Excitation-Contraction Uncoupling and Sarcopenia

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Abstract

The molecular and cellular mechanisms underlying sarcopenia, the age-related decline in muscle mass and strength, are not fully understood. It has been proposed that a reduced level of calcium ion-supply to contractile muscle proteins via excitation-contraction uncoupling leads to the muscle weakness associated with aging. The basic process of excitation-contraction uncoupling is a result of a larger number of ryanodine receptors being uncoupled to the voltage-sensing dihydropyridine receptor units. Other mechanisms of sarcopenia include reduced protein synthesis, mitochondrial dysfunction, inadequate nutrition, reduction in hormone levels and neuromuscular changes. In animal models of sarcopenia, a drastic decline in the α_{1S}-subunit of the dihydropyridine receptor was observed. However, immunoblot analysis of vastus lateralis specimens from male humans aged 18 to 82 years of age showed no major changes in the relative abundance of this transverse-tubular receptor. In addition, the oligomeric status of the α_{1S}-dihydropyridine receptor was unaltered in aged human fibres. It would appear therefore that dihydropyridine receptor expression is preserved during the aging process of human skeletal muscle fibres. Thus, the assumption that abnormal triadic signal transduction is responsible for sarcopenia can not be transferred from animal models to senescent human muscle without modifications.

Key words: aging, caloric restriction, calsequestrin, dihydropyridine receptor, excitation-contraction uncoupling, insulin-like growth factor-1, ryanodine receptor, sarcopenia.

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Cellular aging is a fundamental biological process [74, 75] and its role in the functional decline of skeletal muscle fibres is poorly understood. This review will focus on the potential relationship between excitation-contraction uncoupling and sarcopenia, a hypothesis originally developed by Delbono and co-workers [106]. We have recently analysed the expression of signal transduction elements involved in calcium homeostasis in animal models of aging [110] and aged human muscle fibres [111] and will discuss the findings with respect to excitation-contraction uncoupling. Besides abnormal triadic signal transduction playing a role in muscle weakness, it has also been postulated that neuronal alterations, hormonal factors, oxidative stress, free radical production and post-translational modification of proteins are responsible for sarcopenia [100]. Moreover, alterations in the capacity to maintain normal cellular homeostasis have been suggested to underlie the reduced cellular function characteristic of the aging process, and to predispose the senescent organism to a host of pathologies such as cancer, heart disease, and a range of muscle and neuro-degenerative disorders [74].

A vast body of literature exists on the mechanisms of excitation-contraction coupling in skeletal muscle [92]. However, excitation-contraction uncoupling has only recently been proposed as a contributing factor to the process of sarcopenia [31-33, 106, 110]. The molecular basis for excitation-contraction uncoupling in muscles from aging mammals is a reduction in the number of α_{1S}-dihydropyridine receptors at the transverse tubules leading to an increase in the percentage of ryanodine receptor calcium release channel units of the junctional sarcoplasmic reticulum being uncoupled to voltage-sensing receptor subunits [31]. Renganathan et al. [106] demonstrated that the main functional consequence of this alteration is a failure in the transduction of sarcolemmal depolarisation into a proper calcium signal and consequently a weakened mechanical response resulting in the loss of muscle strength in the elderly. Interestingly, modulation of excitation-contraction coupling by insulin growth factor-1 (IGF-1) in skeletal

muscle increases the number of transverse-tubular receptors and thereby appears to prevent the age-related decline in the number of α_{1S}-dihydropyridine receptors [33, 107]. The functional significance of these alterations is that IGF-1 prevents the age-related decline in muscle force. In agreement with this finding is a recent study by Zheng et al. [137]. These researchers have demonstrated that IGF-1 regulates the transcription of the α_{1S}-dihydropyridine receptor in skeletal muscle cells by acting on the cAMP-response element-binding protein (CREB) of the promoter [137].

Sarcopenia

The age-related loss of muscle strength and endurance has been termed sarcopenia. The pathophysiology of sarcopenia is characterised by a significant reduction in overall skeletal muscle mass, a decrease in fibre strength and increased fatigability [37, 47]. Sarcopenia occurs in all individuals to some degree as a consequence of aging, but it can be accelerated by a variety of factors including inactivity, poor nutrition and chronic illness. Based on various studies it is estimated that on average five percent of skeletal muscle mass is lost per decade from the fourth decade on [44, 125]. It is estimated that 20-30 billion dollars of health care costs in the United States are spent on problems related to sarcopenia [112].

The molecular and cellular mechanisms that underlie sarcopenia are only beginning to be elucidated. However, it is believed that a combination of neurogenic and myogenic factors determine the pathophysiological pathway leading to sarcopenia [15, 30, 35, 40, 81, 82, 109, 120, 128]. Figure 1 summarises some of these biological factors involved in the age-related loss of muscle mass and strength. This includes decreased protein synthesis of myofibrillar components [4], disturbed ion homeostasis [31], an altered equilibrium of growth factors and hormones [43], progressive denervation [34], decreased capillarisation [30], loss of motor units [14], a shift to slower fibre types [87], mitochondrial alterations [15], abnormal metabolism and a progressive decline in energy intake [128], as well as oxidative stress [100] and increased apoptosis [35]. Based on our current knowledge of the numerous factors involved in the aging process, the single most important cause of sarcopenia appears to be the loss of motor neuron input to skeletal muscle fibres that occurs with age [14,34]. Since innervation is crucial to the maintenance of muscle mass as well as strength, it is likely that denervation represents a central factor in sarcopenia. Myogenic factors refer to contraction-induced injury, selective primary muscle fibre atrophy, and alterations in muscle signal transduction. Contraction-induced injury has been related to increased mechanical frailty and decline in muscle restorative capacity with age [12].

