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Comparative histochemical and morphometric analysis of muscle fibers of the *psoas* muscle in individuals of both genders with aging

Uporedna histohemijska i morfometrijska analiza mišićnih vlakana psoasnog mišića kod osoba oba pola tokom starenja

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Abstract

Background/Aim. There is a reduction of the *psoas* muscle with aging, and histopathological analysis (HPA) on *postmortem* material also shows its significant changes with advancing age. The aim of this study was to determine the presence and distribution of types I and II muscle fibers in the human *psoas* muscle in individuals of different ages and genders. **Methods.** The material consisted of tissue samples of the right *iliopsoas* muscle taken from 30 adult cadavers (18 males and 12 females), aged from 30 to 90 years, divided into three age groups. The material was obtained from the Institute of Forensic Medicine, Faculty of Medicine, University of Niš, Serbia. Hematoxylin and eosin (H&E) staining was used in the HPA of muscle cells. **Results.** The values of astereological parameters (area, perimeter, and Feret's diameter) of type I and type II muscle fibers were greater in male cases in comparison with female ones, although with no statistically significant difference. Based on the histochemical and morphometric analysis, it was concluded that, after 70 years of life, a loss of type II muscle fibers occurred, which was more conspicuous in female cases. **Conclusion.** During aging, the loss of type II muscle fibers, as well as the atrophy of type I and type II muscle fibers, demonstrate similar dynamics in both genders.

Key words:
age factors; histological techniques; muscle atrophy; muscle, skeletal; muscle fibers, skeletal; psoas muscles; gender factors.

Apstrakt

Uvod/Cilj. Veličina psoasnog mišića smanjuje se sa starenjem i histopatološka analiza (HPA) postmortalnog materijala takođe pokazuje značajne promene u njemu u zavisnosti od godina života. Cilj istraživanja bio je da se utvrdi prisustvo i distribucija mišićnih vlakana tipa I i II psoasnog mišića kod osoba različitih starosnih kategorija i različitog pola. **Metode.** Materijal su činili tkivni uzorci desnog bedrenoslabinskog mišića 30 odraslih kadavera (18 muških i 12 ženskih), starosti od 30 do 90 godina, raspoređenih u tri starosne grupe. Materijal je dobijen sa Instituta za sudsku medicinu, Medicinskog fakulteta Univerziteta u Nišu, Srbija. Hematoksilin-eozin (H&E) bojenje je korišćeno u HPA mišićnih ćelija. **Rezultati.** Vrednosti astereoloških parametara (area, perimetar i Feretov dijametar) mišićnih vlakana tipa I i tipa II bile su veće kod kadavera muškog pola u poređenju sa kadaverima ženskog pola, ali bez statistički značajne razlike. Na osnovu histohemijske i morfometrijske analize zaključeno je da nakon navršene 70. godine života dolazi do gubitka mišićnih vlakana tipa II, što je kod kadavera ženskog pola bilo izraženije. **Zaključak.** Sa starenjem, gubitak mišićnih vlakana tipa II, kao i atrofija mišićnih vlakana tipa I i tipa II pokazuju sličnu dinamiku kod oba pola.

Ključne reči:
životno doba, faktor; histološke tehnike; mišići, atrofija; mišići, skeletni; mišići, skeletni, vlakna; mišići, slabinski; pol, faktor.

Introduction

The *iliopsoas* muscle, [*musculus* (*m.*) *iliopsoas*], belongs to the group of inner hip muscles. It consists of the iliacus (*m. iliacus*) and *psoas* muscles (*m. psoas*), and it functions as the chief flexor of the hip joint.

This muscle is specific since it represents a sort of connection between the trunk and lower extremity [1], and since it attaches to the vertebrae, it is responsible together with other muscles for the upright posture and ambulation of humans. Some of the studies in which the authors used computerized tomography imaging to examine the transversal section of

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the *psoas* muscle reported a reduction of its size with aging, and histopathological analysis (HPA) of the muscle on *post-mortem* material also showed significant changes in the muscle with advancing age [2]. Imamura et al. [3] had similar results in 1983 studying the *quadriceps femoris* and plantar flexors of the foot. Morphometric analysis of the muscle was performed mostly on animal models [4, 5].

