Scand J Med Sci Sports 2011: 21: 749–757
doi: 10.1111/j.1600-0838.2011.01377.x

SCANDINAVIAN JOURNAL OF
MEDICINE & SCIENCE
IN SPORTS

Review

Structural, biochemical, cellular, and functional changes in skeletal muscle extracellular matrix with aging

T. W. Kragstrup, M. Kjaer, A. L. Mackey

Institute of Sports Medicine, Department of Orthopedic Surgery M, Bispebjerg Hospital and Center for Healthy Aging, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
Corresponding author: Tue Wenzel Kragstrup, MD, Institute of Sports Medicine, Bispebjerg Hospital, 2400 Copenhagen NV, Denmark. Tel: +45 35 31 60 89, Fax: +45 35 31 39 56, E-mail: t.kragstrup@dadlnet.dk

Accepted for publication 24 June 2011

The extracellular matrix (ECM) of skeletal muscle is critical for force transmission and for the passive elastic response of skeletal muscle. Structural, biochemical, cellular, and functional changes in skeletal muscle ECM contribute to the deterioration in muscle mechanical properties with aging. Structural changes include an increase in the collagen concentration, a change in the elastic fiber system, and an increase in fat infiltration of skeletal muscle. Biochemical changes include a decreased turnover of collagen with potential accumulation of enzymatically mediated collagen cross-links and a buildup of advanced glycation end-product cross-links. Altered mechanotransduction, poorer activation of satellite cells, poorer chemotactic and delayed inflammatory responses, and a change in modulators of the ECM are important cellular changes. It is possible that the structural and biochemical changes in skeletal muscle ECM contribute to the increased stiffness and impairment in force generated by the contracting muscle fibers seen with aging. The cellular interactions provide and potentially coordinate an adaptation to mechanical loading and ensure successful regeneration after muscle injury. Some of the changes in skeletal muscle ECM with aging may be preventable with resistance or weight training, but it is clear that more human studies are needed on the topic.

Muscle mechanical properties deteriorate with normal aging, which contributes to the loss of function and independence in the elderly (Trappe, 2009). The deterioration in muscle mechanical properties is mainly due to a loss of muscle mass and thus of contractile muscle proteins, but the disproportionate larger loss of muscle strength relative to the loss in muscle size suggests that other mechanisms are also involved (Trappe, 2009). While it is likely to be a combination of factors, reviewed recently (Narici & Maffulli, 2010), it is feasible that changes in skeletal muscle extracellular matrix (ECM) also contribute (Trappe, 2009).

The ECM of skeletal muscle is organized into three interconnecting layers: the epimysium surrounding the entire muscle, the perimysium dividing the fascicles within the muscle, and the endomysium surrounding the individual muscle fibers (Borg & Caulfield, 1980). The matrix of all three layers consists primarily of collagenous proteins structured by cross-linking molecules, together with a host of other connective tissue proteins such as elastic fibers, proteoglycans, and different cell types (Trappe, 2009; Narici & Maffulli, 2010). Skeletal muscle ECM is critical for the transmission of force and for the passive elastic response of skeletal muscle, and the structure, biochemical properties, and function of this ECM network demonstrate a dynamic balance among deposition, remodeling, and degradation, particularly in response to mechanical loading (Huijing, 1999; Kjaer, 2004).

The possibility of how muscle ECM could be involved in the age-associated deterioration of skeletal muscle is not clear. A better understanding of these processes could contribute to better preventive measures against deterioration in muscle mechanical properties in the elderly. Despite the importance of the ECM network of the muscle, relatively little information is available on changes in muscle ECM in humans. The majority of the work has been performed in animals, and where similar investigations in humans exist, discrepancies in the outcomes are often present. In light of this, it is clear that there is a pressing need for studies documenting changes to human muscle ECM with aging and, as an extension of this, to what extent exercise can ameliorate unfavorable changes. It is the purpose of this article to review what is reported in the literature on the structural, biochemical, cellular, and functional changes of skeletal muscle ECM with aging, with a particular focus on the collagen component of the ECM, and to identify areas where human evidence in particular is lacking.

Structural changes of muscle ECM with aging

The structural changes in skeletal muscle ECM with aging have primarily been investigated in the main collagenous interstitial component of the ECM.

Methodology

Collagen content of skeletal muscle has been evaluated with the aid of a wide variety of analytical methods, which makes direct comparisons between these studies problematic. To provide another layer of complexity, there are, to date, 28 recognized subtypes of collagen (Gordon & Hahn, 2009), with types I, III, IV, and VI representing the most-studied collagen types in skeletal muscle. In general, the two most common methods to measure collagen content are semiquantitative microscopic assessment of stained muscle cross-sections or a more quantitative biochemical measurement of the content of hydroxyproline. The latter takes advantage of the repeating triplet amino acid sequence of the triple helical structure of collagen, where hydroxyproline often occupies every third residue. However, it is likely that the fibrillar types I and III, with long stretches of uninterrupted triple helix, contain a proportionately higher amount of hydroxyproline than the non-fibrillar type VI collagen, which comprised much shorter triple helical regions. In light of this, it is important to consider the relative contribution of each collagen subtype to the tissue structure when hydroxyproline is used as a measure of collagen content. The microscopic approach requires staining of the collagen component, which can be performed in many ways. Standard histological staining techniques, such as hematoxylin and eosin, picrosirius red, and Gomori's silver impregnation, have all been used where the different chemicals stain the different collagen types in a different manner, although it is likely that type I collagen accounts for most of the collagen observed. Doubt exists, however, as to the specificity of these stains for collagen alone and indeed which collagen types or maturity level are most clearly visible by regular light microscopy, as opposed to polarized light, appears to be somewhat unclear. Further specificity with regard to single collagen types has been achieved by immunohistochemical staining with antibodies specific for collagen types I, III, IV, V, or VI, providing that the antibodies are reliable. Analysis of histological or immunohistochemical staining usually involves capturing images of selected regions (with or without perimysium), which are subjected to computer-generated quantification of the proportion of pixels in the image demonstrating positive staining. While it is impossible to compare the results from such different methods, some general patterns do appear, with the stronger evidence from studies employing several of these methods in the same muscle samples.

