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# Inhibition of the Growth-Related Fiber Type Shift in Rat Soleus Muscle by Hypobaric-Hypoxia

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***Summary.*** **The histochemical type shift of fibers from fast-twitch oxidative glycolytic (FOG) to slow-twitch oxidative (SO) in the rat soleus muscle occurs during postnatal development. Our recent experiments demonstrated that the type shift of fibers from FOG to SO is inhibited by hypobaric-hypoxia. Furthermore, the present study has shown that hypobaric-hypoxia inhibits the growth-related type shift of fibers from FOG to SO in the rat soleus muscle, irrespective of the duration of exposure to hypobaric-hypoxia.**

**Key words**—histochemistry, hypobaric-hypoxia, muscle fiber type, rat, soleus muscle.

## INTRODUCTION

Mammalian skeletal muscle fibers are classified into fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG), or slow-twitch oxidative (SO), according to their enzyme histochemical profiles.[12] The type shift of fibers from FOG to SO in the rat soleus muscle occurs during postnatal development.[10,16] Previously, we found that the growth-related type shift of fibers from FOG to SO in the rat soleus muscle was inhibited by hypobaric-hypoxia.[5,8] In addition, we have shown that this inhibition was induced by hypobaric-hypoxia for 5 weeks, irrespective of the age at which animals were exposed to hypobaric-hypoxia.[6]

In the present study, to determine whether hypoxic-exposure for more than 5 weeks produces similar results, the change in the fiber type distribution of the rat soleus muscle was examined after 10 weeks of hypobaric-hypoxia of 463 torr, equivalent to an altitude of 4,000 m.

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## MATERIALS AND METHODS

### Animals and hypoxic-exposure

Ten male Sprague-Dawley rats were randomly divided into two hypoxic groups of five animals each. The animals in the hypoxic groups were housed in a hypobaric chamber (Yamashita Technology Systems, Tokushima, Japan) and from the postnatal age of 5 or 10 weeks were exposed to hypobaric-hypoxia of 463 torr, equivalent to an altitude of 4,000 m, for 10 weeks. The pressure in the chamber was returned to sea level atmospheric pressure twice a week, during which time food and water were replaced and the chamber was cleaned. The air flow in the chamber was regulated at 20 $l \cdot min^{-1}$. Four normoxic groups of six male rats each were housed at sea level atmospheric pressure and used as controls at 5, 10, 15, and 20 weeks of age. All the animals were housed in a controlled environment with 12 h of daylight 12 h of darkness and maintained in room air at a temperature of $22 \pm 2^{\circ}C$. Standard food and water were provided ad libitum.

### Histochemistry

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). After being weighed, the soleus muscle was removed and weighed while moist. The muscle was immediately frozen in isopentane cooled in a mixture of dry ice and acetone. Serial

**Table 1.** Body weights and soleus muscle weights of normoxic and hypoxic rats

| | n | Body weight (g) | Muscle weight (mg) | Muscle weight (mg/100 g BW) |
|---|---|---|---|---|
| 5 wks normoxia | 6 | 156.1± 7.0 | 61.5± 4.8 | 39.6±4.6 |
| 10 wks normoxia | 6 | 365.1±16.0 | 148.4±12.2 | 40.7±3.5 |
| 15 wks normoxia | 6 | 479.1±21.8 | 175.5±12.8 | 36.7±2.9 |
| 15 wks hypoxia | 5 | 392.6±18.9** | 172.1±13.4 | 43.8±2.9* |
| 20 wks normoxia | 6 | 546.8±31.7 | 194.8±16.2 | 35.7±2.7 |
| 20 wks hypoxia | 5 | 403.7±24.4** | 177.1±20.0 | 43.9±4.7* |

Values are means±standard deviations. n, number of animals analyzed; mg/100 g BW, mg per 100 g body weight. *$p<0.01$, **$p<0.001$ compared with the value in the normoxic group at the corresponding age.