Skeletal muscles consist of connective tissue sheaths enveloping them from the outside (*epimysium*) and numerous fascicles, enveloped and separated one from another by a connective tissue layer called *perimysium.* Muscle fascicles consist of a large number of myofibrils containing contractile proteins, actin, and myosin, arranged in a parallel fashion [6, 7].

A muscle fiber is a multinuclear, syncytial unit shaped like an elongated, narrow and slender cylinder. The nuclei are subcapsular, and there are 4–6 of them per cell on the transversal section [8].

Red muscles, with a greater mitochondrial and lipid content and greater capillary density, are by function intended to maintain posture or to be engaged during longer activities. The color of red muscles is the consequence of a relatively higher content of myoglobin compared to white muscles which contain fewer mitochondria but abundant glycogen, which makes them better suited for anaerobic respiration and sudden and occasional contractions [8].

Human muscles contain red and white muscle fibers arranged in a typical, combined mosaic pattern, resembling a chessboard. Depending on anatomical localization and function, the proportions of type I and type II muscle fibers vary but type II fibers are, nevertheless, predominant with 60%–65%, compared to 35%–40% for type I fibers [9]. Type II muscle fibers are darker in color, while type I fibers are of a paler color [9, 10].

In the earliest studies investigating the difference between type I and II muscle fibers between genders [10], it was concluded that some of the fibers are larger in men. Type II muscle fibers are usually larger in men compared to type I fibers, in contrast to women in whom type I fibers have an equal or greater diameter compared to type II muscle fibers.

In 1970, in the study by Brooke and Kaiser [10], who investigated biceps muscles, conclusions that are still valid in general were drawn about the gender difference in skeletal muscles. The investigation of the *vastus externus* muscle by Lexell et al. [11] in 1988 and Kobayashi [12] in 1991 did not demonstrate any significant differences in the diameter between type I and type II muscle fibers in men and women. As for the *biceps brachii*, a much higher percentage of type II fibers is present in men, while in women, the percentages of both types are about equal. In contrast, the prevalence of type I and II fibers of the *vastus externus* muscle is approximately the same in both genders [13].

It is thought that the established difference in the size of muscle fibers between men and women is determined by the fact that men are relatively higher and heavier than women, with a larger muscle mass, and are more physically active. Androgenous hormones also play a role in the size of muscle fibers [14, 15]. The difference between muscle fibers in men and women depends on the examined muscles as well [16].

The effects of physical exercise and training on the muscle system have been investigated in numerous studies [13, 17]. The results of these studies are in general contradictory, but some general principles do exist. It is evident that physical exercise and training of any kind increase the diameter of muscle fibers. In essence, anaerobic activities lead to hypertrophy of type II muscle fibers, which is frequently seen in sprinters. In long-distance runners, in whom aerobic metabolism is more significant, type I muscle fibers are usually larger.

Power training, for instance, weightlifting, produces significant hypertrophy of type II fibers and lesser (if any) hypertrophy of type I muscle fibers [18]. It is well known that sprinters in general have a greater number of type II muscle fibers compared to sedentary controls, and long-distance runners have a greater number of type I fibers compared to untrained individuals.

Many authors believe that these two groups of runners have a genetically determined composition of muscles as to muscle fiber types and that muscle fiber type conversion is negligible if it occurs at all [17].

In the process of aging, starting from the sixth decade and after 70 years of age, skeletal muscles structurally and functionally change so that after 75 years of age, the power of muscles is reduced by 30–50%. The reason behind this reduction is the reduction of muscle fiber diameter. At 75 years of age, the diameter is reduced by 80% compared to the age of 25 years. Since the power of contraction is not linearly associated with muscle fiber diameter but is proportional to the surface of muscle fiber transversal section, a diameter that is 80% of the normal diameter produces a 60% loss of muscle strength (contraction force) [19].

