

Microcirculatory Changes and Disuse Are Cause of Damage to Muscle Fibres During Aging

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Abstract

Aging induces histological, enzyme-histochemical and ultrastructural changes in skeletal muscle fibres as atrophy, myopathic alterations and damage of the fibre type regulation.

Reduction of the physical activity induces fibre atrophy and mitochondrial alterations with decline in mitochondrial oxidations. Muscle fascicles are reduced in size and perifascicular fatty infiltration is evident. Some fibres show degeneration and necrosis. Preferential atrophy of type 2 fibres is evident in the oldest subjects.

Changes in almost all ultrastructural components of muscle fibres are evident. Occasional derangement of the myofibrillar apparatus, rod formation, numerical reduction and alterations of mitochondria, increase in sarcoplasmic lipid droplets and proliferation of endogenous lysosomes are frequently observed. Microvascular changes in aging muscle are characterized by thickening and duplication of the basement membrane, and by increased thickness of the muscular layer in small arteries.

These alterations result in loss of oxygen flux and energy supply, which induce degenerative myopathic changes in the aging muscle.

Key words: aging, human skeletal muscle, morphology, morphometry.

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In the last decade about forty studies have been published in English journals on skeletal muscle aging. They mainly focus on biochemical, molecular and physiological aspects of the rat muscle fibres. Studies on human muscle in aged subjects have been rarely performed.

A decrease in muscle mass and strength, and a slowing muscle contraction are common features in the human aging. The reduction in muscle mass is predominantly accounted by loss in fibre volume and only partially by fall in fibre number [17].

Experimental studies stressed that the age-related reduction in physical activity and the effects of hypoxia on enzyme activities in skeletal muscle could be important factors in the decline of mitochondrial function [8, 28] and introduced the concept of failure of aging skeletal muscle by accumulation of various mutations in mitochondrial DNA [9, 11, 34].

Despite their importance, clinical signs and symptoms as pain and stiffness are not good indicators of muscle damage. The morphological and morphometric analysis are currently considered the gold standards used to verify and quantify muscle damage.

The present study results from previous morphological and morphometric analysis of the aging human muscle, in which muscle fibre atrophy, changes in fibre type distribution and intramuscular microcirculatory alterations have been described [29, 33].

The purpose of this study is to examine the mechanisms by which muscle fibres may be damaged and have undergone myopathic degenerative changes in the context of the age-related skeletal muscle atrophy.

Methods

Fifty-three healthy and sedentary male subjects, aged 30-89 (years), with no metabolic, ischemic and neuromuscular diseases were selected for this study.

They were divided into four age groups: 30-50 (8 cases), 65-70 (20 cases), 71-80 (15 cases), 81-89 years (10 cases). Open biopsies were taken from the left vastus lateralis muscle under local anaesthesia during surgical reduction of a recent femoral fracture.

Serial transverse sections of paraffine embedded muscle specimens were stained with hematoxylin and eosin, and with immunohistochemical stain for CD34 to identify capillary and small blood vessels.

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Serial transverse cryostatic sections of muscle biopsies cooled in liquid nitrogen, were treated for alkaline myosin ATPase at pH 9.4, DPNH-diaphorase, cytochrome C oxydase and acid phosphatase activities.

Small muscle specimens were fixed in glutaraldehyde-paraformaldehyde mixture in 0.1 % sodium cacodylate buffer. Epoxy-resin embedded ultrathin muscle sections were stained with uranyl acetate and lead citrate and were observed with a Zeiss EM electron microscope.

Quantitative evaluation of fibre diameter, fibre types, cytoplasmic lipid droplets and mitochondria were performed with an automatic Interactive Image Analysis System-IBAS I, II (Kontron, Bildanalyse, Munich, Germany) on light and electron microscopic micrographs.

Diameter area and percentage area were selected for the automatic measurements (calculated in microns and in microns square for the area). Statistical analyses were performed by means of the IBAS I System. Basic statistic data as standard deviation (SD) were also reported. To compare fibre type diameter and mitochondrial size in the studied groups, an analysis of variance was performed.