Primary atrophy of type II fibres without alterations in fibre-type distribution has been reported in aging muscle, however, it has been difficult to determine whether these changes result from aging or disuse [79, 85, 91]. Lexell et al. [85] have shown that there is an age-related loss in fibre numbers in a comprehensive study of the entire vastus lateralis muscle in 43 male cadavers aged 15-83. Between the ages of 20 and 80, there is an approximately fifty percent reduction in the total fibre number and this loss is more rapid after the age of fifty [85]. Based on measurements of cross-sectional area, there is a selective loss of fast-twitch type II fibres as compared to slow-twitch type I fibres. The shift to a relatively higher proportion of type I fibres with aging occurs independent of rigorous endurance exercise as demonstrated in a 20-year longitudinal study of distance runners [124].

Our animal studies on aging skeletal muscle showed a similar shift to slower fibre type characteristics as indicated by an age-related increase in the slow calsequestrin isoform named CSQ_s [110]. Extending these studies to human skeletal muscle, we used the fast CSQ_f isoform of the Ca^{2+}-binding element calsequestrin and the myosin heavy chain isoforms MHC_f and MHC_s as markers of potential fast-to-slow transitions [111]. The fast CSQ_f isoform and high-molecular-mass calsequestrin-like-proteins (CLPs) were relatively comparable in their relative density in microsomal preparations from aging human skeletal musle fibres. Unfortunately, antibodies to the slow/cardiac CSQ_s isoform did not cross-react with human muscle. We could therefore not determine a potential increase in a slow isoform of the ion-regulatory apparatus, as had

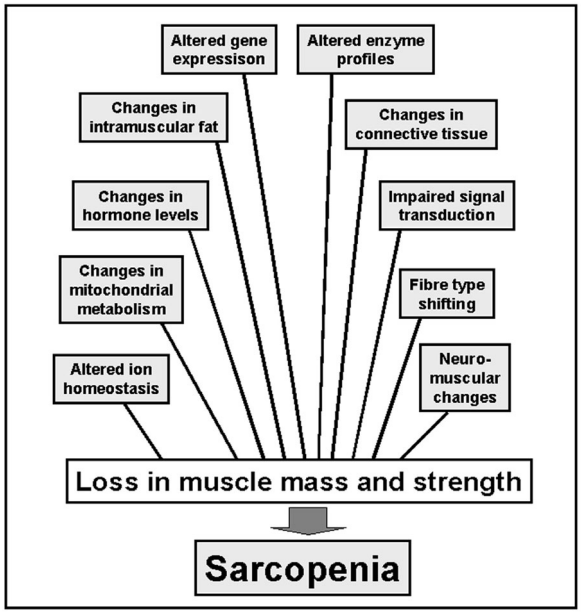

Figure 1. Overview of molecular and cellular factors involved in the degenerative processes leading to sarcopenia, the age-related decline in skeletal muscle mass and strength.

been shown for aged rabbit muscle. Immunoblotting of the MHC isoforms did not indicate a major shift to slower fibre types in senescent fibres [111].

Since there is no conclusive evidence of accelerated muscle protein breakdown in healthy older people [99], aging is probably associated with a reduced capacity of skeletal muscle to synthesize new proteins. This imbalance ultimately leads to reduced muscle mass and protein content. Preliminary studies have revealed that the synthesis rate of myosin heavy chain isoforms is significantly reduced in middle-aged and older men and women [4]. However, the decline in protein synthesis is not uniform for all proteins; there is a greater decline in mitochondrial protein synthesis and MHC synthesis than in sarcoplasmic protein synthesis, which actually increases with age [4, 115].

General mechanisms that may affect skeletal muscle directly or indirectly are oxidative DNA damage and mitochondrial DNA (mtDNA) mutations [100]. Superoxide radical and hydrogen peroxide that are continuously produced in aerobic cells undergo metal ion-catalysed conversion into hydroxyl radicals [25, 50]. These hydroxyl radicals cause oxidative damage, which in turn may be related to the development of mutations in mtDNA. The accumulation of damage to mtDNA by oxidation may be the basis for defects in the oxidative phosphorylation capacity with age. However, the mitochondrial theory of aging has been subject to controversy in recent times. The hypothesis that the aging process is associated with mitochondrial dysfunction and oxidative stress has been investigated in human skeletal muscle; muscle biopsies were taken from old and young male subjects, old subjects showed reduced maximal respiration and creatine-stimulated respiration [123]. However, respiration in isolated mitochondria was similar in young and old subjects. Generation of reactive oxygen species by isolated mitochondria and measures of oxidative defence were not significantly altered with age. Thus the hypothesis of increased oxidative stress in aged muscle could not be confirmed [123].