Due to reduced elasticity, flexibility, and joint diseases of different intensity, older individuals are less active, which is associated with the loss of muscle volume and contraction force. This is supported by the fact that aging individuals have selective atrophy of type II muscle fibers [19]. The effects of poorer nutrition in the elderly have not been sufficiently studied, although it is well known that cachexia is associated with the atrophy of type II muscle fibers [20].

Based on the above-mentioned, there may be an association between aging and loss of muscle mass in both genders. Given the possible relationship between the process of aging and muscle fiber changes in that process in both genders, the aim of this study was as follows: to detect the presence and distribution of types I and II muscle fibers in the human *psoas* muscle of individuals of different ages and genders using the hematoxylin and eosin (H&E) method and to determine their morphological characteristics using immunohistochemical analysis and monoclonal antibody against myosin; to quantify the presence of the dynamics of types I and II muscle fibers in the human *psoas* muscle in both genders during aging, measuring volume density of the fibers, by way of stereological methods in the sections stained immunohistochemically and by the use of a monoclonal antibody against myosin; to quantify the changes in size and shape of muscle fibers in the human *psoas* muscle in both genders during aging, measuring the area, perimeter, and Feret's diameter.

Methods

The study was conducted at the Institute of Anatomy, Institute of Histology and Embryology, Institute of Pathological Anatomy, and Institute of Forensic Medicine, which represent the teaching and scientific bases of the Faculty of Medicine, University of Niš, Serbia.

The study material consisted of tissue samples of the right *iliopsoas* muscle taken from 30 adult cadavers (18 male, 12 female), aged 30 to 90 years, autopsied at the Institute of Forensic Medicine, Faculty of Medicine in Niš, in the period from January to April 2013. The study was conducted abiding by the ethical norms regulating the use of cadaveric material in biomedical research by the Ethics Committee of the University of Niš Faculty of Medicine (Decision no. 01-9337-18). Autopsy findings did not indicate the presence of any pathological changes or traumatic damage to the right *iliopsoas* muscle. The cadavers were divided into three age groups: first (I), with cases aged 30–49 years (n = 10); second (II), with cases aged 50–69 years (n = 10); and third (III), with cases aged 70 years and above (n = 10).

The samples of the right *psoas* major muscle sized 5 × 5.5 mm were taken utilizing the incision perpendicular to the muscle at the level of the mid-distance between the upper border of the twelfth thoracic vertebra (T12) and lower border of the fifth lumbar vertebra (L5).

Histological analysis

Histological analysis, as well as the identification of possible changes of the muscle fibers of the right *iliopsoas* muscle during aging, was based on light microscopy-based assessment of their properties. The tissue of the *psoas* muscle was fixed in 10% buffered formalin during the next 24 hours. The obtained paraffin molds of the *psoas major* muscle were used to obtain up to 5 μm thick tissue sections. We used classical H&E and Periodic Acid-Schiff (PAS) methods to identify the basic structures of the *psoas* muscle. The stained histological sections were then analyzed using light microscopy under 4×, 10×, and 40× magnification. Digital images of the analyzed sections were obtained using a 1.3-megapixel digital camera.

Immunohistochemical analysis

Using immunohistochemical analysis, we established the presence of cells with a positive reaction to applied immunohistochemical markers. The ultravision LP-HRP polymer (Cat. No. TL-125HL) detection technique using a monoclonal antibody against myosin (anti-Myosin, Skeletal Muscle, Clone, MYSNO2, Ready to Use, Termo Scientific Lab Vision, Ca, 1:320) was used in analysis of muscle fibers type II.

Morphometric analysis of muscle fibers

Morphometric analysis of muscle fibers of the *psoas major* muscle was performed in 10 randomly selected visual fields per each analyzed case (270 visual fields in total for 27 analyzed cases). Stereological analysis of type I and type II muscle fibers in the analyzed visual fields was performed by measuring their volume density using a multipurpose test system, M168. Astereological analysis of type I and type II muscle fibers was performed by measuring their area (A_MI and A_MII), perimeter (B_MI and B_MII), and Feret's diameter (D_{FM}I and D_{FM}II) of the profile of transversally sectioned fibers.

