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Original scientific paper

Immunohistochemical Analysis of the Human Psoas Major Muscle with Regards to the Body Side and Aging

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A B S T R A C T

The aim of our study was to explore the age related changes of the fibre type composition of the human psoas major muscle. Moreover, we wanted to compare the fibre type composition of the left and right muscle. Muscle samples were collected from 15 young and 15 old males. Type I, IIA and IIX muscle fibres were typed using myosin heavy chain identification. The serial transverse sections were analysed using a light microscope. Results of our study showed that the age-related atrophy affected all three fibre types. Type IIA fibres were affected most profoundly while type I fibres were affected most weakly. The percentage of the different fibre types did not change during aging. There were no differences in the fibre type composition between the left and right muscle. Human psoas major muscle undergoes normal aging changes with the atrophy of all three fibre types, whereas atrophy most profoundly affects type IIA fibres. No differences in the fibre type composition between the left and right muscle point to the equal engagement of both legs in normal everyday activities of human.

Key words: *human, psoas major muscle, aging, left-right, myosin heavy chain, Immunohistochemistry*

Introduction

Skeletal muscle consists of different fibre types characterized by their specific myosin heavy chain (MHC) isoforms[1]. Immunohistochemical analysis shows that in muscle fibres of human limb muscles three MHC isoforms are expressed: MHC-I, MHC-IIa and MHC-IIx, namely types I, IIA and IIX[2]. Different muscle fibres can be typed using monoclonal antibodies specific for MHC isoforms[3].

During aging skeletal muscle shows reduction of muscle mass and strength and moreover change of contractile properties. Influence of aging on physiological properties of skeletal muscle is specific for particular type of muscle fibre. Age-related loss of muscle mass results primarily from a decrease in the total number of both type I and type II fibres and, secondarily, from a preferential atrophy of type II fibres[1,4,5]. Atrophy of type II fibres lead to a larger proportion of slow type muscle mass in aged muscle. It is evidenced by slower contraction and relaxation times in older muscle, respectively[1]. Muscle mass and strength in humans start to decrease at the age of 50. Between the ages of 50 and 70 reduction amounts 15% per decade and between ages of 70 and 80 there is another 30% of the reduction[6,7,8]. Loss of muscle mass and strength is not always proportional, so it affects leg muscles more profoundly than muscles of the arm[9]. Moreover, Lexell et al. in his study of cross-sections of whole m. vastus lateralis showed decrease in the number of muscle fibres for 39% between ages of 20 and 80 which is proportional with the loss of muscle strength[10]. Aging of skeletal muscle also results in changes of the percentage of given muscle fibres. There can be increase of the percentage of type I muscle fibres[11], some studies didn't find any changes in the percentage of muscle fibres[12], while one study even showed a decrease of the percentage of type I muscle fibres[13].

Some authors compared the fibre type composition of the skeletal muscles between the left and right side of the body. In most cases no significant differences were found

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with regards to the dominant side of the body, while particular differences were found only in the muscles of the hand[14,15,16].

The aim of our study was to explore the aging of the human psoas major muscle, by comparing the muscle fibre size and the distribution of different fibre types between young and old subjects. Moreover, we wanted to compare the fibre type composition between the left and right human psoas major muscle, as to determine differences of the composition of this muscle with regard to the dominant side of the body.

Materials and Methods

Materials

Samples of the psoas major muscles used in this study were obtained from 15 young male subjects (<35 years of age) and 15 old male subjects (>65 years of age) at the Department of Forensic Medicine, University Hospital in Rijeka who had sudden accidental death. Muscle samples were taken within 24 hours from the onset of death. None of them had a history of neuromuscular disease or any other disease that could alter muscle fibre type composition. The local ethics committee of the School of Medicine, University of Rijeka, approved this study. Muscle samples 1x1x1 cm in size were taken from the right and left sides of the body closer to cranial origin of the muscle, alongside the lumbar spine. The tissue specimens were frozen in liquid nitrogen and stored at –80 °C until they were processed for immunohistochemistry.

