Brown fat is essential for cold-induced thermogenesis but not for obesity resistance in aP2-Ucp mice

BOHUMÍR ŠTEFL, ALENA JANOVSKÁ, ZDENĚK HODNÝ, MARTIN ROSSMEISL, MILADA HORAŇOVÁ, IVO SYROVÝ, JAROSLAVA BĚMOVÁ, BĚLA BENDLOVÁ, AND JAN KOPECKÝ

Faculty of Sciences, Charles University, 128 00 Prague; Institute of Endocrinology, 116 91 Prague; and Institute of Physiology, Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic

Brown fat is essential for cold-induced thermogenesis but not for obesity in aP2-Ucp mice. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E527–E533, 1998.—The role of brown adipose tissue in total energy balance and cold-induced thermogenesis was studied. Mice expressing mitochondrial uncoupling protein 1 (UCP-1) from the fat-specific aP2 gene promoter (heterozygous and homozygous aP2-Ucp transgenic mice) and their nontransgenic C57BL/6j littermates were used. The transgenic animals are resistant to obesity induced by a high-fat diet, presumably due to ectopic synthesis of UCP-1 in white fat. These animals exhibited atrophy of brown adipose tissue, as indicated by smaller size of brown fat and reduction of its total UCP-1 and DNA contents. Norepinephrine-induced respiration (measured in pentobarbital sodium-anaesthetized animals) was decreased proportionally to the dosage of the transgene, and the homozygous (but not heterozygous) transgenic mice exhibited a reduction in their capacity to maintain body temperature in the cold. Our results indicate that the role of brown fat in cold-induced thermogenesis cannot be substituted by increased energy expenditure in other tissues.

C57BL/6j mouse strain; uncoupling protein 1; high-fat diet; adipose tissue; mitochondria

MAMMALIAN BROWN ADIPOSE tissue is a well-known site of regulatory thermogenesis, a process that is activated by cold or during arousal from hibernation. However, brown fat is also involved in control of energy balance due to the activation of thermogenesis by excessive energy intake (for references see 11). The potential for energy expenditure depends on uncoupling protein 1 (UCP-1), which increases proton conductivity of the inner mitochondrial membrane as well as heat production in brown fat mitochondria at the expense of ATP synthesis (11). Enhanced levels of this protein prevent obesity (5, 18). The dual role (above) of energy expenditure in brown fat is demonstrated in most animal models of obesity, in which brown fat is in a relatively atrophic state (for references see 11). Genetic ablation of brown fat induced obesity and hyperphagia in mice and reduced their ability to maintain body temperature in the cold (22). However, development of obesity and hyperphagia were eliminated in a thermoneutral environment (24). These experiments indicated (12, 22, 24) that brown fat may also be involved in the control of energy intake.

Recent studies suggested (6, 30) that UCP-1-mediated thermogenesis in brown fat was essential for protection of the organism against cold but not for maintenance of body weight. It was shown that mice lacking UCP-1 (6) or mice that could not induce UCP-1 gene expression due to inactivation of dopamine β-hydroxylase (30) were cold sensitive but not obese. In the first case, the compensatory mechanism could involve (6) the uncoupling protein 2 (UCP-2), the recently described homologue (6, 8, 9) of the brown fat-specific UCP-1. In contrast to the UCP-1 gene, the UCP-2 gene is expressed in various tissues, with the highest expression being found in white fat (6, 8, 9). UCP-2 is very likely involved in the control of total energy balance and adiposity (6, 8, 9) through its expression in lipid-containing cells, which is under control of the adipocyte hormone leptin (27).

Another candidate is uncoupling protein 3 (UCP-3), the most recently described (2, 31) homologue of UCP-1. UCP-3 may regulate thermogenesis in specific tissues such as skeletal muscle and brown fat (in the latter tissue only in rodents and not in humans).

