Arq Neuropsiquiatr 2003;61(3-A):555-560

THE EFFECTS OF AGING ON *BICEPS BRACHII* MUSCLE FIBERS

A morphometrical study from biopsies and autopsies

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ABSTRACT - *Objectives*: In order to study the morphology and size of muscle fibers, cross sections of *biceps brachii* samples from autopsies, up to 9 hours after death, and biopsies of 72 subjects were compared. The subjects aged 13 to 84 years in both sexes. *Methods*: The samples obtained from autopsies (n=47) were from subjects with sudden death, or who died after acute disease without evidence of neuromuscular involvement. The biopsies (n=25) were from patients with symptoms suggestive of inflammatory or metabolic myopathy, not confirmed morphologically. The lesser diameter of muscle fibers was measured using the ATPase reaction. *Results*: Morphological analysis showed that aging changes were present from the sixth decade in autopsies, and consisted of atrophy and/or type-grouping. The statistical models adjusted for females in both autopsies and biopsies were linear straight with no variation in fiber size with increasing age. The models adjusted for males in both groups were quadratic, indicating that age influenced the size of different type fibers. In males type 2 were larger than type 1 fibers, and than fibers in females. *Conclusions*: These values might be useful as controls, helping interpretation of changes in fiber size in samples obtained from biopsies and autopsies.

KEY WORDS: *biceps brachii*, morphometry, aging, autopsy, muscle biopsy.

Efeitos do envelhecimento sobre as fibras do músculo biceps braquial: estudo morfométrico em biópsias e autópsias

RESUMO - *Objetivos*: Para estudar a morfologia e o tamanho das fibras musculares, foram comparadas cortes transversos do bíceps braquial autopsiados, até 9 horas após o óbito, com biopsias musculares, em 72 indivíduos de ambos os sexos e idades entre 13 e 84 anos. *Método:* As amostras das autópsias (n=47) foram obtidas de indivíduos que morreram subitamente, ou após uma doença aguda sem evidência de comprometimento neuromuscular. As biópsias (n=25) foram obtidas de pacientes com sintomas sugestivos de miopatias inflamatória ou metabólica, não confirmadas morfologicamente. O diâmetro menor das fibras foi obtido usando a reação de ATPase. *Resultados:* A análise morfológica mostrou que as mudanças induzidas pelo envelhecimento estiveram presentes a partir da sexta década para autópsias e consistiu de atrofia e grupamento de tipo. O modelo estatístico ajustado para mulheres, para autópsias e biópsias, foi linear e não indicou variação do tamanho das fibras com o aumento da idade. O modelo ajustado para homens, para ambos os casos, foi quadrático, indicando que a idade influenciou o tamanho dos diferentes tipos de fibras. Para homens, as fibras tipo 2 apresentaram-se maiores que as de tipo 1, e maiores que as das mulheres. *Conclusão:* Os valores encontrados podem ser úteis como controles, auxiliando na interpretação de modificações no tamanho das fibras para amostras provindas de biópsia e autópsia.

PALAVRAS-CHAVE: biceps braquial, morfometria, envelhecimento, autópsia, biópsia muscular.

Morphometric data of various types of muscle fibers are very important in the interpretation of changes in their size in biopsies and in autopsies. A number of parameters of fiber size have been used by various investigators in their morphometric studies of muscle, particularly the cross-sectional area[1-4] and the lesser diameter[5-8], or a combination of both[9,10]. It was also observed that most of the

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Received 3 December 2002, received in final form 12 March 2003. Accepted 31 March 2003.

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investigations on cross-sections of human skeletal muscle were made on the *vastus lateralis* and the available quantitative data were based on it[11-13]. For the *biceps brachii* muscle, the most frequently studied for diagnostic purpose, the original study that is the international reference for morphometry, was made by Brooke & Engel[5], in 1969, with biopsies from normal muscle and "muscle from groups of patients who approximate closely to normal".