Statistical analysis

Results are presented as mean values and standard deviation (SD). Independent samples *t*-tests were used to test statistical differences between two samples. The normality of the distribution was validated by the Kolmogorov-Smirnov test. Data were analyzed using SPSS 16.0 (Statistical package for the social sciences, version 16.0, SPSS Inc, Chicago, IL, USA).

Results

The morphological analysis involved histological analysis of transversal sections of the *psoas* muscle tissue stained with H&E and analysis of transversal sections of the *psoas* muscle tissue stained using the immunohistochemical ultravision LP-HRP polymer detection technique.

Classical fascicular structure of skeletal muscles was seen in transversal sections of *m. psoas*, stained with H&E, in all age groups. Connective tissue sheaths within the muscle had usual organization and contents; in the more abundant connective tissue of the *perimysium*, there were nerve elements, arterioles, and venules, while more tender connective tissue of the *endomysium* contained capillaries and rare cells with slender projections enveloping individual muscle cells.

Identification of type II muscle fibers in transversal sections of the *psoas* muscle was performed using the anti-MYSN02 antibody, and immunopositivity was seen as a brown, fine-grained reaction of the sarcoplasm.

In the first age group, MYSN02-immunopositive type II muscle fibers were found in groups, rarely as individual fibers, and between them, there were less numerous type I muscle fibers that did not show a positive reaction. In transversal sections of the muscle, type I and II fibers had an irregular, polygonal shape, with sharper angles seen with type II fibers. Both muscle fiber types were of similar diameter and with typically elongated or oval nuclei.

In the second age group, MYSN02-immunopositive type II muscle fibers were approximately equally prevalent as nonstained type I fibers. Type II muscle fibers were mostly polygonal, with sharp angles, in contrast to type I fibers which were more oval. In some transversal sections of the muscle, individual type II muscle fibers had a characteristic lamellar arrangement of myofibrils in the sarcoplasm, while in most type II muscle fibers immunopositivity manifested as a fine-grained reaction in the sarcoplasm.

In the third age group, MYSN02-immunopositive type II muscle fibers showed polymorphism of shape and thickness –

the cells were oval and with a smaller diameter compared to the nonstained type I muscle fibers. Immunopositive muscle fibers did not demonstrate regular distribution in the form of isolated groups such as in younger age groups; instead, they were irregularly distributed between the nonstained type I fibers.

In all studied age groups, a smaller number of MYSN02-immunopositive type II muscle fibers was seen in female cases, compared to male ones (Figures 1 a–f).

The Kolmogorov-Smirnov tests showed that data were normally distributed. The average age of female cases was statistically significantly higher compared to male cases, (T = 2; SS = 25; p = 0.01) (Figure 2), which had a significant influence on the interpretation of observed gender-related differences when morphometric parameters of the *psoas* muscle fibers were concerned.

The average values of morphometric parameters of type I and type II muscle fibers in male and female cases are shown in Table 1.

