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βAR Signaling Required for Diet-Induced Thermogenesis and Obesity Resistance

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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 (βAR) 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 βARs (β-less mice). β-less mice on a Chow diet had a reduced metabolic rate and were slightly obese. On a high-fat diet, β-less mice, in contrast to wild-type mice, developed massive obesity that was due entirely to a failure of diet-induced thermogenesis. These findings establish that βARs 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*). The latter phenomenon is termed "diet-induced thermogenesis" and is thought to be mediated by 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-less genotypes from existing strains (*11*, *13*, *15*) (fig. S1). This was done to confirm that the phenotype of β-less 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-less and wt mice, unless otherwise indicated, and representative data from males are shown. β-less mice were viable and fertile. On a Chow diet, β-less mice developed mild obesity by 20 weeks compared with wt mice (Fig. 1A). β-less females similarly developed mild obesity (*16*). The increased body weight was attributable mostly to increased body fat in β-less mice (table S1). Leptin levels were also increased in β-less 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. 1 B) and body temperature (*15*) in β-less 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-less 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-less mice was not due to measurable differences in thyroid hormone levels or physical activity (*15*).

We analyzed BAT in β-less versus wt mice because βARs have been shown to stimulate the development and function of this thermogenic adipose tissue (*17*). The interscapular BAT in β-less mice housed at room temperature (22°C) was markedly enlarged and pale in comparison with BAT from wt controls (*16*). The BAT from β-less mice contained large cells with unilocular triglyceride deposits (line 1 mice in Fig. 2A, line 2 mice in fig. S2), similar to BAT from denervated or catecholamine-deficient mice (*10*, *18*). Because β_1 and β_3 ARs stimulate proliferation and differentiation of BAT in vitro (*17*), we also derived mice lacking only these two receptors. Unexpectedly, $\beta_{1,3}$-less mice had normal BAT weight (*16*), and normal BAT appearance by histology (Fig. 2A). Thus, the presence of β_2AR 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-less 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-less mice, but not wt mice (Fig. 2C). Thus,

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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 at room temperature and failed to induce 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 ±

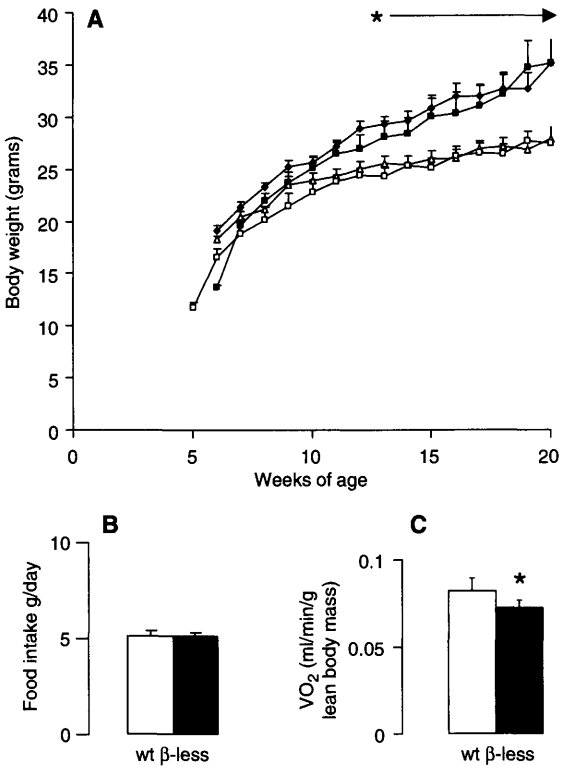

Fig. 1. Phenotype of β-less mice. (**A**) Body weight curve of wt (line 1 and line 2 mice, open symbols, n = 8 in each line) and β-less (line 1 and line 2 mice, filled symbols, n = 8 in each line) male mice on a standard Chow diet (*15*). Data are presented as the mean ± SEM, and differences reached statistical significance after 14 weeks (*P < 0.05 from 15 to 20 weeks). (**B**) Food intake (mean ± SEM) measured over 14 days in 14- to 16-week-old wt (open bars) and β-less (filled bars) mice (n = 8, two experiments). (**C**) Twenty-four–hour oxygen consumption (VO_2), corrected for lean body mass, in age- and weight-matched male wt (open bars) versus β-less mice (filled bars) housed at room temperature (n = 8 each group, *P < 0.05 compared to wt). Data are presented as the mean ± SEM.

