve the quality of life in hypogonadal frail elderly men (672).

In post-menopausal women, however, hormone replacement therapy increased strength without a change in muscle size (711, 963). Studies on postmenopausal homozygotic twins have shown that at least part of the attenuation of the loss of muscle power during estrogen-based hormone replacement therapy is attributable to a better preservation of specific tension of muscle fibers (731). Prevention of the reduction in muscle power during menopause by hormone replacement therapy can also be associated with an attenuated loss of muscle mass, particularly when the therapy is combined with exercise (831). Although this indicates that exercise training adds to the effects of hormone replacement therapy in post-menopausal women, the effects of training on muscle strength have been reported to be blunted in those with, compared with the gains in those without hormone replacement therapy (686). This, suggests that exercise without hormone replacement therapy is preferable and that adding hormone replacement therapy to an exercise programme does not elicit further gains.

While in women estrogen-based hormone replacement therapy improves muscle strength mainly by an increase in specific tension, in older men with low testosterone levels the increased force of single fibers after 5-month testosterone treatment resulted from an increase in fiber size, rather than in specific tension or alterations in cross-bridge kinetics (293). Similar to animal studies with clenbuterol and nandrolone decanoate, the benefits of testosterone treatment were particularly evident in type I fibers (293). Interestingly, the effects were more pronounced during weekly treatment with testosterone than a treatment with alternating months without testosterone administration (293). In women the benefits of hormone replacement therapy remained evident several years after cessation of the therapy (687). Although these observations are promising, care has to be taken about the dose because of the potential detrimental effects on the cardiovascular system (860).

The benefits of hormone replacement therapy may be genotype dependent. For instance, the gain in muscle strength in post-menopausal women was larger in those with the angiotensin-I converting enzyme insertion allele (ACE-I) than those without this allele (963). While hormone replacement therapy may thus have benefits for skeletal muscle function, it is questionable whether this should be advised to any woman, as it is associated with an increased risk of cardiovascular disease, thrombosis and cancer (765, 860).

### Growth hormone

Growth hormone (GH) is the primary pituitary controller of skeletal muscle growth that stimulates the release of insulin-like growth factors (IGFs) by the liver into the blood. GH does not only affect muscle mass via IGF-I, but can also have a direct effect on skeletal muscle (see (297)). After the age

of 50, the secretion of GH declines and 50% of citizens over 65 have been reported to be partially or totally GH deficient (779, 782). In aging rodents (842) and humans (296) there is a loss of pulsatile secretion of GH. The associated lowering of circulating IGF levels suggests that GH therapy of the aged might reverse the aging-related changes in skeletal muscle (779, 782). This is supported by rodent studies showing that GH deficiency causes muscle atrophy, GH treatment stimulates muscle cell hypertrophy (61, 479), reverses aging-related changes in protein synthesis rate and membrane conductance (196, 842), and has been shown to have beneficial effects in elderly humans (435, 606, 782). While GH and IGF-I replacement to adults with growth hormone deficiency has proven beneficial, this is not an unequivocal finding, possibly due to differences in dosage, treatment time and method of dosing (955). Given these equivocal observations and the potential risk of cardiovascular complications, particularly when the recipient is not growth hormone deficient, more studies are required to elucidate the risks and benefits of treatment, but overall it seems people with GH deficiency do benefit from GH and IGF-I treatment.

### Thyroid hormone

The aging-related reduction in muscle power is not only attributable to a loss of force generating capacity, but also a slowing of the muscle. While considerable attention has been given to ways to increase muscle force generating capacity in the older person by interventions such as anabolic steroid treatment and hormone replacement therapies with or without exercise training, little attention has been given to combatting the other component of power: the slowing of the muscle.

Thyroid hormone is a nuclear hormone that via its interaction with the thyroid hormone receptor acts as a transcription factor that binds to the promoter regions of the myogenic regulatory factors that play an important role in regulation of the expression of muscle specific genes (983). Treatment with thyroid hormone induced a significant slow-to-fast transition in fiber type composition in the soleus muscle and an increase in the shortening velocity of the fibers in both male and female old rats (984). Thyroid hormone treatment is thus a promising avenue to help restore muscle power in the older person. However, while thyroid treatment in rats induced a transient improvement in cardiac function, continued treatment caused a transition to pathological cardiac hypertrophy with impaired cardiac function (207). Also in humans hyperthyroidism is detrimental for cardiac health (957), but treatment with a single intravenous bolus of thyroid hormone had a beneficial effect on cardiovascular function in patients with congestive heart failure (369). As thyroid hormone treatment is already used in clinical practice and has, at least transiently, a beneficial effect on cardiac function, the use of thyroid hormone to regain muscle power in old age by the induction of a slow-to-fast transition is something that deserves further attention. It would be particularly important to assess the safe dose and duration of treatment.

Thus, caloric restriction appears to be a potent intervention to delay the aging process and does result in significant increases in life expectancy. It is maybe not so much a tool to regain lost muscle mass and power, as it has potential to delay the aging-related loss of muscle mass and function. However, results obtained in experimental studies in rats raised in a pathogen free environment may not be translated to the human situation.

Overall, muscle plasticity is maintained into old age even though the response to altered functional demands is slower in old muscle. However, rather than seeing this as a problem, new ways can be

developed to capitalize on this characteristic of skeletal muscle to accelerate recovery from hospitalization. Hormone replacement therapies appear to work best in combination with exercise, particularly in those suffering from hypogonadism. Older non-hypogonadic people are advised not to be given hormone replacement therapy because of the detrimental side-effects, such as increased risk of cancer and cardiovascular complications. Thyroid hormone bears promise as 1) it induces a slow-to-fast transition and 2) is already used in the clinic to treat cardiovascular conditions. However, before implementation to combat muscle weakness, clarification is required on the save dose and duration of treatment as prolonged treatment and too high doses of thyroid hormone have severe adverse effects on e.g. cardiac function.

### ***Low grade inflammation and interventions***

In 1999, Ferrucci et al. studied the association between the development of disability and serum IL-6 levels in 1,029 subjects aged 71 years or older without impairment in mobility and activities of daily living. Four years later, it was found that subjects with elevated IL-6 levels were more likely to develop disability (286) and high circulating levels of both IL-6 and C reactive protein (CRP) were also associated with higher mortality (373). Since then, other secondary muscle wasting conditions associated with cancer or chronic diseases (e.g. COPD and diabetes), have been shown to be closely associated with elevated levels of TNF- $\alpha$ , IL-1, and IL-6 which activate the ubiquitin proteasome proteolytic pathway (639, 968). Thus, it was hypothesized that the low-grade systemic inflammation observed in old age plays an important role in the pathogenesis of sarcopenia (202, 496). The mechanisms underlying the aging-associated low-grade inflammation have still not been fully understood, but dysregulation of the immune response, sedentary lifestyle, subclinical pathological conditions, such as increases of adipose tissue, presence of undetected infections, chronic health disturbance, and altered nutrition may all contribute. However, it is well established that older people exhibit the pro-inflammatory condition characterized by slightly elevated cytokine levels that lie far below the levels of acute infections (668). In this section, most research results were mainly reviewed from studies of aging healthy subjects, but results related to aging-related diseases and disabilities have also been reviewed.

### **Observational studies**

Multiple cross-sectional and longitudinal studies have revealed that inflammatory markers, such as IL-6, TNF- $\alpha$  and CRP, increase during aging and correlate with disability, mortality, and the decline in muscle mass, muscle strength, physical performance and physical function (60, 287, 371, 490, 830, 956). Both CRP and IL-6 have been reported to be positively associated with total fat mass and inversely associated with fat-adjusted appendicular lean mass. Even when adjusted for sarcopenia, obesity remained significantly associated with high CRP and IL-6 concentrations (143). High IL-6 (>5 pg/mL) and CRP (>6.1  $\mu$ g/mL) were reported to be associated with a 2 to 3 fold greater risk of losing more than 40% of muscle strength in 986 men and women aged 74.6 $\pm$ 6.2 years after adjustment for confounders (802). Elevated CRP was documented to associate with reduced hand grip strength and chair stand performance in women but only chair stand performance in men in a study with 1,926 men and 2,260 women aged 65.3 $\pm$ 9.0 years (368). In a clinical study of 270 adult patients with newly diagnosed biopsy-proven myopathy, 18% of patients were aged 70 years and older, and half of those patients had been diagnosed with inflammatory myopathies, which is significantly higher

than the proportion of 28% in patients aged 18-69 years. Thus, inflammatory myopathies were the dominant type of myopathy in the elderly compared with the non-elder group, indicating that low-grade inflammation plays an important etiological role in sarcopenia (251). In an experimental study, significantly higher plasma levels of fibrinogen and  $\alpha_2$ -macroglobulin were observed in old male Wistar rats aged 22 months compared with 8-month-old rats, and a negative correlation between fibrinogen and albumin plasma concentrations in old rats revealed a low grade inflammatory state (692).

In addition, the molecular signature of sarcopenia identified increased expression of genes involved in mediating cellular responses to inflammation and apoptosis, including complement component C1QA, C/EBP- $\beta$ , and FOXO3A in a study of human subjects (329). In an experimental study of rats aged 6, 12, 18, 21, 24 and 27 months, the global gene expression profiles in muscle demonstrated a progressive and rapid decline in muscle mass after the age of 18 months, which was associated with an up-regulation of the E3 ligase MuRF1, down-regulated mitochondrial energy metabolism, perturbed neuromuscular junction patency, increased absolute neutrophil count and serum globulin concentrations. Taken together, the loss of mitochondria and increased inflammation are presented upon initiation of sarcopenia, and restoring mitochondriogenesis and reducing inflammation may accordingly alleviate the sarcopenia phenotype (416).

#### *Molecular pathways of low-grade inflammation in sarcopenia*

Pro-inflammatory cytokines, such as TNF $\alpha$ , TWEAK, or IL-1, signal into two established pathways: the NF- $\kappa$ B pathway and the p38 MAP kinase. These two signaling mediators are required to up-regulate the expression of the key E3 ligases, MuRF1, which mediates sarcomeric breakdown, and atrogin-1/MAFbx, which control protein synthesis by ubiquitination of eIF3c. MuRF-1 and atrogin-1 are over-expressed in catabolic and muscle wasting conditions (80, 261, 334, 566, 774, 881). For a detailed review of proteolytic pathways involved in sarcopenia see section 'Regulation of muscle protein synthesis/degradation'.

In old mice, significantly higher total protein concentration, phosphorylation and ubiquitination of I $\kappa$ B $\alpha$  have been observed, preceding NF- $\kappa$ B activation (760). I $\kappa$ B $\alpha$  may also have roles beyond acting as an inhibitor of NF- $\kappa$ B, such as acting as a cotranscription factor affecting the transcription of various histone deacetylases (HDACs) that regulate muscle atrophy through histone acetylation regulation (64, 760). On the other hand, elevated levels of TNF- $\alpha$  decrease the mRNA abundance of PGC-1 $\alpha$ , leading to an increased muscle protein degradation, since PGC1- $\alpha$  inhibits NF- $\kappa$ B and FoxO3 (614). Pro-inflammatory cytokines increase the synthesis of prostaglandin-E2 (PGE2) by inducing the activation of cyclooxygenase 2 (COX2), a rate-limiting enzyme in the synthesis of PGE2 that regulate skeletal muscle protein synthesis negatively (704, 942).

The aging-related low grade inflammation offers different intervention strategies aiming at alleviating sarcopenia. The pentameric CRP (pCRP) may undergo dissociation to a proinflammatory, monomeric CRP (mCRP) which via its proinflammatory effects (induced ROS generation as a major component of inflammatory tissue damage) may amplify inflammation and contribute to aggravation of tissue damage. It has been demonstrated that mCRP induces IL-8 secretion in human neutrophils via intracellular peroxynitrite signaling following the activation of NF- $\kappa$ B and activator protein-1, being

a major source of nitrosative stress. By blocking the dissociation of pCRP to mCRP, the mCRP proinflammatory activity can be prevented (460, 871).

Both TNF- $\alpha$  mRNA and protein levels have been reported elevated in skeletal muscle from frail elderly men and women, TNF- $\alpha$  mRNA and protein levels decreased in response to 3 months resistance training in the old, but did not change in the young control group. In addition, muscle protein synthesis rate in the old exercise group was inversely related to levels of TNF- $\alpha$  protein (352). In a randomized controlled trial, Thompson and co-workers confirmed significant effects of resistance training in old subjects, *i.e.*, serum IL-6 decreased 29% and 33% after 12 and 24 weeks exercise, respectively, and increased after detraining although IL-6 levels remained lower than baseline by 13% even after 2 weeks detraining (873).

Ibuprofen, a commercially available NSAIDs, which inhibits cyclooxygenase activities, has additional anti-inflammatory properties due to modulation of leucocyte activity by reducing cytokine production or inhibition of the NF- $\kappa$ B signaling pathway (738). Chronic administration of Ibuprofen has been found to prevent low grade inflammation in old rats and result in a maintained anabolic effect of food intake and a significant decrease in the aging-related loss of muscle mass (756). An aging-related increase in fibrinogen levels coupled to an increase of  $\alpha$ 2-macroglobulin (4.3 fold) and a 15% decrease of plasma albumin levels were observed in old rats, but ibuprofen alleviated these effects on fibrinogen, albumin and  $\alpha$ 2-macroglobulin levels. In addition, ibuprofen decreased plasma IL6 and IL1 $\beta$  levels by 60% and 46%, respectively, and increased muscle mass significantly (756). Further, ibuprofen increased food intake and muscle protein synthesis, indicating that the treatment restored protein synthesis by inhibiting the low-grade inflammation (702). On the other hand, Ibuprofen activates the PGC-1 $\alpha$  pathway that inhibits apoptosis and oxidative damage and counteracts the activity of NF- $\kappa$ B (31, 148). NSAID users have been reported with a lower risk of sarcopenia compared with non-users after adjusting for potential confounders, indicating that long-term NSAID use might have a protective effect against the loss of muscle mass and function, after adjusting for potential confounders (506).

### **Targeting senescent cells**

Cellular senescence refers to the essentially irreversible growth arrest that occurs when cells experience potentially oncogenic insults or damage, including inflammatory or metabolic stress (543, 868, 992). Senescent cells remain viable and metabolically active. Some senescent cells secrete pro-inflammatory cytokines, chemokines, proteases, and inducers of stem cell dysfunction, termed the senescence-associated secretory phenotype (SASP)(172, 173). A number of inducers, including DNA damage, oncogenic mutations, dysfunctional telomeres, fatty acids, ceramides, ROS, mitogens, and cytokines (491-493, 867) can act alone or in combination to push cells into the senescent cell fate through pathways involving p16<sup>Ink4a</sup>/Rb (retinoblastoma), p53/p21, and possibly other pathways. These contribute to widespread changes in gene expression and chromatin remodeling which underlie senescence-associated growth arrest, the SASP, and changes in morphology. In these respects, cellular senescence is effectively a cell fate reminiscent of differentiation, replication, or apoptosis. Intracellular autocrine loops reinforce progression to irreversible replicative arrest, heterochromatin formation, and initiation of the SASP over a matter of days to weeks. In addition to removing cells from the progenitor and stem cell pool, senescence may

contribute to local and systemic tissue dysfunction and chronic disease predisposition through the SASP and associated chronic sterile inflammation and extracellular matrix degradation. By these mechanisms, senescence appears to contribute to aging-related dysfunction and multiple chronic diseases (438, 465, 543, 691, 867, 868, 992). Diseases in which senescent cells accumulate at sites of pathology include atherosclerosis, Alzheimer's and other neurodegenerative diseases, renal disease, osteoarthritis, osteoporosis, diabetes, obesity, chronic lung disease, retinal disease, and cancers, among many others (71, 146, 175, 338, 340, 341, 400, 638, 920, 973). Cellular senescence in humans is associated with physical dysfunction: abundance of senescent cells in thigh subcutaneous adipose tissue in older women with reduced grip strength, 400 meter walk time, and 4 meter gait speed is higher than in older women with less physical dysfunction (439).

### Genetic targeting of senescent cells

Eliminating senescent cells enhances healthspan and blunts aging-related declines in activity, sarcopenia, fat loss, and cataracts in progeroid mice expressing a drug-inducible suicide gene, ATTAC, driven by a senescence-induced promoter, the p16<sup>Ink4a</sup> promoter (INK-ATTAC mice)(894). Senescent cells can be cleared from INK-ATTAC mice using AP20187, an agent that activates the apoptosis-inducing caspase-8 moiety in the ATTAC fusion protein, which is only expressed by the p16<sup>Ink4a</sup>-positive senescent cells in these mice and that does not affect normal cells. We found that senescent cells can be cleared from not only progeroid, but also naturally-aged INK-ATTAC mice by AP20187 (973). This alleviated aging-related metabolic dysfunction, enhancing insulin sensitivity. Decreasing senescent cells in naturally-aged INK-ATTAC mice alleviates cardiovascular dysfunction and other aging-related phenotypes, as well as improving cardiovascular function in atherosclerotic, high fat-fed INK-ATTAC mice (341, 378). These studies provide strong evidence that targeting senescent cells can alleviate a range of aging- and chronic disease-related disorders.

### SASP inhibitors

Several classes of drugs reduce expression of SASP components, including JAK1/2 inhibitors (e.g., ruxolitinib) (973, 974), glucocorticoids (232), rapamycin (499), and metformin (179). These agents may not inhibit secretion of every SASP factor: at least in the case of rapamycin, secretion of a subset of these factors is decreased. Administration of rapamycin to middle-aged or ruxolitinib to even very old mice reduces frailty and restores activity and strength (281, 974). Furthermore, ruxolitinib alleviates aging-related stem cell and metabolic dysfunction (974). Ruxolitinib alleviated frailty-like "constitutional" symptoms in older subjects with myelofibrosis, including improved 6 minute walking time, which can depend on muscle strength, compared to placebo (500), even though the ruxolitinib did not appear to improve the underlying myeloproliferative disorder, as assessed by bone marrow clonal analysis. Thus, SASP inhibitors appear to counter some of the effects of senescent cells in mice and humans, including muscle weakness and frailty. However, actually eliminating senescent cells may prove to be a better strategy for long-term pharmacological treatment of dysfunction caused by accumulation of senescent cells.

### Senolytics

Senolytic agents are drugs that specifically eliminate senescent cells (486). To discover these drugs, a hypothesis-driven approach was used (227). Senescent cells are resistant to apoptosis (304, 919),



protecting them from the pro-apoptotic microenvironment resulting from their own SASP and their active DNA damage responses and heightened metabolic flux. It was reasoned that pro-survival pathways may be active in senescent cells, presenting a target that could, if interfered with, allow senescent cells to “commit suicide”. From proteomic analyses, survival pathways were identified that could confer resistance to apoptosis to senescent cells. Indeed, targeting these pathways by RNA interference caused selective removal of senescent cells. Based on this information, drugs that inhibit these pathways were selected. These agents induced apoptosis in mouse and human senescent cells, but not non-senescent cells in culture. The first senolytic agents identified were dasatinib, an inhibitor of tyrosine kinase anti-apoptotic pathways, and quercetin, a flavonoid that inhibits AKT-, Bcl-2-, and p21/serpine-related pro-survival pathways. Subsequently, based on the senescent cell pro-survival pathways identified earlier, it was demonstrated that navitoclax, a Bcl-2 family inhibitor, is senolytic (147, 994), as were A1331852, another Bcl-2 family inhibitor, fisetin and other agents (466, 510, 993). The senolytic drugs identified so far tend to be cell-type specific, reflecting differences in the pro-survival pathways activated in senescent cells originating from different cell types (994, 995). Therefore, combinations of agents, for example dasatinib plus quercetin, can be used to target a broader range of senescent cell types than individual agents. When administered to mice, this combination reduced senescent cell burden and alleviated a range of diseases and disorders, much as when senescent cells are eliminated genetically from INK-ATTAC mice. These aging- and disease-related disorders included decreased mobility, frailty, and osteoporosis in progeroid mice, impaired mobility following radiation damage to a leg in young mice, cardiac dysfunction in old mice, and vascular hyporeactivity and calcification in high fat-fed ApoE<sup>-/-</sup> mice, among others (769, 995). Since muscle-related dysfunction is prominent among the phenotypes enhanced by genetic removal of senescent cells in INK-ATTAC mice, by pharmacologically removing senescent cells with senolytics in wild-type mice, and by SASP inhibitors in wild-type mice, targeting senescent cells may be a therapeutic option for aging- and disease- related loss of muscle function, provided clinical trials show these agents also clear senescent cells, are safe, and are effective in humans. Such interventions have the advantage of potentially simultaneously alleviating the range of co-morbidities typically encountered in elderly subjects who present with muscle dysfunction, weakness, and immobility. Hopefully, clinical trials designed to test this possibility will start soon.

### ***Evidence for an irreversible aging process***

Aging is associated with a decline in physiological functions, including that of skeletal muscle, ultimately resulting in death. The origin of aging is an evolutionary enigma (201), but there are multicellular organisms, such as the hydra (718) and the jellyfish *Turritopsis dohrnii* (612), that have been reported to be immortal. Cells have an extensive machinery to repair damage, where already 150 DNA-repair genes were known in 2005 (962), giving the impression that ‘Organisms are programmed for survival, not death’ (467). In fact, there are investigators who expect that in the future we will be able to develop interventions that stop the ageing process (604, 906, 954), or can reset the aging clock (870), thus suggesting that ageing is reversible. However, our experience till now is that we age and ultimately die and the question thus arises whether there is anything that can at least slow down the aging process, and in particular for the context of this review, muscle aging.

The aging process is often said to result in a 1% decline in muscle mass per year after the age of 50. This does, however, not consider that in comparison to the muscle mass in the previous year this decrease amounts to an exponential, rather than a linear, increase in percentage loss of muscle mass year by year (**Fig. 19**; (200)) and explains why in longitudinal studies the rate of muscle wasting or other physiological functions often exceeds 1% per year. Such observations do not apply to muscle alone, but seem to be an inherent phenomenon affecting all organ systems, as reflected by similar aging-related patterns in swimming, running and chess performance (72).

It is often stated that muscle plasticity is maintained in old age and that gains of 30% muscle mass or aerobic capacity can be achieved at any age. It should be noted, however, that the absolute increase decreases with increasing age and a hundred-year old can never attain the muscle mass (s)he had when (s)he was twenty (illustrated in **Fig. 19A**). In fact, in old humans (706, 932) and rodents (46, 48, 78) the hypertrophic response is attenuated and may even result in an impaired rather than improved force generating capacity (204). The impaired muscle plasticity is not limited to an impaired hypertrophic response, but also the muscular adaptations to chronic electrical stimulation are delayed in old rats (911). In addition, it has been found in rats that lifelong exercise started in old age resulted in a reduced, rather than extended life expectancy in rats (255) corresponding with the observation that improvements in survival in humans over the last century are negligible, or even absent, after the age of 100 (239). This also illustrates that the 'rejuvenating' effect of exercise decreases with increasing age (**Fig. 19A**). These observations suggest that there is 1) a threshold age beyond which strenuous exercise becomes detrimental, rather than beneficial and 2) that there is an inherently irreversible aging process (**Fig. 19A**; (200)).

## **6. AGING AND NEUROMUSCULAR DISORDERS, ADDITIVE AGING EFFECTS AND AGING AS AN ETIOLOGICAL FACTOR**

Aging represents an extremely complex biological process where intrinsic changes are accumulating with time resulting in an increasing probability of death and the development of disease (999). The distinction between normal aging and muscle disease is however difficult, since if the changes in muscle structure and function observed in all humans between the age of 30 and 90 would be considered pathological if observed in a young person over a six-month period. It may accordingly be argued that aging represents a disease in itself (473), but general changes in skeletal muscle structure and function during aging will be considered a physiological phenomenon rather than a disease in this review as stated in the introduction and named *sarcopenia*. Pathological changes observed in old age have been divided into different groups (999): *i) Aging-dependent changes/diseases* affecting all individuals to varying degrees, such as osteopenia and osteoarthritis. *ii) Aging-related diseases* with a very long latency period and becoming manifest at old age such as some neoplastic diseases, *iii) Intercurrent diseases* which are observed at all ages, but are more common in old age due to a decline in homeostatic mechanisms, such as severe pneumonia, *iv) Wear-and-tear disease and remnants of incomplete healed former disease* such as old cardiac infarction and hearing loss, and *v) Age-dependent chronic non-lethal disease* which develop at an early age and remains present at old age such as chronic skin disease. However, the distinction between these four categories is not trivial for many diseases where old age may be an etiological or contributing factor to disease or disease progression. In this section we will focus on

diseases affecting different parts of the motor unit where aging is playing an important role, *i.e.*, diseases primarily affecting the motoneurone, neuromuscular junction and muscle cell.

### ***Motoneuron disease***

#### ***Post-polio syndrome***

The muscle weakness and atrophy in acute poliomyelitis are proportional to the motoneurone loss caused by viral invasion of the nervous system and anterior horn cell destruction. The initial motoneurone loss will be, at least partially, compensated for by extensive collateral sprouting if there are remaining intact motoneurons reinnervating previously denervated muscle fibers and motor unit reorganization. The “post-polio syndrome” (PPS) is characterized by recurrence of weakness, atrophy and fatigue in muscles previously affected by poliomyelitis (*e.g.*, (6, 191, 366)). This condition is only observed in patients with a history of poliomyelitis, with a complete or partial recovery of muscle function after the acute disease, and with at least a 15-year period of stable muscle function prior to the onset of symptoms that affect daily activities. PPS was described for the first time more than a century ago (*e.g.*, (140, 747)), and has received increasing scientific attention due to the increased number of patients with PPS, caused by epidemic poliomyelitis in the early 1950s (for refs. see (365)).

PPS has been suggested to be secondary to failing compensatory and adaptive mechanisms in response to chronic overuse, premature motoneuron aging, or a consequence of the motoneurone loss associated with normal aging (see (6)). Due to the extensive collateral sprouting by intact motoneurons in severely affected muscles, remaining motor units are recruited excessively during activities of daily living. This “overuse” of motor units has been suggested to be an important etiological factor underlying PPS (6). The overused motor units typically show increased territories, type grouping, type I muscle fiber predominance, muscle fiber hypertrophy, frequent fiber branching of large size fibers, and an increased number of muscle fibers with internal nuclei (see (86-88, 354, 355, 365, 531, 884), altered motoneurone firing frequencies, lower capillary densities, and increased diffusion distances, along with altered contractile and metabolic properties in the overused motor units may lead to a shortage of substrate during muscle work. This in turn could explain the muscle fatigue and pain in patients with PPS (86, 355).

More than half of the motoneurons of an extremity muscle may be lost before the patient experiences any muscle weakness (see (86)). However, in a patient with a prior polio lesion, even a relatively modest loss of motor units may have a dramatic effect on muscle function and muscle size, due to the small number of very large and overused remaining motor units. Since the onset of PPS is typically found in middle-aged or older patients with a previous polio lesion, the relatively sudden reappearance of muscle weakness may be related to the combined effect of a small number of remaining very large and overused motor units and the normal aging-related loss of motor units. Although the aging-related loss of motor units is relatively small in quantitative terms, it will have a dramatic effect on muscle function in muscles with few remaining motor units containing a large number of muscle fibers.

#### ***Amyotrophic Lateral Sclerosis (ALS)***

ALS is a rapidly progressive disease with muscle wasting, spasticity and hyperreflexia resulting from the degeneration of upper and lower motoneurons. Recent studies have suggested that post-translational modifications of proteins and other cellular components play an important role in several neurodegenerative diseases. Specific interest has been focused on modifications by oxidation, nitration, and glycation of proteins and neurofilaments in the pathophysiology of ALS (for refs. see (822)). It is of specific interest to note that spinal motoneurons show higher susceptibility to the neurotoxicity induced by reducing sugars, the accumulation of reactive oxygen species (ROS) and advanced glycation endproducts (AGE) than do spinal non-motoneurons. Further, the accumulation of proteins modified by oxidative stress has been reported in the spinal cord of patients with ALS. This progressive disease, which typically has its onset in middle age, may be associated with a defective or inefficient defense system against oxidative stress (62, 822). Glutathione (GSH) levels have been reported to be reduced in specific regions in the central nervous system in patients with ALS, while other cellular enzymatic defense mechanisms remain intact. This is of specific interest since glutathione has been reported to protect against the dose-dependent neurotoxicity induced by reducing sugars and the concomitant formation of AGE.

It has been suggested that the increased sensitivity of spinal motoneurons may be related to a relatively inefficient GSH system due to a less efficient detoxification of GSH, reduction by GSH reductase, or transportation of oxidized GSH to the extracellular space in spinal motoneurons (822). The effectiveness of the natural antioxidant glutathione has been established in the prevention of AGE in collagen or lens crystallins (550) as well as in the reversal of glycation-induced impairment of myosin function (741). It has been suggested that the prevention by this AGE formation inhibitor will decrease as this compound becomes increasingly oxidized during the aging process. Thus, an aging-related decrease in the prevention of AGE formation by antioxidants, such as glutathione, may provide one aging-related factor of importance for the expected age of onset of ALS as well as of sarcopenia. As discussed above, the aging-related changes in microvasculature may also play significant role for the progressive motoneurone loss in patients with ALS.

## ***Myopathies***

### ***Myosinopathies***

The coding sequence for the adult MyHC isoforms is among the most heavily amplified in mammalian species, and the large size of the 17p13.1 locus within the MyHC gene would predict a prominent role for MyHC mutations in human myopathy (826). There is a significant need for improved molecular diagnostic tools together with structure function analyses of myosin for screening patients with myosinopathies, since a large proportion of patients evaluated for skeletal muscle weakness still fail to receive specific diagnosis. Experimental animal studies have shown distinct patterns of muscle dysfunction in IIx and IIb MyHC null mice, while heterozygous mice are clinically normal (3). Missense mutation in the  $\beta$ /slow MyHC gene causes a familial hypertrophic cardiomyopathy-like disease in heterozygous mice, indicating that missense mutations are more deleterious than nulls (323). In humans, central core disease is seen in type I fibers in patients with dominantly inherited mutations in the  $\beta$ /slow MyHC gene (276). An autosomal dominant myopathy with a missense mutation in the type IIa MyHC inform has been reported, i.e., a mutation in a highly conserved region of the motor domain in the myosin head, resulting in the replacement of a glutamic

acid with a lysine amino acid (613). This myopathy is characterized by a mild myopathy in childhood and adolescence with a progression in middle age. The mechanism underlying the late progression of this myopathy is not known. However, it is interesting to note that the missense mutation results in the substitution of one glutamic acid with a lysine. Lysines are the primary targets for posttranslational modifications of proteins by glycation (for refs. see (740)). Thus an impaired ability of the natural antioxidant glutathione discussed above may accordingly have a negative impact on the structure and function of the Ila MyHC isoform in old age and contribute to, or cause, the late onset and progression of this specific myopathy.

### Acquired muscle wasting in critically ill intensive care unit (ICU) patients

Intensive care and ICUs have undergone significant development during the past 65 years due to improvements in medical technology, progress in therapeutics, and improved understanding of pathophysiology and pathogenesis. Furthermore, evidence-based medicine has resulted in significant changes in the treatment of critically ill ICU patients, moving towards fewer and less invasive interventions and more humane care (951). Today, critical care is one of the fastest growing hospital disciplines. Because of the growing need for critical care, ICUs have been predicted to occupy one third of hospital beds by 2020 (951). In parallel with lowered mortalities, neuromuscular dysfunction induced by ICU-treatment has become increasingly apparent. The most common and important types of neuromuscular dysfunction are acquired paralysis of limb muscles (critical illness myopathy, CIM) and impaired respiratory muscle function (ventilator induced diaphragmatic dysfunction, VIDD) with enormous negative consequences for patient quality of life and health care costs. The two factors that most strongly predict morbidity and mortality in the ICU are old age and muscle wasting, which are likely synergistic. Furthermore, the molecular characteristics of CIM resemble those of sarcopenia. In addition, intensive care is one of the most costly disciplines in modern health care. In particular, costs for patients suffering from CIM and VIDD are enormous. For instance, CIM is observed in ~30% of ICU patients mechanically ventilated for  $\geq 5$  days. CIM is associated with 3-fold higher ICU costs (not including post-ICU costs). Mechanical ventilation can be lifesaving and ~40% of ICU patients are mechanically ventilated for a median duration of 5-7d, but weaning from mechanical ventilation is time-consuming, comprising ~40% of the time spent on the ventilator. Additional problems in weaning are observed in 20-30% of these patients due to VIDD, resulting in prolonged intensive support with an increased risk of secondary pulmonary complications and mortality, with elderly ICU patients being highly overrepresented (997). These patients account for ~30% of overall ICU costs or a staggering \$64 billion in the US annually. Currently, there is no effective treatment for CIM other than supportive care and intensive rehabilitation. Several observations suggest risk for CIM is likely related to senescent cell burden. CIM is associated with: 1) older age, 2) inflammation, which is a risk factor for CIM, with increased circulating IL-6 and other SASP factors, 3) protein aggregation, and 4) insulin resistance. Similarly, cellular senescence increases with aging, is associated with inflammation through the SASP that is partly driven by IL-6 as well as protein aggregation, and predisposes to insulin resistance, muscle weakness, and wasting. Thus, targeting senescent cells offers a promising intervention strategy in ICU patients suffering from CIM and VIDD.

## **7. SUMMARY AND FUTURE PERSPECTIVES**

The impaired muscle function during aging is related to both quantitative and qualitative changes in muscle structure and function. The ongoing denervation and incomplete reinnervation of skeletal muscles have been described in multiple experimental and clinical studies since half a century, although the mechanisms underlying the aging-related motoneurone loss are complex, include multiple factors which are far from fully understood. However, aging-related changes in microvasculature, oxidative stress, and protein modifications with negative effects on motoneurone transport mechanisms, structure, function and survival have been forwarded as significant etiological factors. The aging-related denervation-reinnervation process has a strong impact on quantitative changes in muscle such as muscle fiber loss and atrophy, resulting in overall muscle wasting and loss of muscle function in old age. However, qualitative changes in skeletal muscle affecting protein function, repair processes, the loss in the co-ordinated expression of contractile, SR and mitochondrial proteins, and the overall decreased resilience to stress in old age most probably have an even more important role for the impaired muscle function associated with old age. Thus, the impact of aging on skeletal muscle structure and function represents an extremely complex biological process that is not easily targeted by a singular intervention strategy; there are currently a number of interventions strategies which have the potential to improve muscle function and significantly improve quality of life among the growing population of elderly citizens, such as interventions targeting the low grade inflammation, interventions improving the proliferative capacity of satellite cells, and the elimination senescent cells. All these interventions together with less sedentary life style and optimal nutrition have the potential to improve muscle function with significant positive effects on mobility, reducing the risk of fall-related accidents, improving quality of life, albeit having little effect on overall life span. In this context, drugs targeting senescent cells secreting pro-inflammatory cytokines, chemokines, proteases, and inducers of stem cell dysfunction, termed the senescence-associated secretory phenotype (SASP) represent a new and highly promising intervention strategy in experimental models and being close to the introduction in clinical trials.