Collagen content

Based on a histological analysis of hematoxylin and eosin-stained sections, an early human study (Inokuchi et al., 1975) found that the collagen content of a non-limb muscle, the rectus abdominis, increases with age, peaking in men in their 50s and declining thereafter (measured in individuals up to 80 years of age). A gender difference was, however, evident as no decline with advancing age was observed in the female samples studied. Several studies of animals (Mohan & Radha, 1980; Alnaqeeb et al., 1984; Kovanen et al., 1987; Zimmerman et al., 1993; Gosselin et al., 1998; Hindle et al., 2009; Ramaswamy et al., 2011) have later shown though that the collagen content of limb skeletal muscle if anything increases with age. Rats considered old in these studies were at least 24 months of age, which corresponds to around 60 human years (Pass & Freeth, 1993). Histological approaches evaluating collagen on cryosections in these studies include picrosirius red (Alnaqeeb et al., 1984; Hindle et al., 2009; Ramaswamy et al., 2011), Gomori's silver impregnation (Kovanen et al., 1987), and immunohistochemistry (Kovanen et al., 1987; Hindle et al., 2009; Ramaswamy et al., 2011), while collagen concentration measured as hydroxyproline content of muscle homogenate was used by Alnaqeeb et al. (1984), Gosselin et al. (1998), Mohan and Radha (1980), and Zimmerman et al. (1993). Collagen concentration measured as the concentration of hydroxyproline in whole muscle was observed to be in the region of 30% greater in old rats compared with middle-aged rats (Alnaqeeb et al., 1984; Zimmerman et al., 1993). The area staining positive for picrosirius red relative to the total cross-sectional area observed by histology has been reported to be more than fivefold increased in very old rats (36–38 months) compared with the 3-month-old young rats (Ramaswamy et al., 2011). Alnaqeeb et al. (1984) studied rats of many different ages and analyzed the collagen level in the skeletal muscle of these rats also by picrosirius red histology, absolute collagen content, and collagen concentration (collagen weight measured as hydroxyproline per muscle weight). The increase in picrosirius red staining was observed between 277-day-old and 508-day-old rats and 508-day-old and 758-day-old rats (Alnaqeeb et al., 1984). Interestingly, the absolute collagen content was unchanged between the 188-day-old and the 299-day-old rats, whereas it was reported to increase in senility, while collagen concentration decreased between 188-day-old and 299-day-old rats and then increased again in senility to a level just above that of the 188-day-old rats (Alnaqeeb et al., 1984). The initial decrease in collagen concentration, together with the unchanged absolute collagen content, could be explained by an increase in the size of the muscle fibers between the 188-day-old and the 299-day-old rats. The subsequent increase in both collagen concentration and absolute collagen content

suggest, however, that the amount of collagen between the 288-day-old and the senile rats had increased rather than a decrease in the size of the muscle fibers (Alnaqeeb et al., 1984). A weakness to this theory is that the absolute collagen content measured was from both muscle and tendon, thereby limiting the possibility to draw conclusions directly about skeletal muscle alone. Alnaqeeb et al. (1984) further demonstrated that the increase in collagen levels of skeletal muscle occurs in both the endomysium and the perimysium. One study indicates that the increase in collagen concentration with aging may depend on muscle fiber type, with slow-twitch fibers being most affected (Zimmerman et al., 1993). Gao et al. (2008) studied the epimysium alone by scanning electron microscopy and found no differences between young and old rats in the ultrastructure and thickness of the epimysium or size of the collagen fibrils. In contrast to most of the animal studies, it was demonstrated in humans that intramuscular endomysial collagen, measured as hydroxyproline concentration, was unchanged with age (Babraj et al., 2005; Haus et al., 2007). The reason for this discrepancy is not clear, but it may be related to the different portions of muscle studied. The human studies used muscle biopsies containing primarily endomysial collagen with limited amounts of perimysium and no epimysium, while most of the animal studies used excised whole muscles containing all ECM layers. Taking the animal studies together, it appears that there may be an increase in the collagen content of skeletal muscle with aging. However, it is clear that further work is required to establish whether such a change occurs in humans and in which structural connective tissue layer of the muscle this change occurs. Variation in the perimysium is greater than variation in endomysium and thus is the main ECM contributor to differences in muscle mechanical properties (Purslow, 2001). Whereas the endomysium is crucial for force transmission between muscle fibers, the function of the perimysium is thought to be the coordination of shape change in muscle contraction (Purslow, 2002), and a reduction in this capacity may contribute to the deterioration in muscle mechanical properties with aging.

Aging or reduced activity?

One of the difficulties in studying the effects of aging is the role that inactivity plays in altering the outcome measures. Because older individuals are generally more inactive than younger counterparts, it is often difficult to discern which effects can be attributed to aging per se and which are simply due to altered activity levels (Lazarus & Harridge, 2007). In animals though, two studies (Zimmerman et al., 1993; Gosselin et al., 1998) have demonstrated that exercise training did not prevent the rise in total muscle collagen level shown with aging, while another study (Kovanen et al., 1987) found that trained senile rats in fact had higher concentrations of muscle collagen than the untrained senile rats. These studies indicate that the increase in ECM seen with aging is not just a result of decreasing exercise activity with aging. The role of exercise on ECM quality and extent of cross-linking with age, however, remains to be evaluated.

