

Brown fat thermogenesis in cold-acclimated rats is not abolished by the suppression of thyroid function

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Zaninovich, Angel A., Marcela Raíces, Inés Rebagliati, Conrado Ricci, and Karl Hagmüller. Brown fat thermogenesis in cold-acclimated rats is not abolished by the suppression of thyroid function. *Am J Physiol Endocrinol Metab* 283: E496–E502, 2002. First published May 15, 2002; 10.1152/ajpendo.00540.2001.—The effects of long-term cold exposure on brown adipose tissue (BAT) thermogenesis in hypothyroid rats have been examined. Thyroid ablation was performed in normal rats after 2 mo of exposure to 4°C, when BAT hypertrophy and thermogenic activity were maximal. After ablation, hypothyroid and normal controls remained in the cold for 2 additional months. At the end of the 4-mo cold exposure, all untreated hypothyroid rats were alive, had normal body temperature, and had gained an average 12.8% more weight than normal controls. Long-term cold exposure of hypothyroid rats markedly increased BAT weight, mitochondrial proteins, uncoupling protein (UCP)-1, mRNA for UCP-1, and oxygen consumption to levels similar to those seen in cold-exposed normal rats. The results indicate that thyroid hormones are required for increased thermogenic capacity to occur as an adaptation to long-term cold exposure. However, cold adaptation can be maintained in the absence of thyroid hormone.

cold acclimation; uncoupling protein-1; hypothyroidism; oxygen consumption; norepinephrine

THE IMPORTANCE OF THE BROWN ADIPOSE TISSUE (BAT) thermogenic response to acute cold stress has been widely recognized (for reviews see Refs. 21, 22, 30). Numerous complex phenomena take place in BAT after exposure to low temperatures, leading to the synthesis of mitochondrial proteins involved in heat production. A critical role in this process is the synthesis of uncoupling protein-1 (UCP-1), strongly promoted by norepinephrine (NE) through a synergism between α - and β -adrenergic receptors (24, 32, 40). The presence of the thyroid hormones is essential to initiate BAT heat production, because triiodothyronine (T_3) potentiates the action of NE on UCP-1 gene transcription (2–4, 37). This explains the development of hypothermia in hypothyroid rats soon after exposure to low tempera-

tures, an effect corrected by the administration of thyroid hormones but not by the administration of NE (39).

Thyroid hormones are active regulators of basal metabolic rate (BMR) and energy expenditure by a number of mechanisms (9, 12, 38, 39). However, earlier studies had suggested that the thyroid hormone may not be necessary for a sustained normothermia after acclimation to cold has been achieved (for review see Ref. 13). Thus Hsieh and Carlson (25) observed a high metabolic rate and normal life survival in hypothyroid rats adapted to cold before thyroidectomy. Despite numerous studies, the influence of thyroid function on the maintenance of normothermia in rodents exposed to prolonged low temperature is not clearly understood.

The purpose of the present study was to assess the role of the thyroid hormones in BAT thermogenesis during long-term cold exposure. After a period of time in the cold, rats were made hypothyroid. Hypothyroid and normal controls were then maintained for an additional period of time in the cold, and thereafter BAT was studied. Accordingly, hypothyroidism was induced after BAT hypertrophy and thermogenic activity were maximally activated. In contrast, room temperature-adapted hypothyroid rats are unsuitable for these studies, because they rapidly develop hypothermia in the cold.

The data presented here show that, although BAT is unable to initiate a thermogenic process in the cold without thyroid hormone, it can maintain a high BAT thermogenic capacity and capacity for a response to NE in hypothyroid rats living in a cold environment, resulting in normothermia and normal survival. The data confirm the accepted role for thyroid hormones in the initial phase of BAT thermogenesis.

EXPERIMENTAL PROCEDURES

Animals and Materials

Male and female Wistar rats with an initial body weight of 108–130 g were used. They had free access to tap water and

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rat chow. All drugs and reagents, unless otherwise specified, were purchased from Sigma Chemical (St. Louis, MO). Radioactive ^{131}I was obtained from the Atomic Energy Commission of Argentina. The antibody against UCP-1 was purchased from Calbiochem (San Diego, CA). The antibody was free of cross-reactivity against UCP-2 or UCP-3, as determined by the manufacturer. The cDNA probe against rat UCP-1 was kindly provided by Dr. J. E. Silva, McGill University, Montreal, Canada.

