Original article

MOLECULAR METABOLISM

Integration of body temperature into the analysis of energy expenditure in the mouse

Gustavo Abreu-Vieira [1,2], Cuiying Xiao [1], Oksana Gavrilova [3], Marc L. Reitman [1,*]

ABSTRACT

Objectives: We quantified the effect of environmental temperature on mouse energy homeostasis and body temperature.
Methods: The effect of environmental temperature (4–33 °C) on body temperature, energy expenditure, physical activity, and food intake in various mice (chow diet, high-fat diet, *Brs3*$^{-/y}$, lipodystrophic) was measured using continuous monitoring.
Results: Body temperature depended most on circadian phase and physical activity, but also on environmental temperature. The amounts of energy expenditure due to basal metabolic rate (calculated via a novel method), thermic effect of food, physical activity, and cold-induced thermogenesis were determined as a function of environmental temperature. The measured resting defended body temperature matched that calculated from the energy expenditure using Fourier's law of heat conduction. Mice defended a higher body temperature during physical activity. The cost of the warmer body temperature during the active phase is 4–16% of total daily energy expenditure. Parameters measured in diet-induced obese and *Brs3*$^{-/y}$ mice were similar to controls. The high post-mortem heat conductance demonstrates that most insulation in mice is via physiological mechanisms.
Conclusions: At 22 °C, cold-induced thermogenesis is ~120% of basal metabolic rate. The higher body temperature during physical activity is due to a higher set point, not simply increased heat generation during exercise. Most insulation in mice is via physiological mechanisms, with little from fur or fat. Our analysis suggests that the definition of the upper limit of the thermoneutral zone should be re-considered. Measuring body temperature informs interpretation of energy expenditure data and improves the predictiveness and utility of the mouse to model human energy homeostasis.

Keywords Thermoneutrality; Basal metabolic rate; Cold-induced thermogenesis; Body temperature; Energy expenditure; Heat conductance

1. INTRODUCTION

The mouse is a mainstay of obesity research, useful for obtaining mechanistic understanding of clinical observations and for guiding clinical investigation, while allowing experiments not possible in humans. For example, identification of brain regions, neurotransmitters, and connectivity has been determined predominantly in rodents [1] and the mouse is often used to identify pharmaceutical targets [2] and to test candidate drugs. It is crucial to know when human and mouse biology are similar in order to increase the predictive value of mouse models. Humans and mice differ in mass by ~3000-fold. Since body surface area scales to the 2/3 power of mass and surface area is a determinant of heat exchange, thermal biology can be different between mice and humans. Inter-species metabolic rate is proposed to scale to the $^{3}/_{4}$ power of body mass and mass-specific metabolic rate therefore to the $-^{1}/_{4}$ power of body mass, meaning the mouse has a ~7-fold higher mass-specific metabolic rate than a human [3–5]. Adult humans live in or near their thermoneutral zone, so body heat is generated predominantly as a byproduct of metabolic processes with a small contribution from adaptive thermogenesis. Upon cold challenge, large mammals maintain an unchanged core body temperature (Tb), while reducing their surface temperature, increasing the size of a substantial thermal 'shell' and further insulating the core.

Small mammals, such as mice, have disproportionately greater heat loss, and thus their physiology is oriented more towards heat generation than heat dissipation [6]. Mice are typically studied below their thermoneutral zone [7,8]. Small mammals have a minimal thermal shell and their Tb, while also tightly regulated, varies much more than in large mammals [9]. In general, as a mammal's body size becomes smaller, the animal will exhibit more variability in Tb [10], greater rates of change

[1]Diabetes, Endocrinology, and Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA [2]Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 106 91 Stockholm, Sweden [3]Mouse Metabolism Core, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA

*Corresponding author. National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Building 10-CRC, Room 5-5940, 10 Center Drive, Bethesda, MD 20982, USA. Tel.: +1 301 496 6442.

E-mails: gustavo.vieira@su.se (G. Abreu-Vieira), cuiyingx@niddk.nih.gov (C. Xiao), OksanaG@BDG10.NIDDK.nih.gov (O. Gavrilova), marc.reitman@nih.gov (M.L. Reitman).

