Effects of Cold Acclimation and Fasting on Thyroxine 5′-Deiodinase in Brown Adipose Tissue of *ob/ob* Mice (43460)

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Abstract. **Gradual acclimation to mild cold for 6 weeks increases the total activity of thyroxine 5′-deiodinase in brown adipose tissue (BAT) of genetically obese (*ob/ob*) mice to a level greater than that in similarly acclimated lean mice. This increase is largely due to the growth of the BAT in the *ob/ob* mouse, because specific activity of the enzyme is only slightly increased. In similarly cold-acclimated lean mice, the specific activity of thyroxine 5′-deiodinase was not altered. BAT mitochondrial GDP binding increased to the same high level in the gradually cold-acclimated *ob/ob* mouse as in cold-acclimated lean mice. We conclude that the growth and maintenance of BAT in the cold-acclimated *ob/ob* mouse, as in the cold-acclimated lean mouse, does not require greatly increased activity of thyroxine 5′-deiodinase. Fasting for 48 hr did not alter thyroxine 5′-deiodinase activity of BAT in either lean or *ob/ob* mice. The fasting-induced increase in activity seen by others in lean mice is probably due to thermoregulatory stimulation of BAT occasioned by the low environmental temperature at which the fasting occurred.** [P.S.E.B.M. 1992, Vol 200]

Thyroxine 5′-deiodinase in brown adipose tissue (BAT) is responsible for the intracellular generation of 3,5,3′-triiodothyronine (T_3) from thyroxine (T_4), and this is believed to play an important role in control of the thermogenic capacity of BAT for thermogenesis, in particular, of the ability of BAT to raise the concentration of the unique mitochondrial uncoupling protein (UCP) when it is stimulated (1–4). The most potent stimulus to an increased activity of T_4 5′-deiodinase in BAT is exposure of a rat or mouse to cold (5–8), a response that requires a functional sympathetic innervation of BAT (9–11). However, despite a marked circadian rhythm in sympathetic nervous system activity in BAT of mice (12) and in thermogenic activity of BAT in mice (13), there is no corresponding variation in T_4 5′-deiodinase activity (13). Moreover, functional denervation of BAT of rats (9) or mice (10) does not alter the basal activity of this enzyme. Thus, control by norepinephrine appears to be of importance only during the intense stimulation engendered by exposure to cold. Other factors, such as levels of insulin, glucagon, and thyroxine, appear to exert regulatory influences on the basal activity of this enzyme (11, 14).

In the genetically obese, *ob/ob*, mouse, BAT is usually in a relatively atrophied state and unresponsive to norepinephrine. Acute exposure of the *ob/ob* mouse to cold has a much less than normal stimulatory effect on BAT thermogenesis (low increase in mitochondrial GDP binding) (15, 16), on mRNA for UCP (17), on mitochondrial concentration of UCP (16), and on T_4 5′-deiodinase activity (6, 7), and these mice are extremely cold intolerant. Because acute exposure to cold does increase the secretion of norepinephrine in BAT of the *ob/ob* mouse (18) and because injected norepinephrine has a smaller than normal effect to increase BAT T_4 5′-deiodinase activity (19), we have concluded that attenuation of the initial cold-induced burst in T_3 production, secondary to refractoriness to norepinephrine, is the reason for the attenuation of the other trophic responses of this tissue to cold.

Several treatments improve the low thermogenic capacity of the *ob/ob* mouse and the responsiveness of its BAT to cold. These include adrenalectomy (7) and

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gradual acclimation to mild cold (15, 16). We have demonstrated that adrenalectomy of the *ob/ob* mouse enhances both the cold-induced increase in T_4 5′-deiodinase activity and the thermogenic (increase in mitochondrial GDP binding) and trophic (increase in total UCP content) responses of BAT to acute exposure to cold (7). However, although somewhat improved, the cold intolerance of these mice persisted and the long-term trophic response of BAT could not be assessed (7). Gradual acclimation to mild cold allows a normal or even supranormal trophic response of BAT of the *ob/ob* mouse to cold, as shown by the delayed but exaggerated increase in protein and cytochrome oxidase contents and in mitochondrial GDP binding and UCP concentration (15, 16). The effect of this procedure on T_4 5′-deiodinase activity of BAT is, however, not known, and it is not clear whether enhanced activity of this enzyme accompanies the exaggerated trophic response. One objective of the present experiments was to find out whether gradual acclimation of the *ob/ob* mouse to cold was accompanied by an increase in T_4 5′-deiodinase activity in BAT.