## 8. ABBREVIATIONS

ActRIIB/IIA=activating type IIB and type IIA receptors

AGE=advanced glycation endproducts

ALK=activating receptor like kinase

ALS=amyotrophic lateral sclerosis

ATP=adenosine triphosphate

BDNF=brain-derived neurotrophic factor

BMP=bone and morphogenetic proteins

BN=brown Norway

CD=capillary density

C:F=capillary to fiber ratio

CIM=critical illness myopathy

CoQ=coenzyme Q

COPD=chronic obstructive pulmonary disease

COX2=cyclooxygenase 2

CRP=C-reactive protein

CS=corticosteroids  
DHOD=di-hydroorotate dehydrogenase  
DHPR=dihydropyridine receptor  
EC=excitation-contraction  
EDL=extensor digitorum longus  
EM= electron microscopy  
EMG=electromyography  
ETFDH=electron-transfer flavoprotein dehydrogenase  
ExICU=experimental intensive care unit  
FCM=fuzzy c-means  
FCSA=fiber cross-sectional area  
FGFR=fibroblast growth factor receptor  
FOXO3=forkhead box protein 3  
GEC=general elliptical cylinder  
GDF=growth and differentiation factors  
GH=growth hormone  
HIF-1 $\alpha$ =hypoxia inducible factor-1  
HSP=heat shock protein  
ICU=intensive care unit  
IGF=insulin growth factor  
IL=interleukin  
JAK/STAT=janus kinase/signal transducers and activators of transcription  
MAPK=mitogen-activated protein kinases  
mCRP=monomeric C-reactive protein  
MDV=mitochondria-derived vesicles  
MND=myonuclear domain  
mtDNA=mitochondrial DNA  
MuRF=muscle-specific RING-B-box  
MUSA=muscle ubiquitin ligase of the SCF complex in atrophy (also known as Fbxo30)  
MyHC=myosin heavy chain  
MyLC=myosin light chain  
MYOD=myogenic differentiation  
NBR1=neighbor of BRCA1 gene 1 protein  
nDNA=nuclear DNA  
NMB=neuromuscular blockade  
NMBA=neuromuscular blocking agents  
NMJ=neuromuscular junction  
NO=nitric oxide  
NSAID=non steroidal anti-inflammatory drugs  
PAX7=transcription factor paired box 7  
pCRP=pentameric C-reactive protein  
PGC1 $\alpha$ = peroxisome proliferator-activated receptor  $\gamma$ , coactivator  
PPS=postpolio syndrome  
QoL=quality of life

RC=respiratory chain  
ROS=reactive oxygen species  
RR=ryanodine receptor  
SASP=senescence-associated secretory phenotype  
SIRT1=sirtuin1  
SPRY1=sprouty 1  
SR=sarcoplasmic reticulum  
TGF $\beta$ =transforming growth factor  $\beta$   
TNF=tumor necrosis factor  
tRNA=transfer RNA  
VEGF=vascular endothelial growth factor  
VIDD=ventilator induced diaphragm dysfunction  
3D=three dimensional

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## 10. REFERENCES

1. **Aagaard P, Suetta C, Caserotti P, Magnusson SP, and Kjaer M.** Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scandinavian journal of medicine & science in sports* 20: 49-64, 2010.
2. **Abou-Khalil R, Mounier R, and Chazaud B.** Regulation of myogenic stem cell behavior by vessel cells: the "menage a trois" of satellite cells, periendothelial cells and endothelial cells. *Cell Cycle* 9: 892-896, 2010.
3. **Acakpo-Satchivi LJ, Edelmann W, Sartorius C, Lu BD, Wahr PA, Watkins SC, Metzger JM, Leinwand L, and Kucherlapati R.** Growth and muscle defects in mice lacking adult myosin heavy chain genes. *J Cell Biol* 139: 1219-1229, 1997.
4. **Acin-Perez R, Fernandez-Silva P, Peleato ML, Perez-Martos A, and Enriquez JA.** Respiratory active mitochondrial supercomplexes. *Mol Cell* 32: 529-539, 2008.
5. **Acosta MJ, Vazquez Fonseca L, Desbats MA, Cerqua C, Zordan R, Trevisson E, and Salviati L.** Coenzyme Q biosynthesis in health and disease. *Biochim Biophys Acta* 1857: 1079-1085, 2016.
6. **Agre JC, Rodriquez AA, and Tafel JA.** Late effects of polio: critical review of the literature on neuromuscular function. *Arch Phys Med Rehabil* 72: 923-931., 1991.
7. **Aguennouz M, Vita GL, Messina S, Cama A, Lanzano N, Ciranni A, Rodolico C, Di Giorgio RM, and Vita G.** Telomere shortening is associated to TRF1 and PARP1 overexpression in Duchenne muscular dystrophy. *Neurobiol Aging* 32: 2190-2197, 2011.
8. **Ahmed SK, Egginton S, Jakeman PM, Mannion AF, and Ross HF.** Is human skeletal muscle capillary supply modelled according to fibre size or fibre type? *Exp Physiol* 82: 231-234, 1997.



9. **Akaaboune M, Culican SM, Turney SG, and Lichtman JW.** Rapid and reversible effects of activity on acetylcholine receptor density at the neuromuscular junction in vivo. *Science* 286: 503-507, 1999.
10. **Akasaki Y, Ouchi N, Izumiya Y, Bernardo BL, Lebrasseur NK, and Walsh K.** Glycolytic fast-twitch muscle fiber restoration counters adverse age-related changes in body composition and metabolism. *Aging Cell* 13: 80-91, 2014.
11. **Akoumianaki E, Lyazidi A, Rey N, Matamis D, Perez-Martinez N, Giraud R, Mancebo J, Brochard L, and Marie Richard JC.** Mechanical ventilation-induced reverse-triggered breaths: a frequently unrecognized form of neuromechanical coupling. *Chest* 143: 927-938, 2013.
12. **Allen DL, Roy RR, and Edgerton VR.** Myonuclear domains in muscle adaptation and disease. *Muscle Nerve* 22: 1350-1360, 1999.
13. **Allen DL, Yasui W, Tanaka T, Ohira Y, Nagaoka S, Sekiguchi C, Hinds WE, Roy RR, and Edgerton VR.** Myonuclear number and myosin heavy chain expression in rat soleus single muscle fibers after spaceflight. *J Appl Physiol* 81: 145-151, 1996.
14. **Almanda AE, Wagers, A.J.** Molecular circuitry of stem cell fate in skeletal muscle regeneration, ageing and disease. *Nature reviews: Molecular cell biology* 5: 267-279, 2016.
15. **Alnaqeeb MA, Al Zaid NS, and Goldspink G.** Connective tissue changes and physical properties of developing and ageing skeletal muscle. *J Anat* 139: 677-689., 1984.
16. **Altun M, Besche HC, Overkleeft HS, Piccirillo R, Edelmann MJ, Kessler BM, Goldberg AL, and Ulfhake B.** Muscle wasting in aged, sarcopenic rats is associated with enhanced activity of the ubiquitin proteasome pathway. *J Biol Chem* 285: 39597-39608, 2010.
17. **Alway SE, Degens H, Krishnamurthy G, and Chaudhrai A.** Denervation stimulates apoptosis but not Id2 expression in hindlimb muscles of aged rats. *J Gerontol A Biol Sci Med Sci* 58: 687-697, 2003.
18. **Amati-Bonneau P, Valentino ML, Reynier P, Gallardo ME, Bornstein B, Boissiere A, Campos Y, Rivera H, de la Aleja JG, Carroccia R, Iommarini L, Labauge P, Figarella-Branger D, Marcorelles P, Furby A, Beauvais K, Letournel F, Liguori R, La Morgia C, Montagna P, Liguori M, Zanna C, Rugolo M, Cossarizza A, Wissinger B, Verny C, Schwarzenbacher R, Martin MA, Arenas J, Ayuso C, Garesse R, Lenaers G, Bonneau D, and Carelli V.** OPA1 mutations induce mitochondrial DNA instability and optic atrophy 'plus' phenotypes. *Brain* 131: 338-351, 2008.
19. **Anderson MJ, and Cohen MW.** Fluorescent staining of acetylcholine receptors in vertebrate skeletal muscle. *J Physiol* 237: 385-400, 1974.
20. **Andersson SG, Zomorodipour A, Andersson JO, Sicheritz-Ponten T, Alsmark UC, Podowski RM, Naslund AK, Eriksson AS, Winkler HH, and Kurland CG.** The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* 396: 133-140, 1998.
21. **Andreu AL, Hanna MG, Reichmann H, Bruno C, Penn AS, Tanji K, Pallotti F, Iwata S, Bonilla E, Lach B, Morgan-Hughes J, and DiMauro S.** Exercise intolerance due to mutations in the cytochrome b gene of mitochondrial DNA. *N Engl J Med* 341: 1037-1044, 1999.
22. **Ansved T, and Edstrom L.** Effects of age on fibre structure, ultrastructure and expression of desmin and spectrin in fast- and slow-twitch rat muscles. *J Anat* 174: 61-79., 1991.
23. **Ansved T, and Larsson L.** Effects of ageing on enzyme-histochemical, morphometrical and contractile properties of the soleus muscle in the rat. *J Neurol Sci* 93: 105-124, 1989.
24. **Ansved T, and Larsson L.** Quantitative and qualitative morphological properties of the soleus motor nerve and the L5 ventral root in young and old rats. Relation to the number of soleus muscle fibers. *J Neurol Sci* 96: 269-282, 1990.
25. **Ansved T, Larsson, L.** Histochemical properties of aging rat skeletal muscle. *Pathobiology of the Aging Rat* 521-534, 1994.
26. **Ansved T, Wallner P, and Larsson L.** Spatial distribution of motor unit fibres in fast- and slow-twitch rat muscles with special reference to age. *Acta Physiol Scand* 143: 345-354, 1991.

27. **Anzil AP, Bieser A, and Wernig A.** Light and electron microscopic identification of nerve terminal sprouting and retraction in normal adult frog muscle. *J Physiol* 350: 393-399, 1984.
28. **Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, Cooper M, Laznik D, Chinsomboon J, Rangwala SM, Baek KH, Rosenzweig A, and Spiegelman BM.** HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature* 451: 1008-1012, 2008.
29. **Arany Z, Lebrasseur N, Morris C, Smith E, Yang W, Ma Y, Chin S, and Spiegelman BM.** The transcriptional coactivator PGC-1beta drives the formation of oxidative type IIX fibers in skeletal muscle. *Cell Metab* 5: 35-46, 2007.
30. **Armstrong RB, Ogilvie RW, and Schwane JA.** Eccentric exercise-induced injury to rat skeletal muscle. *J Appl Physiol* 54: 80-93, 1983.
31. **Asanuma M, and Miyazaki I.** Nonsteroidal anti-inflammatory drugs in experimental parkinsonian models and Parkinson's disease. *Current pharmaceutical design* 14: 1428-1434, 2008.
32. **Ash CE, and Merry BJ.** The molecular basis by which dietary restricted feeding reduces mitochondrial reactive oxygen species generation. *Mechanisms of ageing and development* 132: 43-54, 2011.
33. **Atkinson RA, Srinivas-Shankar U, Roberts SA, Connolly MJ, Adams JE, Oldham JA, Wu FC, Seynnes OR, Stewart CE, Maganaris CN, and Narici MV.** Effects of testosterone on skeletal muscle architecture in intermediate-frail and frail elderly men. *J Gerontol A Biol Sci Med Sci* 65: 1215-1219, 2010.
34. **Ausoni S, Gorza L, Schiaffino S, Gundersen K, and Lomo T.** Expression of myosin heavy chain isoforms in stimulated fast and slow rat muscles. *J Neurosci* 10: 153-160., 1990.
35. **Ausoni S, Gorza L, Schiaffino S, Gundersen K, and Lømo T.** Expression of myosin heavy chain isoforms in stimulated fast and slow rat muscles. *J Neurosci* 10: 153-160, 1990.
36. **Baehr LM, West DW, Marcotte G, Marshall AG, De Sousa LG, Baar K, and Bodine SC.** Age-related deficits in skeletal muscle recovery following disuse are associated with neuromuscular junction instability and ER stress, not impaired protein synthesis. *Aging (Albany NY)* 8: 127-146, 2016.
37. **Baker BA, and Cutlip RG.** Skeletal muscle injury versus adaptation with aging: novel insights on perplexing paradigms. *Exercise and sport sciences reviews* 38: 10-16, 2010.
38. **Balagopal P.** Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol* 273: E790-E800, 1997.
39. **Balagopal P, Rooyackers OE, Adey DB, Ades PA, and Nair KS.** Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol* 273: E790-800, 1997.
40. **Balagopal P, Schimke JC, Ades P, Adey D, and Nair KS.** Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. *Am J Physiol Endocrinol Metab* 280: E203-208., 2001.
41. **Balice-Gordon RJ.** Age-related changes in neuromuscular innervation. *Muscle & Nerve Supplement* 5: S83-S87, 1997.
42. **Balice-Gordon RJ, Breedlove SM, Bernstein S, and Lichtman JW.** Neuromuscular junctions shrink and expand as muscle fiber size is manipulated: in vivo observations in the androgen-sensitive bulbocavernosus muscle of mice. *J Neurosci* 10: 2660-2671, 1990.
43. **Balice-Gordon RJ, and Lichtman JW.** In vivo observations of pre- and postsynaptic changes during the transition from multiple to single innervation at developing neuromuscular junctions. *J Neurosci* 13: 834-855, 1993.
44. **Balice-Gordon RJ, and Lichtman JW.** In vivo visualization of the growth of pre- and postsynaptic elements of neuromuscular junctions in the mouse. *J Neurosci* 10: 894-908, 1990.
45. **Balice-Gordon RJ, and Lichtman JW.** Long-term synapse loss induced by focal blockade of postsynaptic receptors. *Nature* 372: 519-524, 1994.
46. **Ballak SB, Buse-Pot T, Harding PJ, Yap MH, Deldicque L, de Haan A, Jaspers RT, and Degens H.** Blunted angiogenesis and hypertrophy are associated with increased fatigue resistance and unchanged aerobic capacity in old overloaded mouse muscle. *Age* 38: 39, 2016.

47. **Ballak SB, Degens H, de Haan A, and Jaspers RT.** Aging related changes in determinants of muscle force generating capacity: a comparison of muscle aging in men and male rodents. *Ageing Res Rev* 14: 43-55, 2014.
48. **Ballak SB, Jaspers RT, Deldicque L, Chalil S, Peters EL, de Haan A, and Degens H.** Blunted hypertrophic response in old mouse muscle is associated with a lower satellite cell density and is not alleviated by resveratrol. *Exp Gerontol* 62: 23-31, 2015.
49. **Bandy B, and Davison AJ.** Mitochondrial mutations may increase oxidative stress: implications for carcinogenesis and aging? *Free Radic Biol Med* 8: 523-539, 1990.
50. **Banker BQ, Kelly SS, and Robbins N.** Neuromuscular transmission and correlative morphology in young and old mice. *J Physiol* 339: 355-377, 1983.
51. **Bannister RA, Pessah IN, and Beam KG.** The skeletal L-type Ca(2+) current is a major contributor to excitation-coupled Ca(2+) entry. *J Gen Physiol* 133: 79-91, 2009.
52. **Barik A, Lu Y, Sathyamurthy A, Bowman A, Shen C, Li L, Xiong WC, and Mei L.** LRP4 is critical for neuromuscular junction maintenance. *J Neurosci* 34: 13892-13905, 2014.
53. **Barker D, and Ip MC.** Sprouting and degeneration of mammalian motor axons in normal and de-afferented skeletal muscle. *Proc Roy Soc Lond B* 163: 538-554, 1966.
54. **Barrientos A, Casademont J, Rotig A, Miro O, Urbano-Marquez A, Rustin P, and Cardellach F.** Absence of relationship between the level of electron transport chain activities and aging in human skeletal muscle. *Biochem Biophys Res Commun* 229: 536-539, 1996.
55. **Barrientos A, Fontanesi F, and Diaz F.** Evaluation of the mitochondrial respiratory chain and oxidative phosphorylation system using polarography and spectrophotometric enzyme assays. *Curr Protoc Hum Genet* Chapter 19: Unit19 13, 2009.
56. **Bass A, Gutmann E, and Hanzlikova V.** Biochemical and histochemical changes in energy supply enzyme pattern of muscles of the rat during old age. *Gerontologia* 21: 31-45, 1975.
57. **Bassey EJ, Bendall MJ, and Pearson M.** Muscle strength in the triceps surae and objectively measured customary walking activity in men and women over 65 years of age. *Clin Sci* 74: 85-89, 1988.
58. **Bassey EJ, Fiatarone MA, O'Neill EF, Kelly M, Evans WJ, and Lipsitz LA.** Leg extensor power and functional performance in very old men and women. *Clin Sci (Lond)* 82: 321-327, 1992.
59. **Baumer A, Zhang C, Linnane AW, and Nagley P.** Age-related human mtDNA deletions: a heterogeneous set of deletions arising at a single pair of directly repeated sequences. *Am J Hum Genet* 54: 618-630, 1994.
60. **Bautmans I, Onyema O, Van Puyvelde K, Pleck S, and Mets T.** Grip work estimation during sustained maximal contraction: validity and relationship with dependency and inflammation in elderly persons. *J Nutr Health Aging* 15: 731-736, 2011.
61. **Beach RK, and Kostyo JL.** Effect of growth hormone on the DNA content of muscles of young hypophysectomized rats. *Endocrinology* 82: 882-884, 1968.
62. **Beal MF, and Matthews RT.** Coenzyme Q10 in the central nervous system and its potential usefulness in the treatment of neurodegenerative diseases. *Mol Aspects Med* 18: S169-179., 1997.
63. **Becker C, Lord SR, Studenski SA, Warden SJ, Fielding RA, Recknor CP, Hochberg MC, Ferrari SL, Blain H, Binder EF, Rolland Y, Poiraudeau S, Benson CT, Myers SL, Hu L, Ahmad QI, Pacuch KR, Gomez EV, and Benichou O.** Myostatin antibody (LY2495655) in older weak fallers: a proof-of-concept, randomised, phase 2 trial. *Lancet Diabetes Endocrinol* 3: 948-957, 2015.
64. **Beharry AW, Sandesara PB, Roberts BM, Ferreira LF, Senf SM, and Judge AR.** HDAC1 activates FoxO and is both sufficient and required for skeletal muscle atrophy. *J Cell Sci* 127: 1441-1453, 2014.
65. **Bekedam MA, van Beek-Harmsen BJ, Boonstra A, van Mechelen W, Visser FC, and van der Laarse WJ.** Maximum rate of oxygen consumption related to succinate dehydrogenase activity in skeletal muscle fibres of chronic heart failure patients and controls. *Clin Physiol Funct Imaging* 23: 337-343, 2003.

66. **Belanger AY, McComas AJ, and Elder GB.** Physiological properties of two antagonist human muscle groups. *Eur J Appl Physiol Occup Physiol* 51: 381-393, 1983.
67. **Beltran Valls MR, Wilkinson DJ, Narici MV, Smith K, Phillips BE, Caporossi D, and Atherton PJ.** Protein carbonylation and heat shock proteins in human skeletal muscle: relationships to age and sarcopenia. *J Gerontol A Biol Sci Med Sci* 70: 174-181, 2015.
68. **Berg HE, Dudley GA, Haggmark T, Ohlsen H, and Tesch PA.** Effects of lower limb unloading on skeletal muscle mass and function in humans. *J Appl Physiol* 70: 1882-1885, 1991.
69. **Berg HE, Larsson L, and Tesch PA.** Lower limb skeletal muscle function after 6 wk of bed rest. *J Appl Physiol* 82: 182-188, 1997.
70. **Berger A, Mayr JA, Meierhofer D, Fotschl U, Bittner R, Budka H, Grethen C, Huemer M, Kofler B, and Sperl W.** Severe depletion of mitochondrial DNA in spinal muscular atrophy. *Acta Neuropathol* 105: 245-251, 2003.
71. **Bernet JD, Doles JD, Hall JK, Kelly Tanaka K, Carter TA, and Olwin BB.** p38 MAPK signaling underlies a cell-autonomous loss of stem cell self-renewal in skeletal muscle of aged mice. *Nat Med* 20: 265-271, 2014.
72. **Berthelot G, Len S, Hellard P, Tafflet M, Guillaume M, Vollmer JC, Gager B, Quinquis L, Marc A, and Toussaint JF.** Exponential growth combined with exponential decline explains lifetime performance evolution in individual and human species. *Age* 34: 1001-1009, 2012.
73. **Bhuiyan MS, Pattison JS, Osinska H, James J, Gulick J, McLendon PM, Hill JA, Sadoshima J, and Robbins J.** Enhanced autophagy ameliorates cardiac proteinopathy. *J Clin Invest* 123: 5284-5297, 2013.
74. **Billia F, Hauck L, Konecny F, Rao V, Shen J, and Mak TW.** PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci U S A* 108: 9572-9577, 2011.
75. **Bishop DL, Misgeld T, Walsh MK, Gan WB, and Lichtman JW.** Axon branch removal at developing synapses by axosome shedding. *Neuron* 44: 651-661, 2004.
76. **Blake AJ, Morgan K, Bendall MJ, Dallosso H, Ebrahim SB, Arie TH, Fentem PH, and Bassey EJ.** Falls by elderly people at home: prevalence and associated factors. *Age Ageing* 17: 365-372, 1988.
77. **Blau HM, Webster C, and Pavlath GK.** Defective myoblasts identified in Duchenne muscular dystrophy. *Proceedings of the National Academy of Sciences of the United States of America* 80: 4856-4860, 1983.
78. **Blough ER, and Linderman JK.** Lack of skeletal muscle hypertrophy in very aged male Fischer 344 x Brown Norway rats. *J Appl Physiol* 88: 1265-1270., 2000.
79. **Bodine SC, Garfinkel A, Roy RR, and Edgerton VR.** Spatial distribution of motor unit fibers in the cat soleus and tibialis anterior muscles: local interactions. *J Neurosci* 8: 2142-2152., 1988.
80. **Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, and Glass DJ.** Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704-1708, 2001.
81. **Boffoli D, Scacco SC, Vergari R, Solarino G, Santacroce G, and Papa S.** Decline with age of the respiratory chain activity in human skeletal muscle. *Biochim Biophys Acta* 1226: 73-82, 1994.
82. **Bonaldo P, and Sandri M.** Cellular and molecular mechanisms of muscle atrophy. *Dis Model Mech* 6: 25-39, 2013.
83. **Booth FW, and Criswell DS.** Molecular events underlying skeletal muscle atrophy and the development of effective countermeasures. *Int J Sports Med* 18 Suppl 4: S265-269, 1997.
84. **Booth FW, and Kirby CR.** Changes in skeletal muscle gene expression consequent to altered weight bearing. *Am J Physiol* 262: R329-332, 1992.
85. **Boreham CA, Watt PW, Williams PE, Merry BJ, Goldspink G, and Goldspink DF.** Effects of ageing and chronic dietary restriction on the morphology of fast and slow muscles of the rat. *J Anat* 157: 111-125., 1988.
86. **Borg J, Borg K, Edstrom L, Grimby L, Henriksson J, Larsson L, and Tollback A.** Motoneuron and muscle fiber properties of remaining motor units in weak tibialis anterior muscles in prior polio. *Ann N Y Acad Sci* 753: 335-342, 1995.

87. **Borg K, Borg J, Edstrom L, and Grimby L.** Effects of excessive use of remaining muscle fibers in prior polio and LV lesion. *Muscle Nerve* 11: 1219-1230., 1988.
88. **Borg K, and Edstrom L.** Prior poliomyelitis: an immunohistochemical study of cytoskeletal proteins and a marker for muscle fibre regeneration in relation to usage of remaining motor units. *Acta Neurol Scand* 87: 128-132., 1993.
89. **Borgesius NZ, de Waard MC, van der Pluijm I, Omrani A, Zondag GC, van der Horst GT, Melton DW, Hoeijmakers JH, Jaarsma D, and Elgersma Y.** Accelerated age-related cognitive decline and neurodegeneration, caused by deficient DNA repair. *J Neurosci* 31: 12543-12553, 2011.
90. **Bossola M, Pacelli F, Costelli P, Tortorelli A, Rosa F, and Doglietto GB.** Proteasome activities in the rectus abdominis muscle of young and older individuals. *Biogerontology* 9: 261-268, 2008.
91. **Bosutti A, Egginton S, Barnouin Y, Ganse B, Rittweger J, and Degens H.** Local capillary supply in muscle is not determined by local oxidative capacity. *J Exp Biol* 218: 3377-3380, 2015.
92. **Bota DA, and Davies KJ.** Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. *Nat Cell Biol* 4: 674-680, 2002.
93. **Bota DA, Van Remmen H, and Davies KJ.** Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. *FEBS Lett* 532: 103-106, 2002.
94. **Bottinelli R, Betto R, Schiaffino S, and Reggiani C.** Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres. *J Physiol (Lond)* 478: 341-349, 1994.
95. **Brack AS, Bildsoe H, and Hughes SM.** Evidence that satellite cell decrement contributes to preferential decline in nuclear number from large fibres during murine age-related muscle atrophy. *J Cell Sci* 118: 4813-4821, 2005.
96. **Brack AS, and Munoz-Canoves P.** The ins and outs of muscle stem cell aging. *Skeletal muscle* 6: 1, 2015.
97. **Bradlee ML, Mustafa J, Singer MR, and Moore LL.** High-Protein Foods and Physical Activity Protect Against Age-Related Muscle Loss and Functional Decline. *J Gerontol A Biol Sci Med Sci* 73: 88-94, 2017.
98. **Brandstater ME, Lambert, E.H.** A histochemical study of the spatial arrangement of muscle fibers in single motor units within rat tibialis anterior motor units. *Bull Am Ass EMG Electrodiag* 82: 15-16, 1969.
99. **Brandstater ME, Lambert, E.H.** Motor unit anatomy. Type and spatial arrangement of muscle fibers. Basel: Karger, 1973, p. 14-22.
100. **Brierley EJ, Johnson MA, James OF, and Turnbull DM.** Effects of physical activity and age on mitochondrial function. *QJM* 89: 251-258, 1996.
101. **Brill MS, Lichtman JW, Thompson W, Zuo Y, and Misgeld T.** Spatial constraints dictate glial territories at murine neuromuscular junctions. *J Cell Biol* 195: 293-305, 2011.
102. **Brocca L, McPhee JS, Longa E, Canepari M, Seynnes O, De Vito G, Pellegrino MA, Narici M, and Bottinelli R.** Structure and function of human muscle fibres and muscle proteome in physically active older men. *J Physiol* 595: 4823-4844, 2017.
103. **Brocca L, Toniolo L, Reggiani C, Bottinelli R, Sandri M, and Pellegrino MA.** FoxO-dependent atrogenes vary among catabolic conditions and play a key role in muscle atrophy induced by hindlimb suspension. *J Physiol* 595: 1143-1158, 2017.
104. **Brody IA.** Regulation of isometric contraction in skeletal muscle. *Exp Neurol* 50: 673-683., 1976.
105. **Bromley DB.** The Psychology of Human Ageing. *Penguin Books Ltd Middlesex, England* 1971.
106. **Brooks NE, Schuenke MD, and Hikida RS.** Ageing influences myonuclear domain size differently in fast and slow skeletal muscle of rats. *Acta Physiol (Oxf)* 197: 55-63, 2009.
107. **Brooks SV, and Faulkner JA.** Contractile properties of skeletal muscles from young, adult and aged mice. *J Physiol* 404: 71-82., 1988.
108. **Brooks SV, and Faulkner JA.** Contraction-induced injury: recovery of skeletal muscles in young and old mice. *Am J Physiol* 258: C436-442., 1990.

109. **Brooks SV, and Faulkner JA.** Isometric, shortening, and lengthening contractions of muscle fiber segments from adult and old mice. *Am J Physiol* 267: C507-513, 1994.
110. **Brooks SV, and Faulkner JA.** Skeletal muscle weakness in old age: underlying mechanisms. *Med Sci Sports Exerc* 26: 432-439, 1994.
111. **Brown M.** Change in fibre size, not number, in ageing skeletal muscle. *Age Ageing* 16: 244-248, 1987.
112. **Brown M, Ross TP, and Holloszy JO.** Effects of ageing and exercise on soleus and extensor digitorum longus muscles of female rats. *Mech Ageing Dev* 63: 69-77, 1992.
113. **Brown MC, Holland RL, and Hopkins WG.** Motor nerve sprouting. *Ann Rev Neurosci* 4: 17-42, 1981.
114. **Brown MC, Hopkins WG, and Keynes RJ.** Comparison of effects of denervation and botulinum toxin paralysis on muscle properties in mice. *J Physiol* 327: 29-37, 1982.
115. **Brownlee M.** Advanced protein glycosylation in diabetes and aging. *Annu Rev Med* 46: 223-234, 1995.
116. **Bruce SA, Newton D, and Woledge RC.** Effect of age on voluntary force and cross-sectional area of human adductor pollicis muscle. *Q J Exp Physiol* 74: 359-362., 1989.
117. **Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, and Greenberg ME.** Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303: 2011-2015, 2004.
118. **Brunetti M, Miscena A, Salviati A, and Gaiti A.** Effect of aging on the rate of axonal transport of choline-phosphoglycerides. *Neurochemical research* 12: 61-65, 1987.
119. **Bruusgaard JC, Johansen IB, Egner IM, Rana ZA, and Gundersen K.** Myonuclei acquired by overload exercise precede hypertrophy and are not lost on detraining. *Proc Natl Acad Sci U S A* 107: 15111-15116, 2010.
120. **Bruusgaard JC, Liestol K, Ekmark M, Kollstad K, and Gundersen K.** Number and spatial distribution of nuclei in the muscle fibres of normal mice studied in vivo. *J Physiol* 551: 467-478, 2003.
121. **Bruusgaard JC, Liestol K, and Gundersen K.** Distribution of myonuclei and microtubules in live muscle fibers of young, middle-aged, and old mice. *J Appl Physiol* 100: 2024-2030 Epub 2006 Feb 2006, 2006.
122. **Bua E, Johnson J, Herbst A, Delong B, McKenzie D, Salamat S, and Aiken JM.** Mitochondrial DNA-deletion mutations accumulate intracellularly to detrimental levels in aged human skeletal muscle fibers. *Am J Hum Genet* 79: 469-480, 2006.
123. **Bucala R, Model P, and Cerami A.** Modification of DNA by reducing sugars: a possible mechanism for nucleic acid aging and age-related dysfunction in gene expression. *Proc Natl Acad Sci U S A* 81: 105-109, 1984.
124. **Burke RE, Levine DN, Tsairis P, and Zajac FE, 3rd.** Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J Physiol* 234: 723-748, 1973.
125. **Burke RE, Levine DN, and Zajac FE, 3rd.** Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. *Science* 174: 709-712., 1971.
126. **Caccia MR, Harris JB, and Johnson MA.** Morphology and physiology of skeletal muscle in aging rodents. *Muscle Nerve* 2: 202-212., 1979.
127. **Cammarato A, Li XE, Reedy MC, Lee CF, Lehman W, and Bernstein SI.** Structural basis for myopathic defects engendered by alterations in the myosin rod. *J Mol Biol* 414: 477-484, 2011.
128. **Campbell MJ, McComas AJ, and Petito F.** Physiological changes in ageing muscles. *J Neurol Neurosurg Psychiatry* 36: 174-182., 1973.
129. **Campello S, and Scorrano L.** Mitochondrial shape changes: orchestrating cell pathophysiology. *EMBO Rep* 11: 678-684, 2010.
130. **Canepari M, Pellegrino MA, D'Antona G, and Bottinelli R.** Single muscle fiber properties in aging and disuse. *Scand J Med Sci Sports* 2009.
131. **Canto C, Jiang LQ, Deshmukh AS, Matakı C, Coste A, Lagouge M, Zierath JR, and Auwerx J.** Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab* 11: 213-219, 2010.

132. **Cantor S, Zhang W, Delestree N, Remedio L, Mentis GZ, and Burden SJ.** Preserving neuromuscular synapses in ALS by stimulating MuSK with a therapeutic agonist antibody. *eLife* 7: 2018.
133. **Capel F, Rimbert V, Lioger D, Diot A, Rousset P, Mirand PP, Boirie Y, Morio B, and Mosoni L.** Due to reverse electron transfer, mitochondrial H<sub>2</sub>O<sub>2</sub> release increases with age in human vastus lateralis muscle although oxidative capacity is preserved. *Mech Ageing Dev* 126: 505-511, 2005.
134. **Cardasis CA.** Ultrastructural evidence of continued reorganization at the aging (11-26 months) rat soleus neuromuscular junction. *Anat Rec* 207: 399-415, 1983.
135. **Cardasis CA, and LaFontaine DM.** Aging rat neuromuscular junctions: a morphometric study of cholinesterase-stained whole mounts and ultrastructure. *Muscle Nerve* 10: 200-213., 1987.
136. **Cardasis CA, and Padykula HA.** Ultrastructural evidence indicating reorganization at the neuromuscular junction in the normal rat soleus muscle. *Anat Rec* 200: 41-59, 1981.
137. **Carlson BM.** Denervation and the aging of skeletal muscle. *Basic and Applied Myology* 14: 135-139, 2004.
138. **Carnio S, LoVerso F, Baraibar MA, Longa E, Khan MM, Maffei M, Reischl M, Canepari M, Loeffler S, Kern H, Blaauw B, Friguet B, Bottinelli R, Rudolf R, and Sandri M.** Autophagy impairment in muscle induces neuromuscular junction degeneration and precocious aging. *Cell Rep* 8: 1509-1521, 2014.
139. **Carpenter S, and Karpati G.** Necrosis of capillaries in denervation atrophy of human skeletal muscle. *Muscle Nerve* 5: 250-254., 1982.
140. **Carrieu M.** Des amyotrophies spinalis secondaries. Contribution à l'étude de la diffusion des lésions irritatives du système nerveux. *Montpellier* 86: 11-87, 1875.
141. **Casarin A, Giorgi G, Pertegato V, Siviero R, Cerqua C, Doimo M, Basso G, Sacconi S, Cassina M, Rizzuto R, Brosel S, M MD, Dimauro S, Schon EA, Clementi M, Trevisson E, and Salviati L.** Copper and bezafibrate cooperate to rescue cytochrome c oxidase deficiency in cells of patients with SCO2 mutations. *Orphanet journal of rare diseases* 7: 21, 2012.
142. **Cerutti R, Pirinen E, Lamperti C, Marchet S, Sauve AA, Li W, Leoni V, Schon EA, Dantzer F, Auwerx J, Viscomi C, and Zeviani M.** NAD(+)-dependent activation of Sirt1 corrects the phenotype in a mouse model of mitochondrial disease. *Cell Metab* 19: 1042-1049, 2014.
143. **Cesari M, Kritchevsky SB, Baumgartner RN, Atkinson HH, Penninx BW, Lenchik L, Palla SL, Ambrosius WT, Tracy RP, and Pahor M.** Sarcopenia, obesity, and inflammation--results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors study. *The American journal of clinical nutrition* 82: 428-434, 2005.
144. **Chabi B, Ljubcic V, Menzies KJ, Huang JH, Saleem A, and Hood DA.** Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell* 7: 2-12, 2008.
145. **Chai RJ, Vukovic J, Dunlop S, Grounds MD, and Shavlakadze T.** Striking denervation of neuromuscular junctions without lumbar motoneuron loss in geriatric mouse muscle. *PLoS One* 6: e28090, 2011.
146. **Chakkalakal JV, Jones KM, Basson MA, and Brack AS.** The aged niche disrupts muscle stem cell quiescence. *Nature* 490: 355-360, 2012.
147. **Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, Janakiraman K, Sharpless NE, Ding S, Feng W, Luo Y, Wang X, Aykin-Burns N, Krager K, Ponnappan U, Hauer-Jensen M, Meng A, and Zhou D.** Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med* 22: 78-83, 2016.
148. **Chaturvedi RK, and Beal MF.** PPAR: a therapeutic target in Parkinson's disease. *J Neurochem* 106: 506-518, 2008.
149. **Chawla RK, Parks JS, and Rudman D.** Structural variants of human growth hormone: biochemical, genetic, and clinical aspects. *Annu Rev Med* 34: 519-547, 1983.
150. **Cheek DB.** The control of cell mass and replication. The DNA unit--a personal 20-year study. *Early Hum Dev* 12: 211-239, 1985.

