Age-related morphometric characteristics of human skeletal muscle in male subjects

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Objective: The purpose of the present study is to investigate the age-related changes in muscle biopsies from the quadriceps femoris in male subjects of different ages.
Methods: A histological and histochemical study was performed on specimens from the quadriceps femoris from 8 males divided into two groups, under 50 and over 70 years of age. The following measurements were performed: a) number of type 1 and 2 fibres, b) diameter of type 1 and 2 fibres, c) percentage of the number and mean diameter of the two types in the interior and the peripheral area of fascicles.
Results: The proportion of type 2 fibres decreased significantly with age ($p < 0.005$), especially in the periphery of the fascicles, but the proportion of type 1 fibres was not significantly changed. The correlation of fibre size with age showed that type 2 fibres decrease in size with age. This finding was more evident in the periphery of the fascicles ($p < 0.05$).
Conclusion: We found that type 2 skeletal muscle fibres decreased in size and proportion with increasing age. The existence of these age changes should be taken into account in the interpretation of muscle biopsies of aged individuals.

Key words: morphometry, histochemistry, muscle biopsy, age, fibre type.

Introduction

The earliest studies on ageing and muscle fibre composition suggested that type I fibre percentage increases with ageing [1, 2]. Others, particularly Grimby and Saltin, were in disagreement [3]. They examined muscle biopsies of persons aged up to 66 years and found no age-related changes in type 1 distribution. In addition, gender-related alterations of muscle function have been reported with increasing age but there are very few data on gender-related morphological changes in ageing individuals [2, 5]. The existence of these age and gender changes should be taken into account in the interpretation of muscle biopsies of aged individuals and must be investigated in detail.

The purpose of the present study is to investigate the age-related changes in muscle biopsies from the quadriceps femoris in male subjects of different ages. Our aim was to determine whether ageing was associated with quantitative changes in fibre size, fibre type composition and fibre type distribution of the two fibre types in and around the muscle fascicle.

Material and methods

A histological and histochemical study was performed on specimens from the quadriceps femoris taken during autopsy on 8 males who had died suddenly. The patients were divided into two groups. The first one contained 4 patients under the age of 50 and the second group comprised 4 men over the age of 70. The age of the subjects varied from 17 to 82 years.

No subject had a history of neuromuscular or other disease that could have a direct or indirect effect on the peripheral nervous system or the skeletal muscles. The samples were taken immediately post mortem.

Cryostat 10 μm sections were stained for routine (pH 9.4) ATPase. We performed ATPase staining using a modified protocol described by Dubowitz and Brooke [6]. It has been shown that in autopsy material a clear definition of the two fibre types is possible [7]. With the aid of an automatic image analysis system, Image-Pro Plus (Version e4,5.1-Media Cybernetic) the following measurements were performed: a) number of type 1 and 2 fibres, b) diameter of type 1 and 2 fibres, c) percentage of the number and mean diameter of the two types in the interior and the peripheral area of fascicles. Typically, at least 400 fibres were analysed for each section. The size of muscle fibres was assessed by measuring the "smallest fibre diameter" [6]. The findings for each muscle were evaluated in relation to age group.

For the statistical analysis, Student's t-test and linear regression, as well as Spearman's non-parametric method and analysis of variance (ANOVA) were applied.

Results

Proportion of the two fibre types in and around the muscle fascicle

The proportion of type 2 fibres decreased significantly with age ($p < 0.005$), but the proportion of type 1 fibres was not significantly changed (Table I). A clearly greater proportion of type 2 fibres was found in the periphery of the fascicles ($p < 0.001$) in the elderly group. The difference in percentage between internal and peripheral type 2 was 8.8.

Mean diameter of two fibre types in the peripheral and the internal part of the fascicle

The mean diameter of both type 1 and type 2 was smaller in the periphery of the fascicle, although the difference was not significant (Table II).

The correlation of fibre size with age showed that the type 2 fibres decrease in size with age both in the peripheral and the internal part of the fascicle. This finding was more evident in the periphery of the fascicles ($p < 0.05$) (Table III).

