

Opinion

Thermoneutrality, Mice, and Cancer: A Heated Opinion

Bonnie L. Hylander¹ and Elizabeth A. Repasky^{1,*}

The ‘mild’ cold stress caused by standard sub-thermoneutral housing temperatures used for laboratory mice in research institutes is sufficient to significantly bias conclusions drawn from murine models of several human diseases. We review the data leading to this conclusion, discuss the implications for research and suggest ways to reduce problems in reproducibility and experimental transparency caused by this housing variable. We have found that these cool temperatures suppress endogenous immune responses, skewing tumor growth data and the severity of graft versus host disease, and also increase the therapeutic resistance of tumors. Owing to the potential for ambient temperature to affect energy homeostasis as well as adrenergic stress, both of which could contribute to biased outcomes in murine cancer models, housing temperature should be reported in all publications and considered as a potential source of variability in results between laboratories. Researchers and regulatory agencies should work together to determine whether changes in housing parameters would enhance the use of mouse models in cancer research, as well as for other diseases. Finally, for many years agencies such as the National Cancer Institute (NCI) have encouraged the development of newer and more sophisticated mouse models for cancer research, but we believe that, without an appreciation of how basic murine physiology is affected by ambient temperature, even data from these models is likely to be compromised.

Room Temperature: So Much More Than a Thermometer Reading

Thermoregulation has been a topic of intense interest to physiologists for over a century and it is now well understood that maintenance of a constant core body temperature requires significant vascular, neural, and biochemical activity as well as conscious and unconscious behavioral adaptations all of which depend on ambient temperature [1–4]. Physiologists have found major differences between the thermal physiology of mice, other mammals, and humans [3,5–12], such that at ambient temperatures in which humans feel comfortable, mice experience chronic mild cold stress directly impacting their metabolism and thermoregulatory status. Laboratory mice are almost always housed and studied at mildly cool temperatures well below their ‘thermoneutral zone’ which is the ambient temperature in which metabolic heat production is minimal and the mouse does not need to work to keep warm or cold. It is the ambient temperature at which a stable core temperature can be maintained by the basal metabolism of the animal at rest and by ‘adjustments in insulation, posture, and skin blood flow’ [6]. For healthy mice, this **thermoneutral temperature** (TT, see Glossary) is generally 30–32 °C, and it has been known for many decades that, given a choice, mice will choose to spend the majority of time in this range [13].

Importantly, mice rely on metabolic heat generation by ‘adaptive **thermogenesis**’ to a much greater degree than do humans. Adaptive thermogenesis, also known as non-shivering thermogenesis, is a mechanism of metabolic heat production which involves stimulation of the

Trends

Several mouse models show significant differences in experimental outcomes at standard sub-thermoneutral (ST, 22–26 °C) versus thermoneutral housing temperatures (TT, 30–32 °C), including models of cardiovascular disease, obesity, inflammation and atherosclerosis, graft versus host disease and cancer.

NE levels are higher, anti-tumor immunity is impaired, and tumor growth is significantly enhanced in mice housed at ST compared to TT. NE levels are reduced, immunosuppression is reversed and tumor growth is slowed by housing mice at TT.

Housing temperature should be reported in every study such that potential sources of data bias or non-reproducibility can be identified.

Our opinion is that any experiment designed to understand tumor biology and/or having an immune component could potentially have different outcomes in mice housed at ST versus TT and this should be tested.

¹Roswell Park Cancer Institute, Department of Immunology, Elm and Carlton Streets, Buffalo, NY 14263-0001, USA

*Correspondence: elizabeth.repasky@roswellpark.org (E.A. Repasky).