Results

The cases were divided into four age groups. The mean age of the studied subjects, the mean of the muscle fibre diameter, type I fibre type percentage and the percentage of cases showing myopathic and microcirculatory changes are reported in the Table 1.

Fibre diameter and fibre type composition

In the 30-50 age group, vastus lateralis muscle fiber morphology and composition is normal.

The mean fibre diameter is 67,9 mm and the histochemical fiber characterisation shows 35.3% of type I fibres, as usually reported in the vastus lateralis muscle from healthy young subjects [20].

In the 61-70 age group there is no significant reduction in the mean fibre diameter, but type I fibre predominance with type II fibre atrophy becomes apparent (fig 1B). Morphological fascicles are considerably reduced in size but perifascicular alterations in capillaries and small blood vessels are very rare.

In the 71-80 and 81-89 age groups, a significant reduction in the fibre diameter is observed.

Muscle fatty and connective infiltration is evident. Some fibres show lipid storage in cytoplasmic vacuoles.

The number of type I fibres is predominant and atro-

phy of type II fibres is evident in all the studied biopsies (fig 1C and 1D). In these age groups, myopathic structural changes of muscle fibres and microcirculatory lesions are seen in 35 and 70% and in 54 and 70%, respectively.

Structural fibre alterations and myopathic changes

Fibres with myopathic changes are present in 71-80 and 81-89 age groups in different percentages (8% and 14% respectively).

Histological changes as internal nuclei in adult muscle fibres, fibre degeneration and vacuolation, necrosis and mononuclear cell infiltration and phagocytosis are generally considered myopathic.

Fibres positive for acid phosphatase activity reveal activation or proliferation of endogenous lysosomes which are considered an expression of degeneration.

The presence of degeneration or necrosis of muscle fibres (fig 1A) and positive fibres for acid phosphatase activity is seen exclusively in the older age groups.

Ultrastructural features of fibre degeneration are consistent in the 71-80 and 81-89 age groups. Derangement of myofibrillar apparatus, enlargement of sarcoplasmic reticulum and the presence of sarcoplasmic lysosomes and lipid vacuoles are ultrastructural indicators of fibre degeneration. The changes consisted in disorganisation of myofilaments and streaming of Z band material (Fig 2B), rod formation and presence of subsarcolemmal aggregation of parallel tubules containing thin granules. These fibres show frequently internal pyknotic nuclei and sarcolemmal irregular papillary projections containing lysosomes, lipid droplets and glycogen particles (fig 2A). Groups of lysosomes are frequently seen in subsarcolemmal infoldings and between normal or disarrayed myofibrils (fig 2C).

Mitochondria

Morphometric analysis demonstrated that the size and the amount of mitochondria vary with age. In Table 2, morphometric data and variance analysis on muscle mitochondria in the aging man are reported. There is a significant reduction in mitochondrial size and amount per fibre area with aging. Major morphological changes in mitochondria are represented by the development of intracrystal plates and changes in the matrix density. Small abnormal mitochondria with simple or complex intracrystal paracrystalline inclusions are observed (fig. 2D). A

Table 1. Histological and enzyme-histochemical changes in M. Vastus lateralis with age. Statistical significance: * $P < 0.01$; ** $P < 0.001$.

Group (years)	Number of cases	Mean age (years)	Mean fiber diameter	%type I fibres	Myopathic changes number (%) of cases	Capillary changes number (%) of cases
30-50	8	42.0	68.0 $\pm$ 7.2	35.3 $\pm$ 4.2	0	0
65-70	20	67.2	66.0 $\pm$ 5.6	64.6 $\pm$ 4.6	1 (5)	2 (10)
71-80	15	76.0	46.6 $\pm$ 8.2	70.2 $\pm$ 5.8	5 (35)	8 (54)
81-89	10	85.6	41.6 $\pm$ 6.2	74.6 $\pm$ 8.0	7 (70)	7 (70)

