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Chronic intermittent cold exposure did not affect β -hydroxyacyl-CoA dehydrogenase activity in rat soleus muscle

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Abstract

The effects of 4-week intermittent cold exposure on enzyme activities concerning citric acid cycle and β -oxidation were studied in soleus muscles in female Wistar rats. The rats were randomly divided into control (CNT, n=5) and cold-acclimated (CA, n=6) groups. All rats were housed under condition of control temperature (24 ± 1 °C). The rats of CA 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 differ between the two groups. The weight of interscapular brown adipose tissue significantly increased by 71% after the cold exposure ($P < 0.05$). The activity of β -hydroxyacyl-CoA dehydrogenase (HAD), the key enzyme of β -oxidation, did not change after the cold exposure. The activity of citrate synthase (CS) tended to increase by 12% after the cold exposure, but the difference was not significant. The HAD/CS ratio showed, but not significantly, lower value in the CA than CNT. These results suggest that the present type of cold exposure does not improve the capacity of fatty acid utilization in muscle mitochondria.

Key words: β -hydroxyacyl-CoA dehydrogenase; cold acclimation; citrate synthase; skeletal muscle

When the homeotherms are exposed to a cold atmosphere, thermogenesis in the skeletal muscles as well as in other organs is enhanced [1,2,6,7]. In hind-limb muscles, an increase in the oxidative enzyme activity was observed after chronic cold exposure [4,9,10]. In our previous study, succinate dehydrogenase activity was increased in soleus and deep portion of gastrocnemius muscles, but not in superficial portion of gastrocnemius muscle after cold acclimation (5 °C for 4 weeks) [10]. It is therefore possible that an increase in oxidative enzyme activity is observed after cold acclimation in the skeletal muscles that are mainly composed of oxidative fibers.

Adan *et al* (1995) determined the energy sources used to maintain muscle shivering during acute cold exposure (4 °C) for up to 2 h [1]. In their study, substrate shifted from glucose and/or glycogen to triacylglycerol (TG). Therefore it is postulated that the capacity of TG utilization, i.e., β -oxidation of mitochondria, is enhanced after repetitive intermittent exposure to cold atmosphere, even though duration

of daily exposure is short, around 2 hours. However, the capacity of β -oxidation in skeletal muscle after intermittent cold exposure has not yet been determined.

The present study, therefore, observed the activity of β -hydroxyacyl-CoA dehydrogenase (HAD) in rat soleus muscle after intermittent cold exposure for 4 weeks.

Methods

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-acclimated group (CA; 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. The animals were cared for 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.

Muscle samples Under pentobarbital anaesthesia (50 mg/kg i. p.), soleus (SOL) muscle and interscapular brown adipose tissue (BAT) were rapidly excised, washed in cold saline, made free of surrounding connective tissue, weighed and frozen in liquid nitrogen. The muscle samples were stored at -80 °C until biochemical analyses. Muscle homogenates (5% (W/V)) were obtained from around 50 mg of frozen tissue homogenized for two interrupted 15-s bursts with Polytron homogenizer (set at 15,000 rpm) in ice-cold buffer (10mM Tris-HCl, pH 7.0; 175mM KCl, 10mM glutathione (reduced form);

2mM EDTA). After centrifugation at 600 g for 10 min at 0°C, the supernatant was divided into two aliquotes. The aliquotes for the CS activity were stored at -80°C.

Enzyme activity determination Immediately after the sample preparation, the HAD activity was determined using a spectrophotometric assay as previously described by Bass *et al* (1969) [3]. A volume of 100 μ L supernatant was added to 890 μ L of reagents (100 mM triethanolamine-HCl, pH 7.0; 5 mM EDTA; 0.45 mM NADH). The reaction was initiated by adding 10 μ L of 10 mM acetoacetyl-CoA (SIGMA, A-1625). The measurements were carried out at 340 nm on a spectrophotometer (U-2001, Hitachi Co., Tokyo, Japan) at 25°C.

