Raising at thermoneutrality prevents obesity and hyperphagia in BAT-ablated transgenic mice

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Melnyk, Anna, Mary-Ellen Harper, and Jean Himms-Hagen. Raising at thermoneutrality prevents obesity and hyperphagia in BAT-ablated transgenic mice. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R1088–R1093, 1997.—Transgenic mice with ablation of brown adipocytes induced by brown adipocyte-specific expression of diphtheria toxin A chain (DTA) driven by the uncoupling protein (UCP) promoter (UCP-DTA mice) become obese and hyperphagic (Lowell, B. B., V. S. Susulic, A. Hamann, J. A. Lawitts, J. Himms-Hagen, B. B. Boyer, L. P. Kozak, and J. S. Flier. *Nature* 366: 740–742, 1993). A deficit in energy expenditure for brown adipose tissue (BAT) thermogenesis in these mice is presumed to contribute to the development of obesity. The objective of the present study was to obviate any deficit in BAT thermogenesis by raising transgenic and control mice at thermoneutrality (35°C), where both would have equally inactive BAT, to see whether this would prevent the obesity and the hyperphagia. Transgenic and control mice were raised from weaning (3 wk of age) to 8 wk of age at either 24 or 35°C. Raising at 35°C completely prevented development of obesity of UCP-DTA mice, as indicated by their normal carcass fat, normal weights of four major white adipose tissue depots, and normal size of white adipocytes. As seen before, transgenic mice raised at 24°C had excess weight gain by 6 wk of age and by 8 wk had doubled carcass fat, an obesity characterized by increased white adipocyte size with no increase in number of adipocytes. The treatment also prevented hyperphagia of UCP-DTA mice, consistent with the hypothesized role of BAT thermogenesis in control of thermoregulatory feeding (Himms-Hagen, J. *Proc. Soc. Exp. Biol. Med.* 208: 159–169, 1995). UCP-DTA mice thus differ from genetically obese mice (*ob/ob*, *db/db*) for which raising at thermoneutrality is known not to prevent either the obesity or the hyperphagia. Both the obesity and the hyperphagia of UCP-DTA mice appear to be due to their deficit in BAT thermogenesis.

uncoupling protein; energy expenditure; food intake; temperature; thermoregulation; brown adipose tissue

ASSOCIATION OF OBESITY with atrophy of brown adipose tissue (BAT) in laboratory rodents has been recognized for many years (9, 10), and BAT thermogenesis is thought to play an important role in regulation of energy balance (20). However, the hypothesis that a deficit in energy expenditure due to a low level of thermogenesis in BAT might contribute to the development of obesity has remained unproven. Convincing evidence for this hypothesis has now been provided by the creation of a transgenic mouse with partial ablation of brown adipocytes (16), achieved by using regulatory elements of the uncoupling protein gene (UCP) expressed uniquely in brown adipocytes, to drive expression of diphtheria toxin A chain (DTA). UCP-DTA mice have been found to become obese at an early age, later becoming also hyperphagic (16). Eventually they become massively obese and have the insulin resistance, hyperglycemia, hyperinsulinemia, and hyperlipidemia characteristic of noninsulin-dependent diabetes mellitus (7, 8). When fed a high-fat diet, they remain hyperphagic and exhibit the usual increased efficiency of deposition of body fat characteristic of animals eating such a diet (8). Similar to most other animal models of obesity, they overexpress tumor necrosis factor-α (7) and leptin (6) in their white adipose tissue (WAT). However, in contrast to other genetically obese mice, such as *ob/ob* and *db/db*, they do not exhibit hypercorticosteronemia, are not stunted, and are fertile (6, 16, 17).

That development of obesity would ensue when BAT was ablated was predicted by the hypothesis that the mice would suffer from a deficit in energy expenditure (16). However, hyperphagia was an unexpected consequence of the lack of BAT and suggested a role for BAT in control of food intake. A hypothesis for a regulatory role of BAT thermogenesis in control of meal size in animals living at temperatures at which BAT thermogenesis is required for thermoregulation has now been developed, based on the hyperphagia of the UCP-DTA mice and other evidence from the literature (11, 12). The hypothesis predicts a failure to terminate meals normally, hence overeating, when BAT function is impaired.

To find out whether the development of both obesity and hyperphagia in the UCP-DTA transgenic mouse was indeed due to lack of BAT thermogenesis and not to some other impairment, we raised mice under conditions in which BAT thermogenesis would be expected to be virtually absent, that is, at thermoneutrality. When normal mice live at the environmental temperatures usually used for rodents in animal care facilities (21–24°C) they are partially cold-acclimated. Sympathetic nervous system activity in their BAT is much higher than in mice living at 35°C (18, 22), and their BAT has a much greater content of UCP than that of mice living at thermoneutral environmental temperature (between 32–35°C; Ref. 1). Thus it was predicted that removal of the external stimulus of cold to BAT thermogenesis by raising the UCP-DTA transgenic mice at thermoneutrality would annul the deficit in BAT thermogenesis that occurs when the mice are raised at 21–24°C and would prevent both the development of the obesity and the hyperphagia.

