愛知教育大学研究報告，49（芸術・保健体育・家政・技術科学編），pp. 111～114, March, 2000

Age-Related Changes on Morphological and Enzymatic Properties in the Extensor Digitorum Longus Muscle of Senescence Accelerated P6 Mice

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(Received September, 1999)

ABSTRACT

Age-related changes on morphological and metabolic properties in the extensor digitorum longus muscle were studied in 20-, 40-, 50-, and 60-week-old female senescence accelerated P6 mice (SAMP6). A decrease in the cross-sectional area of muscle fibers was observed at 50 and 60 weeks of age. However, SDH activity of muscle fibers did not change with aging. These results indicate that the size and SDH activity of muscle fibers in female SAM have different degeneration stages.

INTRODUCTION

Recently, a murine model of accelerated senescence was developed by Takeda et al[15]. Senescence accelerated mice (SAM) are characterized by genetically accelerated process of aging, and have a short life span (358 days of median survival time). Therefore, this model provides a unique system to study aging process. However, few studies on skeletal muscles and their fibers for this model have been reported[5][8][9]. The purpose of this study was to examine morphological and metabolic changes in fibers of the extensor digitorum longus muscle in female SAMP6 with aging.

MATERIALS AND METHODS

SAMP6 obtained from Chest Disease Research Institute, Kyoto University were bred and maintained in our facility. Female SAMP6 were housed in standard plastic cages housing five to six animals per cage and supplied with a standard diet and drinking water ad libitum. Room temperature was kept at 22℃ with a light-dark cycle of 12-12 hours. Twenty 20-, 40-, 50-, and 60-week-old SAMP6 were killed by neck dislocation, and the extensor digitorum longs (EDL) muscles were gently dissected from surrounding tissue. The muscle was wet-weighed and quickly frozen in isopentane cooled in liquid nitrogen. Several transverse sections were cut at 10μm thick in a microtome cryostat maintained at −25℃ and mounted on coverslips. The sections were subsequently processed for determination of succinate dehydrogenase (SDH) activity. A microphotometric technique was used for quantitative SDH measurements[11]. Cross-sectional areas of muscle fibers were measured on the same sections used for SDH measurements. Approximately 100 fibers per muscle were analyzed for measurements of SDH activity and cross-sectional area. In addition, the number of muscle fibers was counted in the same section.

Data are presented as mean ± SE. A one way analysis of variance (ANOVA) was used for age-related changes. If significant difference was observed by ANOVA, Scheffe (post hoc) test was used to determine the differences amang individual groups. The significant level was set at 0.05.

RESULTS

Table 1 shows changes in body weights and EDL muscle weights with aging. The body weight increased with aging, although these changes were not statistically significant. The absolute weights were not significantly different among groups. The relative weights decreased with aging, although these changes were not statistically significant.

Table 2 shows changes in numbers and cross-sectional areas of EDL muscle fibers with aging.

Table 1. Body weights, tissue weights and relative tissue weights in extensor digitorum longus (EDL) muscles in each aged group

	20wk	40wk	50wk	60wk
Body weight (g)	29.8±0.4	30.7±1.6	32.8±1.2	33.6±0.7
EDL weight (mg)	10.3±0.5	10.2±1.3	10.3±0.4	10.3±0.7
Relative EDL weight (mg/100g body weight)	34.4±1.3	33.2±4.0	31.5±1.6	30.3±1.9

Values are means±SE.

Table 2. Numbers and cross-sectional areas of extensor digitorum longus muscle fiber in each aged group

	20wk	40wk	50wk	60wk
Fiber number	676±84	630±27	493±20	569±30
Cross-sectional area (μm^2)	1460±28	1345±33	1097±17*	1177±22*

Values are means±SE.

*Significant difference from 20-and 40-weeks-old SAM ($p<0.05$)

The number of fibers per muscle cross-sectional area decreased with aging, although these changes were not statistically significant. However, the cross-sectional area of muscle fibers at 50 and 60 weeks was significantly lower than that at 40 weeks.

Fig. 1 shows mean SDH activities of EDL muscle fibers. There were no significant changes in mean SDH activities of muscle fibers with aging.