Experiments

Experiment 1: long-term cold exposure. For measurement of BAT UCP-1, UCP-1 mRNA, and mitochondrial oxygen consumption, rats were placed in individual cages either in a cold room at 4°C or at room temperature (~24°C) lighted between 0600 and 2000 and with unrestricted access to tap water and food pellets. The composition of the diet was 26% protein containing the essential amino acids, 6% fat, and 44% carbohydrates calculated from the nitrogen-free extract. Other components were 6% fiber, vitamins, and 7% ash containing several minerals. Seventy-six percent of the nitrogen was present in digestible form (Pilar Nutrients, Buenos Aires, Argentina). After 2 mo of cold exposure, groups of rats were made hypothyroid by intraperitoneal injection of ^{131}I , and the others were left intact and served as euthyroid controls. Hypothyroid and normal rats were then maintained for an additional 2 mo in the cold. At the end of 4 mo of cold exposure, a group of five hypothyroid rats was injected subcutaneously with thyroxine (T_4 ; 1.5 $\mu\text{g}/100$ g body wt) in divided doses daily for 3 days. All animals were killed by cervical dislocation, blood was collected, and interscapular BAT was removed, weighed, properly cleaned of adhering muscle and white fat, and immediately processed in the manner described in *Technical Procedures*.

Experiment 2: acute cold exposure. Other groups of rats were made hypothyroid at room temperature and thereafter were placed in the cold room at 4°C for 14 h. Normal and T_4 -treated rats were left in the cold room for 24 h, and their controls remained in the warm room. BAT was obtained for the measurement of mitochondrial oxygen consumption and UCP-1 content.

Experiment 3: Methimazole treatment and measurement of hepatic mitochondrial α -glycerophosphate dehydrogenase activity in cold-acclimated rats. Despite the fact that ^{131}I treatment has been shown to be an effective means to abolish thyroid gland function (5), some of these animals might still have had minimal amounts of T_4 or T_3 in serum that might distort the interpretation of results. To eliminate any possible residual thyroid hormone production, a group of six ^{131}I -treated hypothyroid rats in the cold room was given a second antithyroid treatment with 2-mercapto-1-methyl-imidazole (methimazole; 0.03%) in the drinking water. The treatment began 1 mo after the injection of ^{131}I (3 mo after commencement of cold exposure) and was extended for 1 mo. Animals were killed, and BAT and liver were removed and processed.

The absence of T_3 activity was additionally examined in groups of cold-acclimated normal or hypothyroid rats by measuring the T_3 -dependent liver mitochondrial α -glycerophosphate dehydrogenase (α -GPD) enzyme activity. This enzyme is a reliable marker of thyroid status (29), and its gene has recently been cloned (16). BAT mitochondrial α -GPD activity was also determined.

Technical Procedures

Isolation of BAT mitochondria. BAT was homogenized on ice in buffer containing sucrose (0.25 M), *N*-tris-methyl-2-

aminoethanesulfonic acid (K-*TES*; 5 mM), disodium ethylenediaminetetraacetic acid (EDTA; 2 mM), and bovine serum albumin (2%; pH 7.2). Mitochondria were isolated by differential centrifugation as described by Cannon and Lindberg (8). The final pellet was taken to 1-ml volume in sucrose buffer (0.24 M). Protein content was measured in an aliquot of this suspension by use of the method of Gornall et al. (19) and expressed as milligrams per milliliter.

