Comparative Medicine
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Vol 63, No 5
October 2013
Pages 386–391

Original Research

The Hidden Cost of Housing Practices: Using Noninvasive Imaging to Quantify the Metabolic Demands of Chronic Cold Stress of Laboratory Mice

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Laboratory mice routinely are housed at 20 to 22 °C—well below the murine thermoneutral zone of 29 to 34 °C. Chronic cold stress requires greater energy expenditure to maintain core body temperature and can lead to the failure of mouse models to emulate human physiology. We hypothesized that mice housed at ambient temperatures of 20 to 22 °C are chronically cold-stressed, have greater energy expenditure, and have high glucose utilization in brown adipose tissue. To test our hypotheses, we used indirect calorimetry to measure energy expenditure and substrate utilization in C57BL/6J and Crl:NU-*Foxn1*^{nu} nude mice at routine vivarium (21 °C), intermediate (26 °C), and heated (31 °C) housing temperatures. We also examined the activation of interscapular brown adipose tissue, the primary site of nonshivering thermogenesis, via thermography and glucose uptake in this region by using positron emission tomography. Energy expenditure of mice was significantly higher at routine vivarium temperatures compared with intermediate and heated temperatures and was associated with a shift in metabolism toward glucose utilization. Brown adipose tissue showed significant activation at routine vivarium and intermediate temperatures in both hirsuite and nude mice. Crl:NU-*Foxn1*^{nu} mice experienced greater cold stress than did C57BL/6J mice. Our data indicate mice housed under routine vivarium conditions are chronically cold stress. This novel use of thermography can measure cold stress in laboratory mice housed in vivaria, a key advantage over classic metabolic measurement tools. Therefore, thermography is an ideal tool to evaluate novel husbandry practices designed to alleviate murine cold stress.

Abbreviations: BAT, brown adipose tissue; EPR, entropy production rate; PET, positron emission tomography; ROI, region of interest; RQ, respiratory quotient; VCO_2, volume of carbon dioxide produced; VO_2, volume of oxygen consumed.

Laboratory mice routinely are housed at 'room temperature,' that is, 20 to 22 °C. Room temperature is within the recommendations of the *Guide for the Care and Use of Laboratory Animals*[14] but is well below the murine thermoneutral zone of 29 to 34 °C.[3,17] Systemic physiologic cold stress creates a much greater energy demand on mice than humans due to the surface area to volume ratio. Mice housed at routine vivarium temperatures have greater oxygen consumption and feed intake than at thermoneutral temperatures (30 °C).[5,27] Ultimately, this difference may adversely affect translational research, sometimes in unpredictable ways.[11,17] For example, mice housed at temperatures below their thermoneutral zone have a blunted response to LPS-induced fever and lack the classic early-phase hypothermia, demonstrating impaired immune function.[22] In another example, blood pressure and heart rate are significantly elevated at routine vivarium temperatures compared with thermoneutral temperatures,[23] again demonstrating that rodent physiology is perturbed under such housing conditions.

Received: 30 Nov 2012. Revision requested: 22 Jan 2013. Accepted: 18 Apr 2013.
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Mammals defend their body temperature through a series of mechanisms that progressively increase in energy cost: behavior,[10] insulative response,[7] and thermogenesis.[3] Behavioral thermoregulation is the principle mechanism that enables the survival of small rodents, however behavioral adaptations of laboratory rodents housed in barren cages are limited compared with those of their wild counterparts; wild rodents adapt to cold stress through techniques like seeking shelter, burrowing, and building nests.[9,10] The insulative response shunts blood from peripheral sites toward core organs to conserve heat.[7] Once the low-energy cold-adaptive responses are overwhelmed, mammals maintain core body temperature by increasing energy expenditure via shivering and nonshivering thermogenesis.[9,19] Rodents, arctic mammals, and infant mammals primarily rely on nonshivering thermogenesis to preserve core body temperature.[2]

Nonshivering thermogenesis is achieved through mitochondria-rich brown adipose tissue (BAT). The largest deposits of rodent BAT are located in the interscapular region. BAT produces heat rapidly via the oxidative combustion of glucose and triglycerides.[2,6] BAT is rich in β_3-adrenergic receptors, and its activation is mediated primarily by the sympathetic nervous system.[2] BAT responds within minutes to the sensation of cold. When active, rodent BAT is highly metabolic and can receive as much as 40% of the cardiac outflow.[7]

Given our group's experience with preclinical metabolic imaging,[8] we hypothesized that the cold stress imposed by routine husbandry temperatures (21 °C) induces a global shift toward glucose-dependent metabolism that is driven by nonshivering thermogenesis. Moreover, we hypothesized that athymic nude (Crl:NU-*Foxn1*^{nu}) mice housed at room temperatures experience significantly greater energy expenditure and glucose-dependent metabolism than do hirsute (C57BL/6J) mice.