Immunohistochemistry

Serial muscle cryosections (Leica-CM1850, Vienna, Austria) were prepared on glass slides with a 5 ìm thickness. To identify MHC isoforms, three monoclonal antibodies were used: BA-F8 (1:200) specific to slow or MHC-I isoform (stains slow, type I muscle fibres), SC-71 (1:500) specific to MHC-IIa isoform (stains both fast, IIA and IIX muscle fibre types, but more intensively IIA muscle fibres) and BF-35 (undiluted) specific to all MHC isoforms except MHC-IIx (stains all fibre types except IIX, IIX fibres remains unstained). Those monoclonal antibodies were kindly provided by Prof. Stefano Schiaffino (University of Padua, Italy). The slides were incubated overnight with primary antibodies at 4 °C in a humidified environment. After washing them in PBS-Tween 20 for 3x5 min, the sections were incubated at room temperature for 15 min with biotinylated anti-mouse IgG and after that for 15 min with peroxidase-labelled streptavidin (DAKO, Copenhagen, Denmark). After washing the sections with PBS, the bound antigen-antibody complex was visualized with 3.3'-diaminobenzidine in chromogen solution (DAB+Chromogen, DAKO, Copenhagen, Denmark).

Fibre typing and morphometry

The five view fields of the muscle samples obtained from the right and left sides of the body were examined under an Olympus BX50 microscope (Olympus, Tokyo, Japan) and photographed at a magnification of 100x using a Sony video camera (SONY SSC-DC58AP, Japan) connected to a personal computer (Figure 1). The photographic images were analysed using an image analysis system that employed ISSA v.2.5. software (V.A.M.S., Zagreb, Croatia). The percentage of fibre types, fibre type's distribution and fibre cross-sectional area (CSA) were calculated.

Statistics

Statistica 7.1 (StatSoft) computer software (StatSoft Inc., Tulsa, USA) was used for statistical analysis. The results were displayed at the mean and at a 95% confidence interval. In order to make a comparison between the groups, a repeated measures analysis of variance (ANOVA) was used. The Scheffe test was used post hoc to identify specific differences between groups. Statistical significance was set at the $p<0.05$ and $p<0.01$ levels.

Results

Aging of the human psoas major muscle

Our study showed that both examined groups (young and old) had significantly higher percentage of IIA fibres than type I fibres ($F=6.3864$; $p<0.01$) and type IIX fibres

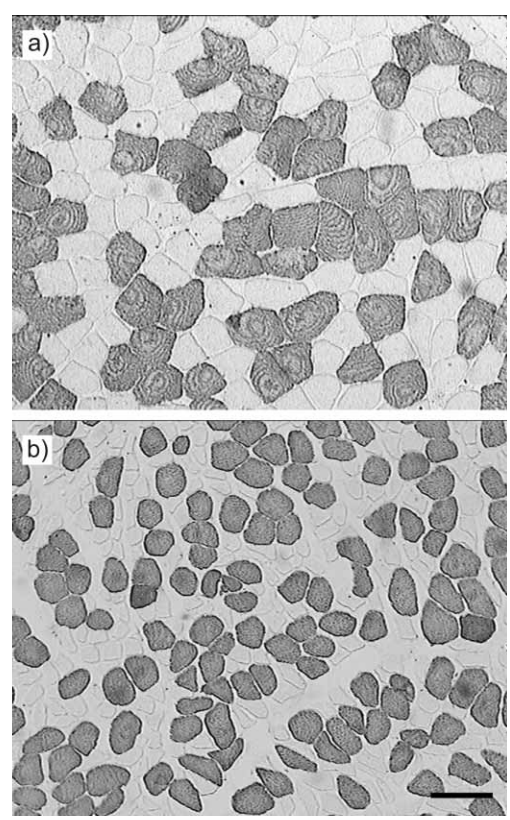

Fig. 1. Human psoas major muscle of a (a) young and (b) old man. Type I muscle fibres are stained dark using monoclonal antibodies against MHC-I isoform. Scale bar is 100 μm.

(F=6.3864; p<0.01) in the psoas major muscle, respectively. Moreover, type I fibres were present in a significantly higher percentage than type IIX fibres (F=6.3864; p<0.01). Between the examined groups, significantly higher percentage of type IIA muscle fibres was found in the young subjects, compared to type IIA muscle fibres of old subjects (F=6.3864; p<0.05). Moreover, percentage of the type I and IIX muscle fibres did not differ significantly between age groups (Figure 2).