Western blot analysis. SDS-PAGE electrophoresis was done according to Laemmli (27). Samples were treated with cracking buffer and heated for 3 min at 100°C. Mitochondrial protein (80, 15, or 20 μg) was loaded in each lane. After electrophoresis, gels were electrotransferred to nitrocellulose Hybond-C membranes (Amersham, Arlington Heights, IL). For Western blot assays, nitrocellulose Hybond sheets were blocked with 5% powdered milk in TBST buffer (50 mM Tris·HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween 20) for 1–2 h. The membranes were incubated with polyclonal antisera anti-UCP-1 (Calbiochem) diluted in blocking solution for 2 h, subjected to three 5-min washes with TBST, and incubated with a second antibody linked to horseradish peroxidase (Amersham) and visualized by enhanced chemiluminescence detection (Renaissance, NEN-Du Pont). Quantifications were carried out with the PC-Software Image Scion.

Northern blot analysis. Northern blots were performed as described before (1), using a 1.1-kb cDNA probe against rat UCP-1 (34). Total RNA (15 μg) from BAT was electrophoresed onto 1.4% formaldehyde gels and then blotted onto Hybond N+ nylon membranes (Amersham) according to the manufacturer's procedures. The probe was labeled using the Prime a Gene labeling kit (Promega). Membranes were hybridized overnight with the UCP-1 ^{32}P -labeled probe at 65°C in Church's buffer. Sequential stringent washes were performed (2 \times SSC, 1 \times SSC, and 0.1 \times SSC with 0.1% SDS) at 65°C, and blots were exposed to X-OMAT Kodak film. Equal RNA loading was checked in the EtBr staining of the electrophoresis.

BAT mitochondrial oxygen consumption. BAT mitochondrial oxygen consumption was measured as described elsewhere (7). It was determined polarographically in an oxygenograph (Gilson Medical Electronic, Middleton, WI) with the use of a Clark electrode at 30°C. The reaction medium had a volume of 0.9 ml and contained potassium chloride (100 mM), K-*TES* (20 mM), magnesium chloride (2 mM), EDTA (1 mM), and 2% BSA, pH 7.2. An aliquot of the mitochondrial suspension was added to the respiration medium (final concentration 1.5 mg protein/ml medium). Oxygen uptake was initiated in *state 4* (without ADP). After the velocity of oxygen consumption was determined, a phosphate acceptor (0.82 mM ADP) was added to the medium (*state 3*), and respiration was allowed to proceed. Results were expressed in nanograms of oxygen per minute per milligram of protein. α -GDP was used as substrate because of the presence in BAT of a highly active α -GPD enzyme (8).

α -GPD activity. Mitochondrial α -GPD activity was measured spectrophotometrically at 500 nm and 37°C in liver and BAT by the microassay procedure described by Gardner (14).

Other methods. Serum T_4 and T_3 concentrations were measured by a double-antibody technique (5). The limits of sensitivity were 5 nmol/l for T_4 and 0.12 nmol/l for T_3 . Body temperature was determined with a clinical thermometer introduced 4 cm into the rectum and kept in place for 3 min. Statistical analysis was performed by analysis of variance and Duncan's test.

RESULTS

Body Weight

After an initial weight loss, all animals gained weight steadily during acclimation to cold, much more so in male than in female rats. At the end of 4 mo, the mean body weight in normal males was 347 ± 17 g and in hypothyroid males 387 ± 15 g, values about three times higher than the initial body weight. In control rats in the warm, body weight increased twofold during this period (data not shown). Body weight in males exceeded that in females by 30.8% in normal rats and 29.2% in hypothyroid rats ($P < 0.001$). Thyroid ablation did not interrupt weight gain. Body weight in cold-acclimated male and female hypothyroid rats exceeded that in normal rats by an average of 12.8% ($P < 0.001$). Despite differences in body weight, BAT thermogenic parameters showed no sex differences, and the data were pooled. After 4 mo of cold acclimation, all hypothyroid rats were alive and had normal body temperature (Table 1).