To test our hypotheses, we measured energy expenditure (entropy production rate, EPR, cal/min) and metabolic substrate utilization via indirect calorimetry[6] and interscapular BAT heat production via infrared thermography and BAT glucose utilization by fluorodeoxyglucose positron emission tomography (PET) at various environmental temperatures, ranging from routine vivarium temperature (21 °C) to heat-supported temperatures (31 °C). Briefly, indirect calorimetry measures O_2 consumption (VO_2) and CO_2 production (VCO_2) to enable the calculation of the energy expenditure of the organism according to the stoichiometric formulas of biologic combustion:

$$1\,\text{g Glucose} + 0.746\,\text{L}\,O_2 \rightarrow 0.746\,\text{L}\,CO_2 + 0.6\,\text{g}\,H_2O$$
$$1\,\text{g Lipid} + 2.029\,\text{L}\,O_2 \rightarrow 1.430\,\text{L}\,CO_2 + 1.09\,\text{g}\,H_2O$$

With these formulas, indirect calorimetry can also be used to calculate global glucose and lipid utilization using the respiratory quotient (RQ), a unitless ratio between VCO_2:VO_2.[5] An RQ of 0.7 is indicative of the use of lipid as the primary substrate of biologic combustion, whereas an RQ of 1.0 is indicative of the primary use of glucose (for additional information, see reference 6).

Materials and Methods

Animals. C57BL/6J and Crl:NU-*Foxn1*^{nu} (age, 10 to 12 wk; 5 male and 5 female mice for each stock or strain) were housed in an AAALAC-accredited facility. The C57BL/6J mice were produced in an internal breeding colony that is repopulated with mice from the Jackson Laboratory (Bar Harbor, ME) every 20 generations, according to a material transfer agreement. Crl:NU-*Foxn1*^{nu} mice were shipped from Charles River Laboratories (Wilmington, MA) and acclimated for 6 d. C57BL/6J and Crl:NU-*Foxn1*^{nu} mice were group-housed in individually ventilated cages (5 mice per cage, 60 air changes hourly; Innovive, San Diego, CA) with corncob bedding (Innovive). The interior dimensions of each cage were 81 in.^{2} × 5 in. (height). Each cage was enriched with a 1-in.^{2} piece of cotton nesting material. SPF status was monitored quarterly by using dirty-bedding sentinels screened for mouse parvovirus, minute virus of mice, mouse norovirus, mouse hepatitis virus, Sendai virus, lymphocytic choriomeningitis virus, polyomavirus, K virus, pneumonia virus of mice, mouse adenovirus, epizootic diarrhea of infant mice (rotavirus), mouse encephalomyelitis virus, reovirus, ectromelia virus, *Mycoplasma pulmonis*, and endo- and ectoparasites. All mice were fed NIH31 diet (Harlan Teklad, Madison, WI) and offered reverse-osmosis–purified, acidified water ad libitum. The room was maintained at 20 to 21 °C, relative humidity of 30% to 70%, 10 to 15 air changes hourly, and a 12:12-h photoperiod. The use of mice in this study was approved by the IACUC of the University of California, Los Angeles.

Indirect calorimetry. O_2 and CO_2 gas analyzers (models 17625 and 17630, respectively, Vacumed, Ventura, CA) were calibrated prior to each trial. Before each trial, static cages were conditioned on heating plates to routine vivarium temperatures (21 °C), intermediate support (26°, the upper limit of the range recommended in the *Guide*[20]), and heat support (31 °C). Cage temperatures were verified by thermography at the surface level of bedding. Individual mice were separated from their groups and placed in preconditioned static cages. We observed that the mice were active when first placed in the novel cages, thus elevating EPR and obscuring the effects of environmental temperature. We selected the acclimation period after performing a pilot study, which demonstrated that the slope of EPR over time measured at 10-min intervals had stabilized by 1 h (data not shown).

In a crossover study design, the mice were acclimated to static cages between the hours of 0900 and 1000 to account for circadian rhythm effects on the 3 temperature conditions. After 1 h of acclimation, the lids were changed to sealed airtight lids with embedded 3-way stopcocks to allow for cage microenvironment gas sampling. At 1000 and 1100, the 3-way stopcock was opened, and the air content of the cage was sampled for 30 s by using the VO_2 and VCO_2 gas analyzers, and then the 3-way stopcock was closed.

