# Title
Effects of chronic intermittent cold exposure on microvascular angiogenesis in rat soleus muscle

# Author(s)
鈴木 淳一

# Citation
冬季スポーツ研究, 6(1): 1-7

# Issue Date
2003-10

# URL
http://s-ir.sap.hokkyodai.ac.jp/dspace/handle/123456789/6777
Effects of chronic intermittent cold exposure on microvascular angioadaptation in rat soleus muscle

Junichi SUZUKI

Research and Education Center for Winter Sports, Hokkaido University of Education, 5-3 Ainosato, Kita-ku, Sapporo, Hokkaido 002-8502, Japan

Abstract
The effects of chronic intermittent cold exposure on microvascular angioadaptation were studied in soleus muscle of female Wistar rats. The rats were randomly divided into control (n=5) and cold-exposed (n=6) groups. All rats were housed under condition of control temperature (24±1°C). The rats of cold-exposed group were subjected to cold chamber set at 5±1°C. The duration of cold exposure was 2 hours per day, and lasted for 4 weeks from the age of 12 weeks. Body weight at the time of sacrifice did not significantly different between the two groups. Although the density of venular capillaries increased by 29% after cold exposure, there was no significant difference between the two groups. Cold exposure slightly increased the capillary-to-fiber (C:F) ratio of venular capillary by 22%, but the difference was not significant. The C:F ratio of arteriolar and intermediate capillaries did not changed after cold exposure. The C:F ratio of total capillary tended to increase after cold exposure (P=0.0614). Cold exposure did not affect the proportion of each capillary portion. These results suggest that capillary angiogenesis was only slightly facilitated after chronic intermittent cold exposure. Moreover, the present type of cold exposure may not facilitate microvascular remodeling such as arterIALIZATION OF capillary.

Key words: angioadaptation; capillary; cold exposure; skeletal muscle

Thermogenesis in the skeletal muscles as well as in other organs is enhanced when the homeothersms are exposed to a cold atmosphere [1,2,6,7]. Enhanced thermogenesis in the skeletal muscles may need adaptive changes in the oxygen transport system of the muscles. An increase in skeletal muscle capillarity has been observed in previous studies of small homeotherms exposed to cold [4,14].

In a previous investigation, the number of arteriolar portions of capillaries was increased in the soleus muscle of rats reared at 5 °C for 68 generations [15]. Chronic cold exposure for 4 weeks also increased the proportion of arteriolar capillaries in oxidative muscles [16]. The arterial blood, which has abundant oxygen, flows through arteriolar capillary portions. Thus it is possible that an increase in arteriolar capillarity may improve the effective oxygen supply to muscle tissues and enable muscle tissues to promote thermogenesis in cold environments.

As chronic cold exposure, intermittent exposure to cold atmosphere (2 hours per day) for several weeks was reported to enhance a capacity for thermogenesis, identified as significant increase in the weight of brown adipose tissue [17]. It is therefore possible that intermittent cold exposure induces microvascular angioadaptation in hind-leg muscle.

The present study, therefore, observed the capillary geometry, especially the distribution of arteriolar and venular capillaries, in skeletal muscle after intermittent cold exposure for 4 weeks.

Methods
This study was approved by the Animal Care and Use Committee of Hokkaido University of Education and performed in accordance with the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" of the Physiological Society of Japan.

Animals Eleven female Wistar rats (10 week-old) were purchased from Clea Japan Inc. (Tokyo, Japan). After the rats were fed for 14 days
to allow adaptation to the new environment, the rats were randomly divided into two groups; warm control (Cnt; n=5) and cold-exposed group (Cold; n=6). All rats were housed under conditions of control temperature (24±1°C) and a relative humidity of about 50%. Lighting (7:00-19:00) was controlled automatically. All rats were given commercial laboratory chow (CE-2, Clea Japan Inc.) and tap water ad libitum. The rats of CA group were subjected to an individual cage in cold chamber set at 5±1°C. The duration of cold exposure was 2 hours per day, and lasted for 4 weeks from the age of 12 weeks. The Cnt rats were also subjected to an individual cage in warm chamber set at 24±1°C for 2 hours per day.

Sample preparation Under light anaesthesia with ether, the rats were anaesthetized with pentobarbital sodium (50 mg/kg i. p.). A toe pinch response was used to validate adequate anesthesia. Then the left soleus (SOL) muscles were excised and weighed. The muscles were fixed at the length measured when the knee joint was maximally extended and the tibiotarsal joint was fixed at 90 degrees. The muscles were placed in embedding medium, O.C.T. compound (Miles Inc., Elkhart, IN, USA), and rapidly frozen in isopentane cooled to its freezing point (-160°C) with liquid nitrogen. The muscle samples were stored at −80°C until analyses.