A recent report suggests that fasting increases T_4 5′-deiodinase activity in BAT of mice (20), in contrast to its lack of effect (14) or suppressive effect (21) in rats. Another objective of the present experiments was to find out whether fasting could increase T_4 5′-deiodinase activity in BAT of the *ob/ob* mouse, as reported for the lean mouse.

Methods

Female C57BL/6J genetically obese (*ob/ob*) mice and phenotypically lean (+/?) littermates were obtained from Jackson Laboratories, Bar Harbor, ME, at 7–8 weeks of age. In the first experiment (effect of gradual cold acclimation), they were housed at 27°C in individual cages (to prevent conservation of heat by huddling in the cold) with free access to Purina Rodent Chow 5012 and water and with lights on from 0700 to 1900 hr. The metabolizable energy content of the chow was 3.43 kcal/g; it contained 22.5% protein, 53.2% carbohydrate, and 4.5% fat by weight. Eight obese and eight lean mice were transferred to a room maintained at 22°C for 2 weeks and then at 18°C for 2 weeks. They were then moved to a room at 14°C for an additional 2 weeks. Of the mice that remained at 27°C for these 6 weeks, eight lean and eight obese mice were acutely exposed to 14°C for 12 hr before they were sacrificed. Food intake was measured weekly during the acclimation period. In the second experiment (effect of fasting), three phenotypically similar mice were housed per cage at 28°C (to minimize thermoregulatory adjustments during fasting) for 10 months after arrival. Some lean and *ob/ob* mice were fasting for 48 hr before they were sacrificed. Food intake by control mice was measured during this period.

Mice were sacrificed by cervical dislocation at 0900 hr (2 hr after lights on) in the first experiment or at 0800 hr (1 hr after lights on) in the second experiment in the room in which they were housed in order to minimize the stress and sympathetic activation that occurs when the animals are moved. Blood was collected in the second experiment. Rectal temperatures were measured using a probe (Bailey Instruments digital thermometer, probe RET-3) inserted to a depth of 1.8 cm. Interscapular and subscapular BAT was quickly removed and placed in an ice-cold isolation medium (0.25 *M* sucrose, 1 m*M* HEPES, and 0.2 m*M* EDTA, adjusted to pH 7.2 with 1 *M* potassium hydroxide).

BAT was cleaned of adhering muscle and white adipose tissue, weighed, and homogenized in 3.5 ml of isolation medium (glass-Teflon homogenizer). The final volume was measured and 500 μl were rehomogenized (glass-glass homogenizer) and frozen in liquid nitrogen for subsequent storage at −80°C. The remainder of the homogenate was used for isolation of mitochondria as described before (7). GDP binding to isolated mitochondria was measured as described previously (22), except that 1 m*M* ADP was used to assess nonspecific biding. T_4 5′-deiodinase was measured in homogenates as described previously (6, 7). Protein was measured in homogenates and mitochondria using a modified Lowry method as described before (22).

Serum T_4 and T_3 concentrations were measured by radioimmunoassay (23). Thyroid hormone-free mouse serum was prepared from lean and *ob/ob* mice for use in standard curves to control for the abnormal thyroid hormone binding by serum of the *ob/ob* mouse (24).

Statistical analysis used SAS general linear models for unbalanced analysis of variance. The post hoc test used was Tukey's. A value of $P < 0.05$ was used to determine a statistically significant difference.

