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Thermogenesis and Fatty Acid Composition of Brown Adipose Tissue in Rats Rendered Hyperthyroid and Hypothyroid—With Special Reference to Docosahexaenoic Acid

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Abstract: The effects of hyperthyroidism and hypothyroidism on brown adipose tissue (BAT) thermogenesis and phospholipid fatty acid composition were investigated in rats. Chronic triiodothyronine (T_3) treatment (hyperthyroidism) increased the interscapular BAT pad weight, its triacylglycerol content, and its DNA content. It did not affect basal and noradrenaline-stimulated *in vitro* oxygen consumption of BAT expressed per μg DNA, although it significantly increased the oxygen consumption of the whole BAT pad. T_3 treatment had little effect on phospholipid content and phospholipid fatty acid composition. In contrast, chronic methimazole treatment (hypothyroidism) decreased the BAT pad weight and the triacylglycerol content, but did not significantly change the DNA content in comparison with the control. It significantly decreased the noradrenaline-stimulated BAT oxygen consumption expressed per μg DNA and per BAT pad, but did not change the basal oxygen consumption. Methimazole treatment significantly affected phospholipid content and phospholipid fatty acid composition. Among the major fatty acids of BAT, it decreased docosahexaenoic acid (DHA), arachidonic acid, palmitic acid, palmitoleic acid, and oleic acid, and it increased linoleic acid, stearic acid, and eicosapentaenoic acid. A regression analysis revealed a positive relationship between *in vitro* respiration and DHA levels in phospholipids (r=0.404, p<0.05). These results suggest that thyroid hormones have trophic action on BAT and are necessary for BAT thermogenic activity. This study also suggests that DHA is involved in the regulation of BAT thermogenic activity, as we previously indicated. [Japanese Journal of Physiology, 48, 355–364, 1998]

Key words: brown adipose tissue thermogenesis, thyroid hormones, docosahexaenoic acid (DHA), phospholipid fatty acid composition.

Brown adipose tissue (BAT) is a thermogenic organ in mammals that is activated by several physiological conditions such as cold exposure, arousal from hibernation, overfeeding, and nonthermal stress [1]. Bioenergetics of BAT mitochondria is distinct from other cells because the brown adipocytes have uncoupling protein (UCP) in the mitochondria [2], which is responsible for transporting protons into the mitochondria without concomitant ATP formation. Many neuronal, endocrine, and humoral factors have been found to be directly or indirectly involved in the BAT thermogenesis. For example, the involvement of noradrenaline, glucagon, thyroid hormones, adrenocortical hormones, and nitric oxide have been investigated [1, 3, 4]. BAT is richly innervated with sympathetic nerves that release noradrenaline under stimulation. It is generally accepted that the noradrenaline is of primary importance for the peripheral activation of BAT thermogenesis. On the contrary, the role of thyroid hormones on BAT thermogenesis is not yet clear, al-

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though it is known that the thyroid hormones are involved in cold-induced nonshivering thermogenesis [5]. Thyroid hormones are known as lipogenic and lipolytic hormones. These two metabolic events, that is, lipogenesis and lipolysis, are closely related to BAT thermogenesis. Nevertheless, conflicting reports exist on the role of thyroid hormones on thermogenesis in BAT. It has been reported that GDP binding to mitochondria as a criterion for UCP capacity (an index of BAT thermogenesis) is suppressed in the pharmacologically induced hyperthyroid rats [6, 7], and that the stimulation of brown adipocyte respiration by isoproterenol is markedly greater in cells from the hyperthyroid rats in comparison with the euthyroid (having a normally functioning thyroid gland) rats [8]. Some authors have found an enhanced BAT thermogenic activity in the pharmacologically induced hypothyroid animals as estimated by BAT weight, its DNA content, and mitochondrial components [9], but we [10] and others [7, 11] did not. Therefore further studies are necessary to clarify the role of thyroid hormones in the regulation of BAT thermogenic activity.

Recent studies have emphasized the role of tissue phospholipid fatty acid composition on cellular functions. Particularly, n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) in the cellular membranes has been found to be a modulator of membrane functions and mitochondrial bioenergetics [12]. BAT is most active in the postnatal period and is activated by thermal and nonthermal stress in adults. We have shown that the thermal and the nonthermal stress, the postnatal period, and the overfeeding state modify the phospholipid fatty acid composition of BAT [13–18]. Furthermore, we and others have indicated that DHA is involved in BAT thermogenesis [13, 19, 20]. However, reports on the effects of hyperthyroidism and hypothyroidism on fatty acid composition are very scanty, and none addressed the effect on the n-3 polyunsaturated fatty acids pattern. The main objective of the present study is to examine the role of thyroid hormones on BAT thermogenic activity by measuring *in vitro* respiration (oxygen consumption), DNA content, triacylglycerol, and phospholipid contents and fatty acid composition, including n-3 polyunsaturated fatty acids (DHA and eicosapentaenoic acid).

