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Fiber Composition and Capillarity in Growing Guinea Pigs Acclimated to Cold and Cold plus Hypoxia[1] (42524)

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Abstract. The central portion of the medial head of the gastrocnemius of control (normoxic and normothermic), hypoxia-, cold-, and cold plus hypoxia-acclimated guinea pigs was analyzed for capillary supply and fiber composition to elucidate changes in capillarity induced by environmental stresses. The muscle was cut at midbelly, frozen, sectioned, and stained for myosin ATPase. Fiber cross-sectional areas; percentages of slow-twitch oxidative (SO), fast-twitch oxidative–glycolytic (FOG), and fast-twitch glycolytic (FG) fibers; and numbers of capillaries around each fiber type were measured. Growth rates of all four guinea pig groups were similar. Capillarity was not affected by acclimation to hypoxia. Cold and cold plus hypoxia acclimation led to increased numbers of capillaries around the fiber in all three fiber types. In addition, significant increases in the percentage of FOG fibers and concomitant decreases in the percentage of FG fibers compared to controls were found in cold and in cold plus hypoxia indicating that a transformation of fiber type from FG to FOG had occurred. The increase in FOGs at the expense of the FGs did not occur in the guinea pigs grown in a hypoxic environment. The increased total capillarity in those muscles studied was the result of more capillaries around all fiber types and was not due to simple transformation of fibers.

Acclimation to hypoxia, cold, or cold plus hypoxia is accompanied by changes in the respiratory and cardiovascular systems of the growing guinea pig (1–4). In chronic hypoxia, in which total body $\dot{V}O_2$ and blood flow remain unchanged (5, 6), adaptive changes appear in response to a reduction in tissue PO_2. In exposure to cold, which causes an increase in metabolic rate and blood flow, the adaptations respond to an increased need for O_2. In cold plus hypoxia, changes result from increased cellular needs for O_2 in the presence of decreased availability. This situation appears to be the most taxing; however, the mortality rate of guinea pigs acclimated to cold plus hypoxia was not as high as in simple hypoxia (1). Because life at high altitudes includes exposure to these two stresses, hypoxia and cold, in varying degrees, it is important to study the stresses both separately and combined.

Previous work has established that skeletal muscle capillarity remains unchanged in hypoxia-acclimated rodents (7–10). Neither is muscle capillarity different in guinea pigs native to the Andes (3900 m) (8). In contrast, guinea pigs acclimated to 5°C showed increased skeletal muscle capillarity (11). Acclimation to cold plus hypoxia produced an increase in the capillarity of the gastrocnemius muscle greater than that seen in cold alone. However, it did not alter the capillarity in the soleus muscle but its fibers were smaller and thus values of capillary density greater than those in normothermia–normoxia were measured (1). Increases in skeletal muscle capillarity have been reported in response to chronic electrical stimulation (12), prolonged endurance exercise (13), and hyperthyroidism (14). It has been postulated that increased muscle blood flow and increased metabolic need are necessary to stimulate capillary proliferation (15).

The fiber composition of skeletal muscle is known to be a dynamic entity and changes can occur in response to severe metabolic demands (16, 17). Each type of fiber SO (slow-twitch oxidative), FG (fast-twitch glycolytic), and FOG (fast-twitch oxidative–glycolytic) is surrounded by a different number of capillaries. The average number of capillaries around the fiber (CAF) is related to the biochemistry

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of the fiber and it increases linearly with increases in fiber cross-sectional area (FCSA) (18, 19). Capillarity could thus be affected not only by changes in CAF but also by changes in the fiber distribution. The stresses known to induce fiber transformation have also been shown to produce changes in capillarity (12–14, 25, 27). Environmental stress alone has not been shown to induce fiber-type transformation in either humans or animals although changes in capillarity have been measured in animals. Therefore this study was undertaken to see if the capillarity changes measured in response to cold and cold plus hypoxia were accompanied by transformation of fiber type.

