Coll. Antropol. **25** (2001) 2: 545–553
UDC 616.74-007.23:612.66
Original scientific paper

Age-Related Skeletal Muscle Atrophy in Humans: An Immunohistochemical and Morphometric Study

M. Nikolić, D. Malnar-Dragojević, D. Bobinac, S. Bajek, R. Jerković and T. Šoić-Vranić

Department of Anatomy, School of Medicine, University of Rijeka, Rijeka, Croatia

A B S T R A C T

The aim of this study was to determine age-related changes in three human muscles with different function and location in the body. The cross sectional area and the percentage of fibers type I, type IIA and type IIX were studied in vastus lateralis, deltoideus and external intercostal muscle. Muscle samples were obtained from 30 male subjects, aged 20–80 years. Fiber types were defined immunohistochemically, using monoclonal antibodies specific for type I, type IIA and type IIX fibers. Cross sectional area of muscle fibers was analyzed morphometrically by computerized image analysis. All muscle fiber types (I, IIA, IIX) showed the reduction in the fiber size in all three examined muscles. In all muscles the proportion of type I and type IIA was changed, but not in type IIX. With increasing age results showed the increase in proportion of type I, while proportion of type IIA fibers decreased, with vastus lateralis muscle being the most affected. These results suggest that age-related muscle atrophy is not a general phenomenon, and does not affect all muscles equally.

Introduction

Skeletal muscle consists of different fiber types characterized by their specific myosin heavy chain (MHC) isoforms. In adult human skeletal muscle, using monoclonal antibodies specific to MHC isoforms, we can distinct three major fiber types: slow contracting fibers, called type I, containing slow myosin heavy chains, and two different types of fast contracting fibers, called type IIA and type IIX which express IIa and IIx myosin heavy chains, respectively[1].

Muscle fibers are dynamic structures capable of altering their phenotype under various conditions such as: increased or decreased neuromuscular activity[2], mechanical loading or unloading[3], altered hormonal profiles[4] and aging[5].

Received for publication September 4, 2001

Human skeletal muscle mass decreases with increasing age, and this aging atrophy is accompanied by a reduction in muscle strenght[6]. Most studies on aging human muscles have focused upon changes in the vastus lateralis muscle. Lexell[7] has shown a loss of muscle mass in humans of about 10% from age 24 to 50 years, with a further 30% loss for 50 to 80 years. The possible explanations for this reduction in mass are morphological and functional changes in muscles. One of the core arguments about the loss of muscle mass with age is a reduction of the volume of individual fibers, a reduction of the total number of fibers, or a combination of both, with type II muscle fibers being the most affected[8]. This could, to some extent be caused by a slowly progressive neurogenic process and an age-related decline in physical activity[9].

Some results indicate that the arm and leg and leg muscles are not affected in the same way. Loss of muscle strength in leg muscles was about 40% compared with 30% in arm muscles, measured between 30 and 80 years of age[10]. Because of such difference, an important question is what histomorphometric differences exist between human skeletal muscles placed on different positions in human body during aging.

The aim of this study was to determine and compare age-related changes of the percentage of slow and fast muscle fibers, fiber type distribution and fiber size in three differently placed human muscles (arm, leg and respiratory muscle). In order to investigate the expression pattern of all myosin heavy chains we used immunohistochemical method and morphometric analysis.

Materials and Methods

The investigation was approved by the ethics committee of School of Medicine, University of Rijeka.

Material

Muscle samples used for this study were obtained from 30 male subjects, aged 20–80 years, who had suffered sudden accidental death. None had a history of neuromuscular disease, nor was there evidence of pathological abnormalities at the *post-mortem* examination. Muscle samples were taken 12–40 h after death from the superficial portion of the vastus lateralis, deltoid and external intercostal muscle.

Vastus lateralis muscle is a powerful extensor of the knee joint. Deltoid muscle is an arm abductor and it is less exposed to the biomechanical load then vastus lateralis muscle. External intercostal muscle is respiratory muscle and functional completely different.

Samples were divided into six age groups: 21–30, 31–40, 41–50, 51–60, 61–70, 71–80 years. The number of subjects in each age group was 5, and from each individual the muscle samples were taken, from three muscles named before.

Preparative procedure

The sample from the vastus lateralis muscle was taken on the border between the middle and distal third of the femur. The deltoid muscle block was cut from the middle of the muscle, and from the external intercostal muscle the block was taken in mid-axillary line from 8^{th} intercostal space. All samples were taken from the right side of the body.