MATERIALS AND METHODS

Animals and treatments. Eight-week–old male Wistar rats were used in the experiments. We obtained 30 seven-week–old rats, weighing from 150 to 170 g from Shizuoka Laboratory Animal Center, Hamamatsu, Japan. After 1 week, the rats were divided into 3 groups matching their initial body weights. They were housed in wire cages (5/cage) with free access to food (laboratory rat biscuits; Oriental MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water at 25± 1°C and about 50% RH on a 12-h light cycle. Hyperthyroidism was induced by injecting 3,3′,5-triiodo-L-thyronine sodium salt (T_3; Aldrich Chem. Co. Milw., WI 53201) subcutaneously (S.C.), and hypothyroidism was induced by injecting 2-mercapto-1-methylimidazole (methimazole; Sigma Chem. Co., St. Louis, MO 63178 USA) intraperitoneally (I.P.). The duration of treatments was 31 d. A stock solution of T_3 (4%) was prepared by dissolving T_3 in 0.1 N NaOH, and it was diluted with saline before use. A daily single dose of T_3 was 20 μg/100 g bw (0.05 ml/100 g bw S.C.) in the first 17 d of the experiment. Two rats died on day 18; the other rats of this group were apparently healthy. From day 18, the dose was reduced to 5 μg/100 g bw (0.05 ml/100 g bw S.C.) and was continued for the rest of the experiment. Methimazole was directly dissolved in saline, and a daily single dose of 5 mg/100 g bw (0.1 ml/100 g bw I.P.) was administered throughout the experiment. The control rats were administered vehicle (saline 0.1 ml/100 g bw) intraperitoneally. The rats were sacrificed by decapitation, and interscapular BAT was excised and cleaned of adherent tissues. The wet weight of the tissue was measured, the samples for *in vitro* oxygen consumption were immediately transferred to the incubation medium, and the samples for DNA and lipid analyses were preserved at −70°C until analysis. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

Biochemical analysis.

DNA. The DNA content of BAT was measured by a fluorometric method using bisbenzimidazole (Hoechst 33258) after defatting [21]. About 20 mg of interscapular BAT was defatted by homogenizing in cold acetone in a high-speed disintegrator, followed by shaking and centrifugation (1,800×*g*, 10 min). The defatted pellets were then homogenized in 10 ml of 1% sodium dodecyl sulfate by use of a glass homogenizer and incubated at 37°C for 1 h before measuring the DNA content with a spectrofluorometer (650-40, Hitachi Ltd., Tokyo). The incubated homogenate (20 μl) was aliquoted into the cuvette and mixed with 1,980 μl of dye solution (0.1 μg Hoechst 33258/ml TNE buffer consisting of 10 mM Tris, 1 mM EDTA, and 0.1 M NaCl). Calf thymus DNA (Sigma) was used as the standard, and measurements were taken at excitation and emission wavelengths of 365 and 460 nm, respectively.

In vitro respiration. *In vitro* respiration of the tissue was determined by measurement of the oxygen consumption rate of tissue blocks by use of a Clark electrode (Rank Brothers, Cambridge, UK). About 50 mg of interscapular BAT was cut into fine blocks of about 0.5 to 1 mm^3. The tissue blocks were preincubated for 1 h at 37°C in Krebs-Ringer phosphate buffer (pH 7.4) under slow shaking. The buffer contained half the recommended concentration of $CaCl_2$, 5 mM glucose, and 4% bovine serum albumin (Armour Co., Fraction V, dialyzed for 48 h through cellulose membrane against Krebs-Ringer phosphate buffer, pH 7.4). About 25 mg of the preincubated tissue blocks were added to a magnetically stirred electrode chamber having a temperature of 37°C and containing 2 ml of the same air-saturated buffer. The chamber was closed, and basal oxygen consumption was measured for 5 min. After this, Noradrenaline (0.19 mg (−)-Arterenol bitartrate salt (Sigma)/ml saline; 20 μl added to the chamber resulting in 1 μg Noradrenaline base/ml buffer) was added with a Hamilton syringe through a small hole in the cover of the chamber. This concentration of Noradrenaline has been shown to produce maximal stimulation of BAT from euthyroid rats in the same measurement condition [22]. The rate of oxygen consumption of electrode itself in the presence of 2 ml buffer over a period of 10 min was measured routinely before tissue blocks were added. The buffer contained 217 nmol O_2/ml. We did not examine by using different dosages of noradrenaline the possible sensitivity changes of BAT cells to noradrenaline under a different thyroid status, although we have shown previously that even a dose 10 times larger than the one we have used in this study produced no difference in the oxygen consumption of BAT cells from euthyroid rats [22]. It has also been demonstrated that the same dose of noradrenaline produced maximal stimulation of brown adipocytes from hypothyroid and euthyroid rats [23].

