Bull. Fac. Educ. Kumamoto. Univ.
No.62, 209－212, 2013

Effects of Heat Stress on Regenerating Fibers of Aged Rat Soleus Muscle

Yasuharu OISHI

（Received October 1, 2013）

老齢ラットヒラメ筋の再生筋線維に対する熱ストレスの影響

大 石 康 晴

Abstract

This study investigated the effects of heat stress on regenerating fibers of aged rat soleus muscle. Soleus fibers were degenerated by injection of bupivacaine and heat stress was applied 24h after the injection of bupivacaine and every other day thereafter until 14-day of experimental period. Three types of fibers, i.e., fast, slow, and fast+slow hybrid fibers, were observed in the regenerating soleus fibers after 14-day, and no difference of fiber type composition was observed between the bupivacaine-injected（BPVC）and bupivacaine-injected plus heat stressed（BPVC+Heat）soleus muscles. However, fiber size of the regenerating fibers was significantly larger in the BPVC+Heat than in the BPVC soleus muscle.

Our data suggested that heat stress had effect to accelerate the increase in fiber size, but had no effect on fiber type differentiation changing to adult fast or slow type in aged rat regenerating soleus fibers.

Key words : regenerating fiber; heat stress; soleus; fiber type; rat

Abstract 和訳

本研究は、老齢ラットヒラメ筋の再生筋線維に対する熱ストレスの影響について検討した。ブピバカインの注入によりヒラメ筋の筋線維を破壊し、注入 24 時間後とそれ以降一日おきに熱ストレスを加えた。14 日間の実験期間終了後、ヒラメ筋の再生筋線維では fast, slow, fast+slow の 3 タイプの筋線維が確認され、ブピバカイン注入群とブピバカイン注入＋熱ストレス群の間では、筋線維組成に差は見られなかった。一方、筋線維のサイズでは、熱ストレス群の再生筋線維サイズが有意に大きい結果であった。

本研究データは、熱ストレスは再生筋線維のサイズの増加を促進する一方、筋線維のタイプ分化には影響しないことを示唆している。

キーワード：再生筋線維；熱ストレス；ヒラメ筋；筋線維タイプ；ラット

Introduction

Mammalian skeletal muscle has high plasticity to change muscle fiber size, i.e., fiber hypertrophy or atrophy, and fiber type characteristics, or to recover from severe injury. Muscle fibers can regenerate after fiber degeneration and this is closely related to the activation of satellite cell, known as a stem cell in skeletal muscle fiber（Grand and Rudnicki, 2007; Hawke and Garry, 2001）. Regenerate fibers can be observed about 3~4 days after fiber degeneration, at which time embryonic and/or neonatal myosin heavy chain（MyHC）is expressed, and adult fast or slow type of MyHC is gradually expressed thereafter, concomitant with the increase in fiber size（Ciciliot and Schiaffino, 2010）.

Laboratory of Muscle Physiology, Faculty of Education, Kumamoto University

Heat stress to rat skeletal muscle, i.e., elevation of muscle temperature, has effects to protect muscle fiber atrophy or accelerate fiber growth even in the regenerating fibers. We reported that heat stress to soleus fibers degenerated by injection of bupivacaine accelerated the increase in fiber size in 8-week-old young rat, but had no effects to the recovery of fiber type composition during 2-week of recovery period (Oishi et al., 2009). It is unclear whether these effects of heat stress can be applied to aged rat soleus.

The purpose, therefore, of the present study is to clarify the effects of heat stress on the size and types of regenerating fibers in aged rat soleus muscle. We used 65- and 8- week-old rats in the present study.