The muscle samples were frozen in isopentane, cooled in liquid nitrogen, and stored at –80 °C until further analysis.

Immunohistochemistry

Monoclonal antibodies specific for type I (BF-F8), IIA (SC-71) and type IIX (BF-35)[11,12] were used in staining a serial of 10 m transverse cryosections for slow and fast myosin heavy chain isoforms. Slides were incubated with monoclonal antibodies (1:1,000) in phosphate-buffered saline (PBS) at room temperature for 30 minutes. After three wash steps in PBS, slides were incubated at room temperature for 30 min with peroxidase-labeled rabbit anti-mouse IgG (Dako A/S, Copenhagen, Denmark) in PBS (1:40). After being washed twice in PBS, the slides were incubated in 50 ml of diaminobenzidine (DAB) solution (0.5 mol/l Tris HCl, pH 7.6, 15 mg of DAB, 100 l of H_2O_2, 25mg of imidasole) at room temperature for 20 min. Finally, the slides were dried and mounted in Canada balsam.

Fiber typing and morphometry

The fiber type frequencies and cross-sectional areas were analyzed by the computer program for quantitative microscope image analysis »SFORM« (VAMS, Zagreb, Croatia). An Olympus BX50 microscope, a Pulnix 765 video camera, and a Sony Trinitron display were used for obtaining images. One thousand fibers of each muscle sample were measured by tracing along the circumference of the fibers. Mean fiber size with standard deviation (SD) was calculated. Statistical evaluations were performed by t-test for independent samples. A probability of 0.05 or less was accepted as statistically significant ($p < 0.05$).

Results

Muscle fiber size

The size of fiber type I in vastus lateralis muscle was greater than the size of fibers type IIA and IIX in all age groups. In deltoid muscle, the size of fibers type IIA and IIX was greater than the size of fibers type I until 50 years of age. After that age, the reduction of size was stronger in fibers type IIA and IIX. So, the size of fibers type I became larger than fibers type II at older age in spite of progressive decreasing of all fibers. In external intercostal muscle the cross sectional area of fibers type IIA and IIX was greater than fibers type I until 40 years of age. After that age, the size of fibers type I compared to fibers type II became larger too (Table 1).

All muscle fiber types (type I, type IIA and type IIX) in vastus lateralis, deltoid and external intercostal muscle showed a reduction in fiber size with increasing age. This reduction, when the oldest (71–80 yrs.) and the youngest (21–30 yrs.) age groups were compared, was always statistically significant ($p < 0.001$). The size of fibers type IIA and type IIX were decreased more than a half size of normal fiber (21–30 yrs.), Table 1).

Percentage of fiber type I, IIA and IIX

Human vastus lateralis muscle in age of 21–30 was composed of type I (32.11 3%), IIA (60.72 4%), and IIX (7.17 1%) muscle fibers. The results showed age-related changes in proportion of type I and type IIA muscle fibers, but not in IIX. In age of 71–80 the percentage of type I muscle fibers increased to 61.42 7%, respectively ($p < 0.001$), and the percentage of type IIA muscle fibers decreased to 30.91 3%, respectively ($p < 0.001$), (Table 2).

In age of 21–30 human deltoid muscle was composed of type I (49.01 4%), IIA (45.69 5%) and IIX (5.30 1%) muscle fibers. With increasing age results showed the increase in proportion of type I (64.97 7%) fibers ($p < 0.01$), while the proportion of type IIA muscle fibers decreased (29.55 5; $p < 0.01$), (Table 2).

Musculus intercostalis externus in age of 21–30 was composed of 42.65 5% of type I, 50.99 4% of type IIA and 6.36 3% of type IIX muscle fibers. In age of 71–80 the percentage of type I fibers increased to 51.60 5%, and percentage of type IIA fibers decreased to 41.80 4% ($p < 0.05$), (Table 2).