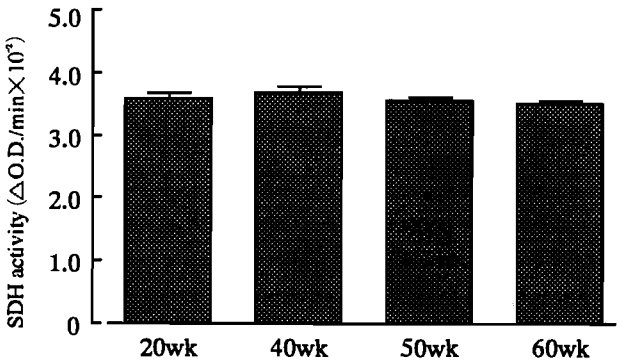

Fig. 1. Succinate dehydrogenase (SDH) activities of extensor digitorum longus muscle fibers in each aged group

DISCUSSION

It is well known that a decrease in the size and number of muscle fibers is induced with aging[1)-3)7)10)]. There was a significant decrease in the size of fibers in the EDL muscle of 50- and 60-week old female SAMP6 in this study. Ishihara et al[9)]. also reported a decrease in the fiber cross-sectional area of the EDL muscle in 60-week old male SAMP6. Furthermore, a decrease in the fiber number of fibers with aging was observed in this study.

Rowe[13)] reported age-related changes in the number and diameter of fibers in hind limb muscles of 750-days-old (107weeks) Inbred 129/Re strain mice. This study showed the changes in the fiber size of EDL muscle at 50 and 60 weeks. Addition-

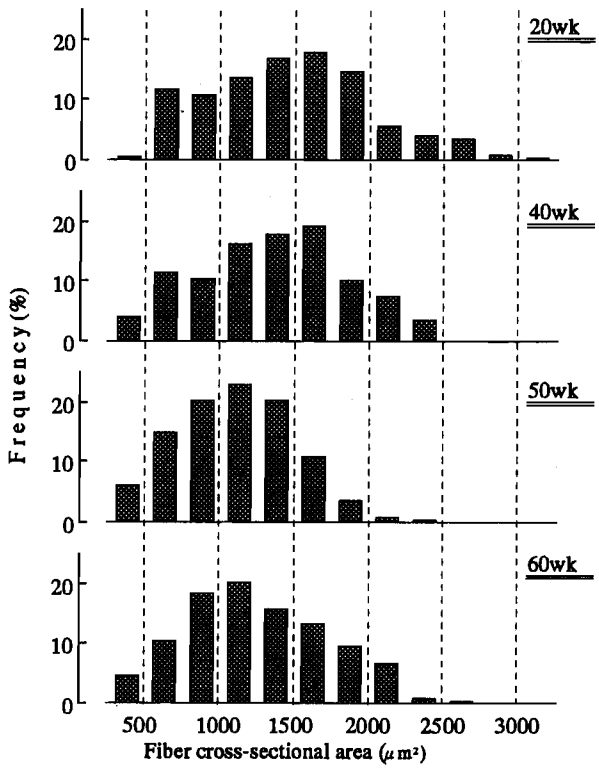

Fig. 2. Frequency distributions in fiber cross-sectional areas of extensor digitorum longus mucle in each aged group

ally, median survival time of SAM is shorter as 358 days (51weeks)[6]. These data suggest that degenerative stages of aging in skeletal muscle of SAMP6 were considered to be acceleration of senescence.

Ishihara et al[7]. demonstrated the selective atrophy and decrease in the fast twitch fibers in the rat EDL muscle with aging. When frequency distributions in the cross-sectional area are consulted (Fig. 2.), age-related changes are clearly induced by the loss of large-sized fibers. Furthermore, a decrease in the total fiber number with aging was observed in this study. Therefore, the atrophy and decrease in muscle fibers observed in this study are may be due to the change of the fast twitch fiber.

SDH activity of muscle fibers did not change with aging (Fig. 1.). A significant correlation was observed between the mean SDH activity and capillary density of muscle unit fibers[4]. Proctor et al[12]. reported that the capillary supply per unit type II fiber area was not affected by age. Since there was a decrease in the fiber size with aging in this study, it was indicated that a decrease in the diffusion distance improved an oxygen supply between blood and site of oxygen use[14]. Therefore, it was considered that a decrease in the diffusion distance by fiber atrophy prevented a decrease in the SDH activity with aging. However, Ishihara et al[9]. reported that a reduction in SDH activity of muscle fibers was observed in 60-week-old male SAMP6. This discrepancy may be due to the difference between sexual specificity and breeding environment.

In summary, it was suggested that size and SDH activity of muscle fibers in female SAM have different degeneration stages.

ACKNOWLEDGMENT

The authors grateful acknowledge the assistance of Mr. Hiroshi Inagaki for his technical assistance.

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (no. 10680022) from the Ministry of Education, Science, Sports and Culture of Japan.

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(平成11年9月10日受理)