Thermography. At the end of each 60-min sampling period, thermography images at 0.5 m from the bedding surface were obtained by using an infrared thermal imaging camera (T400, FLIR, Nashua, NH) to evaluate BAT activation. The camera is equipped with a 320 × 240 pixel detector and manual focus and has a thermal sensitivity of less than 0.045 °C. Using QuickReport version 1.2 (FLIR), we drew a line profile measuring radiated heat over the BAT region; an internal control line profile was drawn at the level of the last rib. We set the emissivity of fur to 0.94 and of the skin of Crl:NU-*Foxn1*^{nu11} to 0.98. The change in temperature in the BAT region was calculated by subtracting the temperature of the control region of interest ($ROI_{control}$) from the temperature at the ROI_{BAT}.

Thermography is prone to erroneous results unless researchers follow a strict, reproducible protocol. For quantification, we used standardized methods of drawing ROI, a high resolution (320 × 240 pixel) to prevent spillover artifacts,[18] standardized distance to the animal, and animals with even fur coats.[19]

Calculations. EPR and RQ were calculated by using previously derived formulas:[6]

$$\text{EPR}\left(\text{cal}/\text{min}\right) = 3.91 \times VO_2 + 1.10 \times VCO_2$$
$$\text{RQ} = VCO_2 \div VO_2$$

Positron emission tomography. To quantify the temperature-dependent uptake of glucose in tissue, C57BL/6J female mice (3 mice per cage) were acclimated individually to vivarium temperatures of 21 °C or were heat-supported at 31 °C in static cages with corncob bedding (Innovive) between 0900 and 1000, injected intraperitoneally with approximately 35 µCi ^{18}F-fluorodeoxyglucose, and underwent a 50-min incubation for fluorodeoxyglucose uptake. Immediately prior to imaging, mice were anesthetized by using 2% isoflurane; loaded into a sealed, heated imaging chamber; and supplied a constant flow of 2% isoflurane mixed with 100% oxygen. PET data, an X-ray radiograph, and a lateral photograph were obtained by using a preclinical PET imaging system (Genisys4, Sofie Biosciences, Culver City, CA). PET images were generated by using a maximum likelihood expectation maximization algorithm[16] for reconstruction using 60 iterations, normalized for detector response, and corrected for isotope decay and photon attenuation. Using the Mouse Atlas Registration System,[15] the

single-projection radiograph, and the lateral photograph,[24,25] we generate an atlas of mouse organ locations and a coregistered standardized ROI_{BAT} for the location of the BAT. PET images of BAT fluorodeoxyglucose uptake were analyzed (AMIDE version 1.0.1; http://freecode.com/projects/amide/releases/339498) to calculate total activity. The total fluorodeoxyglucose uptake in the ROI_{BAT} (defined according to the Mouse Atlas Registration system) was divided by the total injected dose (determined as a whole-body ROI) and expressed as a percentage of the injected dose.

Statistical analysis. The effects of interactions between environmental temperature, sex, and strain or stock on EPR, RQ, and ΔT BAT were tested by using nonlinear mixed-model ANOVA (R Studio):[20]

Y = mouse (sex, strain/stock) + sex + strain/stock + temperature + (sex × strain/stock) + (sex × temperature) + (strain/stock × temperature) + (sex × strain/stock × temperature)

The significance of differences in EPR, RQ, and ΔT BAT between environmental temperatures, sexes, and strain or stock was determined by using a posthoc Tukey test corrected for multiple comparisons (R Studio); a P value of less than 0.05 was considered to be significant. The model assumptions of normality and homogeneity were tested posthoc. The significance of differences in fluorodeoxyglucose uptake by BAT was tested by using the generalized linear model (R Studio). All values are given as least-squares means with standard errors. Because all significant differences retained significance across groups when tested as EPR and RQ data divided by the body weight 0.66, weight was excluded as a factor in our analyses.

Results

Indirect calorimetry. For all groups, EPR increased ($P < 0.001$) with progressively lower temperatures (Figure 1 A). For all groups except C57BL/6J female mice, RQ significantly ($P < 0.001$) increased as environmental temperature decreased (Figure 1 B); RQ in C57BL/6J female mice significantly ($P < 0.01$) increased as temperatures decreased from 31° to 21° (Figure 1 B). According to both ΔT BAT and EPR, female and male mice Crl:Nu-*Foxn1*^{nu} experience significantly ($P < 0.001$) greater metabolic stress than do their C57BL/6J counterparts at all tested temperatures. Nonshivering thermogenesis as measured by ΔT BAT did not vary significantly by sex among C57BL/6J or Crl:Nu-*Foxn1*^{nu} mice. Sex did not alter EPR in C57BL/6J mice, but male Crl:Nu-*Foxn1*^{nu} mice exhibited significantly ($P < 0.001$) greater energy expenditure at 21 °C and 26 °C—but not 31 °C—than did their female counterparts.