The activity of CS was assayed according to Srere (1969) [8]. A volume of 5 μ L supernatant was added to 45 μ L of dilution (20 mM imidazole-HCl buffer, pH 7.0, containing 0.02% BSA)[5]. The diluted sample solution was then added to 900 μ L of reagents (100 mM Tris-HCl buffer, pH 8.0, containing 1 mM DTNB, 10 mM acetyl-CoA). The reaction was started with addition of substrate (50 μ L of 10 mM oxaloacetate). The measurements were carried out at 412 nm on a spectrophotometer at

Table 1. Body and muscle weights and muscle weight-to-body weight values

	CNT (n=5)	CA (n=6)
Initial body weight (g)	206.6 \pm 2.7	209.2 \pm 4.1
Final body weight (g)	233.8 \pm 3.6	229.7 \pm 5.0
Organ weight (mg)		
Whole heart	616.8 \pm 13.6	626.4 \pm 16.9
Left ventricle	411.4 \pm 14.1	420.7 \pm 11.7
Soleus	104.8 \pm 3.6	104.8 \pm 4.1
Gastrocnemius	1200.2 \pm 19.0	1179.5 \pm 22.1
Plantaris	270.5 \pm 3.8	256.2 \pm 3.4*
IBAT	257.8 \pm 18.3	441.1 \pm 35.6*
Organ weight/ body weight (mg/g)		
Whole heart	2.64 \pm 0.05	2.73 \pm 0.06
Left ventricle	1.76 \pm 0.06	2.73 \pm 0.06
Soleus	0.45 \pm 0.01	0.46 \pm 0.01
Gastrocnemius	5.14 \pm 0.06	5.14 \pm 0.09
Plantaris	1.16 \pm 0.02	1.12 \pm 0.01
IBAT	1.11 \pm 0.08	1.91 \pm 0.13*

Values are means \pm SE. CNT, control group; CA, cold-acclimated group; IBAT, interscapular brown adipose tissue. *, significantly different from CNT group at P<0.05.

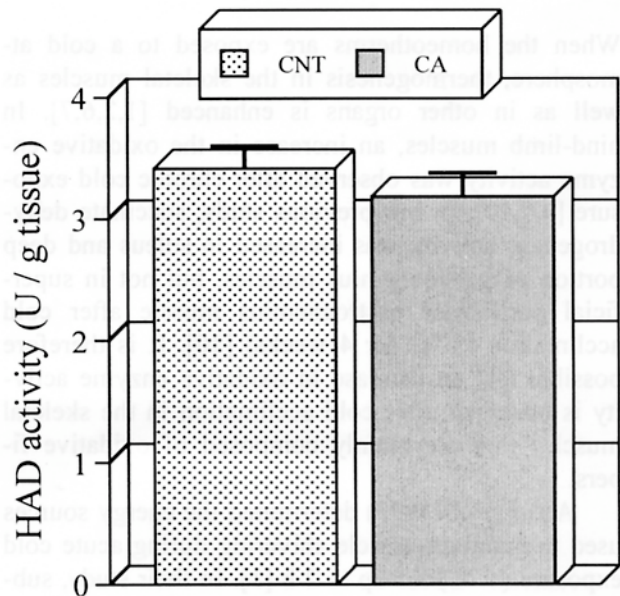


Figure 1
Changes in β -hydroxyacyl-CoA dehydrogenase (HAD) activity after intermittent cold exposure. CNT, control group; CA, cold-acclimated group.

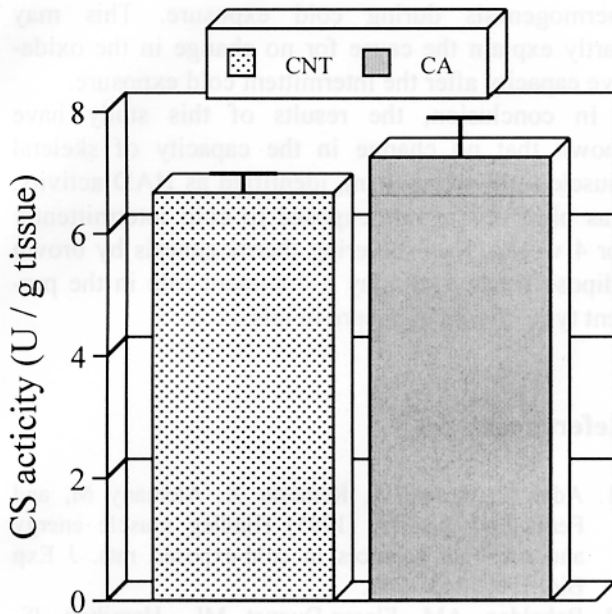


Figure 2
Changes in citrate synthase (CS) activity after intermittent cold exposure. Groups are the same as in Fig. 1.