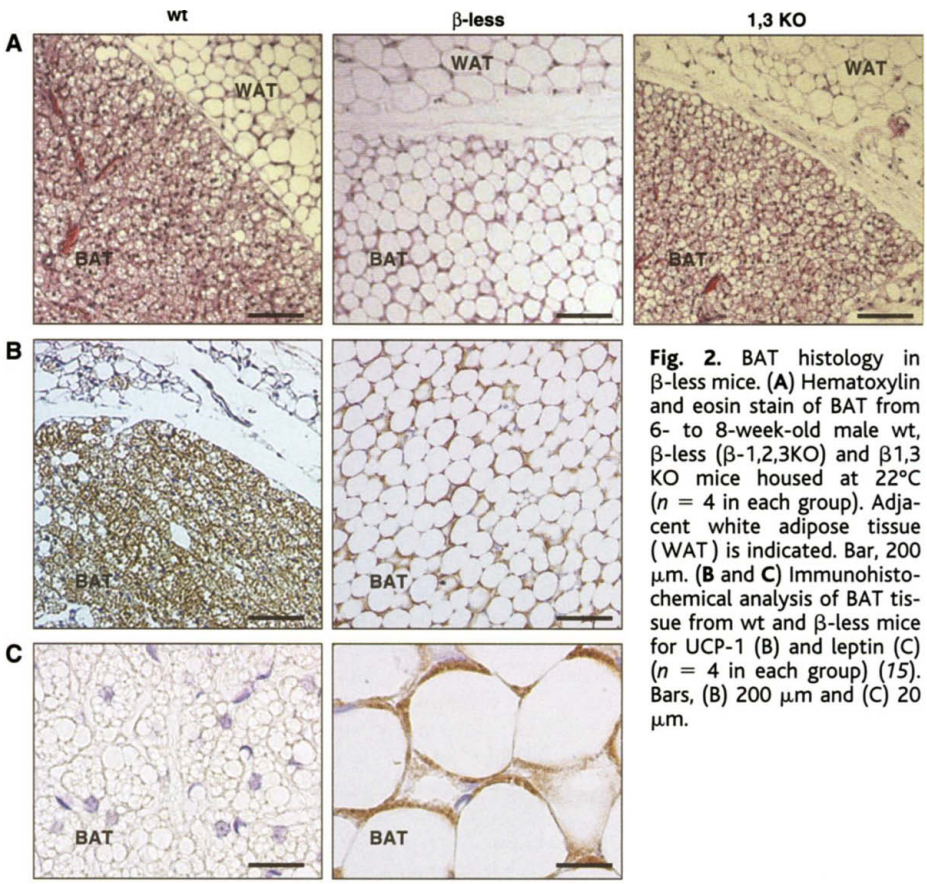

Fig. 2. BAT histology in β-less mice. (**A**) Hematoxylin and eosin stain of BAT from 6- to 8-week-old male wt, β-less (β-1,2,3KO) and β1,3 KO mice housed at 22°C (n = 4 in each group). Adjacent white adipose tissue (WAT) is indicated. Bar, 200 μm. (**B** and **C**) Immunohistochemical analysis of BAT tissue from wt and β-less mice for UCP-1 (B) and leptin (C) (n = 4 in each group) (*15*). Bars, (B) 200 μm and (C) 20 μm.