151. **Chen KD, and Alway SE.** Clenbuterol reduces soleus muscle fatigue during disuse in aged rats. *Muscle Nerve* 24: 211-222, 2001.
152. **Chen KD, and Alway SE.** A physiological level of clenbuterol does not prevent atrophy or loss of force in skeletal muscle of old rats. *J Appl Physiol* 89: 606-612, 2000.
153. **Chen YW, Nader GA, Baar KR, Fedele MJ, Hoffman EP, and Esser KA.** Response of rat muscle to acute resistance exercise defined by transcriptional and translational profiling. *J Physiol* 545: 27-41, 2002.
154. **Cheng S, Degens H, Evans M, Cheng SM, Selanne H, Rittweger J, Heinonen A, Suominen H, Strandberg T, Alen M, and Korhonen MT.** What Makes a 97-Year-Old Man Cycle 5,000 km a Year? *Gerontology* 62: 508-512, 2016.
155. **Chernov AV, Dolkas J, Hoang K, Angert M, Srikrishna G, Vogl T, Baranovskaya S, Strongin AY, and Shubayev VI.** The calcium-binding proteins S100A8 and S100A9 initiate the early inflammatory program in injured peripheral nerves. *J Biol Chem* 290: 11771-11784, 2015.
156. **Chilibeck PD, Paterson DH, Cunningham DA, Taylor AW, and Noble EG.** Muscle capillarization O<sub>2</sub> diffusion distance, and VO<sub>2</sub> kinetics in old and young individuals. *Journal of applied physiology* 82: 63-69, 1997.
157. **Chopek JW, and Gardiner PF.** Life-long caloric restriction: Effect on age-related changes in motoneuron numbers, sizes and apoptotic markers. *Mech Ageing Dev* 131: 650-659, 2010.
158. **Christov C, Chretien F, Abou-Khalil R, Bassez G, Vallet G, Authier FJ, Bassaglia Y, Shinin V, Tajbakhsh S, Chazaud B, and Gherardi RK.** Muscle satellite cells and endothelial cells: close neighbors and privileged partners. *Mol Biol Cell* 18: 1397-1409, 2007.
159. **Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K, Metzger K, Frezza C, Annaert W, D'Adamio L, Derks C, Dejaegere T, Pellegrini L, D'Hooge R, Scorrano L, and De Strooper B.** Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* 126: 163-175, 2006.
160. **Civiletto G, Varanita T, Cerutti R, Gorletta T, Barbaro S, Marchet S, Lamperti C, Viscomi C, Scorrano L, and Zeviani M.** Opa1 overexpression ameliorates the phenotype of two mitochondrial disease mouse models. *Cell Metab* 21: 845-854, 2015.
161. **Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA, and Guo M.** Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* 441: 1162-1166, 2006.
162. **Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancellato R, and Langin D.** Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *Faseb J* 18: 1657-1669, 2004.
163. **Close RI.** Dynamic properties of mammalian skeletal muscles. *Physiol Rev* 52: 129-197, 1972.
164. **Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, and Holloszy JO.** Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. *Journal of gerontology* 47: B71-76, 1992.
165. **Coggan AR, Spina RJ, Rogers MA, King DS, Brown M, Nemeth PM, and Holloszy JO.** Histochemical and enzymatic characteristics of skeletal muscle in master athletes. *Journal of applied physiology* 68: 1896-1901, 1990.
166. **Cogliati S, Enriquez JA, and Scorrano L.** Mitochondrial Cristae: Where Beauty Meets Functionality. *Trends Biochem Sci* 41: 261-273, 2016.
167. **Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, Cipolat S, Costa V, Casarin A, Gomes LC, Perales-Clemente E, Salviati L, Fernandez-Silva P, Enriquez JA, and Scorrano L.** Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell* 155: 160-171, 2013.
168. **Combaret L, Dardevet D, Rieu I, Pouch MN, Bechet D, Taillandier D, Grizard J, and Attaix D.** A leucine-supplemented diet restores the defective postprandial inhibition of proteasome-dependent proteolysis in aged rat skeletal muscle. *J Physiol* 569: 489-499, 2005.



169. **Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, and Rando TA.** Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433: 760-764, 2005.
170. **Conley KE, Jubrias SA, and Esselman PC.** Oxidative capacity and ageing in human muscle. *J Physiol* 526 Pt 1: 203-210, 2000.
171. **Cook JJ, Wailgum TD, Vasthare US, Mayrovitz HN, and Tuma RF.** Age-related alterations in the arterial microvasculature of skeletal muscle. *J Gerontol* 47: B83-88, 1992.
172. **Coppe JP, Patil CK, Rodier F, Krtolica A, Beausejour CM, Parrinello S, Hodgson JG, Chin K, Desprez PY, and Campisi J.** A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. *PLoS One* 5: e9188, 2010.
173. **Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, Nelson PS, Desprez PY, and Campisi J.** Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS biology* 6: 2853-2868, 2008.
174. **Corbin KB, Gardner, E.D.** Decrease in number of myelinated fibers in human spinal roots with age. *Anat Rec* 68: 63-74, 1937.
175. **Cosgrove BD, Gilbert PM, Porpiglia E, Mourkioti F, Lee SP, Corbel SY, Llewellyn ME, Delp SL, and Blau HM.** Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. *Nat Med* 20: 255-264, 2014.
176. **Cottrell L.** Histologic variations with age in apparently normal peripheral nerve trunks. *Arch Neurol Psychiatry* 43: 1138-1150, 1940.
177. **Courtney J, and Steinbach JH.** Age changes in neuromuscular junction morphology and acetylcholine receptor distribution on rat skeletal muscle fibres. *J Physiol* 320: 435-447, 1981.
178. **Couteaux R, Mira JC, and d'Albis A.** Regeneration of muscles after cardiotoxin injury. I. Cytological aspects. *Biol Cell* 62: 171-182, 1988.
179. **Crist CG, Montarras D, and Buckingham M.** Muscle satellite cells are primed for myogenesis but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules. *Cell stem cell* 11: 118-126, 2012.
180. **Cristea A, Karlsson Edlund, P., Lindblad, J., Qaisar, R., Bengtsson, E., Larsson, L.** Effects of ageing and gender on the spatial organization of nuclei in single human skeletal muscle cells. *Aging Cell* 9: 685-697, 2010.
181. **Cristea A, Korhonen MT, Hakkinen K, Mero A, Alen M, Sipila S, Viitasalo JT, Koljonen MJ, Suominen H, and Larsson L.** Effects of combined strength and sprint training on regulation of muscle contraction at the whole-muscle and single-fibre levels in elite master sprinters. *Acta Physiol (Oxf)* 193: 275-289, 2008.
182. **Cristea A, Qaisar R, Edlund PK, Lindblad J, Bengtsson E, and Larsson L.** Effects of aging and gender on the spatial organization of nuclei in single human skeletal muscle cells. *Aging Cell* 9: 685-697, 2010.
183. **Cristea A, Vaillancourt, DE, Larsson, L.** editor. *Aging-related Changes Motor unit Structure and Function*. 2010, p. 55-74.
184. **Croley AN, Zwetsloot KA, Westerkamp LM, Ryan NA, Pendergast AM, Hickner RC, Pofahl WE, and Gavin TP.** Lower capillarization, VEGF protein, and VEGF mRNA response to acute exercise in the vastus lateralis muscle of aged vs. young women. *Journal of applied physiology* 99: 1872-1879, 2005.
185. **Crosbie RH, Lebakken CS, Holt KH, Venzke DP, Straub V, Lee JC, Grady RM, Chamberlain JS, Sanes JR, and Campbell KP.** Membrane targeting and stabilization of sarcospan is mediated by the sarcoglycan subcomplex. *J Cell Biol* 145: 153-165, 1999.
186. **Cui L, Ju Y, Ding L, Trejo-Morales M, and Olfert IM.** Arteriolar and venular capillary distribution in skeletal muscles of old rats. *J Gerontol A Biol Sci Med Sci* 63: 928-935, 2008.
187. **Culican SM, Nelson CC, and Lichtman JW.** Axon withdrawal during synapse elimination at the neuromuscular junction is accompanied by disassembly of the postsynaptic specialization and withdrawal of Schwann cell processes. *J Neurosci* 18: 4953-4965, 1998.
188. **Cullum NA, Mahon J, Stringer K, and McLean WG.** Glycation of rat sciatic nerve tubulin in experimental diabetes mellitus. *Diabetologia* 34: 387-389, 1991.

189. **Cunningham DA, Morrison D, Rice CL, and Cooke C.** Ageing and isokinetic plantar flexion. *Eur J Appl Physiol Occup Physiol* 56: 24-29, 1987.
190. **D'Antona G, Pellegrino MA, Adami R, Rossi R, Carlizzi CN, Canepari M, Saltin B, and Bottinelli R.** The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. *J Physiol* 552: 499-511, 2003.
191. **Dalakas MC.** New neuromuscular symptoms after old polio ("the post-polio syndrome"): clinical studies and pathogenetic mechanisms. *Birth Defects Orig Artic Ser* 23: 241-264, 1987.
192. **Dalkilic I, and Kunkel LM.** Muscular dystrophies: genes to pathogenesis. *Curr Opin Genet Dev* 13: 231-238, 2003.
193. **Davidson YS, Clague JE, Horan MA, and Pendleton N.** The effect of aging on skeletal muscle capillarization in a murine model. *J Gerontol A Biol Sci Med Sci* 54: B448-451, 1999.
194. **Davies CT, White MJ, and Young K.** Electrically evoked and voluntary maximal isometric tension in relation to dynamic muscle performance in elderly male subjects, aged 69 years. *Eur J Appl Physiol Occup Physiol* 51: 37-43, 1983.
195. **De Coster W, De Reuck J, Sieben G, and Vander Eecken H.** Early ultrastructural changes in aging rat gastrocnemius muscle: a stereologic study. *Muscle Nerve* 4: 111-116., 1981.
196. **De Luca A, Pierno S, Cocchi D, and Conte Camerino D.** Growth hormone administration to aged rats improves membrane electrical properties of skeletal muscle fibers. *The Journal of pharmacology and experimental therapeutics* 269: 948-953, 1994.
197. **de Waard MC, van der Pluijm I, Zuiderveen Borgesius N, Comley LH, Haasdijk ED, Rijksen Y, Ridwan Y, Zondag G, Hoeijmakers JH, Elgersma Y, Gillingwater TH, and Jaarsma D.** Age-related motor neuron degeneration in DNA repair-deficient Ercc1 mice. *Acta Neuropathol* 120: 461-475, 2010.
198. **Decary S, Hamida CB, Mouly V, Barbet JP, Hentati F, and Butler-Browne GS.** Shorter telomeres in dystrophic muscle consistent with extensive regeneration in young children. *Neuromuscul Disord* 10: 113-120, 2000.
199. **Degens H.** Age-related changes in the microcirculation of skeletal muscle. *Advances in experimental medicine and biology* 454: 343-348, 1998.
200. **Degens H.** Determinants of skeletal muscle hypertrophy and the attenuated hypertrophic response at old age. *Sports Medicine & Doping Studies* 1: 2012.
201. **Degens H.** The origin of senescence and death: an evolutionary enigma. *Creation Research Society Quarterly* 44: 280-286, 2008.
202. **Degens H.** The role of systemic inflammation in age-related muscle weakness and wasting. *Scand J Med Sci Sports* 20: 28-38, 2010.
203. **Degens H, and Alway SE.** Control of muscle size during disuse, disease, and aging. *Int J Sports Med* 27: 94-99, 2006.
204. **Degens H, and Alway SE.** Skeletal muscle function and hypertrophy are diminished in old age. *Muscle Nerve* 27: 339-347., 2003.
205. **Degens H, Anderson RK, and Alway SE.** Capillarization in skeletal muscle of rats with cardiac hypertrophy. *Med Sci Sports Exerc* 34: 258-266, 2002.
206. **Degens H, Devעי D, Botto-van Bemden A, Hoofd LJ, and Egginton S.** Maintenance of heterogeneity of capillary spacing is essential for adequate oxygenation in the soleus muscle of the growing rat. *Microcirculation* 13: 467-476, 2006.
207. **Degens H, Gilde AJ, Lindhout M, Willemsen PH, Van Der Vusse GJ, and Van Bilzen M.** Functional and metabolic adaptation of the heart to prolonged thyroid hormone treatment. *Am J Physiol Heart Circ Physiol* 284: H108-H115., 2003.
208. **Degens H, Kosar SN, Hopman MT, and de Haan A.** The time course of denervation-induced changes is similar in soleus muscles of adult and old rats. *Appl Physiol Nutr Metab* 33: 299-308, 2008.

209. **Degens H, and McPhee J.** Muscle wasting, dysfunction and inflammaging. In: *Inflammation, Advancing Age and Nutrition: Research and Clinical Interventions*, edited by Rahman I, and Bagchi D Elsevier, 2013, p. 243-250.
210. **Degens H, Moore JA, and Alway SE.** Vascular endothelial growth factor, capillarization, and function of the rat plantaris muscle at the onset of hypertrophy. *Jpn J Physiol* 53: 181-191, 2003.
211. **Degens H, Morse CI, and Hopman MT.** Heterogeneity of capillary spacing in the hypertrophied plantaris muscle from young-adult and old rats. *Adv Exp Med Biol* 645: 61-66, 2009.
212. **Degens H, Ringnald BE, and Hoofd LJ.** Capillarisation, fibre types and myoglobin content of the dog gracilis muscle. *Adv Exp Med Biol* 361: 533-539, 1994.
213. **Degens H, and Rittweger J.** What is new in musculoskeletal interactions? Muscle oxygenation, myonuclear domain, acupuncture, titin and phosphate. *J Musculoskelet Neuronal Interact* 10: 245-248, 2010.
214. **Degens H, Turek Z, and Binkhorst RA.** Compensatory hypertrophy and training effects on the functioning of aging rat m. plantaris. *Mech Ageing Dev* 66: 299-311., 1993.
215. **Degens H, Turek Z, Hoofd L, van't Hof MA, and Binkhorst RA.** Capillarisation and fibre types in hypertrophied m. plantaris in rats of various ages. *Respir Physiol* 94: 217-226, 1993.
216. **Degens H, Turek Z, Hoofd LJ, and Binkhorst RA.** Capillary proliferation related to fibre types in hypertrophied aging rat M. plantaris. *Adv Exp Med Biol* 345: 669-676, 1994.
217. **Degens H, Turek Z, Hoofd LJ, Van't Hof MA, and Binkhorst RA.** The relationship between capillarisation and fibre types during compensatory hypertrophy of the plantaris muscle in the rat. *Journal of anatomy* 180 ( Pt 3): 455-463, 1992.
218. **Degens H, Yu F, Li X, and Larsson L.** Effects of age and gender on shortening velocity and myosin isoforms in single rat muscle fibres. *Acta Physiol Scand* 163: 33-40, 1998.
219. **Del Campo A, Contreras-Hernandez I, Castro-Sepulveda M, Campos CA, Figueroa R, Tevy MF, Eisner V, Casas M, and Jaimovich E.** Muscle function decline and mitochondria changes in middle age precede sarcopenia in mice. *Aging (Albany NY)* 10: 34-55, 2018.
220. **Delbono O, Renganathan M, and Messi ML.** Excitation-Ca<sup>2+</sup> release-contraction coupling in single aged human skeletal muscle fiber. *Muscle Nerve Suppl* 5: S88-92, 1997.
221. **Denis C, Chatard JC, Dormois D, Linossier MT, Geysant A, and Lacour JR.** Effects of endurance training on capillary supply of human skeletal muscle on two age groups (20 and 60 years). *Journal de physiologie* 81: 379-383, 1986.
222. **Dennis RA, Przybyla B, Gurley C, Kortebein PM, Simpson P, Sullivan DH, and Peterson CA.** Aging alters gene expression of growth and remodeling factors in human skeletal muscle both at rest and in response to acute resistance exercise. *Physiol Genomics* 32: 393-400, 2008.
223. **Deruisseau KC, Kavazis AN, and Powers SK.** Selective downregulation of ubiquitin conjugation cascade mRNA occurs in the senescent rat soleus muscle. *Exp Gerontol* 40: 526-531, 2005.
224. **Desai VG, Weindruch R, Hart RW, and Feuers RJ.** Influences of age and dietary restriction on gastrocnemius electron transport system activities in mice. *Arch Biochem Biophys* 333: 145-151, 1996.
225. **Desaki J, and Ezaki T.** Discontinuous capillary segments in the extensor digitorum longus muscle of aged BUF/Mna rats. *Journal of electron microscopy* 51: 425-439, 2002.
226. **Desbats MA, Lunardi G, Doimo M, Trevisson E, and Salviati L.** Genetic bases and clinical manifestations of coenzyme Q10 (CoQ 10) deficiency. *J Inherit Metab Dis* 38: 145-156, 2015.
227. **Desbats MA, Vetro A, Limongelli I, Lunardi G, Casarin A, Doimo M, Spinazzi M, Angelini C, Cenacchi G, Burlina A, Rodriguez Hernandez MA, Chiandetti L, Clementi M, Trevisson E, Navas P, Zuffardi O, and Salviati L.** Primary coenzyme Q10 deficiency presenting as fatal neonatal multiorgan failure. *Eur J Hum Genet* 23: 1254-1258, 2015.
228. **Deschenes MR, Roby MA, Eason MK, and Harris MB.** Remodeling of the neuromuscular junction precedes sarcopenia related alterations in myofibers. *Experimental gerontology* 45: 389-393, 2010.

229. **DeVan AE, and Seals DR.** Vascular health in the ageing athlete. *Experimental physiology* 97: 305-310, 2012.
230. **Deveci D, and Egginton S.** Muscle ischaemia in rats may be relieved by overload-induced angiogenesis. *Exp Physiol* 87: 479-488., 2002.
231. **Di Giulio C, Petruccelli G, Bianchi G, Cacchio M, and Verratti V.** Does hypoxia cause sarcopenia? Prevention of hypoxia could reduce sarcopenia. *Journal of biological regulators and homeostatic agents* 23: 55-58, 2009.
232. **Diao Y, Guo X, Li Y, Sun K, Lu L, Jiang L, Fu X, Zhu H, Sun H, Wang H, and Wu Z.** Pax3/7BP is a Pax7- and Pax3-binding protein that regulates the proliferation of muscle precursor cells by an epigenetic mechanism. *Cell stem cell* 11: 231-241, 2012.
233. **Diaz M, Degens H, Vanhees L, Austin C, and Azzawi M.** The effects of resveratrol on aging vessels. *Exp Gerontol* 85: 41-47, 2016.
234. **DiMauro S, Schon EA, Carelli V, and Hirano M.** The clinical maze of mitochondrial neurology. *Nat Rev Neurol* 9: 429-444, 2013.
235. **Dirks AJ, and Leeuwenburgh C.** Caloric restriction in humans: potential pitfalls and health concerns. *Mech Ageing Dev* 127: 1-7, 2006.
236. **Distefano G, Standley RA, Zhang X, Carnero EA, Yi F, Cornell HH, and Coen PM.** Physical activity unveils the relationship between mitochondrial energetics, muscle quality, and physical function in older adults. *J Cachexia Sarcopenia Muscle* 2018.
237. **Doimo M, Desbats MA, Baldoïn MC, Lenzini E, Basso G, Murphy E, Graziano C, Seri M, Burlina A, Sartori G, Trevisson E, and Salviati L.** Functional analysis of missense mutations of OAT, causing gyrate atrophy of choroid and retina. *Hum Mutat* 34: 229-236, 2013.
238. **Donato AJ, Uberoi A, Wray DW, Nishiyama S, Lawrenson L, and Richardson RS.** Differential effects of aging on limb blood flow in humans. *Am J Physiol Heart Circ Physiol* 290: H272-278, 2006.
239. **Dong X, Milholland B, and Vijg J.** Evidence for a limit to human lifespan. *Nature* 538: 257-259, 2016.
240. **Doyle AM, Mayer, R.F.** Studies of the motor unit in the cat. *Bull Sch Med Univ Maryland* 54: 11-17, 1969.
241. **Drey M, Sieber CC, Degens H, McPhee J, Korhonen MT, Muller K, Ganse B, and Rittweger J.** Relation between muscle mass, motor units and type of training in master athletes. *Clin Physiol Funct Imaging* 36: 70-76, 2016.
242. **Drummond MJ, Addison O, Bruncker L, Hopkins PN, McClain DA, LaStayo PC, and Marcus RL.** Downregulation of E3 ubiquitin ligases and mitophagy-related genes in skeletal muscle of physically inactive, frail older women: a cross-sectional comparison. *J Gerontol A Biol Sci Med Sci* 69: 1040-1048, 2014.
243. **Duchen LW, Excell BJ, Patel R, and Smith B.** Changes in motor end-plates resulting from muscle fibre necrosis and regeneration. A light and electron microscopic study of the effects of the depolarizing fraction (cardiotoxin) of *Dendroaspis jamesoni* venom. *J Neurol Sci* 21: 391-417, 1974.
244. **Dudley GA, Duvoisin MR, Convertino VA, and Buchanan P.** Alterations of the in vivo torque-velocity relationship of human skeletal muscle following 30 days exposure to simulated microgravity. *Aviat Space Environ Med* 60: 659-663, 1989.
245. **Dulhunty A, and Valois A.** Indentations in the terminal cisternae of amphibian and mammalian skeletal muscle fibers. *J Ultrastruct Res* 84: 34-49., 1983.
246. **Dunaevsky A, and Connor EA.** Long-term maintenance of presynaptic function in the absence of target muscle fibers. *J Neurosci* 15: 6137-6144, 1995.
247. **Dunaevsky A, and Connor EA.** Stability of frog motor nerve terminals in the absence of target muscle fibers. *Dev Biol* 194: 61-71, 1998.
248. **Duncan D.** A relation between axone diameter and myelination determined by measurement of myelinated root fibres. *J Comp Neurol* 60: 437-491, 1934.

249. **Durham SE, Bonilla E, Samuels DC, DiMauro S, and Chinnery PF.** Mitochondrial DNA copy number threshold in mtDNA depletion myopathy. *Neurology* 65: 453-455, 2005.
250. **Eccles JC, Sherrington, C.S.** Numbers and contraction values of individual motor units examined in some muscles of the limb. *Proc R Soc Lond B Biol Sci* 106: 326-357, 1930.
251. **Echaniz-Laguna A, Mohr M, Lannes B, and Tranchant C.** Myopathies in the elderly: a hospital-based study. *Neuromuscul Disord* 20: 443-447, 2010.
252. **Eddinger TJ, Cassens RG, and Moss RL.** Mechanical and histochemical characterization of skeletal muscles from senescent rats. *Am J Physiol* 251: C421-430, 1986.
253. **Edgerton VR, and Roy RR.** Regulation of skeletal muscle fiber size, shape and function. *J Biomech* 24: 123-133, 1991.
254. **Edgerton VR, Zhou MY, Ohira Y, Klitgaard H, Jiang B, Bell G, Harris B, Saltin B, Gollnick PD, Roy RR, and et al.** Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. *J Appl Physiol* 78: 1733-1739, 1995.
255. **Edington DW, Cosmas AC, and McCafferty WB.** Exercise and longevity: evidence for a threshold age. *J Gerontol* 27: 341-343, 1972.
256. **Edstrom E, Altun M, Hagglund M, and Ulfhake B.** Atrogin-1/MAFbx and MuRF1 are downregulated in aging-related loss of skeletal muscle. *J Gerontol A Biol Sci Med Sci* 61: 663-674, 2006.
257. **Edstrom L, and Kugelberg E.** Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat. *J Neurol Neurosurg Psychiatry* 31: 424-433., 1968.
258. **Edstrom L, and Larsson L.** Effects of age on contractile and enzyme-histochemical properties of fast- and slow-twitch single motor units in the rat. *J Physiol (Lond)* 392: 129-145, 1987.
259. **Edström L, and Larsson L.** Effects of age on contractile and enzyme-histochemical properties of fast- and slow-twitch single motor units in the rat. *J Physiol* 392: 129-145, 1987.
260. **Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, Mallozzi C, Jacobi C, Jennings LL, Clay I, Laurent G, Ma S, Brachat S, Lach-Trifilieff E, Shavlakadze T, Trendelenburg AU, Brack AS, and Glass DJ.** GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. *Cell Metab* 22: 164-174, 2015.
261. **Egerman MA, and Glass DJ.** Signaling pathways controlling skeletal muscle mass. *Critical reviews in biochemistry and molecular biology* 49: 59-68, 2014.
262. **Egginton S.** Physiological factors influencing capillary growth. *Acta Physiol* 202: 225-239, 2011.
263. **Egginton S, Badr I, Williams J, Hauton D, Baan GC, and Jaspers RT.** Physiological angiogenesis is a graded, not threshold, response. *J Physiol* 589: 195-206, 2011.
264. **Egginton S, and Gaffney EA.** Tissue capillary supply - it's quality not quantity that counts! *Exp Physiol* 95: 971-979, 2010.
265. **Einsiedel LJ, and Luff AR.** Effect of partial denervation on motor units in the ageing rat medial gastrocnemius. *J Neurol Sci* 112: 178-184., 1992.
266. **Emma F, Montini G, Parikh SM, and Salviati L.** Mitochondrial dysfunction in inherited renal disease and acute kidney injury. *Nat Rev Nephrol* 12: 267-280, 2016.
267. **Englander LL, and Rubin LL.** Acetylcholine receptor clustering and nuclear movement in muscle fibers in culture. *J Cell Biol* 104: 87-95, 1987.
268. **Englund DA, Kirn DR, Koochek A, Zhu H, Trivison TG, Reid KF, von Berens A, Melin M, Cederholm T, Gustafsson T, and Fielding RA.** Nutritional Supplementation With Physical Activity Improves Muscle Composition in Mobility-Limited Older Adults, The VIVE2 Study: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Gerontol A Biol Sci Med Sci* 73: 95-101, 2017.
269. **Erskine RM, and Degens H.** Muscle growth, repair and preservation. A mechanistic approach. In: *Nutrition and enhanced sports performance Muscle building, endurance and strength*, edited by Bagchi D, Nair S, and Sen KS. London: Academic press, 2013, p. 247-263.
270. **Evans WJ.** Effects of aging and exercise on nutrition needs of the elderly. *Nutrition reviews* 54: S35-39, 1996.

271. **Evans WJ.** Exercise and protein metabolism. *World review of nutrition and dietetics* 71: 21-33, 1993.
272. **Evans WJ.** Exercise, nutrition and aging. *J Nutr* 122: 796-801, 1992.
273. **Evans WJ.** Exercise, nutrition, and aging. *Clinics in geriatric medicine* 11: 725-734, 1995.
274. **Faber JE, Zhang H, Lassance-Soares RM, Prabhakar P, Najafi AH, Burnett MS, and Epstein SE.** Aging causes collateral rarefaction and increased severity of ischemic injury in multiple tissues. *Arterioscler Thromb Vasc Biol* 31: 1748-1756, 2011.
275. **Fahim MA, Holley JA, and Robbins N.** Scanning and light microscopic study of age changes at a neuromuscular junction in the mouse. *Journal of neurocytology* 12: 13-25, 1983.
276. **Fananapazir L, Dalakas MC, Cyran F, Cohn G, and Epstein ND.** Missense mutations in the beta-myosin heavy-chain gene cause central core disease in hypertrophic cardiomyopathy. *Proc Natl Acad Sci U S A* 90: 3993-3997, 1993.
277. **Faulkner JA, Brooks SV, and Zerba E.** Muscle atrophy and weakness with aging: contraction-induced injury as an underlying mechanism. *J Gerontol A Biol Sci Med Sci* 50 Spec No: 124-129, 1995.
278. **Faulkner JA, Jones DA, and Round JM.** Injury to skeletal muscles of mice by forced lengthening during contractions. *Q J Exp Physiol* 74: 661-670, 1989.
279. **Faulkner JA, Larkin LM, Claflin DR, and Brooks SV.** Age-related changes in the structure and function of skeletal muscles. *Clin Exp Pharmacol Physiol* 34: 1091-1096, 2007.
280. **Fauteck SP, and Kandarian SC.** Sensitive detection of myosin heavy chain composition in skeletal muscle under different loading conditions. *Am J Physiol* 268: C419-424, 1995.
281. **Fedorov YV, Jones NC, and Olwin BB.** Regulation of myogenesis by fibroblast growth factors requires beta-gamma subunits of pertussis toxin-sensitive G proteins. *Mol Cell Biol* 18: 5780-5787, 1998.
282. **Feng G, Krejci E, Molgo J, Cunningham JM, Massoulié J, and Sanes JR.** Genetic analysis of collagen Q: roles in acetylcholinesterase and butyrylcholinesterase assembly and in synaptic structure and function. *J Cell Biol* 144: 1349-1360, 1999.
283. **Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, and Sanes JR.** Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* 28: 41-51., 2000.
284. **Ferrara N, and Davis-Smyth T.** The biology of vascular endothelial growth factor. *Endocr Rev* 18: 4-25., 1997.
285. **Ferrington DA, Husom AD, and Thompson LV.** Altered proteasome structure, function, and oxidation in aged muscle. *FASEB J* 19: 644-646, 2005.
286. **Ferrucci L, Harris TB, Guralnik JM, Tracy RP, Corti MC, Cohen HJ, Penninx B, Pahor M, Wallace R, and Havlik RJ.** Serum IL-6 level and the development of disability in older persons. *J Am Geriatr Soc* 47: 639-646, 1999.
287. **Ferrucci L, Penninx BW, Volpato S, Harris TB, Bandeen-Roche K, Balfour J, Leveille SG, Fried LP, and Md JM.** Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. *J Am Geriatr Soc* 50: 1947-1954, 2002.
288. **Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, and Evans WJ.** High-intensity strength training in nonagenarians. Effects on skeletal muscle. *JAMA* 263: 3029-3034, 1990.
289. **Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, and Evans WJ.** Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* 330: 1769-1775, 1994.
290. **Finer JT, Simmons RM, and Spudich JA.** Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* 368: 113-119, 1994.
291. **Fischbach GD, and Robbins N.** Effect of chronic disuse of rat soleus neuromuscular junctions on postsynaptic membrane. *J Neurophysiol* 34: 562-569, 1971.
292. **Fitts RH, Costill DL, and Gardetto PR.** Effect of swim exercise training on human muscle fiber function. *J Appl Physiol* 66: 465-475, 1989.

293. **Fitts RH, Peters JR, Dillon EL, Durham WJ, Sheffield-Moore M, and Urban RJ.** Weekly versus monthly testosterone administration on fast and slow skeletal muscle fibers in older adult males. *J Clin Endocrinol Metab* 100: E223-231, 2015.
294. **Fitts RH, Winder WW, Brooke MH, Kaiser KK, and Holloszy JO.** Contractile, biochemical, and histochemical properties of thyrotoxic rat soleus muscle. *Am J Physiol* 238: C14-20., 1980.
295. **Fleg JL, and Lakatta EG.** Role of muscle loss in the age-associated reduction in VO<sub>2</sub> max. *J Appl Physiol* 65: 1147-1151., 1988.
296. **Florini JR.** Hormonal control of muscle growth. *Muscle Nerve* 10: 577-598, 1987.
297. **Florini JR, Ewton DZ, and Coolican SA.** Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev* 17: 481-517, 1996.
298. **Fluck M, and Hoppeler H.** Molecular basis of skeletal muscle plasticity--from gene to form and function. *Rev Physiol Biochem Pharmacol* 146: 159-216, 2003.
299. **Folker ES, and Baylies MK.** Nuclear positioning in muscle development and disease. *Frontiers in physiology* 4: 363, 2013.
300. **Fox MA, Sanes JR, Borza DB, Eswarakumar VP, Fassler R, Hudson BG, John SW, Ninomiya Y, Pedchenko V, Pfaff SL, Rheault MN, Sado Y, Segal Y, Werle MJ, and Umemori H.** Distinct target-derived signals organize formation, maturation, and maintenance of motor nerve terminals. *Cell* 129: 179-193, 2007.
301. **Frantzell A, Ingelmark, B.E.** Occurrence and distribution of fatmin human muscles at various age levels. *Acta Soc Med Upsalien* 56: 59-87, 1951.
302. **Frezza C, Cipolat S, Martins de Brito O, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De Strooper B, and Scorrano L.** OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 126: 177-189, 2006.
303. **Fridén J, and Lieber RL.** Segmental muscle fiber lesions after repetitive eccentric contractions. *Cell Tissue Res* 293: 165-171, 1998.
304. **Fridman JS, and Lowe SW.** Control of apoptosis by p53. *Oncogene* 22: 9030-9040, 2003.
305. **Frontera WR, Grimby L, and Larsson L.** Firing rate of the lower motoneuron and contractile properties of its muscle fibers after upper motoneuron lesion in man. *Muscle Nerve* 20: 938-947, 1997.
306. **Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, and Roubenoff R.** Aging of skeletal muscle: a 12-yr longitudinal study. *J Appl Physiol* 88: 1321-1326, 2000.
307. **Frontera WR, Hughes VA, Lutz KJ, and Evans WJ.** A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. *J Appl Physiol* 71: 644-650., 1991.
308. **Frontera WR, Meredith CN, O'Reilly KP, Knuttgen HG, and Evans WJ.** Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J Appl Physiol* 64: 1038-1044, 1988.
309. **Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, and Roubenoff R.** Skeletal muscle fiber quality in older men and women. *Am J Physiol Cell Physiol* 279: C611-618., 2000.
310. **Fuglevand AJ, and Segal SS.** Simulation of motor unit recruitment and microvascular unit perfusion: spatial considerations. *J Appl Physiol* 83: 1223-1234, 1997.
311. **Fujisawa K.** Some observations on the skeletal musculature of aged rats. I. Histological aspects. *J Neurol Sci* 22: 353-366., 1974.
312. **Funai K, Parkington JD, Carambula S, and Fielding RA.** Age-associated decrease in contraction-induced activation of downstream targets of Akt/mTor signaling in skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 290: R1080-1086, 2006.
313. **Galea V.** Changes in motor unit estimates with aging. *J Clin Neurophysiol* 13: 253-260, 1996.
314. **Gallegly JC, Turesky NA, Strotman BA, Gurley CM, Peterson CA, and Dupont-Versteegden EE.** Satellite cell regulation of muscle mass is altered at old age. *J Appl Physiol* 97: 1082-1090 Epub 2004 Apr 1030, 2004.
315. **Galler S, Hilber K, Gohlsch B, and Pette D.** Two functionally distinct myosin heavy chain isoforms in slow skeletal muscle fibres. *FEBS Lett* 410: 150-152, 1997.

