Effects of Cold Acclimation on Cold-Induced Changes in Lipid Metabolism of Rat Brown Adipose Tissue

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Abstract Effects of acute cold exposure (0°C, 12 h) on lipid metabolism of the interscapular brown adipose tissue (BAT) were studied in warm controls (25°C) (WC) and cold-acclimated rats (5°C, 4 weeks) (CA). 1) We confirmed that cold acclimation brought about decreased BAT triglyceride (TG) level and increased BAT phospholipid (PL) level. The indexes of unsaturation, such as unsaturation index and polyunsaturated fatty acids/saturated fatty acids ratio, of bulky fatty acids (FA) (palmitic, stearic, oleic, and linoleic acids) decreased in TG, while those increased in PL. Rare FA (eicosadienoic acid, homo- γ -linoleic acid, lignoceric acid) level, which were about five times higher in PL than TG in WC, decreased in both TG and PL in CA. 2) Effect of acute cold exposure in WC: The level as well as the amount of TG in BAT was greatly reduced and the indexes of unsaturation in TG-FA increased. The most part of reduced TG (85%) was explained by the bulky FA. Rare FA explained only 1.1% of reduced TG. PL level in BAT did not change, but its amount decreased. The indexes of unsaturation increased in PL-FA. 3) Effect of acute cold exposure in CA: CA was placed in warm temperature of 25°C before cold exposure. In contrast with the cold-reduced TG in WC, either the level or the total amount of TG in BAT did not change, but the indexes of unsaturation in TG-FA decreased. The level as well as the total amount of PL in BAT increased. The arachidonic index and arachidonate in PL-FA decreased, but the indexes of unsaturation did not change. These results indicate as a whole that acute cold exposure as well as cold acclimation influences BAT lipid metabolism in FA compositions as well as amount of TG and PL, suggesting that such changes are related to thermogenic activity of this tissue.

Key words: brown adipose tissue, fatty acid composition, cold exposure, phospholipid, triglyceride.

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Brown adipose tissue (BAT) is a major site for cold-induced nonshivering thermogenesis (NST), utilizing mainly fatty acids (FA) as energy substrates. Cold acclimation causes an enhanced thermogenic capacity of this tissue [1]. Several reports have indicated the significant changes in FA composition of BAT triglycerides (TG) as well as phospholipids (PL) in the cold-acclimated rats [2-4], suggesting a preferential utilization of FA such as palmitic and linoleic acids for thermogenesis in BAT [5-7] and significance of PL-FA profiles in BAT function by means of influencing cell membranes [8, 9]. Recent study [10] disclosed that rare fatty acids (FA) were of quantitative significance in BAT of hamsters during hibernation and arousal from hibernation. In the nonhibernating animals such as rats the changes of BAT-rare FA have not been studied during cold acclimation and furthermore only relative compositional changes for other BAT-FA than rare FA have been scrutinized. In the present study we tried to estimate the quantitative significance of FA including rare FA by analyzing both FA compositions and amount of TG as well as PL during cold exposure of rats.

MATERIALS AND METHODS

Animals. Male Wistar-strain rats, weighing about 180 g, were obtained from Shizuoka Laboratory Animal Center, Hamamatsu. Two experiments were designed. For the first experiment concerning prolonged cold exposure the rats were housed individually at $25 \pm 1^\circ\text{C}$ and about 50% relative humidity (warm controls, WC) or $5 \pm 1^\circ\text{C}$ (cold-acclimated rats, CA) for 4 weeks under the artificial lighting from 7:00 to 19:00 and provided *ad libitum* with laboratory rat chow (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) and tap water.

For the second experiment concerning acute cold exposure the animals were exposed to cold of 0°C for 12 h to assess acute effect of cold on BAT lipid metabolism. CA were placed at 25°C for 12 h, followed by cold exposure at 0°C for 12 h (CA-W-C). BAT from CA-W-C were compared with that of CA placed at 25°C for 24 h (CA-W).

Sampling of tissues. The animals were killed by decapitation at the end of the experiments, and the interscapular BATs were removed, cleaned of the adhering tissues and kept at -70°C until analyzed.