Materials and Methods. *Animals.* Male guinea pigs (*Cavia porcellus*), Hartley strain (Camm Research Laboratories, Inc., Wayne, NJ), were raised in four different environments (Table I) for periods of continuous exposure ranging from 2 to 18 weeks. Initially, each group consisted of 46–50 animals but the number decreased as the experiment progressed and 7 or 8 animals were sacrificed at 2, 4, 6, 10, 14, and 18 weeks of exposure. The body weight (body wt) range for the normoxic and normothermic (control) guinea pigs was 89–1274 g. The experimental guinea pigs were between 2 and 3 weeks of age and between 200 and 250 g at the beginning of the respective exposure. Their maximum body weight at sacrifice was 1074 g. The growth rate of guinea pigs under all experimental conditions was similar to the growth rate of control animals but the body weight range of experimental animals was not as large as those for controls.

Environmental conditions. Hypoxia was produced by reducing the oxygen fraction of air (FIO_2) in the chamber by nitrogen replacement over a 5-day period to a final partial pressure of approximately 80 Torr (Table I). Cold (6°C) was produced by Freon decompression in a radiator inside the environmental chamber while control animals were kept at 22°C.

TABLE I. ENVIRONMENTAL CONDITIONS OF TEMPERATURE, FIO_2, AND PIO_2 IN GUINEA PIGS IN CONTROL, COLD, COLD PLUS HYPOXIA, AND HYPOXIA[a]

Groups of guinea pigs studied	Temperature (°C)	FIO_2	PIO_2 (Torr)
Control	22	0.21	133
Cold	5	0.21	133
Cold + hypoxia	6	0.134	85
Hypoxia	22	0.126	80

[a] Values given are means.

Procedures. Guinea pigs were anesthetized with sodium pentobarbital (35 mg/kg ip). The gastrocnemius–plantaris muscles were removed and immediately weighed. The medial head of the gastrocnemius was separated from the surrounding tissue, cut transversely at the widest point, and quickly frozen in isopentane cooled in liquid nitrogen. The central portion of the medial head of the gastrocnemius was the only area analyzed. Ten-micrometer-thick sections were cut at −20°C in a cryostat. The plane of section was at right angle to the longitudinal axis of the fiber. The tissue sections were stained for myofibrillar ATPase at pH 9.4 and after preincubation at pH's of 4.5 and 4.0 (20). The oxidative capacity of the fibers was assessed using DPNH tetrazolium reductase and was correlated with the myosin ATPase stains (20). Capillaries were visualized in the myosin ATPase stain at a preincubation pH between 3.8 and 4.0. An explanation of the method of identification for the fast-twitch oxidative–glycolytic, fast-twitch glycolytic, and slow-twitch oxidative fibers in the guinea pig has been previously reported (8).

Three to four randomly selected photomicrographs, each covering an area of tissue equal to 0.287 mm^2, were projected on an x–y digitizer (GTCO Corporation, Rockville, MD) on line with a Data General Nova 4 computer. Fiber density and the mean percentages of each fiber type present were determined directly by counting an average of 250 fibers. FCSA and the fiber diameter of each individual muscle fiber for each fiber type were determined by use of the computer. CAF was counted for each fiber and was grouped by fiber type. Only vessels with a diameter smaller than 10 μm were counted as capillaries.

Regressions by least-squares and correlation coefficients were calculated for each separate environment, for each fiber type, and for CAF. Comparisons of lines with unequal slopes (21) and Student t tests were used to test the significance of differences between the means.

Results. In all four groups of animals, muscle weight increased linearly with body weight (Table II). CAF increased linearly with FCSA in each of the three fiber types in all guinea pigs. However, the absolute values of CAF for SO, FG, and FOG fibers, at a given FCSA, were different under those experimental conditions in which cold was present. In cold-acclimated and cold plus hypoxia-acclimated animals, CAF values were significantly greater than CAF values for control animals at any given FCSA (Fig. 1). The same difference in CAF for FOG and FG fibers can be appreciated by comparing CAF for small (2000 μm^2) versus large (4000 μm^2) fibers (Fig. 2). For SO fibers only the small size was compared as these fibers do not become as large as the FG and FOG fibers in the normal mature guinea pig. The interpolated values for CAF, rounded off to the nearest half-capillary, are given. The CAF was not determined in hypoxia-acclimated guinea pigs as there was no increase in capillary density.