Furthermore, our study showed that in both age groups type I muscle fibres had a significantly bigger CSA than type IIA fibres (F=49.556; p<0.01) and type IIX fibres (F=49.556; p<0.01). In young subjects type IIA fibres had a significantly bigger CSA than type IIX fibres (F=49.556; p<0.01) while in old subjects there were no differences between CSA of type IIA and IIX muscle fibres. Comparison of the CSAs of all muscle fibre types between two age groups showed that all muscle fibres of young subjects had significantly bigger CSA than the muscle fibres of the same type of old subjects (F=49.556; p<0.01) (Figure 3).

Fibre type composition of the human psoas major muscle with regard to the body side

Our study showed that in young as well as in old subjects the percentage of all muscle fibres examined did not differ significantly between the left and right side.

In both (left and right) psoas major muscles, type IIA fibres were present in a significantly higher percentage than type I fibres (young subjects – F=1.6629; p<0.01, old subjects – F=1.0665; p<0.01) and type IIX fibres (young subjects – F=1.6629; p<0.01, old subjects – F=1.0665; p<0.01). Moreover, type I fibres were on both sides present in a significantly higher percentage than type IIX fibres (young subjects – F=1.6629; p<0.01, old subjects – F=1.0665; p<0.01) (Figure 4).

Similarly to distribution of the muscle fibres, results showed no differences between left and right side when CSAs of all examined fibre types was compared. Type I fibres on both sides had a significantly bigger CSA than type IIA fibres (young subjects – F=7.8063; p<0.01, old subjects – F=1.0752; p<0.01) and type IIX fibres (young subjects – F=7.8063; p<0.01, old subjects – F=1.0752; p<0.01). Moreover, in the group of young subjects type IIA fibres had a significantly bigger CSA than type IIX fibres (F=7.8063; p<0.01) on both sides. No difference in CSAs of type IIA and IIX fibres was found in both muscles of old subjects (Figure 5).

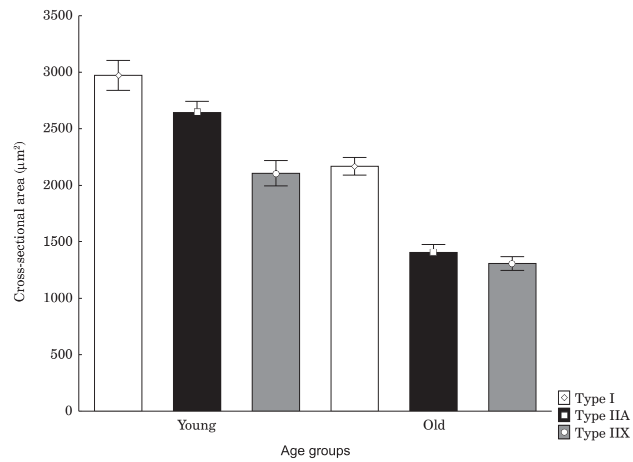

*Fig. 3. The cross-sectional area of the muscle fibre types of the human psoas major muscle in young and old subjects. Values are mean ±0.95 confidence interval. *Significantly different from the rest fibres in the same age group (p<0.01). **Significantly different from the type IIX fibres in the same age group (p<0.01). † Significantly different from the same fibre type of the old subjects (p<0.01).*

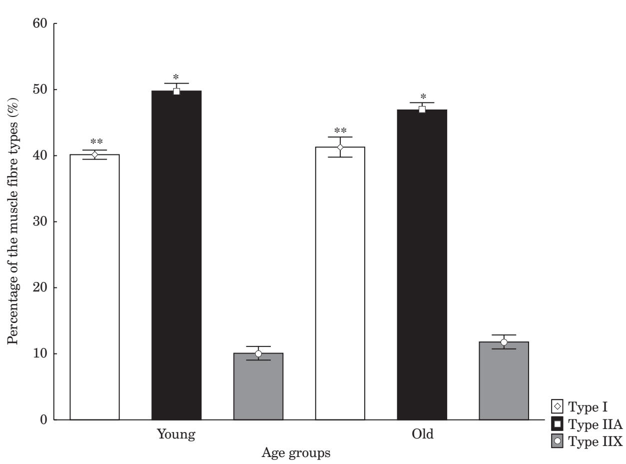

*Fig. 2. The percentage of the muscle fibre types of the human psoas major muscle in young and old subjects. Values are mean ±0.95 confidence interval. *Significantly different from the rest fibres in the same age group (p<0.01). **Significantly different from the type IIX fibres in the same age group (p<0.01). † Significantly different from the type IIA fibres of the old subjects (p<0.01).*

