Effect of Chronic Hypoxia on Skeletal Muscle Fiber Type in Adult Male Rats

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The effects of chronic hypoxia on muscle fiber distribution were investigated in 10-week-old male Wistar rats. Samples of the soleus and the plantaris muscles were extracted after the animals were exposed to normobaric hypoxia (10 % O₂) for 4 weeks. Histochemical myosin-based classification of skeletal muscle fibers was used. There was no evidence of transformation between different fiber types in either muscles. These results differ from those of previous studies. The rats used in those studies were younger, and it is difficult to distinguish the conversion of fiber types that occurs during normal development from other factors. These data suggest that chronic hypoxia does not affect muscle fiber type in adult rats.

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Interest in the adaptive responses to hypoxia has led to the idea that the effect of an hypoxic environment on skeletal muscles may be similar to the effect of physical training. This is because moderately and severely exercising muscles are believed to be relatively hypoxic. Endurance training can increase oxidative capacity both in animal and human skeletal muscle (Holloszy et al., 1984). Exposure to hypoxia has also been said to increase activities of some oxidative enzymes in mammalian muscles (Hochachka et al., 1982; Holm et al., 1973; Reynafarje, 1962). However, longitudinal studies of human skeletal muscle have shown that chronic exposure to very high altitude can decrease the activities of some enzymes (Green et al., 1989; Howald et al., 1990).

Intensive endurance training can cause type IIb (low oxidative) fibers to convert to type IIa (high oxidative) fibers in humans (Jansson et al., 1978) and in animals (Green et al., 1984). The results of studies of the effect of hypoxia on fiber type distribution in

skeletal muscle are not consistent. Taguchi et al. (1985) reported that hypoxia induced transformation of rat skeletal muscle fibers fast twitch glycolytic (FG) to fast twitch oxidative glycolytic (FOG). On the other hand, Bigard et al. (1991) recognized the changes of fiber types from IIa to IIb after hypoxic exposure. In contrast, Green et al. (1989) found no effect of hypoxia on relative muscle fiber distribution in human skeletal muscle. This inconsistency may be related to differences in species and age of the animals, type of muscle, degree of hypoxia, and duration of hypoxic exposure. In addition, fiber types of rat soleus muscle change during the course of normal postnatal development (Kugelberg, 1976). Therefore, because young rats were used in previous studies (Taguchi et al., 1985; Bigard et al., 1991), it is difficult to distinguish natural maturation from other factors that could affect the transformation of muscle fibers.

The purpose of the present study is to clarify the effect of hypoxia on fiber type distribution in the

slow soleus and fast plantaris muscles of adult rats.

METHODS

Male Wistar rats of about 280 g were used. They were separated at random into two groups at the age of 10 weeks. Rats in the control group ($n = 10$) were housed in individual cages and maintained in a normoxic environment. Rats in the experimental group ($n = 15$) were continuously exposed to 10 % O_2 in a chamber for 4 weeks. Hypoxic environment were made by flowing pure nitrogen gas into the chamber at a constant speed. To prevent increase in CO_2 the chamber was ventilated and the CO_2 concentration was kept below 0.1 %. The chamber was opened for about 1 hour a day, so the cage could be cleaned, water and food restocked, and the rats weighed. Hypoxic rats were given chow and water ad libitum. Normoxic rats were given the same amount of food that hypoxic rats had eaten on the previous day, to keep the two groups at equal body weight.

Five rats from each group were selected at random for histochemical studies. After the experimental period the animals were anesthetized with sodium pentobarbital. The soleus and plantaris muscles were removed and weighed. Then they were frozen in isopentane that was cooled with liquid nitrogen, and stored at $-80^\circ C$ until they were analyzed. Serial cross sections ($10\ \mu m$ thick) were cut at $-20^\circ C$ on a cryostat microtome, mounted on cover glass, and air dried at room temperature. Sections were stained for myofibrillar ATPase after preincubation at pH 10.3 (10 min, $37^\circ C$), pH 4.6 (2–4 min, $25^\circ C$), and pH 4.4 (2 min, $25^\circ C$), to divide the muscle fibers into types I, IIa, IIb, and IIc, according to Brooke and Kaiser (1970). The percentage of each type was calculated by counting all fibers that could be identified by type.

The groups were compared with Student's *t* test. $P < 0.05$ was taken to indicate a statistically significant difference.