From 21 to 31 °C, EPR increased significantly ($P < 0.001$) more in male Crl:Nu-*Foxn1*^{nu} mice (175%; SE 5.51) than in male C57BL/6J mice (98% SE 7.62) and in female Crl:Nu-*Foxn1*^{nu} mice (115%; SE, 6.04) than in female C57BL/6J mice (89% SE 7.3; Figure 1 A). Similarly, RQ increased significantly ($P < 0.001$) more in Crl:Nu-*Foxn1*^{nu} mice (female: 17%; SE, 3.14; male: 26%; SE, 3.28) than in C57BL/6J mice (female: 10%; SE, 3.19; male: 20%; SE, 3.40) from 21 to 31 °C.

Thermography. In all groups, nonshivering thermogenesis (as measured by ΔT BAT) significantly ($P < 0.001$) increased as environmental temperature decreased (Figure 2 A through C).

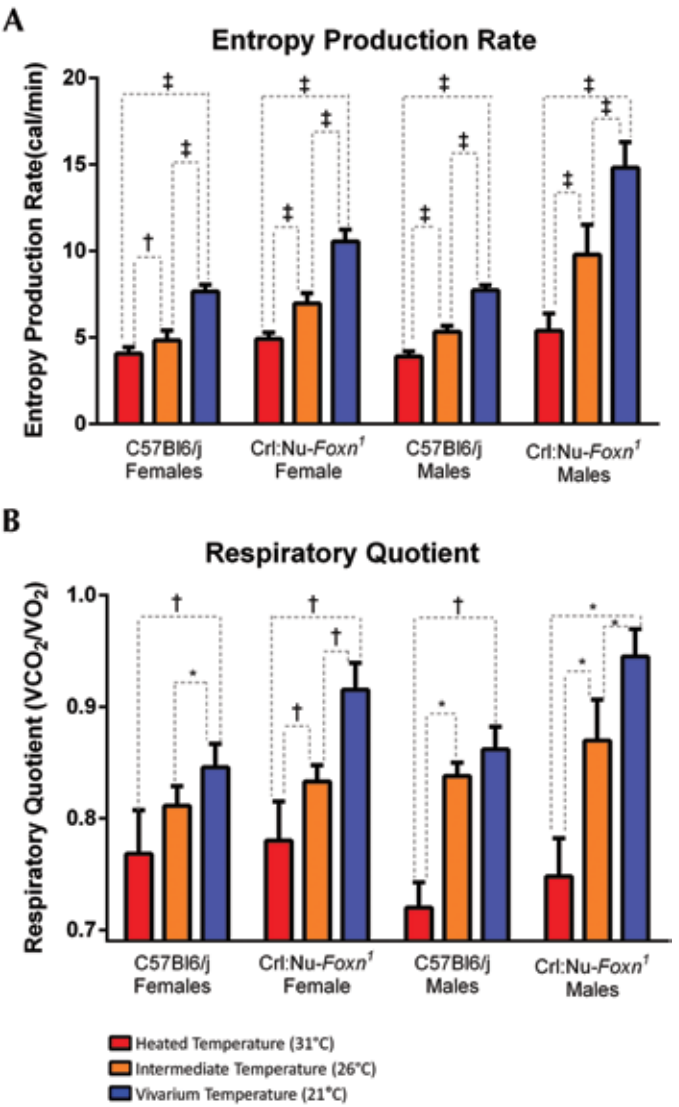

Figure 1. Routine vivarium temperatures increase energy expenditure and induce a switch to glucose-dependent metabolism in mice. (A) When exposed to routine vivarium temperatures (21 °C), mice ($n = 5$) exhibit higher energy expenditure, as measured by indirect calorimetry. (B) At routine vivarium temperatures, there is a shift in global energy fuel utilization from lipid toward carbohydrate. Errors bars represent standard error. Value significantly (*, $P < 0.050$; †, $P < 0.010$; ‡, $P < 0.001$) different from that for mice at routine vivarium temperatures.

Regardless of sex, Crl:NU-*Foxn1*^{nu} mice exhibited significantly ($P < 0.001$) greater nonshivering thermogenesis than did C57BL/6J mice.

^{18}F-Fluorodeoxyglucose uptake by BAT. ROI_{BAT} uptake of the injected dose of the glucose analog fluorodeoxyglucose significantly ($P < 0.001$) increased from 1.45% ± 0.15% at 31 °C to 3.05% ± 0.16% at 21 °C (Figure 3 A through C).