Results

During gradual acclimation to mild cold, *ob/ob* mice did not increase their food intake above their already hyperphagic level (Table I). Lean mice, in contrast, were hyperphagic in the cold, even during the first 2 weeks at the relatively mild "cold" of 22°C, and at 14°C they ate the same large amount as *ob/ob* mice (Table I). Lean mice gained slightly more weight in the cold than lean mice in the warm, whereas *ob/ob* mice did not gain weight in the cold, in contrast to the large increase in weight of these mice in the warm (Table II).

Gradual acclimation to mild cold induced the expected exaggerated growth of BAT. Whereas the protein content of BAT of lean mice just doubled after cold acclimation, the protein content of BAT of the *ob/ob* mice increased almost 4-fold (Fig. 1). The total T_4 5′-deiodinase activity in BAT of cold-acclimated lean mice also doubled, that is, its specific activity did not change significantly (Figs. 2 and 3). The total T_4 5′-deiodinase

Table I. Food Intake of Lean and *ob/ob* Mice during Gradual Acclimation to Mild Cold[a]

Weeks	Temperature warm or cold	Lean		Obese	
		Warm (n = 16)	Cold (n = 8)	Warm (n = 16)	Cold (n = 8)
1–2	27°C or 22°C	11.3 ± 0.4	15.7[b] ± 0.4	20.0[c] ± 0.4	22.1[c] ± 1.0
3–4	27°C or 18°C	9.7 ± 0.6	15.0[b] ± 0.7	17.0[c] ± 0.7	18.3[c] ± 1.0
5–6	27°C or 14°C	10.9 ± 0.5	18.4[b] ± 0.7	18.1[c] ± 1.2	16.2 ± 1.4

[a] Data are expressed as kcal/24 hr.
[b] Significant effect of cold, comparing mice of the same type.
[c] Significant effect of obesity, comparing mice at the same temperature.

Table II. Final Body Weights and Temperatures of Lean and *ob/ob* Mice

	Lean			Obese		
	Acclimation[a]		Cold exposure[b] (n = 8)	Acclimation[a]		Cold exposure[b] (n = 8)
	Warm (n = 8)	Cold (n = 8)		Warm (n = 8)	Cold (n = 8)	
Body wt (g)	20.1 ± 0.3	22.5[c] ± 0.6	20.1 ± 0.5	50.5[d] ± 1.7	38.2[c,d] ± 2.3	50.9[d] ± 1.2
Wt gain (g) in 6 weeks	3.1 ± 0.4	4.4[c] ± 0.2		17.7[d] ± 1.6	3.3[c] ± 2.7	
Body temperature (°C)	36.9 ± 0.2	36.7 ± 0.2	34.9[c] ± 0.5	35.0[d] ± 0.2	33.0[c,d] ± 0.27	21.9[c,d] ± 2.2
BAT wet wt (g)	0.18 ± 0.01	0.29[c] ± 0.02	0.16 ± 0.01	1.52[d] ± 0.12	1.23[d] ± 0.08	1.30[d] ± 0.07

[a] Acclimation to warm refers to mice maintained throughout at 27°C. Acclimation to cold refers to mice successively acclimated over three 2-week periods to 22°C, 18°C, and 14°C.
[b] Exposure to cold refers to mice maintained at 27°C for 6 weeks, then exposed to 14°C for 12 hr.
[c] Significant effect of cold, comparing mice of the same type.
[d] Significant effect of obesity, comparing mice at the same temperature.

