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Influence of Hypobaric-Hypoxia on Fiber Type Distribution of the Rat Extensor Digitorum Longus Muscle

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Summary. **Fiber type distributions of the fast-twitch extensor digitorum longus muscle in rats were examined after exposure to hypobaric-hypoxia for 5 weeks from the postnatal age of 5 weeks or for 10 weeks from the postnatal age of 10 weeks. Muscle fibers were classified into fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), or slow-twitch oxidative (SO). The percentage of FOG fibers was increased and that of FG fibers was decreased in the hypoxic rats at 10 weeks of age, while there was no difference in the percentage of SO fibers between the normoxic and hypoxic rats. There was no difference in the fiber type distribution between the normoxic and hypoxic rats at 20 weeks of age. These results indicate an age-specific response of hypobaric-hypoxia on the fiber types of the rat extensor digitorum longus muscle.**

Key words—extensor digitorum longus muscle, histochemistry, hypobaric-hypoxia, muscle fiber type, rat.

INTRODUCTION

Previous studies,[4,7,8,24,28] have demonstrated the adaptability of capillary density, myoglobin concentration, and oxidative enzyme activity in skeletal muscles by short- or long-term hypoxia, or chronic hypoxic exposures. In addition, our recent studies[6,10,14] revealed the adaptation of histochemical fiber types in the slow-twitch muscle after exposure to hypoxia and found an increased percentage of oxidative (fast-twitch oxidative glycolytic) fibers in the soleus muscle of the developing rat following exposure to hypoxia.

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On the other hand, a few investigations[1,15,19,22] carried out on the adaptation of histochemical fiber types in the fast-twitch muscle after exposure to hypoxia and conflicting interpretations have been given for the results obtained. Therefore, this study examined the fiber type distribution of the fast-twitch extensor digitorum longus muscle in rats after exposure to hypobaric-hypoxia. The rats were exposed to hypobaric-hypoxia for 5 weeks from 5 weeks of age (developmental stage) or for 10 weeks from 10 weeks of age (adult stage) for comparisons with our previous results using the rat slow-twitch soleus muscle.[12,13]

MATERIALS AND METHODS

Animals and hypobaric-hypoxic exposure

Thirty male Sprague-Dawley rats were assigned to the normoxic (n=18) and hypoxic (n=12) groups. The rats in the hypoxic group were housed in a hypobaric-hypoxic chamber and exposed to hypobaric-hypoxia of 463 torr, equivalent to an altitude of 4,000 m. The rats at 5 weeks of age were exposed to hypobaric-hypoxia for 5 weeks (developmental stage, n=6) and those at 10 weeks of age for 10 weeks (adult stage, n=6). The pressure in the hypobaric-hypoxic chamber was returned to sea level atmospheric pressure for 1 h three times a week, during which time body weight was measured, the chamber was cleaned, and food and water were replaced. The air flow in the hypobaric-hypoxic chamber was maintained at 20 l·min^{-1}. The rats in the normoxic group were housed at sea level atmospheric pressure and used as controls at 5 weeks (n=6), 10 weeks (n=6), or 20 weeks (n=6). All animals were housed in a controlled envi-

ronment with a constant dark-light cycle (dark time 0700-1900) and maintained at a temperature of 22±2℃. Standard food and water were provided *ad libitum*. All experiments were approved by the Laboratory Animal Care Committee of Kyoto University, Japan.

Histochemistry

The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg • kg^{-1}). After being weighed, the extensor digitorum longus muscle was removed and immediately frozen in isopentane cooled in a mixture of dry ice and acetone. Serial transverse sections, 10 μm thick, of the widest portion of the muscle midbelly were cut on a cryostat at −20℃. The sections were stained for adenosine triphosphatase following alkaline and acid preincubations, succinate dehydrogenase, and α-glycerophosphate dehydrogenase activities. The muscle fibers were classified into fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), or slow-twitch oxidative (SO) as described previously (Fig. 1).[20] The fiber type distribution was calculated by counting the number of each type of fiber in the entire transverse section of the muscle.

Statistics

Values were expressed as mean ± standard deviation. Student's *t*-test was used to determine any significant differences between the normoxic and hypoxic groups.

RESULTS

Body weight and muscle weight

The body weight, extensor digitorum longus muscle weight, and relative muscle weight in the normoxic group at 5 weeks of age were 153±8 g, 0.08±0.01 g, and 0.054±0.005 g/100 g body weight, respectively.