Despite the existence of a variety of metabolic pathways for nonshivering thermogenesis (17), the discovery of the UCP family (see above) strengthened the concept (3) that the efficiency of mitochondrial energetics in various tissues may be a major variable involved in the control of energy expenditure. However, a further insight is required into the control mechanisms that contribute to energy expenditure of individual tissues via the UCP-dependent thermogenesis. Therefore, experiments have been performed using transgenic mice expressing the UCP-1 gene from the aP2 gene promoter (aP2-Ucp mice) in brown and white fat (18–20). aP2-Ucp mice are partially resistant to obesity induced by genetic (18), dietary (19, 20), or age-related (18) factors or by gold thioglucose (Kopecky et al., unpublished observations). However, indirect evidence suggested partial involution of brown fat in the heterozygous transgenic animals (20). In this report, in addition to the nontransgenic and the heterozygous transgenic mice studied before (18–20), homozygous transgenic mice were also included. Biochemical analysis and indirect calorimetry indicated a reduction of brown fat-mediated thermogenesis in the transgenic mice. Due to the drastic reduction of brown fat, the homozygous (but not the heterozygous) animals became cold sensitive. Our results supported the view (6, 30) that cold-induced thermogenesis in brown fat could...
not be substituted by energy expenditure in other tissues.

**MATERIALS AND METHODS**

Animals. C57BL/6j male mice with aP2-Ucp transgene (copy no. 7; see Refs. 18 and 19) and their nontransgenic littermate controls were identified by Southern blot analysis as described before (18). The mice were housed 4–5 per cage (unless noted otherwise; see below) and maintained under a 12:12-h light-dark cycle (light starting at 6 AM) at 20°C. After weaning at 4 wk of age, the animals were given ad libitum water and standard mouse chow diet. At 3 mo of age, some transgenic and nontransgenic animals were assigned to a high-fat (HF) diet; the other mice were fed chow diet. The composition of the diets was as described before (19). The animals were kept on the specified diet until measurements of energy expenditure at 5–11 mo of age. Mice were caged singly, one day before the experiment on the effect of acute exposure to 4°C (Fig. 2) or from 3 mo of age for determination of the individual food intake (Table 1) and the effect of a 4-wk adaptation to cold (the adaptation started with 5-mo-old mice at 15°C for the 1st wk and continued for 3 wk at 6°C; 5–6 animals in each subgroup).

Indirect calorimetry measurements. Energy expenditure was measured by an open-circuit indirect calorimetry using a breathing mask in a thermostated (32°C) metabolic chamber (1 100-ml volume), and an oxygen analyzer (Spirolyt; Junkalor, Dessau, Germany). In some experiments (see RESULTS), basal metabolic rate was measured in awake, unrestrained animals after overnight fasting. All other experiments were performed with pentobarbital sodium-anesthetized mice (100 mg/kg). Minimum metabolic rate (MMR) was estimated during the first 20 min after the induction of anesthesia. The mouse was then injected intraperitoneally with L-norepinephrine D-bi-

Biochemical techniques. Interscapular brown adipose tissue was dissected from the animals killed by cervical dislocation, and tissue homogenate was prepared using a glass–glass homogenizer in a medium containing 10 mM tris(hydroxy-methyl)aminomethane (Tris)-Cl and 2 mM EDTA, pH 7.4. Protein concentration and the content of UCP-1 antigen in the whole tissue homogenate (after the removal of material sedimented at 500 g, during 5 min of centrifugation) were estimated as described previously (20). DNA in brown fat was measured in fasted mice as described before (20), except that, rather than using the tissue homogenate, 5-µg tissue fragments were digested (at 56°C, overnight) in 75 µl of a medium containing 10 mM Tris-Cl, 5 mM EDTA, 0.5% sodium deoxyse sulfate, and protease K at a concentration of 50 µg/ml (and 15-µl aliquots of the digest were taken for the fluorometry, in a final volume of 1.5 ml). This modification resulted in 1.5-fold higher values of the DNA than before (20). Plasma concentrations of the total and free triiodothyronine (T3) have been measured in fasted mice (19) by competitive radioimmunoassay (using RIA kit for human serum; Immunotech, Prague, Czech Republic; the recovery of added T3 was between 93 and 105%).

