Adaptive Changes in the Thermogenesis of Rats by Cold Acclimation and Deacclimation

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Summary: Male Wistar rats, aged 6 weeks, were maintained at 25°C for 9 to 11 weeks (W group), at 10°C for 9 to 11 weeks (C group), and at 25°C for 2 weeks after exposure to 10°C for 9 weeks (D group). Thermogenesis at 10°C was significantly greater than at 25°C. Thermogenesis per body mass in the C group was greater than in the W and D groups. The RQ value at 10°C was greater than at 25°C in the W group,

whereas the opposite was observed in the C and D groups. It is suggested that a large part of enhanced thermogenesis, caused by cold acclimation for 9 weeks, is lost because of a decreased secretion of calorogenic hormones, in spite of a slight decrease in BAT mass, during deacclimation for 2 weeks. [Japanese Journal of Physiology, 48, 505–508, 1998]

Key words: cold acclimation, deacclimation, thermogenesis.

In warm-acclimated rats, cold shivering occurs earlier than cold-acclimated rats when exposed to the same level of cold. However, when animals are repeatedly exposed to a cold environment, cold shivering is attenuated with a compensatory increase in nonshivering thermogenesis, and an increased capacity for the maintenance of core temperature is observed [1, 2]. Cold exposure in rats induces brown adipose tissue (BAT) hyperplasia accompanied by increased blood flow, increased mitochondriogenesis, and an increase in the amount of uncoupling protein 1 (UCP1) in BAT, which is mediated by the sympathetic nervous system [3, 4]. However, these adaptive changes in physiological responses to cold gradually disappear over a period of several weeks after the cessation of cold exposure [5, 6]. These adaptive changes to cold exposure and thus the effects of deacclimation are affected naturally by the period and frequency of cold exposure.

A great many investigations on cold acclimation in rats, including adaptive changes in thermogenesis, have been reported, but the number of studies on the effects of deacclimation is limited [2, 3]. In this study we attempted to elucidate how thermogenic activity in cold-acclimated rats that had once cold-acclimated

but that now appear to be deacclimated completely differs from that in warm-acclimated rats.

Male Wistar rats, aged 5 weeks and weighing 100 to 200 g, purchased from Japan Clea (Tokyo, Japan) were housed in plastic cages, two per cage, and maintained at $25 \pm 1^\circ\text{C}$ in a 12:12 light–dark cycle for 1 week. They were fed with MF rat food and water ad libitum. The rats were divided into a warm-acclimated group (W group) and a cold-acclimated group (C group). All rats (14) in the W group were maintained at $25 \pm 1^\circ\text{C}$ for 9 weeks. All rats (24) in the C group were maintained at $10 \pm 1^\circ\text{C}$ for 9 weeks. Four rats in the W group and 4 in the C group were sacrificed at 9 weeks to determine the masses of adrenal gland and interscapular BAT. The rats in the C group were randomly allotted into the following two groups so that both groups contained rats of similar body mass: a cold-acclimated group (10 rats) and a deacclimated group (D group, 10 rats). All rats in the C group were maintained at $10 \pm 1^\circ\text{C}$ for a further 2 weeks. All rats in the D group were transferred to a $25 \pm 1^\circ\text{C}$ environment and maintained for 2 weeks. Body mass and food intake were measured at the end of the light cycle every week. All rats were fasted for 12 h before

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Fig. 1. Changes in body mass and food intake in warm-acclimated, cold-acclimated, and deacclimated rats. □, warm-acclimated rats (exposure to 25°C for 9 or 11 weeks); ●, cold-acclimated rats (exposure to 10°C for 9 or 11 weeks); △, deacclimated rats (exposure to 25°C for 2

weeks after exposure to 10°C for 9 weeks). Values are means $\pm$ SD. Significant difference ($p < 0.05$): ^a warm-acclimated rats vs. cold-acclimated rats; ^b cold-acclimated rats vs. deacclimated rats; ^c warm-acclimated rats vs. deacclimated rats.

dissection. Each rat was weighed and anesthetized with ether. Interscapular BAT and adrenal glands were dissected immediately and weighed individually.

Metabolic rates were determined by measuring $\dot{V}O_2$ and $\dot{V}CO_2$ output. Each rat was put in a metabolic chamber, an indirect open circuit calorimeter of 6 l, maintained at 10 or 25°C in the late period of the light cycle, and kept there under no restraint. To observe the differences in metabolic rate at 10 and 25°C in each group and to minimize the effect of room temperature during the initial measurements of metabolic rate on the next measurement, the metabolic rates were measured for 40 min at 10°C in the C group and at 25°C in the W and D groups, respectively. After the metabolic rates were measured at each habitat for each group, the rats were moved to another area and metabolic rates were remeasured. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured from the entry of the rat into the chamber until the end of its stay there. O_2 and CO_2 concentrations of the air flowing in and out of the chamber were measured by using a zirconia solid electrolyte galvanic cell and an infrared spectrometer, respectively. $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated by multiplying the airflow rate per unit of time by the differences in concentration between the air and collected gas from the chamber. The respiratory quotient (RQ) was obtained by dividing $\dot{V}CO_2$ by $\dot{V}O_2$. $\dot{V}O_2$ was converted to a metabolic rate by multiplying it by the caloric value of the respiratory quotient.