316. **Gan WB, Bishop DL, Turney SG, and Lichtman JW.** Vital imaging and ultrastructural analysis of individual axon terminals labeled by iontophoretic application of lipophilic dye. *J Neurosci Methods* 93: 13-20, 1999.
317. **Gan WB, and Lichtman JW.** Synaptic segregation at the developing neuromuscular junction. *Science* 282: 1508-1511, 1998.
318. **Gardetto PR, Schluter JM, and Fitts RH.** Contractile function of single muscle fibers after hindlimb suspension. *Journal of applied physiology* 66: 2739-2749, 1989.
319. **Gardner E.** Decrease in human neurones with age. *Anat Rec* 77: 529-536, 1940.
320. **Gaugler M, Brown A, Merrell E, DiSanto-Rose M, Rathmacher JA, and Reynolds THT.** PKB signaling and atrogene expression in skeletal muscle of aged mice. *J Appl Physiol (1985)* 111: 192-199, 2011.
321. **Gava P, Kern H, and Carraro U.** Age-associated power decline from running, jumping, and throwing male masters world records. *Experimental aging research* 41: 115-135, 2015.
322. **Gavin TP, Ruster RS, Carrithers JA, Zwetsloot KA, Kraus RM, Evans CA, Knapp DJ, Drew JL, McCartney JS, Garry JP, and Hickner RC.** No difference in the skeletal muscle angiogenic response to aerobic exercise training between young and aged men. *J Physiol* 585: 231-239, 2007.
323. **Geisterfer-Lowrance AA, Christe M, Conner DA, Ingwall JS, Schoen FJ, Seidman CE, and Seidman JG.** A mouse model of familial hypertrophic cardiomyopathy. *Science* 272: 731-734, 1996.
324. **Gensler S, Sander A, Korngreen A, Traina G, Giese G, and Witzemann V.** Assembly and clustering of acetylcholine receptors containing GFP-tagged epsilon or gamma subunits: selective targeting to the neuromuscular junction in vivo. *Eur J Biochem* 268: 2209-2217, 2001.
325. **Germani A, Di Carlo A, Mangoni A, Straino S, Giacinti C, Turrini P, Biglioli P, and Capogrossi MC.** Vascular endothelial growth factor modulates skeletal myoblast function. *Am J Pathol* 163: 1417-1428, 2003.
326. **Ghosh P, Mora Solis FR, Dominguez JM, 2nd, Spier SA, Donato AJ, Delp MD, and Muller-Delp JM.** Exercise training reverses aging-induced impairment of myogenic constriction in skeletal muscle arterioles. *Journal of applied physiology* 118: 904-911, 2015.
327. **Gibson MC, and Schultz E.** Age-related differences in absolute numbers of skeletal muscle satellite cells. *Muscle & nerve* 6: 574-580, 1983.
328. **Gill JF, Santos G, Schnyder S, and Handschin C.** PGC-1alpha affects aging-related changes in muscle and motor function by modulating specific exercise-mediated changes in old mice. *Aging Cell* 17: 2018.
329. **Giresi PG, Stevenson EJ, Theilhaber J, Koncarevic A, Parkington J, Fielding RA, and Kandarian SC.** Identification of a molecular signature of sarcopenia. *Physiol Genomics* 21: 253-263, 2005.
330. **Gliemann L, Olesen J, Bienso RS, Schmidt JF, Akerstrom T, Nyberg M, Lindqvist A, Bangsbo J, and Hellsten Y.** Resveratrol modulates the angiogenic response to exercise training in skeletal muscles of aged men. *Am J Physiol Heart Circ Physiol* 307: H1111-1119, 2014.
331. **Goethals S, Ydens E, Timmerman V, and Janssens S.** Toll-like receptor expression in the peripheral nerve. *Glia* 58: 1701-1709, 2010.
332. **Goldman D, Bateman RM, and Ellis CG.** Effect of decreased O<sub>2</sub> supply on skeletal muscle oxygenation and O<sub>2</sub> consumption during sepsis: role of heterogeneous capillary spacing and blood flow. *Am J Physiol Heart Circ Physiol* 290: H2277-2285, 2006.
333. **Goldspink G, Scutt A, Martindale J, Jaenicke T, Turay L, and Gerlach GF.** Stretch and force generation induce rapid hypertrophy and myosin isoform gene switching in adult skeletal muscle. *Biochem Soc Trans* 19: 368-373., 1991.
334. **Gomes MD, Lecker SH, Jagoe RT, Navon A, and Goldberg AL.** Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc Natl Acad Sci U S A* 98: 14440-14445, 2001.
335. **Gonzalez E, Messi ML, and Delbono O.** The specific force of single intact extensor digitorum longus and soleus mouse muscle fibers declines with aging. *J Membr Biol* 178: 175-183, 2000.



336. **Gonzalez E, Messi ML, Zheng Z, and Delbono O.** Insulin-like growth factor-1 prevents age-related decrease in specific force and intracellular Ca<sup>2+</sup> in single intact muscle fibres from transgenic mice. *J Physiol* 552: 833-844, 2003.
337. **Gonzalez M, Ruggiero FP, Chang Q, Shi YJ, Rich MM, Kraner S, and Balice-Gordon RJ.** Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions. *Neuron* 24: 567-583, 1999.
338. **Gopinath SD, Webb AE, Brunet A, and Rando TA.** FOXO3 promotes quiescence in adult muscle stem cells during the process of self-renewal. *Stem cell reports* 2: 414-426, 2014.
339. **Gorza L, Gundersen K, Lomo T, Schiaffino S, and Westgaard RH.** Slow-to-fast transformation of denervated soleus muscles by chronic high-frequency stimulation in the rat. *J Physiol* 402: 627-649, 1988.
340. **Gospodarowicz D, Greene G, and Moran J.** Fibroblast growth factor can substitute for platelet factor to sustain the growth of Balb/3T3 cells in the presence of plasma. *Biochem Biophys Res Commun* 65: 779-787, 1975.
341. **Gospodarowicz D, Weseman J, and Moran J.** Presence in brain of a mitogenic agent promoting proliferation of myoblasts in low density culture. *Nature* 256: 216-219, 1975.
342. **Gospillou G, Bourdel-Marchasson I, Rouland R, Calmettes G, Franconi JM, Deschodt-Arsac V, and Diolez P.** Alteration of mitochondrial oxidative phosphorylation in aged skeletal muscle involves modification of adenine nucleotide translocator. *Biochim Biophys Acta* 1797: 143-151, 2010.
343. **Gospillou G, Sgarlato N, Kapchinsky S, Purves-Smith F, Norris B, Pion CH, Barbat-Artigas S, Lemieux F, Taivassalo T, Morais JA, Aubertin-Leheudre M, and Hepple RT.** Increased sensitivity to mitochondrial permeability transition and myonuclear translocation of endonuclease G in atrophied muscle of physically active older humans. *FASEB J* 28: 1621-1633, 2014.
344. **Gospillou G, Sgarlato N, Norris B, Barbat-Artigas S, Aubertin-Leheudre M, Morais JA, Burelle Y, Taivassalo T, and Hepple RT.** The relationship between muscle fiber type-specific PGC-1 $\alpha$  content and mitochondrial content varies between rodent models and humans. *PLoS One* 9: e103044, 2014.
345. **Grabowska W, Sikora E, and Bielak-Zmijewska A.** Sirtuins, a promising target in slowing down the ageing process. *Biogerontology* 18: 447-476, 2017.
346. **Grady RM, Starr DA, Ackerman GL, Sanes JR, and Han M.** Syne proteins anchor muscle nuclei at the neuromuscular junction. *Proceedings of the National Academy of Sciences of the United States of America* 102: 4359-4364, 2005.
347. **Gray SD, and Renkin EM.** Microvascular supply in relation to fiber metabolic type in mixed skeletal muscles on rabbits. *Microvasc Res* 16: 406-425, 1978.
348. **Greaser ML, Moss RL, and Reiser PJ.** Variations in contractile properties of rabbit single muscle fibres in relation to troponin T isoforms and myosin light chains. *J Physiol (Lond)* 406: 85-98, 1988.
349. **Green H, Goreham C, Ouyang J, Ball-Burnett M, and Ranney D.** Regulation of fiber size, oxidative potential, and capillarization in human muscle by resistance exercise. *Am J Physiol* 276: R591-R596, 1999.
350. **Greenman MJ.** The number, size and axis-sheath relation of large myelinated fibers in the peroneal nerve of the inbred albino rat under normal conditions, in disease and after stimulation. *J Comp Neurol* 27: 403-420, 1917.
351. **Greggio C, Jha P, Kulkarni SS, Lagarrigue S, Broskey NT, Boutant M, Wang X, Conde Alonso S, Ofori E, Auwerx J, Canto C, and Amati F.** Enhanced Respiratory Chain Supercomplex Formation in Response to Exercise in Human Skeletal Muscle. *Cell Metab* 25: 301-311, 2017.
352. **Greiwe JS, Cheng B, Rubin DC, Yarasheski KE, and Semenkovich CF.** Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. *Faseb J* 15: 475-482, 2001.
353. **Gremeaux V, Gayda M, Lepers R, Sosner P, Juneau M, and Nigam A.** Exercise and longevity. *Maturitas* 73: 312-317, 2012.
354. **Grimby G, and Stalberg E.** Dynamic changes in muscle structure and electrophysiology in late polio with aspects on muscular trainability. *Scand J Rehabil Med Suppl* 30: 33-44, 1994.

355. **Grimby L, Tollback A, Muller U, and Larsson L.** Fatigue of chronically overused motor units in prior polio patients. *Muscle Nerve* 19: 728-737, 1996.
356. **Groen BB, Hamer HM, Snijders T, van Kranenburg J, Frijns D, Vink H, and van Loon LJ.** Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes. *Journal of applied physiology* 116: 998-1005, 2014.
357. **Gunther S, Kim J, Kostin S, Lepper C, Fan CM, and Braun T.** Myf5-positive satellite cells contribute to Pax7-dependent long-term maintenance of adult muscle stem cells. *Cell stem cell* 13: 590-601, 2013.
358. **Gurahian SM, and Goldberg SJ.** Fatigue of lateral rectus and retractor bulbi motor units in cat. *Brain Res* 415: 281-292., 1987.
359. **Gutmann E, and Hanzlikova V.** Motor unit in old age. *Nature* 209: 921-922, 1966.
360. **Gutmann E, Hanzlikova V, and Vysokocil F.** Age changes in cross striated muscle of the rat. *J Physiol* 216: 331-343., 1971.
361. **Gutmann E, and Young JZ.** The re-innervation of muscle after various periods of atrophy. *J Anat* 78: 15-43, 1944.
362. **Hagen JL, Krause DJ, Baker DJ, Fu MH, Tarnopolsky MA, and Hepple RT.** Skeletal muscle aging in F344BN F1-hybrid rats: I. Mitochondrial dysfunction contributes to the age-associated reduction in VO<sub>2</sub>max. *J Gerontol A Biol Sci Med Sci* 59: 1099-1110, 2004.
363. **Haidet GC, and Parsons D.** Reduced exercise capacity in senescent beagles: an evaluation of the periphery. *The American journal of physiology* 260: H173-182, 1991.
364. **Hall ZW, and Ralston E.** Nuclear domains in muscle cells. *Cell* 59: 771-772, 1989.
365. **Halstead LS, Grimby, G.** *Post-polio syndrome*. Philadelphia: Hanley & Belfus, Inc, 1995, p. 1-230.
366. **Halstead LS, and Rossi CD.** Post-polio syndrome: clinical experience with 132 consecutive outpatients. *Birth Defects Orig Artic Ser* 23: 13-26, 1987.
367. **Hamalainen N, and Pette D.** Expression of an alpha-cardiac like myosin heavy chain in diaphragm, chronically stimulated, and denervated fast-twitch muscles of rabbit. *J Muscle Res Cell Motil* 18: 401-411, 1997.
368. **Hamer M, and Molloy GJ.** Association of C-reactive protein and muscle strength in the English Longitudinal Study of Ageing. *Age* 31: 171-177, 2009.
369. **Hamilton MA, Stevenson LW, Fonarow GC, Steimle A, Goldhaber JI, Child JS, Chopra IJ, Moriguchi JD, and Hage A.** Safety and hemodynamic effects of intravenous triiodothyronine in advanced congestive heart failure. *Am J Cardiol* 81: 443-447, 1998.
370. **Harada Y, Noguchi A, Kishino A, and Yanagida T.** Sliding movement of single actin filaments on one-headed myosin filaments. *Nature* 326: 805-808, 1987.
371. **Haren MT, Malmstrom TK, Miller DK, Patrick P, Perry HM, 3rd, Herning MM, Banks WA, and Morley JE.** Higher C-reactive protein and soluble tumor necrosis factor receptor levels are associated with poor physical function and disability: a cross-sectional analysis of a cohort of late middle-aged African Americans. *J Gerontol A Biol Sci Med Sci* 65: 274-281, 2010.
372. **Harman D.** The aging process. *Proc Natl Acad Sci U S A* 78: 7124-7128, 1981.
373. **Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH, Jr., Heimovitz H, Cohen HJ, and Wallace R.** Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *The American journal of medicine* 106: 506-512, 1999.
374. **Hartung V, and Asmussen G.** [The effect of age on the extra- and intrafusal muscle fibers in the soleus muscle of the rat]. *Z Mikrosk Anat Forsch* 102: 677-693, 1988.
375. **Hashizume K, and Kanda K.** Differential effects of aging on motoneurons and peripheral nerves innervating the hindlimb and forelimb muscles of rats. *Neuroscience research* 22: 189-196, 1995.
376. **Hashizume K, Kanda K, and Burke RE.** Medial gastrocnemius motor nucleus in the rat: age-related changes in the number and size of motoneurons. *J Comp Neurol* 269: 425-430., 1988.
377. **Haus JM, Carrithers JA, Trappe SW, and Trappe TA.** Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. *Journal of applied physiology* 103: 2068-2076, 2007.

378. **Hausburg MA, Doles JD, Clement SL, Cadwallader AB, Hall MN, Blackshear PJ, Lykke-Andersen J, and Olwin BB.** Post-transcriptional regulation of satellite cell quiescence by TTP-mediated mRNA decay. *eLife* 4: e03390, 2015.
379. **Hauton D, Winter J, Al-Shammari AA, Gaffney EA, Evans RD, and Egginton S.** Changes to both cardiac metabolism and performance accompany acute reductions in functional capillary supply. *Biochim Biophys Acta* 1850: 681-690, 2015.
380. **Hawke TJ, and Garry DJ.** Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol* 91: 534-551, 2001.
381. **Head B, Griparic L, Amiri M, Gandre-Babbe S, and van der Bliet AM.** Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. *J Cell Biol* 187: 959-966, 2009.
382. **Hebert SL, Marquet-de Rouge P, Lanza IR, McCrady-Spitzer SK, Levine JA, Middha S, Carter RE, Klaus KA, Therneau TM, Highsmith EW, and Nair KS.** Mitochondrial Aging and Physical Decline: Insights From Three Generations of Women. *J Gerontol A Biol Sci Med Sci* 70: 1409-1417, 2015.
383. **Heilmann C, and Pette D.** Molecular transformations in sarcoplasmic reticulum of fast-twitch muscle by electro-stimulation. *Eur J Biochem* 93: 437-446, 1979.
384. **Heininger K.** Aging is a deprivation syndrome driven by a germ-soma conflict. *Ageing Res Rev* 1: 481-536, 2002.
385. **Hemenway D, Solnick SJ, Koeck C, and Kytir J.** The incidence of stairway injuries in Austria. *Accid Anal Prev* 26: 675-679., 1994.
386. **Hepple RT.** Mitochondrial involvement and impact in aging skeletal muscle. *Front Aging Neurosci* 6: 211, 2014.
387. **Hepple RT, Hagen JL, Krause DJ, and Jackson CC.** Aerobic power declines with aging in rat skeletal muscles perfused at matched convective O<sub>2</sub> delivery. *Journal of applied physiology* 94: 744-751, 2003.
388. **Hepple RT, Mackinnon SL, Goodman JM, Thomas SG, and Pyley MJ.** Resistance and aerobic training in older men: effects on VO<sub>2</sub>peak and the capillary supply to skeletal muscle. *Journal of applied physiology* 82: 1305-1310, 1997.
389. **Hepple RT, and Vogell JE.** Anatomic capillarization is maintained in relative excess of fiber oxidative capacity in some skeletal muscles of late middle-aged rats. *Journal of applied physiology* 96: 2257-2264, 2004.
390. **Heredia DJ, Schubert D, Maligireddy S, Hennig GW, and Gould TW.** A Novel Striated Muscle-Specific Myosin-Blocking Drug for the Study of Neuromuscular Physiology. *Frontiers in cellular neuroscience* 10: 276, 2016.
391. **Hesser BA, Henschel O, and Witzemann V.** Synapse disassembly and formation of new synapses in postnatal muscle upon conditional inactivation of MuSK. *Molecular and cellular neurosciences* 31: 470-480, 2006.
392. **Hill AV.** The dimensions of animals and their muscular dynamics. *Sci Prog* 38: 209-230, 1950.
393. **Hill RR, and Robbins N.** Mode of enlargement of young mouse neuromuscular junctions observed repeatedly in vivo with visualization of pre- and postsynaptic borders. *Journal of neurocytology* 20: 183-194, 1991.
394. **Hill RR, Robbins N, and Fang ZP.** Plasticity of presynaptic and postsynaptic elements of neuromuscular junctions repeatedly observed in living adult mice. *Journal of neurocytology* 20: 165-182, 1991.
395. **Hippenmeyer S, Huber RM, Ladle DR, Murphy K, and Arber S.** ETS transcription factor Erm controls subsynaptic gene expression in skeletal muscles. *Neuron* 55: 726-740, 2007.
396. **Hirst TC, and Ribchester RR.** Segmentation of the mouse fourth deep lumbrical muscle connectome reveals concentric organisation of motor units. *J Physiol* 591: 4859-4875, 2013.
397. **Hofmann PA, Greaser ML, and Moss RL.** C-protein limits shortening velocity of rabbit skeletal muscle fibres at low levels of Ca<sup>2+</sup> activation. *J Physiol (Lond)* 439: 701-715, 1991.
398. **Hofmann PA, Hartzell HC, and Moss RL.** Alterations in Ca<sup>2+</sup> sensitive tension due to partial extraction of C- protein from rat skinned cardiac myocytes and rabbit skeletal muscle fibers. *J Gen Physiol* 97: 1141-1163, 1991.
399. **Hoh JF.** Muscle fiber types and function. *Curr Opin Rheumatol* 4: 801-808, 1992.

400. **Holdt LM, Sass K, Gabel G, Bergert H, Thiery J, and Teupser D.** Expression of Chr9p21 genes CDKN2B (p15(INK4b)), CDKN2A (p16(INK4a), p14(ARF)) and MTAP in human atherosclerotic plaque. *Atherosclerosis* 214: 264-270, 2011.
401. **Holloway GP, Holwerda AM, Miotto PM, Dirks ML, Verdijk LB, and van Loon LJC.** Age-Associated Impairments in Mitochondrial ADP Sensitivity Contribute to Redox Stress in Senescent Human Skeletal Muscle. *Cell Rep* 22: 2837-2848, 2018.
402. **Homsher E, Wang F, and Sellers JR.** Factors affecting movement of F-actin filaments propelled by skeletal muscle heavy meromyosin. *Am J Physiol* 262: C714-723, 1992.
403. **Hook P, Larsson, L.** Actomyosin interactions in a novel single fiber *in vitro* motility assay. *Journal of Muscle Research and Cell Motility* 21: 357-365, 2000.
404. **Hook P, Li X, Sleep J, Hughes S, and Larsson L.** In vitro motility speed of slow myosin extracted from single soleus fibres from young and old rats. *J Physiol (Lond)* 520 Pt 2: 463-471, 1999.
405. **Hook P, Sriramoju V, and Larsson L.** Effects of aging on actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. *Am J Physiol Cell Physiol* 280: C782-788, 2001.
406. **Hook P, Sriramoju, V., Larsson, L.** Effects of aging on actin sliding speed on myosin from single mouse, rat and human skeletal muscle cells. *Am J Physiol Cell Physiol* 280: C782-C788, 2001.
407. **Hoppeler H, and Vogt M.** Muscle tissue adaptations to hypoxia. *J Exp Biol* 204: 3133-3139, 2001.
408. **Houmard JA, Weidner ML, Gavigan KE, Tyndall GL, Hickey MS, and Alshami A.** Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging. *J Appl Physiol (1985)* 85: 1337-1341, 1998.
409. **Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsyuba E, Knott G, Williams RW, and Auwerx J.** Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature* 497: 451-457, 2013.
410. **Hudlicka O, Brown M, and Egginton S.** Angiogenesis in skeletal and cardiac muscle. *Physiol Rev* 72: 369-417, 1992.
411. **Hudlicka O, Tyler KR, Wright AJA, and Ziada AMAR.** Growth of capillaries in skeletal muscles. *Prog Appl Microcirc* 5: 44-61, 1984.
412. **Hughes SM, Cho M, Karsch-Mizrachi I, Travis M, Silberstein L, Leinwand LA, and Blau HM.** Three slow myosin heavy chains sequentially expressed in developing mammalian skeletal muscle. *Dev Biol* 158: 183-199, 1993.
413. **Hwee DT, Baehr LM, Philp A, Baar K, and Bodine SC.** Maintenance of muscle mass and load-induced growth in Muscle RING Finger 1 null mice with age. *Aging Cell* 13: 92-101, 2014.
414. **Hvid L, Aagaard P, Justesen L, Bayer ML, Andersen JL, Ortenblad N, Kjaer M, and Suetta C.** Effects of aging on muscle mechanical function and muscle fiber morphology during short-term immobilization and subsequent retraining. *Journal of applied physiology* 109: 1628-1634, 2010.
415. **Hvid LG, Suetta C, Nielsen JH, Jensen MM, Frandsen U, Ortenblad N, Kjaer M, and Aagaard P.** Aging impairs the recovery in mechanical muscle function following 4 days of disuse. *Exp Gerontol* 52: 1-8, 2014.
416. **Ibebunjo C, Chick JM, Kendall T, Eash JK, Li C, Zhang Y, Vickers C, Wu Z, Clarke BA, Shi J, Cruz J, Fournier B, Brachat S, Gutzwiller S, Ma Q, Markovits J, Broome M, Steinkrauss M, Skuba E, Galarneau JR, Gygi SP, and Glass DJ.** Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia. *Mol Cell Biol* 33: 194-212, 2013.
417. **Imamura K, Ashida H, Ishikawa T, and Fujii M.** Human major psoas muscle and sacrospinalis muscle in relation to age: a study by computed tomography. *J Gerontol* 38: 678-681, 1983.
418. **Ingram DK.** Age-related decline in physical activity: generalization to nonhumans. *Med Sci Sports Exerc* 32: 1623-1629, 2000.
419. **Irion GL, Vasthare US, and Tuma RF.** Age-related change in skeletal muscle blood flow in the rat. *J Gerontol* 42: 660-665, 1987.
420. **Ishihara A, Naitoh H, and Katsuta S.** Effects of ageing on the total number of muscle fibers and motoneurons of the tibialis anterior and soleus muscles in the rat. *Brain Res* 435: 355-358., 1987.

421. **Jackson MJ.** Reactive oxygen species in sarcopenia: Should we focus on excess oxidative damage or defective redox signalling? *Mol Aspects Med* 50: 33-40, 2016.
422. **Jacobs JM, and Love S.** Qualitative and quantitative morphology of human sural nerve at different ages. *Brain* 108: 897-924., 1985.
423. **Jakobsson F, Borg K, Edstrom L, and Grimby L.** Use of motor units in relation to muscle fiber type and size in man. *Muscle Nerve* 11: 1211-1218., 1988.
424. **Janacek J, Cebasek V, Kubinova L, Ribaric S, and Erzen I.** 3D Visualization and Measurement of Capillaries Supplying Metabolically Different Fiber Types in the Rat Extensor Digitorum Longus Muscle During Denervation and Reinnervation. *J Histochem Cytochem* 57: 437-447, 2009.
425. **Jandreski MA, Sole MJ, and Liew CC.** Two different forms of beta myosin heavy chain are expressed in human striated muscle. *Hum Genet* 77: 127-131, 1987.
426. **Jang YC, and Van Remmen H.** Age-associated alterations of the neuromuscular junction. *Exp Gerontol* 46: 193-198, 2011.
427. **Janssen I, Heymsfield SB, Wang ZM, and Ross R.** Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol* 89: 81-88, 2000.
428. **Ji LL, and Kang C.** Role of PGC-1alpha in sarcopenia: etiology and potential intervention - a mini-review. *Gerontology* 61: 139-148, 2015.
429. **Jimenez-Moreno R, Wang ZM, Gerring RC, and Delbono O.** Sarcoplasmic reticulum Ca<sup>2+</sup> release declines in muscle fibers from aging mice. *Biophys J* 94: 3178-3188, 2008.
430. **Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, and Youle RJ.** Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *J Cell Biol* 191: 933-942, 2010.
431. **Jirmanová I.** Ultrastructure of motor end-plates during pharmacologically-induced degeneration and subsequent regeneration of skeletal muscle. *J Neurocytol* 4: 141-155, 1975.
432. **Jones BA, and Fangman WL.** Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. *Genes Dev* 6: 380-389, 1992.
433. **Jones NC, Fedorov YV, Rosenthal RS, and Olwin BB.** ERK1/2 is required for myoblast proliferation but is dispensable for muscle gene expression and cell fusion. *J Cell Physiol* 186: 104-115, 2001.
434. **Jones NC, Tyner KJ, Nibarger L, Stanley HM, Cornelison DD, Fedorov YV, and Olwin BB.** The p38alpha/beta MAPK functions as a molecular switch to activate the quiescent satellite cell. *J Cell Biol* 169: 105-116, 2005.
435. **Jorgensen JO, Pedersen SA, Thuesen L, Jorgensen J, Ingemann-Hansen T, Skakkebaek NE, and Christiansen JS.** Beneficial effects of growth hormone treatment in GH-deficient adults. *Lancet* 1: 1221-1225, 1989.
436. **Joseph AM, Adhietty PJ, Wawrzyniak NR, Wohlgemuth SE, Picca A, Kujoth GC, Prolla TA, and Leeuwenburgh C.** Dysregulation of mitochondrial quality control processes contribute to sarcopenia in a mouse model of premature aging. *PLoS One* 8: e69327, 2013.
437. **Jozsa L, Jarvinen M, Kvist M, Lehto M, and Mikola A.** Capillary density of tenotomized skeletal muscles. I. Experimental study in the rat. *Eur J Appl Physiol Occup Physiol* 44: 175-181, 1980.
438. **Justice J, Miller JD, Newman JC, Hashmi SK, Halter J, Austad SN, Barzilai N, and Kirkland JL.** Frameworks for Proof-of-Concept Clinical Trials of Interventions That Target Fundamental Aging Processes. *J Gerontol A Biol Sci Med Sci* 2016.
439. **Justice JN, Gregory H, Tchkonja T, LeBrasseur NK, Kirkland JL, Kritchevsky SB, and Nicklas BJ.** Cellular senescence biomarker p16INK4a+ cell burden in thigh adipose is associated with poor physical function in older women. *J Gerontol A Biol Sci Med Sci* 2017.
440. **Kadenbach B, Munscher C, and Muller-Hocker J.** Mutations of mitochondrial DNA support human aging. *Aging (Milano)* 5: 73-75, 1993.
441. **Kadi F, Charifi N, Denis C, and Lexell J.** Satellite cells and myonuclei in young and elderly women and men. *Muscle Nerve* 29: 120-127, 2004.

442. **Kalamgi RC, and Larsson L.** Mechanical Signaling in the Pathophysiology of Critical Illness Myopathy. *Frontiers in physiology* 7: 23, 2016.
443. **Kalamgi RC, Salah H, Gastaldello S, Martinez-Redondo V, Ruas J, Fury W, Bai Y, Gromada J, Sartori R, Guttridge DC, Sandri M, and Larsson L.** Mechano signaling pathways in an experimental intensive critical illness myopathy model. *J Physiol* 2016.
444. **Kanda K, and Hashizume K.** Changes in properties of the medial gastrocnemius motor units in aging rats. *J Neurophysiol* 61: 737-746., 1989.
445. **Kang H, Tian L, Mikesch M, Lichtman JW, and Thompson WJ.** Terminal Schwann cells participate in neuromuscular synapse remodeling during reinnervation following nerve injury. *J Neurosci* 34: 6323-6333, 2014.
446. **Kang H, Tian L, Son YJ, Zuo Y, Procaccino D, Love F, Hayworth C, Trachtenberg J, Mikesch M, Sutton L, Ponomareva O, Mignone J, Enikolopov G, Rimer M, and Thompson W.** Regulation of the intermediate filament protein nestin at rodent neuromuscular junctions by innervation and activity. *J Neurosci* 27: 5948-5957, 2007.
447. **Kang I, Chu CT, and Kaufman BA.** The mitochondrial transcription factor TFAM in neurodegeneration: emerging evidence and mechanisms. *FEBS Lett* 592: 793-811, 2018.
448. **Kano Y, Shimegi S, Furukawa H, Matsudo H, and Mizuta T.** Effects of aging on capillary number and luminal size in rat soleus and plantaris muscles. *J Gerontol A Biol Sci Med Sci* 57: B422-427, 2002.
449. **Kano Y, Shimegi S, Takahashi H, Masuda K, and Katsuta S.** Changes in capillary luminal diameter in rat soleus muscle after hind- limb suspension. *Acta Physiol Scand* 169: 271-276., 2000.
450. **Kasper CE, and Xun L.** Cytoplasm-to-myonucleus ratios in plantaris and soleus muscle fibres following hindlimb suspension. *J Muscle Res Cell Motil* 17: 603-610, 1996.
451. **Katayama M, Tanaka M, Yamamoto H, Ohbayashi T, Nimura Y, and Ozawa T.** Deleted mitochondrial DNA in the skeletal muscle of aged individuals. *Biochem Int* 25: 47-56, 1991.
452. **Katschinski DM.** Is there a molecular connection between hypoxia and aging? *Exp Gerontol* 41: 482-484, 2006.
453. **Kawabe Y, Wang YX, McKinnell IW, Bedford MT, and Rudnicki MA.** Carm1 regulates Pax7 transcriptional activity through MLL1/2 recruitment during asymmetric satellite stem cell divisions. *Cell stem cell* 11: 333-345, 2012.
454. **Kawakami Y, Akima H, Kubo K, Muraoka Y, Hasegawa H, Kouzaki M, Imai M, Suzuki Y, Gunji A, Kanehisa H, and Fukunaga T.** Changes in muscle size, architecture, and neural activation after 20 days of bed rest with and without resistance exercise. *Eur J Appl Physiol* 84: 7-12, 2001.
455. **Kawamura Y, O'Brien P, Okazaki H, and Dyck PJ.** Lumbar motoneurons of man II: the number and diameter distribution of large- and intermediate-diameter cytons in "motoneuron columns" of spinal cord of man. *J Neuropathol Exp Neurol* 36: 861-870., 1977.
456. **Kawamura Y, Okazaki H, O'Brien PC, and Dyck PJ.** Lumbar motoneurons of man: I) number and diameter histogram of alpha and gamma axons of ventral root. *J Neuropathol Exp Neurol* 36: 853-860., 1977.
457. **Kelley KA, Friedrich VL, Jr., Sonshine A, Hu Y, Lax J, Li J, Drinkwater D, Dressler H, and Herrup K.** Expression of Thy-1/lacZ fusion genes in the CNS of transgenic mice. *Brain research Molecular brain research* 24: 261-274, 1994.
458. **Kent-Braun JA, and Ng AV.** Skeletal muscle oxidative capacity in young and older women and men. *J Appl Physiol (1985)* 89: 1072-1078, 2000.
459. **Khan NA, Auranen M, Paetau I, Pirinen E, Euro L, Forsstrom S, Pasila L, Velagapudi V, Carroll CJ, Auwerx J, and Suomalainen A.** Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. *Embo Mol Med* 6: 721-731, 2014.
460. **Khreiss T, Jozsef L, Potempa LA, and Filep JG.** Loss of pentameric symmetry in C-reactive protein induces interleukin-8 secretion through peroxynitrite signaling in human neutrophils. *Circ Res* 97: 690-697, 2005.
461. **Kihara M, Zollman PJ, Schmelzer JD, and Low PA.** The influence of dose of microspheres on nerve blood flow, electrophysiology, and fiber degeneration of rat peripheral nerve. *Muscle Nerve* 16: 1383-1389, 1993.