transverse sections, 10-$\mu$m thick, of the widest portion of the muscle belly were cut in a cryostat maintained at −20°C. The sections were stained for adenosine triphosphatase following alkaline preincubation at pH 10.3, succinate dehydrogenase, and $\alpha$-glycerophosphate dehydrogenase activities. The soleus muscle fibers were classified into FOG, SO, or intermediate (INT) as described previously.[3] The muscle fiber type distribution was calculated by counting the number of each type of muscle fiber in the entire transverse sections of the muscle. The cross-sectional areas of each type of muscle fiber were measured on the sections stained for adenosine triphosphatase activity, using a digitizer connected to a computer. For each muscle sample, more than 100 fibers were measured and at least 50 fibers of FOG or SO measured.

### Statistics

Means and standard deviations were calculated from individual values using standard procedures. Values were represented as means ± standard deviations. A one-way analysis of variance was used to determine any significant differences among the groups.

## RESULTS

### Body weight and muscle weight

The body weight was lower in the hypoxic group than in the normoxic group at 15 and 20 weeks (Table 1). There was no significant difference in the soleus muscle weight between the normoxic and hypoxic groups at 15 and 20 weeks, while the soleus muscle weight per body weight was higher in the hypoxic group than in the normoxic group at 15 and 20 weeks (Table 1).

**Table 2.** Total fiber numbers of the soleus muscle of normoxic and hypoxic rats

| | n | Muscle fiber number |
|---|---|---|
| 5 wks normoxia | 6 | 2448±236 |
| 10 wks normoxia | 6 | 2373±207 |
| 15 wks normoxia | 6 | 2438±222 |
| 15 wks hypoxia | 5 | 2503±183 |
| 20 wks normoxia | 6 | 2531±256 |
| 20 wks hypoxia | 5 | 2344±309 |

Values are means±standard deviations. n, number of animals analyzed.

### Total fiber number

There was no significant difference in the total fiber number of the soleus muscle between the normoxic and hypoxic groups at 15 and 20 weeks (Table 2).

### Fiber type distribution

In the soleus muscle, the percentage of FOG fibers was higher and that of SO fibers was lower in the hypoxic group than in the normoxic group at 15 and 20 weeks (Figs. 1 and 2). There was no significant difference in the percentage of INT fibers between the normoxic and hypoxic groups at 15 and 20 weeks (Fig. 2).