RNA analysis. Total RNA was isolated (4) from adipose tissue samples, and UCP-2 mRNA was analyzed on Northern blots (20) using the full-length UCP-2 gene cDNA (the 1,180-base pair EcoR I restriction fragment derived from mouse UCP-2 gene cDNA in the pCR2.1 vector; a gift from Dr. Bradford B. Lowell, Harvard Medical School, Boston, MA); UCP-1 was analyzed as before (18, 20). Radioactivity was evaluated by PhosphorImager SF (Molecular Dynamics). The blots were rehybridized with the 1,200-base pair Pst I restriction fragment of rat glyceraldehyde 3-phosphate dehydroge-

**RESULTS**

Effect of transgenic dosage on total energy balance and brown fat. It has been demonstrated previously (19)

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**Table 1.** Body weight, cumulative energy intake, feed efficiency, and brown fat in 7-mo-old mice

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Cumulative Energy Intake, kcal</th>
<th>Feed Eff., g body wt gain/100 kcal</th>
<th>Brown Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 17</td>
<td>Difference</td>
<td>Week 0</td>
</tr>
<tr>
<td><strong>HF diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>25.3 ± 0.4</td>
<td>48.5 ± 1.3</td>
<td>23.2 ± 1.3</td>
<td>1.82 ± 0.02</td>
</tr>
<tr>
<td>tg/–</td>
<td>25.0 ± 0.7</td>
<td>36.4 ± 0.8*</td>
<td>11.5 ± 0.8*</td>
<td>1.80 ± 0.73</td>
</tr>
<tr>
<td>tgg/tg</td>
<td>23.2 ± 0.9</td>
<td>30.6 ± 1.2†‡</td>
<td>7.5 ± 1.5†‡</td>
<td>1.69 ± 3.6</td>
</tr>
<tr>
<td><strong>Chow diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>25.2 ± 0.7</td>
<td>31.1 ± 1.7</td>
<td>5.9 ± 1.1</td>
<td>1.75 ± 0.53</td>
</tr>
<tr>
<td>tg/+</td>
<td>25.5 ± 0.9</td>
<td>29.6 ± 1.5</td>
<td>4.1 ± 1.0</td>
<td>1.75 ± 0.29</td>
</tr>
<tr>
<td>tgg/tg</td>
<td>23.5 ± 0.8</td>
<td>27.0 ± 0.7†‡</td>
<td>3.6 ± 0.7†‡</td>
<td>1.80 ± 0.99</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>tg/–</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>tgg/tg</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5–6 mice. UCP, uncoupling protein. At 3 months of age (week 0), subgroups of nontransgenic (+/+) and heterozygous (tg/–) or homozgyous (tgg/tg) aP2-Ucp transgenic mice were assigned high-fat (HF) or chow diet. Individual food intake and feed efficiency (Feed Eff.) were measured over 17 wk of experiment, as described before (19). Interscapular brown fat was dissected and characterized as specified in MATERIALS AND METHODS. Significant differences between subgroups, within each type of diet and column, are indicated: * difference between +/+ and tg/–; † difference between +/+ and tgg/tg; ‡ difference between tg/– and tgg/tg. P value, significance of post hoc test of the effect of diet within each genotype. NS, not significant.
that mice heterozygous for the aP2-Ucp transgene accumulated less body fat than the nontransgenic mice when fed HF diet for 6 mo, despite similar energy intake in both genotypes. To further enhance the effect of the transgene, the homozygous transgenic mice have also been studied (Table 1). In mice fed a chow diet, there was only a marginal effect of the transgene on body weight gain (>4 mo of the feeding experiment), with a smaller gain in transgenic vs. nontransgenic mice. Furthermore, there was no significant difference between the heterozygous and homozygous animals. On the other hand, development of obesity in mice fed a HF diet (see also Ref. 19) was reduced proportionally to the dosage of the transgene, with the greatest reduction observed in the homozygous animals. Because cumulative energy intake was similarly high in all the subgroups (Table 1), feed efficiency was higher in the HF compared with the chow diet fed mice. Under both feeding conditions, feed efficiency was inversely related to the dosage of the transgene. Most prominent was the difference in the feed efficiency between the nontransgenic and the heterozygous transgenic mice fed HF diet.

