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Age-Related Muscle Dysfunction

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Abstract

Aging is associated with a progressive decline of muscle mass, strength, and quality, a condition described as sarcopenia of aging. Despite the significance of skeletal muscle atrophy, the mechanisms responsible for the deterioration of muscle performance are only partially understood. The purpose of this review is to highlight cellular, molecular and biochemical changes that contribute to age-related muscle weakness.

Keywords

actin; myosin; aged muscle; enzymatic activity; oxidative modifications

Sarcopenia

Aging is associated with a progressive decline of muscle mass, strength, and quality, a condition described as sarcopenia. These age-related changes are observed in healthy, active adults who are 50 years and older (Hughes et al., 2002). The prevalence of sarcopenia in older adults under the age of 70 years is about 25% and increases to 40% in adults 80 years or older (Baumgartner et al., 1998). Sarcopenia represents a risk factor for frailty, loss of independence, and physical disability (Roubenoff, 2000). Loss of mobility resulting from muscle loss predicts major physical disability and mortality, and is associated with poor quality of life, social needs, and health care needs (Fried and Guralnik, 1997). The economic impact of sarcopenia and its detrimental correlates are immense (Janssen et al., 2004). Thus, understanding the mechanisms leading to muscle dysfunction (e.g., weakness) at advanced age represents a high public health priority. The purpose of this article is to highlight cellular, molecular, and biochemical changes that contribute to age-related muscle dysfunction.

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Muscle physiology- short review

For skeletal muscle, the control of force has to be accurate and precise with the contractile machinery being switched on and off rapidly to allow for complex coordinated movements. Action potentials initiated at the neuromuscular junction propagate along the length of the fiber and the transverse tubules. As the wave of depolarization passes down the transverse tubules there is an interaction with the sarcoplasmic reticulum that results in the release of calcium, initiating the interaction of actin and myosin and muscle contraction. This process is known as excitation-contraction coupling. Thus, age-related structural changes or chemical modifications in proteins that affect excitation-contraction coupling are likely to influence muscle function.

The basic contractile unit of muscle, the myofibril, consists of a linear array of sarcomeres, which contains interdigitating myosin and actin filaments. The force-generating and catalytic function of myosin is located in its “head” region, which contains the catalytic domain (with sites for ATP hydrolysis and interaction with actin), and the light chain (LC) domain, which contains the essential (ELC) and regulatory (RLC) light chains. The actin filaments contain specific sites of interaction with myosin. Interaction of actin with the myosin head in the presence of ATP results in sliding of actin filaments past myosin filaments toward the center of the sarcomere (contraction). The hydrolysis ATP (biochemical steps) during this cyclic interaction of actin with myosin is accompanied by a sequence of structural transitions in both proteins. In the absence of ATP and/or the presence of ADP, the myosin head forms a strong complex with actin. Binding of ATP to myosin produces a weaker complex. The release of phosphate (by product of ATP hydrolysis) from myosin causes a structural transition in the catalytic and light chain binding domains, generates force, and initiates a new cycle. Force can decrease if the system spends too much time in the weak-binding states, or the strong-binding states are weakened. Velocity can decrease if the system spends too much time in the strong-binding states. Thus, age-related structural or chemical changes in actin and myosin that affect actomyosin ATPase activity by affecting the weak-to-strong actomyosin transition are likely to change muscle function.

Age-related changes in muscle contractility

Despite the significance of skeletal muscle atrophy and weakness as inevitable concomitants of old age, the underlying molecular mechanisms responsible for these impairments are only partially understood. Age-associated muscle atrophy is the result of a combination of individual-fiber atrophy, along with a decrease in the total number of fibers, with a preferential loss of type II (fast-twitch, glycolytic, fibers with myosin heavy chain type II isoforms) muscle fibers (Thompson, 1994). In addition to the loss in muscle mass and consequent loss in muscle force, muscles of old animals show a deficit of ~20% in specific force (force generation normalized for muscle cross-sectional area), suggesting *qualitative* (e.g., dysfunctional proteins) as well as *quantitative* deficiencies associated with aging (Brooks and Faulkner, 1994; Thompson, 1999, 2002).