Analytical methods. The tissue lipids were extracted according to the method by Islam *et al.* [11]; two times extraction by chloroform-methanol (2:1 v/v). Amounts of TG and PL in the extract were determined using the commercial kits of TG-Test Wako and PL-Test Wako (Wako Pure Chemicals Industries, Ltd., Osaka), respectively. The same extract was used for FA analysis. Briefly, 4 to 5 ml of the extract was evaporated to dryness under N_2 in the water bath at 30°C , then taken up in 50 μl of extraction solution. Ten μl of the solution was used directly for transesterification of TG-FA [12]. The remaining solution was applied to TLC for separating PL [13] and its FA was transesterified [12]. These transesterified FA solutions were dried under N_2 in the water bath at 40°C , then

taken up in 30 μ l of extraction solution for PL-FA and 150–300 μ l for TG, and analyzed by Hitachi Model 663-30 Gas Chromatograph equipped with Hitachi Model 883A Data Processor. A 2m glass column packed with Chromosorb W-AW-DMCS-FFAP (Gaskuro Kogyo Inc., Tokyo) was used as stationary phase and operated at 220°C. Quantitative standardization of the chromatograph was based on analysis of FA methyl esters standard mixture from NU-Chek-Prep, Inc.

Calculations. FA composition was expressed by mol% for FA compositions and weight % for quantitative analysis. Unsaturation index (UI): the average number of double bonds per FA molecule as indicated by $\sum M_i N_i / 100$ (M_i , mol% of each FA; N_i , number of double bonds of each FA). Arachidonate index (AI): arachidonate mol%/linoleate mol%. AI is equated with an effect on the elongation-desaturation pathway [14].

Statistics. Statistical significance was determined by analysis of variance and Student's *t*-test and the difference was assumed to be significant at $p < 0.05$.

Abbreviations. C14, myristate; C16, palmitate; C16-1, palmitoleate; C18, stearate; C18-1, oleate; C18-2, linoleate; C18-3, linolenate; C20, arachidate; C20-1, gadoleate; C20-2, eicosadienoate; C20-3, bishomo- γ -linolenate acid; C20-4, arachidonate; C22, behenate; C24, lignocerate; SA, saturated FA; MU, monounsaturated FA; PU, polyunsaturated FA.

RESULTS

Effect of cold acclimation

1. **Body and BAT weights (Table 1).** The initial body weight did not differ between WC and CA. The final body weight at the experiment was less in CA ($p < 0.05$), indicating smaller weight gain in this group. BAT weight was greater in CA ($p < 0.001$) than in WC in terms of both whole tissue pad and the weight per 100 g body weight.

2. **Amounts of TG and PL in BAT (Table 1).** TG level in BAT was lower in CA ($p < 0.001$) than in WC, but the tissue content was greater in CA ($p < 0.001$) than in WC. This was due to the increased weight of BAT in CA. PL was greater in CA ($p < 0.001$) in terms of both level and total amount in BAT than the values in WC.

3. **FA compositions (Fig. 1).** Comparing FA compositions of TG and PL in BAT from WC, arachidonate, SA and PU were higher ($p < 0.001$), resulting in greater AI and UI ($p < 0.001$) in PL than in TG. Rare FA as a whole was about five times higher in PL ($p < 0.001$) than that in TG.

In TG-FA, as shown in Fig. 1A, arachidonate, AI and SA were higher, while UI, MU and rare FA were lower in CA than WC.

In PL-FA (Fig. 1B) MU and rare FA were lower, but arachidonate, PU, UI, and PU/SA were higher in CA than WC.

Fig. 1. Fatty acids (FA) compositions of BAT of WC and CA. Horizontal bar indicates standard error. WC, warm controls housed at 25°C. CA, cold-acclimated rats housed at 5°C for 4 weeks. * Significantly different vs WC. TG, triglyceride; PL, phospholipid. C14, myristate; C16, palmitate; C16-1, palmitoleate; C18, stearate; C18-1, oleate; C18-2, linoleate; C18-3, linolenate; C20-1, gadoleate; C20-2, arachidonate; Rare FA (C20-3, eicosadienoate; C20-3, bishomo- γ -linolenic acid; C24, lignocerate); SA, saturated FA; MU, monounsaturated FA; PU, polyunsaturated FA. AI, arachidonate index (C20-4/C18-2); UI, unsaturation index.