Methods

Experimental design. Male Wistar rats aged 65-week-old were divided into two groups: heat stress (Heat group, n=5) or sedentary control (Control group, n=5). The left soleus muscle of both group were injected one time with 0.5 ml of bupivacaine (Marcain, Fujisawa Yakuhin Kogyo, Osaka, Japan) to degenerate-regenerate the muscle fibers and the right soleus was served as a contralateral control with no treatment. Heat stress was applied only to the rats in the Heat group under a non-anesthetic condition, i.e., the lower half of the rat body was immersed in water maintained at 42 ± 1℃ for 30 min 24 h after the injection of bupivacaine and every other day thereafter. Then the left and right soleus muscles in the Heat group were referred as bupivacaine-heat (BPVC+Heat) and heat (Heat), respectively, and those in the Control group as bupivacaine (BPVC) and contralateral control (Con). Fourteen days after bupivacaine injection, the soleus muscles of all rats were removed and subjected to immuno-histochemical analysis, following by previous reports (Kami & Senba, 2002; Oishi et al., 2008; Oishi et al., 2009), to determine the fiber type composition and fiber area. Some young rats aged 8-week-old were used to compare the fiber type composition of the soleus muscle with the aged rats.

Statistical analyses. All data are presented as means ± SEM. Significant main effects and interactions were determined using two-way ANOVA. When there was a significant, Fisher's post-hoc test was used to determine group differences. Statistical significance was established at $p < 0.05$.

Results

Change of fiber type composition with aging.

Figure 1 shows the immuno-histochemical staining for the fast type fiber in the soleus muscle from young (8-week-old, sedentary control, Fig. 1A) and aged (65-week-old, sedentary control, Fig. 1B) rats. The fast fiber was observed by 15~30% in young, but by 1~5% in aged rats. This may suggest the fiber type transition from fast-to-slow during normal aging process.

Fiber type composition of the regenerating soleus fibers of aged rat. In the bupivacaine injected soleus muscles in the BPVC and BPVC+Heat groups, regenerating fibers were observed at about day 4 and gradually becoming fast or slow fiber by expressing myosin heavy

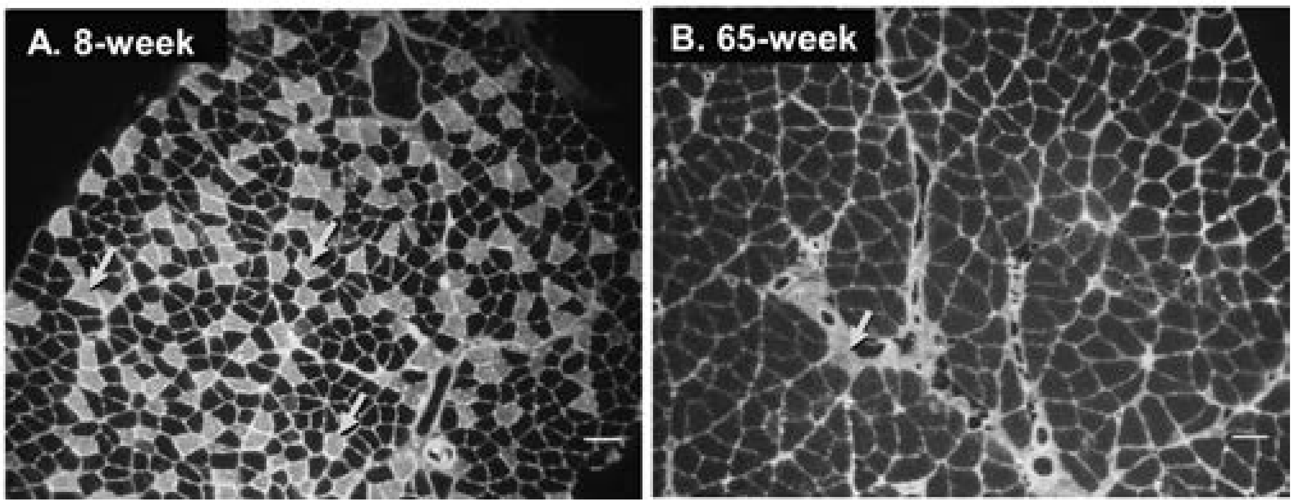

Figure 1. Immunohistochemically stained fast type fibers in sedentary young (8-week) and aged (65-week) rat soleus muscle. Arrows in each panel indicated the fast type fibers. Bar in each panel, 100 µm.