Age-associated qualitative- and quantitative-deterioration of muscle contractility is observed at the whole muscle level and at the single fiber level. Two experimental approaches are

available to evaluate single skeletal muscle fiber contractility, the intact and the permeabilized muscle fiber preparations. The intact single muscle fiber has a membrane with a functional excitation-contraction coupling system. In contrast, the permeabilized fiber lacks the excitation-contraction coupling system and the proteins involved in energy metabolism. The intact fiber preparation provides information of two protein complexes critical for effective excitation-contraction coupling: dihydropyridine and ryanodine receptors. In contrast, the permeabilized fiber offers more direct information on the contractile proteins (e.g., myosin, actin, myosin light chains, troponin). Literature supports excitation-contraction coupling contributing to age-related decline in muscle contractility. For instance, there are decreases in dihydropyridine and ryanodine receptors that translate membrane depolarization into intracellular Ca2+ release, a decrease in the dihydropyridine and ryanodine receptors ratio, and a decrease in functional dihydropyridine receptors leading to excitation-contraction uncoupling in old skeletal muscle (Payne and Delbono, 2004). Investigations focused on the permeabilized individual muscle fiber show that the two principal contractile parameters, specific force (P_o) and unloaded shortening velocity (V_o), decrease progressively with age (Thompson et al., 2001; Thompson and Brown, 1999).

The extent and time course of the deterioration of muscle function depends on many factors, such as the fiber type composition of the specific muscle studied, the selected age group of the rats, and the rodent strain. There is significant muscle atrophy, and reductions in the force generating capacity and the structure of myosin in type IIB skeletal muscle fibers (semimembranosus muscle) with aging when comparing young, old, and, very old rats (Lowe et al., 2001; Zhong et al., 2006). In contrast, the age-associated atrophy and significant functional declines of the type I fibers (soleus muscle) occur later (well into senescence) (Thompson and Brown, 1999)

Age and structural changes-contractile proteins

Electron paramagnetic resonance (EPR) is a high-resolution spectroscopic method which, in combination with site-specific spin labeling of Cys_{707} of myosin, detects changes in the structure of myosin associated with relaxation and contraction. In particular, EPR can determine quantitatively the fraction of myosin molecules in the weak- and strong-binding structural states discussed above. EPR studies show that the age-related decrease in the specific force (24% – 27%) is associated with decrease in the fraction (24% – 30%) of myosin heads in the strong-binding, force-generating structural state (Lowe et al., 2001; Lowe et al., 2004b; Thompson et al., 2001; Zhong et al., 2006). These findings support structural changes in myosin during contraction with age.

Age and myosin ATPase activity

As noted above, hydrolysis of ATP is directly related to contractile function and structural changes in the head region of myosin during contraction. Two experimental approaches are used to determine ATPase activity, myofibrils and purified actin and myosin. Myofibrils preserve the distinct organization and interaction between the contractile proteins. In contrast, purified actin and myosin molecules allow the determination of enzymatic changes in each of these proteins, independently of the other and without interference from other myofibrillar proteins.

Myosin ATPase activity can be determined at various salt concentrations (high and low salt concentrations), which uncover important information about the proteins. Myofibrillar ATPase activity at high salt concentration eliminates effects of actin and other proteins, and is sensitive to post-translational changes in myosin. Age-related molecular changes in myosin are observed in high-salt ATPase activities of myofibrils and myosin (Lowe et al., 2001; Prochniewicz et al., 2005). Like single muscle fibers described previously, the age-induced changes are muscle-specific. These findings suggest that chemical modification of myosin occurs with age.