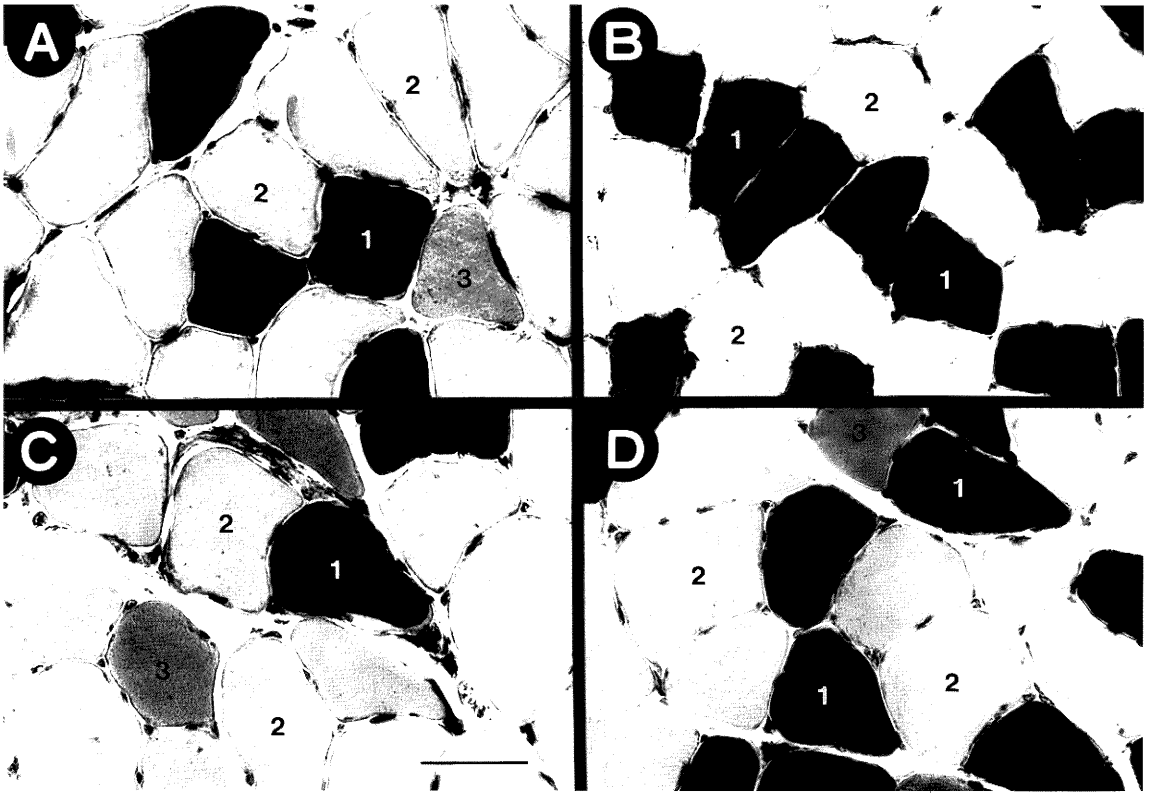


**Fig. 1.** Transverse sections of the soleus muscle of normoxic and hypoxic rats at 15 and 20 weeks. The sections were stained for adenosine triphosphatase activity following alkaline preincubation at pH 10.3. **A**, normoxia at 15 wks; **B**, hypoxia at 15 wks; **C**, normoxia at 20 wks; **D**, hypoxia at 20 wks; *1*, fast-twitch oxidative glycolytic; *2*, slow-twitch oxidative; *3*, intermediate. *Bar*, 50 $\mu$m for all panels.

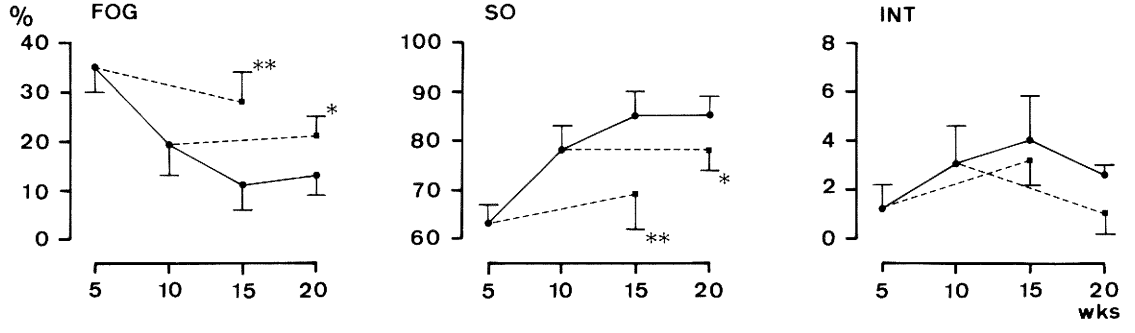


**Fig. 2.** Percentages of fast-twitch oxidative glycolytic (FOG), slow-twitch oxidative (SO), and intermediate (INT) fibers in the soleus muscle of normoxic *(solid lines)* and hypoxic *(dashed lines)* rats. Values are means±standard deviations. *$p<0.05$, **$p<0.01$ compared with value in the normoxic group at the corresponding age.

