Original article

MOLECULAR METABOLISM

Stress-induced activation of brown adipose tissue prevents obesity in conditions of low adaptive thermogenesis

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ABSTRACT

Background: Stress-associated conditions such as psychoemotional reactivity and depression have been paradoxically linked to either weight gain or weight loss. This bi-directional effect of stress is not understood at the functional level. Here we tested the hypothesis that pre-stress level of adaptive thermogenesis and brown adipose tissue (BAT) functions explain the vulnerability or resilience to stress-induced obesity.

Methods: We used wt and triple β1,β2,β3−Adrenergic Receptors knockout (β-less) mice exposed to a model of chronic subordination stress (CSS) at either room temperature (22 °C) or murine thermoneutrality (30 °C). A combined behavioral, physiological, molecular, and immunohistochemical analysis was conducted to determine stress-induced modulation of energy balance and BAT structure and function. Immortalized brown adipocytes were used for in vitro assays.

Results: Departing from our initial observation that βARs are dispensable for cold-induced BAT browning, we demonstrated that under physiological conditions promoting low adaptive thermogenesis and BAT activity (e.g. thermoneutrality or genetic deletion of the βARs), exposure to CSS acted as a stimulus for BAT activation and thermogenesis, resulting in resistance to diet-induced obesity despite the presence of hyperphagia. Conversely, in wt mice acclimatized to room temperature, and therefore characterized by sustained BAT function, exposure to CSS increased vulnerability to obesity. Exposure to CSS enhanced the sympathetic innervation of BAT in wt acclimatized to thermoneutrality and in β-less mice. Despite increased sympathetic innervation suggesting adrenergic-mediated browning, norepinephrine did not promote browning in βARs knockout brown adipocytes, which led us to identify an alternative sympathetic/brown adipocytes purinergic pathway in the BAT. This pathway is downregulated under conditions of low adaptive thermogenesis requirements, is induced by stress, and elicits activation of UCP1 in wt and β-less brown adipocytes. Importantly, this purinergic pathway is conserved in human BAT.

Conclusion: Our findings demonstrate that thermogenesis and BAT function are determinant of the resilience or vulnerability to stress-induced obesity. Our data support a model in which adrenergic and purinergic pathways exert complementary/synergistic functions in BAT, thus suggesting an alternative to βARs agonists for the activation of human BAT.

Keywords Subordinate; Energy expenditure; UCP1; P2RX5; Purinergic

1. INTRODUCTION

Metabolic diseases (e.g. obesity, type 2 diabetes) are rising exponentially to a pandemic level. A substantial number of obese individuals manifest psychiatric comorbidity, experience stressful life events, and typically report more medical complaints and poorer quality of life [1,2]. Stress and negative affect are increasingly recognized as risk factors for eating disorders and obesity [3,4]. Despite major advances in our understanding of the signaling pathways linking food intake and energy homeostasis [5,6], we possess very few therapeutic answers to the obesity epidemic [7,8]. Brown adipose tissue (BAT), a thermogenic organ likely to play a major role in energy balance, obesity and diabetes [9,10], is emerging as a novel target for anti-obesity and diabetes pharmacotherapies. The mechanisms of BAT activation are increasingly investigated after the (re) discovery of functional BAT in adult humans [11−14] and the

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identification of beige adipocytes [15–17]. It is accepted that the thermogenic function of brown adipocytes is regulated by sympathetic nervous system release of norepinephrine that, through β adrenergic receptors (β-ARs) induced lipolysis, culminates in UCP1 (uncoupling protein 1) activation and heat generation [9,18–20]. βARs agonists are powerful activators of BAT in mice and humans [21]. However, their relative lack of selectivity resulting in cardiovascular activation has refocused the therapeutic interest in identifying alternative non-adrenergic mechanisms of BAT activation [7,8,10]. Cold-elicited increase in brown/beige adipocyte activity in humans seems stronger compared to pharmacological stimulation with βARs agonists [22–25], thus suggesting a contribution of additional mediators other than NE/βAR to BAT mediated thermogenesis. Notably, humans experience only a modest basal activation of BAT as a result of their high body weight/surface area and predominant exposure to thermoneutral comfort zone, which results in low sympathetic tone to fat pads and low norepinephrine/βAR signaling compared to rodents [9,26,27]. Accordingly, identifying an alternative activator of the thermogenic program that can be activated under conditions of low BAT activity and/or a βARs-independent mechanism of browning is of enormous translational relevance to generate therapies without sympathomimetic-like side effects. In this study, departing from the original observation that β-ARs are dispensable for cold-induced BAT browning, we demonstrated that resilience to chronic subordination stress-induced obesity [28] is determined by a pre-stress state of low adaptive thermogenesis and BAT function, overall providing a functional explanation for the biphasic effect of chronic stress on energy balance [e.g. 29,30]. Furthermore, we identified a sympathetic/brown adipocyte purinergic pathway in mice that is downregulated at thermoneutrality, which is induced by subordination stress and that mediates browning in brown adipocytes. Importantly, we showed that this pathway is conserved in human BAT.