Growth also caused significant changes in the percentage of subgroups of fast-twitch fiber types (FG and FOG), whereas the percentage of slow-twitch fibers remained unchanged. Under the three experimental conditions significant differences ($P < 0.05$) in fiber percentages were found in the FOG and FG populations compared to those in normothermic and normoxic controls (Fig. 3). However, because the slopes of the cold and cold plus hypoxia lines intercept, these particular differences did not occur along the entire range of body weights. The percentage of SO fibers was not affected significantly by these environmental stresses. In cold and cold plus hypoxia the FG fiber percentage decreased and the FOG fiber percentage increased by approximately the same magnitude. In hypoxic animals the changes in FOG and FG fibers with body weight were of lesser magnitude than those seen under all other conditions: the percentage of FG fiber increase and the percentage of FOG fiber decrease were less than those in normoxic controls. This suggests a stunting of the normal growth pattern of transformation of fiber type in response to hypoxia. These changes suggest that the FG fibers transformed into FOG fibers in cold and cold plus hypoxia as the percentages of SO fibers remained unchanged.

TABLE II. RELATIONSHIP BETWEEN MUSCLE WEIGHT AND BODY WEIGHT IN THE GASTROCNEMIUS OF THE GUINEA PIG

Group	Equation	r
Control	$y = 0.043 + (2.83 \times 10^{-3})x$	0.98
Cold	$y = 0.152 + (2.4 \times 10^{-3})x$	0.97
Cold + hypoxia	$y = 0.119 + (2.48 \times 10^{-3})x$	0.97
Hypoxia	$y = 0.047 + (2.84 \times 10^{-3})x$	0.98

Discussion. Previous work has established that significant changes in the capillarity of the gastrocnemius muscle occur as a result of growth and its accompanying increase in muscle fiber size. Although capillary density, i.e., the number of capillaries per square millimeter of cross section, decreases hyperbolically as fiber cross-sectional area increases, the total number of capillaries in the muscle cross section increases (19). Hence, in the growing guinea pig, the average number of capillaries around the fiber increases linearly as a function of the average fiber size (19).

When cold and cold plus hypoxia are imposed upon growing guinea pigs, an increase in the development of new capillaries occurs in skeletal muscle (1, 11). Increases in muscle capillarity are probably related to hypoxia at the tissue level but a certain threshold must be reached for these changes to occur (22). We and other investigators have not detected changes in skeletal muscle capillarity with the degree of environmental hypoxia used in these and similar experiments (8, 9, 10, 23). Therefore, we must conclude that the critical degree of tissue hypoxia was probably not reached in skeletal muscle in resting animals in simple ambient hypoxia not exceeding an altitude of 5100 m or a PIO_2 of 80 Torr. However, Kayar and Banchero (3) have measured a transient increase in the capillarity of the myocardium of guinea pigs in hypoxia which suggests that cardiac tissue may at least transiently be at a PO_2 below threshold.

Increased capillarity occurs when the tissue demands for oxygen are high enough to produce hypoxia and increased muscle blood flow (15). Endurance exercise (13), chronic electrical stimulation (12, 24), exposure to cold (11), and exposure to cold plus hypoxia (1) have all been shown to stimulate growth of capillaries in skeletal muscle. Thyroxin has been thought