sympathetic nervous system to release norepinephrine (NE) and epinephrine, resulting in increased metabolic activity needed for heat generation in brown adipose tissue (BAT) [10,12]. NE binding to **adrenergic receptors** on brown fat cells causes increased expression of uncoupling protein 1 (UCP-1) in the mitochondria such that most of the energy produced by oxidative respiration immediately generates heat rather than ATP. Indeed, it was shown long ago that heat production through this form of thermogenesis in mice can be triple that of basal metabolism, and is greater than any other animal investigated [14,15]. Living with chronic cold stress and the need for increased metabolic production of heat has considerable physiologic consequences because for each 1 °C drop in ambient temperature it takes about 46.3 kcal/m²/24 h to maintain normal body temperature [13]. Because of the large energetic drain associated with adaptive thermogenesis, mice in nature actively strive to minimize heat loss under cold conditions by seeking thermoneutral temperatures, building warmer nests, and aggregating in larger numbers. Thyroid hormone also plays a crucial role in adaptive thermogenesis. The full thermogenic response of BAT requires ligation of nuclear thyroid hormone receptors (transcription factors) which increases the sensitivity of BAT cells to adrenergic stimulation allowing UCP-1 expression [16,17].

While it is obvious that mice have a very small body surface area to volume ratio compared to humans, it is not generally realized that this results in rapid heat loss to their environment whenever ambient temperature falls below thermoneutrality. In addition, because of their small size, mice cannot bear enough fur or fat insulation to prevent heat loss to their environment. This fact underlies the approximately sevenfold higher metabolic rate of a mouse compared with that of a human [18]. Moreover, it is important to take into consideration that thermogenesis is accompanied by increased production of NE [10,19] which, because of the widespread distribution of adrenergic receptors on different cell types, can have pleiotropic effects.

What About Mice Housed in Colonies for Research: *In Vivo Veritas*?

In the laboratory, mice are able to consume unlimited food to meet the energetic requirements of adaptive thermogenesis. However, Martin *et al.* [20] have voiced strong concerns that ‘control’ mice are in fact ‘sedentary, obese and glucose intolerant’ and cautioned that this has great potential to ‘confound data interpretation on outcomes of human studies.’ The authors note that lack of exercise and unlimited access to food are major culprits. Others have recently begun to recognize the underlying role of cool ambient temperatures in causing overeating and metabolic disorders [7,21,22]. To maintain laboratory mice under conditions ‘judged to be scientifically, technically and humanely appropriate’, guidelines are put forth in an internationally recognized document, *The Guide for the Care and Use of Laboratory Animals* [23], produced by the National Research Council (NRC). The Guide governs all aspects of housing conditions for experimental animals in research facilities (e.g., nutrition, ambient temperature, housing density, etc.) and states that mice should be housed between 20–26 °C. While a mildly cool room temperature helps to maintain the thermal comfort of animal care workers, it is also thought to be beneficial for mouse husbandry because it results in increased litter size and viability [15]. However, it was recently published that mouse reproductive fitness is actually maintained even when they are housed up to 28 °C [24]. What is not generally appreciated is the fact that the Guide states in several different places that variations in these microenvironmental housing factors could affect behavior, physiology (reproduction), phenotype and, possibly, experimental outcomes. However, because these decisions are under the purview of the animal care staff, they are ‘under the radar’ of most researchers and are not generally considered in terms of experimental outcomes. This dichotomy between the mandated, standard temperature (**sub-thermoneutral temperature**, ST) and the thermoneutral temperature (TT) is acknowledged in the *Guide*, which recommends that mice be provided with nesting materials or shelter (see also [25,26]). However, these measures are not always in place, and even when they are, mice are often seen to ‘huddle’ to minimize heat loss and exhibit signs of cold stress [6].

Glossary

Adrenergic receptors: a family of G-protein-coupled receptors present on the surface of almost all cells in the body, including immune cells and cancer cells. Differential receptor expression on different cell types mediates different responses. Elevation in norepinephrine (NE) during chronic cold stress thus has potential to act on many cell types in addition to adipose tissue because NE is widely released during a sympathetic response.

Energy balance: this describes the overall comparison between intake of energy resources (e.g., from diet) and the expenditure of energy (e.g., for immune responses, work/exercise, or thermoregulation).

Sub-thermoneutral temperatures (ST): environmental temperatures which are below the thermoneutral zone. At these temperatures, mice experience chronic cold stress and must expend large amounts of energy to generate enough heat to maintain normal body temperature. Mice use adaptive thermogenesis primarily to generate sufficient heat to maintain body temperature, whereas humans rely largely on normal metabolism and vascular activity because they are able to adjust their thermal environment to reduce cold stress.