Discussion

We demonstrated that mice are physiologically stressed under routine vivarium temperatures despite being housed within the regulations set by the *Guide for the Care and Use of Laboratory Animals*.[14] Through indirect calorimetry and RQ calculations,[6] we

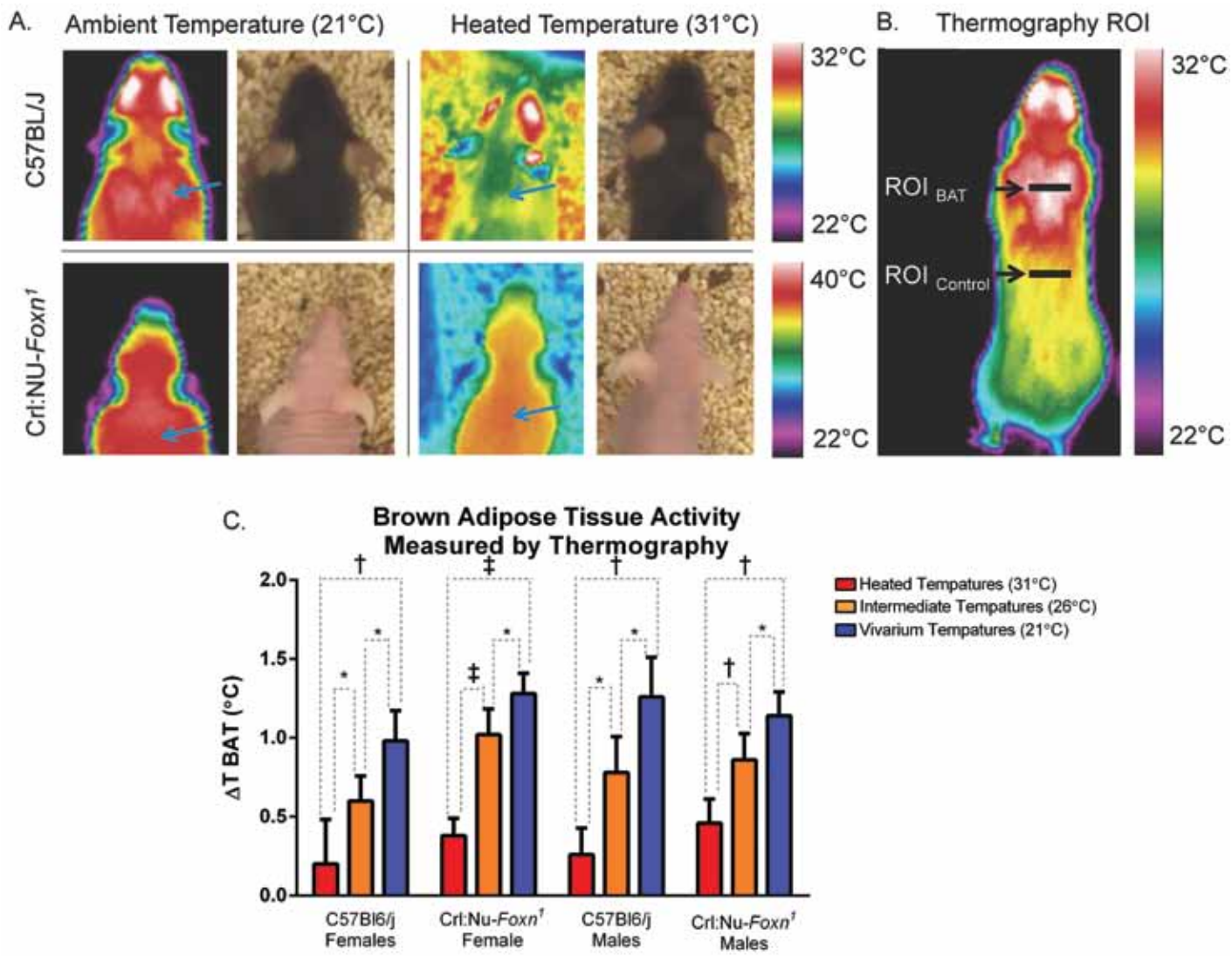

Figure 2. Routine vivarium temperatures induce nonshivering thermogenesis. (A) Mice exhibit greater nonshivering thermogenesis (blue arrow) under routine vivarium temperatures, as visualized by infrared thermography. Nonshivering thermogenesis is quantified by (B) drawing a line profile indicating a region of interest (ROI) across the brown adipose tissue (BAT) region and subtracting an internal control region (black arrows). (C) ΔT BAT is greater under routine vivarium temperatures (21 °C) than under intermediate (26 °C) and heated (31 °C) vivarium temperatures. Error bars represent standard error. Value significantly (*, $P < 0.050$; †, $P < 0.010$; ‡, $P < 0.001$) different from that for mice at routine vivarium temperatures.

show that mice respond to this temperature by fundamentally shifting their global glucose utilization. By using noninvasive in vivo ^{18}F-fluorodeoxyglucose PET imaging, we demonstrate that the metabolic shift towards glucose metabolism is being driven (at least in part) by BAT. The increased EPR under routine vivarium temperatures mirrors the temperature-dependent metabolic rate increase seen in steel-box calorimeter-based experiments.[12] Using another noninvasive imaging technology, infrared thermography, we demonstrate that nonshivering thermogenesis occurs concurrently with high glucose BAT uptake. Through these 3 noninvasive, independent metrics of metabolism, we demonstrate that routine vivarium temperatures drive high glucose metabolism to fuel nonshivering thermogenesis, which accounted for nearly half of the energy expenditures of hirsute mice housed at 21 °C.