Under isometric conditions (muscle contracts without a change in length), simultaneous measurements of force and ATPase activity in single fibers with type IIB myosin heavy chain of adult and aged rats show that fibers from aged rats generate ~20 lower maximum force without changes in the ATPase activity (Lowe et al., 2002). This result indicates a decrease in the energetic efficiency, a partial uncoupling between ATPase activity and force generation, during contraction in aged muscle. In contrast, under unloaded shortening conditions (isotonic) in the same muscle, aging results in a decrease (16%) in the myofibrillar ATPase activity and a similar decrease in the shortening velocity (Lowe et al., 2004a). These findings (ATPase activity under isometric and isotonic conditions) suggest that the age-related inhibition of contractility may in part be due to changing actin-myosin interactions.

Biochemical experiments on purified actin and myosin from adult and aged rats show a decrease in V_{max} (activity extrapolated to infinite actin concentration) and K_m (the concentration of actin at half V_{max}) (Prochniewicz et al., 2005) Subsequent mixing of the purified proteins (actin and myosin) from adult and old muscle in four combinations shows that the age-related decrease in V_{max} is primarily due to changes in myosin. This decrease in V_{max} is consistent with an earlier study showing age-related alterations in the structural states of myosin (transitions from weak to strong interactions). However, the age-related decrease in K_m is due to changes in both proteins, as actin from adult muscle attenuates the age-related decrease in K_m for myosin from old muscle. The data from these experiments suggest that changes in actin, together with changes in myosin, are involved in the molecular mechanism of age-related deterioration of muscle contractility.

Age-related post-translational modifications of skeletal muscle proteins

The ‘free radical theory’ of aging, formulated 50 years ago, proposes that aging and associated degenerative diseases can be attributed to deleterious effects of reactive oxygen species (Harman, 1956). A current version of this theory is the ‘oxidative stress theory’ of aging. The ‘oxidative stress theory’ states that “*A chronic state of oxidative stress exists in cells of aerobic organisms even under normal physiological conditions because of an imbalance of proxidants and antioxidants. This imbalance results in a steady-state accumulation of oxidative damage in a variety of macromolecules. Oxidative damage increases during aging, which results in a progressive loss in the functional efficiency of various cellular processes*.” (Sohal and Weindruch, 1996)

With aging, it has been shown that under basal conditions, oxidant production is increased in old age and the redox state of muscle from aged animals shifts to a more oxidative environment. While some antioxidants are increased in aging muscle, the extent of increase is muscle-specific and not global to all enzymes (Ji, 2002). Thus, the burden of defending against the increased load of free radicals may be greater than the compensatory change in antioxidants. If the antioxidant system is inadequate and key skeletal muscle proteins are modified, the proteasome must remove damaged proteins. Our research group and others have shown that proteasome function in muscle declines with aging (Ferrington et al., 2005; Husom et al., 2004). Thus, the fundamental changes in cell redox status and the ability to remove free radical damaged proteins likely contribute to the age-related changes in muscle contractility discussed above.

Skeletal muscle is particularly vulnerable to oxidative stress. This is due, in part, to the rapid and coordinated changes in energy supply and oxygen flux that occurs during contraction, resulting in increased electron flux and leakage from the mitochondrial electron transport chain. Skeletal muscle also contains a high concentration of myoglobin, a heme-containing protein known to confer greater sensitivity to free radical-induced damage to surrounding macromolecules by converting hydrogen peroxide to other more highly reactive oxygen species (ROS) (Ostdal et al., 1997). The greater concentration of myoglobin in type I fibers (slow-twitch) compared to type II fibers (fast-twitch) suggests the potential for fiber-type specific differences in susceptibility to oxidative stress and may explain the age-related changes in muscle phenotype (Thompson, 1999).

