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Original scientific paper

Muscle Loss in Elderly

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A B S T R A C T

Aging is associated with loss of skeletal muscle mass, strength and endurance. The aim of this study was to determinate age related changes in human muscles with different function and location in the body (vastus lateralis muscle and intercostal internus muscle). Our results suggest that age related muscle atrophy affect both human skeletal muscles. Also, the results showed the increase in percentage of muscle fibers with high oxidative activity during aging.

Key words: *aging, skeletal muscle*

Introduction

Skeletal muscles allow the movements and accomplishment of daily physical activities as well as sport performances. Indeed, muscles play important roles in living organisms. Skeletal muscle consists of different fiber types characterized by their specific myosin heavy chain (MHC) isoforms. Using monoclonal antibodies specific to MHC isoforms, in adult human skeletal muscles 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 size changes across the human lifespan, initially showing rapid increases due to grow and later gradual decreases due to aging. Whereas the initial stage of development involves increase in the number of muscle fibers before birth, later growth occurs primarily by enlargement of size in the postmitotic muscle cells. The stage of young adulthood in the twenties involves a period of peak musculoskeletal function, middle age in the forties and fifties involves some slowing of contraction, but changes in absolute muscle strength are minor until the sixth decade of life[2,3].

Loss of muscle mass with age in humans is well documented. Investigation of individual muscles shows that after thirties, there is decrease in cross sectional areas of the thigh, decreased muscle density, and increased intramuscular fat[4]. Most studies on aging human muscles have focused upon changes in the vastus lateralis muscle. Lexell 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[5].

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 two differently placed human muscles (leg muscle and respiratory muscle). The changes in enzymatic activity of aging muscles were also histochemically demonstrated.

Materials and Methods

This research was approved by the Ethical Committee of School of Medicine, University of Rijeka.

Material

Muscle samples used for this investigation were obtained from thirty male subjects 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 vastus lateralis muscle. Also, muscle samples were taken from internal intercostal muscles.

Samples were divided into two age groups: fifteen young men (age range 20–32), and fifteen elderly men (age range 74–87).

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Preparative procedure

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

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

Immunohistochemistry

10 μm thick transverse cryosections of vastus lateralis and internal intercostal muscles were stained for slow and fast myosin heavy chain isoforms using monoclonal antibodies specific for type I (BF-F8), IIA (SC-71) and for all but type IIX (BF-35). Slides were incubated with monoclonal antibodies (1:1000) in phosphate-bufered saline (PBS) for 30 minutes at room temperature. After three washing steps in PBS, slides were incubated for 30 minutes at room temperature with peroxidase-labelled rabbit anti human IgG (Dako A/S Copenhagen, Denmark) in PBS (1:40). After washing in PBS, slides were incubated in 50 ml of diaminobenzidine (DAB) solution (0.5 M Tris HCl, pH 7.6, 15 mg of DAB, 100 ml of H_2O_2, 25 mg of imidazol) for 20 minutes at room temperature. Finally, slides were dried and mounted in Canada balsam[6].

Histochemistry

10 μm thick transverse cryosections of vastus lateralis and internal intercostal muscles were stained to demonstrate the activity of nicotinamide-adenine dinucleotide tetrazolium reductase (NADH-TR)-indicator of oxidative capacity, and α glycerophosphodehydrogenase (αGPDH)--indicator of glycolytic capacity[7].

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 ($p<0.05$, $p<0.001$).

Results

Muscle fiber size

The size of fiber type I in m. vastus lateralis was greater than the size of fibers type IIA and IIX in young and old subjects. In internal intercostal muscle the cross sectional area of fibers type IIA and IIX was greater than fibers type I in young subjects. In old age, the size of fibers type I compared to fibers type II became larger (Figure 1).

All muscle fiber types (type I, type IIA and type IIX) in vastus lateralis and internal intercostal muscles showed a reduction in fiber size with increasing age. This reuction was always statistically significant ($p<0.001$). In old age the size of fibers type IIA and type IIX were dereased more than a half size of young muscle fibers (Figure 1).

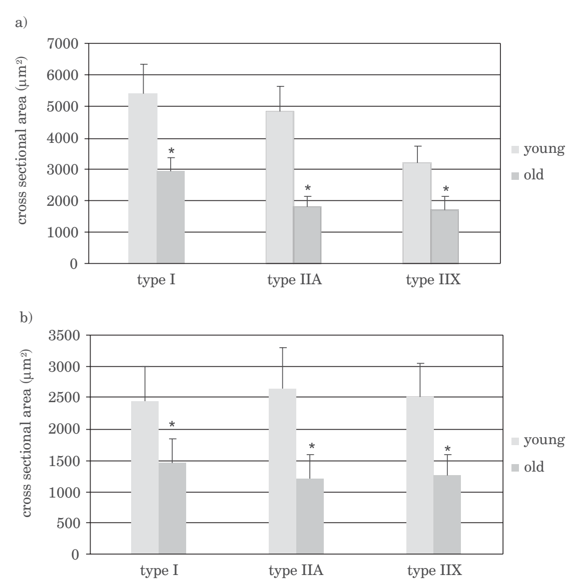
Percentage of fiber type I, IIA and IIX.

