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AGEING AFFECTS THE MYO-NULCEAR DOMAIN IN THE SLOW AND FAST MUSCLE FIBER DEPICTING THE MYOFIBERS-SPECIFIC DISSIMILARITIES WITH THE AGE

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Abstract

Myonuclei, the satellite cells that are present beneath sarcolemma, play an essential part in the maintenance and growth of myofibres. The myonuclear domain controls the cytoplasm of satellite cells. Scientists have established that atrophic variation exists in both skeletal muscles fibres with increasing age. It is critical to determine the relationship between ageing and the myonuclear domain in human skeletal muscles, and in the current study we analysed the impact of age on fibre specific relationships of the myonuclear domain. The myofibres was evaluated in Vastus lateralis muscles of the young adults (n = 9) and older adults (n = 8). We found no sizeable variations in myofibres composition of young and old subjects. Fibre cross-sectional area of type I fibre was similar, but substantial atrophic changes (12%) were observed in type II fibres in older adults compared to young ones. The older adults acquired a lot more myonuclei, resulting in a considerably reduced size of the myonuclear domain of type II fibres. We surmised that myofibres-specific dissimilarities occur due to ageing effects on a myonuclear domain, probably due to the diminishing capability of the myonuclear content of fast muscle fibres. This phenomenon can be helpful in devising the combating strategies to prevent it.

Keywords: *Ageing, Myonuclear domain, Skeletal muscle, Vastus lateralis*

INTRODUCTION

Human skeletal muscles have a unique feature that enables them to regulate themselves (1). They can either increase or decrease their size depending on the physiological demands (2). Myonuclei are satellite cells that are present beneath sarcolemma (3). They play an essential part in the maintenance and growth of myofibres (4). Myonuclei are mainly responsible for controlling the limited area of cytoplasm known as the myonuclear domain (MD) (5). Resistance training and overload workouts can stimulate myonuclei growth (6). It is believed that the myonuclear domain remains the same after exercise. Some studies advocated that muscle growth can be maintained without the addition of new myonuclei (4).

There are a few variations in the dimensions of MD in fibre types in skeletal muscles. This variation in size is fibre specific in most of the species. Fast-twitch fibres acquired less myonuclear content than slow-twitch fibres. Therefore, fast fibres possess a considerable myonuclear domain when compared with slow-twitch fibre (7). With increasing age, the preservation of MD is unclear yet; scientists have established that atrophic variation exists in both fibres, but fast-twitch fibres are most likely vulnerable to these variations (8). Latest scientific studies claim that atrophied muscles that undertake a course of exercise do not reduce myonuclei. Therefore, retraining the myonuclear content led to decreased MD (9). In a few other scientific studies, this MD is preserved in young people. However, it is unclear in the elderly. Researchers concluded that MD was not maintained in untrained older people (10). Yet another research established that muscle fibre size improves in older people in response to overload exercises. However, monocular contents remain insignificant. (11). Consequently, older people have a much larger myonuclear domain rather than young adults.

In the existence of such controversial data, the goal of the investigation was to look into the consequences of growing age & skeletal muscles fibre types (slow twitch & fast-twitch) on MD in skeletal muscles of the human. The nucleus determined MD to cytoplasm ratio. Here we conducted this study to find potential factors contributing to muscle mass loss with increasing age. We hypothesized that type II (fast-twitch) fibres are more vulnerable to morphological alteration associated with ageing than type I (slow-twitch) fibres. Understanding these changes linked with ageing is critical to formulating possible solutions to eliminate & prevent sarcopenia.

METHODOLOGY

SUBJECTS

Eight old (4 females, 4 male) and nine young (4 females, 5male) willingly took part in the current study. Their particular physical attributes are revealed in Table I. They had offered written and oral agreement after presenting the primary research purpose and related procedures, as per recommendations of the regional ethics panel. In addition, they had explained the time frame of the research, associated hazards & advantages. The participants also had the right to distance themself anytime from the study. They had additionally guaranteed their privacy.

Table I. Physical Characteristics of Participants

	Older adults		Young adult	
	Male	**Female**	**Male**	**Female**
Age (Years)	67±4	62±5	29±3	27±4
Height (cm)	171±5	167±6	172±2	167±3
Weight (kg)	77±5	69±4	70±4	63±2