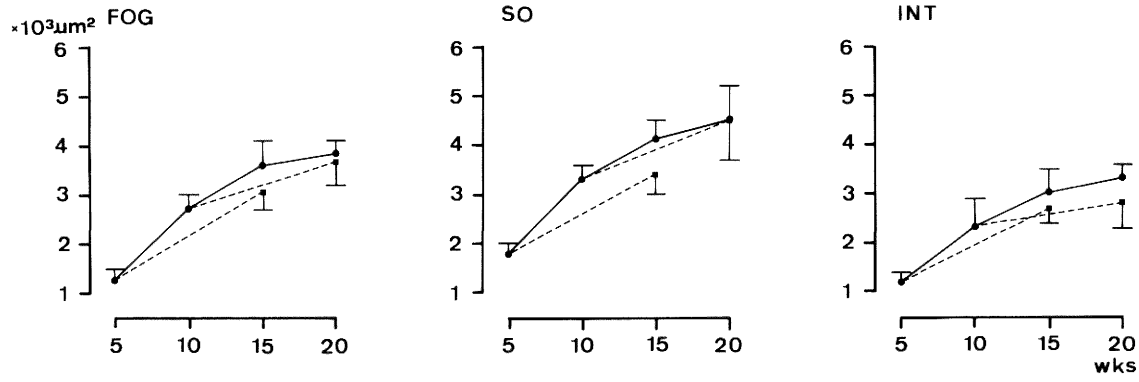


**Fig. 3.** Cross-sectional areas of fast-twitch oxidative glycolytic (FOG), slow-twitch oxidative (SO), and intermediate (INT) fibers in the soleus muscle of normoxic *(solid lines)* and hypoxic *(dashed lines)* rats. Values are means±standard deviations.

### Fiber cross-sectional area

There was no significant difference in the fiber cross-sectional area of the soleus muscle between the normoxic and hypoxic groups at 15 and 20 weeks (Fig. 3).

## DISCUSSION

Numerous investigations have been performed on alterations in the histochemical fiber type distribution of the skeletal muscle exposed to short- or long-term hypoxia, or chronic-hypoxia. However, data on the effect of hypoxia on muscle fiber types are contradictory because of the differences in muscles and animals and/or of hypoxic-conditions. Previous studies[14,15] observed no changes in the fiber type distribution in the rat and guinea pig soleus muscles by exposure to hypoxia. In contrast, other studies using birds,[9] rats,[1] and humans[11,13] have found that hypoxia produces adaptative changes in the fiber type distribution in the vastus lateralis, extensor digitorum longus, tibialis anterior, and plantaris muscles.

Our previous studies[4,8,17] have shown that hypobaric-hypoxia causes some metabolic alterations in neuromuscular systems. In the present study, the type shift of fibers from FOG to SO in the rat soleus muscle which occurs during postnatal development was inhibited for 10 weeks of hypobaric-hypoxia from 5 and 10 weeks of age. This is in agreement with our previous findings[6] that inhibition in the growth-related changes of histochemical fiber types and of myosin heavy chain isoforms in the rat soleus muscle was found, irrespective of the age at which the animals were exposed to 5 weeks of hypobaric-hypoxia. Therefore, we conclude that hypobaric-hypoxia inhibits the type shift of fibers from FOG to SO in the rat soleus muscle, irrespective of the age and duration of exposure to hypobaric-hypoxia.

In this study, we could not elucidate the physiological meanings of inhibition in the growth-related type shift of fibers from FOG to SO in the soleus muscle. Our previous study[7] showed that there was no effect of hypobaric-hypoxia on functional parameters including maximal twitch force, contraction time, and fatiguability of the rat soleus muscle, although hypoxic rats had a higher percentage of FOG fibers than controls. Further studies are needed to clarify the physiological meanings of inhibition in the growth-related type shift of fibers.

Male patients with chronic obstructive lung disease had a higher percentage of type II (FOG) fibers in the muscle than normal, and FOG fiber population decreased towards normal after isovolmic hemodilution.[2] Similarly, we found that hypoxia-acclimatized rats had a higher percentage of FOG fibers than normoxic animals, while the hypoxia-acclimatized animals showed a similar muscle fiber type distribution as found in normoxic animals after they were bred at sea level atmospheric pressure for 5 weeks.[5] Therefore, it is suggested that a higher percentage of FOG fibers in the muscle is found only under hypoxic-conditions and is due to an acute response to hypoxia.

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