TABLE 1
RELATIONSHIP BETWEEN AGE AND MUSCLE FIBER AREA IN VASTUS LATERALIS, DELTOID AND EXTERNAL INTERCOSTAL MUSCLE (MEAN VALUES ± SD)

M. Vastus Lateralis						
Age (years)	Area (m^2)					
	Type I		Type IIA		Type IIX	
21–30	5416.51	918.54	4836.93	828.11	3932.38	418.15
31–40	5121.44	802.22	5001.38	638.12	3222.99	526.44
41–50	4850.52	902.13	3981.72	512.52	3322.26	420.22
51–60	3870.54	651.22	3657.69	451.25	2189.39	351.13
61–70	2926.32	485.62	2437.56	398.22	2050.38	322.14
71–80	2931.61	633.87*	1792.65	450.55*	1715.01	502.18*

M. Deltoideus						
Age (years)	Area (m^2)					
	Type I		Type IIA		Type IIX	
21–30	2862.64	561.65	3160.99	591.55	2910.85	412.25
31–40	2665.79	542.82	2852.47	495.31	2623.54	328.93
41–50	2560.56	502.63	2486.17	325.28	2634.76	321.52
51–60	2465.33	356.21	2339.66	412.58	2364.25	395.84
61–70	1965.58	321.21	1921.84	365.44	1856.44	321.66
71–80	1692.90	419.59*	1222.55	456.52*	1357.69	445.33*

M. Intercostalis Externus						
Age (years)	Area (m^2)					
	Type I		Type IIA		Type IIX	
21–30	2456.43	541.79	2652.32	459.56	2502.43	498.23
31–40	2455.50	413.82	2334.24	469.63	2456.62	463.55
41–50	2340.64	321.25	1896.65	325.26	1988.36	365.22
51–60	1882.97	402.05	1789.95	369.21	1700.55	351.33
61–70	1578.68	352.89	1633.98	395.66	1533.54	312.22
71–80	1471.37	325.95*	1223.99	215.33*	1253.46	321.22*

statistically significant difference as compared to the age group 21–30 ($p < 0.001$)

Shape and distribution of muscle fibers

All muscle fiber types in young and middle aged (20–50 yr.) vastus lateralis, deltoid and external intercostal muscle were distributed in a chessboard fashion (Figure 1), whereas in the age 70 to 80 years fibers type I and type IIA were clustered in groups, respectively. It was seen the area of either slow or fast muscle fibers (Figure 2).

The shape of some muscle fibers was changed too. Hypotrophic, atrophic and irregular fibers of type II were frequent in the aging vastus lateralis and deltoid muscle. Also, in these muscles we have demonstrated the existence of hypertrophic fibers of type I (Figure 3). Atrophic and hypertrophic fibers were not found in external intercostal muscle.

TABLE 2
RELATIONSHIP BETWEEN AGE AND PERCENTAGE OF FIBER TYPES IN VASTUS LATERALIS, DELTOID AND EXTERNAL INTERCOSTAL MUSCLE (MEAN VALUES ± SD)

M. Vastus Lateralis			
Age (years)	Percentage (%)		
	Type I	Type IIA	Type IIX
21–30	32.11 3	60.72 4	7.17 1
31–40	38.05 6	54.16 6	7.79 2
41–50	41.57 5	50.36 3	8.07 3
51–60	47.19 4	46.60 2	6.21 3
61–70	51.02 3	42.91 3	6.07 2
71–80	61.42 7***	30.91 3***	7.67 2
M. Deltoideus			
Age (years)	Percentage (%)		
	Type I	Type IIA	Type IIX
21–30	49.01 4	45.69 5	5.30 1
31–40	50.40 5	44.64 6	4.96 2
41–50	52.13 3	41.83 5	6.04 2
51–60	57.48 6	36.45 4	6.07 3
61–70	58.94 5	34.35 4	6.71 2
71–80	64.97 7**	29.55 5**	5.48 2
M. Intercostalis Externus			
Age (years)	Percentage (%)		
	Type I	Type IIA	Type IIX
21–30	42.65 5	50.99 4	6.36 3
31–40	45.70 6	47.29 5	7.01 3
41–50	48.93 4	44.45 3	6.62 2
51–60	50.27 4	43.87 3	5.86 1
61–70	49.94 6	43.26 5	6.80 2
71–80	51.60 5*	41.80 4*	6.60 2

* statistically significant difference as compared to the age group 21–30 ($p < 0.05$)
** statistically significant difference as compared to the age group 21–30 ($p < 0.01$)
*** statistically significant difference as compared to the age group 21–30 ($p < 0.001$)