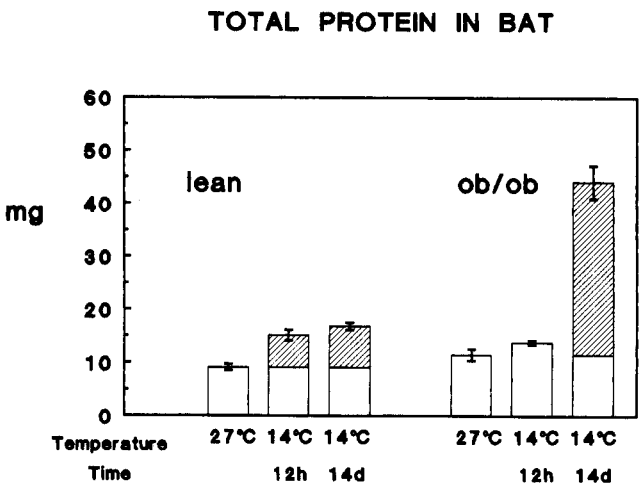

Figure 1. Effect of acute exposure to 14°C for 12 hr and of gradual acclimation to mild cold on total protein in BAT. The cross-hatched portions of the bars denote a significant effect of cold exposure or acclimation, comparing the same type of mouse. The value for obese mice that were acclimated to cold is significantly greater than that for similarly acclimated lean mice. The wet weight of BAT of these mice is in Table II.

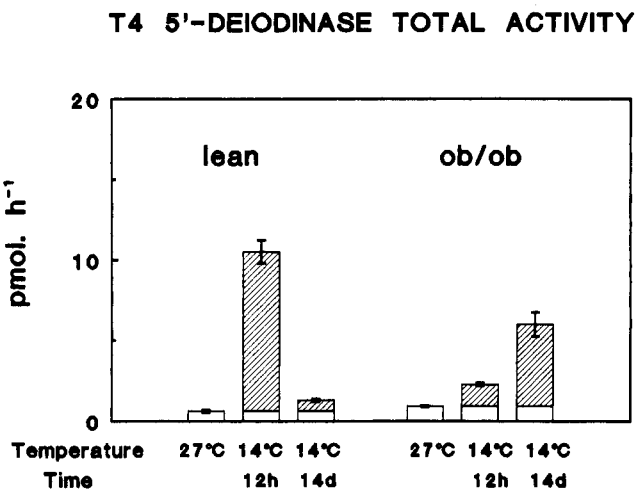

Figure 2. Effect of acute exposure to 14°C for 12 hr and of gradual acclimation to mild cold on the total activity of T_4 5′-deiodinase in BAT. The cross-hatched portions of the bars denote a significant effect of cold exposure or acclimation, comparing the same type of mouse. The value for obese mice exposed to cold for 12 hr is significantly lower than that for similarly cold-exposed lean mice. The value for cold-acclimated obese mice is significantly greater than that for similarly cold-acclimated lean mice.

activity in BAT of *ob/ob* mice increased 6.5-fold (Fig. 2), and in this case, the specific activity did increase significantly (Fig. 3), although only to a relatively small extent (1.7 times). BAT mitochondrial GDP binding also doubled in lean mice, whereas in *ob/ob* mice the increase was much greater (about 10 times) due to the very low level in the warm-acclimated mice: the level achieved in the cold-acclimated state was, in fact, not different in lean and *ob/ob* mice (Fig. 4).

In contrast to the long-term effects of gradual acclimation to cold, acute exposure to 14°C for 12 hr of

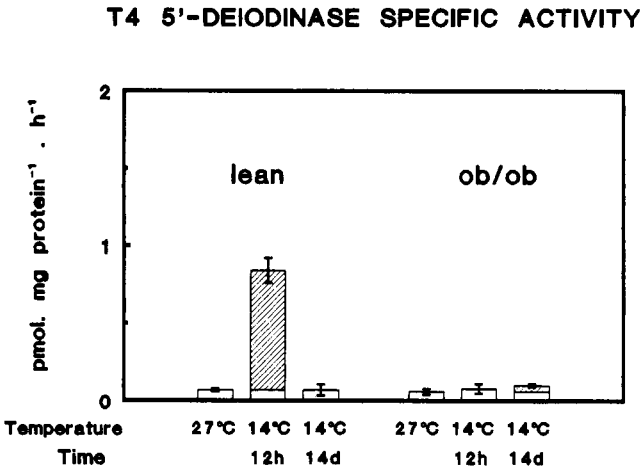

Figure 3. Effect of acute exposure to 14°C for 12 hr and of gradual acclimation to mild cold on the specifiic activity of T_4 5′-deiodinase in BAT. The cross-hatched portions of the bars denote a significant effect of cold exposure or acclimation, comparing the same type of mouse. The value for obese mice exposed to cold is significantly lower than that for similarly cold-exposed lean mice.