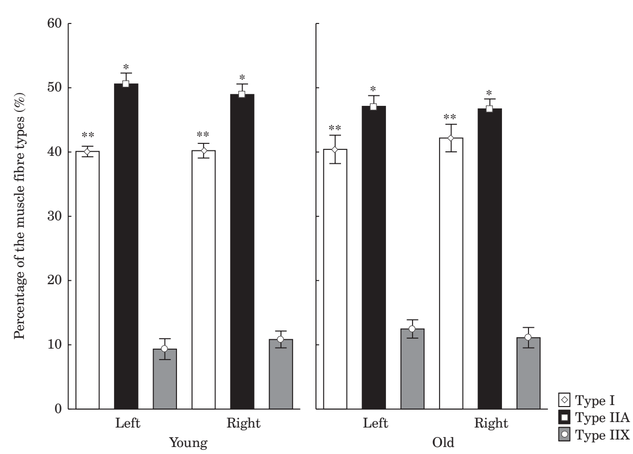

*Fig. 4. The percentage of the muscle fibre types of the left and right human psoas major muscle in young and old subjects. Values are mean ±0.95 confidence interval. *Significantly different from the rest fibres of the same muscle in the same age group (p<0.01). **Significantly different from the type IIX fibres of the same muscle in the same age group (p<0.01).*

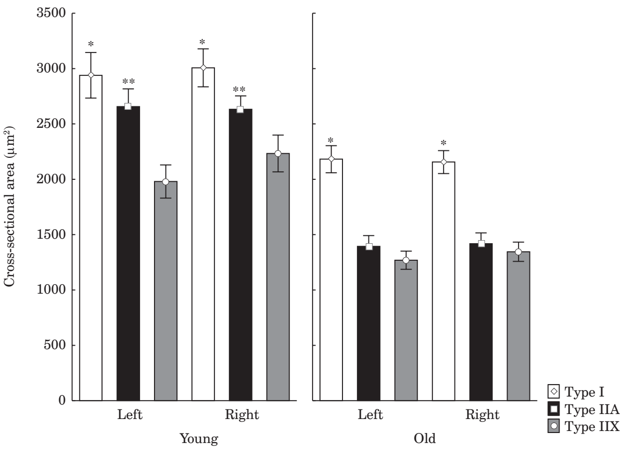

*Fig. 5. The cross-sectional area of the muscle fibre types of the left and right human psoas major muscle in young and old subjects. Values are mean ±0.95 confidence interval. *Significantly different from the rest fibres of the same muscle in the same age group (p<0.01). **Significantly different from the type IIX fibres of the same muscle in the same age group (p<0.01).*

Discussion

Aging of the human psoas major muscle

Our study showed in both age groups (young and old) in the psoas major muscle a predominance of type IIA muscle fibres. Type I muscle fibres were present in smaller percentage and type IIX muscle fibres were far less present. Aging of skeletal muscle results in changes of the percentage of given muscle fibres. There can be increase of the percentage of type I muscle fibres[11], percentages of given fibre types can remain unchanged[12], or there can be even a decrease of the percentage of type I muscle fibres[13]. Therefore, we could place our results in a group of studies that didn't find any changes in the percentage of the muscle fibre types during aging. However, by comparing the percentage of the same muscle fibre types between the young and old subject, our study showed significantly higher percentage of type IIA muscle fibres in a group of young subjects, which indicates a decrease of the percentage of the type IIA muscle fibres in a group of old subjects. Additionally, our study showed a small increase of type IIX muscle fibres in elderly subject which contributes to opinion that there were no changes in proportion of the fast and slow muscle fibres in the psoas major during aging.