METHODS

Animals. A colony of UCP-DTA mice was established with six female UCP-DTA mice (provided by Dr. Bradford B. Lowell, Beth Israel Hospital and Harvard Medical School, Boston, MA) and six male FVB/N mice (from Taconic, Germantown, NY). Breeding pairs were housed at 24°C with free access to food (Agway R-M-H 4020 chow, 14.5% of energy from

fat) and water and lights on for 12 h/day. Some male mice were killed at 3 wk of age. At weaning (at exactly 3 wk of age) other male mice were separated into groups of the same genotype and housed at 24°C or in a transparent incubator maintained at 35°C in the same room for the next 5 wk (usually 3 mice per cage, although occasionally a mouse was alone in a cage for a short time until other mice in the colony were weaned). Body weights and food intake were measured weekly. These mice were killed at 8 wk of age.

Body composition and tissue weights. Mice were killed by cervical dislocation and exsanguination. Interscapular BAT and four WAT depots (epididymal, mesenteric, retroperitoneal, inguinal) were removed and weighed. Samples of inguinal WAT were fixed in a solution of osmium tetroxide for later analysis of size and number of white adipocytes (14). Remaining tissues were frozen for later analysis.

Carcasses were autoclaved, then homogenized in water in a Waring heavy-duty commercial blender. Lipid content of samples was measured after Soxhlet extraction with petroleum ether. Lipid content of the WAT depots removed was assumed to be 90% of the wet weight, and this amount was added to the estimate of carcass fat.

Serum assays. Cholesterol and triacylglycerol were measured by enzymatic assays using Sigma diagnostic kits as before (16).

Tissue assays. Protein, DNA, and cytochrome oxidase were measured in tissue homogenates as before (13). UCP was measured by a solid-phase radioimmunoassay as before (13) with the use of mouse UCP as a standard. The UCP/cytochrome oxidase ratio ($\mu g \cdot \mu mol^{-1} \cdot min^{-1}$) was calculated as a measure of the concentration of UCP in BAT mitochondria. Total number of cells in WAT was calculated from the DNA content (7 pg DNA per nucleus). DNA content measures all cell types, not only mature white adipocytes but also vascular cells, interstitial cells, and preadipocytes.

Statistical analysis. All data are expressed as means ± SE. Instat software was used for analysis of variance followed by Tukey-Kramer post hoc tests.

RESULTS

Body weights and food intake. Weight gain was similar in control and transgenic mice up to 5 wk of age when they were raised at the usual animal facility temperature of 24°C, but body weights of transgenic mice exceeded those of control mice after this age (Fig. 1*A*). Raising at 35°C did not impair growth of control mice (Fig. 1*A*), and weight gain of transgenic mice raised at 35°C paralleled that of control mice at this same temperature during the entire study period (Fig. 1*A*). Food intake was similar in control and transgenic mice up to 4 wk of age when they were raised at 24°C, but food intake of transgenic mice exceeded that of control mice after this age (Fig. 1*B*). Housing at 35°C reduced food intake of control mice to 50% of that eaten by control mice at 24°C (Fig. 1*B*). Transgenic mice housed at 35°C ate the same low amount as control mice at this temperature, only 40% of that eaten by the hyperphagic transgenic mice at 24°C (Fig. 1*B*).

Development of obesity. Carcass analysis showed the expected development of obesity in the 8-wk-old transgenic mice raised at 24°C, with a doubling of carcass fat content (Fig. 2*A*). Transgenic mice were not obese at 3 wk of age (Fig. 2*A*). Raising transgenic mice at 35°C completely prevented the development of obesity at 8 wk of age (Fig. 2*A*). A normal age-associated increase in body fat occurred in both control and transgenic mice raised at 35°C (Fig. 2*A*). Weights of all four WAT depots studied were much greater in the 8-wk-old transgenic mice raised at 24°C than in 8-wk-old control mice at this temperature (data for inguinal WAT shown in Fig. 2*B*). Raising transgenic mice at 35°C completely prevented the exaggerated increase in weight of all four WAT depots between 3 and 8 wk of age (data for inguinal WAT shown in Fig. 2*B*).

Adipocyte number was normal but adipocyte size was increased in inguinal WAT of transgenic mice at 24°C; they had a hypertrophic obesity (Fig. 3). The increase in size of the adipocytes was prevented in transgenic mice raised at 35°C (Fig. 3*B*). (Adipocyte size and number could not be assessed in the 3-wk-old mice because not