Injury

One situation in which there undoubtedly is an accumulation of endomysial connective tissue in humans and in rodents is following muscle injury (Mackey et al., 2004; Brack et al., 2007), a feature that is usually resolved as the muscle regenerates. It has shown that existing collagen structures around individual damaged fibers are preserved and that considerable collagen remodeling takes place (Koskinen et al., 2002). It appears thus that remodeling of existing collagen can be accompanied by fibrosis in cases of severe injury, but that effective resolution of fibrosis is required for restoration of muscle mechanical properties to the pre-injury state. In the context of aging muscle, it is possible that incomplete repair with repeated cycles of injury may accumulate and contribute to sarcopenia. Human data on this topic are limited, but recent observations of extensive evidence of ECM remodeling in the skeletal muscle of young healthy men, 30 days after injury, associated with a sustained elevation in numbers of macrophages and satellite cells (Mackey et al., 2011), suggest a combined contribution of the ECM cells and their environment to muscle regeneration. Future efforts to investigate the potential for a role for the ECM in the progression of sarcopenia could prove insightful.

Elastic fibers

Little information is available with regard to ECM components other than collagen, although elastic fibers have been the topic of a single study (Rodrigues & Rodrigues Junior, 2000) where it was suggested that the density of mature elastic fibers in animals shows a progressive increase with age, whereas the amount of resistant oxytalan fibers decreases. The age-related changes of the elastic fiber system promote elasticity and reduce resistance. This could expose the collagen of skeletal muscle to deformation or rupturing, which may compromise the transmission of tensile muscle strength to the tendons. The change in the elastic fiber system promoting elasticity seems to contradict the stiffening effect of the changes in collagen. However, it should be noted that the study by Rodrigues and Rodrigues Junior (2000) is based on microscopic evaluations of tissue sections, and further studies on the elastic component of muscle ECM are clearly required before conclusions can be drawn on the contribution to muscle function with aging.

Fat infiltration

Another structural change in the skeletal muscle ECM with aging is fat infiltration, known as myosteatosis (Harridge et al., 1999; Baumgartner, 2000; Kent-Braun et al., 2000; Taaffe et al., 2009). Kent-Braun et al. (2000) found that the non-contractile area in leg anterior compartment in older subjects was about 15% and was approximately 2.5-fold larger than in young subjects. Taaffe et al. (2009) found that detraining in elderly humans was accompanied by an increase in the amount of skeletal muscle fat infiltration with a concomitant decrease in strength and that these changes could be reversed with retraining. What role the fat infiltration seen with detraining in elderly humans plays in muscle mechanical properties is unknown. It might be due to cytokines and chemokines secreted from the infiltrating adipocytes. Adipose tissue can thus favor a state of inflammation where both adipocytes and resident macrophages contribute with pro-inflammatory cytokines (interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha) and chemokines (monocyte chemotactic protein-1/chemokine (C-C motif) ligand 2) (Neels & Olefsky, 2006). High levels of IL-6, for example, have been found to be associated with loss of muscle strength but not sarcopenia (Schaap et al., 2006).

Biochemical changes in ECM of aging skeletal muscle

The most evident biochemical changes of the skeletal muscle ECM with aging are related to collagen fiber biosynthesis, degradation rates of the different collagen types, and the formation of collagen cross-links.

Collagen subtypes

The collagen types found in skeletal muscle are numerous, and the importance of the individual collagens is clearly evident where mutations in specific collagen types, for example, collagen type VI, occur, even manifesting as myopathies (Myllyharju & Kivirikko, 2004). In addition to disease, exercise and aging have also been observed to result in changes in levels of specific collagen types. In a small group of healthy young individuals, indices of endurance capacity were negatively associated with endomysial content of collagen type III and basement membrane collagen type IV (Mackey et al., 2005). It has been reported that the relative proportion of type I collagen increased and that of type III collagen decreased with age in animals, as measured by immunohistochemical staining (Kovanen & Suominen, 1989; Hindle et al., 2009). This decrease of type III collagen with age was also reported by Goldspink et al. (1994) using in situ hybridization. Furthermore, a recent study by Ramaswamy et al. (2011) found a thickened basal lamina using electron microscopy with increased immunoreactivity for collagen IV and laminin. The various isoforms of collagen have varying strength and functional characteristics (Kjaer, 2004), and thus alterations in the collagen network may very likely contribute to the deterioration in muscle mechanical properties seen with aging. Further study on this topic is required though to understand the significance of changed proportions of collagen subtypes for muscle mechanical properties with aging.

Collagen synthesis

Collagen synthesis has generally been investigated by measuring mRNA expression of specific collagen types, levels of enzymes involved in collagen synthesis, or using the flooding-dose method. The in situ hybridization measure of mRNA is a measure of tissue distribution of collagen gene expression. The flooding dose method is a measure of the collagen synthesis rate measuring the incorporation of isotope-labeled amino acids in body tissues (Laurent, 1982). Kovanen and Suominen (1989) indirectly described an unchanged synthesis of collagen with senescence by looking at the activity of collagen biosynthesis enzymes in animal skeletal muscle. However, Goldspink et al. (1994) found an age-related decline in collagen expression in animal skeletal muscle using in situ hybridization, a finding that was supported by others (Mays et al., 1991) using a flooding-dose method to study collagen turnover in rat tissues. In contrast to the animal studies, a single human study (Babraj et al., 2005) has shown that the rate of skeletal muscle collagen synthesis is greater in elderly men than in young, when using a flooding-dose method to determine collagen synthesis. The animal and human data are thus conflicting. The animal studies (Mays et al., 1991; Goldspink et al., 1994) suggest a decreased collagen synthesis with age, while the only human study (Babraj et al., 2005) that has studied collagen protein synthesis with aging suggests an increased collagen synthesis.

