Original article

MOLECULAR METABOLISM

Physiological role for leptin in the control of thermal conductance

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ABSTRACT

Objective: To investigate the role played by leptin in thermoregulation, we studied the effects of physiological leptin replacement in leptin-deficient *ob/ob* mice on determinants of energy balance, thermogenesis and heat retention under 3 different ambient temperatures.
Methods: The effects of housing at 14 °C, 22 °C or 30 °C on core temperature (telemetry), energy expenditure (respirometry), thermal conductance, body composition, energy intake, and locomotor activity (beam breaks) were measured in *ob/ob* mice implanted subcutaneously with osmotic minipumps at a dose designed to deliver a physiological replacement dose of leptin or its vehicle-control.
Results: As expected, the hypothermic phenotype of *ob/ob* mice was partially rescued by administration of leptin at a dose that restores plasma levels into the physiological range. This effect of leptin was not due to increased energy expenditure, as cold exposure markedly and equivalently stimulated energy expenditure and induced activation of brown adipose tissue irrespective of leptin treatment. Instead, the effect of physiological leptin replacement to raise core body temperature of cold-exposed *ob/ob* mice was associated with reduced thermal conductance, implying a physiological role for leptin in heat conservation. Finally, both leptin- and vehicle-treated *ob/ob* mice failed to match energy intake to expenditure during cold exposure, resulting in weight loss.
Conclusions: The physiological effect of leptin to reduce thermal conductance contributes to maintenance of core body temperature under sub-thermoneutral conditions.

Keywords Thermoregulation; Thermal conductance; Energy expenditure; Energy intake; Body temperature; Leptin

1. INTRODUCTION

The ability to regulate core body temperature within normothermic limits across a wide range of environmental temperatures is a defining characteristic of all mammals. Free-living animals are routinely exposed to thermal environments that can change rapidly and dramatically on both a daily and seasonal basis, and the energy needs associated with defense of core body temperature in these changing environments can be substantial. Owing to their greater surface-area to-body mass ratio and low thermal inertia, the challenge posed by a cool environment on small mammals is far greater than in larger endothermic animals in terms of the need for both heat production and retention [1]. Unless the energy homeostasis system can meet the challenges imposed by changing thermogenic needs, the amount of body fuel stored as adipose tissue will fluctuate dramatically. In response to chronic cold exposure, for example, the rate of energy expenditure must increase sharply to generate the additional heat needed to avert a drop in core body temperature, and unless energy intake increases proportionately, a progressive decline in body fat mass will result. Simultaneously, the efficiency with which body heat is retained must also increase if core body temperature is to be maintained in a cold environment [1]. Living in a warm environment poses the opposite challenge, as a declining need for heat production will ultimately cause weight gain and increased body temperature unless the decline in thermogenesis is offset by the combined effects of reduced energy intake and an increased rate of heat dissipation to the environment (thermal conductance).

A growing body of evidence suggests that leptin plays a key role in not only the regulation of energy homeostasis but thermoregulation as well [2]. For example, leptin-deficient *ob/ob* mice are characterized not only by hyperphagia and obesity, but also mild hypothermia when housed at room temperature, and they become profoundly hypothermic when exposed directly to cold environments [3–6]. Surprisingly, this thermoregulatory failure is not due to a failure to increase energy expenditure in response to changing ambient temperatures, as they do so in a manner that resembles the response of wild-type mice [7,8]. To explain this paradoxical finding, a recent report suggested that the mechanism underlying leptin's effect to raise defended body temperature involves an increase of the body temperature threshold for activating thermogenesis rather than a stimulatory effect on thermogenesis *per se* [9]. An alternative possibility is that leptin plays a physiological role to limit thermal conductance, which describes the

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Abbreviations: sc, subcutaneous; BAT, brown adipose tissue; Ucp1, uncoupling protein-1; DIO, diet-induced obesity

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ease with which heat flows from the body core to the environment and is an essential aspect of mammalian thermoregulation [1,10–12]. According to this hypothesis, hypothermia in *ob/ob* mice is predicted to be secondary in part to their failure to reduce thermal conductance as a consequence of leptin deficiency. A major goal of the current work was to test this hypothesis.

In addition, whereas *ob/ob* mice increase energy expenditure appropriately in response to a cold challenge, they are unable to adjust energy intake to meet changing energy needs associated with housing across a range of temperatures and consequently lose excessive weight in the cold, while gaining excessive weight in warm environment [7,8,13]. Thus, adaptive changes of energy intake and thermal conductance, but not energy expenditure, appear to require intact leptin signaling [8].

To further clarify the physiological role played by leptin in both thermoregulation and the coupling of thermoregulatory needs to adaptive changes in energy homeostasis, leptin-deficient *ob/ob* mice were infused subcutaneously with either vehicle or a dose of leptin designed to restore plasma levels to those of wild-type controls, and were housed at ~thermoneutrality (30 °C), room temperature (22 °C), or at a cool temperature (14 °C). During this time, comprehensive measures of energy balance and core temperature were continuously obtained and measures of thermal conductance determined.