Significant variations in muscle fiber sizes have been reported on muscles autopsied between 12 and 72 hours after death[10, 14-16]. In mice, Gosdspink et al.[17] suggested that the development of rigor during 5 hours *post-mortem* had no effect on the fiber size in frozen sections. On the other hand, some investigators have found significant differences in quantitative data of samples obtained from biopsies and autopsies[14,18].

Since we could not find any morphometric data on muscle fibers obtained from autopsies less than 12 hours after death, and there are no quantitative studies of skeletal muscle in our region, we aimed, in this study, to compare the variability between muscle fiber sizes in samples obtained from adult human biopsies in which no morphological abnormality was observed, and autopsies up to 9 hours after death, without neuromuscular disorders, in order to determine their size. We also hoped to provide parameters which could be used as control and thus help in the interpretation of changes in size of muscle fibers in biopsies and autopsies.

METHOD

Seventy-two muscle samples obtained from the belly of the left *biceps brachii* of 47 autopsies and 25 biopsies were used in this study. There were 38 females and 34 males, whose ages ranged from 13 to 84 years (autopsy-female - n 24 - mean 41.6 ± 18.57 years; biopsy-female - n 14 - mean 37.5 ± 14.1 years; autopsy-male - n 23 - mean 49.3 ± 18.12 years; biopsy-male - n 11 - mean 30.2 ± 13.8 years). The biopsies were from patients whose symptoms, basically myalgia, suggested inflammatory or metabolic myopathy, which were not confirmed. In autopsies, subjects had sudden death or died after a short acute illness without involvement of the neuromuscular system; their muscles were morphologically normal, or showed mild fiber size variability. Muscles were obtained between 3 and 9 hours after death. In all cases, the muscle samples were immediately frozen in liquid nitrogen. They were oriented in order to obtain transverse sections, which were cut at 10 μm, on cryostat at -20°C. Each section was placed upon a glass cover-slip and stained with Hematoxilin & Eosin and submitted to histochemical reaction to demonstrate myofibrillar adenosine triphosphatase (ATPase) activity incubated at pH 4.3, 4.6 and 9.4. In biopsies, additional staining reactions included periodic acid Schiff (PAS), oil red O, modified Gomori trichrome, and succinic dehydrogenase. In ATPase stained sections, the fiber outlines were traced onto an Image Analyzer Kontron - KS300, Carl Zeiss connected to an IBM-PC computer. In each muscle sample, at least 150 fibers were measured to obtain the lesser diameter. The results were submitted to statistical evaluation applying the regression analysis, and they were assessed comparing the ages grouped in decades.

Morphometric data were grouped according to sex and origin of tissue (autopsy or biopsy). To study the relationship between the size of type 1 and type 2 fibers as well as the age, a multiple regression model was fitted for each group.

The software SAS (version 6.04) was used for the statistical study. The data were analyzed using linear regression techniques. The models were assessed through verification of adjustment and usual assumptions.

Residual plots were used to check these assumptions. If the assumptions were not satisfied we tried to fit a more appropriate model, with quadratic terms.

Regression analysis was used to assert the size of the fibers, assuming a correlation between the fiber size and the age.

Four models, one for each subgroup (autopsy-male; autopsy-female; biopsy-male; biopsy-female), were fitted. Then, more restrict models were fitted, assuming that in each sex, the size of the fibers were equal, i.e., without statistical difference between the models (autopsy-male x biopsy-male) and (autopsy-female x biopsy-female).

If the previous hypothesis is accepted, another model could be tested: that all subgroups are equal. These tests are based on Square Sum of the Residuals[19].

This study was approved by the Ethical Committee of the University Hospital of the School of Medicine of Ribeirão Preto - University of São Paulo.

RESULTS

Morphological data showed that in subjects aged from 13 to 50 years of both groups (autopsy and biopsy), muscle fibers were uniform, with sub-sarcolemmal nuclei and the mosaic arrangement of various staining intensities in myosin ATPase preparations was present (Fig 1). About 50% of these subjects presented a relative predominance of a specific type fiber, such as type 1 fibers for women and type 2 fibers for men (Fig 2). From the sixth decade onwards, some angulated and atrophic fibers as well as type grouping could be found affecting mainly type 2 fibers in both sexes in autopsy and biopsy groups (Fig 3), the latter showing some of those changes in the fifth decade.