Discussion

Our study has shown that the cross sectional area of all muscle fiber types in all three examined muscles continuously is decreasing from the youngest age group to the oldest. Comparing the atrophy of three different muscle fiber types it was seen the atrophy of fast contracting fibers type IIA and IIX is stronger and their size is decreased for approximately 60% at the age 71–80. The size reduction of fiber type IIA is little bit more expressed than of type IIX. The atrophic changes are less expressed in slow contracting fibers type I. The size of fiber type I is decreased in the oldest age for approximately 40% from the youngest

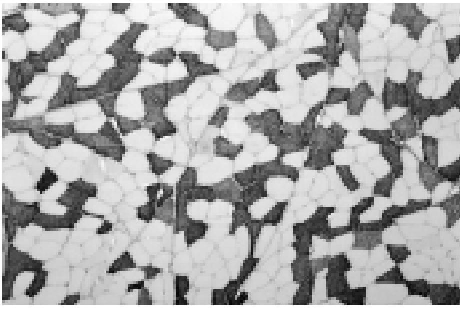

Fig. 1. Deltoid muscle of a male (29 years). Mosaic distribution of muscle fiber types. Fiber types were defined immunohistochemically using monoclonal antibody specific for type IIA fibers (dark); 100.

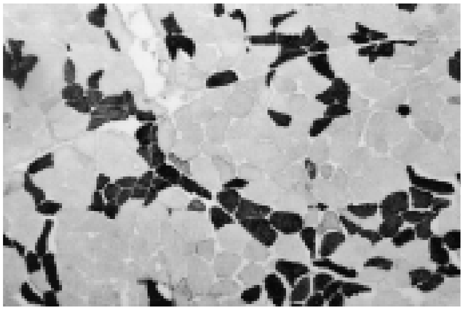

Fig. 2. Deltoid muscle of a male (72 years). Clustering of fiber types. Fiber types were defined immunohistochemically using monoclonal antibody specific for type IIA fibers (dark); 100.

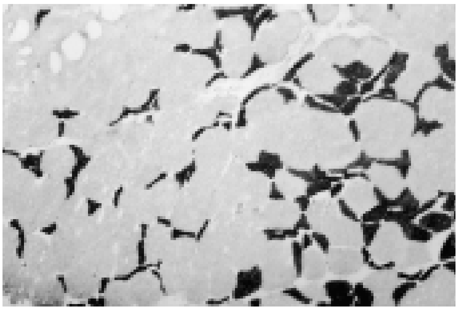

Fig. 3. Vastus lateralis muscle of a male (80 years). Type I fibers are quite large and regular in shape while type II fibers are much smaller and irregular. Fiber types were defined immunohistochemically using monoclonal antibody specific for type IIA fibers (dark); 100.

age. If we compare the fiber size of examined skeletal muscles we can see more intensive changes in vastus lateralis muscle. The atrophy of all muscle fibers is less expressed in external intercostal muscle.

Former studies examined age-related muscle atrophy in vastus lateralis muscle only. Reported results are different. The results of investigations on m. vastus lateralis point to various extent of changes in the fiber size during the aging process. Namely, while the area of fast-twitch type II fibers decreases significantly with age, the area of slow-twitch type I fibers seems to be affected to a far lesser degree[13,14]. Some other studies even suggest that the process of aging does not affect the size of type I fibers area at all[8,15]. The third group of studies described that the size of both type I and type II fibers is affected by aging to a similar extent[16]. Furthermore, it has been suggested that the muscles of the leg react differently from those of the arm to the aging process, i.e. that the age-related atrophy affects them more[17]. In our case the muscle fiber atrophy is stronger in vastus lateralis muscle. This was observed especially in both fibers type II which are a little bit more affected than the same fibers in deltoid or intercostal muscle.

Vastus lateralis muscle is dominantly fast contracting muscle with the prevalence of fibers type II (68%). Other two muscles are not so markedly fast contracting muscles. Among two of muscle fibers type II, percentage of fibers type IIX is less than 10% in all three skeletal muscles. According to our results with increasing age the percentage of fiber type I is increasing and these muscles become slow contracting muscles that means the prevalence of fibers type I. In the same time the percentage of fibers type IIA is decreasing proportionally. This increasing of percentage of fibers type I is very expressed in vastus lateralis muscle and at age group 71–80 years the percentage of fibers type I is changed from 32% to 61%. These changes are explained as conversion fibers type IIA to fibers type I because of changes of myosin isoforms. The percentage of type IIX fibers remains more or less the same in all groups. This pattern of changes is less expressed in deltoid muscle and the least expressed in external intercostal muscle. These results indicate that weight-bearing muscles undergo greater changes than the non-weight-bearing ones. At the same time during aging the changes in external intercostal muscle are the least what can be connected with always the same functional activity in the lifetime while the muscle activity of the arm and leg muscles is decline.