2. EXPERIMENTAL PROCEDURES

2.1. Animals and diet

β1,β2,β3 adrenergic receptor knockout (β-less) mice and their specific wt background strain were previously developed and described by Bachman et al. [19]. Mice were maintained in a fully controlled animal facility (12:12 h light:dark cycle at 22 ± 2 °C). Homozygous breeding pairs were established and pups were weaned in groups of same-sex and same-genotype siblings. Animal experiments were conducted at University of Minnesota (USA) and approved by the Institutional Animal Care and Use Committee, University of Minnesota. Mice were fed a standard (D12405B, Research Diet 3.85 kcal/g, 10% kcal from fat) or a high fat (D12451, Research Diet, 4.73 kcal/g, 45% kcal from fat).

2.2. Overview of the experimental procedures

Experimental male mice were transferred from the animal facility to an adjacent fully controlled environmental room and housed in a 12:12 h light:dark cycle at 14 ± 2 °C, 22 ± 2 °C or 30 ± 2 °C (wt only) according to the experimental conditions detailed below. Mice were allowed one month to acclimate to 14 ± 2 °C or 30 ± 2 °C before performing any experimental procedure.

2.2.1. Mild cold experiment in β-less mice

Mice acclimated for one month to 14 °C were then tested in the indirect calorimetry maintained at 14 °C for the entire duration of the recording. Mice were then allowed 4 days recovery from the calorimetry assessment, after which they were fasted overnight to then undergo a glucose tolerance test. Mice were sacrificed 4 days later at 9 AM.

2.2.2. Chronic subordination stress in wt mice acclimatized to 22 °C or 30 °C and in β-less acclimatized to 22 °C

For all the social stress experiments, the experimental phase consisted of a baseline phase of 5 days, during which all mice received standard diet, and of a stress phase during which all mice received standard diet on the first week of stress and high fat diet for the following weeks according to our published methods [28,31,32]. This enables the establishment of the social hierarchy without any confounding factor due to metabolic effect of the diet. Body weight and food intake were monitored regularly throughout the experimental procedure. Mice underwent body composition analysis once during baseline, and once during the last week of stress prior to the indirect calorimetry assessment (calorimetry maintained at the respective housing temperature for the entire duration of the recording). After 4 days of recovery from indirect calorimetry, all mice underwent an overnight fasting followed by the measurement of their blood glucose levels. Mice were sacrificed 4 days later at 9 AM.

2.2.3. Behavioral experiments

A separate subset of wt and β-less mice acclimatized to 22 °C was used and subjected to same chronic subordination stress general procedure described above. In addition, locomotor activity was measured in home-cage throughout the duration of the procedure. During the last week of stress, mice were given overnight a two-bottle choice for sucrose preference test, and, 3 days later, they were tested in the forced swim test. Mice were sacrificed immediately after the completion of the forced swim test to collect blood and allow for corticosterone analysis in response to heterotypical stress.

2.3. Chronic subordination stress

The stress protocol was conducted as previously described [28,31,32]. Briefly individual β-less and wt mice were transferred as intruder in the home cage of a CD1 aggressive dominant. Dominant and intruder mice were allowed to freely interact for a maximum of 10 min. All wt and β-less mice included in the study were subordinated by CD1 males. After the interaction, resident and intruder mice were separated by a perforated partition, which allowed continuous sensory contact but no physical interaction. During the social interaction, offensive behaviors of the animals were manually recorded and mice social status was determined as previously established and detailed [28,31,32]. The partition was removed daily (between 8:30 and 9:30 AM), for a maximum of 10 min. Only dyads that reliably showed a stable dominant/subordinate hierarchy and in which the subordinate showed no attack after day 4 were included in the study. Age and weight-matched mice, housed in groups of 3 siblings, were included as the control group according to our standard validated protocol [28,31]. Animals included in the stress group were sibling of the animals used as controls.

2.4. Behavioral and endocrine assessment of depression-like behavior

2.4.1. Home-cage activity

Locomotor activity was determined throughout the experiments in subordinate mice by means of an automated system that used small passive infrared sensors positioned on the top of each cage (ActiMeter, TechnoSmart, Rome, Italy) [31].