UCP-1 in hypothyroid BAT was increased by cold acclimation. Figure 1 shows that mitochondrial UCP-1 synthesis in both hypothyroid and normal rats was markedly increased by acclimation to cold. Figure 1A shows results after loading 80 μ g of protein per lane. There was an overexpression of UCP-1 in both hypothyroid and normal rats. However, the high intensity of the UCP-1 band prevented comparison of the levels of UCP-1 from rats exposed to acute (24-h) cold or with rats left in the warm. To overcome this effect, lanes were loaded with 15 μ g of protein. The results, shown in Fig. 1B, illustrate that, regardless of the thyroidal state, cold acclimation induced a marked increase in UCP-1 synthesis compared with rats exposed to 1 day of cold, whereas no UCP-1 was detected in rats left in the warm. T_4 replacement did not increase the UCP-1

response. Figure 1C depicts the UCP-1 in normal rats and in rats treated with ^{131}I alone or ^{131}I plus methimazole. Both hypothyroid groups had levels of UCP-1 similar to those observed in normal rats, thus confirming the deep hypothyroid state in rats treated with the radioisotope alone.

BAT mRNA Levels

Prolonged cold exposure of normal and hypothyroid rats produced similar and significant increases in BAT UCP-1 mRNA (Fig. 2). T_4 treatment failed to alter the results.

Mitochondrial Oxygen Consumption in State 4 and State 3

Cold acclimation increased about four times the state 4 oxygen uptake in BAT mitochondria of hypothyroid and normal rats (Fig. 3). The administration of T_4 did not change the results, which suggests that the hypothyroid BAT was under maximal stimulation. This was confirmed by the same values of maximal oxygen consumption in the presence (state 3) or the absence (state 4) of ADP. These findings are consistent with total uncoupling of the mitochondria. Other indexes of increased BAT thermogenesis in cold-acclimated hypothyroid rats were a fourfold rise in BAT weight and a sevenfold increase in mitochondrial proteins, compared with values in the warm (Table 1).

Effects of Acute (24 h) Cold Exposure

Both normal and T_4 -treated rats responded with a 100% increment in BAT oxygen consumption (Fig. 3) and a significant increase in UCP-1 content (Fig. 1) compared with control rats left in the warm. Hypothyroid rats, however, failed to increase BAT oxygen consumption after 14 h in the cold.

Table 1. BAT size, mitochondrial proteins, and colonic temperatures in rats acclimated to 4°C for 4 mo or left in the warm

	Normal		Hypothyroid		Hypothyroid ¹
	24°C (n = 5)	4°C (n = 16)	24°C (n = 5)	4°C (n = 31)	4°C (n = 8)
	<i>Experiment 1²</i>				
BAT weight, mg	203 ± 18*	409 ± 33†	150 ± 15*	556 ± 18†	596 ± 12
Mitochondrial protein, mg/ml	4.8 ± 0.8*	18.1 ± 0.6†	3.2 ± 0.6*	22.2 ± 0.8†	20.3 ± 1.0
Temperature, °C	37.4 ± 0.4	37.7 ± 0.2	37.5 ± 0.3	37.8 ± 0.2	37.6 ± 0.4
Serum levels, nmol/l					
T_4	47.3 ± 2.7	53.8 ± 3.3*	10.4 ± 1.2	9.3 ± 2.1†	5.8 ± 1.3
T_3	0.82 ± 0.08	0.85 ± 0.07*	0.15 ± 0.02	0.14 ± 0.02†	0.12 ± 0.02
	<i>Experiment 3³</i>				
	α -GPD activity in the cold, ΔOD , 500 $\text{nm} \cdot \text{min}^{-1} \cdot \text{mg protein} \times 10^{-2}$				
Liver		4.2 ± 0.3*		1.6 ± 0.4†	
BAT		446 ± 19‡		375 ± 25§	

Values are means \pm SD; n = no. of rats studied. ¹These 8 of the 31 hypothyroid rats shown in this table were additionally analyzed as a single group, because serum thyroxine (T_4) and triiodothyronine (T_3) values were on the limits of sensitivity. The limits of detection were 5 nmol/l for T_4 and 0.12 nmol/l for T_3 . ²Data correspond to cold-acclimated rats whose mitochondrial oxygen consumption is presented in Fig. 3. Rats were exposed to 4°C for 2 mo before thyroid ablation. ³ α -Glycerophosphate dehydrogenase (α -GPD) activity in cold-acclimated normal and hypothyroid rats used in experiment 3; 6 rats per group were used. Brown adipose tissue (BAT) weight, mitochondrial proteins, and oxygen consumption in these rats were similar to those found in other cold-acclimated rats, and uncoupling protein (UCP)-1 is shown in Fig. 1C. OD, optical density. *P* values: * vs. † < 0.001; ‡ vs. § < 0.002 (ANOVA and Duncan's test).