We also demonstrate through calculations of EPR, RQ, and ΔT BAT that nude mice were significantly more metabolically stressed than were hirsute mice at all tested temperatures. For nude mice, energy expenditure at 21 °C was more than twice that at heated temperatures. Although it should be no surprise that nude mice experience greater cold stress in light of previous observations that they have high feed consumption[26] and lack of insulation,[3] "there is surprisingly little information on their thermoregulatory characteristics.[13]" These results exemplify a key and commonly underappreciated aspect of research using mice: different strains, transgenic mice with altered metabolism, and mice with different phenotypes can experience different amounts of physiologic stress relevant to the ambient temperature. Our results echo the conclusions of earlier reviews: it is difficult to draw conclusions from metabolism-dependent data from mice that experience different degrees of metabolic stress under routine husbandry conditions.[3,13]

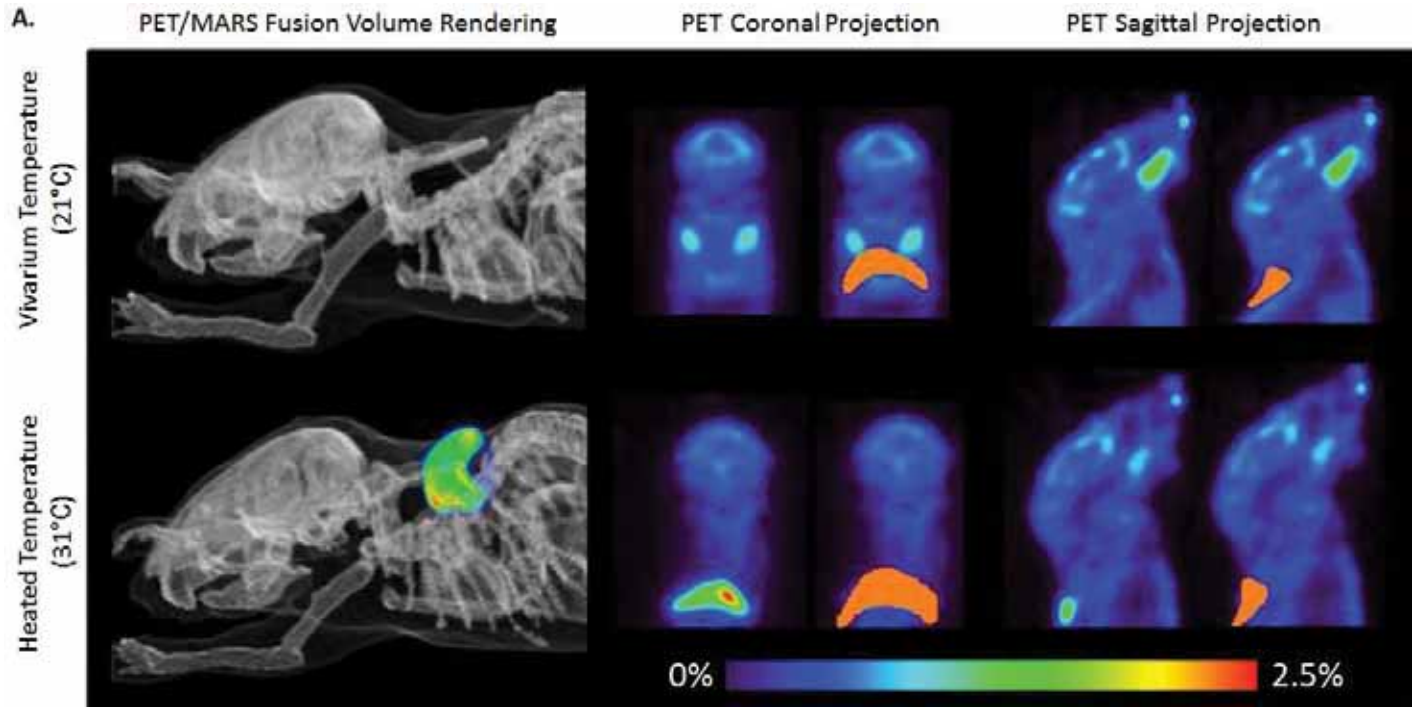

Figure 3. Routine vivarium temperatures increase glucose-dependent metabolism in brown adipose tissue. (A) Fluorodeoxyglucose PET scan volume rending merged with the Mouse Atlas Registration system and 0.2-mm slice coronal and sagittal projections demonstrate high glucose uptake in brown adipose tissue (yellow arrow) of C57BL/6J females mice at routine vivarium temperatures (21 °C) compared with heated temperatures (31 °C); a scale bar indicating percentage of injected dose is included. Standardized BAT regions (orange; generated by using the Mouse Atlas Registration system) are depicted in the right image of each pair of projections.