At 10 weeks of age, the body weight was lower in the hypoxic group than in the normoxic group (normoxia, 332±17 g; hypoxia, 255±23 g, $p<0.001$). The muscle weight was lower in the hypoxic group than in the normoxic group (normoxia, 0.17±0.01 g; hypoxia, 0.13±0.02 g, $p<0.001$), while there was no difference in the relative muscle weight between the normoxic and hypoxic groups (normoxia, 0.051±0.004 g/100 g body weight; hypoxia, 0.051±0.003 g/100 g body weight).

These results were similar for rats at 20 weeks of age. The body weight was lower in the hypoxic group than in the normoxic group (normoxia, 496±34 g; hypoxia, 410±28 g, $p<0.001$). The muscle weight was lower in the hypoxic group than in the normoxic group (normoxia, 0.22±0.03 g; hypoxia, 0.18±0.01 g, $p<0.05$), while there was no difference in the relative muscle weight between the normoxic and hypoxic groups (normoxia, 0.044±0.006 g/100 g body weight; hypoxia, 0.045±0.003 g/100 g body weight).

Total fiber number

The total fiber number in the entire transverse section of the extensor digitorum longus muscle in the normoxic group at 5 weeks of age was 2825±90.

There was no difference in the total fiber number of the muscle between the normoxic and hypoxic groups at 10 weeks (normoxia, 2850±154; hypoxia, 2854±277) or at 20 weeks of age (normoxia, 2717±218; hypoxia, 2702±466).

Fiber type distribution

The percentages of FOG, FG, and SO fibers of the extensor digitorum longus muscle in the normoxic group at 5 weeks of age were 48.2±2.1, 48.9±2.0, and 2.9±1.1, respectively.

The percentage of FOG fibers was significantly higher and that of FG fibers was significantly lower in the hypoxic group than in the normoxic group at 10 weeks of age, while there was no difference in the percentage of SO fibers between the normoxic and hypoxic groups (Fig. 2). On the other hand, there was no difference in the fiber type distribution between the normoxic and hypoxic groups at 20 weeks of age (Fig. 2).

DISCUSSION

It is widely accepted that hypoxia markedly increases capillarization, mitochondrial density, myoglobin concentration, and oxidative enzyme activity in the skeletal muscle to maintain adequate levels of tissue oxygenation.[2-5,7,21,27] Spinal motoneurons innervating the skeletal muscle also exhibit increased oxidative enzyme activity in adaptation to hypoxia.[9,25]

Our previous[6,10,14] studies have shown the alteration of histochemical fiber types in the slow-twitch soleus muscle following exposure to hypoxia and found an increased percentage of oxidative (fast-twitch oxidative glycolytic) fibers. In those papers, we concluded that hypoxia inhibited the growth-