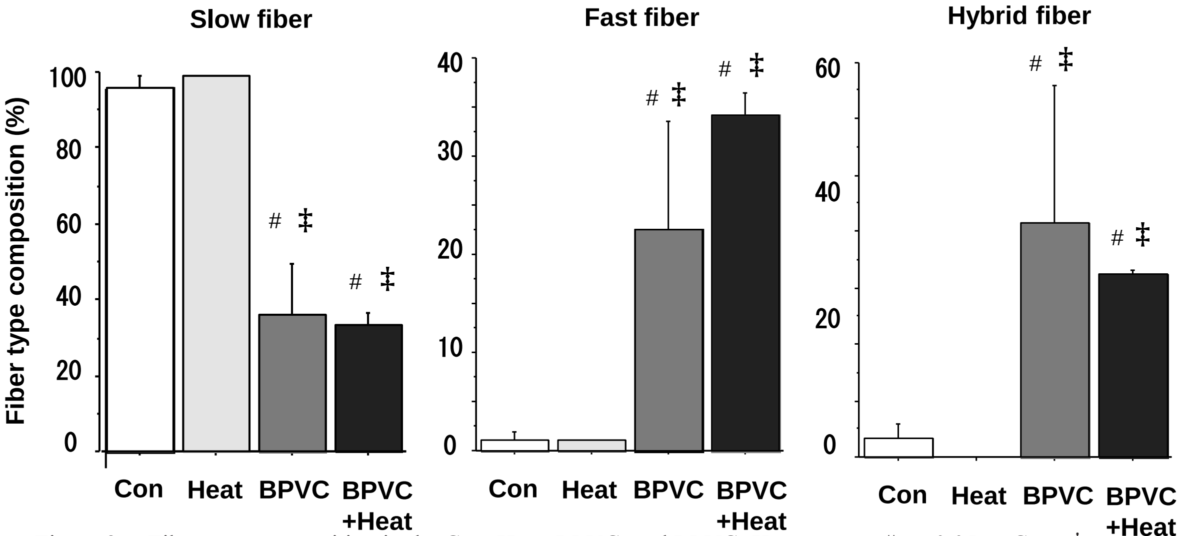

Figure 2. Fiber type composition in the Con, Heat, BPVC, and BPVC+Heat groups. #, p<0.05 vs Con; ‡, vs Heat.

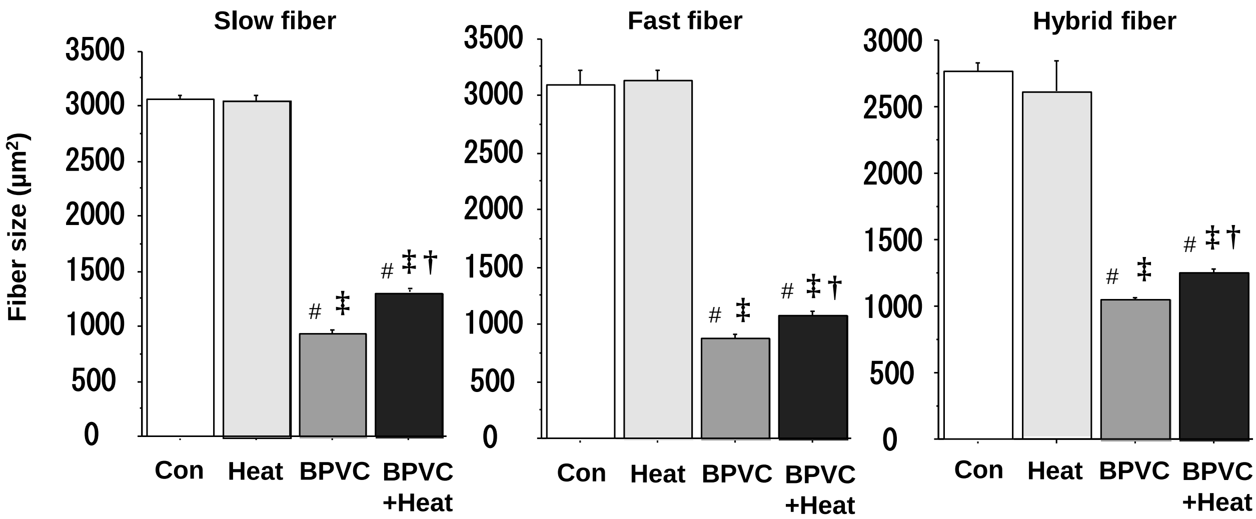

Figure 3. Muscle fiber size in the Con, Heat, BPVC, and BPVC+Heat groups. #, p<0.05 vs Con; ‡, vs Heat; †, vs BPVC.