Poggi[13] described the same pattern of changes as we are, i. e. point to an increase of percentage of type I fibers during aging in vastus lateralis muscle. Another authors reported decrease in the number of fast and slow fibers with no affection of fiber type proportion[8].

Larsson has opinion that this increase of the percentage of type I fibers can be explained by selective loss of only type II fibers during aging, which does not affect the type I fibers[18]. In addition to this selective loss of type II fibers due to the process of denervation, as a second possible explanation for the increased number of type I fibers, it has been suggested that aging causes fast-to-slow transitions. Namely, if denervated type II fibers are reinnervated by »type I neurons« they are then transformed from type II to type I fibers[5].

Morphological changes in the anterior horn of the spinal cord, as well as those in the peripheral axon in older humans and animals, can be accountable for the old-age muscle atrophy as well[19]. The morphological finding supporting these investigations, i.e. the atrophy of motor neurons in the spinal cord of older humans, is that some types of muscle fibers tend to cluster, and do not show the mosaic distribution observed in younger people. This is actually the process of denervation with subsequent re-enervation by the neighboring axon, whose motor neuron has not been affected by atrophy, thus producing clusters of one type fibers in older people[7,20]. Investigations based on quantitative electromyography revealed a reduction in the number of functional motor units in aging human skeletal muscles[21,22]. This loss being more prominent in larger and faster motor units, i.e. type 2 motor units[23]. These investigations point to an increase in the size of the remaining motor units[24,25]. Furthermore, morphological investigations have shown that the number of motor neurons in the lumbo-sacral cord is reduced after sixty years of life; in some cases this number accounts for as little as 50% of the number found in young people[26].

We believe that the atrophy of motor neurons may also be the cause of hypotrophic and atrophic type II fibers, which are, in addition, irregular, angular in shape. The described fibers were found in the muscles of elderly people. Moreover, hypertrophic type I fibers were found in the aged limb muscles, which can be explained by a compensatory muscle mechanism responding to type II muscle fibers atrophy[27]. However, the fact that no atrophic or hypertrophic fibers were found in m. intercostalis externus could be linked to the different function of respiratory muscles in comparison to that of the limbs.

Pette and Staron (1997) suggest that age-related changes could be muscle specific[28]. Monemi and his coworkers (1998) conclude that while limb muscles are affected by the age-related atrophy, the masticatory ones are not[29]. Kirkeby and Garbarsch also suggest that m. vastus lateralis and m. masseter are not affected by the aging process to the same extent[16].

Using immunohistochemical and morphometric analyses we have revealed a different extent of changes in muscles due to the aging process. The age-related atrophy affects the limb muscles, especially those with high proportion of type II fibers, to a far grater extent than the respiratory muscles. The underlying cause of this finding could be the reduction in the functional demand of limb muscles with age, which is not the case with respiratory muscles, whose function remains virtually unchanged during aging.

We believe that our study will contribute to a better understanding of the changes in human skeletal muscles during aging, and supply evidence for the fact that aging does not affect all muscles in the same way.