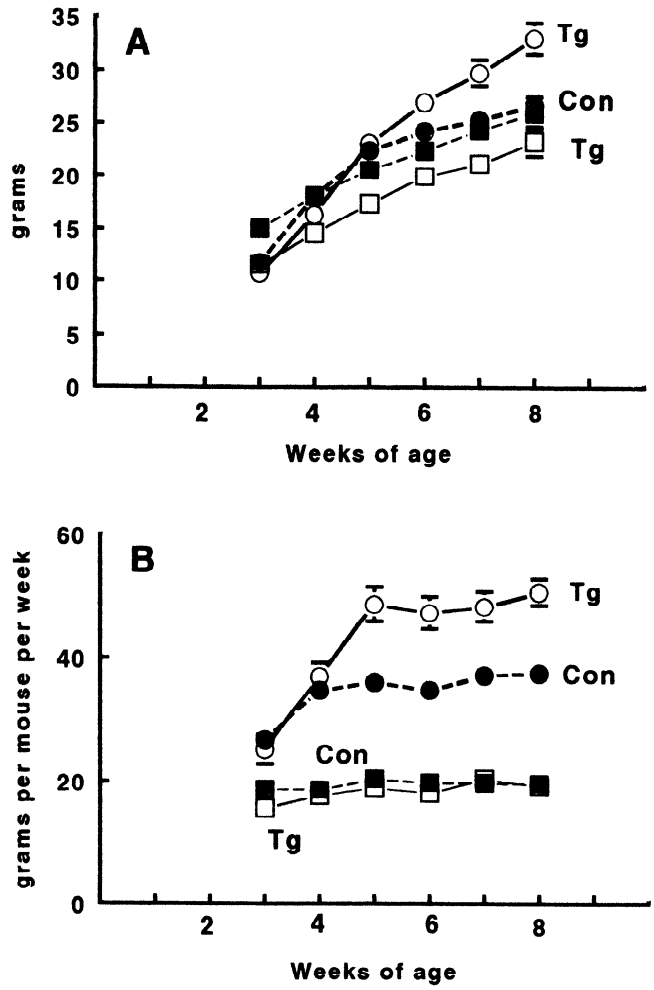

Fig. 1. Growth and food intake of mice from 3 to 8 wk of age. Solid symbols, control (Con) mice; open symbols, transgenic (Tg) mice. Mice at 24°C, circles; mice at 35°C, squares. Numbers of mice: control at 24°C, 18; transgenic at 24°C, 16; control at 35°C, 14; transgenic at 35°C, 11. *A*: means ± SE for weekly body weights. *B*: weekly food intake for these same mice. Body weights of mice killed at 3 wk of age [not shown here, but included in Figs. 2–6 were control 11.9 ± 0.55 g (n = 10) and transgenic 9.0 ± 0.41 g (n = 10)]. Body weights of transgenic mice at 24°C are significantly greater than those of control mice at 24°C at 6 wk and older. Food intake of transgenic mice at 24°C is significantly greater than that of control mice at 24°C from 5 wk of age. There are no significant differences between control and transgenic mice at 35°C.

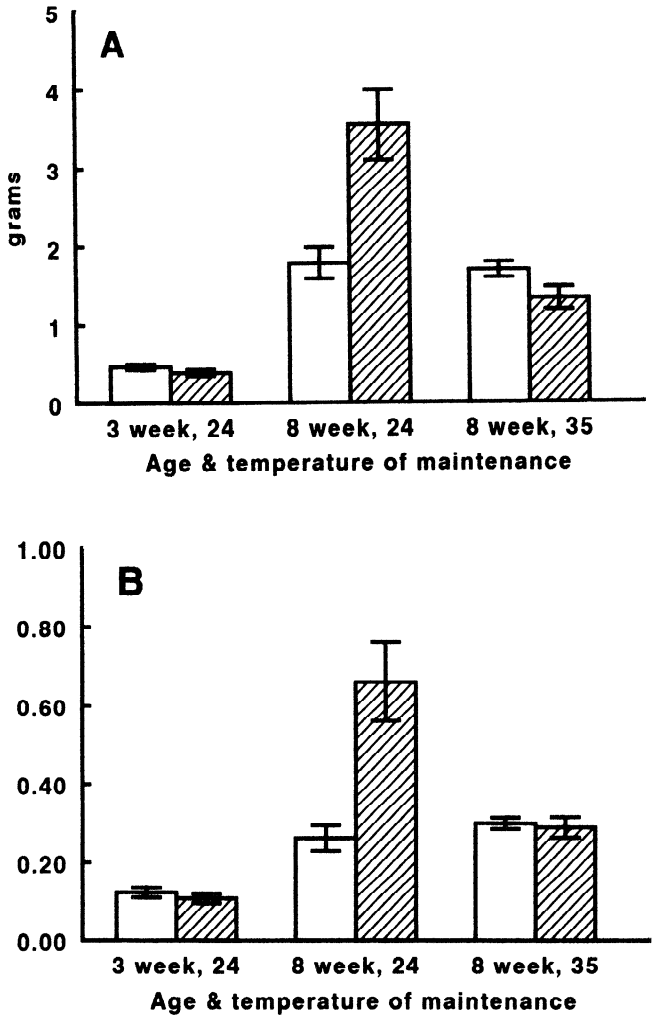

Fig. 2. Carcass fat weight and inguinal white adipose tissue (WAT) weight. Control mice, open bars; transgenic mice, hatched bars. For numbers of mice, see Fig. 1 legend. *A*: total carcass fat. *B*: weight of inguinal WAT. Total carcass fat is significantly greater in 8-wk-old transgenic mice at 24°C than in 8-wk-old control mice at 24°C ($P < 0.001$). There are no other significant differences between control and transgenic mice at the same age and temperature. Body fat increased with age (8 vs. 3 wk) and was not influenced by temperature in control mice. Body fat also increased with age in transgenic mice, but was significantly lower in transgenic mice at 35°C than in transgenic mice at 24°C. Inguinal WAT weighed significantly more in 8-wk-old transgenic mice at 24°C than in 8-wk-old control mice at 24°C ($P < 0.001$). Weights for 8-wk-old control and transgenic mice at 35°C were not significantly different from each other or from 8-wk-old control mice at 24°C. Weights of depots from 3-wk-old control and transgenic mice are not significantly different from each other.