**Data is presented as mean and SD.*

MUSCLE SEMPLING

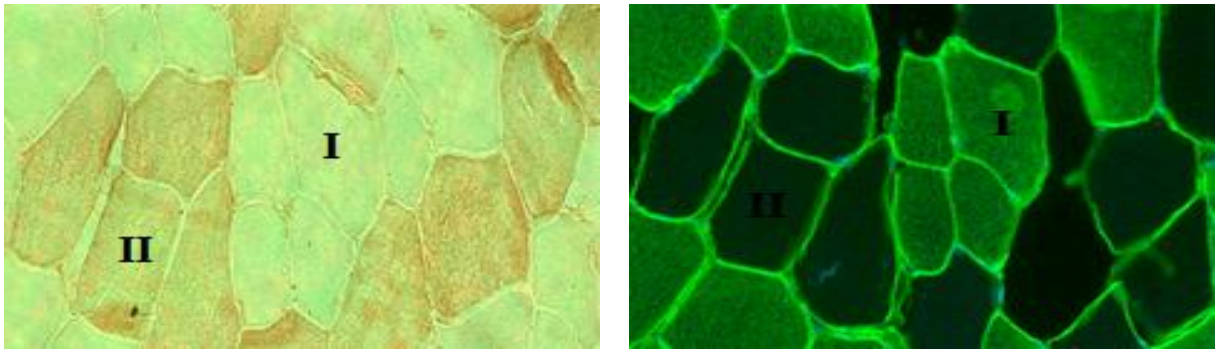
Muscle biopsies were obtained from the Vastus lateralis close to the muscle belly in the same lower leg. It was anesthetised by utilizing 1 Percent lidocaine, and on average, 70 mg biopsies were acquired using Weil- Blakesley forceps. After that, Biopsies were fitted in embedded medium (Tissues, Tek A long way Laboratories). These installing frost biopsies were protected at - 80 Celsius for an additional assessment (4, 19).

IMMUNO-HISTO CHEMICAL ANALYSIS

The cryostat was utilized to dissection Vastus lateralis into 6μm cross-sections, approximately minus twenty Celsius. The pre-incubation conditions were alkaline (10.6) and acidity (4.3). The cross-sections were immune-stained to detect the presence of various myofibrillar ATPase isoforms.

Immunostaining cross-sections estimated the myonuclear content with mAB against the Leu 19 antigen. To get counter-staining, the DAPI was introduced. The myonuclei were shown blue-stained.

To assess primary antibody binding, the peroxidase anti-peroxidase method was used. Light microscopy and a computerized image analysis system (IBASIBAS Kontron electronic) were used to assess the mix section. Captured images of materials (on average, 225) were used to estimate fibre cross-sectional area, fibre type, and Myonuclei.



N2:261 Staining A4:840 Staining

Fig. 1. Recognition of Muscle fibre types

CROSS-SECTIONAL AREA AND MYONUCLEAR DOMAIN

The CSA was calculated by a video capture microscope and NIH image software. The nucleolus/cytoplasm ratio was used for calculating the myonuclear domain. The myonuclear domain was analyzed in slow and fast-twitch fibres in the young and old groups.

STATISTICAL ANALYSIS

The data is displayed as a mean & standard deviation. Changes in morphological parameters were calculated in %age. The data were analyzed by unpaired t-test. SPSS software was implemented for statistical calculations. P-value <0.05 is considered statistically significant.

RESULTS

FIBRE TYPE COMPOSITION

Vastus lateralis muscle constitutes both types of slow and fast fibres. No statistically significant difference was found in the fibre type composition of young and old subjects. However, Old subjects tend to have a higher percentage of Type I fibres than young Subjects (Fig. 2).

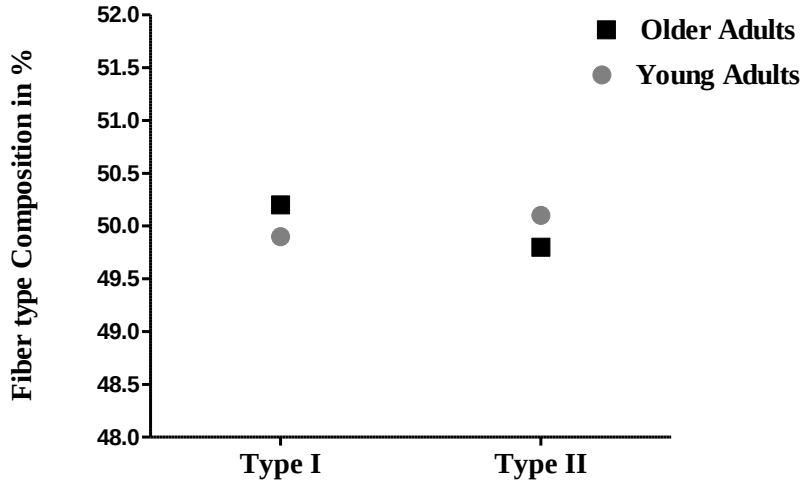


Fig. 2. Graph representing the fibre type composition (%) in young and old adults

FIBRE CROSS-SECTIONAL AREA

The fibre cross-sectional area of type I fibre in young was similar to the corresponding type I fibres in the old group. However, significant atrophic changes (12%) were seen in type II fibres in old adults in comparison with the young adults' group (Fig. 3).