We should highlight several underlying assumptions of indirect calorimetry: biologic combustion of substrates is complete; animals are healthy and not in an acidotic or alkalotic state; and the stoichiometric oxidative reactions known to occur at the cellular level translate to the entire animal.[6] We reported EPR independent of weight for 2 reasons: first, because we incorporated a crossover design and used paired statistics, weight factors out between environmental groups, making it irrelevant and second, lean tissue has different metabolism than does fat.[6] Body fat percentage must be measured to appropriately quantitate EPR per unit of body weight.[2] Quantification methods such as CT and tissue-level methods rely on imperfect assumptions and add statistical noise through propagation of error. This statistical noise can overpower biologic signals, especially in very small animals.[2] For these reasons, we reported EPR on a per-animal basis, as recommended in an earlier review.[2]

Chronic cold stress is a major, systemic confounder in rodent research, and the laboratory animal community should devise ways to address this issue. The scientific community already knows that some murine models, such as the LPS-induced fever model[22] and cardiovascular research,[23] are markedly affected by mild cold stress. Cold stress limits the ability of these models to emulate human pathophysiology. Beyond the few known affected models, the potential effect of chronic cold stress on research using mice is staggering. Rather than investigating cold stress in a piecemeal fashion for each field of science, addressing the issue in a systemic, vivarium-wide fashion is a more prudent approach.

The laboratory animal community is ideally suited to quantify and address the issue of chronic cold stress in laboratory mice. One possible solution is raise macroenvironment (room) temperatures, although this practice risks heat exhaustion as temperatures approach thermoneutrality,[14] and it may prove difficult to accommodate the diverse metabolic phenotypes of laboratory mice solely by raising room temperature.[13] Complicating the matter, life stage, age, pregnancy, and lactation change the thermoneutral zone.[13] Other extrinsic factors not tested here, such as drafts from ventilated caging systems,[1] also may alter the thermoregulatory needs of mice. Given that mouse thermoneutral zones only span 1 to 3 °C[13] and in view of the diversity in housing systems and murine metabolic phenotypes, raising the housing temperature to any one specific value cannot meet the requirements for all conditions. A second approach is to create temperature gradients at the microenvironment level to allow the mice to dynamically choose appropriate temperature ranges.[2] Although feasible, this approach likely would be expensive, given the need to invest in new housing systems.

The third and most promising approach is to provide increased cage complexity—shelters, more malleable bedding (as compared with corncob bedding), and nesting material—to allow mice to behaviorally thermoregulate. A series of experiments demonstrated that thermotactic behavior could largely be mitigated by adding nesting material to the cage.[9] The most promising solution is providing additional nesting material, which alleviates heat-seeking behavior and promotes species-specific behavior (nest building), is inexpensive, and can readily be incorporated into current husbandry pratices.[9]

This is an exciting and unique time for the laboratory animal community, because we now have tools to assess novel strategies for mitigating cold stress. With the proliferation of PET and thermography technology, we can noninvasively measure in vivo metabolism and evaluate new approaches. Thermography is espe-

cially exciting when compared with traditional methods used to measure metabolism (for example, bomb calorimeter) because it allows researchers to assay metabolism in the mouse's home cage. To our knowledge, this report represents the first use of thermography to evaluate cold-induced activation of BAT with regard to housing practices of laboratory rodents. We hope the methods we have outlined will act as a guideline of how to use these technologies to assay the effects of housing modifications on murine physiology.

Acknowledgments

We recognize and thank the numerous people involved in the development of this manuscript: Dr Kenneth Roos for consultation on indirect calorimetry and for providing essential equipment to perform the experiments, Dr Waldemar Ladno and Darin Williams for operation and expertise on imaging equipment, Alexandra David for preparation of figures, and Gautam Gustav Bastian for statistical consultation. We also thank the Crump Institute for Molecular Imaging at UCLA for providing laboratory space and the Division of Laboratory Animal Medicine for maintaining the health of the animals.