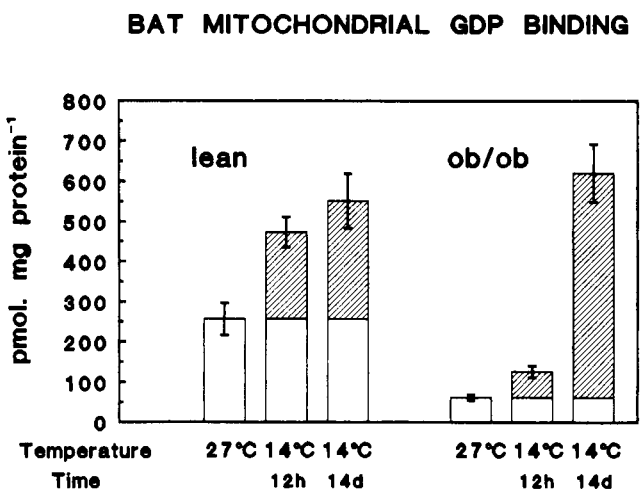

Figure 4. Effect of acute exposure to 14°C for 12 hr and of gradual acclimation to mild cold on BAT mitochondrial GDP binding. The cross-hatched portions of the bars denote a significant effect of cold exposure or acclimation, comparing the same type of mouse. Values for *ob/ob* mice are significantly different from those for lean mice for the groups at 27°C and the groups exposed to 14°C for 12 hr.

mice previously acclimated to 27°C increased protein content of BAT in lean but not obese mice (Fig. 1), brought about a very large increase in both total and specific activity of T_4 5′-deiodinase activity in lean mice, but not in obese mice (Figs. 2 and 3), and increased mitochondrial GDP binding to a high level in lean mice, but only to a much lower level in obese mice (Fig. 4).

Fasting for 48 hr induced weight loss that was greater in *ob/ob* mice than in lean mice (Table III). The energy deficit was greater in the *ob/ob* mice, because if allowed to eat *ad libitum*, their food intake during the 48 hr would have been almost double that of the lean mice (Table III). Body temperature of the lean mice was decreased by fasting, whereas the low temperature of the *ob/ob* mice was not decreased any further. As expected, the serum concentrations of both T_3 and T_4 were decreased by fasting (Table III). Fasting did not alter either specific activity or total activity of T_4 5′-deiodinase in BAT in either lean or *ob/ob* mice (Table IV). The specific activity was the same in BAT of lean and *ob/ob* mice, but the total activity was greater in the obese mice because of the greater size of the BAT (Table IV). BAT mitochondrial GDP binding was decreased by fasting the lean mice, indicative of suppressed thermogenesis, but the low level of binding in the *ob/ob* mice was not further decreased by fasting.

Discussion

The exaggerated growth (increase in protein) of BAT of the *ob/ob* mouse when it is very gradually acclimated to mild cold has been seen previously by us (15) and by others (16). So has the increase in BAT mitochondrial GDP binding to the elevated level seen in cold-acclimated lean mice (15, 16), now known to be associated with a normal increase in mitochondrial UCP concentration (16). The growth is associated with an exaggerated increase in mitochondrial mass (indicated by the increase in cytochrome oxidase content) (15, 16), believed to be the principal basis for the improved thermogenic capacity of BAT of the cold-acclimated *ob/ob* mouse (16). There was, however, no evidence for any large increase in BAT T_4 5′-deiodinase activity preceding the enhanced mitochondriogenesis in the *ob/ob* mouse. We are, of course, unable to exclude a brief transient increase in activity at some time during the 6 weeks of gradual acclimation. However, when the mice were fully acclimated, the increase in total activity was only slightly larger than the increase in size of the BAT, that is, the increase in specific activity was only 1.7 times. Thus, although the impaired acute cold-induced increase in T_4 5′-deiodinase activity in BAT of the *ob/ob* mouse, seen in the present experiments as well as in our previous studies (6, 7), can account for the delay in growth of BAT (7, 16), including the selective increase in UCP concentration in the mitochondria (16), long-term adaptive growth appears to occur without major changes in T_4 5′-deiodinase activity. Moreover, the maintenance of the hypertrophied state of BAT in the cold-acclimated state, with a high level of mitochondrial protein, including an elevated level of UCP, can occur in the presence of a normal level of T_4 5′-deiodinase activity, equal to that of the warm-acclimated state, in both lean and *ob/ob* mice. Thus, long-term mechanisms involved in the control of growth and mitochondriogenesis in BAT during cold-acclimation presumably do not involve enhanced activity of T_4 5′-deiodinase in either lean or *ob/ob* mice.