Aging is associated with changes in several trophic factors [135]. There is an age-associated decrease in total and free serum testosterone levels in healthy men [122]. In addition, growth hormone (GH) levels decline with age [134]. The relative density of circulating IGF-1 molecules are lower in older men than young men [134]. After reaching a peak in the 20s, serum dehydroepiandrosterone (DHEA) levels decline, and this rate of decline accelerates after the eighth decade of life. This has led to the hypothesis that the age-related changes in body composition, insulin sensitivity, and diseases of old age may be related to DHEA deficiency [103].

Currently, a comprehensive approach to the prevention and treatment of sarcopenia remains out of reach. However, in terms of anabolic hormonal interventions, both estrogen and testosterone can be replaced, making them inviting targets for therapeutic trials [13]. Pharmacological doses of testosterone increase skeletal muscle mass and strength in both young and elderly men [7, 56, 117, 126]. Many studies have now documented that exercise training can partially reverse sarcopenia, and that people who retain a high level of physical activity throughout their lives maintain a higher level of physical functioning and live longer [43, 101]. Fiatarone et al. [42] have demonstrated that even frail elderly nursing home patients in their 90s retain the plasticity of muscle in response to training. Another important countermeasure is to ensure adequate intake of energy and protein. At the cellular level, a better understanding of nerve-muscle interactions will allow for more rational interventions in the aging neuromuscular system [34].

Excitation-contraction coupling versus uncoupling

The two major membrane components involved in skeletal muscle excitation-contraction coupling are the transverse tubules and the sarcoplasmic reticulum [46]. Transverse tubules are infoldings of the muscle periphery and contain the voltage sensing units of the surface membrane. The intracellular calcium sequestering and calcium releasing membrane system is represented by the specialized endoplasmic reticulum which contains calcium-transporting ATPase complexes of the SERCA type and the ryanodine receptor (RyR) calcium release channel. In skeletal muscle, the calcium release channels span the narrow gap where the sarcoplasmic reticulum and the transverse tubules are positioned within approximately 15 nm of each other. Morphological and biochemical evidence has demonstrated a physical linkage of a sub-population of RyR1 isoforms of the junctional calcium release channels with the transverse-tubular dihydropyridine receptor tetrads [8, 9].

Different physiological mechanisms of excitation-contraction coupling appear to exist in mammalian skeletal muscle versus cardiac tissue. Figure 2a, b shows a diagrammatic comparison between these two modes of signal transduction at the triad junction. In a cardiac muscle cell, action potentials passing through the surface membrane activate voltage-sensitive L-type calcium channels that rapidly open, allowing extracellular calcium to flow into the cytoplasm. These voltage-sensitive calcium channels are represented by the α_{1C}-subunit of the cardiac dihydropyridine receptor. The calcium ions entering the heart cell activate the RyR2 type of calcium-release channels in the neighbouring sarcoplasmic reticulum and the subsequent efflux of calcium from the lumen activates other RyR2 calcium release channel units, thereby reinforcing calcium release. This is known as 'calcium-induced calcium release' (CICR) and, in the case of cardiac muscle, this term refers to activation of the release channels by calcium coming from both outside

the cell and from within the sarcoplasmic reticulum (Figure 2a). The rise in cytoplasmic calcium activates the contractile apparatus, producing force, and the calcium is subsequently both taken up into the sarcoplasmic reticulum by SERCA2 type pumps and removed from the cytoplasm by Na^{+}/Ca^{2+} exchangers and surface Calcium-pumping ATPases [6].