enough material was available.) The enlarged inguinal WAT depot of transgenic mice raised at 24°C (Fig. 2*B*) also had more total cells (from DNA assay) (Fig. 4*A*) and more total protein (Fig. 4*B*) than that of control mice, changes not seen in the transgenic mice raised at 35°C (Fig. 4, *A* and *B*).

Interscapular BAT. Interscapular BAT of transgenic obese mice generally had less total protein and mitochondrial mass (as assessed by cytochrome oxidase content) than control mice (Fig. 5, *A* and *B*) and a lower UCP content (Fig. 6*A*). In control mice, the expected age- and temperature-dependent decline in UCP content of BAT was seen (Fig. 6*A*); control mice raised at 35°C had very little UCP in their BAT, and transgenic mice raised at this temperature had even less (Fig. 6*A*).

The UCP/cytochrome oxidase ratio, a measure of UCP concentration relative to that of other mitochondrial proteins, was highest in the 3-wk-old mice and lowest in the mice raised at 35°C (Fig. 6*B*). Thus UCP concentration in mitochondria decreased with age, much more so in the mice raised at 35°C than in the mice raised at 24°C. The UCP/cytochrome oxidase ratio in BAT mitochondria of transgenic mice was, however, the same as in control mice in all groups (Fig. 6*B*); the transgenic mice suffer from depletion of mitochondria in their BAT, not from a lower concentration of UCP in the mitochondria that are present.

Serum lipids. There were no differences in either serum cholesterol concentration or in serum triacylglycerol concentration between transgenic and control mice at any age or temperature (data not shown).

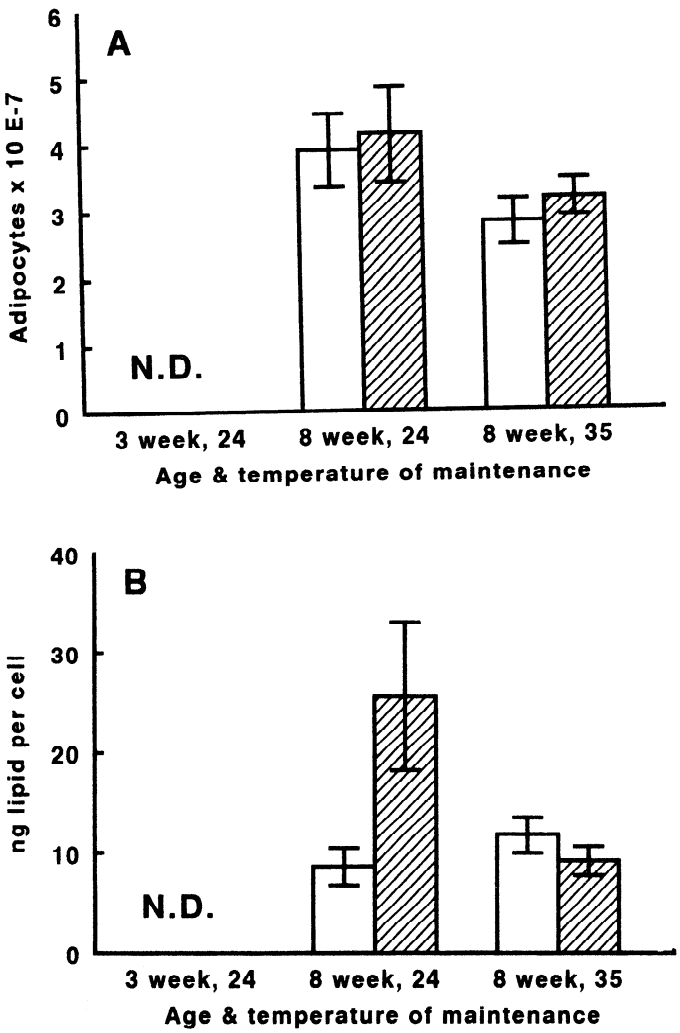

Fig. 3. Number (*A*) and size (*B*) of white adipocytes in inguinal white adipose depot. Control mice, open bars; transgenic mice, hatched bars. ND, not done because too little tissue was available. There are no significant differences in white adipocyte number. Adipocyte size is significantly greater in 8-wk-old transgenic mice at 24°C than in 8-wk-old control mice at 24°C ($P < 0.05$). There is no difference in adipocyte size between 8-wk-old transgenic mice at 35°C and 8-wk-old control mice at 35°C. × 10 E-7, total number of cells multiplied by 10^{-7} for *y*-axis value.