Lipid analysis. The total lipids were extracted as described previously [13]. About 50 mg of BAT was homogenized in a glass homogenizer with 6 ml chloroform-methanol (2 : 1 v/v) and centrifuged, followed by reextracting the residue by homogenizing the pellets with 2 ml of the same mixture of solvents. Phospholipid and triacylglycerol contents in the complete extract were determined by use of the PL-Test Wako and TG-test Wako reagent kits (Wako Pure Chem. Ind., Osaka, Japan). For fatty acid analyses, 6 ml of the complete extract was evaporated to dryness under nitrogen gas in a water bath at 30°C. The dried extract was taken up in 60 μl of chloroform-methanol (2 : 1 v/v) and applied to thin-layer chromatography for separating the phospholipids. The fractionated phospholipids were then removed by scratching and transesterified by adding 5 ml BF_3-methanol. The ester was extracted twice with pentane and dried under nitrogen gas in a water bath at 40°C. The dried transesterified phospholipids were taken up in 10 μl of chloroform-methanol (2 : 1 v/v). Fatty acid analyses were performed by using a Hitachi Model G-3900 gas chromatography equipped with a Hitachi Model D-2500 data processor. A bonded, flexible, fused silica column (30 m×0.25 mm I.D., polyimide film thickness<0.25 μm, J & W Scientific, USA) was used. The temperature of the injection inlet and the detector FID were set at 250°C, and the oven temperature was set at 140°C. The temperature program of the column was 10°C/min from 140°C at the initial injection to 240°C. The hydrogen carrier flow rate was 1.2 ml/min, the nitrogen make-up gas 15 ml/min, and the airflow 400 ml/min. One microliter of sample was injected with a split ratio of about 25 : 1. The hold time was 30 min. Quantitative standardization of the chromatograph was based on the analysis of fatty acid methyl ester standard mixtures (Funakoshi, Tokyo, Japan). Fatty acid contents are expressed in terms of mol%. The unsaturation index (the number of double bonds per fatty acid molecule) was calculated by this formula:

$$\text{Unsaturation index} = (\Sigma M_i N_i)/100 ,$$

where M_i is mol% of each fatty acid and N_i is the number of double bonds of each fatty acid. The arachidonate index was calculated in this way:

Arachidonate index
= arachidonate mol%/linoleate mol%.

Data analysis. Data were imported into Stat View 4.0 for the Macintosh. Data were expressed as mean±SE. The significance of differences between different groups were analyzed by using ANOVA followed by Fisher's PLSD test. A linear regression of peak values of noradrenaline-stimulated *in vitro* oxygen consumption (per μg DNA) on fatty acid composition was performed on the data of euthyroid, hyperthyroid, and hypothyroid rats, and a *t*-test was used to determine whether the mean slope deviated significantly from 0. We considered differences significant at $p<0.05$.

RESULTS

Thyroid status and food intake and body weight

T_3 treatment increased daily food intake compared with the control, and methimazole treatment de-

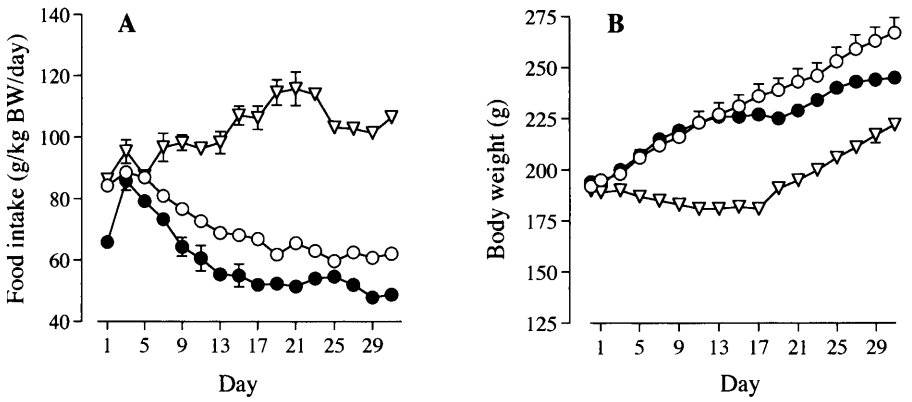

Fig. 1. Effects of thyroid state on food intake (A) and regular body weight change (B). Values are means±SE for rat cages. SE bars smaller than the symbols are not presented in the figure. T_3 or methimazole was administered to the rats for 31 d. An ANOVA run on the daily food intake of control (○), of the T_3-treated group (▽), and of the methimazole-treated group (●) indicated significant differences between groups (control vs. T_3-treated group, $p<0.001$, and control vs. methimazole-treated group, $p<0.01$). An ANOVA run on the change in daily body weight indicated a significant difference between control and the T_3-treated groups ($p<0.001$), but the difference between control and methimazole-treated groups was significant only in the second half of the experiment (day 15 to day 31) ($p<0.05$).

creased it (Fig. 1A). The average daily food intake in the last week of the experiment was 70% higher (102 g/kg/d) in the T_3-treated group and 17% lower (50 g/kg/d) in the methimazole-treated group than in the control group (60 g/kg/d). The food intake in the control group decreased with time, indicating, as previously reported [24], that food consumption per kg bw decreases with age. An ANOVA run on the daily food intakes of the control, T_3-treated, and methimazole-treated groups indicated significant differences between groups (control vs. T_3-treated group, $p<0.001$, and control vs. methimazole-treated group, $p<0.01$).