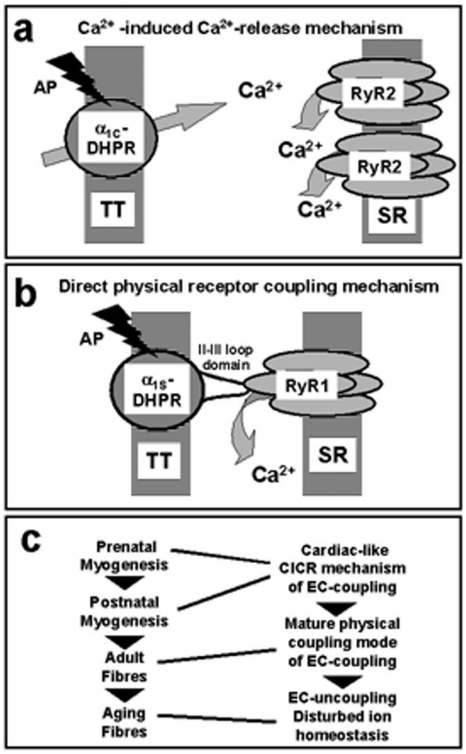

Figure 2. Diagrammatic representation of the predominant signal transduction mechanisms underlying excitation-contraction (EC) coupling at the triad junction between the transverse tubules (TT) and the sarcoplasmic reticulum (SR). The potential changes triggered by a surface action potential (AP) are translated into Ca^{2+}-release from the sarcoplasmic reticulum lumen by different mechanisms in cardiac versus skeletal muscle fibres. While voltage-sensing by the α_{1C}-subunit of the dihydropyridine receptor (DHPR) triggers a Ca^{2+}-induced Ca^{2+}-release mechanism via the RyR2 isoform of the ryanodine receptor in cardiac muscle (a), direct physical interactions between the II-III loop domain of the α_{1S}-DHPR and a cytoplasmic region of the RyR1 Ca^{2+}-release channel mediate this physiological coupling mechanism in mature skeletal muscle fibres (b). The flow chart of panel (c) summarises the proposed developmental and pathophysiological changes in signal transduction during myogenesis and sarcopenia, respectively. Developing muscle fibres appear to exhibit a more cardiac-like Ca^{2+}-induced Ca^{2+}-release (CICR) mechanism as compared to the direct physical interactions between the two receptor classes in mature skeletal muscles. As outlined in this review, aged fibres of animal models show disturbed calcium homeostasis and partial uncoupling between excitation and muscle contraction leading to loss in fibre strength.

Excitation-contraction coupling in vertebrate skeletal muscle differs substantially from that in cardiac muscle, but is mediated by homologous protein species. In skeletal muscle, the action potentials pass along the surface membrane and into the transverse tubular network, which is far more extensive than in cardiac muscle. The surface depolarization is sensed by the α_{1S}-subunit of the dihydropyridine receptors, which acts as a physiological voltage-sensor [36, 92, 113]. Importantly, these ion channels only mediate a small and relatively slow influx of extracellular calcium [92], and instead directly activate the calcium-release channels by physical means (Figure 2b). Adult vertebrate skeletal muscle can contract vigorously in the absence of extracellular calcium, showing that calcium inflow is not essential for initiating calcium release [36, 108]. Interestingly, invertebrate skeletal muscle and neonatal vertebrate skeletal muscle are more like cardiac muscle, requiring inflow of extracellular calcium for full contraction [28, 92]. This phenomenon is outlined in the flow chart of Figure 2c. Developmental studies have shown that a cardiac-like excitation-contraction coupling mechanism exists in skeletal muscle fibres during myogenesis, followed by the establishment of a direct physical coupling mechanism in adult fibres, and an age-related increase in abnormal signal transduction and disturbed ion homeostasis.

It has been postulated that the molecular mechanism underlying excitation-contraction uncoupling in muscles from aging mammals is a reduction in the number of dihydropyridine receptors being present in the transverse tubules, leading to an increase in the percent of ryanodine receptors uncoupled to the voltage sensor [31, 32, 80, 89]. The main functional consequence of this alteration is a failure in the transduction of sarcolemmal depolarisation into a calcium signal and a mechanical response. The reduction in dihydropyridine receptors may alter the signalling cascade leading to an impaired muscle force development, resulting in muscle weakness in the elderly [32]. Figure 3 outlines the pathophysiological mechanism involved in excitation-contraction uncoupling resulting in decreased calcium cycling through the lumen of the sarcoplasmic reticulum. Caloric restriction in rats can prevent the reduced expression of dihydropyridine receptors associated with aging [90]. The contribution of alterations in excitation-contraction coupling has been explored in single fibres from the vastus lateralis muscle. The samples were obtained from young, middle aged and old subjects exhibiting similar physical activity [31]. Dihydropyridine-sensitive calcium currents are reduced in skeletal muscle fibres of aged

human and mice [31, 106]. The pathophysiological effect is a significant reduction in the overall amount of calcium available for triggering mechanical responses in aged skeletal muscle [27, 31]. A lack in dihydropyridine receptors leads to a significant reduction in the peak intracellular calcium concentration recorded in response to maximum cell activation [31]. In analogy to these experimental data, biochemical and pharmacological studies have confirmed a drastic reduction in the number of α_{1S}-dihydropyridine receptors in aging muscles [106, 111]. However, the lack of alterations in the dissociation constant of the receptor for the high affinity dihydropyridine ligand PN200-110 rules out changes in the pharmacological properties of the receptor in muscles from aging mammals [106]. In aged skeletal muscle the calcium-sequestering activity of the sarcoplasmic reticulum may be altered [89]. However, the phosphorylation-dependent regulation and mechanisms of reduced calcium uptake remain controversial [29, 46]. Perhaps a modulation of RyR1 units by luminal sarcoplasmic reticulum proteins such as calsequestrin, or the coupling of calcium release channels by its many modulatory subunits such as calmodulin or the FK-506 binding protein [93], may be altered in aging skeletal muscle [89].

Intense or prolonged contractile activity can reduce muscle force output that recovers after the cessation of activity. This sequential pattern of events is typically referred to as muscle fatigue. The length of the recovery period is one of several characteristics that distinguish different types of fatigue, in particular, low-frequency fatigue (LFF) and high-frequency fatigue (HFF) [66, 67]. HFF is characterized by a rapid loss of force that recovers very quickly once muscle activation is reduced or stopped. Conversely, LFF is characterized by a loss of force primarily at lower stimulation frequencies, from which recovery is slow. It has been suggested that LFF may be due to a wider variety of mechanisms including metabolic, mitochondrial, and/or sarcoplasmic reticulum disruptions. Currently, several mechanisms are likely candidates to explain the reductions in calcium channel activity of the sarcoplasmic reticulum following contractile activity. They include elevated calcium concentrations, alterations in metabolic homeostasis within the micro-compartmentalised triadic space, and modification by reactive oxygen species. Westerblad et al. [131] have suggested that reductions in force can be explained by a reduction in the sarcoplasmic reticulum calcium release units. Consistent with a reduction in calcium release is a general loss of a proper regulation of overall calcium homeostasis where intracellular calcium is elevated significantly. Lamb et al. [77] have shown that raised intracellular calcium abolishes excitation-contraction coupling and alters the structure of the junctional triad structure. The effect of calcium-induced uncoupling may actually serve to preserve muscle cell integrity during extreme conditions.