462. **Kim I, Rodriguez-Enriquez S, and Lemasters JJ.** Selective degradation of mitochondria by mitophagy. *Arch Biochem Biophys* 462: 245-253, 2007.
463. **Kim JH, Torgerud WS, Mosser KH, Hirai H, Watanabe S, Asakura A, and Thompson LV.** Myosin light chain 3f attenuates age-induced decline in contractile velocity in MHC type II single muscle fibers. *Aging Cell* 11: 203-212, 2012.
464. **Kimball SR, O'Malley JP, Anthony JC, Crozier SJ, and Jefferson LS.** Assessment of biomarkers of protein anabolism in skeletal muscle during the life span of the rat: sarcopenia despite elevated protein synthesis. *Am J Physiol Endocrinol Metab* 287: E772-780, 2004.
465. **Kirkland JL.** Inflammation and cellular senescence: potential contribution to chronic diseases and disabilities with aging. Inflammation and cellular senescence: potential contribution to chronic diseases and disabilities with aging. *Public Policy and Aging Report* 23: 12-15, 2013.
466. **Kirkland JL, and Tchkonja T.** Cellular Senescence: A Translational Perspective. *EBioMedicine* 21: 21-28, 2017.
467. **Kirkwood TB.** Evolution of ageing. *Mech Ageing Dev* 123: 737-745, 2002.
468. **Kishino A, and Yanagida T.** Force measurements by micromanipulation of a single actin filament by glass needles. *Nature* 334: 74-76, 1988.
469. **Klitgaard H, Mantoni M, Schiaffino S, Ausoni S, Gorza L, Laurent-Winter C, Schnohr P, and Saltin B.** Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with different training backgrounds. *Acta Physiol Scand* 140: 41-54, 1990.
470. **Knox CA, Kokmen E, and Dyck PJ.** Morphometric alteration of rat myelinated fibers with aging. *J Neuropathol Exp Neurol* 48: 119-139., 1989.
471. **Ko C-P, and Chen L.** Synaptic remodeling revealed by repeated in vivo observations and electron microscopy of identified frog neuromuscular junctions. *J Neurosci* 16: 1780-1790, 1996.
472. **Kobzik L, Reid MB, Brecht DS, and Stamler JS.** Nitric oxide in skeletal muscle. *Nature* 372: 546-548., 1994.
473. **Kohn RR.** Aging and age-related disease: normal processes. In: *Relations between normal aging and disease*, edited by Johnson HA. New York: Raven Press, 1985, p. 1-44.
474. **Kolesar JE, Safdar A, Abadi A, MacNeil LG, Crane JD, Tarnopolsky MA, and Kaufman BA.** Defects in mitochondrial DNA replication and oxidative damage in muscle of mtDNA mutator mice. *Free Radic Biol Med* 75: 241-251, 2014.
475. **Komiya Y.** Slowing with age of the rate of slow axonal flow in bifurcating axons of rat dorsal root ganglion cells. *Brain Res* 183: 477-480., 1980.
476. **Kong XC, Barzaghi P, and Ruegg MA.** Inhibition of synapse assembly in mammalian muscle in vivo by RNA interference. *EMBO Rep* 5: 183-188, 2004.
477. **Korhonen MT, Cristea A, Alen M, Hakkinen K, Sipila S, Mero A, Viitasalo JT, Larsson L, and Suominen H.** Aging, muscle fiber type, and contractile function in sprint-trained athletes. *J Appl Physiol* 101: 906-917, 2006.
478. **Korkusko OV, Sarkisov KG, and Frajfel'd VE.** Die Altersbesonderheiten des Systems der Mikrozirkulation in den Skelettmuskeln und ihre Rolle in der Arbeitsfaehigkeit des Muskels beim Altern der Menschen. *Z Altersforsch* 37: 147-153, 1982.
479. **Kostyo JL.** Rapid effects of growth hormone on amino acid transport and protein synthesis. *Ann N Y Acad Sci* 148: 389-407, 1968.
480. **Kottlors M, and Kirschner J.** Elevated satellite cell number in Duchenne muscular dystrophy. *Cell Tissue Res* 340: 541-548, 2010.
481. **Koubassova NA, and Tsaturyan AK.** Molecular mechanism of actin-myosin motor in muscle. *Biochemistry (Mosc)* 76: 1484-1506, 2011.
482. **Kovanen V, and Suominen H.** Effects of age and life-time physical training on fibre composition of slow and fast skeletal muscle in rats. *Pflugers Arch* 408: 543-551., 1987.

483. **Kovanen V, Suominen H, and Peltonen L.** Effects of aging and life-long physical training on collagen in slow and fast skeletal muscle in rats. A morphometric and immuno- histochemical study. *Cell Tissue Res* 248: 247-255., 1987.
484. **Krnjevic K, Miledi, R.** Motor units in rat diaphragm. *J Physiol (Lond)* 140: 427-439, 1958.
485. **Kron SJ, and Spudich JA.** Fluorescent actin filaments move on myosin fixed to a glass surface. *Proc Natl Acad Sci U S A* 83: 6272-6276, 1986.
486. **Kudla AJ, Jones NC, Rosenthal RS, Arthur K, Clase KL, and Olwin BB.** The FGF receptor-1 tyrosine kinase domain regulates myogenesis but is not sufficient to stimulate proliferation. *J Cell Biol* 142: 241-250, 1998.
487. **Kugelberg E, Edstrom L, and Abbruzzese M.** Mapping of motor units in experimentally reinnervated rat muscle. Interpretation of histochemical and atrophic fibre patterns in neurogenic lesions. *J Neurol Neurosurg Psychiatry* 33: 319-329., 1970.
488. **Kugelberg E, and Lindegren B.** Transmission and contraction fatigue of rat motor units in relation to succinate dehydrogenase activity of motor unit fibres. *J Physiol (Lond)* 288: 285-300, 1979.
489. **Kugelberg E, and Thornell LE.** Contraction time, histochemical type, and terminal cisternae volume of rat motor units. *Muscle Nerve* 6: 149-153., 1983.
490. **Kuikka LK, Salminen S, Ouwehand A, Gueimonde M, Strandberg TE, Finne-Soveri UH, Sintonen H, and Pitkala KH.** Inflammation markers and malnutrition as risk factors for infections and impaired health-related quality of life among older nursing home residents. *Journal of the American Medical Directors Association* 10: 348-353, 2009.
491. **Kuilman T, Michaloglou C, Mooi WJ, and Peeper DS.** The essence of senescence. *Genes Dev* 24: 2463-2479, 2010.
492. **Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, Aarden LA, Mooi WJ, and Peeper DS.** Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133: 1019-1031, 2008.
493. **Kuilman T, and Peeper DS.** Senescence-messaging secretome: SMS-ing cellular stress. *Nat Rev Cancer* 9: 81-94, 2009.
494. **Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, and Prolla TA.** Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* 309: 481-484, 2005.
495. **Kulakowski SA, Parker SD, and Personius KE.** Reduced TrkB expression results in precocious age-like changes in neuromuscular structure, neurotransmission, and muscle function. *Journal of applied physiology* 111: 844-852, 2011.
496. **Kuller LH.** Serum levels of IL-6 and development of disability in older persons. *J Am Geriatr Soc* 47: 755-756, 1999.
497. **Kumar A, Harris TE, Keller SR, Choi KM, Magnuson MA, and Lawrence JC, Jr.** Muscle-specific deletion of rictor impairs insulin-stimulated glucose transport and enhances Basal glycogen synthase activity. *Mol Cell Biol* 28: 61-70, 2008.
498. **Kuo YT, Shih PH, Kao SH, Yeh GC, and Lee HM.** Pyrroloquinoline Quinone Resists Denervation-Induced Skeletal Muscle Atrophy by Activating PGC-1alpha and Integrating Mitochondrial Electron Transport Chain Complexes. *PLoS One* 10: e0143600, 2015.
499. **Laberge RM, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L, Curran SC, Davalos AR, Wilson-Edell KA, Liu S, Limbad C, Demaria M, Li P, Hubbard GB, Ikeno Y, Javors M, Desprez PY, Benz CC, Kapahi P, Nelson PS, and Campisi J.** MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nat Cell Biol* 17: 1049-1061, 2015.
500. **Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, and Auwerx J.** Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127: 1109-1122, 2006.



501. **Lakatta EG.** Cardiovascular regulatory mechanisms in advanced age. *Physiol Rev* 73: 413-467, 1993.
502. **Lal S, Chithra P, and Chandrakasan G.** The possible relevance of autoxidative glycosylation in glucose mediated alterations of proteins: an in vitro study on myofibrillar proteins. *Mol Cell Biochem* 154: 95-100., 1996.
503. **Lamberts SW, van den Beld AW, and van der Lely AJ.** The endocrinology of aging. *Science* 278: 419-424, 1997.
504. **Lamming DW, Mihaylova MM, Katajisto P, Baar EL, Yilmaz OH, Hutchins A, Gultekin Y, Gaither R, and Sabatini DM.** Depletion of Rictor, an essential protein component of mTORC2, decreases male lifespan. *Aging Cell* 13: 911-917, 2014.
505. **Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon AB, Richardson A, Ahima RS, Guertin DA, Sabatini DM, and Baur JA.** Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science* 335: 1638-1643, 2012.
506. **Landi F, Marzetti E, Liperoti R, Pahor M, Russo A, Martone AM, Colloca G, Capoluongo E, and Bernabei R.** Nonsteroidal anti-inflammatory drug (NSAID) use and sarcopenia in older people: results from the iSIRENTE study. *Journal of the American Medical Directors Association* 14: 626 e629-613, 2013.
507. **Landing BH, Dixon LG, and Wells TR.** Studies on isolated human skeletal muscle fibers, including a proposed pattern of nuclear distribution and a concept of nuclear territories. *Human pathology* 5: 441-461, 1974.
508. **Lankford EB, Epstein ND, Fananapazir L, and Sweeney HL.** Abnormal contractile properties of muscle fibers expressing beta-myosin heavy chain gene mutations in patients with hypertrophic cardiomyopathy. *J Clin Invest* 95: 1409-1414, 1995.
509. **Lanza IR, Befroy DE, and Kent-Braun JA.** Age-related changes in ATP-producing pathways in human skeletal muscle in vivo. *J Appl Physiol (1985)* 99: 1736-1744, 2005.
510. **Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ, McConnell JP, and Nair KS.** Endurance exercise as a countermeasure for aging. *Diabetes* 57: 2933-2942, 2008.
511. **Lanza IR, Zabielski P, Klaus KA, Morse DM, Heppelmann CJ, Bergen HR, 3rd, Dasari S, Walrand S, Short KR, Johnson ML, Robinson MM, Schimke JM, Jakaitis DR, Asmann YW, Sun Z, and Nair KS.** Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. *Cell Metab* 16: 777-788, 2012.
512. **Lapierre LR, Kumsta C, Sandri M, Ballabio A, and Hansen M.** Transcriptional and epigenetic regulation of autophagy in aging. *Autophagy* 11: 867-880, 2015.
513. **Laplante M, and Sabatini DM.** Regulation of mTORC1 and its impact on gene expression at a glance. *J Cell Sci* 126: 1713-1719, 2013.
514. **Larsson L.** *Ageing in mammalian skeletal muscles.* New York: Praeger, 1982, p. 60-97.
515. **Larsson L.** Histochemical characteristics of human skeletal muscle during aging. *Acta Physiol Scand* 117: 469-471, 1983.
516. **Larsson L.** Is the motor unit uniform? *Acta Physiol Scand* 144: 143-154, 1992.
517. **Larsson L.** Physical training effects on muscle morphology in sedentary males at different ages. *Med Sci Sports Exerc* 14: 203-206, 1982.
518. **Larsson L.** A technique for measuring contractile properties in single chemically skinned human muscle fibres obtained from percutaneous biopsies. *J Neurol Sci* 98: 430, 1990.
519. **Larsson L, and Ansved T.** Effects of age on the motor unit. A study on single motor units in the rat. *Ann N Y Acad Sci* 515: 303-313, 1988.
520. **Larsson L, and Ansved T.** Effects of ageing on the motor unit. *Prog Neurobiol* 45: 397-458, 1995.
521. **Larsson L, Ansved T, Edstrom L, Gorza L, and Schiaffino S.** Effects of age on physiological, immunohistochemical and biochemical properties of fast-twitch single motor units in the rat. *J Physiol (Lond)* 443: 257-275, 1991.
522. **Larsson L, Biral D, Campione M, and Schiaffino S.** An age-related type IIB to IIX myosin heavy chain switching in rat skeletal muscle. *Acta Physiol Scand* 147: 227-234, 1993.

523. **Larsson L, and Edstrom L.** Effects of age on enzyme-histochemical fibre spectra and contractile properties of fast- and slow-twitch skeletal muscles in the rat. *J Neurol Sci* 76: 69-89, 1986.
524. **Larsson L, Edstrom L, Lindegren B, Gorza L, and Schiaffino S.** MHC composition and enzyme-histochemical and physiological properties of a novel fast-twitch motor unit type. *Am J Physiol* 261: C93-101, 1991.
525. **Larsson L, Grimby G, and Karlsson J.** Muscle strength and speed of movement in relation to age and muscle morphology. *J Appl Physiol* 46: 451-456, 1979.
526. **Larsson L, Hook P, and Pircher P.** Regulation of human muscle contraction at the cellular and molecular levels. *Ital J Neurol Sci* 20: 413-422, 1999.
527. **Larsson L, Li X, Berg HE, and Frontera WR.** Effects of removal of weight-bearing function on contractility and myosin isoform composition in single human skeletal muscle cells. *Pflugers Arch* 432: 320-328, 1996.
528. **Larsson L, Li X, Edstrom L, Eriksson LI, Zackrisson H, Argentini C, and Schiaffino S.** Acute quadriplegia and loss of muscle myosin in patients treated with nondepolarizing neuromuscular blocking agents and corticosteroids: mechanisms at the cellular and molecular levels [see comments]. *Crit Care Med* 28: 34-45, 2000.
529. **Larsson L, Li X, and Frontera WR.** Effects of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. *Am J Physiol* 272: C638-649, 1997.
530. **Larsson L, Li X, Muller U, and Yu F.** Myosin isoform composition and maximum velocity of unloaded shortening in single skeletal muscle cells from man and rat: Specific reference to ageing and thyroid hormone treatment. *Journal of Muscle Research and Cell Motility* 17: 129-130, 1996.
531. **Larsson L, Li X, Tollback A, and Grimby L.** Contractile properties in single muscle fibres from chronically overused motor units in relation to motoneuron firing properties in prior polio patients. *J Neurol Sci* 132: 182-192, 1995.
532. **Larsson L, Li X, Yu F, and Degens H.** Age-related changes in contractile properties and expression of myosin isoforms in single skeletal muscle cells. *Muscle Nerve Suppl* 5: S74-78, 1997.
533. **Larsson L, Li XP, Berg HE, and Frontera WR.** Effects of removal of weight-bearing function on contractility and myosin isoform composition in single human skeletal muscle cells. *Pflug Arch Eur J Phy* 432: 320-328, 1996.
534. **Larsson L, and Moss RL.** Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. *J Physiol* 472: 595-614, 1993.
535. **Larsson L, Muller U, Li X, and Schiaffino S.** Thyroid hormone regulation of myosin heavy chain isoform composition in young and old rats, with special reference to IIX myosin. *Acta Physiol Scand* 153: 109-116, 1995.
536. **Larsson L, and Roland A.** [Drug induced tetraparesis and loss of myosin. Mild types are probably overlooked]. *Lakartidningen* 93: 2249-2254, 1996.
537. **Larsson L, and Salviati G.** Effects of age on calcium transport activity of sarcoplasmic reticulum in fast- and slow-twitch rat muscle fibres. *J Physiol (Lond)* 419: 253-264, 1989.
538. **Larsson L, and Salviati G.** A technique for studies of the contractile apparatus in single human muscle fibre segments obtained by percutaneous biopsy. *Acta Physiol Scand* 146: 485-495, 1992.
539. **Larsson L, Sjodin B, and Karlsson J.** Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22--65 years. *Acta Physiol Scand* 103: 31-39, 1978.
540. **Larsson L, and Yu F.** Gender-related differences in the regulatory influence of thyroid hormone on the expression of myosin isoforms in young and old rats. *Acta Physiol Scand* 159: 81-89, 1997.
541. **Laskowski MB, Olson WH, and Dettbarn WD.** Initial ultrastructural abnormalities at the motor end plate produced by a cholinesterase inhibitor. *Exp Neurol* 57: 13-33, 1977.
542. **Lass A, Kwong L, and Sohal RS.** Mitochondrial coenzyme Q content and aging. *Biofactors* 9: 199-205, 1999.

543. **LeBrasseur NK, Tchkonja T, and Kirkland JL.** Cellular Senescence and the Biology of Aging, Disease, and Frailty. *Nestle Nutrition Institute workshop series* 83: 11-18, 2015.
544. **Leduc-Gaudet JP, Auger MJ, St Jean Pelletier F, and Gousspillou G.** Towards a better understanding of the role played by mitochondrial dynamics and morphology in skeletal muscle atrophy. *J Physiol* 593: 2993-2994, 2015.
545. **Leduc-Gaudet JP, Picard M, St-Jean Pelletier F, Sgarbiato N, Auger MJ, Vallee J, Robitaille R, St-Pierre DH, and Gousspillou G.** Mitochondrial morphology is altered in atrophied skeletal muscle of aged mice. *Oncotarget* 6: 17923-17937, 2015.
546. **Lee AS, Anderson JE, Joya JE, Head SI, Pather N, Kee AJ, Gunning PW, and Hardeman EC.** Aged skeletal muscle retains the ability to fully regenerate functional architecture. *Bioarchitecture* 3: 25-37, 2013.
547. **Lee C, Klopp R, Weindruch R, and Prolla T.** Gene expression profile of aging and its retardation by caloric restriction. *Science* 285: 1390-1393, 1999.
548. **Lee WJ, McClung J, Hand GA, and Carson JA.** Overload-induced androgen receptor expression in the aged rat hindlimb receiving nandrolone decanoate. *Journal of applied physiology* 94: 1153-1161, 2003.
549. **Lee YI, Li Y, Mikesch M, Smith I, Nave KA, Schwab MH, and Thompson WJ.** Neuregulin1 displayed on motor axons regulates terminal Schwann cell-mediated synapse elimination at developing neuromuscular junctions. *Proc Natl Acad Sci U S A* 113: E479-487, 2016.
550. **Lehman TD, and Ortwerth BJ.** Inhibitors of advanced glycation end product-associated protein cross-linking. *Biochim Biophys Acta* 1535: 110-119., 2001.
551. **Leiter JR, Upadhaya R, and Anderson JE.** Nitric oxide and voluntary exercise together promote quadriceps hypertrophy and increase vascular density in female 18-mo-old mice. *Am J Physiol Cell Physiol* 302: C1306-1315, 2012.
552. **Lenhare L, Crisol BM, Silva VRR, Katashima CK, Cordeiro AV, Pereira KD, Luchessi AD, da Silva ASR, Cintra DE, Moura LP, Pauli JR, and Ropelle ER.** Physical exercise increases Sestrin 2 protein levels and induces autophagy in the skeletal muscle of old mice. *Exp Gerontol* 97: 17-21, 2017.
553. **Lewis SE, Kelly FJ, and Goldspink DF.** Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow- and fast-twitch skeletal muscles of the rat. *Biochem J* 217: 517-526, 1984.
554. **Lexell J, Henriksson-Larsen K, Winblad B, and Sjostrom M.** Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. *Muscle Nerve* 6: 588-595., 1983.
555. **Lexell J, Taylor CC, and Sjostrom M.** What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *J Neurol Sci* 84: 275-294, 1988.
556. **Lezza AM, Boffoli D, Scacco S, Cantatore P, and Gadaleta MN.** Correlation between mitochondrial DNA 4977-bp deletion and respiratory chain enzyme activities in aging human skeletal muscles. *Biochem Biophys Res Commun* 205: 772-779, 1994.
557. **Li C, White SH, Warren LK, and Wohlgemuth SE.** Skeletal muscle from aged American Quarter Horses shows impairments in mitochondrial biogenesis and expression of autophagy markers. *Exp Gerontol* 102: 19-27, 2018.
558. **Li M, and Larsson L.** Force-generating capacity of human myosin isoforms extracted from single muscle fibre segments. *J Physiol* 588: 5105-5114, 2010.
559. **Li M, Li M, Marx JO, and Larsson L.** There is no slowing of motility speed with increased body size in rat, human, horse and rhinoceros independent on temperature and skeletal muscle myosin isoform. *Acta Physiol* 202: 671-681, 2011.
560. **Li M, Lionikas A, Yu F, Tajsharghi H, Oldfors A, and Larsson L.** Muscle cell and motor protein function in patients with a IIa myosin missense mutation (Glu-706 to Lys). *Neuromuscul Disord* 16: 782-791, 2006.
561. **Li M, Ogilvie H, Ochala J, Artemenko K, Iwamoto H, Yagi N, Bergquist J, and Larsson L.** Aberrant post-translational modifications compromise human myosin motor function in old age. *Aging Cell* 14: 228-235, 2015.

562. **Li P, Meng X, Bian H, Burns N, Zhao KS, and Song R.** Activation of sirtuin 1/3 improves vascular hyporeactivity in severe hemorrhagic shock by alleviation of mitochondrial damage. *Oncotarget* 6: 36998-37011, 2015.
563. **Li X, and Larsson L.** Maximum shortening velocity and myosin isoforms in single muscle fibers from young and old rats. *Am J Physiol* 270: C352-360, 1996.
564. **Li Y, Lee Y, and Thompson WJ.** Changes in aging mouse neuromuscular junctions are explained by degeneration and regeneration of muscle fiber segments at the synapse. *J Neurosci* 31: 14910-14919, 2011.
565. **Li Y, and Thompson WJ.** Nerve terminal growth remodels neuromuscular synapses in mice following regeneration of the postsynaptic muscle fiber. *J Neurosci* 31: 13191-13203, 2011.
566. **Li YP, Chen Y, John J, Moylan J, Jin B, Mann DL, and Reid MB.** TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *FASEB J* 19: 362-370, 2005.
567. **Lichtman JW, Magrassi L, and Purves D.** Visualization of neuromuscular junctions over periods of several months in living mice. *J Neurosci* 7: 1215-1222, 1987.
568. **Lidell EGT, Sherrington, C.S.** Recruitment and some factors of reflex inhibition. *Proc R Soc Lond B Biol Sci* 97: 488-518, 1925.
569. **Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, and Spiegelman BM.** Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* 418: 797-801, 2002.
570. **Lin S, Xing H, Zang T, Ruan X, Wo L, and He M.** Sirtuins in mitochondrial stress: Indispensable helpers behind the scenes. *Ageing Res Rev* 44: 22-32, 2018.
571. **Lingle CJ, and Steinbach JH.** Neuromuscular blocking agents. *Int Anesthesiol Clin* 26: 288-301, 1988.
572. **Lionikas A, Blizard DA, Gerhard GS, Vandenberg DJ, Stout JT, Vogler GP, McClearn GE, and Larsson L.** Genetic determinants of weight of fast- and slow-twitch skeletal muscle in 500-day-old mice of the C57BL/6J and DBA/2J lineage. *Physiol Genomics* 21: 184-192, 2005.
573. **Lionikas A, Blizard DA, Vandenberg DJ, Glover MG, Stout JT, Vogler GP, McClearn GE, and Larsson L.** Genetic architecture of fast- and slow-twitch skeletal muscle weight in 200-day-old mice of the C57BL/6J and DBA/2J lineage. *Physiol Genomics* 16: 141-152, 2003.
574. **Lionikas A, Blizard DA, Vandenberg DJ, Stout JT, Vogler GP, McClearn GE, and Larsson L.** Genetic determinants of weight of fast- and slow-twitch skeletal muscles in old mice. *Mamm Genome* 17: 615-628, 2006.
575. **Liu G, Mac Gabhann F, and Popel AS.** Effects of fiber type and size on the heterogeneity of oxygen distribution in exercising skeletal muscle. *PLoS One* 7: e44375, 2012.
576. **Liu J, Höglund, A.-S., Karlsson Edlund, P., Lindblad, J., Qaisar, R., Bengtsson, E., Larsson, L.** Myonuclear domain size and myosin isoform expression in muscle fibers from mammals representing a 100,000-fold difference in body size. *Experimental Physiology* Accepted for publication: 2008.
577. **Liu JX, Hoglund AS, Karlsson P, Lindblad J, Qaisar R, Aare S, Bengtsson E, and Larsson L.** Myonuclear domain size and myosin isoform expression in muscle fibres from mammals representing a 100,000-fold difference in body size. *Exp Physiol* 94: 117-129, 2009.
578. **Liu W, Klose A, Forman S, Paris ND, Wei-LaPierre L, Cortes-Lopez M, Tan A, Flaherty M, Miura P, Dirksen RT, and Chakkalakal JV.** Loss of adult skeletal muscle stem cells drives age-related neuromuscular junction degeneration. *eLife* 6: 2017.
579. **Liu VW, Zhang C, and Nagley P.** Mutations in mitochondrial DNA accumulate differentially in three different human tissues during ageing. *Nucleic Acids Res* 26: 1268-1275, 1998.
580. **Lo Verso F, Carnio S, Vainshtein A, and Sandri M.** Autophagy is not required to sustain exercise and PRKAA1/AMPK activity but is important to prevent mitochondrial damage during physical activity. *Autophagy* 10: 1883-1894, 2014.
581. **Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, Sinha M, Dall'Osso C, Khong D, Shadrach JL, Miller CM, Singer BS, Stewart A, Psychogios N, Gerszten RE, Hartigan AJ, Kim MJ, Serwold**

- T, Wagers AJ, and Lee RT.** Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell* 153: 828-839, 2013.
582. **Lokireddy S, Wijesoma IW, Teng S, Bonala S, Gluckman PD, McFarlane C, Sharma M, and Kambadur R.** The ubiquitin ligase Mul1 induces mitophagy in skeletal muscle in response to muscle-wasting stimuli. *Cell Metab* 16: 613-624, 2012.
583. **Lopez-Martin JM, Salviati L, Trevisson E, Montini G, DiMauro S, Quinzii C, Hirano M, Rodriguez-Hernandez A, Cordero MD, Sanchez-Alcazar JA, Santos-Ocana C, and Navas P.** Missense mutation of the COQ2 gene causes defects of bioenergetics and de novo pyrimidine synthesis. *Hum Mol Genet* 16: 1091-1097, 2007.
584. **Lopez S, Miro O, Martinez E, Pedrol E, Rodriguez-Santiago B, Milinkovic A, Soler A, Garcia-Viejo MA, Nunes V, Casademont J, Gatell JM, and Cardellach F.** Mitochondrial effects of antiretroviral therapies in asymptomatic patients. *Antiviral therapy* 9: 47-55, 2004.
585. **Lourenco dos Santos S, Baraibar MA, Lundberg S, Eeg-Olofsson O, Larsson L, and Friguet B.** Oxidative proteome alterations during skeletal muscle ageing. *Redox biology* 5: 267-274, 2015.
586. **Lowe DA, Surek JT, Thomas DD, and Thompson LV.** Electron paramagnetic resonance reveals age-related myosin structural changes in rat skeletal muscle fibers. *Am J Physiol Cell Physiol* 280: C540-547., 2001.
587. **Lowe DA, Warren GL, Snow LM, Thompson LV, and Thomas DD.** Muscle activity and aging affect myosin structural distribution and force generation in rat fibers. *J Appl Physiol* 96: 498-506, 2004.
588. **Lowey S, Waller GS, and Trybus KM.** Function of skeletal muscle myosin heavy and light chain isoforms by an in vitro motility assay. *J Biol Chem* 268: 20414-20418, 1993.
589. **Lowey S, Waller GS, and Trybus KM.** Skeletal muscle myosin light chains are essential for physiological speeds of shortening. *Nature* 365: 454-456, 1993.
590. **Lu J, Tapia JC, White OL, and Lichtman JW.** The interscutularis muscle connectome. *PLoS biology* 7: e32, 2009.
591. **Lushaj EB, Johnson JK, McKenzie D, and Aiken JM.** Sarcopenia accelerates at advanced ages in Fisher 344xBrown Norway rats. *J Gerontol A Biol Sci Med Sci* 63: 921-927, 2008.
592. **Lyons CN, Mathieu-Costello O, and Moyes CD.** Regulation of skeletal muscle mitochondrial content during aging. *J Gerontol A Biol Sci Med Sci* 61: 3-13, 2006.
593. **Lyons PR, and Slater CR.** Structure and function of the neuromuscular junction in young adult mdx mice. *Journal of neurocytology* 20: 969-981, 1991.
594. **Madeo F, Zimmermann A, Maiuri MC, and Kroemer G.** Essential role for autophagy in life span extension. *J Clin Invest* 125: 85-93, 2015.
595. **Magrassi L, Purves D, and Lichtman JW.** Fluorescent probes that stain living nerve terminals. *J Neurosci* 7: 1207-1214, 1987.
596. **Mahoney J, Sager M, Dunham NC, and Johnson J.** Risk of falls after hospital discharge. *J Am Geriatr Soc* 42: 269-274, 1994.
597. **Mahoney JE, Sager MA, and Jalaluddin M.** Use of an ambulation assistive device predicts functional decline associated with hospitalization. *J Gerontol A Biol Sci Med Sci* 54: M83-88, 1999.
598. **Maier F, and Bornemann A.** Comparison of the muscle fiber diameter and satellite cell frequency in human muscle biopsies. *Muscle & nerve* 22: 578-583, 1999.
599. **Malena A, Pantic B, Borgia D, Sgarbi G, Solaini G, Holt IJ, Spinazzola A, Perissinotto E, Sandri M, Baracca A, and Vergani L.** Mitochondrial quality control: Cell-type-dependent responses to pathological mutant mitochondrial DNA. *Autophagy* 12: 2098-2112, 2016.
600. **Maltin CA, Delday MI, Baillie AG, Grubb DA, and Garlick PJ.** Fiber-type composition of nine rat muscles. I. Changes during the first year of life. *Am J Physiol* 257: E823-827, 1989.
601. **Mammucari C, Gherardi G, Zamparo I, Raffaello A, Boncompagni S, Chemello F, Cagnin S, Braga A, Zanin S, Pallafacchina G, Zentilin L, Sandri M, De Stefani D, Protasi F, Lanfranchi G, and Rizzuto R.** The mitochondrial calcium uniporter controls skeletal muscle trophism in vivo. *Cell Rep* 10: 1269-1279, 2015.

602. **Man LL, Liu F, Wang YJ, Song HH, Xu HB, Zhu ZW, Zhang Q, and Wang YJ.** The HMGB1 signaling pathway activates the inflammatory response in Schwann cells. *Neural regeneration research* 10: 1706-1712, 2015.
603. **Mancuso M, Filosto M, Bonilla E, Hirano M, Shanske S, Vu TH, and DiMauro S.** Mitochondrial myopathy of childhood associated with mitochondrial DNA depletion and a homozygous mutation (T77M) in the TK2 gene. *Arch Neurol* 60: 1007-1009, 2003.
604. **Mansueto G, Armani A, Viscomi C, D'Orsi L, De Cegli R, Polishchuk EV, Lamperti C, Di Meo I, Romanello V, Marchet S, Saha PK, Zong H, Blaauw B, Solagna F, Tezze C, Grumati P, Bonaldo P, Pessin JE, Zeviani M, Sandri M, and Ballabio A.** Transcription Factor EB Controls Metabolic Flexibility during Exercise. *Cell Metab* 25: 182-196, 2017.
605. **Manta P, Vassilopoulos D, and Spengos M.** Nucleo-cytoplasmic ratio in ageing skeletal muscle. *Eur Arch Psychiatry Neurol Sci* 236: 235-236, 1987.
606. **Marcus R, Butterfield G, Holloway L, Gilliland L, Baylink DJ, Hintz RL, and Sherman BM.** Effects of short term administration of recombinant human growth hormone to elderly people. *J Clin Endocrinol Metab* 70: 519-527, 1990.
607. **Markofski MM, Dickinson JM, Drummond MJ, Fry CS, Fujita S, Gundermann DM, Glynn EL, Jennings K, Paddon-Jones D, Reidy PT, Sheffield-Moore M, Timmerman KL, Rasmussen BB, and Volpi E.** Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women. *Exp Gerontol* 65: 1-7, 2015.
608. **Marques MJ, Conchello JA, and Lichtman JW.** From plaque to pretzel: fold formation and acetylcholine receptor loss at the developing neuromuscular junction. *J Neurosci* 20: 3663-3675, 2000.
609. **Martin TP, Bodine-Fowler S, and Edgerton VR.** Coordination of electromechanical and metabolic properties of cat soleus motor units. *Am J Physiol* 255: C684-693., 1988.
610. **Martin TP, Bodine-Fowler S, Roy RR, Eldred E, and Edgerton VR.** Metabolic and fiber size properties of cat tibialis anterior motor units. *Am J Physiol* 255: C43-50., 1988.
611. **Martinez-Lopez N, Tarabra E, Toledo M, Garcia-Macia M, Sahu S, Coletto L, Batista-Gonzalez A, Barzilai N, Pessin JE, Schwartz GJ, Kersten S, and Singh R.** System-wide Benefits of Intermeal Fasting by Autophagy. *Cell Metab* 26: 856-871 e855, 2017.
612. **Martinez DE.** Mortality patterns suggest lack of senescence in hydra. *Exp Gerontol* 33: 217-225, 1998.
613. **Martinsson T, Oldfors A, Darin N, Berg K, Tajsharghi H, Kyllerman M, and Wahlstrom J.** Autosomal dominant myopathy: missense mutation (Glu-706 --> Lys) in the myosin heavy chain IIa gene. *Proc Natl Acad Sci U S A* 97: 14614-14619., 2000.
614. **Marzetti E, Calvani R, Cesari M, Buford TW, Lorenzi M, Behnke BJ, and Leeuwenburgh C.** Mitochondrial dysfunction and sarcopenia of aging: from signaling pathways to clinical trials. *Int J Biochem Cell Biol* 45: 2288-2301, 2013.
615. **Marzetti E, and Leeuwenburgh C.** Skeletal muscle apoptosis, sarcopenia and frailty at old age. *Exp Gerontol* 41: 1234-1238, 2006.
616. **Masiero E, Agatea L, Mammucari C, Blaauw B, Loro E, Komatsu M, Metzger D, Reggiani C, Schiaffino S, and Sandri M.** Autophagy is required to maintain muscle mass. *Cell Metab* 10: 507-515, 2009.
617. **Massague J.** TGFbeta signalling in context. *Nat Rev Mol Cell Biol* 13: 616-630, 2012.
618. **Mathieu-Costello O, Agey PJ, Quintana ES, Rousey K, Wu L, and Bernstein MH.** Fiber capillarization and ultrastructure of pigeon pectoralis muscle after cold acclimation. *J Exp Biol* 201: 3211-3220, 1998.
619. **Mathieu-Costello O, Ju Y, Trejo-Morales M, and Cui L.** Greater capillary-fiber interface per fiber mitochondrial volume in skeletal muscles of old rats. *Journal of applied physiology* 99: 281-289, 2005.
620. **McCann CM, Nguyen QT, Santo Neto H, and Lichtman JW.** Rapid synapse elimination after postsynaptic protein synthesis inhibition in vivo. *J Neurosci* 27: 6064-6067, 2007.