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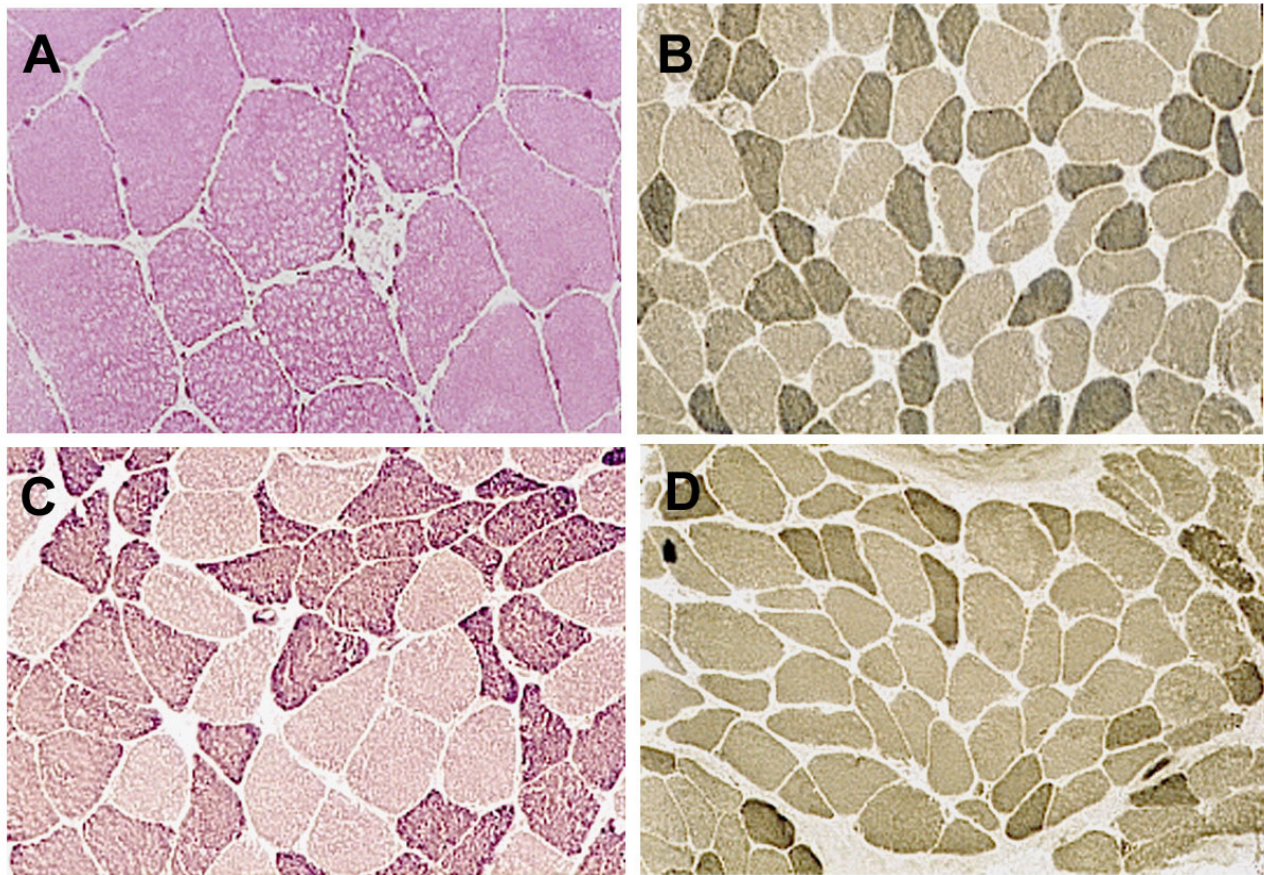


Figure 1. A, a necrotic fibre with sarcoplasmic disgregation between some atrophic vacuolated and normal fibres. Vastus lateralis muscle. Subject aged 84 years. H&E, X 400; B, normal fibre type distribution in vastus lateralis muscle with preferential type 2 fibre atrophy Type 2 fibres are dark. Subject aged 67 years. Myosin ATPase pH 9.6, X 150; C, type 2 fibre atrophy. Some fibres show angular appearance. Subject aged 68 years. Myosin ATPase pH 9.6, X 160; D, numerous type 1 muscle fibres and interspersed rare atrophic type 2 fibres. Subject aged 86 years. Myosin ATPase pH 9.6, X 150.