Based on morphometric analysis, the data for the male subgroups were quadratic, for all different type

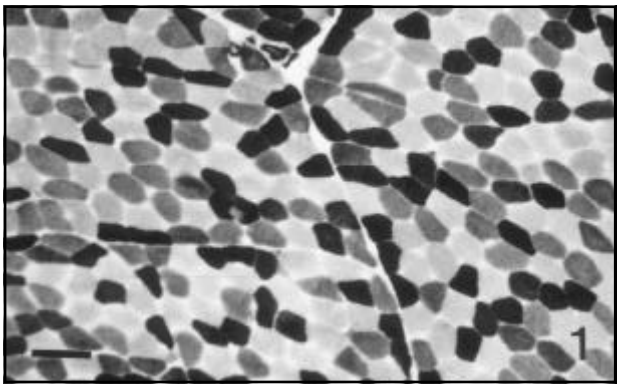

Fig 1. Cross section of autopsied biceps brachii *from a 14 year-old male, showing normal distribution of types 1 (black), 2B (intermediate) and 2A (white) fibers. Myofibrillar ATPase pre-incubation pH 4.6 (bar =100㎜).*

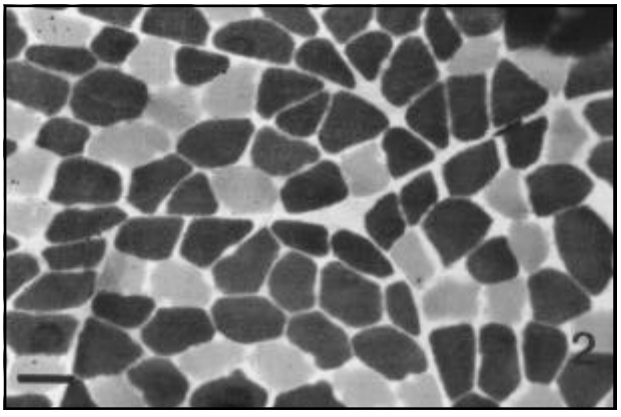

Fig 2. Cross section of a 39-year-old male biceps brachii *muscle from an autopsy subject obtained 6 hours after the death showing a predominance of type 2 fibers (black). Myofibrillar ATPase pre-incubation pH 9.4 (bar=100㎜).*

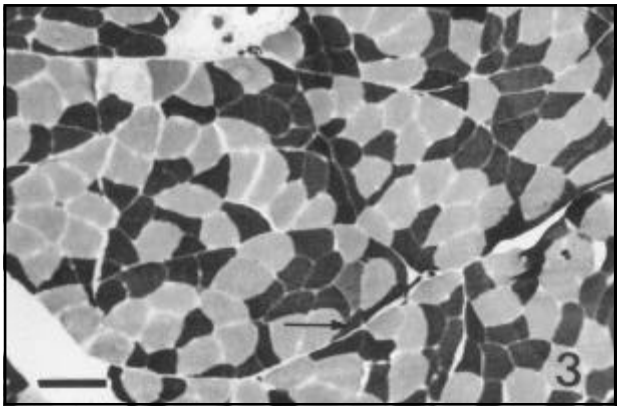

Fig 3. A light micrograph showing a cross section of a 57-year-old male biceps brachii *muscle obtained 3 hours after the death. Note angulated (arrow) and atrophic (arrowhead) fibers, predominantly involving the type 2 fibers (black). Myofibrillar ATPase pre-incubation pH 9.4 (bar =100㎜).*

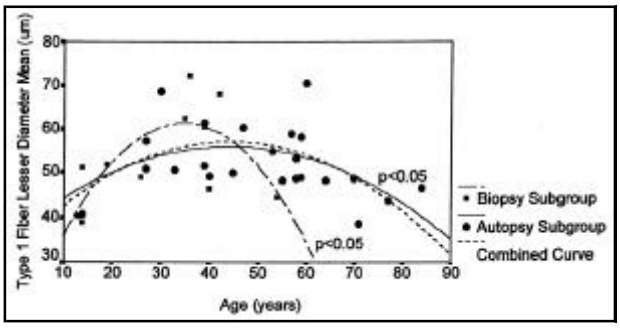

Fig 4. Relationship between age and type 1 fiber sizes (lesser diameter - ㎜) in males.