Interventions: feeding, mechanical loading, and hormones

In an investigation of the effect of feeding on muscle collagen synthesis, Babraj et al. (2005) demonstrated that the rates of collagen synthesis remained at postabsorptive values after nutrition provision. The feeding regime consisted of the ingestion of 20 g of an essential amino acids solution during insulin clamping, and muscle biopsies were taken before and 3 h after the start of the study. This suggests that skeletal muscle collagen synthesis is not regulated by feeding, which is in contrast to the stimulating effect that nutrition has on myofibrillar protein synthesis in skeletal muscle (Babraj et al., 2005). It does not appear that nutritional supplementation can be used as a countermeasure to the biochemical changes of skeletal muscle ECM with aging. A recent study (Heinemeier et al., 2009) investigating the role of

loading on muscle and tendon ECM in rats reported that unloading of muscle tissue does not reduce gene expression levels of collagen or collagen-inducing growth factors, whereas loading resulted in enhanced collagen gene expression levels. In addition to this, Kovanen and Suominen (1989) found that muscle collagen synthesis was higher for older trained rats when compared with older untrained rats. Human data are sparse, but a recent study in young men (Holm et al., 2010) demonstrating a robust increase in rates of muscle collagen synthesis following exercise suggests that enhanced muscle collagen synthesis is a positive physiological adaptation to exercise. Furthermore, in a second human study (Doessing et al., 2010), administration of growth hormone resulted in a substantial upregulation of collagen at the mRNA level, a finding that is especially relevant for aging given the dramatic age-associated decline in circulating levels of growth hormone (Corpas et al., 1993). When the animal and human data are taken together, it appears that exercise acts as a positive regulatory stimulus on muscle ECM synthesis. In addition, growth hormone, as one example of a hormonal stimulant, is especially potent. However, the extent of degradation and cross-linking of muscle collagen is equally important in drawing conclusions as to the optimum quantity and quality of muscle ECM components in the elderly.

Collagen degradation

Very few studies have attempted to study both synthesis and degradation, making it difficult to draw any conclusions regarding net gain or loss of collagen with aging. Mohan and Radha (1980) showed that the collagen of skeletal muscle of old animals is less susceptible to collagen-degrading enzymes when compared with young, and that the activity of these enzymes decreases with age. Another animal study (Mays et al., 1991) demonstrated that the proportion of degraded collagen was unchanged with aging, whereas a human study (Babraj et al., 2005) proposed an increase in collagen degradation because of a combined increase in collagen synthesis and unchanged collagen concentration. In a comparison of old and young rat soleus muscle, the most remarkable findings pertained to genes known to have a biological role in the ECM and cell adhesion (Pattison et al., 2003). It was speculated that the down-regulation of these genes in the old rats was more related to the lower activity levels, and that any accumulation of ECM with age is likely to be a result of reduced degradation rather than increased synthesis (Pattison et al., 2003). At a cellular level, it has recently been described that metalloproteinases from fibroblasts are regulated by proteins in the ECM, while gelatinases from myoblasts are regulated by mechanical deformation (Cha & Purslow, 2010). Together, this information suggests that altered exposure to mechanical loading is an important regulating factor in age-related changes in collagen-degradation processes.

Cross-linking

Various animal studies (Palokangas et al., 1992; Zimmerman et al., 1993; Rodrigues et al., 1996; Gosselin et al., 1998) have shown that enzymatically mediated collagen cross-linking in muscle increases with aging, and that physical exercise training seems to reduce the level of these cross-links in old animals (Zimmerman et al., 1993; Gosselin et al., 1998). This suggests that maintaining a low level of collagen cross-linking is beneficial regarding muscle mechanical properties in the elderly, and that this can be accomplished with exercise. In contrast to the findings in the animal studies, two human studies by Haus et al. (2007) and by Takahashi et al. (1995) have demonstrated that enzymatically mediated muscle collagen cross-linking is unchanged with aging, while AGE cross-links on the other hand demonstrate an increase with aging. The accumulation of AGE cross-links with aging can be explained by the longer exposure time of protein residues to glucose, which increases the chances of AGE cross-link formation. Inhibition of the glycation process is currently the subject of intense research (Bailey, 2001). The enzymatically mediated collagen cross-links may not be as important for the deterioration in muscle mechanical properties with aging as first estimated, but this cannot be ruled out entirely. The discrepancy between the human studies and the animal studies might again be a result of the different portions of muscle studied. Taken together, the accumulation of the cross-linking components could contribute to the increased stiffening seen with aging. Stiffening of the ECM could have a substantial effect on the impairment in force generated by the contracting muscle fibers (Bailey, 2001). While the respective roles of enzymatically mediated collagen cross-links and AGE collagen cross-links in aging skeletal muscle remain to be clarified, it appears that the accumulation of AGE cross-links in particular has the potential to exert a negative influence on skeletal muscle mechanical properties. It will be interesting to follow the outcome of future studies investigating the ability of exercise training to attenuate this accumulation in aging muscle.

Cellular changes in ECM of aging skeletal muscle

Other factors of importance include changes in mechanotransduction, satellite cell function, interactions between cells in the ECM, and modulators of the ECM.