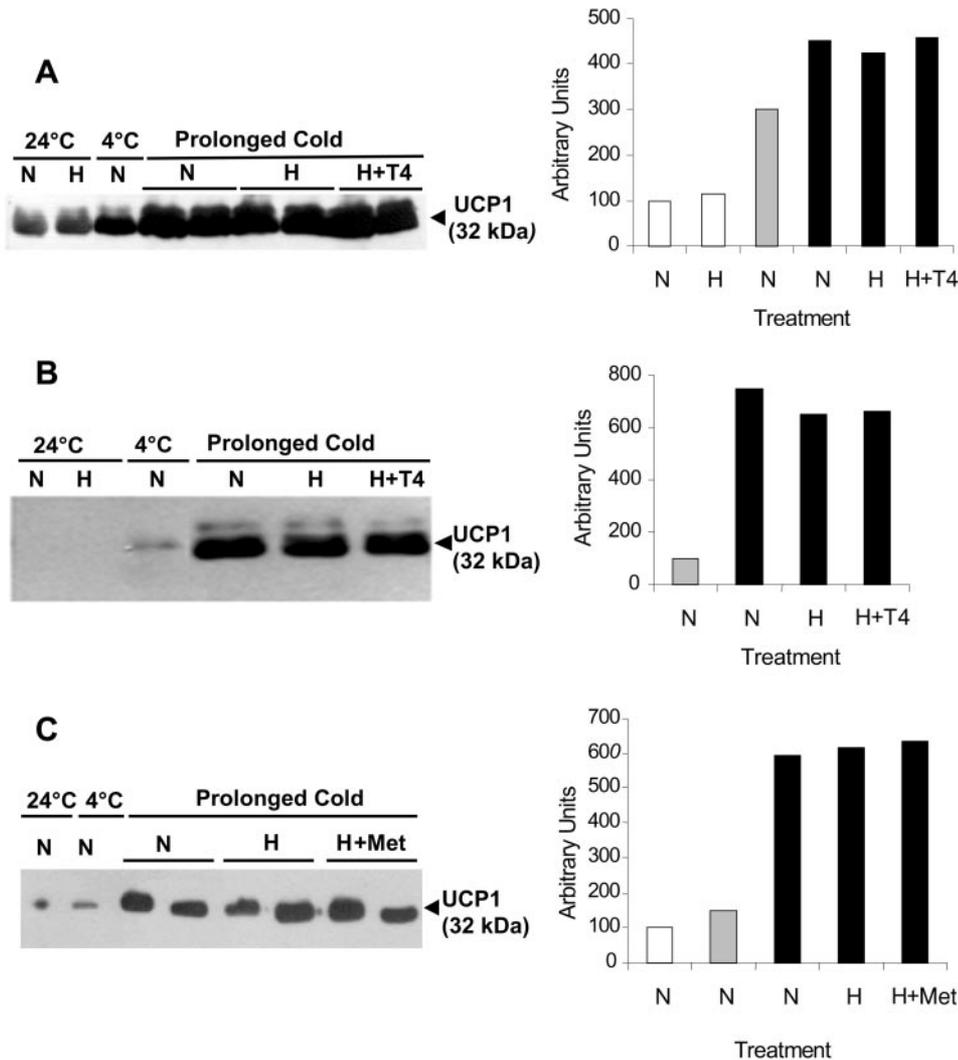


Fig. 1. Western blot assay of brown adipose tissue (BAT) mitochondrial protein from normal (N), hypothyroid (H), or thyroxine (T_4)-treated hypothyroid (H+ T_4) rats exposed to 4°C for periods of 4 mo or 24 h or left in the warm. Areas under "4°C" correspond to rats exposed to 24-h cold. Areas under "Prolonged Cold" correspond to rats exposed to cold for 4 mo. Met, methimazole. Open bars in histograms represent 24°C, gray bars 4°C, and filled bars prolonged cold exposure. Each lane was loaded with mitochondrial protein from a single rat. A: lanes were loaded with 80 µg of mitochondrial protein; the intensity of uncoupling protein (UCP)-1 prevents comparison with groups under other temperature regimens. B: lanes were loaded with 15 µg of protein, allowing assessment of the effects of temperature on UCP-1 synthesis. C: UCP-1 of normal, ^{131}I -, or ^{131}I - + methimazole-treated rats. Twenty micrograms of mitochondrial protein were loaded. Each filled bar in A and C in histograms of prolonged cold exposure rats represents the mean value of the 2 respective samples shown in gel.

Effects of Methimazole Treatment

Methimazole treatment reduced slightly more serum T_4 levels in cold-acclimated hypothyroid rats but did not alter oxygen consumption (not shown) or UCP-1 synthesis (Fig. 1C).

Changes in α -GPD Activity

Liver mitochondrial α -GPD activity in cold-acclimated hypothyroid rats was reduced to one-third that of normal rats (Table 1). The enzyme activity was also decreased in BAT, but α -GPD in BAT is >100 times more potent than in liver. In fact, Table 1 shows that the