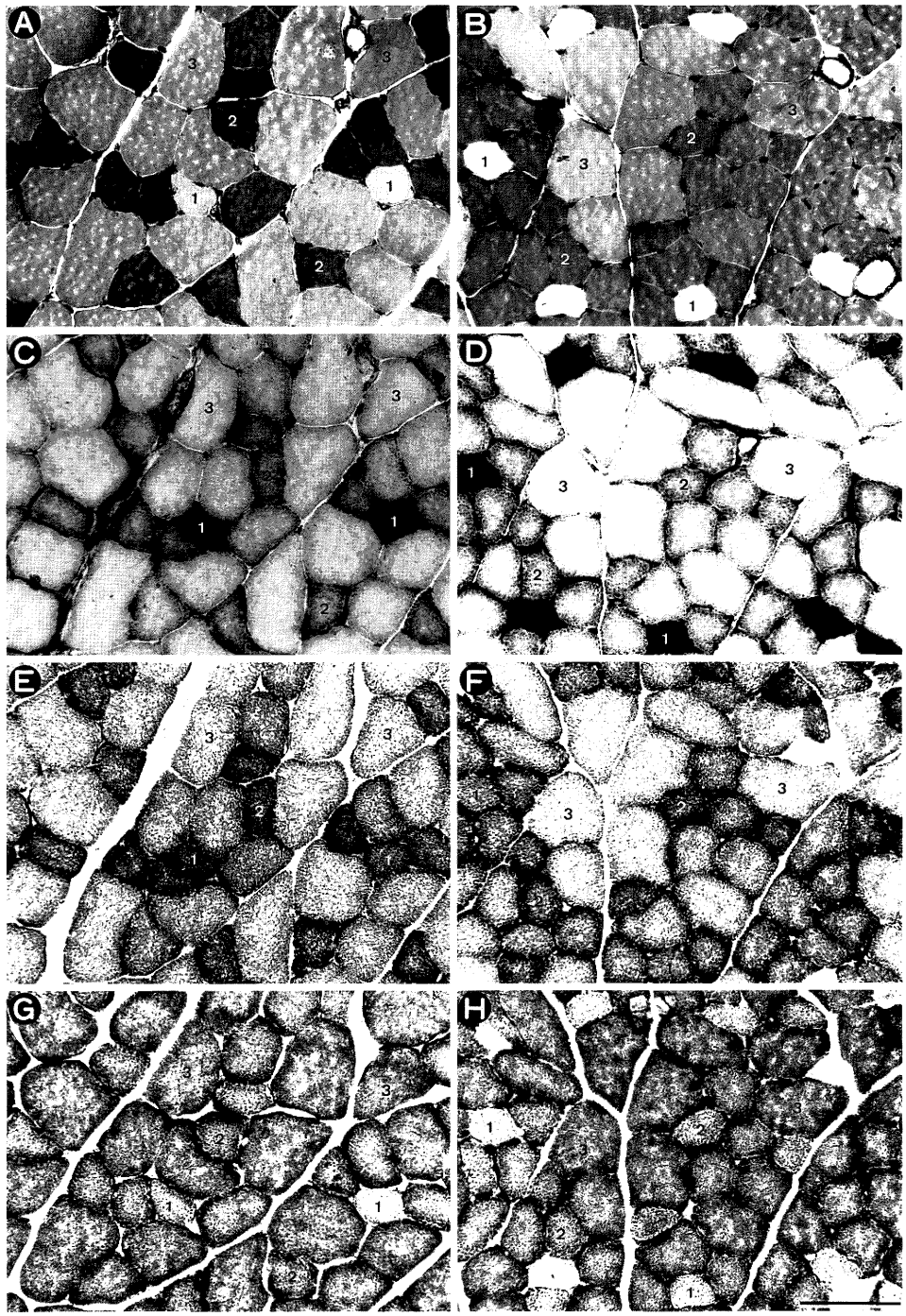

Fig. 1. Serial transverse sections of the extensor digitorum longus muscle in normoxic (*left*) and hypoxic (*right*) rats at 10 weeks of age. The sections are stained for adenosine triphosphatase activities following alkaline (**A** and **B**) and acid (**C** and **D**) preincubations, and for succinate dehydrogenase (**E** and **F**) and α-glycerophosphate dehydrogenase (**G** and **H**) activities. 1, slow-twitch oxidative; 2, fast-twitch oxidative glycolytic; 3, fast-twitch glycolytic. *Bar* indicates 100 μm for all panels.