The initial body weights of control, T_3-treated, and methimazole-treated rats were 192±2.7, 188±2.2, and 194±1.7 g, respectively. Differences in the initial body weights among the groups were not statistically significant. The T_3 treatment and the methimazole treatment both decreased the regular body weight gain compared with the control. The decrease in the body weight gain was more pronounced in the T_3-treated group than in the methimazole-treated group (Fig. 1B). An ANOVA run on the changes in daily body weight of the control, the T_3-treated group and the methimazole-treated group indicated significant differences between the control and the T_3-treated groups ($p<0.001$), but the difference between the control and the methimazole-treated groups was found to be significant only in the second half of the experiment (day 15 to day 31) ($p<0.05$). The body weights of control, T_3-treated, and methimazole-treated rats at sacrifice were 271±7.3, 228±3.8 g (16% lower than the control, $p<0.001$), and 247±3.9 g (9% lower than the control, $p<0.01$), respectively.

Effect of thyroid status on the tissue weights and compositions

Despite a decrease in the body weight, the interscapular BAT pad wet weight, its triacylglycerol, and its DNA contents were greater in the T_3-treated group than in the control group. The phospholipid content of BAT was unchanged. On the other hand, interscapular BAT pad wet weight, its phospholipid, and its triacylglycerol contents were smaller in the methimazole-treated group than in the control group. The DNA content was unchanged (Table 1). Epididymal white adipose tissue weight was smaller in both the T_3-treated (2.2±0.12 g, $p<0.0001$) and methimazole-treated (3.1±0.10 g, $p<0.0001$) groups than in the control group (4.4±0.20 g).

In vitro respiration of BAT

Neither T_3 treatment nor methimazole treatment changed the basal *in vitro* oxygen consumption of BAT expressed per μg DNA (76.3±11.22 pmol/min/μg DNA with T_3 treatment, 77.9±10.16 pmol/min/μg DNA with methimazole treatment, and 88.9±14.17 pmol/min/μg DNA with no treatment). To compare relative thermogenic rates per BAT pad between rats in different thyroid states with different body sizes, values were corrected on the basis of body weight of 250 g. T_3 treatment increased the basal *in vitro* oxygen consumption of BAT (54.0±6.17 nmol/min/pad, $p<0.05$), and no change was noted with methimazole treatment (32.0±4.33 nmol/min/pad) vs. 37.5±5.45 nmol/min/pad without treatment. Noradrenaline stimulated the oxygen consumption in all groups, and the peak value was recorded from 10 to 15 min after stimulation. However, the noradrenaline-stimulated increase in oxygen consumption when expressed per μg DNA was not affected in the T_3-treated group and

Table 1. Effect of thyroid state on brown adipose tissue (BAT) weight and its components.

	BAT pad weight (mg)		Phospholipid (mg)		Triacylglycerol (mg)		DNA (μg)	
	Whole pad	Pad/250 g bw	/100 mg BAT	/pad	/100 mg BAT	/pad	/mg BAT	/pad
Control	173 ±7.9	161 ±9.2	4.14 ±0.324	7.45 ±0.790	70.12 ±1.928	122.64 ±8.501	2.7 ±0.15	461.9 ±17.18
T_3	248 ±6.0**	272 ±8.9**	3.86 ±0.302	9.52 ±0.770	67.54 ±1.686	167.63 ±7.175**	2.7 ±0.13	668.4 ±31.37**
Methimazole	115 ±6.2**,##	117 ±6.8**,##	4.05 ±0.327	4.35 ±0.459*,##	50.21 ±3.486**,##	58.50 ±5.087**,##	3.7 ±0.26*,#	409.6 ±19.80##

* $p<0.01$, ** $p<0.001$ vs. control; # $p<0.01$, ## $p<0.001$ vs. T_3-treated group.