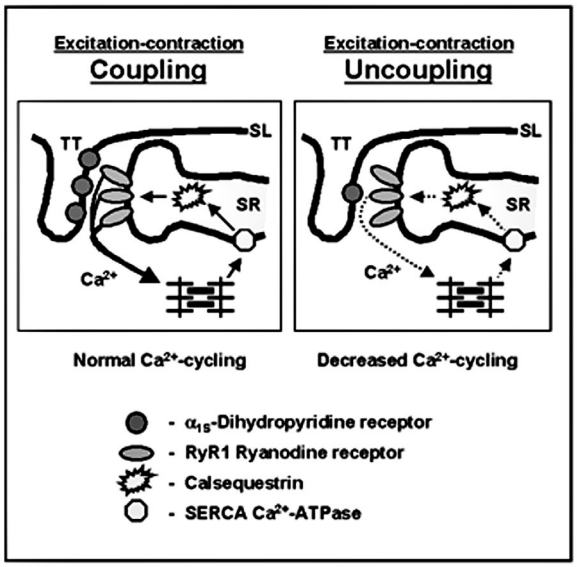

Figure 3. Diagrammatic representation of the excitation-contraction (EC) uncoupling hypothesis of sarcopenia. Shown is in the left panel the normal coupling process in adult skeletal muscle fibres between the α_{1S}-subunit of the dihydropyridine receptor and the RyR1 Ca^{2+}-release channel complex at the triad junction between the invaginations of the sarcolemma, the transverse tubules (TT), and the junctional sarcoplasmic reticulum (SR). In contrast, in the right panel is outlined the pathophysiological consequences of a drastic reduction in the α_{1S}-dihydropyridine receptor. A decreased ratio between the voltage sensor and the Ca^{2+}-release channel leads to decreased overall Ca^{2+}-cycling through the Ca^{2+}-uptake complex of SERCA Ca^{2+}-ATPases and the Ca^{2+}-reservoir complex of luminal calsequestrin clusters. Reduced levels of Ca^{2+}-ion supply to the contractile apparatus via EC-uncoupling may then trigger muscle weakness associated with the aging process.

Reductions in signal transduction and calcium release may provide an opportunity to limit releasable calcium while sequestration continues.

It is believed that part of the reduction in calcium release may be of metabolic origin. Glycogen particles are associated with the sarcoplasmic reticulum membrane system in skeletal muscle [38 ,49], and its reduction is associated with the onset of muscle fatigue [5]. Lactic acid accumulation has long been considered a potential contributor to muscle fatigue [26, 78, 94]. Lactate significantly inhibits calcium release from the sarcoplasmic reticulum by a variety of factors. Magnesium is a potent inhibitor of the sarcoplasmic reticulum calcium channel, both in isolated vesicular preparations and in single-fibre experiments [93]. It is probable that cellular magnesium concentrations are modified as a result of prolonged contractile activity,

but it remains to be established how they influence the sarcoplasmic reticulum calcium release process and muscle fatigue.

Reactive oxygen species have the potential to disrupt normal muscle function by targeting specific proteins for modification. Many reports have indicated that free radicals may play a significant role in muscle damage and fatigue that occurs with exhaustive type of exercise [114, 118]. Endogenous sulfhydryl groups (SH) localized to the sarcoplasmic reticulum calcium release channel are thought to play a role in its regulation [1]. Oxidation of SH groups trigger the opening of the ryanodine receptor calcium release channel, while reduction of the disulfide bond induces closure. Thus SH oxidation is a viable mechanism for modifying excitation-contraction coupling during oxidative stress.

The voltage-sensing dihydropyridine receptor

The skeletal muscle dihydropyridine receptor is an oligomeric complex with a α_{1S}-α_2/δ-β-γ configuration, whereby the α_{1S}-subunit represents the principal pharmacological binding site and calcium channel pore [24]. The transverse-tubular receptor serves as a voltage sensor for asymmetric charge movement, an event consistent with depolarisation to the contractile threshold [108]. The main α_{1S}-subunit consists of four repeats with three cytoplasmic loops between each segment [23]. The II-III loop domain has been shown to play a central role in communication between the voltage sensor and the calcium release channel units [86], as diagrammatically shown in Figure 2b. Tetrad particles of the transverse tubules are presumed to represent four dihydropyridine receptor units, and they have been located opposite ryanodine receptor tetramers [8, 9]. Slower twitching fibres appear to have a larger proportion of dihydropyridine receptor-unlinked ryanodine receptors as compared to fast-twitch skeletal muscles. With respect to sarcopenia, excitation-contraction uncoupling has been proposed as a process that accounts for a significant proportion of the decay in skeletal muscle force with aging [31]. It has been shown that the number of calcium release channels being uncoupled to the voltage sensor increases with age [106]. This finding is consistent with alterations in dihydropyridine receptor activity and calcium release from the sarcoplasmic reticulum in aged human muscle fibers [31]. Uncoupling between excitation and muscle contraction may result in alterations in the voltage-gated calcium release mechanism, decreases in myoplasmic calcium elevation in response to sarcolemmal depolarisation, reduced calcium supply to contractile proteins and reduced contraction force. Our research has demonstrated a drastic decline in the abundance of the α_{1S}-subunit of the dihydropyridine receptor in aged skeletal muscle of New Zealand white rabbits and HicksCar rats [110]. However, we could not confirm this finding in aged human skeletal muscle fibres [111].