Table 2. Morphometric data and variance analysis on muscle fibre types, lipid droplets and mitochondria in the aging man.

Group (years)	Number of cases	Mean fibre diameter ($\mu\text{m} \pm \text{SD}$)		% lipids/fibre area $\pm \text{SD}$	Mean mitochondrial size ($\mu\text{m} \pm \text{SD}$)	% mitochondria/fibre area $\pm \text{SD}$
		Type I	Type II			
30-50	8	65.26 $\pm$ 7.2	70.69 $\pm$ 8.36	0.50 $\pm$ 0.2	0.14 $\pm$ 0.09	2.53 $\pm$ 0.3
60-70	9	64.64 $\pm$ 5.8	68.01 $\pm$ 5.21	1.09 $\pm$ 0.5	0.13 $\pm$ 0.08	2.27 $\pm$ 0.8
71-80	13	47.88 $\pm$ 8.9	45.56 $\pm$ 9.02	1.52 $\pm$ 0.4	0.10 $\pm$ 0.06	2.04 $\pm$ 0.5
81-89	8	43.48 $\pm$ 8.5	39.59 $\pm$ 6.56	2.72 $\pm$ 2.5	0.09 $\pm$ 0.05	1.97 $\pm$ 0.5

Analysis of variance (F test) between the age groups.

Parameter: Type I fibre diameter, $F=10.70$ $P<.05$ Type II fibre diameter, $F=14.66$ $P<.001$ Mitochondrial size, $F=3.98$ $P<.025$

focal alteration associated with fibre degeneration is re-orientation of intramyofibrillar mitochondria so that their long axis coincided with the long axis of the muscle fibre.

Lipid droplets

The results of morphometric analysis of lipid droplets percentage per fibre area are reported in Table 2. Cytoplasmic lipid accumulation in form of round or oval vacuoles containing osmyophilic material is frequently

observed mainly in the 71-80 and 81-89 age groups. Vacuoles are observed among the myofibrils or beneath the plasma membrane, sometimes interspersed between lysosomes and glycogen granules.

Morphometric analysis of lipid vacuoles per fibre area (Table 1) shows a significant increase in advanced age, particularly in the 81-89 age group.