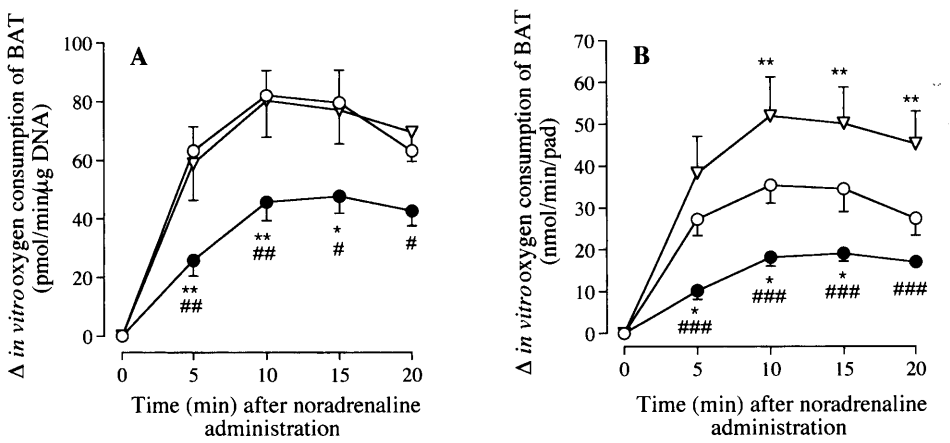

Fig. 2. Noradrenaline-stimulated rate of increase in *in vitro* oxygen consumption per μg DNA (A) and per brown adipose tissue (BAT) pad (B). Values are means±SE. T_3 or methimazole were administered to the rats for 31 d. The weights of BAT pad were corrected on the basis of body weight (expressed per 250 g rat). * $p<0.05$, ** $p<0.01$ vs. control; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs. T_3-treated group. ○, control; ▽, T_3-treated; ●, methimazole-treated.

decreased in the methimazole-treated group compared with the control ($p<0.05$; Fig. 2A). When noradrenaline-stimulated increase in the oxygen consumption was expressed per interscapular BAT pad, it was greater in the T_3-treated group ($p<0.01$) and less in the methimazole-treated group compared with the control ($p<0.05$; Fig. 2B).

Phospholipid fatty acid composition

T_3-treated rats showed no change in saturated, monounsaturated, and polyunsaturated phospholipid fatty acid contents of BAT, nor did they show change in the unsaturation index, the arachidonate index, or the polyunsaturation-saturation ratio compared with the control. In contrast, methimazole-treated rats showed a decrease in the monounsaturated fatty acid content concomitantly with an increase in the polyunsaturated fatty acid content. The polyunsaturation-saturation ratio was increased and the arachidonate index was decreased compared with the control. The saturated fatty acid content and the unsaturation index were unchanged. These results are summarized in Table 2.

The results of fatty acid composition are shown in Fig. 3. In BAT from all three groups, the major phospholipid fatty acids were palmitate (C16:0), palmitoleate (C16:1), stearate (C18:0), oleate (C18:1), linoleate (C18:2), arachidonate (C20:4), and docosahexaenoate (C22:6). T_3 treatment caused no major modification in these fatty acids compared with the control. It caused decreases in eicosapentaenoic acid (C20:5) and docosapentaenoic acid (C22:5) among the fatty acids analyzed. On the other hand, methimazole treatment caused a significant modification of phospholipid fatty acid composition of BAT compared with the control. Myristate (C14:0), C16:0, C16:1, C18:1, eicosadienoate (C20:2), C20:4, C22:5, and C22:6 were decreased, and C18:0, C18:2, and C20:5 were increased.

Relationships between phospholipid fatty acids and BAT respiration

We have previously reported that n-3 polyunsaturated fatty acid DHA (C22:6) is involved in the regulation of *in vitro* respiration of BAT [13, 20]. When noradrenaline-stimulated peak values of oxygen consumption of BAT as expressed per μg DNA were plotted against the proportion of DHA in phospholipids, a positive relationship was evidenced ($r=0.404$, $p<0.05$). No such significant relationship was found when eicosapentaenoic acid and arachidonic acid pro-

Table 2. Thyroid state and the state of saturation of phospholipids.

	SA (mol%)	MU (mol%)	PU (mol%)	UI	PU/SA	AI
Control	45.15±0.499	17.65±0.968	37.20±1.102	1.30±0.021	0.83±0.030	0.42±0.023
T_3	44.84±0.372	17.52±0.479	37.64±0.604	1.29±0.014	0.84±0.019	0.39±0.013
Methimazole	45.14±0.576	11.22±0.378*,#	43.63±0.531*,#	1.30±0.017	0.97±0.022*,#	0.25±0.011*,#

* $p<0.001$ vs. control; # $p<0.001$ vs. T_3-treated group. SA, saturated fatty acids; MU, monounsaturated fatty acids; PU, polyunsaturated fatty acids; UI, unsaturation index; AI, arachidonate index.