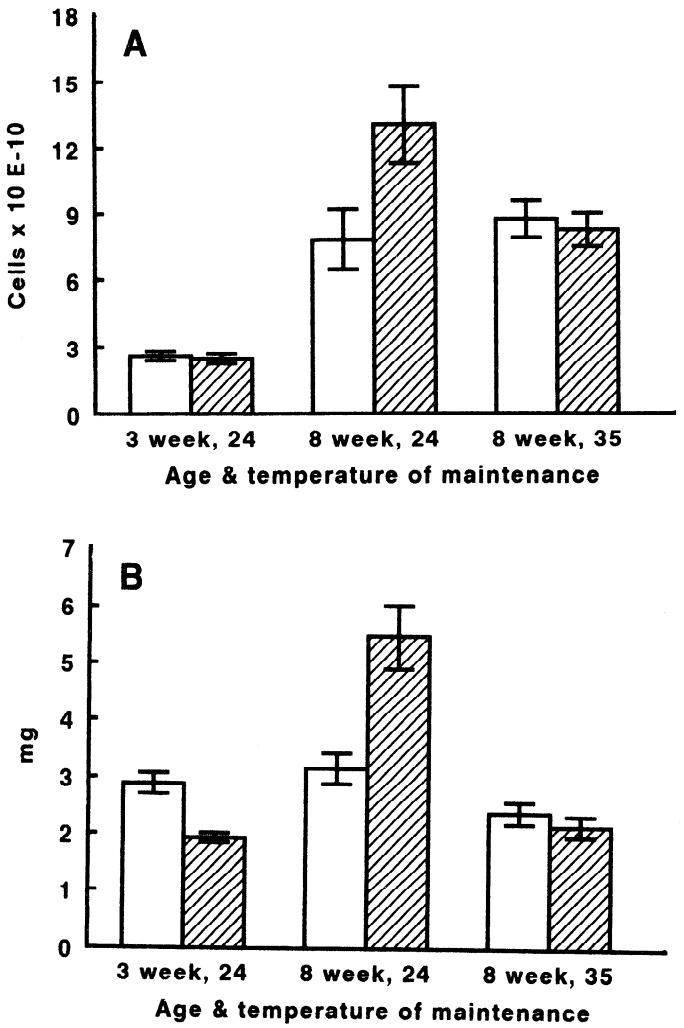

Fig. 4. Total number of cells (from DNA content) (*A*) and total protein content (*B*) in inguinal white adipose depot. Control mice, open bars; transgenic mice, hatched bars. Total number of cells (this means all cell types, not only adipocytes) is significantly greater in transgenic mice at 24°C than in control mice at 24°C ($P < 0.05$) and in all 8-wk-old mice compared with 3-wk-old mice. There are no differences between 8-wk-old control mice at 24°C, 8-wk-old control mice at 35°C, and 8-wk-old transgenic mice at 35°C. Protein content is also significantly greater in 8-wk-old transgenic mice at 24°C than in 8-wk-old control mice at 24°C ($P < 0.001$), in 3-wk-old transgenic mice, or in 8-wk-old transgenic mice at 35°C ($P < 0.001$ for each). There are no other significant differences. × 10 E-10, total number of cells multiplied by 10^{-10} for *y*-axis value.

DISCUSSION

The principal finding in this study is that the development of both the obesity and the hyperphagia in UCP-DTA mice is temperature sensitive. Both are prevented when these mice are raised from weaning to the age of 8 wk at a thermoneutral environmental temperature at which BAT thermogenesis would be at an equally low level in transgenic and control mice. The transgenic mice are able to adjust their energy intake precisely to their needs for growth and maintenance at this relatively high temperature, eating the same amount as control mice and acquiring the same amount of body fat as control mice. However, control mice can precisely adjust their energy intake to meet their needs for growth and maintenance at the low temperature usually used for raising mice (24°C); at this temperature they eat twice as much as at 35°C but acquire the same amount of body fat as mice raised at the higher temperature. UCP-DTA mice, in contrast, are unable to adjust their energy intake precisely to meet their needs for growth and maintenance at a lower temperature and eat 2.6 times as much as they do when raised at 35°C, in excess of their needs, presumed in any case to be lower than in control mice because of the deficit in BAT thermogenesis at this temperature. As a consequence they develop obesity. There appears to be an obligatory link between a mouse's ability to use thermogenesis in its BAT and its ability to adjust its food