621. **McComas AJ, Sica RE, and Campbell MJ.** "Sick" motoneurons. A unifying concept of muscle disease. *Lancet* 1: 321-326, 1971.
622. **McCully KK, Fielding RA, Evans WJ, Leigh JS, Jr., and Posner JD.** Relationships between in vivo and in vitro measurements of metabolism in young and old human calf muscles. *J Appl Physiol (1985)* 75: 813-819, 1993.
623. **McDonagh MJ.** Mechanical properties of muscles from *Xenopus borealis* following maintenance in organ culture. *Comparative biochemistry and physiology* 77: 377-382, 1984.
624. **McKiernan SH, Bua E, McGorray J, and Aiken J.** Early-onset calorie restriction conserves fiber number in aging rat skeletal muscle. *FASEB J* 18: 580-581, 2004.
625. **McMahon TA.** *Muscles, Reflexes, and Locomotion.* Princeton University Press, 1984.
626. **McMartin DN, and O'Connor JA, Jr.** Effect of age on axoplasmic transport of cholinesterase in rat sciatic nerves. *Mech Ageing Dev* 10: 241-248., 1979.
627. **McMullen CA, Ferry AL, Gamboa JL, Andrade FH, and Dupont-Versteegden EE.** Age-related changes of cell death pathways in rat extraocular muscle. *Exp Gerontol* 44: 420-425, 2009.
628. **McPhee JS, French DP, Jackson D, Nazroo J, Pendleton N, and Degens H.** Physical activity in older age: perspectives for healthy ageing and frailty. *Biogerontology* 17: 567-580, 2016.
629. **McPherron AC, Lawler AM, and Lee SJ.** Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 387: 83-90, 1997.
630. **McPherron AC, and Lee SJ.** Double muscling in cattle due to mutations in the myostatin gene. *Proc Natl Acad Sci U S A* 94: 12457-12461, 1997.
631. **Meissner C, Bruse P, and Oehmichen M.** Tissue-specific deletion patterns of the mitochondrial genome with advancing age. *Exp Gerontol* 41: 518-524, 2006.
632. **Mellado W, Slebe JC, and Maccioni RB.** Tubulin carbamoylation. Functional amino groups in microtubule assembly. *Biochem J* 203: 675-681, 1982.
633. **Menshikova EV, Ritov VB, Dube JJ, Amati F, Stefanovic-Racic M, Toledo FGS, Coen PM, and Goodpaster BH.** Calorie Restriction-induced Weight Loss and Exercise Have Differential Effects on Skeletal Muscle Mitochondria Despite Similar Effects on Insulin Sensitivity. *J Gerontol A Biol Sci Med Sci* 73: 81-87, 2017.
634. **Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, Cesari M, and Nourhashemi F.** Proinflammatory cytokines, aging, and age-related diseases. *Journal of the American Medical Directors Association* 14: 877-882, 2013.
635. **Milan G, Romanello V, Pescatore F, Armani A, Paik JH, Frasson L, Seydel A, Zhao J, Abraham R, Goldberg AL, Blaauw B, DePinho RA, and Sandri M.** Regulation of autophagy and the ubiquitin-proteasome system by the FoxO transcriptional network during muscle atrophy. *Nat Commun* 6: 6670, 2015.
636. **Milkiewicz M, Brown MD, Egginton S, and Hudlicka O.** Association between shear stress, angiogenesis, and VEGF in skeletal muscles in vivo. *Microcirculation* 8: 229-241., 2001.
637. **Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, and Imai SI.** Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice. *Cell Metab* 24: 795-806, 2016.
638. **Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T, Nojima A, Nabetani A, Oike Y, Matsubara H, Ishikawa F, and Komuro I.** A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat Med* 15: 1082-1087, 2009.
639. **Mitch WE, and Goldberg AL.** Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *N Engl J Med* 335: 1897-1905, 1996.
640. **Mito T, Ishizaki H, Suzuki M, Morishima H, Ota A, Ishikawa K, Nakada K, Maeno A, Shiroishi T, and Hayashi J.** Transmitochondrial mito-miceDelta and mtDNA mutator mice, but not aged mice, share the same spectrum of musculoskeletal disorders. *Biochem Biophys Res Commun* 456: 933-937, 2015.
641. **Mittal KR, and Logmani FH.** Age-related reduction in 8th cervical ventral nerve root myelinated fiber diameters and numbers in man. *J Gerontol* 42: 8-10., 1987.

642. **Miyazaki K, Ohta Y, Nagai M, Morimoto N, Kurata T, Takehisa Y, Ikeda Y, Matsuura T, and Abe K.** Disruption of neurovascular unit prior to motor neuron degeneration in amyotrophic lateral sclerosis. *Journal of neuroscience research* 89: 718-728, 2011.
643. **Mohan S, and Radha E.** Age-related changes in rat muscle collagen. *Gerontology* 26: 61-67, 1980.
644. **Monemi M, Eriksson PO, Eriksson A, and Thornell LE.** Adverse changes in fibre type composition of the human masseter versus biceps brachii muscle during aging. *J Neurol Sci* 154: 35-48., 1998.
645. **Monti RJ, Roy RR, and Edgerton VR.** Role of motor unit structure in defining function. *Muscle Nerve* 24: 848-866., 2001.
646. **Mooradian AD, and Wong NC.** Molecular biology of aging. Part II: A synopsis of current research. *J Am Geriatr Soc* 39: 717-723., 1991.
647. **Moore F, Weekes J, and Hardie DG.** Evidence that AMP triggers phosphorylation as well as direct allosteric activation of rat liver AMP-activated protein kinase. A sensitive mechanism to protect the cell against ATP depletion. *Eur J Biochem* 199: 691-697, 1991.
648. **Moscoso LM, Merlie JP, and Sanes JR.** N-CAM, 43K-rapsyn, and S-laminin mRNAs are concentrated at synaptic sites in muscle fibers. *Molecular and cellular neurosciences* 6: 80-89, 1995.
649. **Mosole S, Carraro U, Kern H, Loeffler S, Fruhmann H, Vogelaer M, Burggraf S, Mayr W, Krenn M, Paternostro-Sluga T, Hamar D, Cvecka J, Sedliak M, Tirpakova V, Sarabon N, Musaro A, Sandri M, Protasi F, Nori A, Pond A, and Zampieri S.** Long-term high-level exercise promotes muscle reinnervation with age. *J Neuropathol Exp Neurol* 73: 284-294, 2014.
650. **Moss RL, Diffie GM, and Greaser ML.** Contractile properties of skeletal muscle fibers in relation to myofibrillar protein isoforms. *Rev Physiol Biochem Pharmacol* 126: 1-63, 1995.
651. **Mouchiroud L, Houtkooper RH, Moullan N, Katsyuba E, Ryu D, Canto C, Mottis A, Jo YS, Viswanathan M, Schoonjans K, Guarente L, and Auwerx J.** The NAD(+)/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. *Cell* 154: 430-441, 2013.
652. **Mukouyama YS, Gerber HP, Ferrara N, Gu C, and Anderson DJ.** Peripheral nerve-derived VEGF promotes arterial differentiation via neuropilin 1-mediated positive feedback. *Development* 132: 941-952, 2005.
653. **Muller-Delp JM.** Heterogeneous ageing of skeletal muscle microvascular function. *J Physiol* 594: 2285-2295, 2016.
654. **Munscher C, Rieger T, Muller-Hocker J, and Kadenbach B.** The point mutation of mitochondrial DNA characteristic for MERRF disease is found also in healthy people of different ages. *FEBS Lett* 317: 27-30, 1993.
655. **Murdock DG, Christacos NC, and Wallace DC.** The age-related accumulation of a mitochondrial DNA control region mutation in muscle, but not brain, detected by a sensitive PNA-directed PCR clamping based method. *Nucleic Acids Res* 28: 4350-4355, 2000.
656. **Murphy JL, Ratnaike TE, Shang E, Falkous G, Blakely EL, Alston CL, Taivassalo T, Haller RG, Taylor RW, and Turnbull DM.** Cytochrome c oxidase-intermediate fibres: importance in understanding the pathogenesis and treatment of mitochondrial myopathy. *Neuromuscul Disord* 22: 690-698, 2012.
657. **Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL, and Rosenthal N.** Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 27: 195-200, 2001.
658. **Musch TI, Eklund KE, Hageman KS, and Poole DC.** Altered regional blood flow responses to submaximal exercise in older rats. *Journal of applied physiology* 96: 81-88, 2004.
659. **Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, and Sun B.** Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Lett* 470: 113-117, 2000.
660. **Nakai J, Dirksen RT, Nguyen HT, Pessah IN, Beam KG, and Allen PD.** Enhanced dihydropyridine receptor channel activity in the presence of ryanodine receptor. *Nature* 380: 72-75, 1996.
661. **Nederveen JP, Joanisse S, Snijders T, Ivankovic V, Baker SK, Phillips SM, and Parise G.** Skeletal muscle satellite cells are located at a closer proximity to capillaries in healthy young compared with older men. *Journal of cachexia, sarcopenia and muscle* 7: 547-554, 2016.



662. **Nemeth PM, Pette D, and Vrbova G.** Comparison of enzyme activities among single muscle fibres within defined motor units. *J Physiol* 311: 489-495, 1981.
663. **Nemeth PM, Solanki L, Gordon DA, Hamm TM, Reinking RM, and Stuart DG.** Uniformity of metabolic enzymes within individual motor units. *J Neurosci* 6: 892-898, 1986.
664. **Nevitt MC, Cummings SR, and Hudes ES.** Risk factors for injurious falls: a prospective study. *J Gerontol* 46: M164-170, 1991.
665. **Newlands S, Levitt LK, Robinson CS, Karpf AB, Hodgson VR, Wade RP, and Hardeman EC.** Transcription occurs in pulses in muscle fibers. *Genes Dev* 12: 2748-2758, 1998.
666. **Newton JP, Yemm R, and McDonagh MJ.** Study of age changes in the motor units of the first dorsal interosseous muscle in man. *Gerontology* 34: 115-119, 1988.
667. **Nguyen QT, Sanes JR, and Lichtman JW.** Pre-existing pathways promote precise projection patterns. *Nat Neurosci* 5: 861-867, 2002.
668. **Nicklas BJ, and Brinkley TE.** Exercise training as a treatment for chronic inflammation in the elderly. *Exercise and sport sciences reviews* 37: 165-170, 2009.
669. **Nishimune H, Badawi Y, Mori S, and Shigemoto K.** Dual-color STED microscopy reveals a sandwich structure of Bassoon and Piccolo in active zones of adult and aged mice. *Scientific reports* 6: 27935, 2016.
670. **Norman H, Zackrisson H, Hedstrom Y, Andersson P, Nordquist J, Eriksson LI, Libelius R, and Larsson L.** Myofibrillar protein and gene expression in acute quadriplegic myopathy. *J Neurol Sci* 285: 28-38, 2009.
671. **Novak LP.** Aging, total body potassium, fat-free mass, and cell mass in males and females between ages 18 and 85 years. *J Gerontol* 27: 438-443., 1972.
672. **O'Donnell KC, Vargas ME, and Sagasti A.** WldS and PGC-1alpha regulate mitochondrial transport and oxidation state after axonal injury. *J Neurosci* 33: 14778-14790, 2013.
673. **O'Neill BT, Lee KY, Klaus K, Softic S, Krumpoch MT, Fentz J, Stanford KI, Robinson MM, Cai W, Kleinridders A, Pereira RO, Hirshman MF, Abel ED, Accili D, Goodyear LJ, Nair KS, and Kahn CR.** Insulin and IGF-1 receptors regulate FoxO-mediated signaling in muscle proteostasis. *J Clin Invest* 126: 3433-3446, 2016.
674. **O'Sullivan DJ, and Swallow M.** The fibre size and content of the radial and sural nerves. *J Neurol Neurosurg Psychiatry* 31: 464-470., 1968.
675. **Ochala J, Gustafson AM, Diez ML, Renaud G, Li M, Aare S, Qaisar R, Banduseela VC, Hedstrom Y, Tang X, Dworkin B, Ford GC, Nair KS, Perera S, Gautel M, and Larsson L.** Preferential skeletal muscle myosin loss in response to mechanical silencing in a novel rat intensive care unit model: underlying mechanisms. *J Physiol* 589: 2007-2026, 2011.
676. **Ochala J, and Larsson L.** Acquired and hereditary sarcomeric protein diseases. In: *Muscle: Fundamental Biology and Mechanisms and Disease*. . In: *Muscle: Fundamental Biology and Mechanisms and Disease* edited by Joseph A. Hill JTS, H. Lee Sweeney, and Eric N. Olson London, UK. : Academic Press, 2012, p. 1023-1030.
677. **Ochoa J, and Mair WG.** The normal sural nerve in man. II. Changes in the axons and Schwann cells due to ageing. *Acta Neuropathol* 13: 217-239, 1969.
678. **Oda K.** Age changes of motor innervation and acetylcholine receptor distribution on human skeletal muscle fibres. *J Neurol Sci* 66: 327-338, 1984.
679. **Oertel G.** Changes in human skeletal muscles due to ageing. Histological and histochemical observations on autopsy material. *Acta Neuropathol* 69: 309-313, 1986.
680. **Oexle K, Zwirner A, Freudenberg K, Kohlschutter A, and Speer A.** Examination of telomere lengths in muscle tissue casts doubt on replicative aging as cause of progression in Duchenne muscular dystrophy. *Pediatr Res* 42: 226-231, 1997.
681. **Ogilvie H, Cacciani N, Akkad H, and Larsson L.** Targeting Heat Shock Proteins Mitigates Ventilator Induced Diaphragm Muscle Dysfunction in an Age-Dependent Manner. *Frontiers in physiology* 7: 417, 2016.

682. **Oldfors A, Moslemi AR, Jonasson L, Ohlsson M, Kollberg G, and Lindberg C.** Mitochondrial abnormalities in inclusion-body myositis. *Neurology* 66: S49-55, 2006.
683. **Olivieri F, Ahtainen M, Lazzarini R, Pollanen E, Capri M, Lorenzi M, Fulgenzi G, Albertini MC, Salvioli S, Alen MJ, Kujala UM, Borghetti G, Babini L, Kaprio J, Sipila S, Franceschi C, Kovanen V, and Procopio AD.** Hormone replacement therapy enhances IGF-1 signaling in skeletal muscle by diminishing miR-182 and miR-223 expressions: a study on postmenopausal monozygotic twin pairs. *Aging Cell* 13: 850-861, 2014.
684. **Olsen JM, Sato M, Dallner OS, Sandstrom AL, Pisani DF, Chambard JC, Amri EZ, Hutchinson DS, and Bengtsson T.** Glucose uptake in brown fat cells is dependent on mTOR complex 2-promoted GLUT1 translocation. *J Cell Biol* 207: 365-374, 2014.
685. **Omairi S, Matsakas A, Degens H, Kretz O, Hansson KA, Solbra AV, Bruusgaard JC, Joch B, Sartori R, Giallourou N, Mitchell R, Collins-Hooper H, Foster K, Pasternack A, Ritvos O, Sandri M, Narkar V, Swann JR, Huber TB, and Patel K.** Enhanced exercise and regenerative capacity in a mouse model that violates size constraints of oxidative muscle fibres. *eLife* 5: 2016.
686. **Onambele GN, Bruce SA, and Woledge RC.** Oestrogen status in relation to the early training responses in human thumb adductor muscles. *Acta Physiol (Oxf)* 188: 41-52, 2006.
687. **Onambele NG, Skelton DA, Bruce SA, and Woledge RC.** Follow-up study of the benefits of hormone replacement therapy on isometric muscle strength of adductor pollicis in postmenopausal women. *Clin Sci (Lond)* 100: 421-422, 2001.
688. **Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, Van Dorpe J, Hellings P, Gorselink M, Heymans S, Theilmeier G, Dewerchin M, Laudénbach V, Vermylen P, Raat H, Acker T, Vleminckx V, Van Den Bosch L, Cashman N, Fujisawa H, Drost MR, Sciot R, Bruyninckx F, Hicklin DJ, Ince C, Gressens P, Lupu F, Plate KH, Robberecht W, Herbert JM, Collen D, and Carmeliet P.** Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nature genetics* 28: 131-138, 2001.
689. **Orlander J, Kiessling KH, Larsson L, Karlsson J, and Aniansson A.** Skeletal muscle metabolism and ultrastructure in relation to age in sedentary men. *Acta Physiol Scand* 104: 249-261, 1978.
690. **Pallotti F, Chen X, Bonilla E, and Schon EA.** Evidence that specific mtDNA point mutations may not accumulate in skeletal muscle during normal human aging. *Am J Hum Genet* 59: 591-602, 1996.
691. **Palmer AK, Tchkonja T, LeBrasseur NK, Chini EN, Xu M, and Kirkland JL.** Cellular Senescence in Type 2 Diabetes: A Therapeutic Opportunity. *Diabetes* 64: 2289-2298, 2015.
692. **Papet I, Dardevet D, Sornet C, Bechereau F, Prugnaud J, Pouyet C, and Obled C.** Acute phase protein levels and thymus, spleen and plasma protein synthesis rates differ in adult and old rats. *J Nutr* 133: 215-219, 2003.
693. **Parizkova J, Eiselt E, Sprynarova S, and Wachtlova M.** Body composition, aerobic capacity, and density of muscle capillaries in young and old men. *Journal of applied physiology* 31: 323-325, 1971.
694. **Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, and Chung J.** Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature* 441: 1157-1161, 2006.
695. **Park SJ, Gavrilova O, Brown AL, Soto JE, Bremner S, Kim J, Xu X, Yang S, Um JH, Koch LG, Britton SL, Lieber RL, Philp A, Baar K, Kohama SG, Abel ED, Kim MK, and Chung JH.** DNA-PK Promotes the Mitochondrial, Metabolic, and Physical Decline that Occurs During Aging. *Cell Metab* 25: 1135-1146 e1137, 2017.
696. **Parkington JD, LeBrasseur NK, Siebert AP, and Fielding RA.** Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle. *J Appl Physiol (1985)* 97: 243-248, 2004.
697. **Patel SA, Chaudhari A, Gupta R, Velingkaar N, and Kondratov RV.** Circadian clocks govern calorie restriction-mediated life span extension through BMAL1- and IGF-1-dependent mechanisms. *FASEB J* 30: 1634-1642, 2016.

698. **Paturi S, Gutta AK, Katta A, Kakarla SK, Arvapalli RK, Gadde MK, Nalabotu SK, Rice KM, Wu M, and Blough E.** Effects of aging and gender on muscle mass and regulation of Akt-mTOR-p70s6k related signaling in the F344BN rat model. *Mech Ageing Dev* 131: 202-209, 2010.
699. **Pavlath GK, Rich K, Webster SG, and Blau HM.** Localization of muscle gene products in nuclear domains. *Nature* 337: 570-573, 1989.
700. **Payne AM, and Delbono O.** Neurogenesis of excitation-contraction uncoupling in aging skeletal muscle. *Exercise and sport sciences reviews* 32: 36-40, 2004.
701. **Payne GW.** Effect of inflammation on the aging microcirculation: impact on skeletal muscle blood flow control. *Microcirculation* 13: 343-352, 2006.
702. **Pedersen BK.** Anti-inflammation--just another word for anti-ageing? *J Physiol* 587: 5515, 2009.
703. **Pelosi M, Alfo M, Martella F, Pappalardo E, and Musaro A.** Finite mixture clustering of human tissues with different levels of IGF-1 splice variants mRNA transcripts. *BMC Bioinformatics* 16: 289, 2015.
704. **Perkins DJ, and Kniss DA.** Tumor necrosis factor-alpha promotes sustained cyclooxygenase-2 expression: attenuation by dexamethasone and NSAIDs. *Prostaglandins* 54: 727-743, 1997.
705. **Pestronk A, Drachman DB, and Griffin JW.** Effects of aging on nerve sprouting and regeneration. *Exp Neurol* 70: 65-82, 1980.
706. **Petrella JK, Kim JS, Cross JM, Kosek DJ, and Bamman MM.** Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *Am J Physiol Endocrinol Metab* 291: E937-946 Epub 2006 Jun 2013, 2006.
707. **Petrella JK, Kim JS, Cross JM, Kosek DJ, and Bamman MM.** Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *Am J Physiol Endocrinol Metab* 291: E937-946, 2006.
708. **Pette D, and Staron RS.** Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev Physiol Biochem Pharmacol* 116: 1-76, 1990.
709. **Pettigrew FP, and Gardiner PF.** Changes in rat plantaris motor unit profiles with advanced age. *Mech Ageing Dev* 40: 243-259., 1987.
710. **Phillips BE, Williams JP, Greenhaff PL, Smith K, and Atherton PJ.** Physiological adaptations to resistance exercise as a function of age. *JCI insight* 2: 2017.
711. **Phillips SK, Rook KM, Siddle NC, Bruce SA, and Woledge RC.** Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. *Clinical science* 84: 95-98, 1993.
712. **Phillips T, and Leeuwenburgh C.** Muscle fiber specific apoptosis and TNF-alpha signaling in sarcopenia are attenuated by life-long calorie restriction. *Faseb J* 19: 668-670, 2005.
713. **Piasecki M, Ireland A, Coulson J, Stashuk DW, Hamilton-Wright A, Swiecicka A, Rutter MK, McPhee JS, and Jones DA.** Motor unit number estimates and neuromuscular transmission in the tibialis anterior of master athletes: evidence that athletic older people are not spared from age-related motor unit remodeling. *Physiological reports* 4: 2016.
714. **Picard M, Ritchie D, Wright KJ, Romestaing C, Thomas MM, Rowan SL, Taivassalo T, and Hepple RT.** Mitochondrial functional impairment with aging is exaggerated in isolated mitochondria compared to permeabilized myofibers. *Aging Cell* 9: 1032-1046, 2010.
715. **Picard M, Taivassalo T, Gouspillou G, and Hepple RT.** Mitochondria: isolation, structure and function. *J Physiol* 589: 4413-4421, 2011.
716. **Piiper J, and Scheid P.** Diffusion limitation of O2 supply to tissue in homogeneous and heterogeneous models. *Respir Physiol* 85: 127-136, 1991.
717. **Pipalia TG, Koth J, Roy SD, Hammond CL, Kawakami K, and Hughes SM.** Cellular dynamics of regeneration reveals role of two distinct Pax7 stem cell populations in larval zebrafish muscle repair. *Dis Model Mech* 2016.

718. **Piraino S, Boero F, Aeshbach B, and Schmid V.** Reversing the life cycle: medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). *Biol Bull* 190: 302-312, 1996.
719. **Plyley MJ, Olmstead BJ, and Noble EG.** Time course of changes in capillarization in hypertrophied rat plantaris muscle. *J Appl Physiol* 84: 902-907, 1998.
720. **Poggioli T, Vujic A, Yang P, Macias-Trevino C, Uygur A, Loffredo FS, Pancoast JR, Cho M, Goldstein J, Tandias RM, Gonzalez E, Walker RG, Thompson TB, Wagers AJ, Fong YW, and Lee RT.** Circulating Growth Differentiation Factor 11/8 Levels Decline With Age. *Circ Res* 118: 29-37, 2016.
721. **Pollanen E, Sipila S, Alen M, Ronkainen PH, Ankarberg-Lindgren C, Puolakka J, Suominen H, Hamalainen E, Turpeinen U, Konttinen YT, and Kovanen V.** Differential influence of peripheral and systemic sex steroids on skeletal muscle quality in pre- and postmenopausal women. *Aging Cell* 10: 650-660, 2011.
722. **Poole JG, Lawrenson L, Kim J, Brown C, and Richardson RS.** Vascular and metabolic response to cycle exercise in sedentary humans: effect of age. *Am J Physiol Heart Circ Physiol* 284: H1251-1259, 2003.
723. **Power GA, Dalton BH, Behm DG, Vandervoort AA, Doherty TJ, and Rice CL.** Motor unit number estimates in masters runners: use it or lose it? *Medicine and science in sports and exercise* 42: 1644-1650, 2010.
724. **Pratt SJ, Shah SB, Ward CW, Inacio MP, Stains JP, and Lovering RM.** Effects of in vivo injury on the neuromuscular junction in healthy and dystrophic muscles. *J Physiol* 591: 559-570, 2013.
725. **Price FD, von Maltzahn J, Bentzinger CF, Dumont NA, Yin H, Chang NC, Wilson DH, Frenette J, and Rudnicki MA.** Inhibition of JAK-STAT signaling stimulates adult satellite cell function. *Nat Med* 20: 1174-1181, 2014.
726. **Proctor DN, Shen PH, Dietz NM, Eickhoff TJ, Lawler LA, Ebersold EJ, Loeffler DL, and Joyner MJ.** Reduced leg blood flow during dynamic exercise in older endurance-trained men. *J Appl Physiol* 85: 68-75, 1998.
727. **Proctor DN, Sinning WE, Walro JM, Sieck GC, and Lemon PW.** Oxidative capacity of human muscle fiber types: effects of age and training status. *Journal of applied physiology* 78: 2033-2038, 1995.
728. **Pryor WA, and Squadrito GL.** The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol* 268: L699-722, 1995.
729. **Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, and Spiegelman BM.** Activation of PPARgamma coactivator-1 through transcription factor docking. *Science* 286: 1368-1371, 1999.
730. **Putman CT, Sultan KR, Wassmer T, Bamford JA, Skorjanc D, and Pette D.** Fiber-type transitions and satellite cell activation in low-frequency-stimulated muscles of young and aging rats. *J Gerontol A Biol Sci Med Sci* 56: B510-519, 2001.
731. **Qaisar R, Renaud G, Hedstrom Y, Pollanen E, Ronkainen P, Kaprio J, Alen M, Sipila S, Artemenko K, Bergquist J, Kovanen V, and Larsson L.** Hormone replacement therapy improves contractile function and myonuclear organization of single muscle fibres from postmenopausal monozygotic female twin pairs. *J Physiol* 591: 2333-2344, 2013.
732. **Qaisar R, Renaud G, Morine K, Barton ER, Sweeney HL, and Larsson L.** Is functional hypertrophy and specific force coupled with the addition of myonuclei at the single muscle fiber level? *FASEB J* 2011.
733. **Qiu MG, and Zhu XH.** Aging changes of the angioarchitecture and arterial morphology of the spinal cord in rats. *Gerontology* 50: 360-365, 2004.
734. **Quetelet A.** *Sur l'Homme et le Développement de ses Facultés.* Brussels: Haumann, 1836.
735. **Quinlan JG, Iuzzo PA, Lambert EH, and Gronert GA.** Ankle dorsiflexor twitch properties in malignant hyperthermia. *Muscle Nerve* 12: 119-125., 1989.
736. **Quinzii CM, Lopez LC, Gilkerson RW, Dorado B, Coku J, Naini AB, Lagier-Tourenne C, Schuelke M, Salviati L, Carrozzo R, Santorelli F, Rahman S, Tazir M, Koenig M, DiMauro S, and Hirano M.** Reactive oxygen species, oxidative stress, and cell death correlate with level of CoQ10 deficiency. *FASEB J* 24: 3733-3743, 2010.
737. **Radak Z, Takahashi R, Kumiyama A, Nakamoto H, Ohno H, Ookawara T, and Goto S.** Effect of aging and late onset dietary restriction on antioxidant enzymes and proteasome activities, and protein carbonylation of rat skeletal muscle and tendon. *Exp Gerontol* 37: 1423-1430, 2002.

738. **Rainsford KD.** Discovery, mechanisms of action and safety of ibuprofen. *International journal of clinical practice Supplement* 3-8, 2003.
739. **Ralston E, Lu Z, Biscocho N, Soumaka E, Mavroidis M, Prats C, Lomo T, Capetanaki Y, and Ploug T.** Blood vessels and desmin control the positioning of nuclei in skeletal muscle fibers. *J Cell Physiol* 209: 874-882, 2006.
740. **Ramamurthy B, Hook P, Jones AD, and Larsson L.** Changes in myosin structure and function in response to glycation. *Faseb J* 15: 2415-2422., 2001.
741. **Ramamurthy B, Jones AD, and Larsson L.** Glutathione reverses early effects of glycation on myosin function. *Am J Physiol Cell Physiol* 285: C419-424, 2003.
742. **Ramamurthy B, Jones, A. D., Larsson, L.** Glutathione reverses early effects of glycation on myosin function. *Submitted for publication* 2002.
743. **Ramamurthy B, and Larsson L.** Detection of an aging-related increase in advanced glycation end products in fast- and slow-twitch skeletal muscles in the rat. *Biogerontology* 14: 293-301, 2013.
744. **Ramaswamy KS, Palmer ML, van der Meulen JH, Renoux A, Kostrominova TY, Michele DE, and Faulkner JA.** Lateral transmission of force is impaired in skeletal muscles of dystrophic mice and very old rats. *J Physiol* 589: 1195-1208, 2011.
745. **Ratkevicius A, Joyson A, Selmer I, Dhanani T, Grierson C, Tommasi AM, DeVries A, Rauchhaus P, Crowther D, Alesci S, Yaworsky P, Gilbert F, Redpath TW, Brady J, Fearon KC, Reid DM, Greig CA, and Wackerhage H.** Serum concentrations of myostatin and myostatin-interacting proteins do not differ between young and sarcopenic elderly men. *J Gerontol A Biol Sci Med Sci* 66: 620-626, 2011.
746. **Rattan SI.** Synthesis, modifications, and turnover of proteins during aging. *Exp Gerontol* 31: 33-47, 1996.
747. **Raymond M.** Paralyse essentielle de l'enfance: Atrophie musculaire consécutive. *Gaz Med Paris* 4: 225-226, 1875.
748. **Rebeck RT, Karunasekara Y, Board PG, Beard NA, Casarotto MG, and Dulhunty AF.** Skeletal muscle excitation-contraction coupling: who are the dancing partners? *Int J Biochem Cell Biol* 48: 28-38, 2014.
749. **Reid B, Martinov VN, Nja A, Lomo T, and Bewick GS.** Activity-dependent plasticity of transmitter release from nerve terminals in rat fast and slow muscles. *J Neurosci* 23: 9340-9348, 2003.
750. **Reid MB, Khawli FA, and Moody MR.** Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle. *J Appl Physiol* 75: 1081-1087., 1993.
751. **Reiser PJ, Kasper CE, and Moss RL.** Myosin subunits and contractile properties of single fibers from hypokinetic rat muscles. *J Appl Physiol* 63: 2293-2300, 1987.
752. **Renganathan M, Messi ML, and Delbono O.** Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle. *J Membr Biol* 157: 247-253, 1997.
753. **Rexed B.** Contribution to the knowledge of the postnatal development of the peripheral nervous system in man. *Acta Psychiatri Neurol Scand Suppl.* 33: 121-193, 1944.
754. **Rich M, and Lichtman JW.** Motor nerve terminal loss from degenerating muscle fibers. *Neuron* 3: 677-688, 1989.
755. **Rich MM, and Lichtman JW.** In vivo visualization of pre- and postsynaptic changes during synapse elimination in reinnervated mouse muscle. *J Neurosci* 9: 1781-1805, 1989.
756. **Rieu I, Magne H, Savary-Auzeloux I, Averous J, Bos C, Peyron MA, Combaret L, and Dardevet D.** Reduction of low grade inflammation restores blunting of postprandial muscle anabolism and limits sarcopenia in old rats. *J Physiol* 587: 5483-5492, 2009.
757. **Ripoll E, Sillau AH, and Banchemo N.** Changes in the capillarity of skeletal muscle in the growing rat. *Pflugers Arch* 380: 153-158, 1979.
758. **Rivard A, Berthou-Soulie L, Principe N, Kearney M, Curry C, Branellec D, Semenza GL, and Isner JM.** Age-dependent defect in vascular endothelial growth factor expression is associated with reduced hypoxia-inducible factor 1 activity. *J Biol Chem* 275: 29643-29647, 2000.

759. **Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, Magner M, Asahara T, and Isner JM.** Age-dependent impairment of angiogenesis. *Circulation* 99: 111-120, 1999.
760. **Rivas DA, McDonald DJ, Rice NP, Haran PH, Dolnikowski GG, and Fielding RA.** Diminished anabolic signaling response to insulin induced by intramuscular lipid accumulation is associated with inflammation in aging but not obesity. *Am J Physiol Regul Integr Comp Physiol* 310: R561-569, 2016.
761. **Robbins AS, Rubenstein LZ, Josephson KR, Schulman BL, Osterweil D, and Fine G.** Predictors of falls among elderly people. Results of two population- based studies. *Arch Intern Med* 149: 1628-1633, 1989.
762. **Robbins J, Horan T, Gulick J, and Kropp K.** The chicken myosin heavy chain family. *J Biol Chem* 261: 6606-6612, 1986.
763. **Robbins N, and Fahim MA.** Progression of age changes in mature mouse motor nerve terminals and its relation to locomotor activity. *Journal of neurocytology* 14: 1019-1036, 1985.
764. **Robinson MM, Dasari S, Konopka AR, Johnson ML, Manjunatha S, Esponda RR, Carter RE, Lanza IR, and Nair KS.** Enhanced Protein Translation Underlies Improved Metabolic and Physical Adaptations to Different Exercise Training Modes in Young and Old Humans. *Cell Metab* 25: 581-592, 2017.
765. **Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, and Puigserver P.** Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434: 113-118, 2005.
766. **Romanello V, Guadagnin E, Gomes L, Roder I, Sandri C, Petersen Y, Milan G, Masiero E, Del Piccolo P, Foretz M, Scorrano L, Rudolf R, and Sandri M.** Mitochondrial fission and remodelling contributes to muscle atrophy. *EMBO J* 29: 1774-1785, 2010.
767. **Romanello V, and Sandri M.** Mitochondrial Quality Control and Muscle Mass Maintenance. *Front Physiol* 6: 422, 2015.
768. **Rome LC, Sosnicki AA, and Goble DO.** Maximum velocity of shortening of three fibre types from horse soleus muscle: implications for scaling with body size. *J Physiol (Lond)* 431: 173-185, 1990.
769. **Roos CM, Zhang B, Palmer AK, Ogrodnik MB, Pirtskhalava T, Thalji NM, Hagler M, Jurk D, Smith LA, Casaclang-Verzosa G, Zhu Y, Schafer MJ, Tchkonja T, Kirkland JL, and Miller JD.** Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell* 15: 973-977, 2016.
770. **Rosenberg IH.** Summary comments. *The American journal of clinical nutrition* 50 (Suppl): 1231S-1233S, 1989.
771. **Rosenheimer JL.** Ultraterminal sprouting in innervated and partially denervated adult and aged rat muscle. *Neuroscience* 38: 763-770, 1990.
772. **Rosenheimer JL, and Smith DO.** Differential changes in the end-plate architecture of functionally diverse muscles during aging. *J Neurophysiol* 53: 1567-1581., 1985.
773. **Rossiter HB, Howlett RA, Holcombe HH, Entin PL, Wagner HE, and Wagner PD.** Age is no barrier to muscle structural, biochemical and angiogenic adaptations to training up to 24 months in female rats. *J Physiol* 565: 993-1005, 2005.
774. **Roth SM, Metter EJ, Ling S, and Ferrucci L.** Inflammatory factors in age-related muscle wasting. *Curr Opin Rheumatol* 18: 625-630, 2006.
775. **Rowan SL, Purves-Smith FM, Solbak NM, and Hepple RT.** Accumulation of severely atrophic myofibers marks the acceleration of sarcopenia in slow and fast twitch muscles. *Exp Gerontol* 46: 660-669, 2011.
776. **Rowan SL, Rygiel K, Purves-Smith FM, Solbak NM, Turnbull DM, and Hepple RT.** Denervation causes fiber atrophy and myosin heavy chain co-expression in senescent skeletal muscle. *PLoS One* 7: e29082, 2012.
777. **Rowe GC, El-Khoury R, Patten IS, Rustin P, and Arany Z.** PGC-1alpha is dispensable for exercise-induced mitochondrial biogenesis in skeletal muscle. *PLoS One* 7: e41817, 2012.
778. **Roy RR, Monke SR, Allen DL, and Edgerton VR.** Modulation of myonuclear number in functionally overloaded and exercised rat plantaris fibers. *J Appl Physiol* 87: 634-642, 1999.
779. **Rudman D.** Growth hormone, body composition, and aging. *J Am Geriatr Soc* 33: 800-807, 1985.