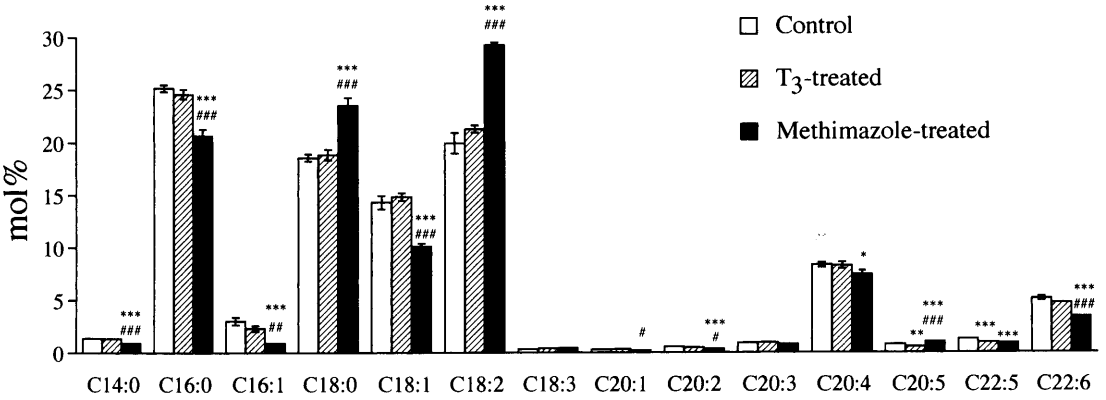

Fig. 3. Phospholipid fatty acid composition of brown adipose tissue. Values are expressed as means±SE. T_3 or methimazole was administered to the rats for 31 d. □, control; ▨, T_3-treated; ■, methimazole-treated. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. control. # $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs. T_3-treated group. C14:0, myristate; C16:0, palmitate; C16:1, palmitoleate; C18:0, stearate; C18:1, oleate; C18:2, linoleate; C18:3, linolenate; C20:1, gadoleate; C20:2, eicosadienoate; C20:3, bis-homo-γ-linolenate; C20:4, arachidonate; C20:5, eicosapentaenoate; C22:5, docosapentaenoate; C22:6, docosahexaenoate.

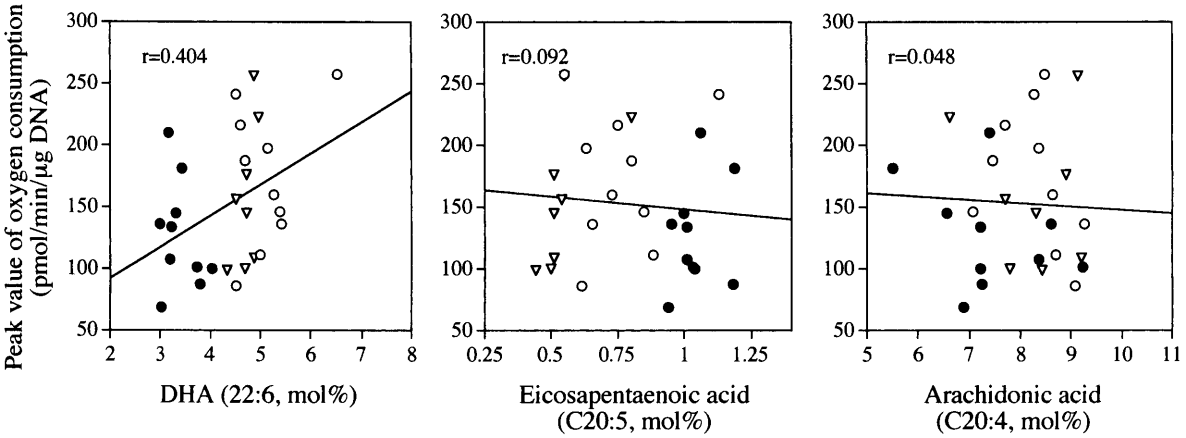

Fig. 4. Correlation between oxygen consumption of brown adipose tissue (peak values) and docosahexaenoic acid, eicosapentaenoic acid, and arachidonic acid levels of phospholipids. The "*r*" value is indicated in each. Data from control (○), T_3-treated group (▽), and methimazole-treated group (●) were plotted for this analysis.

portions in phospholipids were related to the oxygen consumption (Fig. 4).

DISCUSSION

This study shows that thyroid hormones are involved in the regulation of several events closely related to BAT thermogenesis. Thyroid hormones have the potential to indirectly influence BAT thermogenesis by regulating the storage and supply of energy substrate fatty acids (lipogenesis and lipolysis) for BAT thermogenesis. They also influence BAT thermogenesis by regulating brown adipocyte respiration and membrane phospholipid composition.