Other fundamental differences between type I and type II fibers may confer differing degrees of susceptibility to oxidative stress. For example, the major energy pathway utilized in type I fibers occurs through oxidative metabolism, whereas the glycolytic pathway is the primary means for generating energy in type II fibers. Thus, type I fibers likely produce greater ROS via mitochondrial oxidative phosphorylation compared with type II fibers. To counter the effects of ROS, type I fibers have higher antioxidant capacities that prevent or attenuate oxidative damage (Reid and Durham, 2002; Thompson, 1994). Although type II fibers may generate lower levels of ROS during metabolism than do type I fibers, type II fibers may be more susceptible to oxidative stress because their antioxidant defenses are less robust. These fiber-type differences in susceptibility to oxidative stress may be mechanistically related to the aging phenotype (both fiber types are affected, but the time course of change is fiber type-dependent).

Nitration and aging skeletal muscle

In order to evaluate the role of oxidative stress and the skeletal muscle aging phenotype, we compared the protein oxidative damage of skeletal muscle proteins in two muscles, the soleus and semimembranosus, each composed of different skeletal muscle fiber types. Specifically, the soleus muscle is composed of >90% type I fibers, whereas the semimembranosus is composed of >90% type IIB fibers (Armstrong and Phelps, 1984). We used 3-nitrotyrosine (3-NT) as a stable marker of protein oxidative damage. This posttranslational modification can alter protein function and is associated with acute and chronic disease states (Alvarez and Radi, 2003). 3-NT is formed when tyrosine is nitrated by

peroxynitrite, a highly reactive molecule generated by the reaction of nitric oxide with superoxide. Muscle fibers are exposed to periodic fluxes of nitric oxide and superoxide, thus providing favorable conditions for the formation of peroxynitrite. Tyrosine nitration can inhibit protein function for example, by altering a protein's conformation, imposing steric restrictions to the catalytic site, and preventing tyrosine phosphorylation (Cassina et al., 2000). Thus, the functional significance of tyrosine nitration depends on both the site of modification and the extent of the protein population containing functionally significant modifications.

The impact of protein tyrosine nitration on muscle performance has been demonstrated with *in vitro* studies (Callahan et al., 2001). In isolated skeletal muscle preparations, peroxynitrite potentially impaired both energetics and contractility (Callahan et al., 2001). Although these *in vitro* studies did not identify specific protein targets, the extent of functional decline is consistent with age-induced changes in single fiber contractile properties, suggesting that protein nitration may contribute to underlying mechanism for the age-related functional decrement (Thompson and Brown, 1999).

We hypothesized that with aging the semimembranosus (type II) muscle would accumulate a greater amount of oxidized proteins (3-NT) compared to proteins in the soleus (type I) muscle. The five modified proteins, identified by MALDI-TOF Mass Spectrometry and Western immunoblotting included the sarcoplasmic reticulum Ca^{+2}-ATPase (SERCA2a), aconitase, β-enolase, TPI, and carbonic anhydrase III, exhibited an age-dependent increase in 3-NT content in both type I and type II muscles. However, significant levels of 3-NT modification were present at an earlier age in the semimembranosus muscle. The function of these proteins include energy production (TPI, β enolase, aconitase, carbonic anhydrase III), and calcium homeostasis (SR Ca-ATPase)

Interestingly, mitochondrial aconitase is one of the major intracellular targets of nitric oxide, and the decrease in aconitase activity has been attributed to the direct reactions of nitric oxide with the iron-sulfur cluster (Patel et al., 2003). This protein shows oxidative damage during aging in cardiac tissue (Kanski et al., 2005a). In addition, previous studies demonstrated oxidative modifications of carbonic andydrase III *in vivo* with a concomitant decrease in catalytic activities in liver tissue (Cabiscol and Levine, 1995). There is increasing evidence that links β-enolase and TPI as targets for nitration in Alzheimer's disease, and in aging cardiac and skeletal muscle, providing a possible rationale for the observed disturbance in energy metabolism of aging tissue (Castegna et al., 2002; Kanski et al., 2003; Kanski et al., 2005b)

Viner and colleagues demonstrated a progressive age-related accumulation of nitrotyrosine that was selectively limited to the SERCA2a isoform in preparations from both fast- and slow-twitch rat muscle (Viner et al., 1999). Mass spectrometry analysis of tryptic peptides from aged rats identified the sites of nitration at tyrosine residues 294, 295, and 753. The functional impact of modification of these tyrosine residues is suggested by the significant age-dependent loss in Ca^{2+}-ATPase function in slow-twitch muscle preparations containing predominantly SERCA2a. From our work, accumulation of 3-NT in SERCA2a is observed in the soleus (slow-twitch) as well as in the semimembranosus (fast-twitch). Taken together,

these studies provide some insights about the molecular mechanisms responsible for the observed phenotypic changes in skeletal muscle.