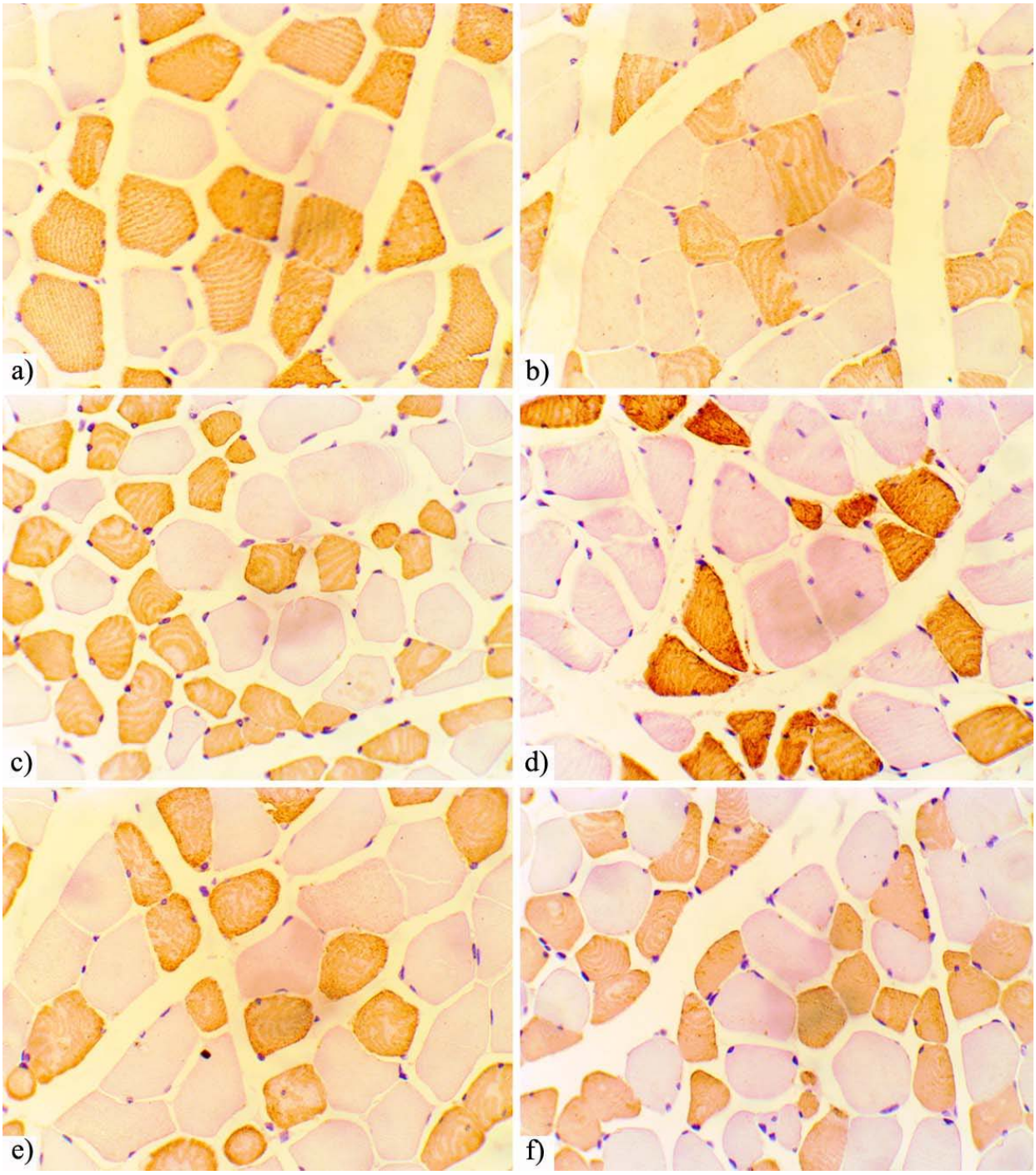

Fig. 1 – MYSN02-immunopositivity in transversal sections of the *musculus psoas*, by age groups and gender: a) a man aged 31 years; b) a woman aged 35 years; c) a man aged 52 years; d) a woman aged 56 years; e) a man aged 73 years; f) a woman aged 75 years. A reduction of the number and thickness of immunopositive type II muscle fibers can be seen with aging, predominantly in men, and a reduced number of type II muscle fibers in women, compared to men (LP-HRP, ×400).