In this study, T_3 treatment for 31 d increased the triacylglycerol content of BAT (37% increase compared

with the control) and decreased the white adipose tissue weight (50% decrease compared with the control). The decrease in white adipose tissue weight may reflect a decrease in its triacylglycerol content. Thyroid hormone is known as a lipogenic hormone as well as a lipolytic hormone. The responsiveness of thyroid hormone-stimulated lipogenesis has been shown to be organ-specific. However, the previous reports on adipose tissues regarding responsiveness to thyroid hormone are contradictory with one another. Some reported that thyroid hormone treatment increased the synthesis of fatty acids in white and brown adipose tissue [25–27], whereas others observed the opposite effect as assessed by the decrease in the activities of fatty acid synthetase on white adipose tissue [28] and no appreciable effect on brown adipose tissue [29]. For energy substrate, BAT does not depend solely on its *de novo* lipogenesis; it also depends on a supply of free fatty acids through blood. Besides the *de novo* synthesis of triacylglycerol, BAT would probably depend on the supply of free fatty acid through blood for thermogenesis in the present chronic state of hyperthyroidism. T_3 treatment indeed elevates the blood level of free fatty acids in warm-adapted and cold-adapted rats, which has previously been demonstrated [30]. It has also been shown that this lipolytic action of T_3 can be partially blocked by a depletion of catecholamines, indicating that white adipose tissue is the main source of free fatty acids in blood. The decrease in white adipose tissue in the T_3-treated rats may be partially explained by this event. On the contrary, an increased level of triacylglycerol content of BAT may reflect active lipogenesis as well as BAT hyperplasia under T_3 stimulation (Table 1).

Thyroid hormone–induced systemic thermogenesis is well documented. However, confusing reports have been made on the contribution of BAT in the thyroid-stimulated systemic thermogenesis. Some reported that T_3 treatment increased the basal oxygen consumption of BAT (an index of BAT thermogenesis) [31], whereas others reported that although T_3 treatment increased the BAT mass and DNA content, it suppressed BAT thermogenic activity as measured by GDP binding to mitochondria [32]. Our study clearly indicated that the interscapular BAT thermogenesis is stimulated by T_3 treatment, as evidenced by increases in the DNA content (cellularity) of BAT and the basal oxygen consumption expressed per pad, corrected on the basis of body size. Although the reasons for the discrepancy are not clearly known, the discrepancy observed in these findings may be due to the differences in the species and the ages of animals and to the doses and durations of thyroid hormone treatments. Furthermore, the noradrenaline-stimulated increase in the rate of BAT thermogenesis expressed per BAT was significantly higher in the T_3-treated group than in the control group (Fig. 2). Although the noradrenaline-stimulated whole pad thermogenesis was higher in the T_3-treated group, thermogenesis per μg DNA (per BAT cells) was unchanged compared with the euthyroid control. In contrast, the isoproterenol-stimulated increase in the rate of BAT thermogenesis expressed per BAT cells has been reported to be higher in rats rendered hyperthyroid by T_3 treatment for 2 d than in the control rats [8]. The discrepancy may be explained by the differences in the agonists and the duration of the T_3 treatment. Furthermore, under the prolonged T_3 treatment the animals may have responded to maintain homeostasis, thereby maintaining the normal responsiveness of BAT cells to agonist.

On the other side, it has been reported that hypothyroid rats have normal-sized BAT [7] and DNA content [11]. In contrast, others have reported that hypothyroid rats have a large-sized BAT and DNA content [9]. In our study, the hypothyroid rats have smaller-sized BAT and normal DNA content. These results are consistent with our previous findings in the thyroidectomized rats [10]. Although the reasons for discrepancies in the results are not readily understood, one possibility may be the difficulty that sometimes arises in judging the borderline between BAT and white adipose tissue by eye inspection and thereby causing a variation in the BAT wet weight. The smaller size of BAT compared with the control in our study, however, could be explained by the decreased triacylglycerol content of BAT cells. In contrast with another study showing an increase in the basal oxygen consumption of interscapular BAT (whole pad) in hypothyroid rats [9], the basal oxygen consumption of BAT expressed per μg DNA and per whole pad and corrected on the basis of body size were unchanged. Moreover, using the same dose of noradrenaline to stimulate BAT from methimazole-induced hypothyroid rats and euthyroid rats, we demonstrated that a noradrenaline-stimulated increase in the rate of BAT thermogenesis expressed per μg DNA and per BAT pad was significantly lower in the methimazole-treated group than in the euthyroid group (Fig. 2). The decrease in the noradrenaline-stimulated BAT thermogenesis is consistent with our previous finding that thyroidectomy completely abolishes the glucagon-stimulated increase in the free fatty acid (fuel for BAT thermogenesis) concentration in Sulzer's drainage vein from BAT [10] and with another study showing that hypothyroidism induced by a low-iodine diet plus 6-n-

propyl-2-thiouracil diminished noradrenaline-stimulated oxygen consumption of brown adipocytes [23]. Our study does not explain if the changes in BAT thermogenic activity under different thyroid status were secondary to the changes in food intake or vice versa. In this regard, it should be mentioned that BAT is established as the principal organ for diet-induced thermogenesis and that excess food intake enhances BAT thermogenic activity [33]. It has also been demonstrated by cutting the excess food consumed during cold acclimation that fully developed nonshivering thermogenic activity could still be acquired [34]. Summarizing all these results, it is concluded that thyroid hormones have trophic action on BAT and that they are necessary for the normal thermogenic activity of BAT.