Abbreviations: Ta, environmental temperature; Tb, core body temperature; dTb, defended body temperature; EE, energy expenditure; TEE, total energy expenditure; PAEE, physical activity energy expenditure; TEF, thermic effect of food; BMR, basal metabolic rate; CIT, cold-induced thermogenesis; RQ, respiratory quotient; LCT, lower critical temperature; HFD, high-fat diet

Received February 17, 2015 • Revision received February 26, 2015 • Accepted March 3, 2015 • Available online 10 March 2015

http://dx.doi.org/10.1016/j.molmet.2015.03.001

in Tb, increased sensitivity to a cool environment (for example in the increase in metabolic rate), a warmer lower bound of the thermoneutral zone, and use of Tb reduction for energy conservation.

The importance of Tb in energy homeostasis has been recognized since at least 1867 (attributed to Sander-Ezn by Ref. [11]) and is acknowledged in reviews [12–14] but is underappreciated in most studies of mouse energy homeostasis. Previously Tb has been invoked to explain phenotypes, but has not been quantitatively integrated into the analysis. Here we examine the effect of environmental temperature (Ta) on energy homeostasis in mice with continuous measurement of Tb, energy expenditure, physical activity, and food intake. Integration of the Tb data indeed provides new insights into the costs of circadian Tb variation, physical activity, and adaptation to the cold.

2. EXPERIMENTAL METHODS

2.1. Animals

Mice were singly housed at 21–22 °C with a 12:12-h dark–light cycle (lights on at 0600 h) in a clean, conventional facility with water and food provided ad libitum. Experiments were approved by the NIDDK Institutional Animal Care and Use Committee. Two groups of six mice were studied in each of three experiments (Table S1). Experiment 1 studied 16–20-week old male chow-fed littermate C57BL/6J and *Brs3*$^{-/y}$ [15] mice. Experiment 2 used 18–19-week old male C57BL/6J mice fed chow (NIH-07, 15 kcal % from fat, Harlan) or a high-fat diet (D12492, 60 kcal % from fat, Research Diets) for the prior 4 weeks. Experiment 3 analyzed 15-week old female chow-fed FVB/N and A-ZIP/F-1 [16] mice.

2.2. Body temperature measurement

G2 E-Mitter transponders (Starr Life Sciences, Oakmont, PA) were implanted intraperitoneally under anesthesia (isoflurane or ketamine 100 mg/kg ip and xylazine 10 mg/kg ip) with flunixin analgesia (2.2 mg/kg sc at operation and daily for two days). Mice were studied at least one week after surgery. Signals were acquired using ER4000 Energizer/Receivers and the manufacturer's software. Body weights are reported after subtraction of the weight of implanted E-Mitters. Body composition was measured by time domain Echo MRI 3-in-1 (Echo Medical Systems, Houston, TX) immediately after euthanasia and removal of the E-Mitter.

2.3. Indirect calorimetry

The indirect calorimetry system (CLAMS using Vital View version 5.0, Columbus Instruments, Columbus, OH) was housed in a temperature-controlled enclosure and used to measure O_2 consumption, CO_2 production, activity (infrared beam break; one beam break is one count), food intake, and Tb (telemetry) from 12 chambers (2.5 L volume, constant flow rate of 0.5 L/min, sampling flow of 0.4 L/min, without bedding or nesting), each sampled every 13 min. Mice were acclimated to the chambers for 3 days at 22 °C, followed in order by one day each at 22 °C, 26 °C, 30 °C, 33 °C, 28 °C, 24 °C, 18 °C, 12 °C, and 4 °C, with the chamber temperature changed at 1200. Data during Ta transition and cage maintenance (1200–1300) were excluded. Food and water were provided ad libitum at all times. Protein oxidation was not measured and the RQ was not corrected for protein oxidation. Each experiment yielded ~960 time points per mouse.