In accordance with the other data (19, 20), transgene-induced involution of interscapular brown fat was detected. Total weight of brown fat and its protein, DNA, and UCP-1 contents (Table 1) was drastically reduced in homozygous (compared with both heterozygous or nontransgenic) animals, under both sets of dietary conditions. In the chow diet-fed mice, no differences could be detected between the heterozygous transgenic and nontransgenic mice (except for a difference in DNA; see also Ref. 20). In the HF diet-fed mice, differences have been observed even between the nontransgenic and the heterozygous mice, reflecting the induction of brown fat thermogenesis in nontransgenic mice fed the HF diet (see Ref. 20). As the specific UCP-1 content (referred to total brown fat weight, protein, or DNA, respectively) was quite similar in all subgroups, the relative atrophy of brown fat in the transgenic mice reflects a decrease of brown fat cellularity (see DISCUSSION). Importantly, results indicated that the resistance to obesity induced by the transgene did not correlate with the capacity for thermogenesis in brown fat.

Energy expenditure in HF and chow diet-fed mice. Energy expenditure has been studied using indirect calorimetry in anesthetized animals (see MATERIALS AND METHODS). In these measurements, the MMR values could reflect differences in whole body energy expenditure due to diet, cold adaptation, or pharmacological treatment (14, 16), whereas the specific capacity for nonshivering thermogenesis in brown fat could be assessed from the stimulatory effect of norepinephrine on oxygen uptake (i.e., NEMR values; see Refs. 17 and 28). Measurements were performed during the early and advanced stages of development of the HF diet-induced obesity (19), in 5- and 11-mo-old mice, respectively (Table 2; for the chow diet-fed mice, see also Fig. 1). Only small differences in body weight could be detected among the younger animals of all three genotypes, with marginal reduction of body weight in the transgenic chow diet-fed mice and a partial suppression of developing obesity in the HF diet-fed transgenic animals (see also Table 1). In the older nontransgenic animals, large dietary obesity developed, which was eliminated completely in the heterozygous animals (the homozygous transgenic mice were not included in this age group).

The magnitude of MMR (Table 2) was similarly high in all subgroups of younger mice. In older nontransgenic animals, MMR was higher in the HF diet compared with the chow diet-fed mice. Such a difference in older animals may be related to a larger body mass of the HF vs. chow diet-fed nontransgenic animals. In mice fed a chow diet, a stimulation of MMR by the transgene was apparent (Table 2; see also below).

In animals of both age groups, and within each set of dietary conditions, NEMR was suppressed by the transgene (Table 2), with the maximum decrease in homozygous animals fed a HF diet (Table 2; the 5-mo-old mice). Also in chow diet-fed mice (10 mo old, experiment not shown), a 1.8-fold lower NEMR was observed in the homozygous vs. heterozygous transgenic mice (19.5 ± 8.3 vs. 34.8 ± 7.9 ml O$_2$/h per animal; n = 4; P < 0.05). This suppression of NEMR is in agreement with the atrophy of brown fat in the transgenic mice (see above). Stimulation of NEMR by HF diet in nontransgenic (but not transgenic) animals was also noticed (Table 2).
which apparently reflected the HF diet-induced proliferation of brown fat in the nontransgenic mice (see also Table 1). Such a stimulation, which was observed in both age groups, resulted in the highest metabolic rate in the HF diet-fed nontransgenic animals (Table 2).

Metabolic rate during aging. With regard to similar energy intake of the transgenic and the nontransgenic mice, resistance to developing obesity of the former should be associated with increased metabolic rate. Only a minute difference in metabolic rate is to be expected between the nontransgenic and transgenic mice and may not be easy to detect (see DISCUSSION). When basal metabolic rates were measured in awake, unrestrained animals (see MATERIALS AND METHODS), no consistent differences between the two genotypes was observed (not shown; see DISCUSSION). Measurements of MMR in the chow diet-fed 5- and 11-mo-old mice (Table 2) indicated a marginal (but not significant) increase in the heterozygous vs. nontransgenic mice. Therefore, the measurements of MMR in chow diet-fed mice (Table 2) have been extended to include more age groups of the nontransgenic and the heterozygous transgenic mice (Fig. 1A). The MMR varied with age (see also Ref. 29), with the older (>6-mo-old) animals showing the higher values. As before (Table 2), the analysis of the additional age groups (Fig. 1A) has revealed subtle but not significant differences between the two genotypes, suggesting a higher resting metabolism in the transgenic mice. All the NEMR values indicate a relative atrophy of brown fat in the transgenic mice (Fig. 1B).