Discussion

Skeletal muscle fibre changes have been reported in ageing humans. However, the age-related patterns of changes in muscle fibres are inconclusive. Some report an increase of type 1 muscle fibres with increasing age [8-11], whereas others report insignificant changes [12, 13] or a decreased area with increased age [14]. Our findings were consistent with those of the cross-sectional studies which showed reduced number and mean area of type 2 muscle fibres during ageing [8, 10, 15-18]. The underlying mechanism of these changes is not clear. It could be a result of the reduced activity of elderly people, although selective involvement of motor neurons, particularly the larger motor neurons innervating type 2 fibres, cannot be excluded [19].

An apparent "grouping" of the muscle fibre types has been observed and these data confirm those of studies in older human skeletal muscles [20]. The mechanisms for the obvious grouping of the fibre types in the ageing human muscle still needs to be fully understood. Presently, the most evident explanation seems to be that the fibre type grouping arises from a continuous process of denervation and partial reinnervation that is claimed to accelerate with advancing age [21]. When we compared our two age groups, we found a greater proportion of type 2 but the difference tended to decrease with age, and that is in accordance with the findings of Sjöström *et al.* [22]. The proportion of two histochemical types of fibres varies systematically within the muscle. A greater proportion of type 2 fibres has been observed in the superficial part [8]. It has been suggested that this phenomenon could be the result of adaptation of the various parts of the muscle to different functional demands. It is known that in the flight muscles of several avian species a ring composed almost entirely of large type 2 fibres surrounds each fascicle. In non-flight muscles the distribution is random [23]. The influence of local factors in the histochemical organization of the fascicle cannot be ignored. It has been suggested that the greater amount of collagen surrounding the fascicle in comparison with the thin layer of collagen around individual fibres creates different mechanical conditions that modulate the physiological and histochemical properties of muscle

Table I. Mean proportion of the two muscle fibre types in the peripheral and the interior part of the fascicle (pm)

Age group	Periphery		Interior	
	type I	type II	type I	type II
group I	37.6	62.4	44.4	55.6
group II	37.1	48.5	43.7	39.7

pm – postmortem

Table II. Mean diameter of the two muscle fibre types in the peripheral and the interior part of the fascicle (pm)

Age group	Periphery		Interior	
	type I	type II	type I	type II
group I	43.8	36.5	46.1	39.7
group II	42.6	35.3	45.2	38.7

pm – postmortem

Table III. Correlation of fibre type diameter with age

Age group	Periphery		Interior	
	type I	type II	type I	type II
group I/ group II	$p = 0.003$	n.s	n.s.	$p = 0.005$

n.s. – no significant

fibres [24]. The role of local factors could also be supported by our findings of reduced muscle fibre diameter in the periphery of the fascicle. This could be indicative of vascular involvement, since perifascicular atrophy in dermatomyositis is considered to be a result of vasculopathy [25]. Blood flow to skeletal muscle is a potentially important factor in the reduction of muscle function associated with ageing (sarcopenia). Reduced capillary density, less maximal blood flow, and a slower hyperaemic flow response have been reported in some, but not all, studies [26]. The possibility cannot be excluded that the histochemical organization of the central and the peripheral part of the muscle fascicle is the result of a combination of the above or other factors. The matter could be further elucidated by a combined physiological, histochemical and biochemical investigation of strictly selected groups of subjects.

The gender-related morphological changes in muscle fibre composition are controversial and there is a small number of references [2, 5]. Fayet *et al.* in their study showed a decrease of the proportion of type 2 fibres in males without significant changes in their size [27]. Similar were the findings of Larsson *et al.* in their study of biopsies from the vastus lateralis muscle of male subjects from 22 to 65 years of age [19]. The differences between males and females may be partially related to gender differences in muscular activity.

In conclusion, we found that type 2 skeletal muscle fibres decreased in size and proportion with increasing age. The differences in the size and shape of the fast-twitch fibres may be a result of decreasing muscle activity, as lack of physical activity or regular exercise may result in age-related disuse atrophy. Training programmes must systematically manipulate training frequency, intensity, and duration to be effective and to improve endurance at any age. The existence of these age and gender changes should be taken into account in the interpretation of muscle biopsies of aged individuals.

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