According to a study by Mayhew et al. [90], caloric restriction can prevent the reduction in the dihydropyridine receptor in aging rats. This implies that caloric restriction preserves the mechanical properties of aging skeletal muscles. Renganathan et al.[106, 107] have shown that L-type calcium channel function can be modulated by insulin-like growth factor-1 (IGF-1). Receptor activation via a phosphorylation cascade appears to involve tyrosine kinase [106]. This inter-receptor signalling is impaired in skeletal muscles from old animals and the alteration may contribute to weakness and fatigue. Transgenic over-expression of IGF-1 results in a significant increase in the dihydropyridine receptor density and no apparent change in expression of ryanodine receptors. This causes an approximately 30% increase in the ratio between dihydropyridine receptors and ryanodine receptors [107]. This phenomenon was seen in both fast- and slow-twitch muscles [33]. Interestingly, antisense RNA amplification showed a significant increase in the level of mRNA encoding for the α_{1S}-subunit of the dihydropyridine receptor in IGF-1-treated primary cultured rat muscle cells [33]. This indicates that IGF-1 regulates the level of the α_{1S}-subunits through activation of gene expression in skeletal muscle cells [129]. Zheng et al. [136] have shown that transgenic over-expression of IGF-1 results in marked increases in skeletal muscle mRNA encoding the α_{1S}-subunit of the dihydropyridine receptor, implying that IGF-1 regulates the expression of this receptor by modulating nuclear transcription. Musaro et al. [98] have generated a model of persistent, functional myocyte hypertrophy using a tissue-restricted transgene encoding a locally acting isoform of insulin-like growth factor-1 that is expressed in skeletal muscle (mIGF-1). The preservation of muscle architecture and age-independent regenerative capacity through localized mIGF-1 transgene expression suggests a potential for novel clinical strategies in the treatment of age and disease-related muscle frailty.

The ryanodine receptor calcium release channel

The ryanodine receptor of the junctional sarcoplasmic reticulum is a central element of excitation-contraction coupling. The importance of a proper linkage between the ryanodine receptor and the transverse-tubular dihydropyridine receptor is illustrated by the fact that calcium release is lost in transgenic mice lacking the voltage sensor [121]. Originally identified as the junctional foot protein, it was later determined to be the sarcoplasmic reticulum calcium release channel from morphological, biochemical and pharmacological studies using the plant alkaloid ryanodine [21, 65, 70, 104]. The monomeric protein contains 5,035 amino acids, with an apparent molecular mass of 565 kDa. The skeletal muscle ryanodine receptor is a high-molecular-mass homo-tetramer [21]. Chemical cross-linking analysis confirmed the oligomeric nature of the

ryanodine receptor, as well as the concept of direct physical interactions between triad receptors during excitation-contraction coupling [97]. Opening and closing of the calcium channel pore is accompanied by major conformational changes [105]. Numerous loosely packed structural domains of the cytoplasmic assembly of the ryanodine receptor appear to be responsible for maintaining the structural integrity of the ion pore and allow calcium ions to flux through the receptor transmembrane assembly [105]. Three mammalian isoforms have been identified and are encoded by three different genes, termed ryr1, ryr2 and ryr3. The corresponding protein isoforms RyR1, RyR2 and RyR3 correspond to the skeletal, cardiac and brain type of ryanodine receptor respectively, the order in which they were identified. Brain tissue contains small amounts of the skeletal and cardiac forms as well as the RyR3 protein, whereas the muscle forms predominate in their respective tissues [59, 65]. Besides four identical subunits of 565kDa, the ryanodine receptor from skeletal muscle also contains the 12 kDa FKBP12 protein. Expression of the FKBP12 subunit of the ryanodine receptor has been shown to increase during postnatal development of rabbit skeletal muscle [51]. FKBP12 modulates calcium channel gating [11] and mutant mice deficient in FKPB12 have apparently normal skeletal muscle but exhibit severe dilated cardiomyopathy [116]. The junctional sarcoplasmic reticulum protein triadin [39, 76] is implicated as a negative regulator of the RyR1 calcium release channel [102]. In addition, a novel 90-kDa junctional face membrane protein [57] has been shown to be tightly associated with the triadic calcium release channel complex [52]. With respect to sarcopenia, both the RyR1 isoform of the calcium release channel and the SERCA1 calcium pump exhibit slower protein turnover in aged skeletal muscle fibres [41]. Increased sensitivity of the ryanodine receptor to caffeine in aging has been reported, suggesting age-induced alterations in channel regulation [132, 133]. Most importantly, however, is the decrease in the coupling ratio between the ryanodine receptor and the α_{1S}-dihydropyridine receptor in aged fibres [107].