2.4. Analysis of energy expenditure components

Each analysis was performed individually for each mouse, at each Ta and each of the light/dark phases (18 data sets per mouse). The total energy expenditure (TEE) was fit by second order regression to the physical activity. Taking the Y-axis intercept as the TEE in the absence of physical activity, the energy expenditure of physical activity (PAEE) was defined as: PAEE = TEE − Y-intercept. Very similar PAEEs were obtained using linear, 2nd, or 4th order regression. The 2nd order regression was used since the fit was slightly better than using linear regression; linear regression is suitable if only the Y-intercept is of interest.

Thermic effect of food (TEF, also known as specific dynamic action) was calculated from the diet manufacturer's data using the consensus thermic effects of fat (2.5%), carbohydrate (7.5%), and protein (25%) [17]. Note that this method of calculating TEF specifically avoids incorporating changes in energy expenditure due to neurobehavioral adaptations to the fed or fasted state. The TEF for chow diet (7022 NIH-07, Harlan; 3.1 metabolizeable kcal/g, 15% calories from fat, 56% calories from carbohydrate, 29% calories from protein) is 11.8% or 0.367 kcal/g. The NIH-07 food quotient was calculated as 0.909 using values of 0.71 for fat, 1.00 for carbohydrate, and 0.835 for protein [17]. Similarly, the TEF for the high fat diet (D12492, Research Diets; 5.24 metabolizeable kcal/g, 60% calories from fat, 20% calories from carbohydrate, 20% calories from protein) is 8.0% or 0.419 kcal/g, with a food quotient of 0.793. In humans, TEF peaks sooner after smaller meals than large ones [18]. Since the time course of TEF in mice is not known, TEF was calculated as the average over each light/dark phase, assuming no time delay.

The basal metabolic rate (BMR) is calculated at thermoneutrality (33 °C) during the light phase. Under these conditions, BMR = TEE–PAEE–TEF. Cold-induced thermogenesis (CIT) was defined as CIT = TEE–PAEE–TEF–BMR.

2.5. Heat conductance after death

The heat conductance in kcal/h/gradient °C (where gradient °C = Tb − Ta) was calculated in mice with implanted E-mitters each minute from 6 to 35 min after death as $= Cp_{system}*(\Delta Tb/\Delta t)/(meanTb - meanTa)$, where Δt is the 1-min time interval ($t_2 - t_1$), ΔTb is the Tb change during the interval ($Tb_2 - Tb_1$), meanTb is the mean Tb of the interval ($Tb_1 + Tb_2$)/2, and meanTa is the mean Ta of the interval ($Ta_1 + Ta_2$)/2 and was constant at 21.6 °C. Thus, the rate of heat loss can also be written: $Cp_{system} * (Tb_2 - Tb_1)/(t_2 - t_1)/([Tb_1 + Tb_2]/2 - [Ta_1 + Ta_2]/2)$. Accounting for the implanted E-mitters, the total heat capacity of the mouse plus E-mitter system, Cp_{system}, is $Cp_{mouse} * (M_{total} - M_{Emitter}) + (Cp_{Emitter} * M_{Emitter})$ cal/°C, where $M_{Emitter}$ is the mass of the E-mitter, 1.111 ± 0.008 g (mean ± SEM, n = 21), M_{total} is the mass of the mouse + $M_{Emitter}$, and $Cp_{Emitter}$ is 0.160 ± 0.015 cal/g/°C (n = 7). The heat capacity of a mouse alone, Cp_{mouse}, was taken as $0.9100 - 0.5231 * m_f$ cal/g/°C, where m_f is the fat fraction [19]. Since the implanted E-mitter precludes m_f measurement by magnetic resonance spectroscopy, m_fs were taken from historical data of mice matched for body weight, sex, and genotype. Compared to using $Cp_{system} = Cp_{mouse}$, incorporating $Cp_{Emitter}$ into Cp_{system} contributed a correction of ≤4%.

2.6. Statistical analysis

Data points represent mean ± SEM of 5–6 mice, unless indicated otherwise. Standard least squares mixed model analysis with mouse as a random effect was performed using JMP 11.0.0 (SAS Institute Inc, Cary, NC).

3. RESULTS

3.1. Determinants of body temperature

We varied Ta from 4 to 33 °C while continuously measuring Tb, TEE, respiratory quotient (RQ), locomotor activity, and food intake in singly