Thermogenesis: the production of heat by an animal. Maintenance of normal body temperature is a crucial homeostatic requirement for survival. When an animal is cold, the brain initiates behavioral responses (e.g., moving to a warmer location) and neural responses (thermogenesis) to conserve and generate heat. Shivering is initiated for short-term heat production in skeletal muscle. Non-shivering (adaptive) thermogenesis occurs in brown adipose tissue (BAT) which expands with chronic exposure to cold. The primary driver of thermogenesis is NE released from sympathetic nerves in BAT.

Thermoneutral zone or temperature (TT): the temperature range within which the heat produced as a byproduct of normal metabolism alone, combined with blood flow movements from the core to the surface of the body, enables an animal to maintain a normal core body temperature of approximately 37 °C. For mice this is in the range of

Researchers have noted a variety of significant differences in mice housed at ST versus TT, including differences in basal metabolism [12], cardiovascular physiology [27,28], the size of organs and tail length [29], and the effects of hypoxia on lipolysis during thermogenesis [30]. Physiological responses such as heart rate and metabolic rate have a linear relationship with the ambient temperature (see review by Maloney *et al.* [22]). We have focused here on comparisons between experimental mice at only ST and TT because differences in experimental models have been recently documented. In the future, it will also be important to determine experimental outcomes at intermediate temperatures. In addition to room temperature, mouse housing density is also known to affect stress levels [31,32] and, because temperatures in the cage are altered by the number of mice/cage [33], there is great opportunity for variation in the degree of chronic cold stress experienced during the course of the experiment. Therefore, investigators should be aware that removing mice during an experiment may affect metabolism, as well as many of the other temperature-sensitive biological/physiological responses that are discussed below. This likely contributes to experimental variability between experiments and labs. Changes in fat tissue also accompany cold stress. In general, BAT increases activity and volume with cold stress [10,34–37], and recently it has been recognized that cells in white adipose tissue (WAT) can be converted to a brown phenotype by cold exposure [38] (reviewed by [39]). Smith *et al.* have recently visualized significant differences in BAT in mice at ST and TT showing that the lipid to water ratio in BAT is reduced in colder temperatures in accord with its role in thermogenesis [40]. In addition, an important role for a layer of dermal white adipose tissue (dWAT) in thermogenesis has been described [41], and it will be important to determine how this fat depot may change between ST and TT. The data discussed below show that the chronic mild cold stress imposed by housing mice at ST versus TT is a fundamental factor affecting murine models of tumor growth and antitumor immunity.

30–32 °C. However, mice are housed at temperatures below this zone in research colonies. The standard ambient housing temperature for research mice is sub-thermoneutral at 20–26 °C, with most institutes using a range from 21–23 °C. In most cases the ambient room temperature used is not reported in the literature.

Feeling Cold, Tumor Immunology, and Therapeutic Efficacy – Not a Good Forecast

We recently compared tumor growth at ST and TT in several syngeneic and carcinogen-induced murine tumor models and found that tumors grow significantly more slowly when mice are housed at TT instead of ST [42]. In addition, spontaneous lung metastases of 4T1 were also significantly reduced in mice at TT. However, when tumors were grown in immunodeficient mice, no differences in growth occurred, implicating the adaptive immune response in improved tumor control at TT. In experiments in which CD8⁺ T cells were depleted, the beneficial effect of TT was lost. At TT, tumors and draining lymph nodes contained significantly greater numbers of CD8⁺ T cells including antigen-specific cells. There were also greater numbers of activated CD8⁺ T cells at TT as judged by CD69, IFN γ , and Glut-1 expression. On the other hand, populations of Tregs (FoxP3⁺ cells) and myeloid-derived suppressor cells (MDSC: CD11b⁺GR-1⁺) were significantly higher at ST than at TT. Thus, the ability of mice to control tumor growth under optimized thermal physiology may be much greater than currently appreciated based on experiments carried out at ST. This shift in the balance of effector and suppressor cells matters because researchers spend considerable time and effort testing ways to improve antitumor immunity, and it is probable that housing conditions themselves are a significant contributing factor to immunosuppression. At thermoneutrality the mechanisms underlying successful antitumor immune responses may differ from those mechanisms at ST. For example, recent evidence shows that exposure of mice to mild cold stress (ST) affects the differentiation pathway of macrophages such that IL-4-mediated ‘alternative activation’ of adipose tissue macrophages (which secrete NE) occurs to promote thermogenesis in BAT and lipolysis in WAT [43]. These alternatively activated macrophages could themselves be another source of immunosuppression.