α -GPD activity in the BAT of hypothyroid rats was >230 times higher than that in liver, which accounts for the enhanced substrate oxidations in BAT mitochondria. In a previous study (6), BAT α -GPD activity was 15-fold that in heart, and it was not affected by changes in temperature or thyroid hormones.

DISCUSSION

The thermogenic effects of thyroid hormones are believed to result from their widespread metabolic

stimulation in many tissues. Their contribution to BAT heat production was studied more intensively after 1980, when a large number of studies investigated the role of BAT in nonshivering thermogenesis during acute cold exposure or after cold acclimation of rodents. It was observed that cold produced rapid and sustained increases in the activity of the mitochondrial conductance pathway in BAT through the synthesis of UCP-1, a process accompanied by hypertrophy and hyperplasia of this tissue (36). These responses were largely under the control of NE inputs from the sympathetic nerve endings. Bianco and Silva (3, 4) showed that the prevention of hypothermia in cold-exposed hypothyroid rats correlated with the degree of UCP-1 concentration and that this effect was T_3 dependent. Their data showed that 5'-deiodinase type II (D2) activity in BAT was essential to generate sufficient intracellular concentration of T_3 through T_4 deiodination, because circulating T_3 alone could not saturate the nuclear T_3 receptor (TR) occupancy that would potentiate the cAMP response to NE stimulation (4). This observation was recently confirmed in D2 gene-deficient mice showing hypothermia despite normal UCP-1 concentration