780. **Rudman D, Chawla RK, and Kutner MH.** Heterogeneity of growth hormone in the nocturnal serum of children. *Pediatr Res* 19: 981-985, 1985.
781. **Rudman D, Feller AG, Cohn L, Shetty KR, Rudman IW, and Draper MW.** Effects of human growth hormone on body composition in elderly men. *Horm Res* 36 Suppl 1: 73-81, 1991.
782. **Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW, and Mattson DE.** Effects of human growth hormone in men over 60 years old. *N Engl J Med* 323: 1-6, 1990.
783. **Russ DW, Boyd IM, McCoy KM, and McCorkle KW.** Muscle-specificity of age-related changes in markers of autophagy and sphingolipid metabolism. *Biogerontology* 16: 747-759, 2015.
784. **Ryan NA, Zwetsloot KA, Westerkamp LM, Hickner RC, Pofahl WE, and Gavin TP.** Lower skeletal muscle capillarization and VEGF expression in aged vs. young men. *Journal of applied physiology* 100: 178-185, 2006.
785. **Rygiel KA, Picard M, and Turnbull DM.** The ageing neuromuscular system and sarcopenia: a mitochondrial perspective. *J Physiol* 594: 4499-4512, 2016.
786. **Sacconi S, Salviati L, Nishigaki Y, Walker WF, Hernandez-Rosa E, Trevisson E, Delplace S, Desnuelle C, Shanske S, Hirano M, Schon EA, Bonilla E, De Vivo DC, DiMauro S, and Davidson MM.** A functionally dominant mitochondrial DNA mutation. *Hum Mol Genet* 17: 1814-1820, 2008.
787. **Safdar A, Little JP, Stokl AJ, Hettinga BP, Akhtar M, and Tarnopolsky MA.** Exercise increases mitochondrial PGC-1 $\alpha$  content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J Biol Chem* 286: 10605-10617, 2011.
788. **Sakita M, Murakami S, and Fujino H.** Age-related morphological regression of myelinated fibers and capillary architecture of distal peripheral nerves in rats. *BMC Neurosci* 17: 39, 2016.
789. **Sakuma K, Kinoshita M, Ito Y, Aizawa M, Aoi W, and Yamaguchi A.** p62/SQSTM1 but not LC3 is accumulated in sarcopenic muscle of mice. *J Cachexia Sarcopenia Muscle* 7: 204-212, 2016.
790. **Salmons S, and Henriksson J.** The adaptive response of skeletal muscle to increased use. *Muscle Nerve* 4: 94-105, 1981.
791. **Salviati G, Betto R, Danieli Betto D, and Zeviani M.** Myofibrillar-protein isoforms and sarcoplasmic reticulum Ca<sup>2+</sup>-transport activity of single human muscle fibres. *Biochem J* 224: 215-225, 1984.
792. **Samorajski T.** Age differences in the morphology of posterior tibial nerves of mice. *J Comp Neurol* 157: 439-445., 1974.
793. **Samuel MA, Valdez G, Tapia JC, Lichtman JW, and Sanes JR.** Agrin and synaptic laminin are required to maintain adult neuromuscular junctions. *PLoS One* 7: e46663, 2012.
794. **Sander HW, Golden M, and Danon MJ.** Quadriplegic areflexic ICU illness: selective thick filament loss and normal nerve histology. *Muscle Nerve* 26: 499-505, 2002.
795. **Sandri M, Barberi L, Bijlsma AY, Blaauw B, Dyar KA, Milan G, Mammucari C, Meskers CG, Pallafacchina G, Paoli A, Pion D, Roceri M, Romanello V, Serrano AL, Toniolo L, Larsson L, Maier AB, Munoz-Canoves P, Musaro A, Pende M, Reggiani C, Rizzuto R, and Schiaffino S.** Signalling pathways regulating muscle mass in ageing skeletal muscle. The role of the IGF1-Akt-mTOR-FoxO pathway. *Biogerontology* 14: 303-323, 2013.
796. **Sanes JR, Johnson YR, Kotzbauer PT, Mudd J, Hanley T, Martinou JC, and Merlie JP.** Selective expression of an acetylcholine receptor-lacZ transgene in synaptic nuclei of adult muscle fibers. *Development* 113: 1181-1191, 1991.
797. **Sanes JR, Marshall LM, and McMahan UJ.** Reinnervation of muscle fiber basal lamina after removal of myofibers. Differentiation of regenerating axons at original synaptic sites. *J Cell Biol* 78: 176-178, 1978.
798. **Sartori R, Gregorevic P, and Sandri M.** TGF $\beta$  and BMP signaling in skeletal muscle: potential significance for muscle-related disease. *Trends Endocrinol Metab* 25: 464-471, 2014.
799. **Sartori R, Milan G, Patron M, Mammucari C, Blaauw B, Abraham R, and Sandri M.** Smad2 and 3 transcription factors control muscle mass in adulthood. *Am J Physiol Cell Physiol* 296: C1248-1257, 2009.

800. **Sartori R, Schirwis E, Blaauw B, Bortolanza S, Zhao J, Enzo E, Stantzou A, Mouisel E, Toniolo L, Ferry A, Stricker S, Goldberg AL, Dupont S, Piccolo S, Amthor H, and Sandri M.** BMP signaling controls muscle mass. *Nat Genet* 45: 1309-1318, 2013.
801. **Scelsi R, Marchetti C, and Poggi P.** Histochemical and ultrastructural aspects of m. vastus lateralis in sedentary old people (age 65--89 years). *Acta Neuropathol* 51: 99-105, 1980.
802. **Schaap LA, Pluijm SM, Deeg DJ, and Visser M.** Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *The American journal of medicine* 119: 526 e529-517, 2006.
803. **Schaefer AM, Sanes JR, and Lichtman JW.** A compensatory subpopulation of motor neurons in a mouse model of amyotrophic lateral sclerosis. *J Comp Neurol* 490: 209-219, 2005.
804. **Schafer MJ, Atkinson EJ, Vanderboom PM, Kotajarvi B, White TA, Moore MM, Bruce CJ, Greason KL, Suri RM, Khosla S, Miller JD, Bergen HR, 3rd, and LeBrasseur NK.** Quantification of GDF11 and Myostatin in Human Aging and Cardiovascular Disease. *Cell Metab* 23: 1207-1215, 2016.
805. **Schaie WK.** Quasi-experimental research designs in the psychology of aging. In: *Handbook of Psychology of Aging*, Eds JE Birren and KW Schaie New York: Van Nostrand Reinhold: 39-58, 1977.
806. **Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, and Sandri M.** Mechanisms regulating skeletal muscle growth and atrophy. *FEBS J* 280: 4294-4314, 2013.
807. **Schiaffino S, and Reggiani C.** Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol Rev* 76: 371-423, 1996.
808. **Schon EA, Rizzuto R, Moraes CT, Nakase H, Zeviani M, and DiMauro S.** A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. *Science* 244: 346-349, 1989.
809. **Schultz AB, Ashton-Miller JA, and Alexander NB.** What leads to age and gender differences in balance maintenance and recovery? *Muscle Nerve Suppl* 5: S60-64, 1997.
810. **Schultz E, and Lipton BH.** Skeletal muscle satellite cells: changes in proliferation potential as a function of age. *Mechanisms of ageing and development* 20: 377-383, 1982.
811. **Sciacco M, Bonilla E, Schon EA, DiMauro S, and Moraes CT.** Distribution of wild-type and common deletion forms of mtDNA in normal and respiration-deficient muscle fibers from patients with mitochondrial myopathy. *Hum Mol Genet* 3: 13-19, 1994.
812. **Scioli MG, Bielli A, Arcuri G, Ferlosio A, and Orlandi A.** Ageing and microvasculature. *Vascular cell* 6: 19, 2014.
813. **Scorrano L.** Multiple functions of mitochondria-shaping proteins. *Novartis Found Symp* 287: 47-55; discussion 55-49, 2007.
814. **Seaberg B, Henslee G, Wang S, Paez-Colasante X, Landreth GE, and Rimer M.** Muscle-derived extracellular signal-regulated kinases 1 and 2 are required for the maintenance of adult myofibers and their neuromuscular junctions. *Mol Cell Biol* 35: 1238-1253, 2015.
815. **Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, and Rudnicki MA.** Pax7 is required for the specification of myogenic satellite cells. *Cell* 102: 777-786, 2000.
816. **Sebastian D, Sorianello E, Segales J, Irazoki A, Ruiz-Bonilla V, Sala D, Planet E, Berenguer-Llargo A, Munoz JP, Sanchez-Feutrie M, Plana N, Hernandez-Alvarez MI, Serrano AL, Palacin M, and Zorzano A.** Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway. *EMBO J* 35: 1677-1693, 2016.
817. **Shankaran M, Shearer TW, Stimpson SA, Turner SM, King C, Wong PY, Shen Y, Turnbull PS, Kramer F, Clifton L, Russell A, Hellerstein MK, and Evans WJ.** Proteome-wide muscle protein fractional synthesis rates predict muscle mass gain in response to a selective androgen receptor modulator in rats. *Am J Physiol Endocrinol Metab* 310: E405-417, 2016.
818. **Sheetz MP, and Spudich JA.** Movement of myosin-coated fluorescent beads on actin cables in vitro. *Nature* 303: 31-35, 1983.
819. **Sheetz MP, and Spudich JA.** Movement of myosin-coated structures on actin cables. *Cell motility* 3: 485-489, 1983.



820. **Shephard RJ.** Physical Activity and Aging. *London:Croom Helm* 24-39, 1978.
821. **Shimpo M, Ikeda U, Maeda Y, Takahashi M, Miyashita H, Mizukami H, Urabe M, Kume A, Takizawa T, Shibuya M, Ozawa K, and Shimada K.** AAV-mediated VEGF gene transfer into skeletal muscle stimulates angiogenesis and improves blood flow in a rat hindlimb ischemia model. *Cardiovascular research* 53: 993-1001, 2002.
822. **Shinpo K, Kikuchi S, Sasaki H, Ogata A, Moriwaka F, and Tashiro K.** Selective vulnerability of spinal motor neurons to reactive dicarbonyl compounds, intermediate products of glycation, in vitro: implication of inefficient glutathione system in spinal motor neurons. *Brain Res* 861: 151-159., 2000.
823. **Shoffner JM, Lott MT, Voljavec AS, Soueidan SA, Costigan DA, and Wallace DC.** Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. *Proc Natl Acad Sci U S A* 86: 7952-7956, 1989.
824. **Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, and Nair KS.** Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci U S A* 102: 5618-5623, 2005.
825. **Short KR, Vittone JL, Bigelow ML, Proctor DN, Coenen-Schimke JM, Rys P, and Nair KS.** Changes in myosin heavy chain mRNA and protein expression in human skeletal muscle with age and endurance exercise training. *Journal of applied physiology* 99: 95-102, 2005.
826. **Shrager JB, Desjardins PR, Burkman JM, Konig SK, Stewart SK, Su L, Shah MC, Bricklin E, Tewari M, Hoffman R, Rickels MR, Jullian EH, Rubinstein NA, and Stedman HH.** Human skeletal myosin heavy chain genes are tightly linked in the order embryonic-IIa-IId/x-ILb-perinatal-extraocular [In Process Citation]. *J Muscle Res Cell Motil* 21: 345-355, 2000.
827. **Sidenius P, and Jakobsen J.** Axonal transport in early experimental diabetes. *Brain Res* 173: 315-330, 1979.
828. **Simon AM, Hoppe P, and Burden SJ.** Spatial restriction of AChR gene expression to subsynaptic nuclei. *Development* 114: 545-553, 1992.
829. **Sindler AL, Reyes R, Chen B, Ghosh P, Gurovich AN, Kang LS, Cardounel AJ, Delp MD, and Muller-Delp JM.** Age and exercise training alter signaling through reactive oxygen species in the endothelium of skeletal muscle arterioles. *Journal of applied physiology* 114: 681-693, 2013.
830. **Singh T, and Newman AB.** Inflammatory markers in population studies of aging. *Ageing Res Rev* 10: 319-329, 2011.
831. **Sipila S, Narici M, Kjaer M, Pollanen E, Atkinson RA, Hansen M, and Kovanen V.** Sex hormones and skeletal muscle weakness. *Biogerontology* 14: 231-245, 2013.
832. **Siu PM, Pistilli EE, Butler DC, and Alway SE.** Aging influences cellular and molecular responses of apoptosis to skeletal muscle unloading. *Am J Physiol Cell Physiol* 288: C338-349, 2005.
833. **Smerdu V, Karsch-Mizrachi I, Campione M, Leinwand L, and Schiaffino S.** Type Iix myosin heavy chain transcripts are expressed in type IIb fibers of human skeletal muscle. *Am J Physiol* 267: C1723-1728, 1994.
834. **Smith CB, Goochee C, Rapoport SI, and Sokoloff L.** Effects of ageing on local rates of cerebral glucose utilization in the rat. *Brain* 103: 351-365, 1980.
835. **Snijders T, Nederveen JP, Joannisse S, Leenders M, Verdijk LB, van Loon LJ, and Parise G.** Muscle fibre capillarization is a critical factor in muscle fibre hypertrophy during resistance exercise training in older men. *Journal of cachexia, sarcopenia and muscle* 2016.
836. **Snyder GK.** Capillary growth in chick skeletal muscle with normal maturation and hypertrophy. *Respir Physiol* 102: 293-301, 1995.
837. **Snyder GK, Farrelly C, and Coelho JR.** Adaptations in skeletal muscle capillarity following changes in oxygen supply and changes in oxygen demands. *Eur J Appl Physiol Occup Physiol* 65: 158-163, 1992.
838. **Sobin SS, Bernick S, and Ballard KW.** Histochemical characterization of the aging microvasculature in the human and other mammalian and non-mammalian vertebrates by the periodic acid-Schiff reaction. *Mech Ageing Dev* 63: 183-192, 1992.

839. **Son Y-J, and Thompson WJ.** Nerve sprouting in muscle is induced and guided by processes extended by Schwann cells. *Neuron* 14: 133-141, 1995.
840. **Son Y-J, and Thompson WJ.** Schwann cell processes guide regeneration of peripheral axons. *Neuron* 14: 125-132, 1995.
841. **Song Z, Moore DR, Hodson N, Ward C, Dent JR, O'Leary MF, Shaw AM, Hamilton DL, Sarkar S, Gangloff YG, Hornberger TA, Spriet LL, Heigenhauser GJ, and Philp A.** Resistance exercise initiates mechanistic target of rapamycin (mTOR) translocation and protein complex co-localisation in human skeletal muscle. *Sci Rep* 7: 5028, 2017.
842. **Sonntag WE, Steger RW, Forman LJ, and Meites J.** Decreased pulsatile release of growth hormone in old male rats. *Endocrinology* 107: 1875-1879, 1980.
843. **Soubannier V, McLelland GL, Zunino R, Braschi E, Rippstein P, Fon EA, and McBride HM.** A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr Biol* 22: 135-141, 2012.
844. **Soubannier V, Rippstein P, Kaufman BA, Shoubridge EA, and McBride HM.** Reconstitution of mitochondria derived vesicle formation demonstrates selective enrichment of oxidized cargo. *PLoS One* 7: e52830, 2012.
845. **Spendiff S, Vuda M, Gouspillou G, Aare S, Perez A, Morais JA, Jagoe RT, Filion ME, Glicksman R, Kapchinsky S, MacMillan NJ, Pion CH, Aubertin-Leheudre M, Hettwer S, Correa JA, Taivassalo T, and Hepple RT.** Denervation drives mitochondrial dysfunction in skeletal muscle of octogenarians. *J Physiol* 594: 7361-7379, 2016.
846. **Spinazzi M, Casarin A, Pertegato V, Ermani M, Salviati L, and Angelini C.** Optimization of respiratory chain enzymatic assays in muscle for the diagnosis of mitochondrial disorders. *Mitochondrion* 11: 893-904, 2011.
847. **Stalberg E, and Fawcett PR.** Macro EMG in healthy subjects of different ages. *J Neurol Neurosurg Psychiatry* 45: 870-878., 1982.
848. **Stienen GJ, Kiers JL, Bottinelli R, and Reggiani C.** Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. *J Physiol (Lond)* 493: 299-307, 1996.
849. **Stiles AR, Simon MT, Stover A, Eftekharian S, Khanlou N, Wang HL, Magaki S, Lee H, Partynski K, Dorrani N, Chang R, Martinez-Agosto JA, and Abdenur JE.** Mutations in TFAM, encoding mitochondrial transcription factor A, cause neonatal liver failure associated with mtDNA depletion. *Molecular genetics and metabolism* 119: 91-99, 2016.
850. **Strauss KA, Jinks RN, Puffenberger EG, Venkatesh S, Singh K, Cheng I, Mikita N, Thilagavathi J, Lee J, Sarafianos S, Benkert A, Koehler A, Zhu A, Trovillion V, McGlincy M, Morlet T, Deardorff M, Innes AM, Prasad C, Chudley AE, Lee IN, and Suzuki CK.** CODAS syndrome is associated with mutations of LONP1, encoding mitochondrial AAA+ Lon protease. *Am J Hum Genet* 96: 121-135, 2015.
851. **Street SF.** Lateral transmission of tension in frog myofibers: a myofibrillar network and transverse cytoskeletal connections are possible transmitters. *Journal of cellular physiology* 114: 346-364, 1983.
852. **Stålberg E, Ekstedt, J.** Single fibre EMG and microphysiology of the motor unit in normal and diseased human muscle. In: *New developments in EMG and Clinical Neurophysiology*, edited by Desmedt J. Basel: S. Karger, 1973, p. 113-129.
853. **Suetta C, Hvid LG, Justesen L, Christensen U, Neergaard K, Simonsen L, Ortenblad N, Magnusson SP, Kjaer M, and Aagaard P.** Effects of aging on human skeletal muscle after immobilization and retraining. *Journal of applied physiology* 107: 1172-1180, 2009.
854. **Suetta C, Magnusson SP, Rosted A, Aagaard P, Jakobsen AK, Larsen LH, Duus B, and Kjaer M.** Resistance training in the early postoperative phase reduces hospitalization and leads to muscle hypertrophy in elderly hip surgery patients--a controlled, randomized study. *J Am Geriatr Soc* 52: 2016-2022, 2004.
855. **Sugiura T, Matoba H, Miyata H, Kawai Y, and Murakami N.** Myosin heavy chain isoform transition in ageing fast and slow muscles of the rat. *Acta Physiol Scand* 144: 419-423., 1992.
856. **Sultan KR, Dittrich BT, Leisner E, Paul N, and Pette D.** Fiber type-specific expression of major proteolytic systems in fast- to slow-transforming rabbit muscle. *Am J Physiol Cell Physiol* 280: C239-247, 2001.

857. **Suzuki M, Okamura T, Shimazu Y, Takahashi H, Eguchi K, Kano K, and Tsuchiya S.** [A study of falls experienced by institutionalized elderly]. *Nippon Koshu Eisei Zasshi* 39: 927-940, 1992.
858. **Swallow M.** Fibre size and content of the anterior tibial nerve of the foot. *J Neurol Neurosurg Psychiatry* 29: 205-213., 1966.
859. **Sweeney HL, Kushmerick MJ, Mabuchi K, Sreter FA, and Gergely J.** Myosin alkali light chain and heavy chain variations correlate with altered shortening velocity of isolated skeletal muscle fibers. *J Biol Chem* 263: 9034-9039, 1988.
860. **Swerdloff RS, and Wang C.** Androgens and aging in men. *Exp Gerontol* 28: 435-446., 1993.
861. **Syrovoy I, and Gutmann E.** Changes in speed of contraction and ATPase activity in striated muscle during old age. *Exp Gerontol* 5: 31-35, 1970.
862. **Syrovoy I, and Hodny Z.** Non-enzymatic glycosylation of myosin: effects of diabetes and ageing. *Gen Physiol Biophys* 11: 301-307., 1992.
863. **Takahashi A, Kureishi Y, Yang J, Luo Z, Guo K, Mukhopadhyay D, Ivashchenko Y, Branellec D, and Walsh K.** Myogenic Akt signaling regulates blood vessel recruitment during myofiber growth. *Mol Cell Biol* 22: 4803-4814, 2002.
864. **Takahashi K.** A clinicopathologic study on the peripheral nervous system of the aged. Sciatic nerve and autonomic nervous system. *Geriatrics* 21: 123-133., 1966.
865. **Tamaki T, Hirata M, and Uchiyama Y.** Qualitative alteration of peripheral motor system begins prior to appearance of typical sarcopenia syndrome in middle-aged rats. *Frontiers in aging neuroscience* 6: 296, 2014.
866. **Tang K, Breen EC, Gerber HP, Ferrara NM, and Wagner PD.** Capillary regression in vascular endothelial growth factor-deficient skeletal muscle. *Physiol Genomics* 18: 63-69, 2004.
867. **Tchkonina T, Morbeck DE, Von Zglinicki T, Van Deursen J, Lustgarten J, Scoble H, Khosla S, Jensen MD, and Kirkland JL.** Fat tissue, aging, and cellular senescence. *Aging Cell* 9: 667-684, 2010.
868. **Tchkonina T, Zhu Y, van Deursen J, Campisi J, and Kirkland JL.** Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J Clin Invest* 123: 966-972, 2013.
869. **Tengan CH, and Moraes CT.** Duplication and triplication with staggered breakpoints in human mitochondrial DNA. *Biochim Biophys Acta* 1406: 73-80, 1998.
870. **Tezze C, Romanello V, Desbats MA, Fadini GP, Albiero M, Favaro G, Ciciliot S, Soriano ME, Morbidoni V, Cerqua C, Loeffler S, Kern H, Franceschi C, Salvioli S, Conte M, Blaauw B, Zampieri S, Salviati L, Scorrano L, and Sandri M.** Age-Associated Loss of OPA1 in Muscle Impacts Muscle Mass, Metabolic Homeostasis, Systemic Inflammation, and Epithelial Senescence. *Cell Metab* 25: 1374-1389 e1376, 2017.
871. **Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, Bannasch H, Scheichl A, Woollard KJ, von Dobschutz E, Kolodgie F, Virmani R, Stark GB, Peter K, and Eisenhardt SU.** Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: in vivo proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *Circulation* 130: 35-50, 2014.
872. **Thomason DB, and Booth FW.** Atrophy of the soleus muscle by hindlimb unweighting. *J Appl Physiol* 68: 1-12, 1990.
873. **Thompson D, Markovitch D, Betts JA, Mazzatti D, Turner J, and Tyrrell RM.** Time course of changes in inflammatory markers during a 6-mo exercise intervention in sedentary middle-aged men: a randomized-controlled trial. *Journal of applied physiology* 108: 769-779, 2010.
874. **Thompson LV, and Brown M.** Age-related changes in contractile properties of single skeletal fibers from the soleus muscle. *J Appl Physiol* 86: 881-886, 1999.
875. **Thompson LV, Durand D, Fugere NA, and Ferrington DA.** Myosin and actin expression and oxidation in aging muscle. *J Appl Physiol* 101: 1581-1587, 2006.
876. **Thompson RW, McClung JM, Baltgalvis KA, Davis JM, and Carson JA.** Modulation of overload-induced inflammation by aging and anabolic steroid administration. *Exp Gerontol* 41: 1136-1148, 2006.

877. **Thompson W, and Jansen JKS.** The extent of sprouting of remaining motor units in partly denervated immature and adult rat soleus muscle. *Neuroscience* 2: 523-535, 1977.
878. **Tidball JG.** Regulation of muscle growth and regeneration by the immune system. *Nature reviews Immunology* 17: 165-178, 2017.
879. **Tierney MT, Aydogdu T, Sala D, Malecova B, Gatto S, Puri PL, Latella L, and Sacco A.** STAT3 signaling controls satellite cell expansion and skeletal muscle repair. *Nat Med* 20: 1182-1186, 2014.
880. **Tintignac LA, Brenner HR, and Ruegg MA.** Mechanisms Regulating Neuromuscular Junction Development and Function and Causes of Muscle Wasting. *Physiol Rev* 95: 809-852, 2015.
881. **Tisdale MJ.** The ubiquitin-proteasome pathway as a therapeutic target for muscle wasting. *The journal of supportive oncology* 3: 209-217, 2005.
882. **Tohgi H, Tsukagoshi H, and Toyokura Y.** Quantitative changes with age in normal sural nerves. *Acta Neuropathol (Berl)* 38: 213-220., 1977.
883. **Tokuhiro E, Dean HJ, Friesen HG, and Rudman D.** Comparative study of serum human growth hormone measurement with NB2 lymphoma cell bioassay, IM-9 receptor modulation assay, and radioimmunoassay in children with disorders of growth. *J Clin Endocrinol Metab* 58: 549-554, 1984.
884. **Tollback A, Knutsson E, Borg J, Borg K, and Jakobsson F.** Torque-velocity relation and muscle fibre characteristics of foot dorsiflexors after long-term overuse of residual muscle fibres due to prior polio or L5 root lesion. *Scand J Rehabil Med* 24: 151-156., 1992.
885. **Tomlinson BE, and Irving D.** The numbers of limb motor neurons in the human lumbosacral cord throughout life. *J Neurol Sci* 34: 213-219., 1977.
886. **Tomonaga M.** Histochemical and ultrastructural changes in senile human skeletal muscle. *J Am Geriatr Soc* 25: 125-131., 1977.
887. **Touvier T, De Palma C, Rigamonti E, Scagliola A, Incerti E, Mazelin L, Thomas JL, D'Antonio M, Politi L, Schaeffer L, Clementi E, and Brunelli S.** Muscle-specific Drp1 overexpression impairs skeletal muscle growth via translational attenuation. *Cell Death Dis* 6: e1663, 2015.
888. **Towne C, Montgomery KL, Iyer SM, Deisseroth K, and Delp SL.** Optogenetic control of targeted peripheral axons in freely moving animals. *PLoS One* 8: e72691, 2013.
889. **Trendelenburg AU, Meyer A, Rohner D, Boyle J, Hatakeyama S, and Glass DJ.** Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. *Am J Physiol Cell Physiol* 296: C1258-1270, 2009.
890. **Trevisson E, DiMauro S, Navas P, and Salviati L.** Coenzyme Q deficiency in muscle. *Curr Opin Neurol* 24: 449-456, 2011.
891. **Trifunovic A, Hansson A, Wredenberg A, Rovio AT, Dufour E, Khvorostov I, Spelbrink JN, Wibom R, Jacobs HT, and Larsson NG.** Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proc Natl Acad Sci U S A* 102: 17993-17998, 2005.
892. **Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlof S, Oldfors A, Wibom R, Tornell J, Jacobs HT, and Larsson NG.** Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429: 417-423, 2004.
893. **Trounce I, Byrne E, and Marzuki S.** Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. *Lancet* 1: 637-639, 1989.
894. **Troy A, Cadwallader AB, Fedorov Y, Tyner K, Tanaka KK, and Olwin BB.** Coordination of satellite cell activation and self-renewal by Par-complex-dependent asymmetric activation of p38alpha/beta MAPK. *Cell stem cell* 11: 541-553, 2012.
895. **Tseng BS, Kasper CE, and Edgerton VR.** Cytoplasm-to-myonucleus ratios and succinate dehydrogenase activities in adult rat slow and fast muscle fibers. *Cell Tissue Res* 275: 39-49, 1994.
896. **Tsuru M, Nagata K, Jimi A, Irie K, Yamada A, Nagai R, Horiuchi S, and Sata M.** Effect of AGEs on human disc herniation: intervertebral disc hernia is also effected by AGEs. *The Kurume medical journal* 49: 7-13, 2002.

897. **Tuffery AR.** Growth and degeneration of motor end-plates in normal cat hind limb muscles. *J Anat* 110: 221-247, 1971.
898. **Turek Z, Olders J, Hoofd L, Egginton S, Kreuzer F, and Rakusan K.** PO<sub>2</sub> histograms in various models of tissue oxygenation in skeletal muscle. *Adv Exp Med Biol* 248: 227-237, 1989.
899. **Turney SG, and Lichtman JW.** Reversing the outcome of synapse elimination at developing neuromuscular junctions in vivo: evidence for synaptic competition and its mechanism. *PLoS biology* 10: e1001352, 2012.
900. **Turney SG, Walsh MK, and Lichtman JW.** In vivo imaging of the developing neuromuscular junction in neonatal mice. *Cold Spring Harbor protocols* 2012: 1166-1176, 2012.
901. **Tymk K, and Mathieu-Costello O.** Structural and functional changes in the microvasculature of disused skeletal muscle. *Frontiers in bioscience : a journal and virtual library* 6: D45-52, 2001.
902. **Tzekova N, Heinen A, and Kury P.** Molecules involved in the crosstalk between immune- and peripheral nerve Schwann cells. *Journal of clinical immunology* 34 Suppl 1: S86-104, 2014.
903. **Wachsmuth M, Hubner A, Li M, Madea B, and Stoneking M.** Age-Related and Heteroplasmy-Related Variation in Human mtDNA Copy Number. *PLoS genetics* 12: e1005939, 2016.
904. **Wadley AJ, Veldhuijzen van Zanten JJ, and Aldred S.** The interactions of oxidative stress and inflammation with vascular dysfunction in ageing: the vascular health triad. *Age* 35: 705-718, 2013.
905. **Wagenmakers AJ, Strauss JA, Shepherd SO, Keske MA, and Cocks M.** Increased muscle blood supply and transendothelial nutrient and insulin transport induced by food intake and exercise: effect of obesity and ageing. *J Physiol* 2015.
906. **Vainshtein A, Desjardins EM, Armani A, Sandri M, and Hood DA.** PGC-1 $\alpha$  modulates denervation-induced mitophagy in skeletal muscle. *Skeletal muscle* 5: 9, 2015.
907. **Valdez G, Tapia JC, Kang H, Clemenson GD, Jr., Gage FH, Lichtman JW, and Sanes JR.** Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. *Proceedings of the National Academy of Sciences of the United States of America* 107: 14863-14868, 2010.
908. **Valdez G, Tapia JC, Kang H, Clemenson GD, Jr., Gage FH, Lichtman JW, and Sanes JR.** Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. *Proceedings of the National Academy of Sciences of the United States of America* 107: 14863-14868, 2010.
909. **Valdez G, Tapia JC, Lichtman JW, Fox MA, and Sanes JR.** Shared resistance to aging and ALS in neuromuscular junctions of specific muscles. *PLoS One* 7: e34640, 2012.
910. **Walsh MK, and Lichtman JW.** In vivo time-lapse imaging of synaptic takeover associated with naturally occurring synapse elimination. *Neuron* 37: 67-73, 2003.
911. **Walters TJ, Sweeney HL, and Farrar RP.** Influence of electrical stimulation on a fast-twitch muscle in aging rats. *J Appl Physiol* 71: 1921-1928., 1991.
912. **van der Meer SF, Jaspers RT, Jones DA, and Degens H.** The time course of myonuclear accretion during hypertrophy in young adult and older rat plantaris muscle. *Ann Anat* 193: 56-63, 2011.
913. **Van Mier P, and Lichtman JW.** Regeneration of single laser-ablated motor axons and the reoccupation of postsynaptic sites followed in living mice. *Soc Neurosci Abs* 15: 334, 1989.
914. **van Norren K, Rusli F, van Dijk M, Lute C, Nagel J, Dijk FJ, Dwarkasing J, Boekschoten MV, Luiking Y, Witkamp RF, Muller M, and Steegenga WT.** Behavioural changes are a major contributing factor in the reduction of sarcopenia in caloric-restricted ageing mice. *J Cachexia Sarcopenia Muscle* 6: 253-268, 2015.
915. **Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, Alderson NL, Baynes JW, Epstein CJ, Huang TT, Nelson J, Strong R, and Richardson A.** Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics* 16: 29-37, 2003.
916. **van Steenis G, and Kroes R.** Changes in the nervous system and musculature of old rats. *Vet Pathol* 8: 320-332, 1971.