In lean mice, the effect of norepinephrine to stim-

Table III. Effect of 48 hr of Fasting on Lean and *ob/ob* Mice

	Lean		Obese	
	Fed ($n = 6$)	Fasting ($n = 6$)	Fed ($n = 6$)	Fasting ($n = 6$)
Body wt (g)	25.8 ± 1.4	21.6[a] ± 0.8	82.9[b] ± 4.4	73.0[b] ± 2.3
Change in body wt in 48 hr (g)	+1.0 ± 0.3	−5.1[a] ± 0.6	+1.0 ± 0.9	−10.5[a,b] ± 2.5
Food intake in 48 hr[c] (kcal/mouse)	43.4 ± 6.0	0	77.9[b] ± 3.5	0
Temperature (°C)	36.7 ± 0.2	34.5[a] ± 0.3	33.5[b] ± 0.9	32.0[b] ± 0.5
Serum T_3 (ng/dl)	95.3 ± 4.6	53.1[a] ± 4.1	83.0 ± 5.2	49.4[a] ± 4.0
Serum T_4 (μg/dl)	2.8 ± 0.3	0.9[a] ± 0.1	2.0 ± 0.1	1.2[a] ± 0.1

[a] Significant effect of fasting comparing mice of the same type.
[b] Significant effect of obesity, comparing mice treated in the same way.
[c] Values for food intake are from measurements made with three mice per cage in each of two cages.

Table IV. Effect of Fasting for 48 hr on BAT of Lean and *ob/ob* Mice

	Lean		Obese	
	Fed ($n = 6$)	Fasting ($n = 6$)	Fed ($n = 6$)	Fasting ($n = 6$)
Wet wt (g)	0.26 ± 0.02	0.26 ± 0.02	3.63[b] ± 0.23	3.53[b] ± 0.36
Protein (mg)	10.7 ± 0.3	7.7[a] ± 0.8	23.8[b] ± 0.9	27.1[b] ± 1.4
T_4 5′-deiodinase				
Total activity (pmol/mg protein)	0.41 ± 0.05	0.32 ± 0.02	1.14[b] ± 0.24	0.84[b] ± 0.10
Sp act (pmol/mg protein/hr)	0.038 ± 0.005	0.042 ± 0.004	0.047 ± 0.009	0.031 ± 0.003
Mitochondrial GDP binding (pmol/mg protein)	77.8 ± 4.0	40.7[a] ± 2.1	32.0[b] ± 1.2	27.8[b] ± 3.4

[a] Significant effect of fasting, comparing mice of the same type.
[b] Significant effect of obesity, comparing mice treated in the same way.