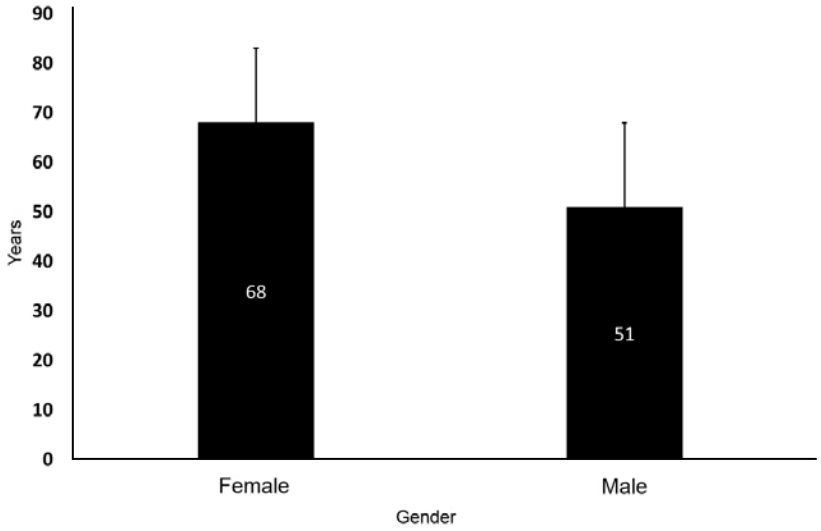

Fig. 2 – Average age of male and female cases.

Table 1

Average values of morphometric parameters of type I and type II muscle fibers in male (n = 14) and female (n = 13) cases

Parameter	Mean ± SD
V_vI (%)	42.57 ± 5.34
female	41.93 ± 8.28
male	
V_vII (%)	25.00 ± 5.63
female	30.54 ± 6.67
male	
A_MI (μm^2)	1,112.88 ± 234.29
female	1,318.85 ± 493.91
male	
B_MI (μm)	126.69 ± 14.83
female	136.34 ± 26.43
male	
$D_{FM}I$ (μm)	47.82 ± 6.16
female	51.50 ± 10.00
male	
A_MII (μm^2)	743.25 ± 223.62
female	931.14 ± 423.21
male	
B_MII (μm)	104.84 ± 17.43
female	117.58 ± 28.03
male	
$D_{FM}II$ (μm)	40.20 ± 7.06
female	45.27 ± 10.97
male	

V_vI – volume density of type I muscle fibers; V_vII – volume density of type II muscle fibers; A_MI – area of type I muscle fibers; B_MI – perimeter of type I muscle fibers; $D_{FM}I$ – Feret's diameter of type I muscle fibers; A_MII – area of type II muscle fibers; B_MII – perimeter of type II muscle fibers; $D_{FM}II$ – Feret's diameter of type II muscle fibers; SD – standard deviation.

The results of the *t*-test showed that there is no statistically significant difference ($p > 0.05$) between the genders regarding given parameters (average area, perimeter, and Feret's diameter of type I and II muscle fibers). The values in the male group were slightly higher than in the female group (Figure 3), suggesting a tendency for higher values in males, but future studies are needed to confirm that.

Finally, average volume density of type II muscle fibers was statistically significantly greater in male cases (T = 2.34; SS = 25; $p = 0.028$) (Figure 4). Nevertheless, since female cases were statistically significantly older than male cases, we could not decisively tell whether the observed difference was the consequence of aging or gender-related differences.

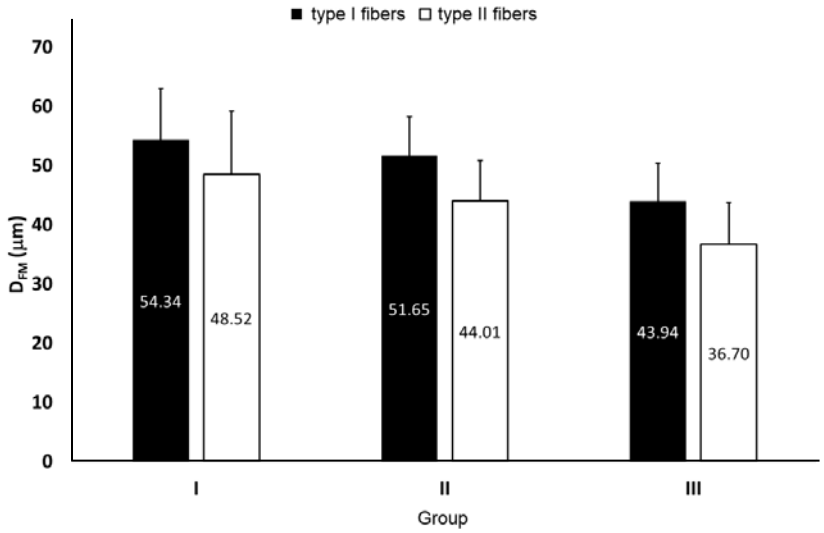

Fig. 3 – Average Feret's diameter of type I and type II muscle fibers (D_{FM}) in the analyzed age groups: I – aged 30–49 years; II – aged 50–69 years; III – more and equal to 70 years.