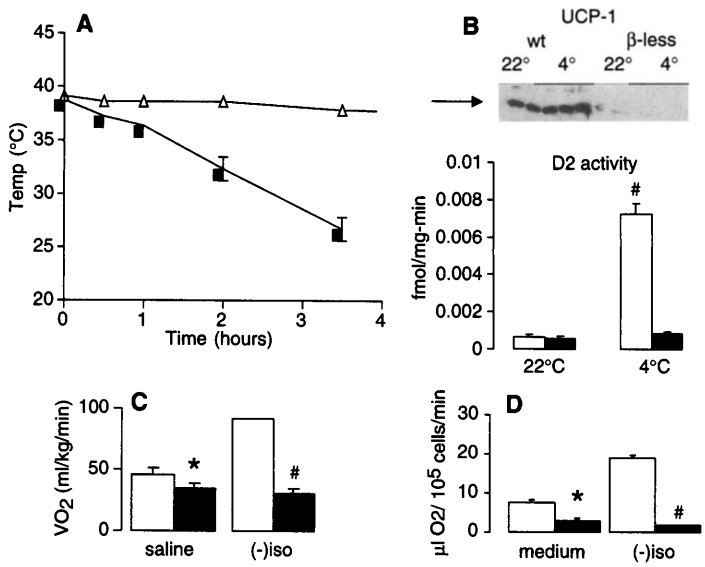

Fig. 3. BAT function in β-less mice. **(A)** Body temperature (mean ± SEM) of male, 6- to 8-week-old weight-matched wt (△) and β-less (■) mice during exposure to 4°C. Cold exposure was terminated after 4 to 6 hours to prevent lethal hypothermia in β-less mice (n = 4, total of 4 experiments). **(B)** UCP-1 expression and type II deiodinase activity during cold exposure. **(Top)** A Western blot for UCP-1 (arrow) with 40 μg of total protein from interscapular BAT taken from wt and β-less mice exposed for 4 hours to 22°C or 4°C. **(Bottom)** Type II deiodinase (D2) activity (mean ± SEM) from BAT taken from mice housed at 22°C and after a 4-hour exposure to 4°C. Baseline D2 activity is not significantly different (0.57 ± 0.123 versus 0.64 ± 0.15 fmol min^{-1} mg^{-1} in wt) but induction after cold exposure is absent in β-less mice (0.84 ± 0.12, open bars, versus 7.3 ± 0.6 fmol min^{-1} mg^{-1} in wt, solid bars, $P < 0.001$ compared to wt at 4°C). **(C)** In vivo oxygen consumption (mean ± SEM) in 8-week-old male mice (wt, solid bars, β-less, open bars) was measured at baseline (saline) and for 4 hours after a maximally effective dose of isoproterenol (1 mg kg^{-1}, subcutaneous injection) (–) (*$P < 0.05$, #$P < 0.01$ versus wt). **(D)** Oxygen consumption (mean ± SEM) of isolated brown adipocytes from wt (solid bars) and β-less mice (open bars) was measured for 10-min periods at baseline, and after treatment with medium or 100 μM isoproterenol (–) (*$P < 0.05$, #$P < 0.01$ versus wt, n = 4 in each group, total of three experiments).

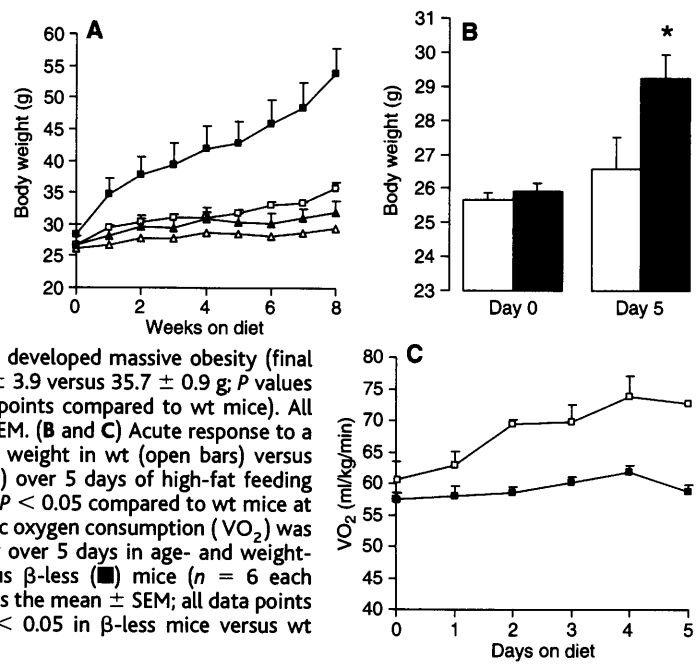

Fig. 4. Response of β-less mice to a high-fat diet. **(A)** Three-month-old male wt control (open symbols) and β-less (filled symbols) mice were fed Chow (12% kcal from fat, triangles) (*15*) or a high-fat (58% kcal from fat, squares) diet (n = 8 in each group) for 8 weeks. The β-less mice, but not wt mice, developed massive obesity (final body weight = 53.84 ± 3.9 versus 35.7 ± 0.9 g; P values are <0.05 at all time points compared to wt mice). All data are the mean ± SEM. **(B and C)** Acute response to a high-fat diet **(B)**. Body weight in wt (open bars) versus β-less mice (filled bars) over 5 days of high-fat feeding (n = 6 in each group, *$P < 0.05$ compared to wt mice at day 5). **(C)** Mass-specific oxygen consumption (VO_2) was measured continuously over 5 days in age- and weight-matched wt (□) versus β-less (■) mice (n = 6 each group, data expressed as the mean ± SEM; all data points were significant to $P < 0.05$ in β-less mice versus wt control mice).

0.02 versus 1.91 ± 0.04 ml min^{-1} per mouse in wt mice, $P < 0.05$) after 5 days of high-fat feeding. Thus, β-less mice have a failure of diet-induced thermogenesis.

The target tissue of this sympathetically driven diet-induced thermogenesis is uncertain and presents a paradox. BAT, because of its UCP1-mediated uncoupled respiration and its intense sympathetic innervation, is thought to be the source of diet-induced thermogenesis (*1*, *5*). Indeed, BAT in β-less mice 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 UCP-1–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.

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