Junctional triad proteins

The junctional face membrane of the sarcoplasmic reticulum and adjacent luminal region contains a number of ion-regulatory proteins including triadin, junctin, JFP-45, JFP-90 and calsequestrin. These proteins have been localized to the triadic region and are thought to participate in the maintenance of the junctional couplings, the regulation of the calcium release channel complex and the facilitation of direct interactions between the dihydropyridine receptor and the ryanodine receptor. Contradictory findings have been published on triadin, the 95 kDa glycoprotein of the triad junction [9, 10]. While initial protein overlay studies suggested that calsequestrin binds to both the ryanodine receptor and the dihydropyridine receptor [22, 73], biochemical binding experiments strongly indicate that triadin acts as a linker between calsequestrin and the calcium release channel [55, 58]. Structurally, triadin appears to consist of one transmembrane domain and a large luminal region [76]. The luminal domain contains a number of positively charged residues and may serve as an anchoring site for calsequestrin in a calcium-dependent manner. Increased expression of triadin has been observed during postnatal development of rabbit skeletal muscle [51]. In aging muscle fibres, the interactions between triadin and calsequestrin clearly decrease [55] suggesting impaired coupling within the calcium regulatory apparatus in sarcopenia. Little is known about the function of the novel 90-kDa junctional face membrane protein, JFP-90 [57]. Chemical crosslinking analysis has shown an overlap between JFP-90 and the ryanodine receptor suggesting potential protein-protein interactions between these two triad components [52]. As illustrated in Figure 4, in human vastus lateralis skeletal muscle the expression of JFP-90 drastically increases with age. In contrast to this finding, in our recent immunoblotting survey of aging human skeletal muscle fibres [111], all other calcium-regulatory elements studied appear not to be changed in sarcopenia. The pathophysiological consequence of the increase in JFP-90 and its potential involvement in the functional decline of aged skeletal muscle fibre is not known. It might represent a compensatory mechanism to counter-act age-related alterations of the triadic signal transduction complex. Zorzato et al. [138] have recently described another integral membrane protein that is highly enriched in the skeletal muscle junctional face membrane. JFP-45 of 45 kDa co-localises with the ryanodine receptor and appears to interact with the voltage sensor and calsequestrin indicating a regulatory role in excitation-contraction coupling. The mRNA encoding JFP-45 was shown to decrease approximately three-fold during

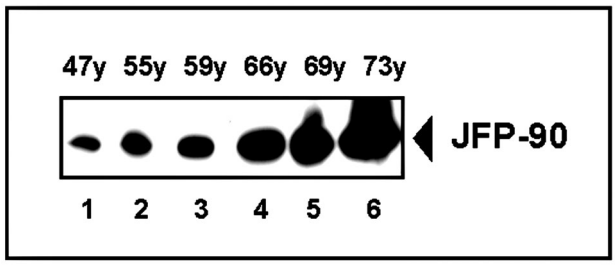

Figure 4. Immunoblot analysis of the junctional face protein of 90 kDa, JFP-90, in aged human skeletal muscle fibres. Shown is an immunoblot of microsomes prepared from human skeletal muscle specimens, decorated with monoclonal antibody VF1c to JFP-90. Sample ages are shown at the top of individual lanes. Human muscle microsomes were prepared and analysed by standard methodology [111].

aging [2].

Calsequestrin and calcium sequestration

Calsequestrin was discovered as the major calcium binding protein of the sarcoplasmic reticulum from skeletal muscle over three decades ago [63, 88]. Dye binding assays suggested that a similar calcium binding protein is present in cardiac muscle [68], and by 1983 the cardiac isoform of calsequestrin had been purified and biochemically characterized [17, 20]. A slow- and a fast-twitch isoform of calsequestrin exist in skeletal muscle. Calsequestrin represents a high capacity, medium-affinity calcium-binding protein and protein clusters form the major calcium-storage site of the luminal terminal cisternae region [69, 119]. Blot overlay assays have confirmed that calsequestrin forms large self-aggregates and also binds tightly to junctin, triadin and the RyR1 isoform of the junctional calcium release channel [55]. The distribution of calsequestrin is not even throughout the lumen of the sarcoplasmic reticulum, but is markedly concentrated in close proximity to the ryanodine receptor [48]. Following calcium binding to calsequestrin, conformational changes in the ryanodine receptor occur and these can be reversed by dissociation of the supramolecular complex. Thus, calsequestrin can be considered as an endogenous regulator of the calcium release channel [64]. Calsequestrin and the ryanodine receptor are mutually coupled, such that the conformational changes of one protein are transmitted to the other [54, 71].