Effect of acute cold exposure

1. *TG and PL in BAT (Table 1).* Acute cold exposure in WC decreased the BAT weight, TG and PL contents, and TG level, but did not change PL level in WC.

In CA exposed to warm (CA-W), BAT weight and TG content were not changed, but PL level as well as total content was decreased. In CA-W exposed to cold the BAT weight and the content as well as the level of TG were not changed (CA-W-C), but the level and total content of PL were increased, although these values were decreased in CA-W.

2. *FA compositions.* TG-FA: In WC, acute cold exposure increased MU, UI, PU/SA, AI, and arachidonate, and decreased SA and rare FA (Fig. 2A). In CA-W, MU and rare FA were increased ($p < 0.01$ and $p < 0.001$, respectively), and SA was decreased ($p < 0.01$) as compared with those in CA (Figs. 1A, 3A). In CA-W, acute cold exposure increased SA, and decreased MU, PU/SA, UI, AI arachidonate and rare FA (Fig. 3A) (CA-W-C).

PL-FA: In WC, acute cold exposure increased PU/SA, and decreased SA, AI, and rare FA (Fig. 2B). In CA-W, arachidonate, MU and AI were increased ($p < 0.02$, < 0.01 , and < 0.001 , respectively) (Figs. 1A, 3B) as compared with those in CA. In CA-W, acute cold exposure decreased these values (Fig. 3B) (CA-W-C).

3. *Quantitative changes in TG-FA during acute cold exposure.* Figure 4 shows the quantitative values of TG-FA in BAT, which were calculated by multiplying the weight of TG by gravimetric (weight) ratio of each FA. About 85% of decrease of BAT-TG content by acute cold exposure was explained by a decrease in bulky FA such as C16, C18, C18:1, and C18:2 (Fig. 4A) and only 1.1% by a decrease in rare FA (Fig. 4B) in WC. No appreciable changes were observed in CA-W by acute cold exposure.

DISCUSSION

TG-FA in BAT. Several reports [2, 4, 15] described no significant changes in TG-FA composition of BAT after cold acclimation, but our previous report [3] showed the significant changes in TG of BAT; increased SA, decreased MU and UI. These changes were also confirmed in the present study. In contrast, it has been reported that cold acclimation induces desaturation of FA in TG of white adipose tissue [2]. The reason for such difference remains unknown, but it may reflect the difference in the function of these two tissues: BAT for thermogenesis and white adipose tissue for fat deposition. Rare FA has been reported to be accumulated during entry into hibernation and to be preferentially utilized during the early phase of arousal in the hamster BAT, providing about 75% of lipids being lost during this phase [10]. However, rare FA would not appear to contribute significantly to an enhanced BAT thermogenesis in rats, because the amount of rare FA of BAT was less than 2% of TG-FA in all experimental groups (Fig. 4B), and rare FA was only 1.1% of the amount of BAT-TG lost during cold exposure in WC (Fig. 4B).

Fig. 2. Effect of acute cold exposure on fatty acids (FA) compositions of BAT of WC, WC-C, warm controls exposed to 0°C for 12 h. Legends are the same as in Fig. 1.

Therefore, it is concluded that cold-induced BAT thermogenesis is mainly supported by bulky FA in TG of BAT. Since TG-FA profiles trended towards a desaturation as shown by increased AI, UI, MU level and PU/SA, and decreased SA level in the acute cold-exposed WC (Fig. 2A), SA may be preferentially utilized during cold-induced thermogenesis in BAT.

FA are the major energy substrates for BAT thermogenesis and BAT is a dominant site for an enhanced nonshivering thermogenesis (NST) in CA. However, it was noted that BAT-TG was not changed in the level and content during acute cold exposure in CA-W (Table 1), while FA compositions in BAT-TG were altered to more saturation as shown by the increased SA level and decreased UI as well as PU/SA (Fig. 3A), suggesting an acute cold-induced modification in BAT-TG metabolism, in spite of an apparent no changes in the level and amount of BAT-TG; i.e., an enhancement of lipogenesis in this tissue. It has been shown that the rate of fatty acid synthesis in BAT is more than 10 times higher in the cold-acclimated rats than in the warm controls [16]. Therefore, the present finding that TG content in BAT was not changed in CA-W during acute cold exposure would not necessarily indicate no utilization of TG. It is most likely that an increased lipogenesis would have replenished the reduced BAT-TG with newly synthesized lipids.