Histological analyses Frozen sections, 10 μm thick, were cut from the mid-belly of the muscle using a cryotome (CM-1500; Leica Japan Co., Tokyo, Japan) at −20°C. The profiles of the arteriolar and venular portions of the capillary were determined using the staining method for alkaline phosphatase (AP) and dipeptidyl peptidase (DPP) IV in the capillary endothelium. The original staining protocol was described by Lojda (1979)[10]. Briefly, tissue sections were incubated in a mixture containing 1mM glycyl-L-proline-4-methoxy-beta-naphthylamine, 3 mM fast blue salt B and 5% (v/v) N,N-dimethylformamide in 0.1 M acetate buffer (pH 7.4) at 4°C for 16 hours. This mixture is reactive to DPP IV in the capillary endothelium, and stains the venular portions of capillaries red. The sections were then transferred to a mixture containing 2.5 mM naphthol AS-MX phosphate, 7 mM variamine blue salt RT and 5% (v/v) N,N-dimethylformamide in 0.1 M Tris-HCl buffer (pH 9.2) at 38°C for 1 hour. This mixture is reactive to AP, and stains the arteriolar portions of capillaries blue. The transitional zone along the capillary length that demonstrated both AP and DPP IV activity was stained a violet color. The validity of this double staining method for differentiation of arteriolar and venular capillary portions was confirmed previously [8,10,11]. The images of incubated sections were digitized using a digital microscope camera (PDMC 1e, Polaroid Co., Cambridge, USA) attached to a light microscope (BX-50, Olympus Co., Tokyo, Japan) and were stored on computer disk. The capillary profiles were identified as either arteriolar (blue), venular (red) or intermediate (violet). The parameters of the measurements were: the capillary density (the number of capillaries per mm²); the capillary-to-fiber ratio (the number of capillaries per mm² divided by the number of muscle fibers per mm²). Non-overlapping microscopic fields were selected at random from each muscle sample when the microscope was set at phase-contrast, i.e., the color of the capillary was not detected by the observer. During the measurements, the observer was blind as to the source (groups) of each slide.

Statistical Analysis All results are expressed as means±SE. Using the Kolmogrov-Smirnoff test, the distribution of all parameters was first tested to determine whether it was compatible with a normal distribution. The Student’s t-test was used to analyze two-sample parametric comparisons. Differences were considered to be statistically significant at P<0.05 and were considered to be a tendency at P<0.1.

Results

Figure 1 shows representative micrographic images of sections that demonstrate capillary profiles from the SOL. Although the density of venular capillaries increased by 29% after the cold exposure, there was no significant difference in capillary density values between the two groups (Fig. 2). Cold exposure slightly increased the capillary-to-fiber (C:F) ratio of venular capillary by 22%, but the difference was not significant (Fig. 3). The C:F ratio of arteriolar and intermediate capillaries did not change after cold exposure. The C:F ratio of total capillary tended to increase after the cold expo-
Cold exposure and muscle capillarity

**Fig. 1** Micrographic images of the soleus muscle stained with alkaline phosphatase and dipeptidyl peptidase IV. Types of capillaries cannot be distinguished, because of grey scale images. Bar represents 50 μm.

Discussion

This study shows that there was a tendency to facilitate microvascular angioadaptation after 4 wk of intermittent cold exposure. The C:F ratio of total capillary tended to increase after the cold exposure in soleus muscle (P=0.0614, Fig. 3).

After chronic cold exposure for 4 weeks, an increase in the arteriolar portion of capillaries was observed in SOL and mixed-fiber portion of gastrocnemius muscles of rats [15]. The capillary-to-fiber ratio, however, remained unchanged after cold acclimation. Therefore, these adaptive changes may be induced by arterIALIZATION of capillaries rather than by capillary angiogenesis. Price et al. [12] have found that, in rat gracilis muscle, the smooth muscle cells in the terminal arterioles "proceed" toward the venular side along capillary pathways as the animals grow. Those authors postulated that the terminal arteriolar growth is facilitated by elevated circumferential wall stress [13]. An enhanced oxidative metabolism during cold exposure [7] may cause arteriolar vasodilation. Because arteriolar vasodilation increases their circumferential wall stress, it is possible that the elongation of terminal arterioles, with a subsequent elongation of arteriolar capillary portions, may be facilitated by chronic cold exposure. Recent studies have reported that mRNA for basic fibroblast growth factor (bFGF) [18] and insulin-like growth factor (IGF)-1 [19] increased in brown adipose tissues of adult rats acclimated to cold. If these factors are also expressed in skeletal muscle tissues by cold acclimation, proliferation of arteriolar smooth muscle cells would be facilitated in those tissues and arteriolar capillary
Fig. 2 The density of arteriolar, intermediate and venular capillaries and total capillary density. All values are represented as means±SE.

In the present study, the proportion of arteriolar and venular capillaries remained unchanged after intermittent cold exposure (Figs. 2 and 3), indicating that arterialization of capillary was not facilitated. This observation suggests that the intermittent cold exposure used in the present study does not produce...
stimuli that facilitate arterIALIZATION of capillary.

It has been reported that a strong shivering when warm-acclimated rats were transferred to 6 °C, but no shivering activity in the leg and back muscles of rats acclimated to cold (6 °C) for 4-6 weeks [6]. This finding suggests that, during continuous cold exposure, shivering thermogenesis (ST) may play a dominant role in the early phase of cold acclimation, and that non-shivering thermogenesis (NST) may become more predominant within 4-6 weeks. In a previous study, the weight of interscapular BAT, contributes predominantly to the NST, markedly increased after intermittent cold exposure (4 °C, 2 h/day for 4 wk)[17]. Therefore it seems possible that, in the later stage of the present cold exposure, the ST, i.e., muscle contraction does not contribute to thermogenesis during cold exposure. This may partly explain the present observation that the C:F
ratio did not significantly increase after the intermittent cold exposure.

In conclusion, the results of this study have shown that capillary angiogenesis was only slightly facilitated after chronic intermittent cold exposure. Moreover, cold exposure used in the present study may not facilitate microvascular remodeling such as arterialization of capillary.

References