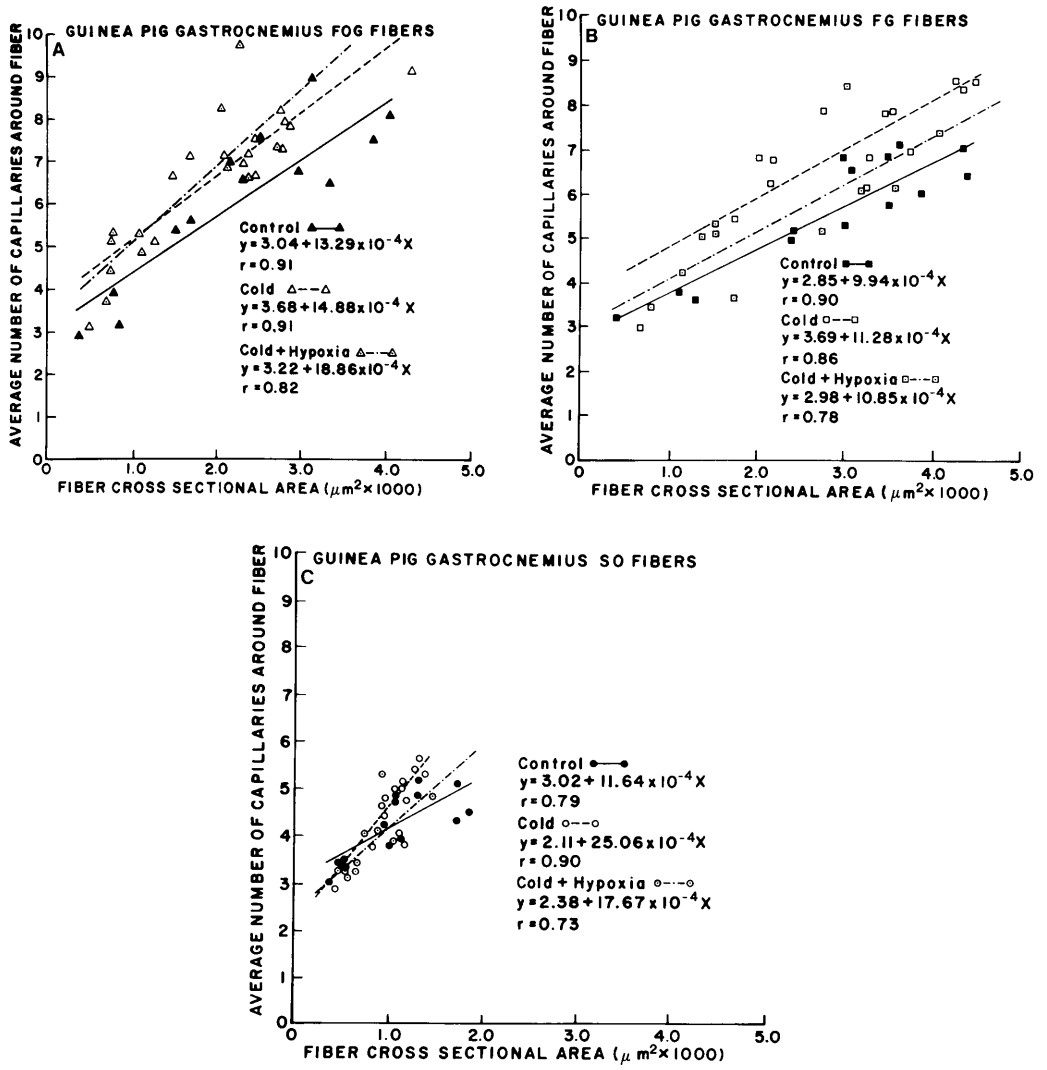

FIG. 1. Relationships between FCSA and average CAF for the FOG, FG, and SO fiber types in the central portion of the medial head of the gastrocnemius in guinea pigs in control, cold, and cold plus hypoxia. All lines in FOG and FG are significantly different from control and from each other ($P < 0.05$).

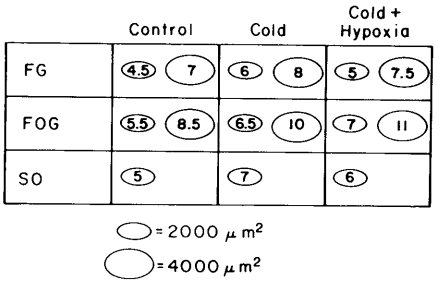

FIG. 2. Interpolated values of CAF for FOG, FG, and SO fibers at two FCSAs: 2000 and 4000 μm^2.

to be implicated in adaptation to a cold environment but data in support of this contention are still controversial. When iatrogenically administered to rats it produced increased capillaries in some muscles (14).