2. MATERIALS AND METHODS

2.1. Animals

Adult male C57/Bl6 mice and leptin-deficient *ob/ob* mice on the C57/Bl6 background strain were obtained from Jackson Laboratories, ME. All studied animals were individually housed in a temperature-controlled room with a 12:12 h light:dark cycle under specific-pathogen free conditions and provided with *ad libitum* access to water and chow unless otherwise stated (PMI Nutrition, MO). All procedures were performed in accordance with NIH Guidelines for the Care and Use of Animals and were approved by the Animal Care Committee at the University of Washington.

2.2. Systemic leptin administration

Adult male *ob/ob* mice were separated into weight-matched groups, respectively and implanted subcutaneously (sc) with an osmotic minipump (Alzet Model 1007D; DURECT Corporation, Cupertino, CA) containing either vehicle (PBS; pH 7.9) or leptin at a dose of 100 ng/h (Dr. A.F. Parlow; National Hormone & Peptide Program, CA) ($n = 7–8$ per group) designed to achieve leptin levels in the physiological range for wild-type mice based on previous studies [14].

2.3. Core temperature

Adult male *ob/ob* mice underwent implantation of body temperature transponders into the peritoneal cavity (Starr Life Science Corp, Oakmont, PA). Following at least a one-week recovery period, animals were acclimated to metabolic cages enclosed in temperature- and humidity-controlled cabinets (Caron Products and Services, Marietta, OH). Signals emitted by body temperature transponders were sensed by a receiver positioned near the cage and analyzed using VitalView software as previously described [8].

2.4. Measurements of energy expenditure, food intake and ambulatory activity

Animals were acclimated to indirect calorimetry cages prior to study and data collection. Energy expenditure measures were determined using a computer-controlled indirect calorimetry system (Promethion®, Sable Systems, Las Vegas, NV) with support from the University of Washington Nutrition Obesity Research Center (NORC) Energy Balance and Glucose Metabolism (EBGM) Core as described in detail previously [8,15]. The calorimetry system consists of 16 metabolic cages (similar to home cages with bedding) that are equipped with water bottles and food hoppers connected to load cells for continuous food and water intake monitoring and housed in a temperature- and humidity-controlled cabinet (Caron Products and Services, Marietta, OH). O_2 consumption and CO_2 production were measured for each animal for 1 min at 10-min intervals. Respiratory quotient (RQ) was calculated as the ratio of CO_2 production to O_2 consumption. Energy expenditure was calculated using the Weir equation [16]. Whole body thermal conductance was calculated as energy expenditure divided by the difference between core and ambient temperature [1,8]. Ambulatory activity was determined simultaneously with the collection of calorimetry data. Consecutive adjacent infrared beam breaks in the x-, y- and z-axes were scored as an activity count, and a tally was recorded every 10 min. Data acquisition and instrument control were coordinated by MetaScreen v.1.6.2 and raw data was processed using ExpeData v.1.4.3 (Sable Systems) using an analysis script documenting all aspects of data transformation.

2.5. Thermal conductance

To quantify whether leptin regulates the ease with which heat flows from the body core to the environment (via convection, conduction, radiation and evaporation), we calculated whole body thermal conductance of *ob/ob* mice receiving either vehicle or leptin at a dose intended to achieve physiological leptin replacement. Thermal conductance is a useful parameter in comparative thermoregulatory studies of rodents [12,17] and is derived from the formula: $C = EE/(Tb - Ta)$, where C = conductance; EE = energy expenditure; Tb = core temperature and Ta = ambient temperature [1,8,10–12,17]. This parameter of heat transfer has long been calculated based on energy expenditure in situations where the mean energy expenditure rate equals the mean heat loss rate as is true over time intervals that involve very little or no net change in core temperature (e.g., 24 h periods) [8].

2.6. Body composition analysis

In a separate cohort of adult male *ob/ob* mice that were not implanted with body temperature transponders, measures of body fat mass and lean mass were determined using quantitative magnetic resonance spectroscopy (QMR) (EchoMRI 3-in-1; Echo MRI, TX) using the University of Washington NORC EBGM Core [18].

2.7. Experimental protocol

After acclimation to cages situated within temperature- and humidity-controlled chambers, to allow for pair-wise comparisons, measures of energy expenditure, food intake, locomotor activity and body temperature were recorded continuously in all mice for 68 h with the temperature maintained at 22.0 ± 0.1 °C before being implanted with a minipump infusing either vehicle or leptin as described above. These same measures were then recorded in animals that were housed at either 30.0 ± 0.1 °C (a temperature that is, or is close to, thermoneutrality in WT mice) [1,19], 22.0 ± 0.1 °C or to 14.0 ± 0.1 °C. Temperature changes occurred gradually over a 4-h interval and mice remained at each temperature for 68 h. A separate group of *ob/ob* mice were subjected to the same protocol described above except that they were not implanted with body temperature transponders to permit measures of body composition.