Human vastus lateralis muscle in young age was composed of type I (32.11±3%), type IIA (60.72±4%), and IIX (7.17±1%) muscle fibers. The results showed age related changes in proportion of type I and IIA muscle fibers but not in IIX. In old age 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 ($p<0.001$) (Figure 2).

In young age m. intercostalis internus was composed of 44.65±5% of type I, 48.95±4% of type IIA and 6.40±3% of type IIX muscle fibers. In old age the percentage of type I fibers increased to 53.60±4%, and percentage of type IIA muscle fibers decreased to 39.80±4%. The results indicated age related changes in proportion of type I and type IIA fibers, but not in IIX muscle fibers (Figure 2).

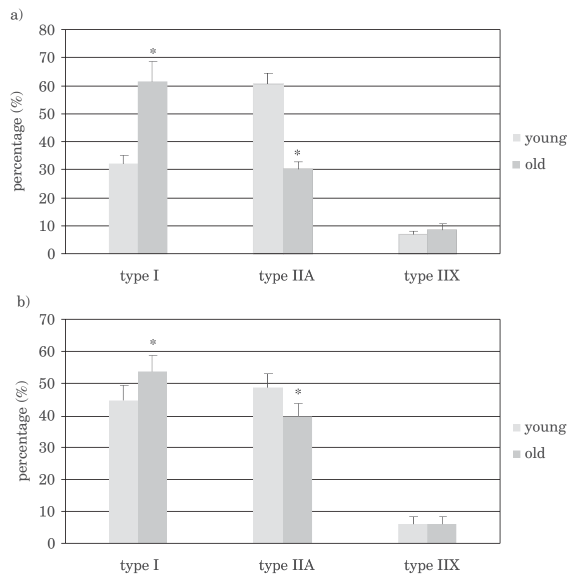
Enzyme histochemistry.

Results showed the increase in percentage of muscle fibers with high oxidative activity during aging (Figures 3 and 4).



*Statistically significant difference as compared to the young age group ($p<0.001$)

Fig. 1. Relationship between age and muscle fibre cross sectional area in a) vastus lateralis and b) intercostalis internus muscle.



* Statistically significant difference as compared to the young age group (p<0.001)

Fig. 2. Relationship between age and percentage of fibre types in a) vastus lateralis and b) intercostalis internus muscle.

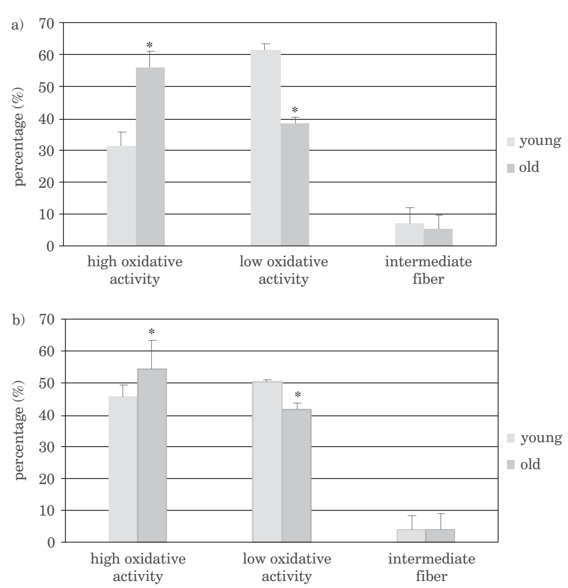
Discussion

Our study focuses on the period of life from the seventh decade onward when muscle strength show a steady decline, which for simple isomeric performance is on the order of approximately 1.5% per year[8]. This condition of aging has been linked to the normal process of loss of muscle tissue, which is related to reduce number of both type I and type IIA muscle fibers, plus an additional factor of muscle fiber atrophy[3], a process called sarcopenia[9].

This investigation has shown that the cross sectional area of all muscle fibers in examined muscles is decreasing from the young age group to the old age group. 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 old age. The atrophic changes are less expressed in slow contracting fibers type I.