ECM signaling to cells

The highly organized network of proteins making up the ECM of skeletal muscle not only performs a vital role in the transmission of force but also serves as an initiator of many signaling pathways in the multitude of cell types residing in the ECM. Exactly how communication between these elements occurs has been closely studied

(Wang et al., 2009). Receptors on cell surface membranes, such as cadherins and integrins, are the contacts through which signals are conducted from the surrounding ECM to the cell nucleus. In this way, fibroblasts, for example, on registration of force from a muscle contraction, conduct the mechanical signals from the cell membrane to the nucleus. Depending on which receptor and pathway are activated, gene expression could be altered to increase or decrease production of collagen or other proteins. In light of this, it can be speculated that any changes in the concentration or type of cell surface receptors with aging would influence the cell's sensitivity to mechanical signals and the efficiency with which these signals are transferred to the nucleus. Ramaswamy et al. (2011) have recently described increased expression of beta1 integrin, but the information about changes in ECM signaling to cells with aging is still sparse. A few studies investigating signaling between ECM and satellite cells are discussed below.

Satellite cells

The influence of age on ligand density has been shown for the satellite cell, another major resident cell type in direct contact with the ECM of skeletal muscle. Activation of satellite cells is essential for successful muscle regeneration, and one of the few satellite cell activating pathways studied in the context of aging is the Delta–Notch pathway. Carlson et al. (2009) has shown that skeletal muscle from men in the eighth decade contains lower levels of the Notch ligand Delta, and correspondingly lower levels of active Notch, when compared with the skeletal muscle of men in the third decade. A further role for cell-ECM interactions in the context of satellite cells in an aging muscle is in renewal of the satellite cell population. It has been proposed that the asymmetric distribution of receptors present on the myofiber and basement membrane sides of the satellite cell envelope exert opposing signals, which become especially relevant under cell division such that the daughter cell in contact with the basal lamina remains as a satellite cell (replenishing the cell pool) while the daughter cell in contact with the host myofiber differentiates and is incorporated into the host fiber as a new myonucleus (Kuang et al., 2008). Thus, because satellite cells are crucial for muscle regeneration, it appears that the cell niche can not only strongly influence the activation of satellite cells (e.g. via Delta–Notch signaling) but also play a major role in determining the myogenic reserve of a muscle by influencing cell renewal. As an example of a specific muscle ECM component influencing skeletal muscle, Marfan's syndrome is worth examining. This is a connective tissue disorder arising out of fibillin-1 deficiency and is associated with skeletal muscle weakness and an inability to improve muscle mass with physical exercise. Mutations in fibrillin-1 in mice lead to heightened levels of muscle transforming growth factor (TGF)-beta signaling, which appears to exert an inhibitory effect on muscle regeneration following injury (Cohn et al., 2007), possibly through the negative regulatory effects of TGF-beta on satellite cells (Carlson et al., 2009). Blocking TGF-beta improved muscle morphology and enhanced muscle regeneration (Cohn et al., 2007), highlighting the importance of the release and regulation of cytokines, such as TGF-beta, by the ECM.

Inflammatory cells

Other cell types in direct contact with ECM in skeletal muscle include macrophages, endothelial cells, pericytes, and adipocytes. Macrophages play an important role in muscle regeneration by removing damaged material and interacting with satellite cells, encouraging their activation and providing protection from apoptosis (Sonnet et al., 2006). Furthermore, it has been suggested that the different cell populations of muscle ECM are not randomly situated in relation to each other (Christov et al., 2007), and that they actively interact with each other to optimize regeneration (Abou-Khalil et al., 2010). Positioning and movement of cells in the ECM appears thus to be vital for cells to receive and dispatch signals. This aspect of the ECM has not received much attention in the context of aging skeletal muscle. Chemotaxis and migration of macrophages through the ECM requires matrix metalloproteinase (MMP) activity to make a path through the matrix. Alterations in the types of MMP and the relative proportions of their substrates with aging would thus influence muscle regeneration after injury. Indeed, Shavlakadze et al. (2010) reported poorer chemotactic and delayed inflammatory responses for injured muscle of older mice. It should be noted, however, that a very good myogenic response was observed to follow this delayed inflammatory response (Shavlakadze et al., 2010), underlining that old muscle can regenerate muscle well, albeit at a slower rate.

Matricellular proteins

Most of the components of skeletal muscle ECM promote cell adhesion. There is, however, another group of ECM proteins, known as matricellular proteins, of which tenascin-C deficient (TNC) is a member. In contrast to the adhesion-promoting ECM proteins, the role of tenascin-C deficient is in de-adhesion, the process of disassembly of focal adhesion complexes, which is believed to promote a more favorable environment for cell motility, survival and repair (Murphy-Ullrich, 2001; Fluck et al., 2008). Tenascin-C deficient has shown to be strongly upregulated at the protein and gene level in heavily loaded human and animal muscle (Fluck et al., 2000; Crameri et al., 2007; Mikkelsen et al., 2009; Mackey et al., 2011) and around atrophying type II fibers (Schoser et al., 1999). Fluck et al. (2008) further showed that TNC-deficient mice demonstrate an accentuated

atrophy of type II fibers, underlining the importance of this ECM protein in maintenance of muscle with aging and repair after injury. In this respect, modulation of the ECM plays an important role in regulation of aging skeletal muscle through cell-matrix interactions.

Functional changes in skeletal muscle and its ECM with aging

The functional changes in skeletal muscle ECM have been studied not only regarding stiffness but also regarding active tension, isometric force, power, and lateral transmission of force.