Carbonylation and aging skeletal muscle

Introduction of reactive carbonyls into proteins, known as protein carbonylation, is a prominent marker of oxidative stress in aging skeletal muscle (Fano et al., 2001). Due to the production of ROS in skeletal muscle mitochondria, which increase with age and drive these protein modifications, mitochondrial muscle proteins are particularly susceptible to carbonylation (Meany et al., 2007). The age-associated fiber-type differences are observed in the susceptibility to of proteins to carbonylation. Protein carbonylation occurs through two major mechanisms, metal catalyzed oxidation and reaction of nucleophilic amino acid side chains with lipid oxidation products such as 4-hydroxyl-2-nonenal (HNE). In the former mechanism, metals such as copper and iron catalyze the formation of highly-reactive, short-lived hydroxyl radicals that modify nearby amino acids, like proline, arginine, lysine, and threonine (Stadtman and Berlett, 1997). In the latter mechanism, lipid peroxidation leads to the generation of aldehyde-containing byproducts, which covalently modify nucleophilic amino acid side chains on proteins, such as cysteine, histidine and lysine (Stadtman and Berlett, 1997).

We recently investigated differences in mitochondrial protein carbonylation attributed to muscle-type (fast- versus slow-twitch) and age (manuscript in review). We found that fast-twitch muscle had about two times more proteins susceptible to carbonylation, with over 20 of these proteins showing significant increases in carbonylation with age in fast-twitch muscle. Subsequent analysis using Ingenuity Pathway Analysis (IPA) (Mayburd et al., 2006), revealed that these carbonylated proteins belong to pathways and functional classes in muscle already known to be impaired in aging skeletal muscle. Moreover, proteins susceptible to carbonylation having a role in two functions, cellular function/maintenance and cell death, were significantly represented in the fast-twitch muscle, but not in the slow-twitch muscle.

Although several proteins were identified one interesting example is the detection of carbonylated voltage-dependent anion channel (VDAC) protein from fast-twitch muscle, which maps to “Cellular function and maintenance” within the IPA environment. This protein shows a large increase in carbonylation in aged muscle. Along with its binding partner, ADP/ATP translocase protein, which also shows marked increase in carbonylation with age, VDAC enables transport of ions, such as calcium ions (Ca^{+2}), across the inner-mitochondrial membrane, critical to mitochondrial function (Pi et al., 2007). Impaired mitochondrial cycling of Ca^{+2}, has been associated with aging skeletal muscle (Weisleder et al., 2006). One may hypothesize that increased carbonyl modification of these proteins critical to mitochondrial inner membrane transport may contribute to this impaired cellular function in aged fast-twitch muscle.

IPA identified also biochemical pathways represented by proteins showing changes in carbonylation with age which are known to be impaired in aging muscle. One example is the “fatty acid metabolism” pathway. In our dataset, proteins with enzymatic activity mapping to five of the steps in fatty acid metabolism show increased age-dependent carbonylation

and one (Acetyl-CoA acyltransferase (EC 2.3.1.16)) showed decreased abundance of its carbonylated form. Carbonylation of fatty acid proteins suggests a possible role of these modifications in decreasing the function of this pathway with age. A known feature of aging skeletal muscle is the decreased ability of this tissue to oxidize fatty acids for energy generation (Toth et al., 2005). The increased carbonylation of numerous proteins involved in fatty acid metabolism therefore points to a possible link between these modifications causing a decrease in their function, and leading to decreased efficiency in removing lipids.