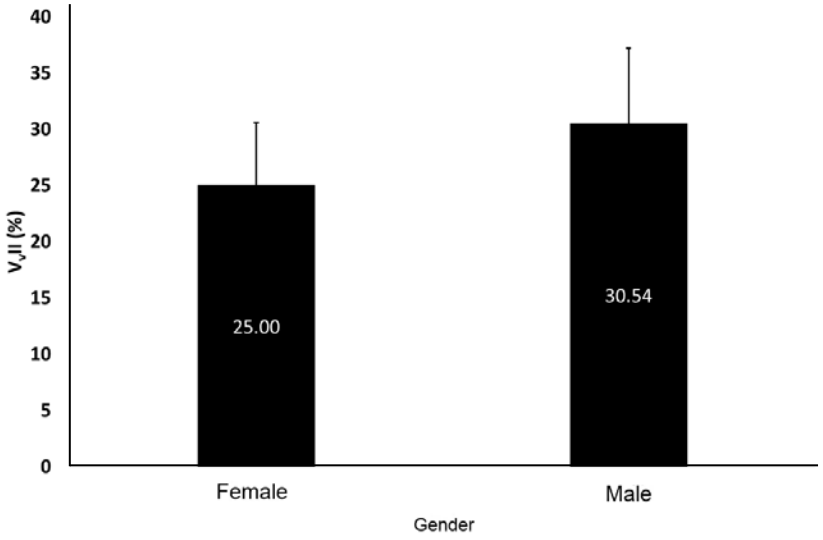

Fig. 4 – Average volume density of type II muscle fibers (V_vII) in male and female cases.

Discussion

The results of our study represent a morphological and morphometric analysis of changes affecting the fibers of the *psoas* muscle during aging in cases of different ages and of both genders.

Morphological analysis of type I and type II muscle fibers of the *psoas* muscle demonstrated that in more advanced ages, changes affecting muscle fibers occurred, especially the type II ones. These changes were quantified in morphometric analysis, and after that statistically analyzed, which provided an insight into their prevalence in cases of different ages, monitoring of their dynamics, and analysis of their interrelatedness during the process of aging.

M. psoas major, the object of our study, is a lower extremity muscle. Its origin is complex and involves lateral parts of the trunk and corresponding intervertebral discs from the twelfth thoracic to the fifth lumbar vertebra. Distally, the muscle joins the *iliacus* muscle forming the *iliopsoas* muscle, which attaches with its terminal tendon to the trochanter minor of the femur. The *m. psoas major* has a flexion role and is involved, together with adjacent muscles, in external rotation and adduction of the hip [21]. The *psoas* muscle, therefore, has an important dynamic and active postural function and belongs to lower extremity muscles which are important for everyday activities, such as walking, climbing the stairs, getting up from a chair, etc. Detection of age-related changes affecting the *psoas* muscle is thus of vital importance in the preservation of mobility and prevention of disability.

Arbanas et al. [21] have immunohistochemically and morphometrically analyzed the samples of the *psoas* muscle from 15 men aged 18 to 35 years in order to study the