Stiffness, active tension, isometric force, and power

Several animal studies (Alnaqeeb et al., 1984; Kovanen & Suominen, 1988; Rodrigues et al., 1996; Gosselin et al., 1998; Gao et al., 2008) have shown that the stiffness of skeletal muscle increases throughout life. Gao et al. (2008) specifically found a stiffening of the epimysium in particular. Alnaqeeb et al. (1984) found that older muscles showed a greater decline in active tension with aging, and Haus et al. (2007) described reductions in peak isometric force and peak power. While it is difficult to isolate the effect of the different changes of the skeletal muscle ECM on these functional changes, Alnaqeeb et al. (1984) found that the increase in stiffness correlated with the increase in collagen concentration, suggesting a positive relationship. Both Zimmerman et al. (1993) and Gosselin et al. (1998) showed increased stiffness and increased collagen cross-linking with aging. Haus et al. (2007) tentatively concluded that AGE cross-links might be the biggest contributor to the deterioration in muscle mechanical properties with aging. Overall, it appears likely that the changes in skeletal muscle ECM contribute to deterioration in function of the aging muscle, but the relative contributions of the various changes are difficult to identify from present knowledge.

Lateral transmission of force

A recently published study has demonstrated changes in the lateral transmission of force with aging (Ramaswamy et al., 2011). They studied both longitudinal and lateral force transmission in young rats (3 months of age), old rats (30–33 months of age), and very old rats (36–38 months of age). The lateral transmission of force was decreased in old rats compared with young rats and in very old rats compared with old rats (Ramaswamy et al., 2011). The lateral transmission of force has been proposed to protect the skeletal muscle fibers against contractile damage and to be important for the subsequent healing (Bloch & Gonzales-Serratos, 2003). A decrease in the lateral transmission of force could thus contribute to the increased vulnerability to injury and to the slower healing after muscle damage seen with aging. Ramaswamy et al. (2011) found that the same loss of lateral force transmission in mdx mice and furthermore demonstrated an acquired loss of dystrophin expression and a thickening of the basal lamina in very old rats. This suggests that the impaired lateral force transmission and possible deterioration in muscle mechanical properties could be a result of changes in the interaction between the skeletal muscle fibers and the skeletal muscle ECM with aging.

Conclusion

The ECM of skeletal muscle is critical for both force transmission and the passive elastic response of skeletal muscle. The literature suggests that muscle ECM changes with age, and that the changes in skeletal muscle ECM contribute to the deterioration in muscle mechanical properties observed with aging (Fig. 1). The results can be difficult to interpret as studies diverge, but it is clear that more human studies are needed on the topic to draw conclusions about the relationship between

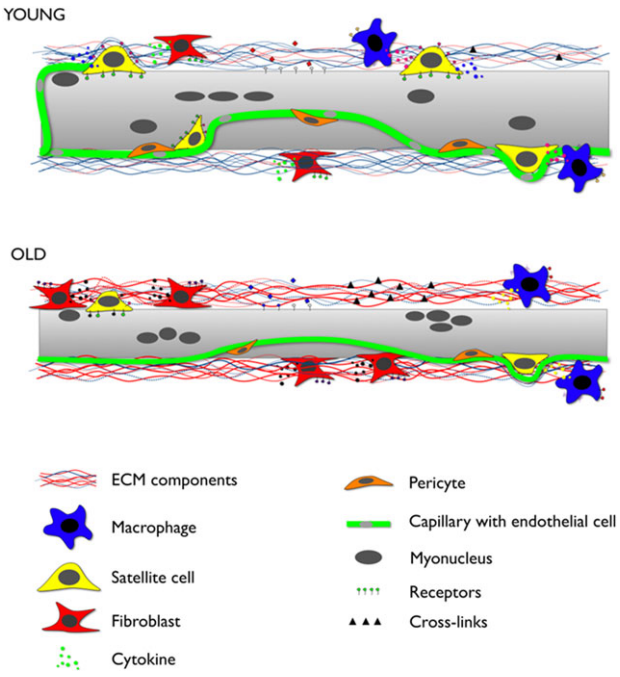

Fig. 1. Illustration of the changes in skeletal muscle extracellular matrix with aging. Structural, biochemical, and cellular changes contributing to the increased muscle stiffness and loss of muscle function with aging. Collagen concentration and the enzymatically mediated collagen cross-links seem to increase, and AGE cross-links appear to build up as a possible consequence of decreased collagen degradation. Changes in the amount of collagen and the relative proportions of different collagen subtypes, for example, type I and type III may also occur with aging. Furthermore, there seems to be an altered mechanotransduction, a poorer activation of satellite cells, poorer chemotactic and delayed inflammatory responses, and a change in modulators of the extracellular matrix.

age-associated changes in muscle ECM and the decline in the quality of muscle mechanical properties in the elderly. Furthermore, the potential of preventive measures, such as exercise training, to counteract these unfavorable changes represents an unexplored area of research, which may provide important insight into the progression of sarcopenia.

Key words: aging, muscle, skeletal, extracellular matrix, exercise.

Acknowledgements

Funding is gratefully acknowledged from MYOAGE (nr. 223576) funded by the European Commission under FP7 and from Nordea Foundation (Healthy Aging grant).

References

Abou-Khalil R, Mounier R, 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 2010: 9: 1–5.

Alnaqeeb MA, Al Zaid NS, Goldspink G. Connective tissue changes and physical properties of developing and ageing skeletal muscle. J Anat 1984: 139 (Pt 4): 677–689.

Babraj JA, Cuthbertson DJ, Smith K, Langberg H, Miller B, Krogsgaard MR, Kjaer M, Rennie MJ. Collagen synthesis in human musculoskeletal tissues and skin. Am J Physiol Endocrinol Metab 2005: 289: E864–E869.