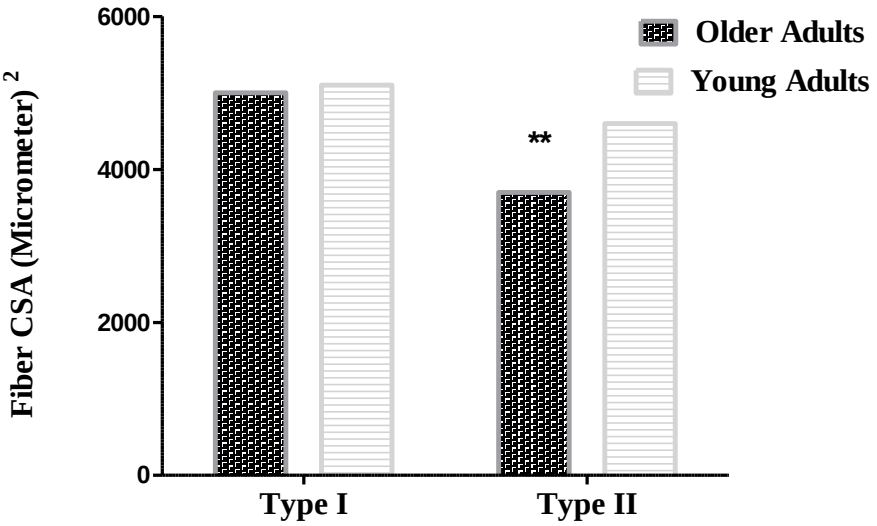


Fig. 3. The graph representing the data with Fibre Cross-sectional area (CSA) (μm) ^{2} of Type I & Type II fibres in Young & Old adults (Statistical significance * at P < 0.05, ** at P < 0.001)

MYONUCLEI/MUSCLE FIBRE

Type II fibres tend to have a smaller number of myonuclear content than type I fibres in both groups. However, no significant changes were seen in myonuclear content with increasing age (Table II).

Table II. Myonuclear content (m/f) and myonuclear domain in young and old subjects.

Fibre type	Young subjects	Old Subjects
Myonuclei/ muscle fibre in Type I	2.75±0.8	2.77±0.35
Myonuclei/ muscle fibre in Type II	2.34±0.17	2.43±0.28**
Myonuclear domain in Type I	2005±215	1975±314
Myonuclear domain in Type II	1905±298	1431±325*

Data is taken as mean± SD.
Statistical significance * at P < 0.05, ** at P < 0.001

MYONUCLEAR DOMAIN

Ageing did not affect the myonuclear domain of Type I fibres, but significant changes were seen in the myonuclear domain of type II fibres. In the old group, the myonuclear domain of type II fibres significantly reduced compared to a myonuclear domain of type II fibres in the young group (Table II).

DISCUSSION

The current study's main goal was to determine how ageing affects the myonuclear domain (MND) in type I and type II fibres. The main finding was a significant decrease in the myonuclear domain of type II fibres as they aged. However, no significant changes in the myonuclear domain of type I fibres were observed.

Ageing is a complex phenomenon. A lot of structural and physiological changes occurs with increasing age. Muscle mass declines, resulting in impairment and reduced functional ability. Functional disability and impairment are caused by age-related sarcopenia (12, 13). The effect of ageing on MD is critical for understanding sarcopenia and developing new methods for preventing and rejuvenating the changes associated with muscle mass loss with ageing. According to recent data, up to 45 percent of people over the age of 65 in the United States are developing sarcopenia (14), and more than 20 percent appear to be

functionally dependent (15). Every year, significant funds are invested in healthcare issues in the United States. Controlling muscle tissue loss would result in billions of dollars saved each year (16).

Interestingly, we have found that slow fibres have more capacity to maintain their fibre type composition than the fast type of fibre with increasing age. A recent review concluded that the primary mechanism behind these adoptions might be related to decreasing physical activity and lack of overload stimulus (13). Besides the changes in fibre type composition, atrophic modifications induced in the skeletal muscles with ageing are common (8, 17, 18). Understanding the mechanism related to decreasing muscle mass is essential to combat the increasing health care cost in older adults and promote physical independence in the elderly (16, 19).

We have demonstrated that there is a slight variation in fibre type composition in young and elderly adults. There is a slight increase in the composition of type I fibre in older adults. It could be due to the type or mode of activities they engage in or a lack of a training threshold required for type II fibres (20). Our results of the fibre type composition are concurrent with the finding of the older studies (21). The type I fibre has a larger CSA than type I in both young and old groups. However, more marked atrophic changes were seen in type II fibres in the elderly.

Interestingly, no atrophic change was seen in type I fibres with increasing age. Interestingly, we found that the myonuclear domain was the same in young and older adults in type I fibre. However, in type II the myonuclear domain was significantly reduced in older adults. It shows that older adults have a more genetic backup for type II fibres, but they lack the appropriate stimulus for their proper functioning. Now the question arises if we provide appropriate stimulus, do they have the ability to respond appropriately to increase muscle mass and strength. The answer is yes. A recent study advocated that strength training was an effective non-pharmacological strategy for rejuvenating muscle mass (11).

CONCLUSION

We concluded that ageing affects the myonuclear domain differently in slow and fast fibres. Type II fibres showed pronounced changes. Appropriate overload training may be the best intervention to combat or slow down these ageing-related changes. Further exploration of this phenomenon can be helpful in devising combating strategies to prevent this age-related phenomenon.

Conflicts of interest:

The authors declared no conflict of interest.

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