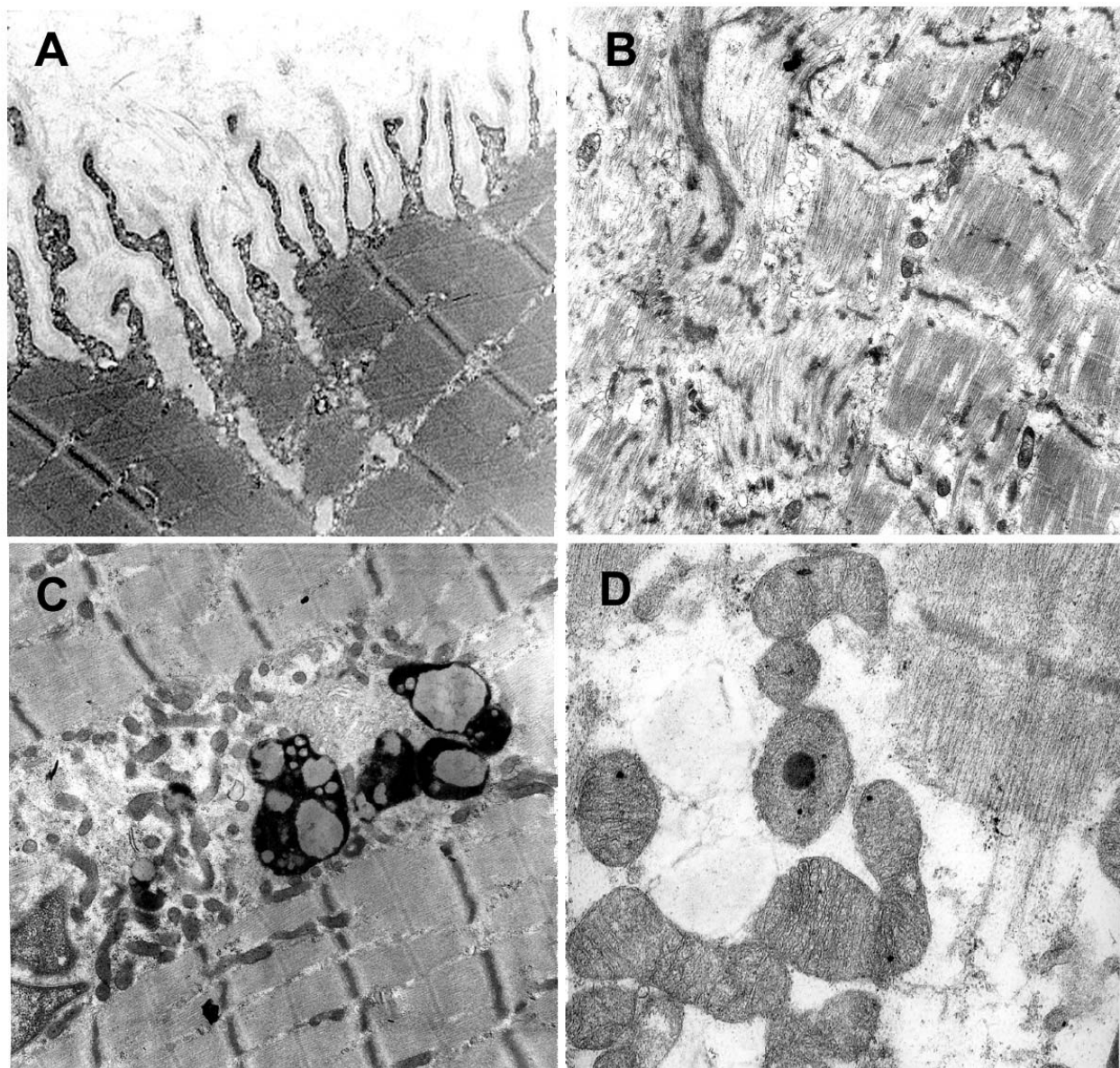


Figure 2. A, sarcolemmal irregular projections in an atrophic muscle fibre. Vastus lateralis muscle. Subject aged 65 years. Electron microscopic micrograph, X 4000; B, Disarrayed myofibrils with streaming of Z line in a degenerating muscle fibre. Subject aged 76 years. Electron microscopic micrograph, X 6000; C, groups of lysosomes and small mitochondria between normal myofibrils. Subject aged 76 years. Electron microscopic micrograph, X 6000; D, small mitochondria with abnormal appearance and intracrystal inclusions. Subject aged 70 years. Electron microscopic micrograph, X 18000.

Capillaries

Morphometric analysis show progressive reduction in intramuscular capillaries with age. Morphological changes in blood vessels, such as arteriosclerosis and thickening and reduplication of the capillary basement membrane (fig 3A and 3B) are frequent in the 71-80 and 81-89 age groups. The capillary lumen is not generally reduced, but the endothelial cells show an increase in the number of pinocytotic vesicles. The small muscular arteries of the oldest subjects show an increased thickness

of the media, with focal deposition of dense, hyaline material between smooth muscle cells.

Discussion

Morphological studies on skeletal muscle in the aging man are very rare and they mainly concern the histochemical and ultrastructural aspects of senescent fibres [20, 23, 29, 33].

Interesting results have been obtained in a recent histological and ultrastructural post-mortem study in extraocular muscles from aging men. All patients over 65 years