Glycation and aging skeletal muscle

According to the glycation hypothesis and oxidative stress theory of aging, accumulation of advanced glycation end products (AGEs) of the Maillard reaction alters the structural properties of proteins and reduces their susceptibility to degradation (Sell and Monnier, 1989) Decreased susceptibility of AGE-proteins to degradation by the proteasome, the function of which is also compromised during aging (Ferrington et al., 2005; Husom et al., 2004), might contribute to the build up of modified proteins.

Although there are many forms of AGEs, N^{ϵ}-(carboxymethyl)lysine (CML), is the major AGE-product *in vivo* and is a useful biomarker of oxidative stress and damage (Fu et al., 1996). For instance, CML accumulates in aging human skin collagen (Dunn et al., 1991), lens crystallins (Dunn et al., 1989), and arterial walls (Schleicher et al., 1997). These findings suggest glycoxidation reactions and oxidative stress may be important mechanisms in age-related degenerative processes. In other words, CML may be involved in the development of age-related deterioration of skeletal muscle function. Generally, muscle shows the least glycation of several tissues, with the basal level of glycation in muscle protein of 0.2 mmol/mol lysine (Brown et al., 2005).

In a study comparing the extensor digitorum longus muscle (fast-twitch muscle) from young and very old rats, the older rats had a significantly greater percentage (10-fold difference) of myofibers that immunolabeled intracellularly for CML compared to the younger group (Snow et al., 2007). The pattern of the AGE immunolabeling had two characteristic appearances at the individual myofiber level. One pattern was intracellular punctuate labeling and the other pattern was labeling at the myofiber periphery. There is a preponderance of evidence that long lived proteins have AGE, however short lived proteins also accumulate AGE when O2 is available (Verzijl et al., 2000). Cellular proteins are more highly glycated than extracellular proteins (Thornalley et al., 2003). Subsequent MALDI-TOF MS analysis identified the AGE-susceptible proteins as creatine kinase, carbonic anhydrase III, β-enolase, actin, and voltage-dependent anion channel 1, with β-enolase showing an accumulation of CML with age in muscle. These CML-modified proteins are critical enzymes involved in energy production. It is thought that β-enolase is a scavenger of AGE because lysines are available for modification at the exposed surface of the protein. This scavenging process may spare other proteins from CML-modification and consequent functional impairment. β-enolase is a good candidate for this role, as glycation of this protein has only a limited impact on cell physiology. Indeed, glycation leads to a decrease of β-enolase activity, but no changes were detected in glycolytic flux (Gomes et al., 2006).

The glycation of myosin has been detected in the skeletal muscle of aged rats (Syrovy and Hodny, 1992). Interestingly, *in vitro* studies on myosin purified from the soleus muscle of young rats showed that glycation of myosin decreases actin motility (Ramamurthy et al., 2001) and also decreases K^+-activated and actin-activated ATPase activities (Avigad et al., 1996). Thus, it has been proposed that the mechanism of this functional loss is modification of lysine-rich nucleotide- and actin-binding regions of the myosin molecule (Ramamurthy et al., 2001).

Aging and oxidative modifications of actin and myosin

As discussed earlier, in aged muscle, there is a reduction in the fraction of myosin heads in the strong-binding structural state, such that there are fewer myosin-actin interactions capable of generating force (Lowe et al., 2001). In addition, a significant age-related inhibition of myosin ATPase, critical for generating force, was reported from investigations of isolated proteins (myosin and actin) from young and old animals (Prochniewicz et al., 2005). Thus, mechanisms that decrease or interrupt the interaction of myosin and actin are likely to explain the age-related reduction in force-generating capacity.