RESULTS

For the first two weeks body weight of hypoxic rats stayed at the value measured before exposure to hypoxia (286.0 ± 5.5 g). By the end of the third week they had lost an average of 18 g. Their weight had decreased to 256.4 ± 20.4 g by the fourth week, but the only statistically significant difference was between the value measured before hypoxia and that measured after three weeks of hypoxia ($P < 0.05$). In contrast, body weight in the normoxic rats did not change through the study. There were no significant differences in body weight between groups throughout the experiment (Fig. 1).

There was also no significant difference in weight

Fig. 1 Changes in body weight of hypoxic and normoxic rats. The difference between body weight measured before the experiment and that measured after three weeks of hypoxia was statistically significant. (* $P < 0.05$).

Fig. 2 Muscle weights in hypoxic and normoxic rats. There were no significant differences.

Table 1 Individual and mean values of relative muscle fiber composition (%) in the soleus muscles of hypoxic and normoxic rats.

	I	IIC	IIa	IIb		I	IIC	IIa	IIb
Hypoxia					Normoxia				
1	81.7	11.9	6.4	0	1	94.3	0.3	5.4	0
2	87.9	3.6	8.5	0	2	95.1	0.5	4.4	0
3	78.9	1.7	19.3	0	3	81.5	9.4	9.0	0
4	83.4	7.4	9.2	0	4	80.5	8.2	11.2	0
5	84.2	8.0	7.8	0	5	—	—	—	—
mean	83.2	6.5	10.2	0	mean	87.9	4.6	7.5	0
SD	3.3	4.0	5.2	0	SD	7.9	4.9	3.2	0

Table 2 Individual and mean values of relative muscle fiber composition (%) in the plantaris muscles of hypoxic and normoxic rats.

	I	IIC	IIa	IIb		I	IIC	IIa	IIb
Hypoxia					Normoxia				
1	5.4	0.1	12.7	81.8	1	9.2	0.3	20.6	68.7
2	9.8	0.7	18.9	70.6	2	9.2	0.2	18.9	71.7
3	10.8	0.3	11.7	77.1	3	13.4	0.9	12.3	73.4
4	7.0	0.1	21.2	71.7	4	9.5	0.2	15.9	74.4
5	6.5	0.4	13.2	79.9	5	7.4	1.1	18.6	72.9
mean	7.9	0.3	15.5	76.2	mean	9.7	0.5	17.3	72.2
SD	2.3	0.2	4.2	4.9	SD	2.2	0.4	3.2	2.2

of the soleus muscle between the normoxic (121.1 ± 7.6 g) and hypoxic (124.7 ± 16.6 g) groups (Fig. 2). Similarly, there was no significant difference in plantaris muscles weights between the two groups (normoxic: 287.6 ± 44.8 g, hypoxic: 281.8 ± 10.9 g) (Fig. 2).

There were no significant differences between groups in fiber type composition of either muscle (Tables 1 and 2). There were no type IIb fibers in soleus muscle. With the exception of type IIb in soleus, type IIC fibers accounted for the smallest percentage of fibers in both muscles. The percentage of type IIC in the plantaris was much lower than in the soleus.

DISCUSSION

Breathing 10 % O₂ causes moderately severe hypoxia, and the rats exposed to hypoxia had lost some weight by the third week of experiment (Fig.

1). This decrease in body weight is not consistent with previous reports of an increase of body mass during hypoxia (Sillau and Banchero, 1977; Taguchi et al., 1985; Itoh et al., 1990). This discrepancy may have been caused by differences in the experimental procedure. Instead of gradually changing the degree of hypoxia, we exposed the rats to 10 % O₂ from the first day of the study. The rats in the present study, therefore, might have been under greater stress than those in previous studies. When both hypoxic and normoxic rats were given food ad libitum like in the previous studies, at the end of the experiment the body weight of the hypoxic rats was lower than that of normoxic rats. Bigard et al. (1991) reported that hypoxic rats ate less than control rats, so the lower body weight of hypoxic rats may have been caused by decreased food intake. Because a reduction in body weight also might have had qualitative and quantitative effects on skeletal

muscles, especially on a postural muscle like soleus, we gave normoxic rats the same amount of food that hypoxic rats had eaten, and there were no significant differences in body weight between groups (Fig. 1).