chain fast or slow isoform. At day 14, the percentage of slow fiber was almost the same between the BPVC (36%) and BPVC+Heat (33%) group (Fig. 2). No differences were also observed for the percentage of fast or fast+slow hybrid fibers between the BPVC and BPVC+Heat groups (Fig.2).

Cross-sectional are (CSA) of the regenerating fibers of aged rat. As shown in Fig. 3, CSA of each fiber type in the Con group was similar with that in the Heat group. CSA of the regenerating fibers in the BPVC treated two groups was reached to the level of 28~48% from that of the Con or Heat soleus fibers. The BPVC+Heat group indicated 21~38% larger CSA in all fiber types than BPVC group (Fig. 3).

Discussion

With aging, the soleus muscle of 65-week-old sedentary control rat was changed toward slower characteristic with the increase in slow type of fibers and decrease in the fast type, compared with the young soleus fibers as shown in Fig.1B. Since we could not observe the significantly atrophied fibers and/or abnormal-shaped fibers in the aged soleus muscle, the change of fiber type composition observed in the aged soleus may be caused by the rearrangement of motor nerve from F (fast) to S (slow) type. Furthermore, since the fiber size of aged (65-week-old) rat soleus muscle did not show the signal of fiber atrophy and was similar with that of young soleus fibers, it is considered that fiber type conversion with aging as indicated in Fig. 1B occurs prior to muscle fiber atrophy with aging.

One important question is whether heat stress has useful effects on the recovery of fiber type composition and fiber size of regenerating soleus muscle in aged rat. As shown in Figs. 2 and 3, compared with the bupivacaine treated two groups (BPVC vs BPVC+Heat), fiber type composition was not different between the groups, but fiber size of all fiber type was significantly larger in the BPVC+Heat than BPVC group. We have reported in our previous paper using 8-week-old young rat that heat stress accelerated the growth of regenerating fibers but not fiber type composition (Oishi et al., 2009). Thus present study also supports this conclusion. However, we could observe in the present study the slow and fast fibers in the regenerating fibers after 14 days of bupivacaine injection, although only the fast+slow hybrid fibers could be detected in regenerating fibers of young rat soleus muscle with the same time period in our previous paper (Oishi et al., 2009). The difference between the young and old rat soleus muscle on the recovery of fiber types, i.e., differentiation to the slow or fast type, is unclear. The effects of heat stress to the fiber type differentiation in the regenerating fibers remain to be elucidated.

Reference

1. Ciciliot S, Schiaffino S. Regulation of mammalian skeletal muscle: basic mechanisms and clinical implications. *Curr Pharm Design* 16: 906-914, 2010.
2. Grand FL, Rudnicki MA. Skeletal muscle satellite cells and adult myogenesis. *Curr Opin Cell Biol* 19: 628-633, 2007.
3. Hawke TJ, Garry DJ. Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol* 91: 534-551, 2001
4. Kami K, Senba E. In vivo activation of STAT3 signaling in satellite cells and myofibers in regenerating rat skeletal muscles. *J Histochem Cytochem* 50: 1579?1589, 2002.
5. Oishi Y, Hayashida M, Tsukiashi S, Taniguchi K, Kami K, Roy R. R., Ohira Y. Heat stress increases myonuclear number and fiber size via satellite cell activation in rat regenerating soleus fibers. *J Appl Physiol* 107: 1612-1621, 2009.
6. Oishi Y, Ogata T, Yamamoto K, Terada T, Ohira T, Ohira Y, Taniguchi K, Roy RR. Cellular adaptations in soleus muscle during recovery after hindlimb unloading. *Acta Physiol* 192: 381-395, 2008.