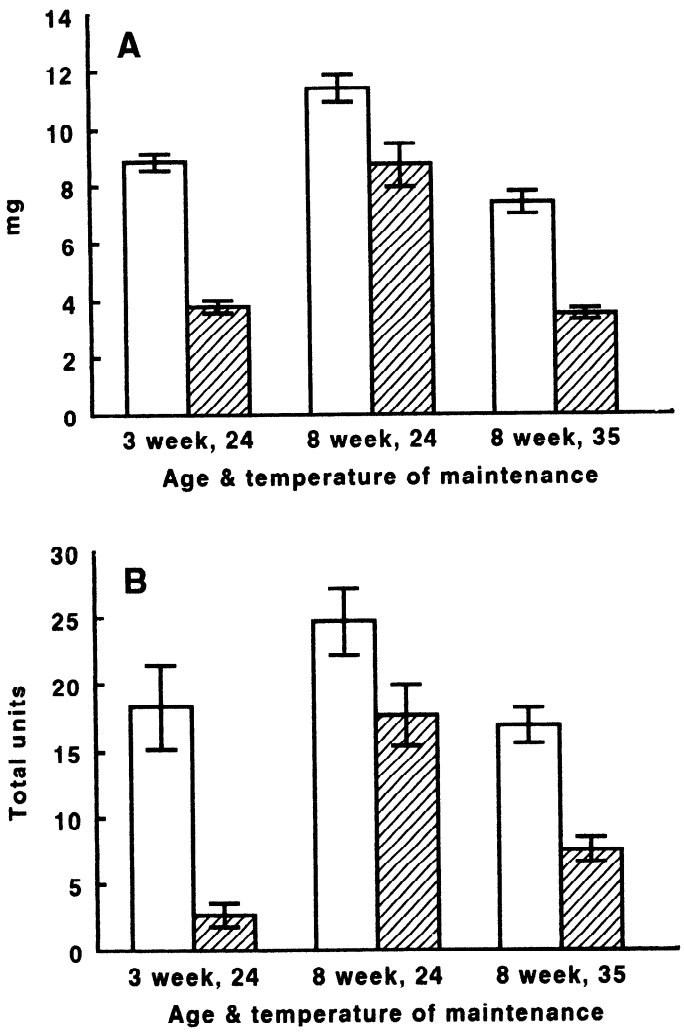

Fig. 5. Total protein (*A*) and total cytochrome oxidase activity (μmol/min) (*B*) in interscapular brown adipose tissue (BAT). Control mice, open bars; transgenic mice, hatched bars. Protein content is significantly lower in transgenic mice compared with control mice at same age and temperature for 3-wk-old mice ($P < 0.001$), for 8-wk-old mice at 24°C ($P < 0.001$), and for 8-wk-old mice at 35°C ($P < 0.001$). Total cytochrome oxidase activity is significantly lower in 3-wk-old transgenic mice than in 3-wk-old control mice ($P < 0.05$) and in 8-wk-old transgenic mice at 35°C than in 8-wk-old control mice at 35°C. It is significantly higher in 8-wk-old transgenic mice at 24°C than in 3-wk-old transgenic mice ($P < 0.01$) or in 8-wk-old transgenic mice at 35°C ($P < 0.01$). The difference between control and transgenic mice at 24°C did not reach statistical significance.

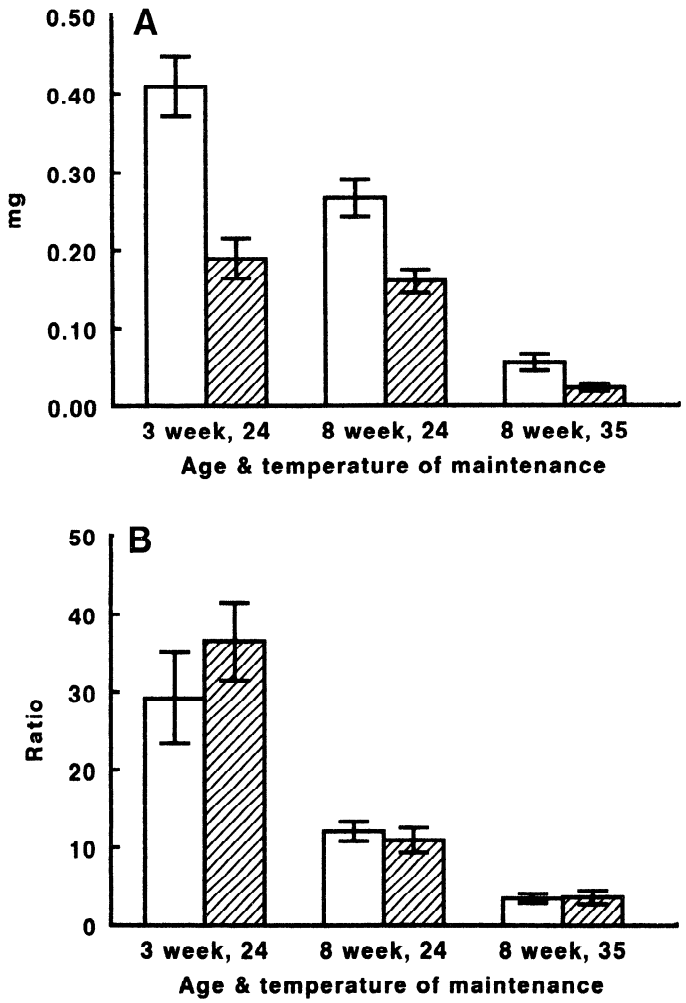

Fig. 6. Uncoupling protein (UCP) content (*A*) and UCP/cytochrome oxidase ratio (*B*) of interscapular BAT. Control mice, open bars; transgenic mice, hatched bars. UCP content is significantly lower in 3-wk-old transgenic mice than in 3-wk-old control mice ($P < 0.001$) and in 8-wk-old transgenic mice at 24°C than in 8-wk-old control mice at 24°C ($P < 0.01$). Difference between 8-wk-old transgenic mice at 35°C and 8-wk-old control mice at 35°C did not reach statistical significance; an unpaired *t*-test revealed a statistically significant difference ($P < 0.016$) between these two very low values, much lower than those for all other groups of mice ($P < 0.001$). There are no significant differences in UCP/cytochrome oxidase ratio between groups of transgenic and control mice at same age and temperature. There is a decrease in the ratio with age ($P < 0.001$) and with the higher temperature ($P < 0.01$).

