β-Adrenergic Receptors, Diet-induced Thermogenesis, and Obesity*

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There is increased awareness that energy expenditure is an important component of weight control and that its dysregulation promotes obesity. This minireview will examine the role of energy expenditure in regulating fat stores, the underlying mitochondrial basis for energy expenditure, and implications of this for potential mechanisms of adaptive thermogenesis and then discuss, in detail, recent evidence regarding the important role of β-adrenergic receptors in diet-induced thermogenesis and prevention of diet-induced obesity.

Energy Balance

Fat is stored when the number of calories consumed exceeds the number of calories expended. In principle, obesity could be caused by increased food intake or decreased energy expenditure or from a combination of the two. Indeed, most single gene mutation models of rodent obesity, whether naturally occurring or genetically engineered, have both increased food intake and decreased energy expenditure. The frequent occurrence of combined abnormalities strongly supports the view that systems exist to match energy expenditure to food intake over time and that dysfunction of both arms is required to produce massive obesity. The power and precision of this system is demonstrated by the fact that most individuals are not obese, despite the thermodynamic reality that a slight mismatch between intake and expenditure is all that is necessary to produce marked weight gain. Although components of this energy balance system have been identified, including leptin, the leptin receptor, α-melanocyte-stimulating hormone, the melanocortin-4 receptor, and now βARs (1–6), many questions still remain. For example, what is the wiring diagram of central neural circuits regulating energy balance? Also, and relevant to this review, what are the efferent pathways, i.e., nerves and hormones, target tissues, and intracellular mechanisms, by which the brain controls energy expenditure?

Evidence That Energy Expenditure Is Defective in Obesity

The strongest support for defects in energy expenditure in obesity comes from monogenic rodent models such as ob/ob, db/db, and melanocortin-4 receptor gene knock-out mice. When food intake of these mutant animals, deficient in either leptin, leptin receptors, or melanocortin-4 receptors, is restricted to that of wild-type controls, a maneuver termed pair feeding, marked obesity still develops (7). Animals with experimentally induced hypothalamic lesions also become obese when pair fed to sham-treated controls (8). In fact, there are few exceptions to the rule that animal models of obesity, whether genetic or lesion-induced, have decreased energy expenditure. The situation in humans is less clear. In large part, this is because of difficulties in studying heterogeneous populations, the impracticality of pair feeding as a research tool, and difficulties in directly measuring energy expenditure and, in particular, in normalizing energy expenditure to account for differences in body size and composition. Despite this, evidence does suggest that obesity in humans is caused, in part, by abnormalities in energy expenditure. In the now classic study conducted with Pima Indians, it was shown that low energy expenditure, normalized for lean body mass, predicted future weight gain (9).

Fat Mass Set Point and Diet-induced Thermogenesis

It has been hypothesized that each individual has a “fat mass” set point and that excursions above or below this set point cause compensatory alterations in energy expenditure aimed at defending that set point (Fig. 1). Support for this comes from a number of studies, including one in which energy expenditure was assessed in humans before and after experimentally imposed alterations in body weight (10). A 10% increase in body weight caused an increase in energy expenditure above that observed for individuals of similar body composition who had never experienced such a weight gain. The converse was true for individuals with a 10% reduction in body weight. Thus, dietary excess is somehow sensed, most likely by the brain, which to prevent excessive weight gain then triggers an increase in energy expenditure. This increase in energy expenditure is often referred to as “diet-induced thermogenesis.” The magnitude of this response is highly variable and is influenced by genetic makeup, as evidenced by the fact that overfeeding of a fixed number of calories to identical twins causes highly variable weight gain between twin pairs but within twin pairs causes similar degrees of weight gain (11). Because the increase in caloric intake was fixed in this study and was equal among individuals, the resistance to increased weight gain observed in many of the twin pairs must be accounted for by increased energy expenditure. Indeed, this was confirmed in another study in which energy expenditure was directly assessed. It was found that variation in diet-induced weight gain was accounted for by variation in the ability of diet to increase energy expenditure (12).

Thermogenesis: Definitions, Practical Considerations, and Intracellular Mechanisms

Alterations in energy expenditure caused by diet or cold exposure are often referred to as “adaptive thermogenesis.” Total energy expenditure can be roughly broken down into three components (Fig. 2): 1) obligatory energy expenditure required to perform cellular and organ functions; 2) adaptive thermogenesis induced by diet or cold exposure; and 3) physical activity. With few exceptions, most rodent models of obesity have defects in adaptive thermogenesis.