Response to cold. To further characterize the effect of the transgene on energy expenditure and brown adipose tissue, the effect of a 4-wk acclimatization to cold (see MATERIALS AND METHODS) has been studied in the chow diet-fed mice. Because the homozygous animals appeared extremely cold sensitive (see below), only the nontransgenic and heterozygous transgenic mice could be compared. The gain of body weight during the 4-wk acclimatization period (0.7 ± 0.5 and 1.1 ± 0.2 g in the nontransgenic and the transgenic mice) was significantly lower compared with the animals kept at 20°C (5.7 ± 0.2 and 5.4 ± 0.9 g in the nontransgenic and the transgenic mice), but no significant effect of the genotype was noticed. The increase of MMR (see Fig. 1 for MMR in the 6-mo-old animals) was similar in both genotypes in cold (1.2- and 1.1-fold, in the nontransgenic and the transgenic animals, respectively), as was the corresponding increase in the NEMR (1.2- and 1.1-fold, respectively).

Further study was focused on the effect of acute cold exposure (transfer from 20 to 4°C; Fig. 2), at which all three genotypes could be compared. The effect was similar in the nontransgenic and in heterozygous transgenic mice, as judged from a similar time course of the decline of deep body temperature. However, the homozygous transgenic mice were much less cold tolerant (Fig. 2), and the fast drop in their body temperature did not allow prolongation of the experiment over 3 h. In fact, even at 20°C, at the beginning of the experiment, body temperature of the homozygous mice was significantly lower (0.8–1.0°C) compared with that of the other two groups of mice, which had similar temperature (Fig. 2). Thus, in accordance with data showing atrophy of brown fat (Table 1) and reduced NEMR (Table 2), the homozygous transgenic mice appeared to lose their capacity to maintain body temperature in cold.
Thyroid status. Because thyroid hormones affect metabolic rate and thermogenesis and because hormone levels are modulated by feeding (for references see 25) and because brown fat is involved in control of plasma T3 in rodents (7, 21), plasma levels of total and free T3 were measured in developing dietary obesity (Table 3). In 7- but not in 5-mo-old mice fed the HF diet since 3 mo of age, higher levels of total T3 were found compared with the chow-fed mice. However, no other significant differences, and namely no differences induced by the transgene, have been observed.

Expression of UCP-2 mRNA in adipose tissue. We also tested if the expression of UCP-1 gene from the aP2-gene promoter could affect the UCP-2 gene expression in brown and white fat (see also Ref. 6). Any change in UCP-2 expression might influence adiposity (9, 15). In accordance with the previously published data, UCP-1 transcript could be detected only in white fat of the transgenic and not in the nontransgenic mice (Fig. 3A; see Refs. 18 and 20), and UCP-2 expression was significantly higher in white compared with brown fat (Fig. 3B; see Refs. 6 and 8). However, in both brown and white fat, no significant effect of the transgene on UCP-2 gene expression could be detected.

DISCUSSION

Previously published data (20) on brown fat morphology and respirometry with tissue fragments suggested functional involution of brown fat in aP2-Ucp transgenic mice. To verify this interesting effect of the expression of UCP-1 from the fat-specific aP2 promoter, further experiments have been performed in which the dosage of the transgene was a new variable. The size of brown fat and its UCP-1 content, the NEMR values, and the cold sensitivity of the homozygous transgenic mice all clearly indicate a transgene-induced atrophy of brown fat. However, despite the fact that NEMR was decreased proportionally to the dosage of the transgene under both dietary conditions, only in the animals fed the HF diet was this decrease reflected in the level of UCP-1 antigen. In the chow diet-fed mice, the UCP-1 antigen content in brown fat was strongly suppressed.