We also found that NE levels were higher in tumors of mice at ST compared to TT [44]. In more recent work, M. Bucsek *et al.* (manuscript in preparation) find that, at ST, tumor growth can be significantly delayed by administration of propranolol (a β -adrenergic receptor antagonist), and

this effect is also CD8⁺ T cell-dependent, suggesting a causative role for NE-mediated stress signaling in dampening antitumor immunity. In addition to a role for adrenergic stress in causing immunosuppression, there may also be a role for abnormal **energy balance** in mildly cold-stressed mice. It has been noted by others [45] that the increase in fuel consumption required for activation of the immune system (up to 2000 kJ/day) is enough to disrupt most other homeostatic needs of the body. It is likely that with the burden of rapid tumor growth, expansion of immune cell populations would compete for energy needed for thermogenesis such that, ultimately, immunosuppression may protect the ability of the organism to maintain body temperature. Much more research will be necessary to dissect these potential mechanisms and their role in ultimately shaping an effective antitumor immune response. Related to this point, it is important to note that, in all of our experiments comparing ST with TT, the core temperatures of mice maintained at 22 °C remained normothermic for several weeks. Finally, while core temperature (approximately 37 °C) was also found to be similar in mice held at 20 °C and 25 °C by Uchida *et al.* [46], these authors found that skin temperature is lower in mice held at 20 °C. Thus, researchers should be aware that the tumor microenvironment of subcutaneous tumor models in mice held at ST could be hypothermic while in mice held at TT, the temperature of a subcutaneous tumor could be 30 °C if not higher because the tumor may be perfused by core temperature blood circulating to the surface for heat loss (although this would still be cooler than normal body temperature). The actual impact of internal tumor temperature in different sites of the body on tumor growth has not yet been addressed.

Because dendritic cell (DC) function is crucial for T cell activation, our group also examined how housing temperature might impact these antigen-presenting cells. The results suggest that activated DCs from tumor bearing mice at ST are less able to stimulate T cell proliferation than are DCs from tumor-bearing mice at TT [47]. Together, these studies from our group show that the balance of pro-tumorigenic cells (Tregs, MDSC) and antitumorigenic cells (CD8⁺ CTLs) is different at ST and TT, while antigen presentation may be suppressed at ST leading to reduced immune surveillance.

While the above discussion deals with immune responses in cancer, it is important to consider that the degree of autoimmunity is also likely to be influenced by housing temperature. We would predict, for example, that autoimmunity, which is often expected following immunotherapy for cancer, may be enhanced at TT but suppressed in mouse models at ST.

Being Too Cold Can Be Helpful in Diseases in Which Immunosuppression Is Desired

While there is a need to stimulate antitumor immunity for control of tumor growth, in other diseases, such as in graft versus host disease (GVHD) following allogeneic hematopoietic cell transplantation, suppression of the immune response is beneficial. There is a significant research effort to develop treatments for GVHD using mouse models. However, it has been known for decades that the transplantation of T cells derived from murine bone marrow alone is not sufficient to develop the severity of GVHD seen in patients. To achieve that degree of GVHD, researchers must also transplant additional T cells (from spleen or lymph nodes). This suggests differences in the mechanisms of GVHD between the mouse model and human patients. However, our group has recently published [48] that severe GVHD does develop from transplant of bone marrow alone (i.e., without additional T cells) if mice are housed at TT instead of ST or if mice at ST are treated with a β 2-AR receptor antagonist. Furthermore, administering a β 2-AR agonist to mice at TT suppresses development of GVHD. These results suggest that elevated NE levels at ST interfere with obtaining a full understanding of the GVHD phenotype in this mouse model, and again demonstrate that immunological responses are generally impaired in mice housed at ST. Further research will be necessary to determine how reducing GVHD through stress signaling could impact possible tumor recurrence in this model.

