A COMPARATIVE STUDY ON THE AGING PROCESS OF MUSCLES' CAPILLARY SYSTEM: DIAPHRAGM AND RECTUS ABDOMINIS IN RATS. A FUTURE MODEL FOR PHYSICAL ACTIVITY STUDIES?

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SUMMARY

In the present study, the age-related changes of the capillary system were investigated in the diaphragm and rectus abdominis muscles of 1, 8, and 18-month-old rats. The activation patterns of these muscles differ in the sense that the diaphragm is regularly mobilized many times at each minute during the entire life of the animal, while the rectus abdominis, although mobilized during breathing, is much less and more irregularly activated. The muscular fibers were pre-incubated in pH 4.35 and stained by the Padykula and Herman method to study of ATP-ase myofibrillary activity, enabling the separation of the fibers into 3 groups: slow oxidative (I); glycolitic fast oxidative (IIA) and fast oxidative (IIB) and the identification of the capillary around the fibers in the same section. The capillary/fiber relationship and the capillary density were obtained. The analysis of the capillary/fiber relationship and of the area between the 2 muscular groups showed us different patterns of capillary development, in aging, of diaphragm muscle's non-fatigable fibers (I and IIA) in comparison to rectus abdominis. These age-related changes of the diaphragm and rectus abdominis muscles could be related to continuous diaphragm contraction and could be a natural model of permanent exercise and muscle aging.

Keywords: Aging, Diaphragm, Exercise.

INTRODUCTION

Exercise is considered as a potentially protective factor on muscular aging[(1)], and may eliminate age differences.

Structural differences in diaphragm due to the aging process have been described[(2,3,4)] but few studies have addressed changes on capillary pattern, both at the diaphragm muscle (DIA) and rectus abdominis anterior muscle (RA) during aging, but it is possible to imagine some degree of relationship between capillary organization and fibers oxidative ability.

Little is known about the capillary/ muscle relationship of skeletal muscles and the changes occurring due to age and its functional mobility pattern. Thus, this study was conducted to evaluate age changes on capillary/ fiber relationship and the capillary density of two muscles differently activated: the DIA, which regularly contracts many times at a minute, throughout an animal's life, and the RA, the activation of which being much less frequent.

MATERIALS AND METHODS:

Thirty Wistar male rats were used in this investigation. Groups of 10 animals were sacrificed 1, 8, or 18 months after birth, being named for this study purposes as young, adult and old groups, respectively. Mean weight of animals (in grams ± SD) was 68 ± 10.06, 420 ± 37.15 and 458 ± 43.34 g, respectively. The animals received water and standard ration *ad libitum*. Due to technical problems, particularly related to the achievement of the exact color to enable the identification of capillary types of fiber at the same slide, only the material corresponding to 13 rats could be used for final study.

Tissue Isolation

The animals were sacrificed through ether inhalation. After death, a RA transversal section and all right costal DIA was obtained. Strips representing DIA and RA were fixed in "Swiss roll" and submitted to hexane previously refrigerated in liquid nitrogen (-70oC). Serial sections of 10*μ* were then performed.

Staining

Various preparations for pre-incubation in different pHs were tested to allow the observation, in the same section, of the three kinds of fibers and of the capillaries around fibers. This was achieved with a pre-incubation in a 4.35 pH.

Sections were then submitted to pre-incubation in a 4.35 pH and submitted to Padykula-Herman technique for ATPase microfibrilar activity[(5)].

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Morphometric Evaluation

The sections were submitted to study under open-camera microscope and recorded in hard copies. Records were then analyzed for determining fibers area by using a planimetric system and a digitalizing table connected to a computer. The number of capillaries around each fiber was manually counted. From 47 to 140 fibers were, in 3 to 16 fields, randomly chosen in each animal. Those data enabled us to know the capillary/ fiber relationship and the capillary density for each kind of fiber (I, IIA and IIB) both for DIA and for RA.

Statistical analysis

Data were analyzed by variance (Kruskal-Wallis). When positive, the results were submitted to analysis for Dunn-modified multiple comparisons. Each age group for DIA and RA by the significance test of Mann-Whitney. Significance was determined at 5%.

RESULTS

Firstly, we could see a similar pattern in the parameters measured both for DIA (Table 1) and for RA (Table 2) in young animals when compared at this age (one month). On the other hand, when we analyze the aging pattern, we could see a difference in DIA and RA muscles. In both muscles and at all ages, IIB fibers (fast fatigable) presented a reduced vascularization pattern in comparison to slow non-fatigable (I) and fast fibers (IIA) (Tables 1 and 2).

In DIA, in the C/F relationship and in DC we could notice an aging pattern characterized by objective changes between the young and adult groups, but this was not seen between the adult and the old groups. In RA, we could see on C/F relationship and on CD an aging pattern characterized by objective changes among young, adult and old groups.

When we assessed the young, adult and old groups by the Mann-Whitney test, we could notice a more intense aging pattern in RA when compared to DIA.