PL-FA in BAT. Previous reports [3, 4] indicated the general trend towards a desaturation of the PL in BAT from cold-acclimated rats. The present results are in agreement with these studies. In light of studies claiming that a rise in unsaturated FA in PL would exert beneficial changes in the cellular membrane functions [8, 9], the cold-induced changes in FA compositions of BAT-PL may be involved in an enhanced BAT function through their effects on membrane flexibility in ion transport, permeability, enzyme activity, and resistance to cell damage.

It is interesting to note that FA compositions of BAT-PL were significantly changed in response to acute cold exposure of 12 h (Figs. 2B, 3B). Biosynthesis of PL has been shown to be a rapid process in a matter of hours [17, 18]. In this context, it is interesting to note that during the first 24 h of cold exposure, hypertrophy and new formation of BAT mitochondria, and furthermore, great extension of BAT cell surfaces by deep vaginations and formation of cell processes were produced [19]. The present changes in PL-FA profiles (Figs. 2B, 3B) as well as PL contents (Table 1) observed during acute cold exposure are considered to reflect such rapid alterations of membrane components in BAT.

Cold acclimation activates the sympathetic nervous system, causing an increase in the noradrenaline excretion in the urine [20]. Noradrenaline level in plasma is also raised during exposure of rat to cold temperature [21, 22]. It has been reported that noradrenaline could significantly stimulate PL turnover in BAT [23]. Thus, it is inferred that noradrenaline is one of the factors which are responsible for the changes in FA compositions of BAT-PL by cold acclimation and acute cold exposure.

The physiological significance of the changes in FA composition and amount

Fig. 3. Effect of acute cold exposure on fatty acids (FA) compositions of BAT of cold-acclimated rats exposed to warm. CA-W, cold-acclimated rats transferred to warm (25°C) for 24 h; CA-W-C, cold-acclimated rats transferred to warm for 12 h and exposed to 0°C for 12 h. Legends are the same as in Fig. 1.

Table 1. Effect of acute cold exposure on BAT weights, TG, and PL contents.

	Body weight (g)		BAT		TG		PL	
	Initial	Final	mg	/100 g body weight	mg/pad	/100 mg BAT	mg/pad	/100 mg BAT
I. WC								
WC (10)	179±4.9	279±4.2	164±6.0	59±2.1	84±6.3	51±3.3	3.6±0.21	2.2±0.13
WC-C (10)			111±7.2*	40±2.9*	25±3.0*	20±2.0*	2.4±0.16*	2.2±0.08
II. CA								
CA (10)	184±3.4	252±8.3*	452±12.9	188±7.3	150±7.6	32±1.2	16.7±0.52	3.6±0.12
CA-W (10)			408±17.4	163±7.6†	157±12.4	38±2.0†	10.4±0.40†	2.6±0.12†
CA-W-C (10)			440±16.5	181±5.2	162±10.9	37±1.7†	14.7±0.62†*	3.4±0.19*

(): Number of animals; BAT, interscapular brown adipose tissue; TG, triglyceride; PL, phospholipids; WC, warm controls; WC-C, WC exposed to 0°C for 12 h; CA, cold-acclimated rats; CA-W, CA placed at 25°C for 24 h; CA-W-C, CA exposed to 0°C for 12 h after placed at 25°C for 12 h. *Significantly different vs. WC; †Significantly different vs. CA; *Significantly different vs. CA-W.

Fig. 4. Effect of acute cold exposure on amounts of fatty acids of BAT-TG. *Significantly different vs. WC, but in C24 of CA-W indicates significant difference vs. CA. Legends are the same as in Figs. 1-3.

during cold acclimation and acute cold exposure is not completely understood, but it should be considered that these changes are not accidental, but adaptive ones.

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