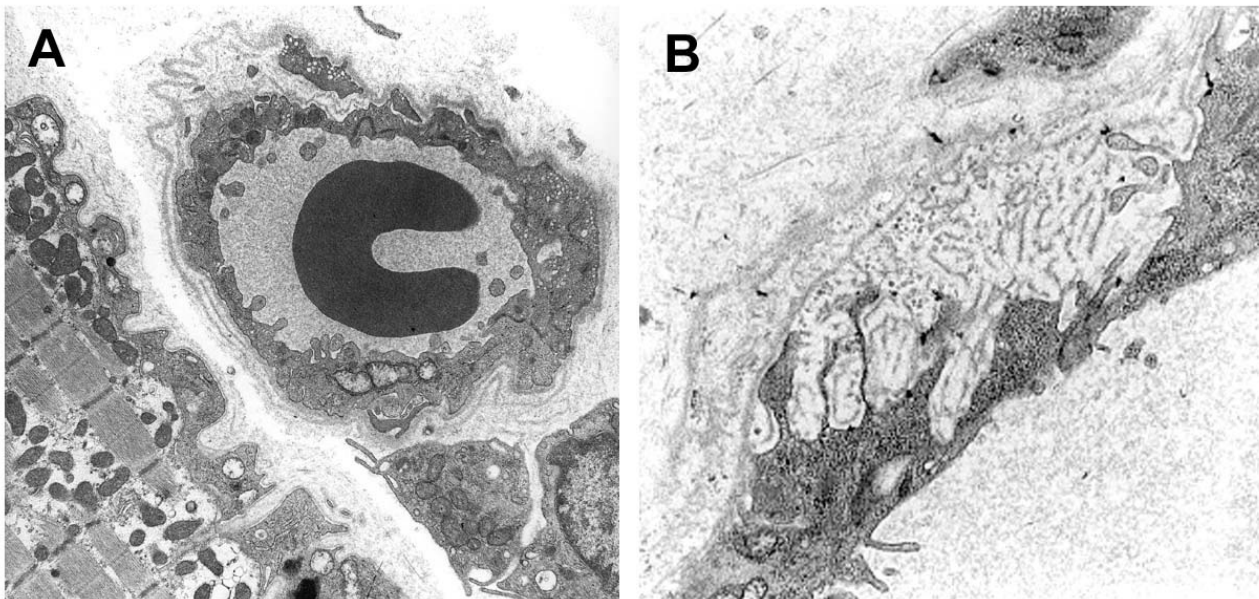


Figure 3. A, intramuscular capillary with irregular basement membrane tickening. Electron microscopic Micrograph, X 4000; B, at the high magnification, reduplication of the capillary basement membrane is evident. Subject aged 84 years. Electron microscopic micrograph, X 10000.

had definite age-related changes and subjects between 70 and 80 years revealed muscle fibre atrophy and variation in fibre size, with increase of fibrous and adipose interstitial tissues. Moreover, they have evident myopathic changes including cytoplasmic vacuolation, cytoplasmic bodies and increased number of ringbinden, similar to those observed in the present study on limb muscles [23].

The present study describe the morphological and ultrastructural characteristics of aged human muscle in absence of overt metabolic, vascular and neuromuscular diseases.

Particular attention was paid to the study of fibre atrophy and of degenerative myopathic changes, and to their possible relation with senile microvascular alterations of muscle.

Different basic morphological changes characterise the aging skeletal muscle: increasing of muscle fibre atrophy and predominant involvement of type II fibres; incidental patterns of denervation; myopathic damage of muscle fibres; structural changes in the myofibrillar apparatus and reduction in mitochondrial percentage and volume, and progressive intramuscular microcirculatory lesions. The fibre atrophy in aging muscle may be due to a reduction in physical activity and disuse [8]. These changes are probably strictly related to reduced mitochondrial function in older subjects.

In the last years, experimental biochemical and genetic studies revealed age-associated mitochondrial alterations in rat skeletal muscle. The decline in the respiratory chain activity and in mitochondrial oxidations, and the accumulation of mitochondrial DNA deletions are probably major determinants of age-related muscle alterations [7, 11, 24, 27, 34].

In humans, incidental denervation patterns are probably a consequence of subclinical peripheral nerve alterations or of some effects of aging on the motor unit [13, 31].

The type I fibre predominance observed in older subjects is directly related to the type II atrophy, decrease we found in most elderly subjects.

Fibre type composition and plastic properties of aging muscle have been predominantly studied in experimental rat studies.

The type II fibre atrophy resembles the one observed in rat, in which the analysis of several hindlimb muscles revealed differences of fibre properties within the muscles and confirmed the influence of aging and hypoxia on the different fibre types, with atrophy predominantly confined to type II fibres [30].