intake in accordance with environmental temperatures below thermoneutrality. This link provides support for the hypothesis that BAT thermogenesis plays an important role in thermoregulatory feeding (11, 12), matching energy intake to the mouse's need for fuel for thermogenesis. In contrast to a previous study (16), the weanling mice were not obese, although their BAT was definitely atrophied, as indicated by the reduction in mitochondrial content. In our study, the accelerated weight gain appeared to coincide with the hyperphagia detected at 5 wk of age, as in the previous study (16). However, in the absence of detailed carcass analysis between 3 and 6 wk of age we cannot pinpoint more precisely the age at which obesity started to develop.

Biochemical assessment of BAT of the UCP-DTA transgenic mice suggests that the principal consequence of the expression of the toxigene is a loss of brown adipocytes and the abundant mitochondria contained within them. The result is a reduced capacity for thermogenesis in BAT. The control of UCP concentration in mitochondria in relation to age (it decreases with age from 3 to 8 wk) and environmental temperature (it decreases even further at thermoneutrality) is normal in the cells that are present. Thus the defect in the UCP-DTA mouse lies in its reduced capacity for thermogenesis, not in the central mechanisms that exert control over its BAT.

The obesity of the UCP-DTA mouse at 8 wk of age is hypertrophic, the doubling of body fat achieved by increased adipocyte size with no increase in adipocyte number. The increased number of total cells in WAT, with a concomitant increase in protein content, is interpreted as an increase in vascularity to support the expanded mass of tissue. The transgenic mice raised at 24°C had not yet developed the hypertriglyceridemia or hypercholesterolemia seen in older obese mice (7, 8, 16). We are, therefore, unable to ascertain whether these presumed sequelae of obesity might also have been prevented by the treatment.

In conclusion, both the obesity and the hyperphagia of the UCP-DTA mouse are temperature dependent, apparent only when the mouse lives at a temperature below thermoneutrality, when BAT thermogenesis is switched on. Both fail to develop at thermoneutrality, that is, under conditions where BAT thermogenesis would be switched off. In our study, both apparently developed at the same time, indicating an important role for the hyperphagia in the development of obesity in these transgenic mice in addition to the presumed deficit in energy expenditure in their BAT. Results provide compelling evidence for an important role for BAT thermogenesis in regulation of energy balance in mice living at temperatures below thermoneutrality.

Perspectives

In contrast to the transgenically obese UCP-DTA mouse, other genetically obese mice still become obese when raised at thermoneutrality. Housed at 33–34.5°C, genetically obese *ob/ob* mice are still hyperphagic and exhibit raised metabolic efficiency (4, 18), despite the equally low sympathetic stimulation to BAT thermogenesis in both *ob/ob* and lean mice at this temperature (18, 22) and the normalization of their blood corticosterone levels (18). Genetically obese diabetic mice (*db/db*) likewise remain hyperphagic and exhibit elevated weight gain when housed at 33°C (4, 19) despite normal corticosterone levels (19). These obese mutants eat less at 33°C than at 24°C, but still remain hyperphagic in comparison with control lean mice (4). The difference between the genetically obese mice (*ob/ob* and *db/db*) and the transgenically obese mouse (UCP-DTA) in their response to raising at thermoneutrality can probably be interpreted in the light of what is now known about the genetic defects in the leptin system present in the two mutants. The *ob*

gene codes for a protein, leptin, made exclusively by adipose tissues (23), that circulates in the blood at a level directly related to adipose mass (6) and probably regulates multiple hypothalamic functions, including that involved in regulating food intake, by acting on a specific leptin receptor (2, 3, 15). Leptin itself is absent in the *ob/ob* mouse, whereas the leptin receptor is functionally impaired in the *db/db* mouse (2, 3, 15, 23). In the absence of a functioning leptin system both strains of these mutant mice still become obese and hyperphagic when raised at thermoneutrality, suggesting that the leptin system is important for balancing food intake with the energy needs imposed by different environmental temperatures. The expression of the *ob* gene is indeed known to be temperature dependent, being suppressed in a cold environment (21). We suggest that UCP-DTA mice have a functional leptin system that serves to regulate their energy intake at thermoneutrality, in contrast to *ob/ob* and *db/db* mice. The defect in these mice lies in their deficit in BAT thermogenesis at temperatures below thermoneutrality, leading to lack of influence of BAT thermogenesis on both energy expenditure and energy intake, a lack that is not overcome by the elevated leptin levels seen in these animals (6).