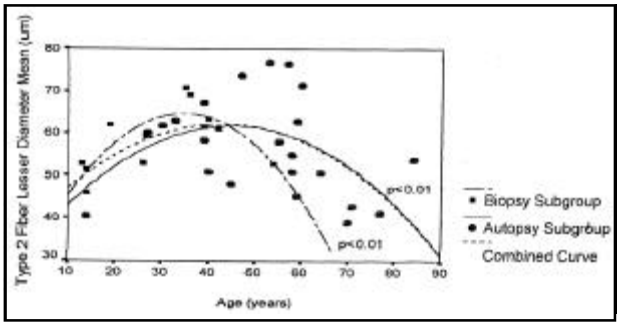

Fig 5. Relationship between age and type 2 fiber sizes (lesser diameter - ㎜) in males.

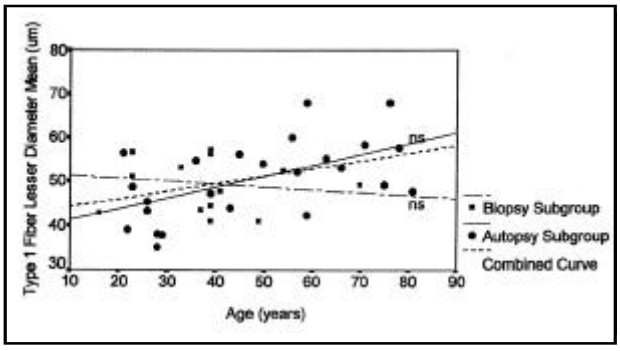

Fig 6. Relationship between age and type 1 fiber sizes (lesser diameter - ㎜) in females.

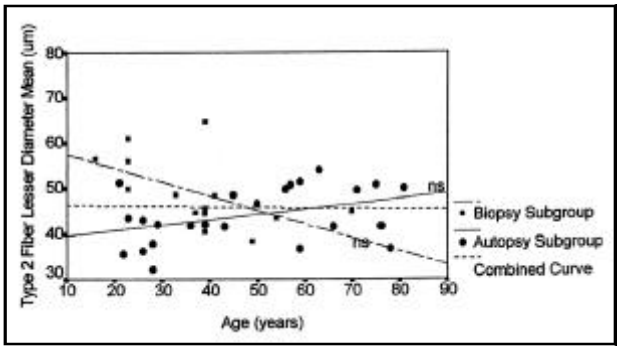

Fig 7. Relationship between age and type 2 fiber sizes (lesser diameter - ㎜) in females.

Table 1. The mean value obtained by aggregated adjustment curve by decade of age and 95% confidence interval in relation to type fibers in males (mm).

Decade	M* (CI)† type 1	M* (CI)† type 2
3rd	41.55 (34.30 – 65.28)	54.92 (36.93 – 72.92)
4th	54.48 (39.27 – 69.69)	59.93 (42.27 – 77.60)
5th	56.75 (41.52 – 71.98)	62.13 (44.45 – 79.82)
6th	56.59 (41.35 – 71.83)	61.53 (43.83 – 79.24)
7th	54.01 (38.77 – 69.24)	58.12 (40.43 – 75.82)
8th	48.99 (33.51 – 64.47)	51.91 (33.94 – 69.89)
9th	41.55 (25.07 – 58.03)	42.90 (23.75 – 62.04)
Mean (SD)‡	52.76 (8.8)	57.35 (10.3)

*M, mean (μm); †CI, 95% confidence interval (μm); ‡SD, standard deviation.