DISCUSSION

Muscle aging is probably associated to multiple factors. Old subjects performing regular daily activities (e.g., walks) seem to show a better capillarization pattern and VO2 kinetics during exercise(6). DIA seems to present a different aging pattern when compared to other striated muscles. From one side, aging is associated to a reduction on maximal tetanic force production in aged rats[7]. On the other hand, DIA seems to present differences in aging compared to the other striated muscles, since high-intensity exercises, up to exhaustion, result in significant fatigue of the quadriceps muscle contractibility, but not of the diaphragm in healthy old subjects, relatively sedentary[8].

Gosselin described that, in 344 Fisher female rats submitted to exercise, fiber size and capillary density did not differ among young and senescent rat, neither exercises affected those measures. Furthermore, diaphragm seems to keep its oxidative ability and microvascularization as aging progresses[9].

In this study, we could see different capillarization patterns in DIA and RA muscles aging. In both groups and at all ages, IIB fibers (fast fatigable) presented a pattern of vascularization reduction compared to slow, non-fatigable fibers (I) or fast (IIA). Probably, the low oxidative ability of IIB fibers could explain the capillarization reduction seen in this kind of fiber.

A limitation in this study is the relatively small sample that was used. This limitation was due to implicit difficulties to adjust pH in order to enable the visualization of the kinds of fibers and capillaries in the same section, as well as due to the fact of working with open-

Fiber		TYPE I		TYPE II-A		TYPE II-B	
Animal	month	C/F	D. Cap	C/F	D. Cap	C/F	D. Cap
AVERAGE	1	3.442	6.867	4.424	8.145	3.993	4.570
S.D.		0807	1.953	0.690	1.065	0.834	1.592
AVERAGE	8	4.740	2.324	5.601	2.431	6.304	1.532
S.D.		0.238	0.275	0.374	0.126	0.376	0.147
AVERAGE	18	4.632	2.294	5.536	2.162	6.782	1.584
D.		0.874	0.538	0.521	0.120	0.622	0.182
K-Wallis		p=0.153	p=0.055	p=0.231	**p=0.025**	**p=0.048**	p=0.057
Wilcoxon		p=0.723	p=0.723	p=1.00	p=0.077	p=0.888	p=1.00

Table 1 - *Variables Measured at Diaphragm Muscle*

Fiber		TYPE I		TYPE II-A		TYPE II-B	
Animal	month	C/F	D. Cap.	C/F	D. Cap.	C/F	D. Cap.
AVERAGE	1	3.768	6.262	4.733	6.500	4.740	2.925
S.D.		0.198	1.386	0.037	2.011	0.021	0.518
AVERAGE	8	3.606	2.422	5.155	1.699	5.999	0.758
S.D.		0.423	0.226	0.375	0.289	0.689	0.130
AVERAGE	18	4.495	2.378	6.690	1.796	7.374	0.737
S.D.		0.213	0.264	0.120	0.207	0.877	0.051
Kruskal-Wallis		**p=0.027**	**p=0.029**	**p=0.015**	**p=0.015**	**p=0.029**	**p=0.030**
Wilcoxon		**p=0.033**	p=0.723	**p=0.033**	p=0.077	p=0.723	p=1.00

Table 2 - *Variables Measured at Rectus Abdominis Muscle*

camera microscopy. However, literature describes other authors working with a similarly small number of animals.
In DIA, we could see an aging model characterized by statistically significant changes among young and adult groups regarding fibers area and capillary density with no statistically significant changes in aging process, on capillaries/ fibers relationship, except for fast fibers (type IIB). When adult and old groups were compared, we could see that no significant changes existed between both groups in regard of any adopted parameters.
In RA, we saw an aging model characterized by statistically significant changes among young, adult and old groups for all kinds of fibers studied. By comparing the adult and old groups, statistically significant changes could be noticed regarding capillary/ fiber relationship for non-fatigable fibers (I and IIA), as well as regarding sectional area of fast fatigable fibers (IIB); there was no statistical significance in the rest of the comparisons, thus we could determine a different aging model for the rectus abdominis anterior muscle compared to DIA.
In the analysis of capillary density in both muscle groups, we could see that the changes on capillary/ fiber relationship were somehow compensated by different changes on fibers areas so that in old animals, capillary density is similar in diaphragm and in rectus abdominis muscles.
Gathered data allow us to conclude that there is an objective difference among diaphragm and rectus abdominis muscles aging models and a potential "protection", especially regarding fatigue-resistant fibers in DIA aging process. The contractibility condition often found in the muscle could be associated to those results.
Anyway, those results, necessarily limited, may encourage other researchers to dedicate themselves to the challenge of finding simple and reliable experimental models of physical activity and their impact on aging process of the locomotive apparatus.

CONCLUSION

Data allow us to conclude that there is a real difference in aging pattern between DIA and RA, especially regarding the capillary pattern of fatigue-resistant fibers. The continuous contraction of DIA, as a natural model of continuous exercise, could be one of the potential explanations for that.

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