REFERENCES

1. SCHIAFFINO, S., C. REGGIANI, Physiol. Rev., 76 (1996) 371. — 2. JAKUBIEC-PUKA, A., J. KORDOWSKA, C. CATANI, U. CARRARO, Eur. J. Biochem., 193 (1990) 623. — 3. GOLDSPINK, G., J. Anat., 194 (1999) 323. — 4. LARSSON, L., X. P. LI, A. TERESI, G. SALVIATI, J. Physiol. (Lond), 481 (1994) 149. — 5. LARSSON, L., T. ANSVED, Prog. Neurobiol., 45 (1995) 397. — 6. GRIMBY, G., B. SALTIN, Clin. Physiol., 3 (1983) 209. — 7. LEXELL, J., D. DOWNHAM, M. SJOSTROM, J. Neurol. Sci., 72 (1986) 211. — 8. LEXELL, J., C. C. TAYLOR, M. SJOSTROM, J. Neurol. Sci., 84 (1988) 275. — 9. LEXELL, J., J. Gerontol., 50A (1995) 11. — 10. FAULKNER, J. A., S. V. BROOKS, E. ZERBA, Annu. Rev. Gerontol. Geriatr., 10 (1990) 147. — 11. SCHIAFFINO, S., L. GORZA, G. PITTON, Dev. Biol., 127 (1988) 1. — 12. SCHIAFFINO, S., L. GORZA, S. SARTORE, J. Muscle Res. Cell. Motil., 10 (1989) 197. — 13. POGGI, P., C. MARCHETTI, R. SCELSI, Anat. Rec., 217 (1987) 30. — 14. LEXELL, J., T. TAYLOR, J. Anat., 174 (1991) 239. — 15. COGGAN, A. R., R. J. SPINA, D. S. KING, J. Gerontol. Sci., 46 (1992) B71. — 16. KIRKEBY, S., C. GARBARSCH, Histol. Histopathol., 15 (2000) 61. — 17. TOMONAGA, M., J. Am. Geriatr. Soc., 25 (1977) 125. — 18. LARSSON, L., B. SJODIN, L. KARLSSON, Acta Physiol. Scand., 103 (1978) 31. — 19. FUJISAWA, K., J. Neurol. Sci., 22 (1974) 353. — 20. LEXELL, J., D. Y. DOWNHAM, Acta Neuropath., 81 (1991) 377. — 21. BROWN, W. F., J. Neurol. Neurosurg. Psychiatry, 35 (1972) 845. — 22. BROWN, W. F., M. J. STRONG, R. SNOW, Muscle Nerve, 11 (1988) 423. — 23. DOHERTY, T. J., A. A. VANDERVOORT, A. W. TAYLOR, W. F. BROWN, Can. J. Appl. Physiol., 18 (1992) 331. — 24. STALBERG, E., P. R. FAWCETT, J. Neurol. Neurosurg. Psyhiatry, 45 (1982) 870. — 25. STALBERG, E., O. BORGES, M. ERICSSON, B. ESSEN-GUSTAVSSON, P. R. FAWSETT, L. O. NORDESJO, Muscle Nerve, 12 (1989) 382. — 26. TOMLINSON, B. E., D. IRVING, J. Neurol. Sci., 34 (1977) 213. — 27. LEXELL, J., C. C. TAYLOR, J. Anat., 174 (1991) 239. — 28. PETTE, D., R. S. STARON, Int. Rev. Cytol., 170 (1997) 143. — 29. MONEMI, M., P. O. ERIKSSON, A. ERIKSSON, L. E. THORNELL, J. Neurol. Sci., 154 (1998) 35.

M. Nikolić

Department of Anatomy, School of Medicine, University of Rijeka, Braće Branchetta 20, 51000 Rijeka, Croatia

DOBNO VEZANA ATROFIJA SKELETNE MUSKULATURE U ČOVJEKA: IMUNOHISTOKEMIJSKA I MORFOMETRIJSKA STUDIJA

S A Ž E T A K

Cilj studije bio je određivanje dobno vezanih promjena u tri mišića različite lokalizacije i funkcije u tijelu čovjeka. Poprečni presjeci te relativni udjeli vlakana tipa I, tipa IIA i tipa IIX ispitivani su u mišićima: m. vastus lateralis, m. deltoideus i m. intercostalis externus. Uzorci mišića dobiveni su od 30 muških ispitanika, dobi 20–80 godina. Tipovi vlakana definirani su imunohistokemijski, korištenjem monoklonalnih antitijela specifičnih za vlakna tipa I, IIA te tipa IIX. Poprečni presjeci mišićnih vlakana analizirani su morfometrijski, analizom kompjuterizirane slike. Svi tipovi mišićnih vlakana (I, IIA, IIX) pokazali su redukciju u veličini vlakna s dobi u sva tri ispitivana mišića. U svim mišićima relativni udio vlakana tipa I i IIA je promijenjen, no ne i tipa IIX. Rezultati su pokazali porast udjela vlakana tipa I s dobi, dok je udio vlakana tipa IIA smanjen, pri čemu su promjene kod m. vastus lateralis bile najviše izražene. Ovi rezultati sugeriraju kako mišićna atrofija povezana s dobi nije opći fenomen jer ona ne pogađa sve mišiće u jednakoj mjeri.