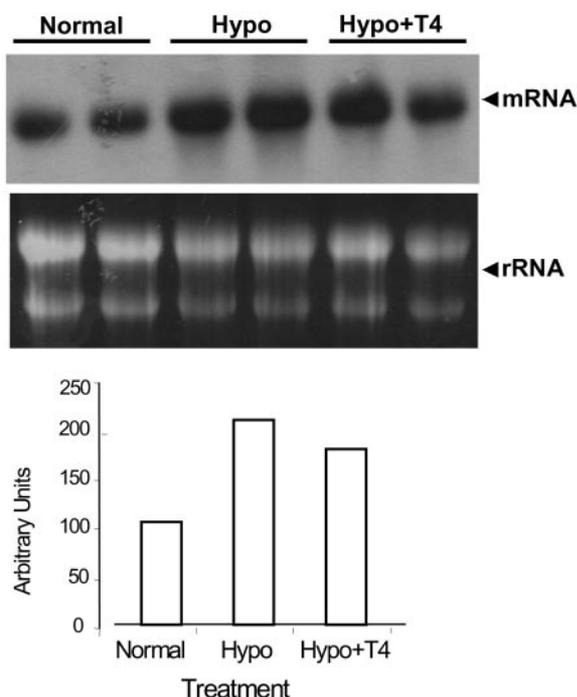


Fig. 2. Northern blot assays in 2 representative experiments per group of cold-acclimated rats. Each lane represents pooled mRNA from 3 rats. Fifteen micrograms of total RNA were loaded per lane. Samples were obtained from normal, hypothyroid (Hypo) and thyroxine (T_4)-treated hypothyroid (Hypo+ T_4) rats. Each bar in the histogram represents the mean value of the 2 respective samples shown in gel. Hypothyroid rats had a rise in mRNA of 215% and T_4 -treated rats of 180% with respect to values in normal rats (100%), as shown in histograms.

(10). The D2 knockout mice are unable to generate enough T_3 to saturate the $TR\alpha_1$ isoform, which appears responsible for NE signal transduction, whereas the $TR\beta$ isoform mediates primarily UCP-1 synthesis (35).

Although there is abundant evidence that thyroid hormones are essential to initiate the thermogenic process in response to low temperature, no solid evidence is available to support a significant role for the thyroid hormones in the maintenance of normothermia in a cold environment. In fact, rats kept outdoors had a significant decrease in both BMR and thyroid function during the cold months (20), and the cold-acclimated mouse maintains a large capacity for nonshivering thermogenesis despite the return of D2 in BAT to a normal low level (26). The findings of high BMR and normal survival in cold-acclimated hypothyroid rats reported by Hsieh and Carlson (25) prompted the authors to suggest that the metabolic response to cold was not directly dependent on the amount of circulating T_4 . In the present study, the antithyroid treatment was given when BAT hypertrophy and thermogenic activity were maximally stimulated by 2-mo exposure to cold. The effects of cold regularly occur after 4 to 6 wk at low temperatures (22).

The presence of a pronounced hypothyroid state was critical to this investigation. Because even small quantities of circulating T_4 would produce T_3 , which could

stimulate BAT response to cold (3), emphasis was laid on determining that these rats were indeed hypothyroid. The hypothyroid state was confirmed by several lines of evidence. First, the concentrations of serum T_4 and T_3 decreased to the limits of sensitivity of the method, particularly in the eight rats segregated from the hypothyroid group shown in Table 1 and in six rats treated with methimazole. Second, further evidence was drawn from the pronounced fall of hepatic α -GPD, which is a reliable index of T_3 activity (29). Another important question is whether negligible circulating levels of both T_4 and T_3 may have been sufficient to stimulate BAT response to cold. Earlier work by Bianco and Silva (3) showed that acute cold exposure of hypothyroid rats whose serum T_4 and T_3 concentrations were as low as those in the present study resulted in a 90% fall of BAT UCP-1 and a drop in body temperature to 29°C. After physiological T_3 replacement, serum T_3 concentration was normalized, but an 86% fall in UCP-1 synthesis occurred. Maximal (100%) saturation of BAT nuclear T_3 receptors is required for the full expression of UCP-1 synthesis (37). This degree of receptor occupancy could not be reached in cold-exposed, T_3 -treated hypothyroid rats even after the administration of >15 times the physiological T_3 replacement and extremely hyperthyroid levels of serum T_3 (3). This phenomenon explains the development of hypothermia during cold exposure of hypothyroid rats treated with physiological amounts of T_3 (6, 7). The foregoing data are consistent with the fact that thyroid hormone production in hypothyroid rats was nil or sufficiently low that it resulted in a deep hypothyroid state. Furthermore, the negligible levels of circulating