Table 3. Plasma triiodothyronine in 5- and 7-mo-old mice

<table>
<thead>
<tr>
<th></th>
<th>Week 9</th>
<th>Week 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total T3, nM</td>
<td>Total T3, nM</td>
</tr>
<tr>
<td>HF diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>1.31 ± 0.27</td>
<td>1.47 ± 0.11</td>
</tr>
<tr>
<td>tg+/+</td>
<td>1.06 ± 0.25</td>
<td>1.36 ± 0.19</td>
</tr>
<tr>
<td>tg/tg</td>
<td>1.46 ± 0.33</td>
<td>1.85 ± 0.17</td>
</tr>
<tr>
<td>Chow diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>0.98 ± 0.16</td>
<td>0.71 ± 0.16</td>
</tr>
<tr>
<td>tg+/+</td>
<td>1.10 ± 0.12</td>
<td>0.80 ± 0.13</td>
</tr>
<tr>
<td>tg/tg</td>
<td>1.09 ± 0.25</td>
<td>0.77 ± 0.19</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>tg+/+</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>tg/tg</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE for mice used in experiment described in Table 1 (see legend of Table 1 for details). Plasma concentrations were estimated at 9 and 17 wk, respectively, from the beginning of feeding experiment. T3, triiodothyronine.

Fig. 3. Northern blot analysis of gene expression in adipose tissue of 7-mo-old chow diet-fed mice. A: total RNA was extracted from epididymal white fat of nontransgenic (lanes 1 and 4) and transgenic (lanes 2 and 3) mice. Detection of transcripts of uncoupling protein 2 [UCP-2; 1.7 kilobase (kb)], glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1.6 kb), and UCP-1 (1.4 kb) was performed as described in MATERIALS AND METHODS. Representative blot is shown. B: quantity of UCP-2 mRNA was estimated as described in MATERIALS AND METHODS in total RNA from brown fat (BAT) and epididymal fat (WAT) of nontransgenic (open bars) and transgenic (filled bars) mice (see also A) and expressed relative to standard RNA. Values are means ± SE (n = 5–6 mice; see Table 1).
only in the homozygous transgenic mice, resulting in cold sensitivity (see also Refs. 6, 22, and 30). It was surprising that, in the chow diet-fed heterozygous vs. nontransgenic mice, the stimulation of metabolic rate by norepinephrine was blunted despite similar 1) increase in the capacity for NEMR by cold adaptation, 2) body temperatures during cold exposure, and 3) UCP-1 content in brown fat. It is known, however, that the activity of UCP-1 is not always proportional to the amount of expressed protein [due to the phenomenon of “masking/unmasking” (for references see 26)].

The mechanism of brown adipose tissue involution remains unknown. Atrophy reflects lower cellularity, and not a lower specific content of UCP-1, in brown adipocytes (see RESULTS). In fact, the levels of UCP-1 transcript per microgram of total brown fat RNA were higher rather than lower in the transgenic mice, due to the overexpression of UCP-1 from the transgene (despite the fact that expression of endogenous UCP-1 was strongly suppressed; see Refs. 18 and 20). Therefore, it is not likely that atrophy of brown fat is a compensatory mechanism for increased thermogenesis in white fat. Furthermore, the content of the ectopic UCP-1 synthesized in white fat of adult heterozygous transgenic mice is about two orders of magnitude lower than in brown adipose tissue of nontransgenic mice (20), and even in homozygous mice the content of the ectopic UCP-1 is no more than twofold higher compared with the heterozygous animals (not shown). Therefore, it is likely that atrophy reflects excessive expression of UCP-1 from the transgene, which results in a collapse of energy metabolism in proliferating brown preadipocytes, leading to either inhibition of the proliferation or cell death. At present, these possibilities are under investigation.