Bailey AJ. Molecular mechanisms of ageing in connective tissues. Mech Ageing Dev 2001: 122: 735–755.

Baumgartner RN. Body composition in healthy aging. Ann N Y Acad Sci 2000: 904: 437–448.

Bloch RJ, Gonzales-Serratos H. Lateral force transmission across costameres in skeletal muscle. Exerc Sport Sci Rev 2003: 31: 73–78.

Borg TK, Caulfield JB. Morphology of connective tissue in skeletal muscle. Tissue Cell 1980: 12: 197–207.

Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 2007: 317: 807–810.

Carlson ME, Suetta C, Conboy MJ, Aagaard P, Mackey A, Kjaer M, Conboy I. Molecular aging and rejuvenation of human muscle stem cells. EMBO Mol Med 2009: 1: 381–391.

Cha MC, Purslow PP. The activities of MMP-9 and total gelatinase respond differently to substrate coating and cyclic mechanical stretching in fibroblasts and myoblasts. Cell Biol Int 2010: 34: 587–591.

Christov C, Chretien F, Abou-Khalil R, Bassez G, Vallet G, Authier FJ, Bassaglia Y, Shinin V, Tajbakhsh S, Chazaud B, Gherardi RK. Muscle satellite cells and endothelial cells: close neighbors and privileged partners. Mol Biol Cell 2007: 18: 1397–1409.

Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin II type 1 receptor blockade attenuates TGF beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med 2007: 13: 204–210.

Corpas E, Harman SM, Blackman MR. Human growth hormone and human aging. Endocr Rev 1993: 14: 20–39.

Crameri RM, Aagaard P, Qvortrup K, Langberg H, Olesen J, Kjaer M. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. J Physiol 2007: 583: 365–380.

Doessing S, Heinemeier KM, Holm L, Mackey AL, Schjerling P, Rennie M, Smith K, Reitelseder S, Kappelgaard AM, Rasmussen MH, Flyvbjerg A, Kjaer M. Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. J Physiol 2010: 588: 341–351.

Fluck M, Mund SI, Schittny JC, Klossner S, Durieux AC, Giraud MN. Mechano-regulated tenascin-C orchestrates muscle repair. Proc Natl Acad Sci U S A 2008: 105: 13662–13667.

Fluck M, Tunc-Civelek V, Chiquet M. Rapid and reciprocal regulation of tenascin-C and tenascin-Y expression by loading of skeletal muscle. J Cell Sci 2000: 113 (Pt 20): 3583–3591.

Gao Y, Kostrominova TY, Faulkner JA, Wineman AS. Age-related changes in the mechanical properties of the epimysium in skeletal muscles of rats. J Biomech 2008: 41: 465–469.

Goldspink G, Fernandes K, Williams PE, Wells DJ. Age-related changes in collagen gene expression in the muscles of mdx dystrophic and normal mice. Neuromuscul Disord 1994: 4: 183–191.

Gordon MK, Hahn RA. Collagens. Cell Tissue Res 2009: 339: 247–257.

Gosselin LE, Adams C, Cotter TA, McCormick RJ, Thomas DP. Effect of exercise training on passive stiffness in locomotor skeletal muscle: role of extracellular matrix. J Appl Physiol 1998: 85: 1011–1016.

Harridge SD, Kryger A, Stensgaard A. Knee extensor strength, activation, and size in very elderly people following strength training. Muscle Nerve 1999: 22: 831–839.

Haus JM, Carrithers JA, Trappe SW, Trappe TA. Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle. J Appl Physiol 2007: 103: 2068–2076.

Heinemeier KM, Olesen JL, Haddad F, Schjerling P, Baldwin KM, Kjaer M. Effect of unloading followed by reloading on expression of collagen and related growth factors in rat tendon and muscle. J Appl Physiol 2009: 106: 178–186.

Hindle AG, Horning M, Mellish JA, Lawler JM. Diving into old age: muscular senescence in a large-bodied, long-lived mammal, the Weddell seal (Leptonychotes weddellii). J Exp Biol 2009: 212: 790–796.

Holm L, van Hall G, Rose AJ, Miller BF, Doessing S, Richter EA, Kjaer M. Contraction intensity and feeding affect collagen and myofibrillar protein synthesis rates differently in human skeletal muscle. Am J Physiol Endocrinol Metab 2010: 298: E257–E269.

Huijing PA. Muscle as a collagen fiber reinforced composite: a review of force transmission in muscle and whole limb. J Biomech 1999: 32: 329–345.

Inokuchi S, Ishikawa H, Iwamoto S, Kimura T. Age-related changes in the histological composition of the rectus abdominis muscle of the adult human. Hum Biol 1975: 47: 231–249.

Kent-Braun JA, Ng AV, Young K. Skeletal muscle contractile and noncontractile components in young and older women and men. J Appl Physiol 2000: 88: 662–668.

Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev 2004: 84: 649–698.