It is well known that chronic hypoxia causes loss of muscle mass and also decreases mean fiber cross-sectional area both in humans (Hoppeler et al., 1990) and in animals (Sillau et al., 1977; Itoh et al., 1990). In the present study, there were no significant differences in the weight of either the soleus or plantaris muscles between groups (Fig. 2). These data indicate that hypoxia has no specific effect on muscle weight. Instead, the previously reported difference in muscle mass between hypoxic and normoxic rats might have resulted from decreased food intake and loss of body weight.

The present data on muscle fiber composition in normoxic rats are similar to those reported previously (Kugelberg, 1976; Green et al., 1984; Luginbuhl et al., 1984). Four weeks of moderately severe hypoxia did not elicit a change in muscle fiber types in the slow soleus muscle (Table 1). This conflicts with some previous results. Taguchi et al. (1985) and Itoh et al. (1990) reported that there was a higher percentage of FOG fibers, and a lower percentage of slow twitch oxidative (SO) fibers in soleus muscle from hypoxic rats than in soleus muscles from normoxic rats. In those studies the rats breathed 12% O₂, so the lack of change in fiber type composition in the present study did not result from insufficient hypoxic stimulation. The rats in the present study breathed hypoxic air for four weeks, and those studied by Itoh et al. (1985) were hypoxic for 10 weeks, but a recent study has shown that 14 weeks of hypoxia does not cause rat soleus muscle fiber types to change (Bigard et al., 1991).

Taguchi et al. (1985) and Itoh et al. (1990) concluded that hypoxia-induced transformation of SO fibers to FOG fibers accounted for the observed difference in the percentage of FOG fibers. However, FOG fibers change into SO fibers during normal post-

natal development in rat soleus (Brooke et al., 1971; Curless and Nelson, 1976; Kugelberg, 1976), so the differences reported by Taguchi et al. (1985) and by Itoh et al. (1990) may reflect hypoxia-induced inhibition of this change. According to Kugelberg (1976), the transformation of FOG fibers to SO fibers in soleus muscle is faster when rats are young (body weight below about 200 g) than when they are older. Younger rats (body weight of about 150 g) were used in the studies by Taguchi et al. (1985) and by Itoh et al. (1990), but in the present study the rats were older and weighed about 280 g. If fiber type transformation in the soleus muscle of the older rats was nearly over, this alone could account for the difference between the present results and those reported previously.

Another reason for the significant differences in muscle fiber distribution between hypoxic and normoxic rats in the previous studies might be a difference in body weight between the experimental groups. As the soleus is a postural muscle, body mass may affect its activity. Increases in muscle activity caused by removal of a synergist muscle (Oakley and Gollnick, 1985), electrical stimulation (Pette, 1986), or physiological exercise (Green et al., 1984; Luginbuhl et al., 1984) can transform the muscle fibers to slow type. It is possible, therefore, that the weight of normoxic rats elicited the natural transition of soleus fibers to slow type. Since hypoxic rats weighed less, there was less stimulus to change fiber types. However, Yamaguchi et al. (unpublished data) suggested that body weight does not have a major effect on the transformation of the developing rat soleus muscle. The activity pattern of motoneurons (Pette and Vrbova, 1985), mechanical factors such as load and stretch (Lowrie et al., 1989), and some hormones such as thyroid hormone (Kelly et al., 1985) and testosterone (Ianuzzo et al., 1977) can also modify the phenotype of rat soleus muscle. However, we do not know if any of these factors mediate the differentiation of the rat soleus muscle during exposure to hypoxia.

We found that chronic hypoxia did not influence the fiber distribution of fast plantaris muscle (Table 2). In contrast, hypoxia has been said to cause transformation of fiber types from fast red to fast white in gastrocnemius and in tibialis anterior (Sillau and Banchemo, 1977), and from type IIa to type IIb in the deep portion of the plantaris muscle (Bigard et al., 1991). In contrast, hypoxia-induced conversion of fast twitch glycolytic fibers to FOG fibers was said to occur in extensor digitorum longus (Taguchi et al., 1985; Itoh et al., 1990) and in plantaris (Taguchi et al., 1985) muscles. One explanation for the differences among these results is that the methods used to identify fiber types were different, and the results of myosin-based and metabolism-based classification methods may not agree (Nemeth and Pette, 1980).

In summary, continuous exposure to moderately severe hypoxia did not cause transformation of muscle fiber types in slow soleus and in fast plantaris muscles of adult male rats. The differences in mediators of conversion of fiber types such as the level of muscle activity and hormonal factors during chronic hypoxia between young and adult rats should be studied.

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