References

1. **Baumans V, Schlingmann F, Vonck M, van Lith HA**. 2002. Individually ventilated cages: beneficial for mice and men? Contemp Top Lab Anim Sci **41:**13–19.
2. **Cannon B, Nedergaard J.** 2004. Brown adipose tissue: function and physiological significance. Physiol Rev **84:**277–359.
3. **Cannon B, Nedergaard J.** 2011. Nonshivering thermogenesis and its adequate measurement in metabolic studies. J Exp Biol **214:**242–253.
4. **Celi FS.** 2009. Brown adipose tissue—when it pays to be inefficient. N Engl J Med **360:**1553–1556.
5. **DeRuisseau LR, Parsons AD, Overton JM.** 2004. Adaptive thermogenesis is intact in B6 and A/J mice studied at thermoneutrality. Metabolism **53:**1417–1423.
6. **Ferrannini E.** 1988. The theoretical bases of indirect calorimetry: a review. Metabolism **37:**287–301.
7. **Foster DO, Frydman ML.** 1979. Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. Can J Physiol Pharmacol **57:**257–270.
8. **Fueger BJ, Czernin J, Hildebrandt I, Tran C, Halpern BS, Stout D, Phelps ME, Weber WA.** 2006. Impact of animal handling on the results of 18F-FDG PET studies in mice. J Nucl Med **47:**999–1006.
9. **Gaskill BN, Gordon CJ, Pajor EA, Lucas JR, Davis JK, Garner JP**. 2013. Impact of nesting material on mouse body temperature and physiology. Physiol Behav **110-111:**87–95.
10. **Gaskill BN, Rohr SA, Pajor EA, Lucas JR, Garner JP.** 2009. Some like it hot: mouse temperature preferences in laboratory housing. Appl Anim Behav Sci **116:**279–285.
11. **Geiger R, Aron RH, Todhunter P.** 2009. The climate near the ground. Landam (MA): Rowman and Littelfield Publishers.
12. **Gordon CJ.** 1985. Relationship between autonomic and behavioral thermoregulation in the mouse. Physiol Behav **34:**687–690.
13. **Gordon CJ.** 2012. Thermal physiology of laboratory mice: defining thermoneutrality. J Therm Biol **37:**654–685.
14. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
15. **Jobe JB, Smith DM, Ball K, Tennstedt SL, Marsiske M, Willis SL, Rebok GW, Morris JN, Helmers KF, Leveck MD, Kleinman K.** 2001. ACTIVE: a cognitive intervention trial to promote independence in older adults. Control Clin Trials **22:**453–479.
16. **Lange K, Carson R.** 1984. EM reconstruction algorithms for emission and transmission tomography. J Comput Assist Tomogr **8:**306–316.
17. **Lodhi IJ, Semenkovich CF.** 2009. Why we should put clothes on mice. Cell Metab **9:**111–112.
18. **Norton P, Horn S, Pellegrino JG, Perconti P**. 2006. Infrared detectors and detector arrays. In: Bronzino JD, editor. Medical devices and systems. Boca Raton (FL): CRC Press.
19. **Overton JM, Williams TD.** 2004. Behavioral and physiologic responses to caloric restriction in mice. Physiol Behav **81:**749–754.
20. **Pinheiro J, Bates D**. 2000. Mixed-effects models in S and S-PLUS. Basel (Switzerland): Springer.
21. **Purohit RC, McCoy MD.** 1980. Thermography in the diagnosis of inflammatory processes in the horse. Am J Vet Res **41:**1167–1174.
22. **Rudaya AY, Steiner AA, Robbins JR, Dragic AS, Romanovsky AA.** 2005. Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. Am J Physiol Regul Integr Comp Physiol **289:**R1244–R1252.
23. **Swoap SJ, Overton JM, Garber G.** 2004. Effect of ambient temperature on cardiovascular parameters in rats and mice: a comparative approach. Am J Physiol Regul Integr Comp Physiol **287:**R391–R396.
24. **Wang H, Stout DB, Chatziioannou AF**. 2012. Mouse atlas registration with nontomographic imaging modalities—a pilot study based on simulation. Mol Imaging Biol **14:**408–419.
25. **Wang H, Stout DB, Taschereau R, Gu Z, Vu NT, Prout DL, Chatziioannou AF.** 2012. MARS: a mouse atlas registration system based on a planar X-ray projector and an optical camera. Phys Med Biol **57:**6063–6077.
26. **Weihe WH.** 1984. The thermoregulation of the nude mouse. Exp Cell Biol **52:**140–144.
27. **Williams TD, Chambers JB, Henderson RP, Rashotte ME, Overton JM.** 2002. Cardiovascular responses to caloric restriction and thermoneutrality in C57BL/6J mice. Am J Physiol Regul Integr Comp Physiol **282:**R1459–R1467.