Before reviewing mechanisms of adaptive thermogenesis, it is important to review the laws that govern the mechanics of energy metabolism. From such analyses, the universe of processes that could potentially mediate adaptive thermogenesis becomes evident (readers are referred to Ref. 13 for an in-depth formulation of this thesis). For nearly all metabolic reactions, a fixed, stoichiometric number of reactants generates a fixed, stoichiometric number of products. As discussed below, this limits mechanisms that could be responsible for adaptive thermogenesis. The oxidation of fixed amounts of fuel leads to fixed amounts of ATP being produced, and the utilization of fixed amounts of ATP leads to fixed amounts of biological work being performed. Thus, the amount of fuel oxidized is linked to the biological work performed by a cell or organism. This tight linkage between fuel oxidation and work is explained by the nature of enzymatic reactions with fixed ratios of reactants/products and also by properties of oxidative phosphorylation put forth by the chemiosmotic theory of Peter Mitchell (reviewed in all textbooks of biochemistry).

As is shown in Fig. 3, oxidation of fuel results in electrons being transferred, by means of NADH and FADH2, to the electron transport chain (ETC). As these electrons move down the ETC, energy is
used by complexes I, III, and IV to pump protons outside the inner membrane, creating a electrochemical potential gradient ($\Delta$GpH$_i$). These protons re-enter via ATP synthase with the resulting energy being used to drive conversion of ADP to ATP. The coupling of fuel oxidation to ATP utilization is due to the following features. 1) Movement of electrons down the ETC results in a fixed number of protons being pumped across the inner membrane (five versus three protons for every electron donated by either NADH or FADH, respectively). 2) Electrons may not move down the ETC in the absence of protons being pumped. 3) Entry of a proton via ATP synthase results in fixed amounts of ATP being produced (one molecule of ATP for every three protons). 4) Protons are unable to enter via ATP synthase if ADP is unavailable. 5) Proton pumps in the ETC are unable to operate when $\Delta$GpH$_i$ is high. Consequently, in the absence of work and hydrolysis of ATP, ADP is unavailable. Movement of electrons down the ETC results in a fixed number of protons being pumped across the inner membrane (five versus three protons for every electron donated by either NADH or FADH, respectively). Electrons may not move down the ETC in the absence of protons being pumped. Entry of a proton via ATP synthase results in fixed amounts of ATP being produced (one molecule of ATP for every three protons). Protons are unable to enter via ATP synthase if ADP is unavailable. Proton pumps in the ETC are unable to operate when $\Delta$GpH$_i$ is high. Consequently, in the absence of work and hydrolysis of ATP, ADP is unavailable. Protons are prevented from re-entering via ATP synthase, hence increasing $\Delta$GpH$_i$. This puts “back pressure” on the ETC proton pumps, preventing electrons from traveling down the ETC, increasing the NADH/NAD ratio and subsequently inhibiting fuel oxidation.

With these rules and limitations in mind, it becomes clear that fuel consumption, and hence energy expenditure, can only be increased by one of two ways. Either 1) utilization of ATP must be increased, thus increasing availability of ADP or 2) “uncoupling” of the tight relationship between fuel oxidation and work must occur, allowing fuels to be oxidized in the absence of ATP being consumed (and ADP being generated). ATP utilization can be increased by physical activity and growth, which are by definition unrelated to adaptive thermogenesis, or by operation of so-called “futile cycles.” Three examples of potentially important futile cycles include 1) the synthesis and degradation of proteins, 2) the pumping and leakage of ions across membranes, and 3) the esterification and lipolysis of fatty acids/triglycerides. Note that for each of these futile cycles, ATP is consumed but “net” work is not performed. Other, potentially important futile cycles may also exist. A thorough discussion of this issue can be found in Ref. 13. Because the activity of futile cycles is difficult to assess in intact organisms, it has been difficult to determine their importance in mediating adaptive thermogenesis. Consequently, there is a void of information in this important area.

A clear example of “uncoupling” as a means of increasing energy expenditure is that brought about by uncoupling protein-1 (UCP1), a mitochondrial inner membrane protein that leaks protons across the mitochondrial inner membrane (14, 15). The energy that had been stored in the mitochondrial proton electrochemical gradient is released in the form of heat and is not used to synthesize ATP (Fig. 3). Thus, UCP1 uncouples the relationship between fuel oxidation and ATP hydrolysis. UCP1 protein is expressed at very high levels in brown adipose tissue, a tissue that is abundant in small rodents. The primary function of brown fat is to produce heat in response to cold exposure. The activity of UCP1 in brown fat is controlled by sympathetic nerve activity. Activation of $\beta$ARs on brown adipocytes acutely increases UCP1 proton leak, a process thought to be mediated by lipolysis-induced increases in fatty acid concentrations (16). The critical role for UCP1 has been revealed by UCP1 gene knock-out mice, which are unable to maintain body temperature during cold exposure (17).