Atrophy of brown fat should interfere with the participation of this tissue in diet- and cold-induced thermogenesis or even control of food intake (see the introduction). However, in contrast to mice ablated of brown fat [due to the expression of the diptheria toxin A chain from the UCP-1 gene promoter (Ref. 22)], ap2-Ucp mice were neither obese nor hyperphagic. A similar feature in both types of mice was that reduction of brown fat resulted in cold sensitivity. A similar phenotype to that of the ap2-Ucp mice could be observed in two other models described recently, i.e., in mice with an inactivated UCP-1 gene (6) and mice that, due to inactivation of dopamine β-hydroxylase, could not induce UCP-1 gene expression (30). As with the ap2-Ucp animals, these two types of mice are cold sensitive but not obese. Like the mice with the ablated brown fat (22), mice with inactivated dopamine β-hydroxylase are also hyperphagic (30). Comparison of all four models described above clearly indicates that cold-induced thermogenesis in brown fat cannot be substituted by other tissues.

Mechanisms for expenditure of energy in tissues other than brown fat (17) may also be stimulated by cold (1, 23), but in the absence of brown fat their capacity may not be sufficient for maintenance of constant body temperature. These mechanisms could involve the recently discovered UCP-2 (6, 8, 9) and UCP-3 (2, 31), which may regulate metabolic rate by controlling the efficiency of mitochondrial energy conversion (3) in a broad range of tissues (UCP-2), or, in a tissue-specific manner, in muscle and brown fat (UCP-3). Cold-induced thermogenesis may depend critically on the norepinephrine stimulation of the β3-adrenergic receptors, which are present predominantly in white and brown adipose tissue but also in the gastrointestinal tract (for references see 28). In the absence of brown fat, the main physiological target would be UCP-2 in white fat, where its gene is mainly expressed (6, 8, 9). However, the capacity of UCP-2-based thermogenesis in the cold would be much lower than that of endogenous UCP-1 in brown fat. In fact, white adipose tissue is known to contribute 5–10% to total energy expenditure (29), i.e., much less than brown fat (Ref. 17) [note also that metabolic rate correlates better with lean body mass than total body weight (Ref. 13)]. Similarly, the relatively low content of the ectopic UCP-1 in white fat of the ap2-Ucp mice (see above) may reduce obesity but may not be sufficiently high to replace thermogenesis mediated by endogenous UCP-1 during cold exposure. Moreover, the activity of ectopic UCP-1 synthesized from the ap2 gene promoter in white fat of the transgenic mice is probably not stimulated by cold, at least at the level of the transgene expression (18).

With regard to the equal food intake of the nontransgenic and transgenic mice fed the two different diets (Table 1), assuming that the only effect of ectopic UCP-1 in adipose tissue is increased energy expenditure, it can be calculated that the reduction in the gain of body weight (which results from a difference in adiposity (see Ref. 20)) in the transgenic vs. nontransgenic mice during 5-mo feeding of the HF diet (Table 1; see also Ref. 19) corresponds to a difference in total metabolic rate of ~1.5 ml O2 per hour per animal. Such a difference, which is difficult to detect due to errors in the measurements, may be reflected by the marginal elevation of MMR measured in anesthetized transgenic animals (Table 2 and Fig. 1). However, it is evident that only minute changes of energy expenditure in white fat (induced by any UCP) may be required, in the long run, for affecting body weight and adiposity.

In the awake animals, an effect of the transgene on basal metabolic rates could not be demonstrated. Paradoxically, in awake mice at 20°C, the deep body temperature (as measured in the morning; see legend to Fig. 2) dropped proportionally to the dosage of the transgene, suggesting a possible effect of the transgene on circadian changes in energy expenditure (10). It also remains to be determined if mechanisms that affect metabolic rate in awake animals, such as activity of the adrenergic system, muscle tonus, motor activity, and transient thermic effect of food, can contribute to the obesity resistance of the ap2-Ucp mice.

In conclusion, this report documents relative atrophy of brown fat and its thermogenic function in ap2-Ucp transgenic mice. Brown adipose tissue appeared to be essential for protecting the organism against cold, even when the thermogenic potential in other tissues is sufficiently high to reduce developing obesity.
REFERENCES