Lexell et al. showed a decrease of a total number of the muscle fibres in vastus lateralis muscle during aging[10]. However, it is to be pointed that he performed his study on the cross section of the whole muscle, which could not be performed in our study and therefore we couldn't determine if there was a decrease of a total number of the muscle fibres in psoas major muscle.

Furthermore, results of our study showed a decrease of the CSAs of all muscle fibre types in the psoas major muscle during aging. The CSAs of all muscle fibre types were significantly smaller in the group of old subjects than the CSAs of muscle fibre types of the young subjects. The CSA of the type IIA fibres of the old subjects was 47% smaller that the one of the young subjects (1406.23 μm^2 versus 2644.65 μm^2) while the CSA of the type IIX fibres was 38% smaller (1306.75 μm^2 versus 2106.80 μm^2) and the CSA of the type I fibres was 28% smaller (2168.96 μm^2 versus 2973.28 μm^2) in the group of old subjects. From presented facts it is obvious that atrophy most profoundly affected type IIA muscle fibres and most weakly type I muscle fibres which corresponded to the data from the literature.

Fibre type composition of the human psoas major muscle with regard to the body side

Results of our study showed no differences in the fibre type composition between the left and right human psoas major muscle. We found no significant differences in the percentage, as well as CSAs of examined muscle fibres, between the left and right side within the given age groups. Sjöström et al. compared left and right tibialis anterior muscle and didn't find differences in the percentages and CSAs of the type I and II muscle fibres as well. Yet, his results showed significantly bigger cross section of whole muscle as well as total number of muscle fibres in the left muscle[14]. However, it is to be pointed again that he performed his study on the cross section of the whole muscle which was not applicable in our study. Similarly to Sjöström, Erzen et al. didn't find any differences in the percentages and CSAs of muscle fibres by comparing the left and right vastus lateralis muscle[15]. Józsa et al. performed his study on the hand and arm muscles. He found no significant differences in the hand muscles but arm muscles had significantly higher number of the type II fibres than type I fibres on the dominant side[16]. From above mentioned it is obvious that our results correspond with the results of studies performed on the muscles of lower extremities where no differences were found between the left and right side. We assume that the reason for such results lies in almost equal engagement of both legs in normal everyday activities of man. On the other hand, man primarily uses its dominant arm in everyday activities so that can explain differences in the composition of the arm muscles found by Józsa et al.

Conclusions

Human psoas major muscle undergoes normal aging changes characterized by the atrophy of type I, IIA and IIX fibre types. Atrophy most profoundly affects type IIA fibres and most weakly type I fibres. The percentage of the different fibre types remains unchanged.

No differences in the fibre type composition between the left and right psoas muscle, points to the equal engagement of both legs in normal everyday activities of man.

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IMUNOHISTOKEMIJSKA ANALIZA VELIKOGA SLABINSKOG MIŠIĆA ČOVJEKA S OBZIROM NA STRANU TIJELA I STARENJE

SAŽETAK

Cilj našeg istraživanja bio je ispitati utjecaj starenja na građu velikoga slabinskog mišića te usporediti građu lijevog i desnog mišića u čovjeka. Uzorci mišića uzeti su od 15 mladih i 15 starih muškaraca. Mišićna vlakna tipa I, IIA i IIX tipizirana su pomoću identifikacije izoformi teških lanaca miozina. Serijski poprečni rezovi analizirani su pomoću svjetlosnog mikroskopa. Rezultati našeg istraživanja pokazali su da starosna atrofija zahvaća sva tri tipa mišićnih vlakna. Atrofija najjače zahvaća vlakna tipa IIA, a najslabije vlakna tipa I. Tijekom starenja nije došlo do promjena u zastupljenosti pojedinog tipa mišićnih vlakana. Također, nismo pronašli razlike u građi između lijevog i desnog mišića. Veliki slabinski mišić pokazuje normalne starosne promjene sa atrofijom svih triju tipova mišićnih vlakana, a atrofija najjače pogađa vlakna tipa IIA. Činjenica da nema razlike u građi lijevog i desnog mišića ukazuje na podjednaku aktivnost oba mišića u normalnim svakodnevnim aktivnostima.