During growth in guinea pigs in normoxia and normothermia there is a change in the distribution of fiber types: a significant increase in the FOG percentage at the expense of the FG fibers. Because FOG fibers are surrounded by a greater number of capillaries than FG fibers, an increase in the capillarity of muscle

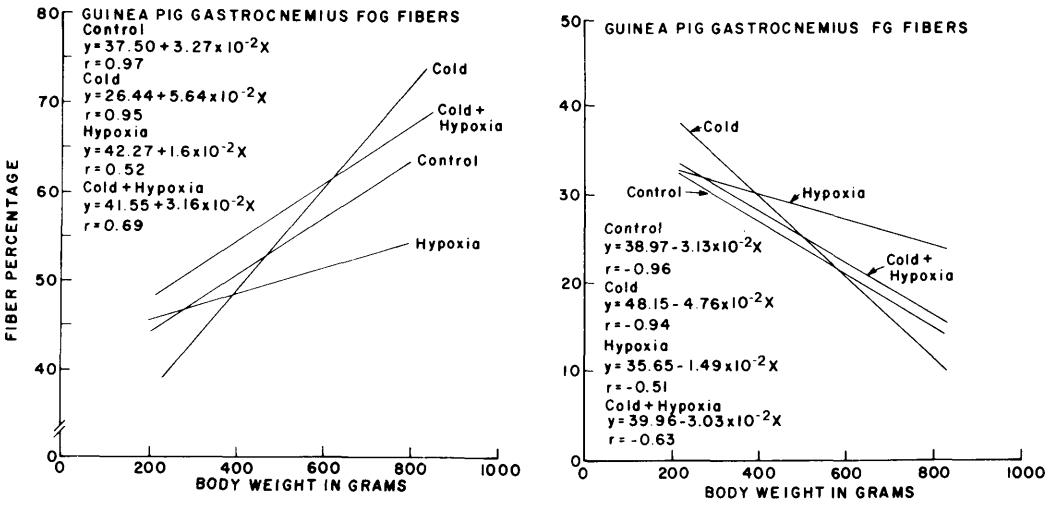

FIG. 3. Relationship between body weight and FOG and FG fiber percentages in the gastrocnemius of guinea pigs in cold, cold plus hypoxia, and hypoxia. Experimental values differ from control ($P < 0.05$).

should occur. However, the magnitude of this effect on capillary density is much less than that caused by the changes in FCSA and the total capillary density decreases with body growth.

A transformation of FG to FOG occurred whenever cold exposure was present, whereas in hypoxia the normal transitions in percentages of FOG and FG fibers were stunted. One may speculate that these changes would favor the increased oxygen demands placed upon skeletal muscle by ambient cold because FOG fibers have a greater capacity for aerobic metabolism than FG fibers. Blake and Banchero (2) have shown that the $\dot{V}O_2$ in guinea pigs exposed to cold is increased two and a half times. In ambient hypoxia the stunted changes would seem to favor anaerobic metabolism and it would be more advantageous if the FG fiber population were maintained or increased because this fiber type is capable of producing the same energy with less oxygen.

It has been demonstrated that cross-innervation alters the phenotypic expression of the fiber and the muscle fibers change with the type of the nerve supply (18). Therefore, the importance of nerve stimulation in the fiber composition of muscle was established. Complete IIB (FG) to IIA (FOG) transformations have been reported with intermittent electrical stimulation of muscle at 10 Hz, 8 hr per day, for a period of 7 weeks (25). The transformed fibers acquired both biochemical and histochemical staining properties of IIA (FOG) fibers. Long-term continuous stimulation (10 Hz) can be shown to convert fast- to slow-twitch fibers and in the process of conversion it is possible that the transforming fibers first exhibit the characteristics of IIA (FOG) fibers (17). As these chronically stimulated fibers undergo transformation and increase their content of oxidative enzymes they also exhibit a decrease in diameter, while glycolytic enzyme activity decreases. Both the cross-innervation and the chronic electrical stimulation studies established means by which fiber-type transformation would take place. It has been shown that long-term endurance exercise (26–28), wherein the muscle is intermittently hypoxic, is yet another stimulus for transformation of fiber type. We have now shown that an environmental condition which greatly stimulates the need for oxygen also influences the expression of fiber type. FG to FOG conversions will occur when oxygen utilization is increased, whereas the normal level of conversion associated with growth will be blunted when oxygen availability in the environment is decreased.

The environmental demands placed on the oxygen delivery system influence the metabolism of skeletal muscle and the fibers exhibit adaptation. These data show that the changes elicited in the overall capillarity of muscle by

environmental stresses are due to both an absolute increase in the number of capillaries of all fiber types and a transformation of FG to FOG fibers whenever cold is present. These changes improve oxygen delivery. This work points to the necessity of studying the human in the same environments as adaptation to altitude may depend upon the stimulus of cold.

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