composition of muscle fibers. The age of this group of cases partly matches the age of the first group of our cases. The authors reported that the *psoas* muscle was mainly composed of type II muscle fibers (60%) – fast-twitching, glycolytic, and undergoing fatigue more quickly compared to type I fibers (40%) – slow-twitching, oxidative, and able to resist fatigue for longer periods of time. Similar to our own findings, they established that type I muscle fibers were characterized by a significantly greater transversal section area compared to type II fibers. Based on the composition of the *psoas* major muscle, Arbanas et al. [21] concluded that this muscle had complex dynamic and postural functions. The results of our morphometric analysis revealed slightly greater volume density values of type I muscle fibers compared to type II fibers of the *psoas* muscle. This could indicate a possible predominance of type I fibers in our study. However, volume density is a stereological parameter, the value of which is influenced by the number and area of the analyzed structure (in this case, a corresponding muscle fiber type). The fact that type I fibers have a significantly greater area of the transversal section compared to type II fibers and that the prevalence of type I fibers was approximately the same as the prevalence of type II fibers can account for a slightly higher value of their volume density compared to type II muscle fibers in our first age group. Our results, therefore, demonstrate a similar prevalence of type I and type II muscle fibers in our first (youngest) age group, which agrees with the results obtained by Arbanas et al. [21].

Histological changes in the skeletal muscles in older individuals are reflected in the reduction of muscle mass with simultaneous increase of fatty and connective tissue, as seen in our study as well. The size of type II muscle fibers is reduced, while the size of type I fibers remains unaffected. The reduction in the size of muscle fibers with advancing age can be attributed to the loss of myosin heavy chains [20, 22]. Moreover, the accumulation of ”ring-like“ and ”torn“ muscle fibers can be seen, then the accumulation of lipofuscin and non-myelinated rod-like structures, as well as the reduction of the number of blood vessels. Neuromuscular damage involves the increase of the size of the motor unit and the reduction of the number of motor neurons in the anterior horns of spinal cord gray matter. Furthermore, the process of aging is associated with the reduced production of new muscle fibers, as a consequence of the reduced activity of myosatellite cells. At the cellular level, muscle alterations associated with aging involve proliferation of the sarcoplasmic reticulum and T-tubular system and disorganization of sarcomeres, myofilaments, and Z-lines [23].

A significantly greater loss of type II muscle fibers compared to type I fibers can be explained by changes in the neuromuscular system associated with aging, as well as the reduction of number and function of myosatellite cells as myogenic stem-cells which may differentiate into new muscle fibers [24, 25].

In our study, the obtained values of stereological parameters of type I and type II muscle fibers were greater in male cases compared to female ones, but these differences were not statistically significant. In addition, the average volume density of type I muscle fibers was greater in female cases, but again the difference did not reach statistical significance. In contrast, the average volume density of type II muscle fibers was statistically significantly greater in male cases. In the literature, there is information about a different distribution of the type I and type II muscle fibers in different genders [10]. It was emphasized that in men both muscle mass and muscle fiber size was greater than in women. Individual muscle fibers are larger in men than in women. Type II muscle fibers are usually larger in men, but type I muscle fibers are of equal or greater diameter in women. It was thought that the gender difference was the consequence of greater height and weight of men compared to women, with greater muscle mass and more physically active [26, 27]. The impact of male sex hormones cannot be neglected either. Androgenous hormones have an impact on muscle fiber size in men; it is well known that testosterone therapy leads to muscle hypertrophy [15]. Bennington and Krupp [16] believed that some of the observed gender-related differences depended on the muscle which was analyzed. For instance, the *biceps brachii* muscle in men contains a markedly higher percentage of type II muscle fibers, while in women, this muscle contains similar percentages of these two muscle fiber types. In our study, we also observed certain differences in some of the muscle fiber parameters between male and female cases. However, with the exception of the volume density of type II muscle fibers, other differences in most of the parameters were not statistically significant. Since female cases were statistically significantly older than male cases, we could not decide with certainty whether the observed difference in the content of type II muscle fibers was the natural consequence of aging or if it was gender-related.

Conclusion

Based on the above elaborations of other authors' and our own study, the following conclusions may be drawn: the loss of type II muscle fibers is associated with a continual (and of similar intensity) atrophy of type I and type II muscle fibers in both men and women. The loss of type II muscle fibers, as well as the atrophy of type I and type II muscle fibers, demonstrate similar dynamics in both genders during aging. The values of astereological parameters (area, perimeter, and Feret's diameter) of type I and type II muscle fibers are higher in male than in female cases but without any statistically significant difference. The average volume density of type I muscle fibers is greater in female cases compared to male ones but with no any statistically significant difference.

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