Table 2. The mean value and 95% confidence interval in relation to type fibers in females (mm).

Fibers	N	Mean (SD)	95% Confidence Interval
Type 1	38	50.24 (7.89)	34.78 – 65.71
Type 2	38	46.02 (7.10)	32.12 – 59.91

N, number of subjects.

fibers, indicating that fiber size was influenced by age (Fig 4 and 5). The maximum mean fitted curve (combined curve: autopsies + biopsies subgroups) is achieved at 44.3 years old to type 1 fibers and at 42.8 years old to type 2 fibers, respectively. Thereafter there is an increasing rate of reduction in the lesser diameter of all type fibers. Between 50 and 60 years this reduction is 4.56% and 5.54% for type 1 and type 2 fibers, and between 70 and 80 years it is 15.18% and 17.36% for type 1 and type 2 fibers, respectively.

The mean value and 95% confidence interval (C.I.) for type 1 and type 2 fiber diameters, by decades, in males are shown in Table 1.

In females the results were not significantly different, i.e., the size of the fibers was similar with increasing age (Fig 6 and 7). In both figures, few autopsied patients older than 77 years and younger than 22 years were observed, while few biopsied patients had 55 years or more and 23 years or less. In autopsies, type 1 fibers mean diameter was 50.35 μm, while type 2 fibers mean diameter was 44.47 μm. In biopsies, mean diameters in type 1 and type 2 fibers were 49.60 μm and 49.21 μm respectively.

Taking into account autopsies and biopsies in females together, the mean value and 95% CI of fiber types, was not significantly different (Table 2).

The comparison between male and female mean fiber diameter with the fiber type, showed that type 2 fibers in males were larger than any fiber in females. In the male group, type 2 fibers were always larger than type 1 fibers. In the female group, the larger fibers were type 1 and the difference between type 1 and type 2 fibers was statistically significant ($p<0.05$).

DISCUSSION

Most of the organs and systems suffer physiologic and structural changes with aging, which are determined by genetic, social, cultural, and environmental factors. We obtained a representative sample of the left *biceps brachii* muscle from autopsies and biopsies, from which the local population profile concerning the trophic aspects was traced. Morphometric data on human skeletal muscle in autopsies with such a short interval of 3 and 9 hours after death is not available. The morphological findings induced by aging have been extensively reported by several authors and the pathogenesis of the changes observed is still debated.

The presence of angulated fibers is a condition usually associated with aging[16], which may have manifested early in some subjects due to their daily life activities. In the present study, angulated fibers were rarely observed before the seventh decade of life, when they appeared in 100% of the subjects of both sexes. Isolated type 2 fiber atrophy was observed in both sexes especially from the 6th decade onwards. The preferential involvement of type 2 fibers in aging has been widely studied by numerous authors[2,4,11,15,16,18,20,21]. Type 2 fibers are involved in diverse circumstances as a secondary phenomenon like disuse atrophy, upper motor neuron lesions, myasthenia gravis and steroid therapy[22-24].

In our material the fiber type-grouping was better characterized in samples obtained from autopsies, possibly because the fragments obtained were larger. As for the muscle analyzed, the *biceps brachii* in humans do not usually present fiber type predominance, but may be more susceptible to neurogenic modifications induced by daily life activities, and cervical spondylosis, than the lower limb muscles, such as the *vastus lateralis*, more frequently analyzed in European countries[18].

Based on previous reports mentioned above, we interpreted the morphological changes observed in

our cases as age-related, particularly in biopsies, whose patients were examined clinically and electrophysiologically, and the clinical notes were reviewed in order to discard any sign of a neurogenic process. In autopsies, however, this possibility can not be entirely ruled out, because neurological examination has not always been performed, but reviewing the clinical notes, the macroscopy and the morphological findings in organs such as heart, liver, kidney, lung and brain, no evidence was found of a systemic disease that could lead to a neurogenic atrophy.

