BAR Signaling Required for Diet-Induced Thermogenesis and Obesity Resistance

Eric S. Bachman,1 Harveen Dhillon,1 Chen-Yu Zhang,1 Saverio Cinti,2 Antonio C. Bianco,3 Brian K. Kobilka,4 Bradford B. Lowell1*

Excessive caloric intake is thought to be sensed by the brain, which then activates thermogenesis as a means of preventing obesity. The sympathetic nervous system, through β-adrenergic receptor (BAR) action on target tissues, is likely the efferent arm of this homeostatic mechanism. To test this hypothesis, we created mice that lack the three known BARs (β-lens, β-lens, and β-lens). These findings establish that BARs are necessary for diet-induced thermogenesis and that this efferent pathway plays a critical role in the body’s defense against diet-induced obesity.

In a prevalent view of body weight homeostasis, it is proposed that dietary excess is sensed by the brain, which, to avoid excessive weight gain, then triggers a reduction in food intake and an increase in energy expenditure (1). Previous attempts have included ablation of the sympathetic nervous system (SNS) and stimulation of βARs on thermogenically active target tissues (2, 3). Brown adipose tissue (BAT), with its uncoupled mitochondrial respiration, is one such target tissue and has been suggested to be an important mediator of diet-induced thermogenesis (4–6). While this model (diet → brain → SNS → βARs → thermogenesis → protection from obesity) has great appeal and is widely cited, there has been no direct demonstration that such a pathway operates and is important in preventing diet-induced obesity. Previous attempts have included ablation of sympathetic nerves (7–9) and the generation of genetically altered mice that are unable to synthesize catecholamines (10). However, neither these perturbations, nor gene knockouts of individual βARs (11–13), have resulted in obesity, possibly because of nonspecific complications caused by loss of all adrenergic signaling (10) and functional redundancy between the three known βARs, which are coexpressed on brown adipocytes (11, 14). To test this model, we created mice that lack the three known βARs (β-less).

Two lines of mice were derived for both wild-type (wt) and β-lens genotypes from existing strains (11, 13, 15) (fig. S1). This was done to confirm that the phenotype of β-lens mice is due to absence of βARs and to rule out genetic background effects. Experiments were performed with both lines of male and female β-lens and wt mice, unless otherwise indicated, and representative data from males are shown. β-lens mice were viable and fertile. On a Chow diet, β-lens mice developed mild obesity by 20 weeks compared with wt mice (Fig. 1A). β-lens females similarly developed mild obesity (16). The increased body weight was attributable mostly to increased body fat in β-lens mice (table S1). Leptin levels were also increased in β-lens mice (7.63 ± 0.9 ng/ml) versus wt mice (3.9 ± 1.03 ng/ml, P < 0.05, n = 3), consistent with increased fat mass. Food intake (Fig. 1B) and body temperature (15) in β-lens mice were similar to those of wt controls. Metabolic rate, however, as indicated by oxygen consumption, was on average 16% lower in 8-week-old β-lens mice (56 ± 1.46 ml kg⁻¹ min⁻¹) compared with weight-matched wt controls (67.34 ± 1.57 ml kg⁻¹ min⁻¹; n = 8, P < 0.05). This decrement in metabolic rate persists when oxygen consumption is expressed per gram of lean body mass (Fig. 1C) and per mouse (1.38 ± 0.05 versus 1.59 ± 0.09 ml min⁻¹ per mouse in wt; P < 0.05, n = 6 in each group). The lower metabolic rate in β-lens mice was not due to measurable differences in thyroid hormone levels or physical activity (15).

We analyzed BAT in β-lens versus wt mice because BARs have been shown to stimulate the development and function of this thermogenic adipose tissue (17). The interscapular BAT in β-lens mice housed at room temperature (22°C) was markedly enlarged and pale in comparison with BAT from wt controls (16). The BAT from β-lens mice contained large cells with unilocular triglyceride deposits (line 1 mice in Fig. 2A, line 2 mice in fig. S2), similar to BAT from denerveated or catecholamine-deficient mice (10, 18). Because β1 and β3 ARs stimulate proliferation and differentiation of BAT in vitro (17), we also derived mice lacking only these two receptors. Unexpectedly, β1-less mice had normal BAT weight (16), and normal BAT appearance by histology (Fig. 2A). Thus, the presence of β1AR alone is sufficient for normal BAT morphology. The BAT-specific thermogenic molecule, uncoupling protein–1 (UCP-1), was abundantly expressed in wt mice, whereas β-lens mice expressed lower levels that were apparent as a cytoplasmic rim around unilocular triglyceride deposits (Fig. 2B). Leptin expression, which is normally restricted to white adipose tissue (WAT), was expressed in BAT of β-lens mice, but not wt mice (Fig. 2C). Thus,
BAT from β-less mice has features of both BAT and WAT. These results indicate that βARs are necessary for normal BAT morphology and that βARs are functionally redundant in BAT.