Koskinen SO, Ahtikoski AM, Komulainen J, Hesselink MK, Drost MR, Takala TE. Short-term effects of forced eccentric contractions on collagen synthesis and degradation in rat skeletal

muscle. Pflugers Arch 2002: 444: 59–72.
Kovanen V, Suominen H. Effects of age and life-long endurance training on the passive mechanical properties of rat skeletal muscle. Compr Gerontol A 1988: 2: 18–23.
Kovanen V, Suominen H. Age- and training-related changes in the collagen metabolism of rat skeletal muscle. Eur J Appl Physiol Occup Physiol 1989: 58: 765–771.
Kovanen V, Suominen H, 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 1987: 248: 247–255.
Kuang S, Gillespie MA, Rudnicki MA. Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell 2008: 2: 22–31.
Laurent GJ. Rates of collagen synthesis in lung, skin and muscle obtained in vivo by a simplified method using [3H]proline. Biochem J 1982: 206: 535–544.
Lazarus NR, Harridge SD. Inherent ageing in humans: the case for studying master athletes. Scand J Med Sci Sports 2007: 17: 461–463.
Mackey AL, Brandstetter S, Schjerling P, Bojsen-Moller J, Qvortrup K, Pedersen MM, Doessing S, Kjaer M, Magnusson SP, Langberg H. Sequenced response of extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. FASEB J 2011: doi: 10.1096/fj.1010-176487
Mackey AL, Donnelly AE, Roper HP. Muscle connective tissue content of endurance-trained and inactive individuals. Scand J Med Sci Sports 2005: 15: 402–408.
Mackey AL, Donnelly AE, Turpeenniemi-Hujanen T, Roper HP. Skeletal muscle collagen content in humans after high-force eccentric contractions. J Appl Physiol 2004: 97: 197–203.
Mays PK, McAnulty RJ, Campa JS, Laurent GJ. Age-related changes in collagen synthesis and degradation in rat tissues. Importance of degradation of newly synthesized collagen in regulating collagen production. Biochem J 1991: 276 (Pt 2): 307–313.
Mikkelsen UR, Langberg H, Helmark IC, Skovgaard D, Andersen LL, Kjaer M, Mackey AL. Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. J Appl Physiol 2009: 107: 1600–1611.
Mohan S, Radha E. Age-related changes in rat muscle collagen. Gerontology 1980: 26: 61–67.
Murphy-Ullrich JE. The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? J Clin Invest 2001: 107: 785–790.
Myllyharju J, Kivirikko KI. Collagens, modifying enzymes and their mutations in humans, flies and worms. Trends Genet 2004: 20: 33–43.
Narici MV, Maffulli N. Sarcopenia: characteristics, mechanisms and functional significance. Br Med Bull 2010: 95: 139–159.
Neels JG, Olefsky JM. Inflamed fat: what starts the fire? J Clin Invest 2006: 116: 33–35.
Palokangas H, Kovanen V, Duncan A, Robins SP. Age-related changes in the concentration of hydroxypyridinium crosslinks in functionally different skeletal muscles. Matrix 1992: 12: 291–296.
Pass D, Freeth G. The rat. ANZCCART News 1993: 6: 1–4.
Pattison JS, Folk LC, Madsen RW, Childs TE, Booth FW. Transcriptional profiling identifies extensive downregulation of extracellular matrix gene expression in sarcopenic rat soleus muscle. Physiol Genomics 2003: 15: 34–43.
Purslow P. The intramuscular connective tissue matrix and cell-matrix interaction in relation to meat toughness. Proc Int Cong Meat Sci Technol 45th Yokohama Japan 2001: 210–219.
Purslow PP. The structure and functional significance of variations in the connective tissue within muscle. Comp Biochem Physiol A Mol Integr Physiol 2002: 133: 947–966.
Ramaswamy KS, Palmer ML, van der Meulen JH, Renoux A, Kostrominova TY, Michele DE, Faulkner JA. Lateral transmission of force is impaired in skeletal muscles of dystrophic mice and very old rats. J Physiol 2011: 589 (5): 1195–1208.
Rodrigues CJ, Rodrigues Junior AJ. A comparative study of aging of the elastic fiber system of the diaphragm and the rectus abdominis muscles in rats. Braz J Med Biol Res 2000: 33: 1449–1454.
Rodrigues CJ, Rodrigues Junior AJ, Bohm GM. Effects of aging on muscle fibers and collagen content of the diaphragm: a comparison with the rectus abdominis muscle. Gerontology 1996: 42: 218–228.
Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am J Med 2006: 119: 526 e9–526 e17.
Schoser BG, Faissner A, Goebel HH. Immunolocalization of tenascin-C in human type II fiber atrophy. J Mol Neurosci 1999: 13: 167–175.
Shavlakadze T, McGeachie J, Grounds MD. Delayed but excellent myogenic stem cell response of regenerating geriatric skeletal muscles in mice. Biogerontology 2010: 11: 363–376.
Sonnet C, Lafuste P, Arnold L, Brigitte M, Poron F, Authier FJ, Chretein F, Gherardi RK, Chazaud B. Human macrophages rescue myoblasts and myotubes from apoptosis through a set of adhesion molecular systems. J Cell Sci 2006: 119: 2497–2507.
Taaffe DR, Henwood TR, Nalls MA, Walker DG, Lang TF, Harris TB. Alterations in muscle attenuation following detraining and retraining in resistance-trained older adults. Gerontology 2009: 55: 217–223.
Takahashi M, Hoshino H, Kushida K, Inoue T. Direct measurement of crosslinks, pyridinoline, deoxypyridinoline, and pentosidine, in the hydrolysate of tissues using high-performance liquid chromatography. Anal Biochem 1995: 232: 158–162.
Trappe T. Influence of aging and long-term unloading on the structure and function of human skeletal muscle. Appl Physiol Nutr Metab 2009: 34: 459–464.
Wang N, Tytell JD, Ingber DE. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. Nat Rev Mol Cell Biol 2009: 10: 75–82.
Zimmerman SD, McCormick RJ, Vadlamudi RK, Thomas DP. Age and training alter collagen characteristics in fast- and slow-twitch rat limb muscle. J Appl Physiol 1993: 75: 1670–1674.