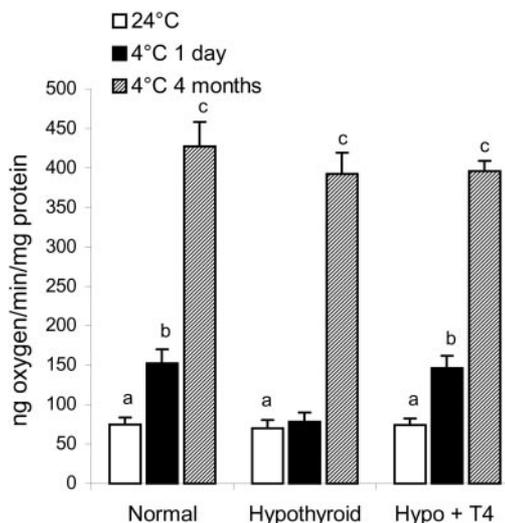


Fig. 3. BAT mitochondrial oxygen consumption in normal ($n = 16$), untreated hypothyroid ($n = 31$), and T_4 -treated hypothyroid ($n = 5$) rats exposed to 4°C for 4 mo. In the acute cold studies (6 rats/group) hypothyroidism was induced at room temperature ($\sim 24^\circ\text{C}$), and rats were placed at 4°C for 14 h, whereas normal and T_4 -treated rats remained in the cold for 24 h. Five animals per group were used for the study of rats adapted to room temperature. Notice that, during acute cold exposure, hypothyroid BAT did not raise oxygen consumption with respect to values in the warm. Probability values: a vs. b and b vs. c < 0.001 (ANOVA and Duncan's test).

thyroid hormones were insufficient to initiate, or even maintain, BAT thermogenesis in a cold environment.

BAT hypertrophy and enhancement of thermogenesis in euthyroid rats during long-term cold acclimation were shown long ago (36), but the maintenance of a sustained hypertrophied state and high indexes of BAT thermogenesis in cold-adapted hypothyroid rats have not been previously reported and were unexpected. The hypothyroid rats were normothermic and had elevated levels of UCP-1, UCP-1 mRNA, and mitochondrial oxygen consumption. The magnitude reached by the thermogenic parameters suggests that this tissue was under maximal stimulation, a contention supported by the failure of exogenous T_4 to promote further increments of UCP-1 synthesis or oxygen consumption. These findings agree with those in which the addition of ADP to the respiration medium failed to stimulate oxygen consumption, an indication that the mitochondria were uncoupled. The results suggest that, despite the absence of T_3 , which is needed for full expression of β -adrenergic receptors, there was sufficient sympathetic signaling to stimulate maximal BAT activation. The adequacy of sympathetic inputs in BAT and other tissues in the absence of T_3 can be seen in the normothermia of cold-acclimated hypothyroid rats, as discussed below.

In a physiological setting, the metabolic activity in tissues other than BAT contributes to normothermia through a variety of pathways, including thyroid hormone modulation of mitochondrial oxygen consumption (9, 12, 15, 31, 38). These effects are exerted on the mitochondria of specific target tissues like liver and skeletal muscle, which are the most important oxygen consumers (33). It has been shown that the stimulation of thermogenesis by the thyroid hormones is related to the activity of the mitochondrial nitric oxide synthase, which is able to regulate mitochondrial and systemic oxygen consumption in different tissues and conditions through the production of nitric oxide (11). We have recently found an increased transcription and activity of liver and muscle mitochondrial nitric oxide synthase in hypothyroid rats and a decrease in hyperthyroid rats (9), which suggests that modulation of this enzyme is a substantial part of thyroid effects on mitochondrial oxygen consumption in the main heat-producing tissues.

From the findings that acclimated hypothyroid rats were normothermic, one can reasonably assume that heat production in other thermogenic tissues was not significantly affected by cessation of T_3 supply. This effect is particularly important in skeletal muscle, where T_3 is essential for the synthesis of UCP-3 (17, 28, 41). We have preliminary data showing that muscle mitochondrial oxygen consumption in cold-acclimated hypothyroid rats was lower than that in normal rats in the cold but twofold higher than that in hypothyroid controls in the warm, whereas consumption in cold-exposed muscle dropped to less than one-half after treatment with reserpine. These findings suggest that the hypothyroid muscle can respond to enhanced caloric demands in the cold with an increase in nonshivering

heat production, mediated predominantly by sympathetic inputs. These findings agree with the reports of Gong et al. (18) and Himms-Hagen and Harper (23), suggesting that uncoupling of mitochondria by UCP-3 is not a major determinant of muscle thermogenesis.

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