All experiments described here were performed in accordance with the guidelines for the Care and Use of Laboratory Animals in Hyogo College of Medi-

cine.

The data are presented as means $\pm$ their standard deviations. Differences were analyzed by using a Student's *t*-test or a one-way analysis of variance (ANOVA) with repeated measurements and *post hoc* Bonferroni tests among groups. A significant difference was accepted at $p < 0.05$.

Figure 1 shows changes in body mass (BM) and food intake in the three groups during the experimental period. BMs at 4, 5, 7, 8, 9, and 10 weeks of cold exposure in the C group were significantly lighter than those in the W group at corresponding weeks. During deacclimation, BM increased with a greater rate of increase in the D group compared with that observed in the C group. BM at 11 weeks in the D group approached that of the W group and was significantly greater than that in the C group. Food intake in the C group was significantly greater than that in the W group throughout the experiment. Food intake in the D group decreased during deacclimation, and at 10 and 11 weeks it was significantly smaller than in the C group.

Table 1 shows BM, masses of adrenal glands, and interscapular BAT at the end of the experiment. Adrenal gland mass and adrenal gland mass per BM (at 9 weeks) in the C group were significantly greater than in the W group. Adrenal mass per BM in the D group was significantly smaller than in the C group. BAT mass and BAT mass per BM in the C and D groups were significantly greater than in the W group. No significant differences between values in the C and D groups were noted. Table 2 shows $\dot{V}O_2$, $\dot{V}CO_2$, meta-

Table 1. Body mass, masses of adrenal gland, and brown adipose tissue.

Group (experimental session)	Body mass (g)	A.G. mass (mg)	A.G./B.M. (mg/g×1,000)	BAT mass (mg)	BAT/B.M. (mg/g)
Warm acclimation (9 weeks)	431.0±28.8	30.84±4.01	71.65±9.33	386.3±41.70	0.88±0.11
Warm acclimation (11 weeks)	443.2±25.3	30.56±2.86	69.10±7.13	426.5±81.40	0.95±0.19
Cold acclimation (9 weeks)	402.9±23.6	35.63±3.33 [#]	88.36±5.82 ^{###}	646.2±72.1 ^{###}	1.61±0.28 ^{###}
Cold acclimation (11 weeks)	405.9±19.7 ^{##}	31.69±3.61	77.92±7.02	711.8±71.0 ^{###}	1.75±0.15 ^{###}
Deacclimation (2 weeks)	422.9±30.6	28.70±3.22	68.08±8.32 ^{\$}	687.9±69.4 ^{###}	1.67±0.18 ^{###}

Values are means±SD. A.G., adrenal gland; B.M., body mass; BAT, brown adipose tissue. [#]*p*<0.05, ^{##}*p*<0.01, ^{###}*p*<0.001: significant differences between warm acclimation vs. cold acclimation or deacclimation at the same age. ^{\$}*p*<0.05: significant differences between cold acclimation and deacclimation at the same age.

Table 2. $\dot{V}O_2$, $\dot{V}CO_2$ metabolic rate, and respiratory quotient.

Group	$\dot{V}O_2$ (ml·100 g ⁻¹ ·min ⁻¹)		$\dot{V}CO_2$ (ml·100 g ⁻¹ ·min ⁻¹)	
	10°C	25°C	10°C	25°C
Warm acclimation	3.36±0.56	2.20±0.56 ^{##}	2.86±0.33	1.76±0.35 [#]
Cold acclimation*	3.67±0.83	2.29±0.33 [#]	2.88±0.56	1.95±0.14 [#]
Deacclimation**	3.03±0.26	1.93±0.27 ^{##}	2.33±0.25	1.70±0.24

Group	M.R. (cal·100 g ⁻¹ ·min ⁻¹)		R.Q.	
	10°C	25°C	10°C	25°C
Warm acclimation	16.34±2.52	10.57±2.54 ^{##}	0.858±0.074	0.807±0.065
Cold acclimation*	17.53±3.81	11.13±1.43 [#]	0.792±0.060	0.860±0.067
Deacclimation**	14.43±1.03	9.45±1.33 ^{##}	0.771±0.100	0.880±0.040