Besides calsequestrin of apparent 63 kDa, another calcium-binding element of the luminal sarcoplasmic reticulum is represented by sarcalumenin of apparent 160 kDa [83]. Sarcalumenin is mostly found in the longitudinal tubules and probably acts both as a calcium binding protein and calcium shuttle. The 53 kDa sarcoplasmic reticulum glycoprotein and sarcalumenin both co-localize with the SERCA type Ca^{2+}-ATPases [83]. With respect to myogenesis, calsequestrin and sarcalumenin drastically increase in abundance during early muscle development [51]. In contrast, the expression of the calcium binding protein calreticulin decreases rapidly during postnatal myogenesis and is only found in a low density in mature skeletal muscle [3]. Several other protein components of the sarcoplasmic reticulum membrane system have been identified including calsequestrin-like proteins (CLPs) [18], the histidine-rich calcium-binding protein [60-62], endoplasmin (GRP94) [19] and a multifunctional protein disulphide isomerase [45]. The origin and significance of the calsequestrin-like proteins are not clear at present [18]. These proteins range in molecular mass from approximately 160 kDa to 210 kDa. Possible roles for the calsequestrin-like proteins include calcium-binding and storage in the junctional sarcoplasmic reticulum alongside calsequestrin, assembly of calsequestrin clusters, anchorage of calsequestrin to the junctional face membrane and/or communication with the calcium release channel complex [95]. With respect to aging, relatively little is known about the fate of these calcium binding elements. Recently, Cai et al. [16] have reported a down-regulation of the cytosolic calcium binding protein parvalbumin in rat fast-twitch muscle during aging. Interestingly, high-intensity exercise could reverse this age-related change.

Because calsequestrin is a central mediator of calcium cycling through the sarcoplasmic reticulum, it has been of interest to determine whether calsequestrin complex formation is modified during pathophysiological down-stream events of muscle aging. Silver staining of electrophoretically separated microsomal proteins from aged muscle showed no major changes in the expression of fast calsequestrin, the ryanodine receptor and junctin. However, a calsequestrin-peroxidase probe (used in a blot overlay assay) revealed a clear tendency of aged rabbit skeletal muscle fibers towards reduced protein interactions within the calsequestrin complex [55]. Thus, abnormal ion homeostasis in aged animal muscle fibres might be due to impaired protein-protein interactions. In our recently published study on the analysis of vastus lateralis specimens from male humans aged 18 to 82 years of age, apart from small inter-individual variations in expression levels, there were no major changes in the relative abundance of fast calsequestrin [111]. Hence, although impairments of other Ca^{2+}-regulatory proteins and/or disturbed protein-protein interactions might be involved in the pathophysiological changes of sarcopenia, calsequestrin expression seems to be preserved during the aging process of human skeletal muscle fibres [111].

Conclusions

The last decade has witnessed an increased interest in the evolutionary role of aging [75] including the molecular and cellular mechanisms of the biological aging process [74]. With respect to age-related changes in skeletal muscle fibres, research has focussed on the physiological and cell biological consequences that result from muscle wasting [100]. There is widespread agreement that sarcopenia is a multi-factorial phenomenon, probably involving abnormal ion handling, impaired signal transduction, free radical production, oxidative stress, mitochondrial damage, hormonal imbalances, metabolic disturbances and protein degradation. The idea that excitation-contraction uncoupling might be involved in sarcopenia leading to a drastic decline in skeletal muscle force with aging is an interesting hypothesis [31]. The analysis of animal models has convincingly demonstrated that a reduction in the number of α_{1S}-dihydropridine receptor subunits leads to an increase in the percentage of ryanodine receptor units being uncoupled to the voltage sensor. This results in an impaired transduction of sarcolemmal depolarization into a calcium signal and thereby weakens the mechanical response. With the exception of the findings of a micro-array study [130], biochemical

and pharmacological analyses confirmed the excitation-contraction uncoupling hypothesis of animal sarcopenia [31-33, 106, 107, 110]. It is encouraging that caloric restriction appears to retard the aging process in laboratory animals [72]. Possibly the reversal of excitation-contraction uncoupling plays a central role in this protective process [33].

In order to increase our general understanding of the molecular and cellular mechanisms contributing to the sarcopenic process, animal models represent an important biomedical tool. The Klotho mouse, recently developed by Utsugi et al. [127], appears to be an excellent model system for studying human aging. This mutant exhibits several aging phenotypes such as arteriosclerosis, osteoporosis, skin atrophy, and pulmonary emphysema [127]. Most importantly, the introduction of high-throughput DNA micro-array technology has decisively advanced aging research [53]. With respect to sarcopenia research, it represents a new tool for measuring the biological aging process on a cell- and tissue-specific basis. The recent gene expression analysis of aged rhesus monkeys has demonstrated a selective up-regulation of transcripts involved in inflammation and oxidative stress, and a down-regulation of genes involved in mitochondrial electron transport and oxidative phosphorylation [72]. Lee et al. [84] could previously show that aging results in a differential gene expression pattern indicative of a marked stress response and lower expression of metabolic and biosynthetic genes in mouse skeletal muscle. Caloric restriction appears to retard these age-related abnormalities by causing a metabolic shift toward increased protein turnover [84]. One of the most promising biochemical pathways discovered of being involved in aging is the insulin/insulin-like growth factor (IGF-1) signalling mechanism. The transcription factor DAF-16 clearly affects expression of a subset of genes that influence the ageing process [96]. The future study of how the insulin/IGF-1 pathway triggers the up-regulating of genes involved in cellular stress-responses and/or the down-regulating of life-shortening genes might ultimately lead to a better understanding of the aging process.

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