UCP2 and UCP3 are homologues of UCP1 that are 56% identical at the amino acid level and are expressed in a number of tissues. Like UCP1, they also have proton leak activity (18–21). However, unlike UCP1, the homologues appear not to play an important role in regulating whole body energy expenditure. This is evident from the observations that UCP2 and UCP3 gene knock-out mice have normal body weight, energy expenditure, responses to high fat feeding, and cold tolerance (22–25). The reason for the lack of effect of UCP homologues on whole body energy expenditure is presently unclear but is likely to be because of the following two issues. 1) Levels of UCP2 and UCP3 protein are 100–700-fold lower than UCP1 (26, 27) and 2) brown adipocytes, the site of UCP1 expression, are highly specialized cells with abundant mitochondria possessing numerous, densely packed cristae (the structure where proton pumping and UCP-mediated proton leak occurs). Also, brown adipocytes are distinguished by the fact that $\beta$AR stimulation, in addition to increasing UCP1 proton leak, simultaneously and independently increases fuel oxidation, hence “feeding” a markedly stimulated proton leak. The net consequence is that brown adipose tissue, when stimulated, importantly impacts on whole body energy expenditure. Nevertheless, UCP2- and UCP3-mediated proton leak does affect the intracellular bioenergetic status of cells in which they are expressed (20, 25, 28) In pancreatic beta cells, UCP2 has been shown to be an important negative regulator of insulin secretion (25, 29). UCP homologues, by virtue of their ability to decrease mitochondrial membrane potential, may also be important negative regulators of superoxide production (23, 24, 30).
The Sympathetic Nervous System: an Important Efferent Pathway Controlling Adaptive Thermogenesis

The sympathetic nervous system is thought to be the efferent pathway by which the brain regulates adaptive thermogenesis. The evidence for this is as follows: 1) cold exposure and diet increase sympathetic nerve activity (31), 2) exogenous administration of norepinephrine and epinephrine stimulates energy expenditure, both in vivo and in isolated tissue preparations, 3) the thermogenic target tissue, brown adipose tissue, is heavily innervated by sympathetic nerves, and 4) thermogenic activity in brown adipose tissue is completely dependent upon intact sympathetic stimulation (32). βARs, and not αARs, are thought to transmit the thermogenic signal to peripheral target tissues because pharmacologic treatment with βAR-selective agonists potently stimulates thermogenesis. There are three βARs that could mediate sympathetically driven thermogenesis; however, the relative importance of each is unknown. One of these receptors, the β3AR, has received significant attention. This subtype is expressed primarily on white and brown adipocytes in rodents and on brown adipocytes in humans. Selective ligands have been developed, and these have marked anti-obesity actions in mice and rats (33, 34). However, the development of agents with similar anti-obesity effects in humans has been problematic. This may be because humans, in contrast to rodents, have fewer brown adipocytes and the human β3AR gene promoter is primarily active in brown but not white adipocytes (35).

Based upon the above mentioned findings plus additional evidence, the following model has emerged: diet → brain → SNS → βARs → thermogenic target tissue (brown fat, muscle?, other tissue?) → thermogenesis → protection from diet-induced obesity (Fig. 4). Despite the appeal of this model, it has been difficult to directly demonstrate its existence and its importance in resisting diet-induced obesity. Previous attempts to experimentally perturb this system have included chemical (6-hydroxydopamine) and immunologic (nerve growth factor antisera) ablation of sympathetic nerves and generation of gene knock-out mice that are unable to synthesize catecholamines (36). Although these animals have shown features of impaired brown adipose tissue function, perturbations such as these have not led to the development of obesity. Common in these prior experiments is the combined absence of αAR and βAR signaling. It is possible that the absence of αAR signaling, which causes dysregulation of blood flow to the skin and consequently marked heat loss (36), masks the important role of βAR signaling in regulating adaptive thermogenesis and whole body energy homeostasis. Further tests of the SNS → βAR → thermogenesis pathway have included gene knock-out of the individual βARs (37–40). However, these experiments have also failed to demonstrate a major role for this pathway in resisting obesity, presumably because of functional redundancy between the three βARs, all of which are co-expressed on white and brown adipocytes.