One mechanism that may play a role in the age-related decline in force generation is oxidative damage to myofibrillar proteins (suggested above with myosin glycation). The results of several investigations suggest that actin and myosin have protein-specific differences in susceptibility to oxidation. For instance, while cysteine oxidation was increased in myosin, actin cysteine oxidation was not altered with age (Prochniewicz et al., 2005). In a systematic study of *in vivo* oxidative modifications of myosin and actin and aging, both nitration and the formation of HNE (4-hydroxy-2-nonenal) adducts were evaluated (Thompson et al., 2006). HNE is a reactive aldehyde that originates from the peroxidation of membranes and forms a mixture of adduct types on the side-chains of cysteine, lysine, and histidine through a Michael-type nucleophilic addition (Davies and Dean, 1997). The protein modifications, HNE (Humphries and Szweda, 1998) and nitrotyrosine (Viner et al., 1999), have been shown to inhibit protein function. The levels of these two markers of oxidative stress, 3NT and HNE, on myosin and actin did not increase with age (Thompson et al., 2006). This finding suggests that accumulation of oxidative damage to these two key myofibrillar proteins does not occur with age.

It should be noted that with aging other oxidative modifications might accumulate and/or a site-specific amino acid modification of critical residues on these proteins could adversely affect function and contribute to muscle weakness. Additionally, an important limitation in the characterization of modified proteins from aged tissue is the fact that the data provide only a snapshot of a dynamic process, as proteins are constantly being synthesized and degraded in most tissues. Lastly, current knowledge about post-translational modification, and the techniques available to measure them, may not permit the quantitative analysis of all potential post-translational modifications of a given protein of interest as well as its functional characterization.

Age-related alterations in protein expression

Another mechanism that may explain age-related muscle dysfunction is a decrease in the interaction of myosin and actin due to a change in stoichiometry between these two critical proteins. Since force generation depends on the interaction of myosin heads with actin molecules, maintaining optimal stoichiometry between myosin and actin is essential. The cellular content of proteins depends on the balance between the two competing processes of protein synthesis and degradation. With aging, there is evidence for decreased myosin heavy chain synthesis rates (Balagopal et al., 1997) and a loss in the regulation of the proteasome, the main protease responsible for degrading myofibrillar proteins (Ferrington et al., 2005; Husom et al., 2004). Thus, changes in rates of synthesis or degradation could lead to protein-specific declines in either actin or myosin content.

The determination of the total MHC and actin content, in both the semimembranosus and the soleus muscles, found age-related decrease in the MHC but not actin content in the semimembranosus only (Thompson, 2006). On the other hand, myosin content was unaffected by age in rat soleus muscle (Thompson, 2006). The significant decline in myosin protein expression in the semimembranosus muscle resulted in a change in the optimal stoichiometry between myosin and actin with age. This change in stoichiometry may decrease the number of active cross-bridges contributing to force generation, providing a mechanism for muscle weakness in the fast-twitch muscle compared to the slow-twitch muscle. Furthermore, the decline in MHC in the semimembranosus muscle supports the age-related muscle specific phenotype (fast-twitch versus slow-twitch discussed earlier). Interestingly, age-related changes of myosin concentration were also indicated as a major determinant of changes in P_0 of human vastus lateralis muscle (D'Antona et al., 2003).

Summary

Clearly, physiological studies detect age-related deterioration of contractility of single fibers. Structural analysis, using EPR, shows structural changes in myosin which are correlated with the age-related decline in force. Biochemical studies reveal age-related molecular changes in myosin and actin, which are most likely due to oxidative modifications. Studies evaluating post-translational modifications of key skeletal muscle proteins reveal modification-specific, protein-specific and muscle-specific changes which are linked to muscle metabolism. Protein expression studies detect age-related changes in stoichiometry of key skeletal muscle proteins in fast-twitch muscle. In view of the muscle-specificity of age-related changes (fast-twitch fibers compared to slow-twitch fibers), understanding of the molecular basis of physiological changes contributing to sarcopenia requires a multidisciplinary experimental approach, in which a specific muscle is studied using a wide range of physiological, biochemical, structural and chemical techniques.

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