It is well documented in both animal and human studies that aging may cause alterations in muscle fibre contractile and cytoskeletal components. Effects of aging on enzyme histochemical, morphometrical and contractile properties of the rat soleus muscle and skeletal muscle fibre number and composition in different muscles of aging rats have been studied [3, 4, 14]. Particular attention was paid to the effects of hypoxia on muscle metabolism and mitochondria in skeletal muscle of rats of different ages [7, 27, 28]. Conclusive hypothesis on muscle damage in old age is controversial. The disuse associated with biochemical and malnutritional factors, the accumulation of mitochondrial DNA mutations, circulatory disturbances and neurogenic phenomena were taken in account [9, 18].

The role of chronic ischemia in the age-related muscle fibre changes

It is long recognised that microcirculatory changes worsen with advancing age as well as age-related arteriosclerotic changes in peripheral small vessel [1-32]. In the present study, the reduction in the intramuscular capillary number with advancing age, and a slight increased thickness of the capillary basement membrane raised the suspicion that the exchange processes may be impaired, independently from any haemodynamic factor [5, 33].

Results from experimental acute and chronic ischemia after temporary blood vessel ligation and after in vivo uncoupling of oxidative phosphorylation revealed alterations in the structure and distribution of muscle capillaries, with variable myofibrillar and mitochondrial changes [15, 19, 22, 25], similar to those seen in human aged muscle. Ischemic changes after temporary aortic ligation in rats occurred in type II earlier than in the type I fibres [19], as in old man.

Finally, in the last decades, numerous biochemical studies on the age-related ischemic muscle damage have been performed. Decline with age of the respiratory chain activity in human skeletal muscle was recently demonstrated [6, 7] and impairment of mitochondrial respiratory chain function and presence of mitochondrial DNA deletion in human aging muscle were seen [10-11]. The correlation between oxidative metabolic reduction and the decrease in mitochondrial percentage per fibre area we observed in the oldest subjects may suggest an adaptation of muscle fibres to a reduced metabolic demand [29].

All studies confirmed that the chronic reduction in muscle blood supply, seemingly an occurrence in aged subjects, results in muscle fibre variable suffering and degeneration with predominantly ultrastructural changes in the myofibrillar apparatus and mitochondria.

The final cell damage process occurs via one of few common final pathways, as loss of the cellular energy supply, loss of the intracellular calcium homeostasis and over-activity of oxidising free radical-mediated reactions. Aged skeletal muscle fibres frequently showed Z line changes, as smearing and streaming which are considered as indicators of the activity of calcium-dependent proteases [2], and ultrastructural changes in sarcoplasmic reticulum which may reflect possible alterations in the calcium-related mechanisms inducing muscle fibre degeneration in the aged subjects.

The present hypothesis is supported by data from the effects of ischemia on cardiac tissue. It was suggested that loss of the fibre membrane control during ischaemia or anoxia increases the cell permeability with consequent energy shortage and progressive fall in the cell energy reserves [16, 26], with final cell damage through damage of sarcoplasmic reticulum calcium pumps and impairment of calcium homeostasis [2].

The results are an important contribution for studies on dietary, endocrine, pharmacological and physical treatments of elderly aged subjects.

Conclusions

The skeletal muscle fibres in the old man show constant histological, enzyme histochemical and ultrastructural changes, indicating at least two main processes in age-related muscle fibre modification.

The first age-related process is the muscle disuse that induce a progressive fibre atrophy. The decline in muscle mass is not entirely accounted for by a fall in muscle fibre number, but it is secondary to volume loss. The reduction in the physical activity and the genetic aging of cells play the major contribution to the muscle atrophy, to the decline in mitochondrial oxidations and to the impairment of the fibre type regulation with reduction of type II fast fibres in old aged subjects.

The second age-related process is the muscle hypoxia due to impairment of blood exchanges secondary to microvascular alterations and to reduction in muscle capillarity demonstrated in senescent muscle.

The loss of an adequate oxygen flux and of the energy supply to the fibres, the increase in intracellular calcium content after sarcoplasmic reticulum alterations and after activation of lysosomal processes, and the production of free radicals within muscle, have been probably cause of the degenerative damage of the aging skeletal muscle [21].

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