Multiple regression analysis allowed the comparison of data obtained in the subgroups, autopsy and biopsy, which better characterized differentiation in fiber size of both sexes. Contrary to the facts pointed by Eriksson et al.[14] and Nygaard & Sanchez[18] who postulated the differentiation in size of skeletal muscle fibers according to the origin of the samples (biopsy or autopsy), this study demonstrated that up to approximately 9 hours after death the changes did not statistically modify the fibers' size. Goldspink et al.[17] proposed that the difference was not significant in the parameters studied up to 5 hours after death in rats; after this period, the fiber undergoes retraction which can reach 15% of the control value. Shorey & Cleland[10] and Braund & Amling[25] in their studies, concluded that there are progressive effects of *rigor mortis* on the muscle fibers, towards swelling, and that the degree of swelling can be related to the interval between the death and the removal of the material[10]. On the other hand, Hergarthy & Hooper[26], in fresh material, proposed that there is retraction of the fiber diameter in sample obtained from necropsies of mice. However, Kirkeby & Garbarsch[21] compared histomorphometric data in the *vastus lateralis* and masseter muscles obtained from autopsies of old, and biopsies of young individuals, and found statistically significant differences between the two muscles but not between the origin of samples (autopsy x biopsy). From these discrepant results it is important to highlight that the interval between the death and the removal of the tissue must be taken into account, because Shorey & Cleland[10] established an interval between 18 and 54 hours after human death, and Hergarthy & Hooper[26] obtained the mice samples 4 hours after death.

In general, the data obtained for type 1 and type 2 fibers in biopsies from both sexes, were respectively contained within the intervals established from the data obtained in autopsies. In the study of Brooke & Engel[5] mean values of lesser diameter for males and females were higher than those observed in our material, but their subjects were younger than ours. Despite the difference, type 2 fibers are larger in males, while type 1 fibers are larger in females.

Studies in which the effects of age on the trophism of muscle fibers were analyzed *post-mortem* in *vastus lateralis* samples, have determined a linear regression model for the fiber area in males[15,16]; the relationship between fiber size and age was not significant, but significant reduction of fiber type 2 size was observed with aging ($p<0.01$). In our study, the regression model adjusted was quadratic and type 2 fibers size tended to reduce with aging, more than type 1 fibers.

In females, the data obtained in the present work were linear, and the relation between fiber size (types 1 and 2) and the age was not significant. From a compilation of various data, Grimby et al.[11] verified a reduction in the area of *vastus lateralis* muscle fibers in female in the 7th and 8th decades compared with data before the 2nd decade. In their studies, the reduction was more severe for type 2 than type 1 fibers up to 80 years old; the estimated reduction values were about 23% for type 2 and 6.8% for type 1 fibers. In our work, it is possible that the number of cases from the 8th decade has still been insufficient, or alternatively, it represents local and occupational factors influencing the maintenance of fiber trophism.

According to Brooke & Engel[5], female fibers should measure 30 to 70 μm and male fibers, 40 and 80 μm. The CI of 95% determined with our data for the various fiber types and sexes, are practically contained in those established by the authors. However, this interval was not maintained in males above 70 and below 30 years, requiring the establishment of an interval of "normality" for this population, at least locally.

We conclude that, for males, the muscle fibers' size changed at various ages, increasing up to about 40 years, decreasing after that. For females, the fibers' size was not statistically modified with aging. The results can be used as a reference for normality in autopsy and biopsy studies.

Acknowledgments - We are grateful to Miss Flávia A. Guedes and to the autopsy technicians for their help collecting the post-mortem specimens. We also thank Dr. Cláudia Sobreira for useful comments on the clinical notes of patients submitted to biopsies and Miss Maria Paula M. Scandar for technical assistance.