It is known that living tissues favorably modulate cellular functions by modifying the membrane phospholipid composition, probably by modifying the unsaturation state of the membrane lipids. In this study, the hyperthyroidism rendered by T_3 treatment did not change the state of membrane phospholipid unsaturation as estimated by the levels of monounsaturated or polyunsaturated fatty acids and by the unsaturation index. Hypothyroidism rendered by methimazole treatment, on the other hand, caused a decrease in monounsaturation and an increase in polyunsaturation without changing the overall unsaturation state as estimated by the unsaturation index in BAT phospholipids (Table 2). We previously reported that the n-3 polyunsaturated fatty acid DHA levels in BAT phospholipid are closely related to the noradrenaline-stimulated *in vitro* oxygen consumption of BAT cells [13, 20]. Subsequently, it was demonstrated that GDP binding to BAT mitochondria from rats fed n-3 polyunsaturated fatty acid supplemented food is markedly stimulated [19], although respiration of BAT cells is unchanged possibly because of a concomitant depletion of n-6 unsaturated fatty acids [35]. It was noted here that the hyperthyroid rats showed no changes in noradrenaline-stimulated increase in *in vitro* respiration when expressed per μg DNA and that DHA in BAT phospholipids was unchanged compared with the control. On the other hand, hypothyroid rats showed a decrease in *in vitro* respiration when expressed per μg DNA, and this was accompanied by decreased levels of DHA and arachidonic acid and an increased level of eicosapentaenoic acid in BAT phospholipids (Fig. 3). Therefore the cause of the decrease in oxygen consumption of BAT from hypothyroid rats may lie in the change of these fatty acid levels, namely, DHA, arachidonate, and eicosapentaenoate. However, a correlation analysis revealed a positive relationship only between the levels of DHA and oxygen consumption (per μg DNA). These results regarding phospholipid fatty acid composition and *in vitro* respiration agree with our previous reports, suggesting that the n-3 polyunsaturated fatty acid DHA plays a role in the regulation of BAT thermogenesis as estimated by *in vitro* oxygen consumption irrespective of the extent of the total unsaturation of phospholipid fatty acids [13, 20]. These results also show that the suppression of normal thyroid functions impairs BAT thermogenic activity possibly by modifying phospholipid fatty acid composition, most notably by depleting the DHA level.

In tissues and cell lines other than BAT, it has been indicated that the mode of action of DHA may involve alteration of cell membrane permeability. Membranes of the rod outer segment, known to be highly enriched in DHA , are very permeable to K^+ [36]. Stillwell *et al.* [37], by using T27A tumor cells and Chow and Jondall [38], by using JURKAT leukemia cells, have reported that the presence of a sufficient amount of DHA in membranes increases membrane permeability. Recently, Stillwell *et al.* [39], have demonstrated by incorporating DHA in hepatocyte mitochondria from BALB/c mice that tightly coupled mitochondria are uncoupled upon the accumulation of a small amount of DHA in the mitochondria. BAT is the typical tissue in which a physiological uncoupling mechanism exists. In brown adipocytes, heat is generated by uncoupling oxidative phosphorylation from electron transfer, facilitated by a high rate of proton movement across the mitochondrial membrane (termed as "proton leak"); therefore fuel oxidation dissipates heat without concomitant ATP formation. As brown adipocytes are packed with mitochondria, mitochondrial phospholipids presumably constitute the major fraction of total phospholipids of BAT in the present study. Changes in membrane DHA levels under various physiological conditions that demand varied levels of heat production would perhaps influence brown adipocyte respiration by changing the mitochondrial membrane permeability with DHA [40]. This view would explain the relationship between the noradrenaline-stimulated BAT respiration expressed per μg DNA and the levels of DHA in phospholipids in the present euthyroid, hypothyroid, and hyperthyroid states.

In conclusion, the experiments presented here show that chronic hyperthyroidism enhances noradrenaline-stimulated BAT thermogenesis by increasing the cellularity and that hypothyroidism decreases BAT thermogenesis by decreasing the thermogenic ability of individual cells. This implies that optimum levels of

thyroid hormones are necessary for brown adipocytes to show the maximum thermogenic activity under adrenergic stimulation and in a basal state. This study also shows that the suppression of normal thyroid functions impairs BAT thermogenic activity possibly by modifying the phospholipid milieu of the tissue, most notably by depleting the DHA level of phospholipids. Furthermore, a positive relationship between DHA levels in BAT phospholipids and *in vitro* respiration of BAT suggests a regulatory role of DHA in BAT thermogenesis, regardless of the degree of unsaturation of membrane phospholipids.

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