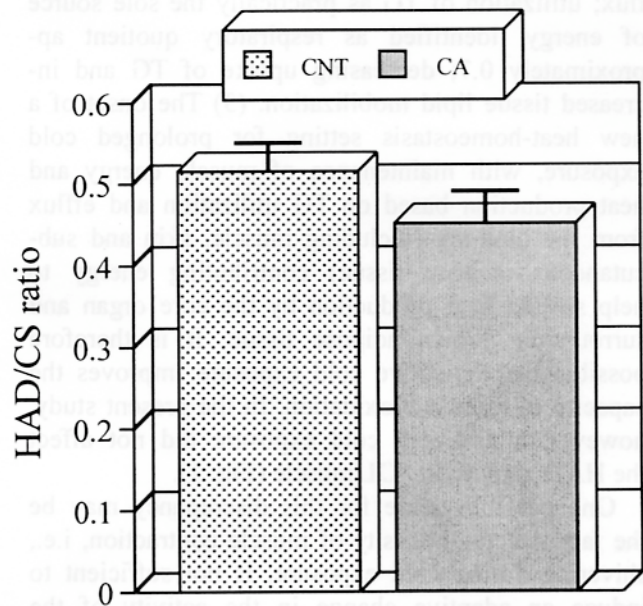


Figure 3
Changes in HAD/CS ratio after intermittent cold exposure. Groups are the same as in Fig. 1.

25°C. The extinction coefficients for NADH and DTNB were 6.22 and 13.6 /mM/cm, respectively. Specific activities were expressed in international units ($\mu\text{mol}/\text{min}$) per gram of tissue wet mass.

Statistical Analysis All results are expressed as means \pm SE. Using the Kolmogorov-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 $P < 0.05$.

Results

Table 1 shows the body and muscle weights of the two groups. Body weight at the time of sacrifice did not significantly differ between the two groups. In the PL muscle, muscle wet weight showed significantly lower value in the CA than in the CNT ($P < 0.05$), but muscle weight-to-body weight ratio did not significantly differ between the two groups. The weight of interscapular BAT and its weight-to-body weight ratio significantly increased by 71% and 72%, respectively, after the cold expo-

sure.

The HAD activity did not change after the cold exposure (Fig. 1). The activity of CS tended to increase by 12% after the cold exposure, but the difference was not significant (Fig. 2). The HAD/CS ratio showed, but not significantly, lower value in the CA than CNT (Fig. 3).

Discussion

This study shows that no change in the skeletal muscle β -oxidative capacity, identified as β -hydroxyacyl-CoA dehydrogenase (HAD) activity, was observed in rats exposed to cold intermittently for 4 weeks.

Adan *et al* (1995) determined the sources of energy used to maintain muscle shivering during acute cold exposure (4 °C) in rats. In their study, three distinct phases were observed in hind-leg substrate utilization [1]. (1) The onset of shivering, with the use of glucose/glycogen and an increase in lactate efflux. Lipid oxidation was practically zero, but the uptake of TG from the blood remained unchanged. (2) A substrate-energy shift, with drastically decreased use of glucose/glycogen, and of lactate ef-

flux; utilization of TG as practically the sole source of energy, identified as respiratory quotient approximately 0.7; decreasing uptake of TG and increased tissue lipid mobilization. (3) The onset of a new heat-homeostasis setting for prolonged cold exposure, with maintenance of muscle energy and heat production based on TG utilization and efflux from the hind-leg (including muscle, skin and subcutaneous adipose tissue) contributing energy to help sustain heat production by the core organ and surrounding brown adipose tissue. It is therefore possible that repetitive cold exposure improves the capacity of muscle β -oxidation. In the present study, however, intermittent cold exposure did not affect the HAD activity in SOL muscle (Fig. 1).

One possible cause for this discrepancy may be the fact that the intensity of muscle contraction, i.e., shivering during cold exposure, is not sufficient to induce an adaptive change in the activity of the HAD. Trembley *et al* (1994) [11] determined the effects of two different modes of training on body fatness and skeletal muscle metabolism in young adults who were subjected to either a 20-week endurance-training (ET) program or a 15-week high-intensity intermittent-training (HIIT) program. The mean estimated total energy cost of the ET program was approximately double the corresponding value for the HIIT program. Despite its lower energy cost, the HIIT program induced a more pronounced reduction in subcutaneous adiposity compared with the ET program. The enhancing effect of training on muscle HAD activity was significantly greater after the HIIT program.

It has been reported that a strong shivering was observed 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 the present study, the weight of interscapular BAT, contributes predominantly to the NST, markedly increased after the intermittent cold exposure (Table 1). 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 cause for no change in the oxidative capacity after the intermittent cold exposure.

In conclusion, the results of this study have shown that no change in the capacity of skeletal muscle lipid metabolism, identified as HAD activity, was observed in rats exposed to cold intermittently for 4 weeks. Non-shivering thermogenesis by brown adipose tissue may play a dominant role in the present type of cold exposure in rats.

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Cold exposure and muscle enzyme activity

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