Can Mild Cold Stress Alter Apoptosis Signaling and Chemotherapeutic Efficacy?

With regard to mild cold stress (and resultant adrenergic signaling in tumor cells), Eng *et al.* [44] found that the level of NE in pancreatic tumors of mice at ST was significantly higher than in tumors of mice at TT. This increased adrenergic signaling induced increased expression of anti-apoptotic molecules, including Bcl-XI, Bcl-2, and Mcl-1, as well as phosphorylated BAD, at ST compared to TT. These changes were recapitulated by treatment of cell lines *in vitro* with a β -AR agonist, thus confirming a direct effect of adrenergic signaling on tumor cells themselves. The tumor-promoting effect of adrenergic signaling has been reviewed recently [49,50], but the ability of ST to cause this degree of stress is generally unrecognized. Moreover, tumors in mice housed at TT were significantly more sensitive to Apo2L/TRAIL, cisplatin, and nab-paclitaxel (Abraxane) than were tumors in mice at ST. Treating tumor-bearing mice housed at ST with propranolol decreased the expression of anti-apoptotic molecules and sensitized tumors to therapy to the same degree as TT housing. Thus, the 'mild' cold stress of standard housing induces a type of environmentally mediated therapeutic resistance similar to sources discussed by others [51]. Along this line, it is relevant that Pasquier and colleagues [52,53] have also recently reported that relieving adrenergic stress in mice at ST improves the antitumor efficacy of chemotherapy, although they did not also conduct experiments at TT. Overall, our data reveal that the elevated NE caused by chronic stress at ST is sufficient to bias the outcomes of experimental therapeutics testing. Because it is becoming clear that the long-term overall efficacy of chemotherapy [54–56], radiation [57], and photodynamic therapy [58] involves the generation of an antitumor immune response, investigators should be aware that, under standard conditions, these responses could be very different at ST and TT.

Cancer, Inflammation, and Metabolic Disorders: Are Mouse Models Too Cold?

In addition to antitumor immunity, cancer research has also pointed to a major role for dysregulated inflammation and metabolism in promoting tumor growth. Nevertheless, are these linkages being influenced by cold stress? A large body of literature links increased dietary fat intake with obesity and cancer progression [59], but it is becoming clear that factors affecting obesity are altered at ST. For example, a UCP-1 knockout mouse model showed deficits in cold-induced non-shivering thermogenesis as expected, but failed to develop the expected obesity phenotype [60,61]. Several groups have now shown that this unexpected lack of the predicted phenotype was because the mice were housed at ST, and when these mice are housed at TT (and are not burning energy through adaptive thermogenesis) they do become obese [61–64]. This effect of ST on obesity has been observed in other models as well [65–67], and it is clear that precise data on the linkage between diet, obesity, and cancer should be re-examined in the context of absence of cold stress. Interestingly, Koizumi and colleagues showed that the reported ability of dietary restriction to reduce the incidence of lymphoma in mice only occurred at ST, and that this effect was lost in mice housed at TT [68], again demonstrating a dichotomy of experimental results at ST versus TT.