917. **van Wessel T, de Haan A, van der Laarse WJ, and Jaspers RT.** The muscle fiber type-fiber size paradox: hypertrophy or oxidative metabolism? *Eur J Appl Physiol* 110: 665-694, 2010.
918. **Wanagat J, Cao Z, Pathare P, and Aiken JM.** Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia. *Faseb J* 15: 322-332, 2001.
919. **Wang E.** Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. *Cancer Res* 55: 2284-2292, 1995.
920. **Wang JC, and Bennett M.** Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. *Circ Res* 111: 245-259, 2012.
921. **Wang Y, and Hekimi S.** Mitochondrial dysfunction and longevity in animals: Untangling the knot. *Science* 350: 1204-1207, 2015.
922. **Wang Y, Oxer D, and Hekimi S.** Mitochondrial function and lifespan of mice with controlled ubiquinone biosynthesis. *Nat Commun* 6: 6393, 2015.
923. **Wang ZM, Leng X, Messi ML, Choi SJ, Marsh AP, Nicklas B, and Delbono O.** Relationship of Physical Function to Single Muscle Fiber Contractility in Older Adults: Effects of Resistance Training with and without Caloric Restriction. *J Gerontol A Biol Sci Med Sci* 2018.
924. **Varanita T, Soriano ME, Romanello V, Zaglia T, Quintana-Cabrera R, Semenzato M, Menabo R, Costa V, Civiletto G, Pesce P, Viscomi C, Zeviani M, Di Lisa F, Mongillo M, Sandri M, and Scorrano L.** The OPA1-dependent mitochondrial cristae remodeling pathway controls atrophic, apoptotic, and ischemic tissue damage. *Cell Metab* 21: 834-844, 2015.
925. **Watanabe H, Ogasawara, M., Suzuki, N., Nishizawa, N., Ambo, K.** Glycation of myofibrillar protein in aged rats and mice. *Biosci Biotechnol Biochem* 56: 1109-1112, 1992.
926. **Watkins SC, and Cullen MJ.** A quantitative comparison of satellite cell ultrastructure in Duchenne muscular dystrophy, polymyositis, and normal controls. *Muscle & nerve* 9: 724-730, 1986.
927. **Welle S, Bhatt K, Shah B, and Thornton C.** Insulin-like growth factor-1 and myostatin mRNA expression in muscle: comparison between 62-77 and 21-31 yr old men. *Exp Gerontol* 37: 833-839, 2002.
928. **Welle S, Bhatt K, and Thornton CA.** High-abundance mRNAs in human muscle: comparison between young and old. *J Appl Physiol* 89: 297-304., 2000.
929. **Welle S, Brooks AI, Delehanty JM, Needler N, Bhatt K, Shah B, and Thornton CA.** Skeletal muscle gene expression profiles in 20-29 year old and 65-71 year old women. *Exp Gerontol* 39: 369-377, 2004.
930. **Welle S, Brooks AI, Delehanty JM, Needler N, and Thornton CA.** Gene expression profile of aging in human muscle. *Physiol Genomics* 14: 149-159, 2003.
931. **Welle S, Thornton C, Jozefowicz R, and Statt M.** Myofibrillar protein synthesis in young and old men. *Am J Physiol* 264: E693-698, 1993.
932. **Welle S, Totterman S, and Thornton C.** Effect of age on muscle hypertrophy induced by resistance training. *J Gerontol A Biol Sci Med Sci* 51: M270-275., 1996.
933. **Verdijk LB, Snijders T, Holloway TM, J VANK, and LJ VANL.** Resistance Training Increases Skeletal Muscle Capillarization in Healthy Older Men. *Med Sci Sports Exerc* 48: 2157-2164, 2016.
934. **Vermeij WP, Dolle ME, Reiling E, Jaarsma D, Payan-Gomez C, Bombardieri CR, Wu H, Roks AJ, Botter SM, van der Eerden BC, Youssef SA, Kuiper RV, Nagarajah B, van Oostrom CT, Brandt RM, Barnhoorn S, Imholz S, Pennings JL, de Bruin A, Gyenis A, Pothof J, Vijg J, van Steeg H, and Hoeijmakers JH.** Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice. *Nature* 537: 427-431, 2016.
935. **Wernig A, and Herrera AA.** Sprouting and remodelling at the nerve-muscle junction. *Prog Neurobiol* 27: 251-291, 1986.
936. **Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, Catalano JV, Deininger M, Miller C, Silver RT, Talpaz M, Winton EF, Harvey JH, Jr., Arcasoy MO, Hexner E, Lyons RM, Paquette R, Raza A, Vaddi K, Erickson-Viitanen S, Sun W, Sandor V, and Kantarjian HM.** The clinical benefit of ruxolitinib across patient

- subgroups: analysis of a placebo-controlled, Phase III study in patients with myelofibrosis. *British journal of haematology* 161: 508-516, 2013.
937. **Vescovo G, Zennaro R, Sandri M, Carraro U, Leprotti C, Ceconi C, Ambrosio GB, and Dalla Libera L.** Apoptosis of skeletal muscle myofibers and interstitial cells in experimental heart failure. *J Mol Cell Cardiol* 30: 2449-2459., 1998.
938. **Wheeler SJ, and Plummer JM.** Age-related changes in the fibre composition of equine peripheral nerve. *J Neurol Sci* 90: 53-66., 1989.
939. **Whipple RH, Wolfson LI, and Amerman PM.** The relationship of knee and ankle weakness to falls in nursing home residents: an isokinetic study. *J Am Geriatr Soc* 35: 13-20, 1987.
940. **White Z, Terrill J, White RB, McMahon C, Sheard P, Grounds MD, and Shavlakadze T.** Voluntary resistance wheel exercise from mid-life prevents sarcopenia and increases markers of mitochondrial function and autophagy in muscles of old male and female C57BL/6J mice. *Skelet Muscle* 6: 45, 2016.
941. **White Z, White RB, McMahon C, Grounds MD, and Shavlakadze T.** High mTORC1 signaling is maintained, while protein degradation pathways are perturbed in old murine skeletal muscles in the fasted state. *Int J Biochem Cell Biol* 78: 10-21, 2016.
942. **Whitehouse AS, Smith HJ, Drake JL, and Tisdale MJ.** Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. *Cancer Res* 61: 3604-3609, 2001.
943. **Whitman SA, Wacker MJ, Richmond SR, and Godard MP.** Contributions of the ubiquitin-proteasome pathway and apoptosis to human skeletal muscle wasting with age. *Pflugers Arch* 450: 437-446, 2005.
944. **Wigston DJ.** Remodeling of neuromuscular junctions in adult mouse soleus. *J Neurosci* 9: 639-647, 1989.
945. **Wigston DJ.** Repeated in vivo visualization of neuromuscular junctions in adult mouse lateral gastrocnemius. *J Neurosci* 10: 1753-1761, 1990.
946. **Viguie CA, Lu DX, Huang SK, Rengen H, and Carlson BM.** Quantitative study of the effects of long-term denervation on the extensor digitorum longus muscle of the rat. *Anat Rec* 248: 346-354, 1997.
947. **Willadt S, Nash M, and Slater CR.** Age-related fragmentation of the motor endplate is not associated with impaired neuromuscular transmission in the mouse diaphragm. *Scientific reports* 6: 24849, 2016.
948. **Williams JL, Cartland D, Rudge JS, and Egginton S.** VEGF trap abolishes shear stress- and overload-dependent angiogenesis in skeletal muscle. *Microcirculation* 13: 499-509, 2006.
949. **Williamson JR, Hoffmann PL, Kohrt WM, Spina RJ, Coggan AR, and Holloszy O.** Endurance exercise training decreases capillary basement membrane width in older nondiabetic and diabetic adults. *Journal of applied physiology* 80: 747-753, 1996.
950. **Winbanks CE, Chen JL, Qian H, Liu Y, Bernardo BC, Beyer C, Watt KI, Thomson RE, Connor T, Turner BJ, McMullen JR, Larsson L, McGee SL, Harrison CA, and Gregorevic P.** The bone morphogenetic protein axis is a positive regulator of skeletal muscle mass. *J Cell Biol* 203: 345-357, 2013.
951. **Vincent JL.** Critical care--where have we been and where are we going? *Crit Care* 17 Suppl 1: S2, 2013.
952. **Viner RI, Ferrington DA, Huhmer AF, Bigelow DJ, and Schoneich C.** Accumulation of nitrotyrosine on the SERCA2a isoform of SR Ca-ATPase of rat skeletal muscle during aging: a peroxynitrite-mediated process? *FEBS Lett* 379: 286-290., 1996.
953. **Wink DA, Nims RW, Darbyshire JF, Christodoulou D, Hanbauer I, Cox GW, Laval F, Laval J, Cook JA, Krishna MC, and et al.** Reaction kinetics for nitrosation of cysteine and glutathione in aerobic nitric oxide solutions at neutral pH. Insights into the fate and physiological effects of intermediates generated in the NO/O<sub>2</sub> reaction. *Chem Res Toxicol* 7: 519-525., 1994.
954. **Viscomi C, Bottani E, Civiletto G, Cerutti R, Moggio M, Fagiolari G, Schon EA, Lamperti C, and Zeviani M.** In vivo correction of COX deficiency by activation of the AMPK/PGC-1alpha axis. *Cell Metab* 14: 80-90, 2011.

955. **Viscomi C, Bottani E, and Zeviani M.** Emerging concepts in the therapy of mitochondrial disease. *Biochim Biophys Acta* 1847: 544-557, 2015.
956. **Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, Nevitt M, and Harris TB.** Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci* 57: M326-332, 2002.
957. **Woeber KA.** Thyrotoxicosis and the heart. *N Engl J Med* 327: 94-98, 1992.
958. **Wohlgemuth SE, Seo AY, Marzetti E, Lees HA, and Leeuwenburgh C.** Skeletal muscle autophagy and apoptosis during aging: effects of calorie restriction and life-long exercise. *Exp Gerontol* 45: 138-148, 2010.
959. **Wokke JHJ, Jennekens FGI, van den Oord CJM, Veldman H, Smit LME, and Leppink GJ.** Morphological changes in the human end plate with age. *J Neurol Sci* 95: 291-310, 1990.
960. **von Maltzahn J, Chang NC, Bentzinger CF, and Rudnicki MA.** Wnt signaling in myogenesis. *Trends Cell Biol* 22: 602-609, 2012.
961. **von Maltzahn J, Jones AE, Parks RJ, and Rudnicki MA.** Pax7 is critical for the normal function of satellite cells in adult skeletal muscle. *Proc Natl Acad Sci U S A* 110: 16474-16479, 2013.
962. **Wood RD, Mitchell M, and Lindahl T.** Human DNA repair genes, 2005. *Mutat Res* 577: 275-283, 2005.
963. **Woods D, Onambele G, Woledge R, Skelton D, Bruce S, Humphries SE, and Montgomery H.** Angiotensin-I converting enzyme genotype-dependent benefit from hormone replacement therapy in isometric muscle strength and bone mineral density. *J Clin Endocrinol Metab* 86: 2200-2204, 2001.
964. **Wright EA, Spink, J.M.** A study of the loss of nerve cells in the central nervous system in relation to age. *Gerontologia* 3: 277-287, 1959.
965. **Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, and Spiegelman BM.** Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115-124, 1999.
966. **Wu Z, Puigserver P, and Spiegelman BM.** Transcriptional activation of adipogenesis. *Current opinion in cell biology* 11: 689-694, 1999.
967. **Wullems JA, Verschueren SM, Degens H, Morse CI, and Onambele GL.** A review of the assessment and prevalence of sedentarism in older adults, its physiology/health impact and non-exercise mobility counter-measures. *Biogerontology* 17: 547-565, 2016.
968. **Wust RC, and Degens H.** Factors contributing to muscle wasting and dysfunction in COPD patients. *International journal of chronic obstructive pulmonary disease* 2: 289-300, 2007.
969. **Wust RC, Gibbings SL, and Degens H.** Fiber capillary supply related to fiber size and oxidative capacity in human and rat skeletal muscle. *Adv Exp Med Biol* 645: 75-80, 2009.
970. **Wust RC, Jaspers RT, van Heijst AF, Hopman MT, Hoofd LJ, van der Laarse WJ, and Degens H.** Region-specific adaptations in determinants of rat skeletal muscle oxygenation to chronic hypoxia. *Am J Physiol Heart Circ Physiol* 297: H364-374, 2009.
971. **Wust RC, Morse CI, de Haan A, Jones DA, and Degens H.** Sex differences in contractile properties and fatigue resistance of human skeletal muscle. *Exp Physiol* 93: 843-850, 2008.
972. **Xie LX, Ozeir M, Tang JY, Chen JY, Jaquinod SK, Fontecave M, Clarke CF, and Pierrel F.** Overexpression of the Coq8 kinase in *Saccharomyces cerevisiae* coq null mutants allows for accumulation of diagnostic intermediates of the coenzyme Q6 biosynthetic pathway. *J Biol Chem* 287: 23571-23581, 2012.
973. **Xu M, Palmer AK, Ding H, Weivoda MM, Pirtskhalava T, White TA, Sepe A, Johnson KO, Stout MB, Giorgadze N, Jensen MD, LeBrasseur NK, Tchkonja T, and Kirkland JL.** Targeting senescent cells enhances adipogenesis and metabolic function in old age. *eLife* 4: e12997, 2015.
974. **Xu M, Tchkonja T, Ding H, Ogronnik M, Lubbers ER, Pirtskhalava T, White TA, Johnson KO, Stout MB, Mezera V, Giorgadze N, Jensen MD, LeBrasseur NK, and Kirkland JL.** JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc Natl Acad Sci U S A* 112: E6301-6310, 2015.



975. **Yamazaki T.** Effects of intermittent weight-bearing and clenbuterol on disuse atrophy of rat hindlimb muscles. *J Jpn Phys Ther Assoc* 8: 9-20, 2005.
976. **Yang L, Licastro D, Cava E, Veronese N, Spelta F, Rizza W, Bertozzi B, Villareal DT, Hotamisligil GS, Holloszy JO, and Fontana L.** Long-Term Calorie Restriction Enhances Cellular Quality-Control Processes in Human Skeletal Muscle. *Cell Rep* 14: 422-428, 2016.
977. **Yang L, Licastro D, Cava E, Veronese N, Spelta F, Rizza W, Bertozzi B, Villareal DT, Hotamisligil GS, Holloszy JO, and Fontana L.** Long-Term Calorie Restriction Enhances Cellular Quality-Control Processes in Human Skeletal Muscle. *Cell Rep* 14: 422-428, 2016.
978. **Yao YM.** Maintenance of axon terminals at synaptic sites in the absence of muscle fibers. In: *Current Issues in Neural Regeneration Research*, edited by Reier PJ, Bunge RP, and Seil FJ. New York: Alan R. Liss, Inc., 1988, p. 167-178.
979. **Yarasheski KE, Zachwieja JJ, and Bier DM.** Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women [published erratum appears in *Am J Physiol* 1993 Oct;265(4 Pt 1):following table of contents]. *Am J Physiol* 265: E210-214, 1993.
980. **Yarasheski KE, Zachwieja JJ, Campbell JA, and Bier DM.** Effect of growth hormone and resistance exercise on muscle growth and strength in older men. *Am J Physiol* 268: E268-276, 1995.
981. **Yin H, Price F, and Rudnicki MA.** Satellite cells and the muscle stem cell niche. *Physiol Rev* 93: 23-67, 2013.
982. **Young A, Stokes M, and Crowe M.** Size and strength of the quadriceps muscles of old and young women. *Eur J Clin Invest* 14: 282-287., 1984.
983. **Yu F, Degens H, and Larsson L.** The influence of thyroid hormone on myosin isoform composition and shortening velocity of single skeletal muscle fibres with special reference to ageing and gender. *Acta Physiol Scand* 167: 313-316, 1999.
984. **Yu F, Degens H, Li X, and Larsson L.** Gender- and age-related differences in the regulatory influence of thyroid hormone on the contractility and myosin composition of single rat soleus muscle fibres. *Pflugers Arch* 437: 21-30, 1998.
985. **Yu F, Hedstrom M, Cristea A, Dalen N, and Larsson L.** Effects of ageing and gender on contractile properties in human skeletal muscle and single fibres. *Acta Physiol (Oxf)* 190: 229-241, 2007.
986. **Yuan Y, Cruzat VF, Newsholme P, Cheng J, Chen Y, and Lu Y.** Regulation of SIRT1 in aging: Roles in mitochondrial function and biogenesis. *Mech Ageing Dev* 155: 10-21, 2016.
987. **Zaglia T, Milan G, Ruhs A, Franzoso M, Bertaggia E, Pianca N, Carpi A, Carullo P, Pesce P, Sacerdoti D, Sarais C, Catalucci D, Kruger M, Mongillo M, and Sandri M.** Atrogin-1 deficiency promotes cardiomyopathy and premature death via impaired autophagy. *J Clin Invest* 124: 2410-2424, 2014.
988. **Zane AC, Reiter DA, Shardell M, Cameron D, Simonsick EM, Fishbein KW, Studenski SA, Spencer RG, and Ferrucci L.** Muscle strength mediates the relationship between mitochondrial energetics and walking performance. *Aging Cell* 16: 461-468, 2017.
989. **Zechner C, Lai L, Zechner JF, Geng T, Yan Z, Rumsey JW, Collija D, Chen Z, Wozniak DF, Leone TC, and Kelly DP.** Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. *Cell Metab* 12: 633-642, 2010.
990. **Zhang C, Linnane AW, and Nagley P.** Occurrence of a particular base substitution (3243 A to G) in mitochondrial DNA of tissues of ageing humans. *Biochem Biophys Res Commun* 195: 1104-1110, 1993.
991. **Zhong S, Lowe DA, and Thompson LV.** Effects of hindlimb unweighting and aging on rat semimembranosus muscle and myosin. *J Appl Physiol* 101: 873-880, 2006.
992. **Zhu Y, Armstrong JL, Tchkonja T, and Kirkland JL.** Cellular senescence and the senescent secretory phenotype in age-related chronic diseases. *Curr Opin Clin Nutr Metab Care* 17: 324-328, 2014.
993. **Zhu Y, Dornebal EJ, Pirtskhalava T, Giorgadze N, Wentworth M, Fuhrmann-Stroissnigg H, Niedernhofer LJ, Robbins PD, Tchkonja T, and Kirkland JL.** New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. *Aging* 9: 955-963, 2017.

994. **Zhu Y, Tchkonina T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, Pirtskhalava T, Giorgadze N, Johnson KO, Giles CB, Wren JD, Niedernhofer LJ, Robbins PD, and Kirkland JL.** Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell* 15: 428-435, 2016.
995. **Zhu Y, Tchkonina T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ikeno Y, Hubbard GB, Lenburg M, O'Hara SP, LaRusso NF, Miller JD, Roos CM, Verzosa GC, LeBrasseur NK, Wren JD, Farr JN, Khosla S, Stout MB, McGowan SJ, Fuhrmann-Stroissnigg H, Gurkar AU, Zhao J, Colangelo D, Dorronsoro A, Ling YY, Barghouthy AS, Navarro DC, Sano T, Robbins PD, Niedernhofer LJ, and Kirkland JL.** The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 14: 644-658, 2015.
996. **Zhuo L, Sun B, Zhang C-L, Fine A, Chiu S-Y, and Messing A.** Live astrocytes visualized by green fluorescent protein in transgenic mice. *Dev Biol* 187: 36-42, 1997.
997. **Zilberberg MD, and Shorr AF.** Prolonged acute mechanical ventilation and hospital bed utilization in 2020 in the United States: implications for budgets, plant and personnel planning. *BMC health services research* 8: 242, 2008.
998. **Zuo Y, Lubischer JL, Kang H, Tian L, Mikesh M, Marks A, Scofield VL, Maika S, Newman C, Krieg P, and Thompson WJ.** Fluorescent proteins expressed in mouse transgenic lines mark subsets of glia, neurons, macrophages, and dendritic cells for vital examination. *J Neurosci* 24: 10999-11009, 2004.
999. **Zurcher C, Slagboom, P.** *Basic aspects of aging*. Washington, D.C.: ILSI Press, 1994.
1000. **Zurcher C, van Zwieten, M.J., Solleveld, H.A., Hollander, C.F.** Aging research. In: *The mouse in biomedical research*, edited by Foster HLS, J.D., Fox, J.G. New York: Academic Press, 1982, p. 11-25.

**Table 1:** Aging-related changes in muscle capillarisation

Species	Muscle	Age	C:F	CD	FCSA	Reference
Mouse (C57BL/1crfa) ♂	Sol EDL	6 vs 28 months	↑	↑		(193)
Mouse (C57BL/6) ♂	Gastrocnemius	3 vs 24 months	=	ND	↓	(274)
Rat Wistar ♀	Plantaris	5 vs 13 vs 25 months	↓ (5=13 > 25)	↓ (5>13>25)	↓ (IIb only 5<13>25)	(215, 216)
Rat Wistar ♀	Soleus	5 vs 25 months	=	=	=	(208)
Rat F344 ♀	Gastrocnemius	6 vs 24 months	=	ND	ND	(773)
Rat Wistar ♂	Soleus Plantaris	3 vs 19 months	↑ ↑	= =	= =	(448)
Rat F344 ♂	Flexor digitorum longus	6-8 vs 26-28 months	=	=	=	(911)
Rat Long Evans ♀	Sol EDL	9 vs 27 months	=	ND	=	(112)
Rat F344FBN (sex not given)	G <sub>w</sub> G <sub>R</sub> Sol	8 vs 28-30 months	= = =	↑ ↓ =	↓ ↑ ↑	(389)
Rat F344FBN ♂	Sol	12 & 24 vs 35 months	=	↑	↓	(619)

	EDL			↑	↓	
Beagle	Gastrocnemius, Semitendinosus, Triceps	2-3 vs 10-13 years	↓	↓	FCSA type II ↓ FCSA type II = FCSA type II ↓	(363)
Human ♂ & ♀	Gastrocnemius	24 vs 64 years	↓	↓	FCSA type II ↓	(164)
Human ♂	Gastrocnemius	26 vs 66 years	=	=	=	(156)
Human ♂	VL	21-30 vs 51-62 years	↓	=	↓	(727)
Human ♂	VL	22 vs 62 years	↑	=	↑	(221)
Human ♂	VL	24 vs 64 years	↓	=	↓	(322)
Human ♂	VL	21 vs 65 years	↓	=	=	(784)
Human ♂	VL	24 vs 70 years	↓	↓	↓	(356)
Human ♂	VL	21 vs 73 years	↓	=	↓	(693)
Human ♂	VL (longitudinal)	65 vs 77 years	↓	ND	=	(306)
Human ♀	VL	22 vs 70 years	↓	=	FCSA type II ↓	(184)
Human ♂	VL	26 vs 72	↓	↓	FCSA type II ↓	(933)

## FIGURE LEGENDS

**Figure 1. Spatial organization of motor unit fibers in young animals.** Camera lucida tracings of glycogen-depleted cross-sections of fast-twitch motor unit fibres from young animals, classified according to their MyHC composition, in 21 cross-sections of tibialis anterior muscle (each section is from a separate rat). The superficial part of the muscle is facing the top of the figure. The type IIa, type IIx and IIb MyHC units are identified by filled circles, open circles, and filled triangles, respectively. The horizontal bar represents 1 mm (the graph is modified from Larsson et al. 1991 (521, 524)).

**Figure 2. Spatial organization of motor unit fibers in old animals.** Camera lucida tracings of glycogen-depleted cross-sections of fast-twitch motor unit fibres from old animals, classified according to their MyHC composition, in 16 cross-sections of tibialis anterior muscle (each section is from a separate rat). The superficial part of the muscle is facing the top of the figure. Motor units including type IIa, type IIx and IIb MyHC muscle fibers are identified by filled circles, open circles, and filled triangles, respectively. Motor units containing more than one type of muscle fiber are identified by two filled circles. The horizontal bar represents 1 mm (the graph is modified from (521, 524)).

**Figure 3. Myelinated neurones in peripheral nerves and ventral roots from young and old rats.** Toluidine semithin transverse sections of the soleus motor nerve from a young (a, 5 months) and an old (b, 24 months) rat and from the L5 ventral root from a young (c, 4 months) and an old (d, 22 months) rat. In d, note myelin ovoids (black arrows), myelin reduplication (unfilled arrow), splitting of myelin sheets (asterisks) thinly myelinated fibers and clusters of regenerating fibers. Calibration bars 1  $\mu$ m (a,b) and 20  $\mu$ m (c,d). redrawn from(24)

**Figure 4. Repeated, vital images of a mouse sternomastoid muscle NMJ illustrate the utility of vital imaging and the stability of the synapse.** Transgenic expression of cyan and green fluorescent proteins (CFP and GFP) labels motor axons and Schwann cells, respectively. The AChRs were labeled with a nonblocking concentration of rhodamine-conjugated  $\alpha$ -BTX (red fluorescence). The axon enters the synaptic site from the left and arborizes above pretzel-shaped AChRs on the surface of the target muscle fiber. Several terminal Schwann cell somata (round, brighter staining objects) are present above the NMJ that extend processes to cover the nerve terminal. No discernable changes are observed in the images taken after a 30-day interval (lower row compared with upper row). Image courtesy of Hyuno Kang and adapted from (445).

**Figure 5. Abrupt morphological changes occur in a small fraction of NMJs over any given interval in aging mice.** Repeated vital examination of NMJs in the sternomastoid muscle of aging mice reveals most junctions undergo no changes. However, abrupt morphological changes occur at a small fraction of junctions between each imaging session. NMJs that show such changes are found to have lost most of the AChRs that had been labeled with  $\alpha$ -BTX in the prior imaging session. Newly synthesized AChRs then appear that are revealed by the reapplication of  $\alpha$ -BTX, "new AChR". The new AChRs are, however, found in altered locations demonstrating a change in NMJ morphology. (A) Within a period of two weeks, a young-appearing junction – with contiguous AChR-rich gutters and matching terminal motor axon branches, a "pretzel" – loses its pre-existing complement of AChRs and comes to have new AChRs whose fragmented appearance becomes more apparent as the fiber grew and

the endplate expanded in size ("new AChR"). Concurrently, the presynaptic nerve terminal becomes varicose. (B) Fragmented, old appearing junctions can also undergo further remodeling – a loss of the existing AChRs, insertion of significant amounts of new receptors – and additional fragmentation of the AChR-rich area occurs in this case. Scale bar = 20 $\mu$ m. Images courtesy of Yue Li and adapted from (564).

**Figure 6.** NMJs on the regenerated muscle fibers undergo remodeling. Repeated vital imaging of AChRs (red), SCs (green) and nerve terminal (blue) immediately following fiber ablation of a muscle fiber (Day 0) using a laser microbeam and after 12 days of recovery. Immediately following the injury to the muscle fiber, the AChR-rich aggregate has a "pretzel"-like appearance apposed by matching terminal branches of a motor axon. Upon regeneration of the ablated muscle fiber (Day 12) and earlier (not shown), the AChR aggregate becomes fragmented and the nerve terminal branches become varicose. This altered morphology is maintained for the remainder of the animal's life. Scale bar = 10 $\mu$ m. Images courtesy of Yue Li and adapted (565).

**Figure 7.** "Fragmentation" of postsynaptic AChR aggregates occurs in aging and in a variety of cases of damage to sternomastoid muscle fibers. AChRs at normal mature, mouse NMJs occur in mostly contiguous "gutters" that resemble "pretzels". In contrast, a large proportion of postsynaptic AChR aggregates in an aged (22M) muscle display a fragmented morphology. Similar fragmentation of postsynaptic AChR aggregates is observed in muscles of dystrophic (mdx) mice (in this case 1M old), in wild-type 1M old muscle 7 days after cardiotoxin-induced damage, and in a young muscle where a single muscle fiber was ablated with a laser microbeam applied on each side of the NMJ. Scale bar = 10  $\mu$ m. Images courtesy of Robert Louis Hastings (CTX) and Yue Li (laser ablation).

**Figure 8.** Fragmentation of AChRs at NMJs occurs after muscle fiber damage, fiber degeneration and regeneration. NMJs in a 6 mo. old mouse sternomastoid muscle. More central, large image is a maximum projection of a confocal stack. A "young" NMJ (left bottom) and an "old" fragmented junction (top right) are both present. The fragmented NMJ is associated with a string of nuclei located centrally within the muscle fiber (see in YZ virtual section to the right and compare with the outline of the fiber). These central nuclei are hallmarks of muscle fiber segments that have undergone damage/degeneration and have subsequently regenerated from myoblasts created by satellite cells. The myonuclei associated with the young NMJs are peripherally located (seen in XZ virtual section). The vertical and horizontal yellow dotted lines indicate the location of YZ and XZ virtual sections, respectively. Scale bar = 10 $\mu$ m.

**Figure 9.** Myonuclear domain (MND) size in muscle fibers from mammalian species with a body mass ranging from 25g to 2500 kg Myonuclear domain size is measured in muscle fibers expressing the type I, IIa, IIax, IIx, IIxb and IIb MyHC isoforms. The MND size in muscle fibers expressing the type ( $\beta$ /slow) MyHC isoform is shown in the inset. Values are means + SD (from (577)).

**Figure 10.** Modelling of fiber and myonuclear domains. I. The different steps of modelling the fiber with a parametric general elliptical cylinder (GEC). (A) The needed model parameters are the center point  $c$ , the lengths of the major, and minor axes  $a$ , and  $b$ , and the angle  $\theta$  between the major axis, and the  $x$ -axis. Here they schematically overlaid on an original

image slice. (B) The result of the fuzzy c-means (FCM) clustering of the pixels, where bright pixels denote likely fiber pixels, and dark pixels denote likely background pixels. (C) A slice of the weight volume G, that is created based on the parameters extracted from the FCM thresholded image slice. (D) A slice of the GEC model of the fiber surface (red), overlaid on the original image slice. The bar length denotes 50  $\mu\text{m}$ . II. (A) A GEC model of a fiber segment. The centroids of the myonuclei are shown as blue spheres merged with the surface rendering. (B) The myonuclear domains extracted using the GEC, and the nuclei centroids. The representation of each myonuclear domain at the muscle cell surface is indicated by specific colors (from (180)).

**Figure 11.** Confocal microscopy images of myonuclei in individual muscle fibres. I. (A) Ordered myonuclei organisation in a type IIa fibre from a young man. (B) Increased variability in myonuclei distances in a type IIa fibre from an old woman. (C) Myonuclei aggregation in a muscle fibre from an old subject (detail from B). (D) Variation in the spatial organisation of nuclei in the lower (D) and upper (F) part of a single muscle fibre expressing the type I MyHC isoform from a young man. (F) Deep groove like structures on the fibre surface harbouring myonuclei from an old subject. Rhodamine phalloidin (red) was used to stain for actin and DAPI (blue) to stain for the DNA (nuclei). All pictures were captured using a Plan-Neofluar 20x/0.5 objective. The horizontal bar denotes 100  $\mu\text{m}$ . II. Myonuclear domain size in type I (A) and type IIa (B) fibers from young men (YM), young women (YW), old men (OM) and old women (OW) (from (180))

**Figure 12.** Increased variability in myonuclear organization in old age. I. Confocal images at the bottom of single muscle fiber segments from a young (A) and an old subject. Nuclei are DAPI labeled (a), the centroids of the nuclei are shown as spheres (b), the basal lamina protein laminin is labeled in yellow (c), and double labeling of nuclei centroids and laminin (d). The horizontal bars denote 50  $\mu\text{m}$ . II. Box plots showing the standard deviation (SD) of the nearest neighbor (NN) distances between myonuclei from individual muscle fibers expressing the type I (A) and type IIa (B) MyHC isoforms in young men (YM), young women (YW), old men (OM) and old women (OW). The boxes represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the median value is indicated in the box. The horizontal bars denote the 10<sup>th</sup> and 90<sup>th</sup> percentiles, and data outside this range are represented as dots (from (180)).

**Figure 13.** The postulated vicious cycle driven by ROS. The classical model of mitochondrial damage by ROS postulates that ROS leaking from the RC cause mutations in mtDNA, which in turn determine the synthesis of dysfunctional RC protein subunits, thus increasing ROS production in a vicious circle.

**Figure 14.** The central role of CoQ in mitochondria homeostasis. CoQ is essential for energy metabolism (at the level of the RC and of the Krebs cycle) lipid and pyrimidine metabolism, controls thermogenesis and apoptosis, and is a major antioxidant.

**Figure 15.** Cellular and biological process controlled by mitochondrial dynamic. Mitochondrial fusion is mediated by mitofusin1/2 (MFN1/2) and OPA1 to produce an elongated mitochondrial network. Elongated mitochondria optimize energy production, prevent apoptosis and reduce mitophagy. Mitochondrial fission is controlled by DRP1, Mff and Fis1 to generate a fragmented mitochondrial network. Mitochondrial fission promotes mitophagy to remove the dysfunctional mitochondria but also induces ROS production that cause Endoplasmic Reticulum (ER) stress and activation of Unfolded Protein Response

(UPR). UPR induces the ATF4-dependent upregulation of FGF21 that is secreted by the muscle and causes a premature senescence of epithelial tissues.

**Figure 16.** The relationship between muscle capillary supply area and aerobic capacity.

Frozen sections of human vastus lateralis muscle biopsies stained with (A) lectin (*Ulex europaeus*) to localise capillaries and (B) succinate dehydrogenase as an index of mitochondrial activity. (C) Capillary domains represent the area of tissue closer to a given capillary (red dots) than neighbouring capillaries. (Ci, Cii) Overlap of domains with fast and slow fibres, respectively. (Ciii) Magnified region to illustrate overlap of domains (blue outlines) and slow fibres (pink outlines). I: type I fibres; II: type II fibres; arrows indicate corresponding capillary locations in each panel; asterisks identify the same fibre in each panel. Scale bars: 100  $\mu\text{m}$  (from (91)).

**Figure 17.** Effects of aging on fiber size and capillary supply. **A)** Fiber cross-sectional area (FCSA) and **B)** local capillary to fiber ratio (number of capillaries supplying a fiber) in normal (blue line) and hypertrophied (red line) plantaris muscles from female Wistar rats of different ages. This picture illustrates 1) maturational increases in FCSA and LCFR between the age of 5 and 13 months, 2) comparing 5- and 25-month-old rats would have shown no age effect on FCSA and LCFR, 3) and that at all these ages there is a maintained hypertrophy and angiogenic response (215, 217).

**Figure 18.** Depicted principal pathways studied in sarcopenia. The red arrows underline which and how the different factors have been shown to change in sarcopenia.

**Figure 19.** Effects of physical exercise on physiological parameters. **A)** Illustration of the impact of aging and training on a physiological parameter (e.g. muscle mass). Note the linear aging-related decrease in the parameter at a rate of 1% per year of the value at 30 years (this is similar to the often-reported annual 1% loss of muscle mass). The vertical arrows indicate a 30% improvement in e.g. aerobic function, muscle mass or muscle strength in response to a training program, where in absolute terms the improvement that can be gained decreases with age, also causing a decreasing 'rejuvenation' with increasing age, as illustrated by the horizontal arrows. This thus shows that 1) even when the relative gains do not change with age the 2) absolute gains and 3) attainable rejuvenation decrease with age. **B)** The data in panel **A** are here expressed as a percentage decline in the physiological parameter from the value it had in the year before. This exponential pattern can be explained by a random accumulation of damage in remaining tissue (from (200)).