REFERENCES

1. Blomstrand E, Celsing F, Fridén J, Ekblom B. How to calculate human muscle fibre areas in biopsy samples: methodological considerations. Acta Physiol Scand 1984;122:545-551.
2. Essén-Gustavsson B, Borges O. Histochemical and metabolic characteristics of human skeletal muscle in relation to age. Acta Physiol Scand 1986; 126:107-114.
3. Glenmark B, Hedberg G, Jansson E. Changes in muscle fibre type from adolescence to adulthood in women and men. Acta Physiol Scand 1992;146:251-259.
4. Poggi P, Marchetti C, Scelsi R. Automatic morphometric analysis of skeletal muscle fibers in the aging man. Anat Rec 1987;217:30-34.
5. Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types: 1. Adult male and female. Neurology 1969;19:221-233.
6. Frontera WR, Meredith CN, O'Reilly KP, Knutt-Gen, HG. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. J Appl Physiol 1988;64:1038-1044.
7. Manfa P, Kalfakis N, Kararizou E, Vassilopoulos D. Size and proportion of fiber types in human muscle fascicles. Clin Neuropathol 1996;15:116-118.
8. Scarpelli M, Montironi R, Tulli D, et al. Quantitative analysis of quadriceps muscle biopsy in systemic sclerosis. Pathol Res Pract 1992;188:603-606.
9. Pernus F; Erzen I. Fibre size, atrophy, and hypertrophy factors in vastus lateralis muscle from 18- to 29-year-old men. J Neurol Sci 1994;121:194-202.
10. Shorey CD, Cleland KW. Morphometric analysis of frozen transverse sections of human skeletal muscle taken post-mortem. Acta Anat 1988;131:30-34.
11. Grimby G, Danneskiold-Samsoe B, Hvid K, Saltin B. Morphology and enzymatic capacity in arm and leg muscles in 78-81 year old men and women. Acta Physiol Scand 1982;115:125-134.
12. Larsson L. Histochemical characteristics of human skeletal muscle during ageing. Acta Physiol Scand 1983;117:469-471.
13. Mahon M, Toman A, Willan PL, Bagnall KM. Variability of histochemical and morphometric data from needle biopsy specimes of human quadriceps femoris muscle. J Neurol Sci 1984;63:85-100.
14. Eriksson PO, Eriksson A, Ringqvist M, Thornell LE. The reliability of histochemical fibre typing of human necropsy muscles. Histochemistry 1980;65:193-205.
15. Lexell J, Taylor CC. Variability in muscle fibre areas in whole human quadriceps muscle: effects of increasing age. J Anat 1991;174:239-249.
16. Lexell J, Taylor CC, Sjöström M. What is the cause of the ageing atrophy? J Neurol Sci 1988;84:275-294.
17. Goldspink G, Gelder S, Clapison L, Overfield P. Pre- and post-rigor fixation of muscle. J Anat 1973;114:1-6.
18. Nygaard E, Sanchez J. Intramuscular variation of fiber types in the brachial biceps and the lateral vastus muscles of elderly men: how representative is a small biopsy sample? Anat Rec 1982;203:451-459.
19. Neter J, Wasserman W, Kutner MH. Applied linear regression models. Boston. Irwin, 1989;3349-3385.
20. Larsson L, Sjödin B, Karlsson J. Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22-65 years. Acta Physiol Scand 1978;103:31-39.
21. Kirkeby S, Garbarsch S. Aging affects different human muscle in various ways: an image analysis of the histomorphometric characteristics of fiber types in human masseter and vastus lateralis muscles from young adults and the very old. Histol Histopathol 2000;15:61-71.
22. Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types: 2. Diseases of the upper and lower motor neuron. Neurology 1969;19:378-393.
23. Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types: 3. Myotonia, myasthenia gravis, and hypokalemic periodic paralysis. Neurology 1969;19:469-477.
24. Banker BQ, Engel AG. Basic reactions of muscle. In Engel AG, Franzini-Armstrong C (eds). Myology. 2. Ed . New York: Mc Graw Hill, 1994;832-888.
25. Braund KG, Amling KA. Muscle biopsy samples for histochemical processing: alterations induced by storage. Vet Pathol 1988;25:77-82.
26. Hegarhty PV, Hooper AC. Sarcomere length and fibre diameter distributions in four different mouse skeletal muscles. J Anat 1971;110:249-257.