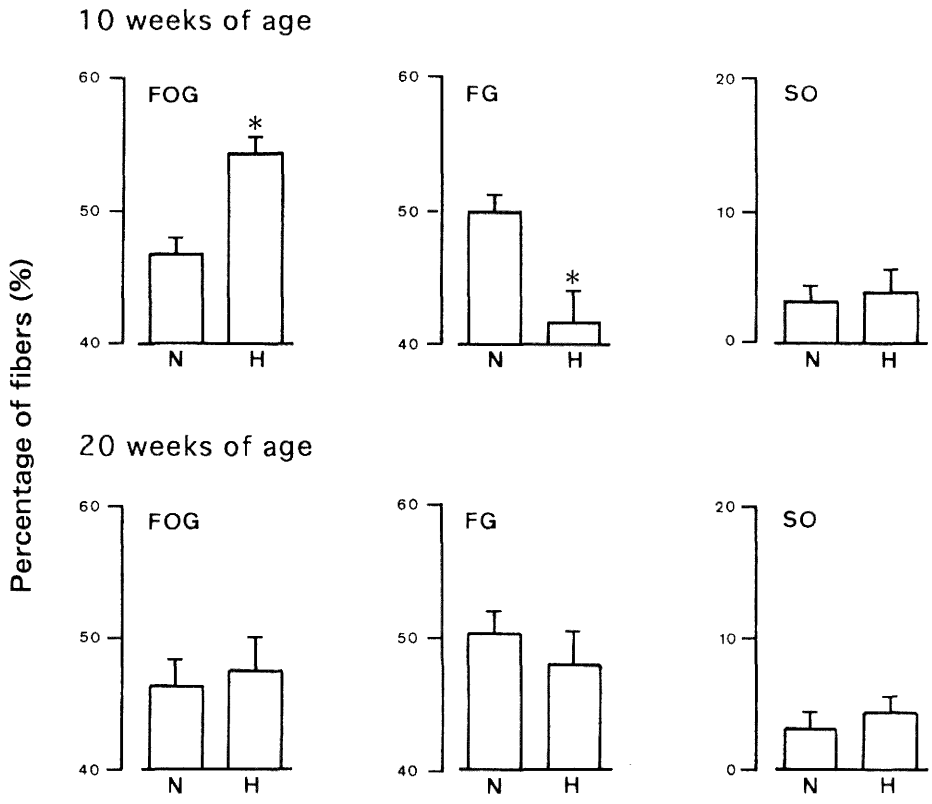

Fig. 2. Percentages of fibers of each type in the extensor digitorum longus muscle in normoxic and hypoxic rats. The hypoxic rats were exposed to hypobaric-hypoxia for 5 weeks from the postnatal age of 5 weeks *(top)* or for 10 weeks from the postnatal age of 10 weeks *(bottom)*. Values are means±standard deviations. FOG, fast-twitch oxidative glycolytic; FG, fast-twitch glycolytic; SO, slow-twitch oxidative; N, normoxia; H, hypoxia. *$p<0.001$ compared with the value in the normoxic group.

related type shift of muscle fibers from FOG to SO in the soleus muscle, which occurs during postnatal growth.[16,23] In addition, we reported that this inhibition was induced by hypoxia, irrespective of the age and the duration at which the animals were exposed to hypoxia.[11-13] Therefore, it is still uncertain whether the increased percentage of oxidative fibers found in the rat soleus muscle following hypoxia is an adaptive change.

A few investigations have been carried out on the response of histochemical fiber types in the fast-twitch extensor digitorum longus muscle after exposure to hypoxia, with conflicting interpretations given for the results obtained. Bigard et al.[1] observed a higher proportion of type IIab (this type is classified into the intermediate type between high-oxidative IIa and low-oxidative IIb) fibers in the extensor digitorum longus muscle in rats after 14 weeks of hypoxic exposure (the pressure was gradually reduced to an equivalent altitude of 4,000 m), indicating that an increased proportion of type IIab fibers reflects a tendency toward the enhancement of high-glycolytic fibers (shift of fiber types from IIa to IIab and IIb). In contrast, León-Velarde et al.[15] observed that the extensor digitorum longus muscle in Andean coots living at high altitude (4,200 m) has a higher proportion of high-oxidative type I fibers and a lower proportion of high-glycolytic type IIb fibers. A recent study,[19] however reported that there was no difference in the fiber type distribution of the extensor digitorum longus muscle in rats following 56 days of hypoxic exposure, equivalent to an altitude between 2,250 m and 2,250 m.

This study found an increased percentage of high-oxidative FOG fibers in the extensor digitorum longus muscle in the hypoxic rats at 10 weeks of age. The type shift of muscle fibers with growth does not occur in the rat extensor digitorum longus muscle after mononeuronal innervation from the single motoneuron to its muscle unit is completed.[17,18,26] In fact, this study showed no difference in the fiber type distribution of the extensor digitorum longus muscle

among the normoxic rats at 5 weeks, 10 weeks, and 20 weeks of age. Therefore, it is concluded that hypoxia induced the type shift of fibers from low-oxidative FG to high-oxidative FOG in the extensor digitorum longus muscle of developing rats. It is expected that the increased percentage of FOG fibers causes a facilitation of oxygen transport and an improvement in the diffusion of oxygen from capillaries to muscle fibers under hypoxic conditions.

On the other hand, the type shift of fibers in the extensor digitorum longus muscle was not found in the hypoxic rats at 20 weeks of age although any gain in body and muscle weights during postnatal growth was inhibited by hypoxia. Older rats may have lower adaptations to hypoxia on the fiber type distribution of the fast-twitch muscle. However, further studies will be needed to elucidate the muscle type- and age-specific responses of hypoxia.

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