Values are means±SD. *Cold acclimation to 10°C for 11 weeks. **Deacclimation to 25°C for 2 weeks after exposure to 10°C for 9 weeks. M.R., metabolic rate; R.Q., respiratory quotient. [#]*p*<0.05, ^{##}*p*<0.01: significant differences between the values at 10°C and those at 25°C.

bolic rate expressed per BM, and RQ in three groups at 10 and 25°C. $\dot{V}O_2$ at 10°C was significantly greater than at 25°C in all groups. The W and C groups showed significantly greater $\dot{V}CO_2$ values at 10°C than at 25°C. RQ at 10°C was greatest in the W group and smallest in the D group, but the order was reversed at 25°C. RQ in the W group at 10°C was greater than at 25°C, whereas RQ values of rats in the C and D groups at 10°C were smaller than at 25°C. However, these differences were statistically insignificant.

The changes in BM during cold acclimation and deacclimation observed in the present experiment accord with previous reports. The lower BM in cold-acclimated rats was caused by greater thermoregulatory thermogenesis in cold environments in spite of a significantly greater caloric intake. Cold-acclimated rats reached their previous body masses after 2 weeks of deacclimation. Significantly greater masses of adrenal gland and BAT in the C group than in the W group indicate that the exposure to 10°C for 9 weeks acts as a

cold stressor and brings about full cold acclimation [3, 7]. Adrenal gland masses decreased to a greater degree than those of BAT during deacclimation for 2 weeks.

Almost any type of stress will cause immediate and marked increases in ACTH secretion accompanied by increased glucocorticoid secretion within minutes, and BAT responds to chronic cold exposure by increasing its mass. Thus the rate of increase or decrease in adrenal mass is greater than in the BAT mass, and different events might be involved in BAT degradation and involution of the adrenal.

It is known that an increased BAT mass and an increased amount of UCP1 in BAT in cold-acclimated rats decreases gradually during a deacclimation of the rats. However, the rate of decrease in BAT mass and UCP1 in BAT because of deacclimation is especially affected by the period of cold acclimation [5,8]. The amount of increased BAT during short-term cold acclimation decreased rapidly after the cessation of cold

exposure, but that obtained during long-term cold acclimation decreased slowly [5]. Thus we assume that the cold acclimation for 9 weeks is a long-term acclimation.

It is known that the ratio of nonshivering thermogenesis to thermogenesis resulting from shivering usually increases during cold acclimation. The increase in resting metabolic rate in cold-acclimated rats might be caused mainly by an increase in the amount of nonshivering thermogenesis because of an increase in BAT mass with an increase in the number of mitochondria and the amount of UCP1 of BAT, induced by the action of noradrenaline released from the sympathetic nervous system [9].

In the present experiment, resting metabolic rates per body mass in cold-acclimated rats at 10 and 25°C were greater than in warm-acclimated rats, whereas resting metabolic rates per body mass in deacclimated rats were much smaller than those in cold-acclimated rats, though BAT mass per body mass was slightly smaller in the D group than in the C group. Enhanced thermogenesis in BAT of cold-acclimated rats in cold was not utilized during deacclimation for 2 weeks, and the capacity for heat production of BAT in deacclimated rats might therefore be functionally deteriorated possibly because of decreased secretions of thyroidal and adrenocortical hormones. These results suggest that enhanced thermogenesis in cold-acclimated rats was maintained in a cold environment, and the greater part of enhanced thermogenesis, obtained during cold acclimation for 9 weeks, was lost as a result of adaptive changes in the endocrine system during deacclimation for 2 weeks.

It is interesting to note that the absolute value of the increase in resting metabolic rate at 10°C from that at 25°C in the D group ($4.98 \text{ cal} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) was much smaller than in the W group ($5.77 \text{ cal} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$). This smaller increase in metabolic rate in deacclimated rats might be caused by increased insulation of the skin obtained during exposure to 10°C for 9 weeks and poor responsiveness in terms of thermogenesis of BAT caused by deacclimation. These results indicate that the magnitude of stress in terms of thermogenesis because of cold exposure in rats in the D group was small when compared with rats in the W group. The smaller RQ value in the C and D groups than in the W group at 10°C might be

caused by an increased ratio of fat metabolism to carbohydrate metabolism associated with an enhanced turnover of free fatty acids, a characteristic of cold acclimation in rats [2]. This similar metabolic pattern in the C and D groups indicates that in rats the effects of cold acclimation for 9 weeks on the utilization of energy source would not disappear for 2 weeks after deacclimation.

We could conclude that enhanced thermogenesis of cold-acclimated rats is lost because of a decreased secretion of calorogenic hormones during deacclimation for 2 weeks, possibly in spite of an increase and a maintenance of BAT UCP1 level.

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