BAT in β-less mice was unresponsive to both physiological (cold exposure) and pharmacological (β-agonist) stimulation. When β-less mice were exposed to cold at 4°C, their core body temperature dropped rapidly (Fig. 3A). Cold sensitivity was accompanied by failure to induce thermogenic mechanisms in BAT. For example, compared to wt mice, β-less mice expressed lower levels of UCP-1 after cold exposure (Fig. 3B). Similarly, induction of type II deiodinase (D2), which is necessary for thermogenesis in BAT through local triiodothyronine (T3) production (19), was absent in β-less mice (Fig. 3B). This demonstrates that βARs are the major mechanism for cold-mediated induction of D2 activity. To demonstrate pharmacologically that β-less mice lack βARs and to determine whether there are additional βARs in BAT, we measured thermogenic responses to the general β-agonist, isoproterenol, in wt and β-less mice. A maximally effective dose of isoproterenol, which stimulated oxygen consumption in wt mice more than twofold, had no effect in β-less mice (Fig. 3C). Similarly, isolated brown adipocytes from β-less mice had no response, in vitro, to treatment with maximal doses of isoproterenol (Fig. 3D). These results confirm that βARs are necessary for normal BAT function and provide evidence against the existence of additional, functional βARs in BAT.

To determine if the βAR component of the SNS mediates resistance to diet-induced obesity, we fed adult β-less and wt mice a high-fat diet (58% of kcal from fat) (15). Both lines of adult β-less mice developed massive obesity (line 2 mice shown, Fig. 4A). The increment in weight gain was twice that predicted from the combined effects of diet and genotype, indicating that a synergistic interaction exists between βAR deficiency and high-fat diet with respect to the development of obesity (table S1). Food intake, measured during the first 2 weeks of high-fat feeding, was comparable in the β-less and wt mice (table S1), indicating that the obesity observed in β-less mice is not due to increased food intake.

To directly determine whether the obesity of β-less mice was due to impaired diet-induced thermogenesis, as suggested by the lack of effect on food intake, we monitored the metabolic rate of mice for 6 days during the transition to a high-fat diet. As observed in our previous set of animals, high-fat feeding over 5 days resulted in significantly greater weight gain in β-less versus wt mice (Fig. 4B). Again, food intake was unaffected by genotype (16). Metabolic rate, as indicated by oxygen consumption, increased by 16.7% in wt mice after 5 days of high-fat feeding (Fig. 4C). However, in β-less mice, this diet-induced increase in oxygen consumption was absent. Similarly, when oxygen consumption was expressed on a per mouse basis, β-less mice had significantly lower rates (1.63 ±
were significant to control mice).

Periods at baseline, and after treatment with medium or 100 mg of total protein from interscapular BAT taken from wt (solid bars) and its UCP1-mediated uncoupled respiration (Fig. 3). Baseline D2 activity is active, and thermogenesis in this tissue was inactive, and thermogenesis in this tissue could not be stimulated by exogenous β-agonists. Also supporting the role of brown fat in diet-induced thermogenesis is the observation that transgenic mice expressing UCP1-diphtheria toxin-A, which have markedly decreased brown fat, are obese (20) and sensitive to diet-induced obesity (6). This finding, however, has been difficult to reconcile with the phenotype of UCP1 gene-knockout mice, which, despite being greatly impaired with respect to cold-induced thermogenesis, are neither obese nor sensitive to diet-induced obesity (21). Thus, UCP1 is required for cold- (22) but not diet-induced thermogenesis, whereas βARs are required for both. This paradox can be explained in one of two ways: Either UCP1-independent, diet-inducible thermogenic mechanisms exist in brown adipocytes, or a target tissue other than brown fat mediates sympathetically driven diet-induced thermogenesis. Other explanations may also be possible.

In summary, our study directly establishes that diet-induced thermogenesis requires the presence of βARs and that diet-induced thermogenesis is a critical mechanism underlying body weight homeostasis.

References and Notes
14. Supplementary material is available on Science Online.
15. E. S. Bachman et al., unpublished data.
22. We thank E. Cereci and C. E. Lee for technical assistance with histological specimens; H. Yamamoto for assistance with telemetry experiments; and B. Spiegelman, J. Flier, and O. Boss for helpful comments. This work was supported by grants from the National Institutes of Health and Eli Lilly and Company.

Supporting Online Material
www.sciencemag.org/cgi/content/full/297/5582/843
D1
Materials and Methods
SOM Text
Figs. S1 and S2
Tables S1 and S2
19 April 2002; accepted 4 June 2002