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REFERENCES

1. **Ashwell, M., G. Jennings, D. Richard, D. M. Stirling, and P. Trayhurn.** Effect of acclimation temperature on the concentration of the mitochondrial "uncoupling" protein measured by radioimmunoassay in mouse brown adipose tissue. *FEBS Lett.* 161: 108–112, 1983.
2. **Chen, H., O. Charlat, L. A. Tartaglia, E. A. Woolf, X. Weng, S. J. Ellis, N. D. Lakey, J. Culpepper, K. J. Moore, R. E. Breitbart, G. M. Duyk, R. I. Tepper, and J. P. Morgenstern.** Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor in *db/db* mice. *Cell* 84: 491–495, 1996.
3. **Chua, S. C., Jr., W. K. Chung, X. S. Wu-Peng, Y. Zhang, S.-M. Liu, L. Tartaglia, R. L. Leibel.** Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science* 271: 994–996, 1996.
4. **Coleman, D. L.** Thermogenesis in diabetes-obesity syndromes in mutant mice. *Diabetologia* 22: 205–211, 1982.
5. **Collins, S., C. M. Kuhn, A. E. Petro, A. G. Swick, B. A. Chrunyk, and R. S. Surwit.** Role of leptin in fat regulation. *Nature* 380: 677, 1996.
6. **Frederich, R. C., B. Löllmann, A. Hamann, A. Napolitano-Rosen, B. B. Kahn, B. B. Lowell, and J. S. Flier.** Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J. Clin. Invest.* 96: 1658–1663, 1995.
7. **Hamann, A., H. Benecke, Y. Le Marchand-Brustel, V. S. Susulic, B. B. Lowell, and J. S. Flier.** Characterization of insulin resistance and NIDDM in transgenic mice with reduced brown fat. *Diabetes* 44: 1266–1273, 1995.
8. **Hamann, A., J. S. Flier, and B. B. Lowell.** Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes, and hyperlipidemia. *Endocrinology* 137: 21–29, 1996.
9. **Himms-Hagen, J.** Brown adipose tissue thermogenesis and obesity. *Prog. Lipid Res.* 28: 67–115, 1989.
10. **Himms-Hagen, J.** Brown adipose tissue thermogenesis and obesity: an interdisciplinary approach. *FASEB J.* 4: 2890–2898, 1990.
11. **Himms-Hagen, J.** Role of brown adipose tissue thermogenesis in control of thermoregulatory feeding in rats: a new hypothesis that links thermostatic and glucostatic hypotheses for control of food intake. *Proc. Soc. Exp. Biol. Med.* 208: 159–169, 1995.
12. **Himms-Hagen, J.** Does thermoregulatory feeding occur in newborn infants? A novel view of the role of brown adipose tissue thermogenesis in control of food intake. *Obesity Res.* 3: 361–369, 1995.
13. **Himms-Hagen, J., J. Cui, E. Danforth, Jr., D. J. Taatjes, S. S. Lang, B. L. Waters, and T. H. Claus.** Effect of CL-316,243, a thermogenic β_3-agonist, on energy balance and brown and white adipose tissues in rats. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35): R1371–R1382, 1994.
14. **Hirsch, J., and E. Gallian.** Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* 9: 110–119, 1968.
15. **Lee, G.-H., R. Proenca, J. M. Montez, K. M. Carroll, J. G. Darvishzadeh, J. I. Lee, and J. M. Friedman.** Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379: 632–635, 1996.
16. **Lowell, B. B., V. S. Susulic, A. Hamann, J. A. Lawitts, J. Himms-Hagen, B. B. Boyer, L. P. Kozak, and J. S. Flier.** Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 366: 740–742, 1993.
17. **Lowell, B. B., V. S. Susulic, A. Hamaan, J. A. Lawitts, J. Himms-Hagen, B. B. Boyer, L. Kozak, and J. S. Flier.** Transgenic ablation of brown adipose tissue. In: *Molecular and Genetic Aspects of Obesity*, edited by G. A. Bray and D. H. Ryan. Baton Rouge: Louisiana State University Press, 1996, p. 593–608.
18. **Romsos, D. R., D. Ferguson, and J. G. Vander Tuig.** Effects of a warm environment on energy balance in obese (ob/ob) mice. *Metabolism* 34: 931–937, 1985.
19. **Saito, M., and G. A. Bray.** Diurnal rhythm for corticosterone in obese (ob/ob) diabetes (db/db) and gold-thioglucose-induced obesity in mice. *Endocrinology* 113: 2181–2185, 1983.
20. **Susulic, V. S., and B. B. Lowell.** Brown adipose tissue and the regulation of body fat stores. *Curr. Opin. Endocrinol. Diabetes* 3: 44–50, 1996.
21. **Trayhurn, P., J. S. Duncan, D. V. Rayner.** Acute cold-induced suppression of *ob* (obese) gene expression in white adipose tissue of mice: mediation by the sympathetic nervous system. *Biochem. J.* 311: 729–733, 1995.
22. **Zaror-Behrens, G., and J. Himms-Hagen.** Cold-stimulated sympathetic activity in brown adipose tissue of obese (*ob/ob*) mice. *Am. J. Physiol.* 244 (*Endocrinol. Metab.* 7): E361–E366, 1983.
23. **Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman.** Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425–432, 1994.