ulate an increase in T_4 5′-deiodinase activity in BAT (19) and to increase the level of mRNA for UCP (25) requires synergistic actions on both α_1- and β-adrenergic receptors, and it seems likely that the two acute responses are closely coordinated, as in rats, In rats, the cold-induced increase in T_3 production by T_4 5′-deiodinase in BAT is believed to be essential for the initiation of the trophic response to cold, bringing about rapid saturation of the nuclear T_3 receptors and a facilitatory amplification of the β-adrenergic-mediated selective increase in level of mRNA for UCP (1–3). T_3 appears to exert this effect at multiple sites, including an enhancement of the initiation of transcription, an enhancement of processing of transcriptional precursors to mature mRNA and an eventual prolongation of the half-life of the mature mRNA for UCP (26). However, in rats the time course of the cold-induced increase in T_4 5′-deiodinase in BAT differs from that in the mouse. In the rat, an initial peak at about 12 hr is followed by a reduction to a level that is still about 100 times that in the basal state, and this high level is maintained for up to 28 days in the cold (9, 27). Thus, a role of continued participation of elevated T_3 production in the maintenance of the hypertrophied state of BAT seems plausible. Also, a role for a continued elevation in T_3 production in the cold-adapted rat in raising T_3 availability for other tissues is suggested by the persistence of a raised resting metabolic rate of these animals when they return to a thermoneutral environment (28, 29). In contrast, in mice during acclimation to cold, the T_4 5′-deiodinase activity of BAT peaks at 12–14 hr, returns to a level only moderately elevated above basal by 24 hr, and is down to the low level in warm-acclimated mice by 14 days (30 and present experiments). This is in keeping with the lack of increase in sympathetic nervous system activity in BAT of the mouse adapted to 16°C, in comparison with that in the mouse adapted to 26°C (18), and with the lack of increase in resting metabolic rate at thermoneutrality of cold-adapted mice (15, 31). Moreover, in mice, again in contrast to rats, the half-life of the mRNA for uncoupling protein is much reduced after 1.5 days in the cold (32), being even smaller than that of mRNA for UCP in BAT of mice returned to thermoneutrality after being cold acclimated (32, 33), which suggests a different regulation by availability of T_3 than in the rat. Although the serum T_3 level does increase in cold-acclimated mice (6, 30), it seems unlikely that this would be sufficient to saturate

BAT nuclear T_3 receptors. Thus, it is clear that the regulatory mechanisms involved in control of growth of BAT differ in rats and mice, possibly because of differences in the way in which they regulate the activity of their sympathetic nervous system in the cold.

Fasting for 48 hr did not alter the T_4 5′-deiodinase activity of BAT of lean or obese mice maintained at 28°C in our experiments. This is in contrast to the results of Wu *et al.* (20) who saw a 3-fold increase in specific activity after 48 hr of fasting and a 5-fold increase by 72 hr. These authors studied mice that weighed the same as those in the present study and weight loss over 48 hr was similar. Also, similar decreases in the concentration of T_3 and T_4 occurred in both studies. Replacement of T_4 did not, in any case, prevent the increase in T_4 5′-deiodinase (20). The different result is most probably due to a difference in housing conditions, such as use of a lower temperature than that used in the present study or to single housing of the mice (not specified in [20]). Thermoneutrality for mice is rather high (29–33°C [34]). Indeed, lean mice at the relatively mild cold temperature of 22°C show signs of cold-acclimation, having BAT with a larger thermogenic capacity with an elevated mitochondrial GDP binding and UCP concentration (35) and a higher sympathetic nervous system activity in their BAT (18). In our present experiments, we saw a substantial stimulation of food intake in mice at this temperature compared with mice living at 27°C, which indicates a stimulatory effect of this degree of mild cold. It is known that if rats are fasted at temperatures much below thermoneutrality, there is activation of BAT, as indicated by a rise in the level of mRNA for UCP, an effect that does not occur if the fasting rats are maintained at thermoneutrality (36). It seems likely that the same occurred in the experiments of Wu *et al.* (20), that is, the fasting mice became cold-stressed and, as a consequence, T_4 5′-deiodinase activity in their BAT increased, just as during the initial phase of cold exposure. Fasting is, in fact, known to suppress sympathetic nervous system activity in BAT of mice (37), and our finding of a decrease in BAT mitochondrial GDP binding in the fasting lean mice indicates that such suppression did indeed occur under the conditions of our experiment. In our experiments, the fasting mice were not only housed at a temperature close to thermoneutrality, but were also housed three per cage. It is known that mice housed in groups at 23°C are less cold stressed and have less active BAT than mice housed singly (38) and that this effect of housing density is seen even at the higher temperature of 28°C (39). Thus, we conclude that fasting in itself does not activate BAT T_4 5′-deiodinase activity of mice and that *ob/ob* mice are like lean mice in this respect.

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