Mice Lacking All Three βARs (β-less Mice)

To address the issues raised above and critically test the role of the SNS → βAR → thermogenesis pathway in resisting diet-induced obesity, we recently created mice that lack all three βARs (β-less mice) (6). This was accomplished by crossing mice lacking β3ARs (37) with mice lacking both β1 and β2ARs (41). Brown adipocytes in these animals were thermogenically inactive as indicated by the presence of large, unilocular lipid droplets, reduced expression of UCP1 protein, and complete resistance to cold exposure-induced increases in UCP1 protein and type II thyroxine deiodinase activity. Also, the addition of isoproterenol, a selective pan-βAR agonist, to isolated brown adipocytes failed to increase oxygen consumption. When β-less mice were placed at 4 °C, their core body temperature dropped rapidly. These findings confirm the previously held view that βAR signaling is required for thermogenic function of brown adipocytes and maintenance of body temperature during cold exposure. However, and of interest, βARs are not required for the differentiation of brown adipocytes. The signals required for brown adipocyte differentiation versus that of white adipocytes are presently unknown but may involve PGC1α (PPAR-γ coactivator-1) (42).

When fed a standard chow diet, β-less mice, compared with wild-type mice, had a small increase in fat stores. However, when these animals were fed a calorically dense diet, high in fat and sucrose, they developed massive obesity (Fig. 5). Over an 8-week period of feeding, wild-type mice fed chow gained 2 g, wild-type mice fed the high calorie diet gained 8 g, β-less mice fed chow gained 4 g, whereas β-less mice fed the high calorie diet gained 25 g. The increase in weight gain in the β-less mice fed the high calorie diet is more than two times that expected from the individual, additive effects of high fat diet and genotype. This indicates that a strong synergistic interaction exists between βAR deficiency...
and high calorie diet feeding with respect to the development of obesity. Finally, the observed weight gain of 25 g in 8 weeks represents the development of extreme obesity and is similar to that observed in leptin-deficient, ob/ob mice. The marked obesity observed in high calorie-fed, β−less mice is due entirely to a defect in diet-induced thermogenesis. This is strongly supported by two lines of evidence. First, the food intake of wild-type and β−less mice fed the high calorie diet was identical, despite the fact that the β−less mice gained 3 times more weight. Second, the feeding of the high calorie diet caused a marked increase in energy expenditure (diet-induced thermogenesis) in the wild-type mice, a response that was completely absent in β−less mice. Thus, β−less mice have complete failure of diet-induced thermogenesis. These findings establish that βARs are required for diet-induced thermogenesis and that this effector pathway plays a critical role in the defenses of the body against diet-induced obesity.

Target Tissue Mediating Sympathetically Driven Diet-induced Thermogenesis Is Unknown

As reviewed above, βAR signaling mediates diet-induced thermogenesis and this diet → βAR → thermogenesis pathway plays a critical role in resisting diet-induced obesity. Because of this, identification of the target tissue and intracellular mechanism mediating diet-induced thermogenesis is an area of intense interest. Many pieces of evidence are consistent with the view that brown adipose tissue is responsible for βAR-mediated diet-induced thermogenesis and that UCP1-driven uncoupled respiration is the intracellular mechanism. Brown adipose tissue in β−less mice has a histological appearance consistent with inactivity, and UCP1 protein expression is greatly reduced. Also transgenic mice expressing diphtheria toxin-A in brown adipocytes (UCP1-DTA mice), which have a 60–70% reduction in the amount of brown fat, are obese (43) and sensitive to diet-induced obesity (44).

On the other hand, arguing strongly against a role for brown fat and, in particular, UCPI is the observation that UCPI gene knock-out mice, despite being sensitive to cold exposure, have normal resting energy expenditure at room temperature, are not obese, and are resistant to diet-induced obesity (17). This is in striking contrast to β−less mice which have reduced resting energy expenditure at room temperature, are mildly obese on chow, and are extremely sensitive to diet-induced obesity.

There are at least two hypotheses which, if true, would explain these important differences between β−less mice and UCPI gene knock-out mice. In the first hypothesis brown adipose tissue and UCPI mediate cold-induced thermogenesis, but another target tissue mediates both cold-induced and diet-induced thermogenesis. UCPI is the intracellular mechanism responsible for cold-induced thermogenesis whereas a UCPI-independent mechanism is responsible for diet-induced thermogenesis. Possible UCPI-independent, brown fat-based mechanisms include UCP2, UCP3, and a number of futile cycles. Identification of a UCPI-independent mechanism mediating diet-induced thermogenesis would be of great interest. It is clear that resolution of the discrepancy between β−less mice and UCPI gene knock-out mice, with respect to diet-induced thermogenesis, will result in new insight regarding the regulation of body weight control.

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