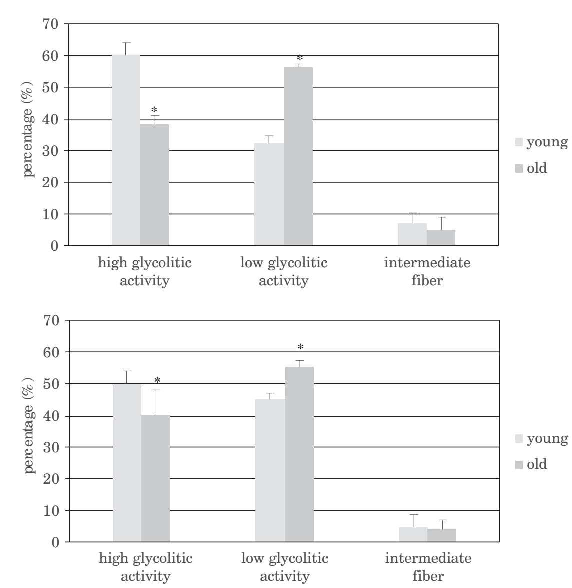
Vastus lateralis is muscle with the prevalence of fibers type II (68%). M. intercostalis internus are not so markedly fast contracting muscle. Among two of muscle fibers type II, percentage of fibers type IIX is less than 10% in all muscles. According to our results the percentage of fiber type I increases with increasing age and these muscles become slow contracting muscles that means the prevalence of fibers type I. In the same time percentage of type IIA fibers decreases proportionally. This increasing of percentage of type I fibers is very expressed in vastus lateralis muscle. These changes are explained as conversion fibers type IIA to fibers type I because of changes of myosin isoforms[1]. The percentage of type IIX fibers remains unchanged during aging. This pattern of changes is less expressed in internal intercostal muscles. These results indicate that weight-bearing muscles undergo greater changes than respiratory muscles.

Also, aging affects the metabolic capacities of skeletal muscles; in particular there are changes in the activities of specific enzymes. Studies on aged human vastus lateralis muscle showed declines in glycogenolytic and glycolytic enzyme activities resulting in reduced respiratory capacities[10]. Our results indicate that enzyme activity of muscle fibers was changed with aging. Results showed the increase in percentage of muscle fibers with high oxidative activity.



* Statistically significant difference as compared to the young age group (p<0.05)

Fig. 3. Relationship between age and percentage of high and low oxidative activity of fibres in a) vastus lateralis and b) intercostalis internus muscle.



* Statistically significant difference as compared to the young age group (p<0.05)

Fig. 4. Relationship between age and percentage of high and low glycolitic activity of fibres in a) vastus lateralis and b) intercostalis internus muscle.

Loss of muscle mass (sarcopenia) with age in humans is well documented. Computed tomography of individual muscles shows that after age 30 there is a decrease in cross sectional areas of the thigh, decreased muscle density, and increased intramuscular fat. These changes are most pronounced in women[4]. Muscle atrophy may result from a gradual and selective loss of muscle fibers. The number of muscle fibers in the section of the vastus lateralis of autopsy specimens is significantly lower in older men compared with younger men[11]. The decline is more marked in type II muscle fibers, which decrease from an average of 60% in sedentary young men to below 30% after age of 80, and is directly related to decreases in strength[12]. A reduction in muscle strength is a major component of normal aging. Same studies indicate that 40% of the female population aged 55–64 years, 45% of women aged 65–74 years, and 65% of women aged 75–84 years were unable to lift 4.5 kg[13]. In addition, similarly high percentage of women in this population reported that they were unable to perform some aspects of normal household work.

Sarcopenia is accelerated with a lack of physical activity. The amount of physical activity generally declines with age. Physically inactive adults undergo a faster and greater loss of muscle mass than physically active adults[14]. Resistance training has been shown to be a powerful intervention in the prevention and treatment of sarcopenia[15].

Our results indicate that age related loss of skeletal muscle mass affected human skeletal muscles, i.e. leg muscles and respiratory muscles.

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GUBITAK MIŠIĆNE MASE U STAROSTI

S A Ž E T A K

Starenje je povezano s gubitkom mišićne mase, snage, te izdržljivosti mišića. Cilj istraživanja bio je utvrditi dobno uvjetovane promjene u humanim skeletnim mišićima koji obavljaju različite funkcije i imaju različit smještaj u tijelu (m. vastus lateralis i m. intercostalis internus). Rezultati dobiveni istraživanjem upućuju da dobno uvjetovana mišićna atrofija zahvaća oba ispitivana mišića. Također, tijekom starenja raste broj mišićnih vlakana koji pokazuju visoku oksidacijsku aktivnost.