Other recent literature demonstrates the links between inflammation and cancer progression [69]. However, the development of inflammation is found to be highly sensitive to cold stress in mice, and exclusively using cold-stressed mice for this research may provide an incomplete picture of these linkages. For example, Tian *et al.* [70] found that, when mice were housed at TT instead of ST, metabolic inflammation was exacerbated, and they found increased inflammation in white adipose tissue and vasculature at TT, which promoted atherosclerosis. These authors conclude that their results 'point to how thermal stress might limit our ability to faithfully model human diseases in mice' [70]. In an accompanying commentary, Ravussin reinforces the point that 'ambient temperature clearly affects phenotypes related to energy homeostasis in rodents' [71]. Interestingly, in addition to being the stress hormone which drives thermogenesis, NE is

also known to inhibit insulin secretion from the pancreatic islets [72] and, notably, Uchida *et al.* [46] found that mice housed at 20 °C had elevated plasma NE in comparison to mice held at 25 °C. These authors also found that a cooler temperature resulted in lower insulin and higher glucose levels and an impaired response in a glucose tolerance test. While 25 °C is still well below thermoneutrality, these data are very important because 20 and 25 °C are both within allowable ambient temperatures as stated by the NRCC Guide [23], highlighting the possibility of significant data variability between laboratories which are following the current guidelines.

Other aspects of insulin resistance and inflammation may need to be reexamined: while Tian *et al.* [70] found increased inflammation in mice at TT, which was associated with promoting atherosclerosis, they did not see an expected further increase in insulin resistance. Together, these data support the opinion that basic assumptions related to obesity, inflammation, and other metabolic disorders and their impact on cancer may differ when mice are housed at TT.

Elenkov *et al.* [73,74] reported that stress hormones induce a shift in pro- and anti-inflammatory cytokines to support a Th2 humoral response and suppress the Th1 cellular response. In mice, it was recently shown that chronic exposure to a stressful sound raised levels of stress hormones (corticosterone and NE) and altered levels of key cytokines, reflecting a shift from a Th1 to a Th2 response, and promoted colon cancer progression [75]. The integral role of inflammation (including inflammatory immune cells, cytokines, and chemokines) in promoting all stages of cancer development and growth is widely recognized and has been recently reviewed [76–78]. In view of the fact that we found significant differences in the immune contexture of tumor-bearing mice at ST and TT, further investigation into how ambient temperature may affect immune cell and cytokine/chemokine networks (profiles) is warranted. At least two studies have reported differences in the induction of cytokines in response to immune challenge [66,79]. Intriguingly, Straub *et al.* [45] outline the possible relationships between chronic inflammatory diseases (which demand that increased energy resources are shifted to the immune response) and many metabolic abnormalities. We can only imagine how these relationships could be exacerbated under conditions of competition for energy when mice are chronically cold stressed.

Concluding Remarks

The outcomes of preclinical mouse studies form the basis for understanding disease biology, immune and therapeutic responses, and determining which therapies to take into clinical trials [80–83]. Many authors have asserted that a major problem in research is irreproducibility [80,81,84,85]. Landis and colleagues point to the general dearth of information on the ‘design, conduct and analysis of the experiments’ [86]. These authors suggest that ‘a core set of research parameters must be defined and should be addressed when reporting the results of animal experiments’ and stated that a ‘concerted effort by all stakeholders, including funding agencies and journals, will be necessary to disseminate and implement best reporting practices throughout the research community.’ This viewpoint is echoed in a recent editorial by the editors of *Nature Neurobiology* who wrote: ‘Factors such as animal housing, handling, food, lighting and noise conditions, all of which effect behavior and brain chemistry, can be varied. The key to reproducibility is accurate reporting of these seemingly mundane details, which potentially have large effects’ [87]. Although housing temperature was not included as a factor in these analyses, studies are accumulating in which experimental outcomes differ depending on the ambient temperature; these are summarized in Figure 1 [7,10,11,19,27,28,40,42,44,46,48,63,66,67,70,79,84,88–94]. In fact, new National Institutes of Health (NIH) requirements regarding Rigor and Transparency in Research specifically mention room temperature as a Relevant Biological Variable that may need to be addressed when designing experiments with mice. Our opinion is that new research must define the full implications of housing-induced thermal stress, not only in cancer research but in all

Outstanding Questions

How important for cancer research are the differences in experimental outcomes in mice maintained at ST versus TT? Would current paradigms regarding key determinants of antitumor immunity be revised if the experiments were repeated at TT?

With the knowledge that mice at ST are significantly immunosuppressed, would it be more informative to test and study immunotherapies at TT?

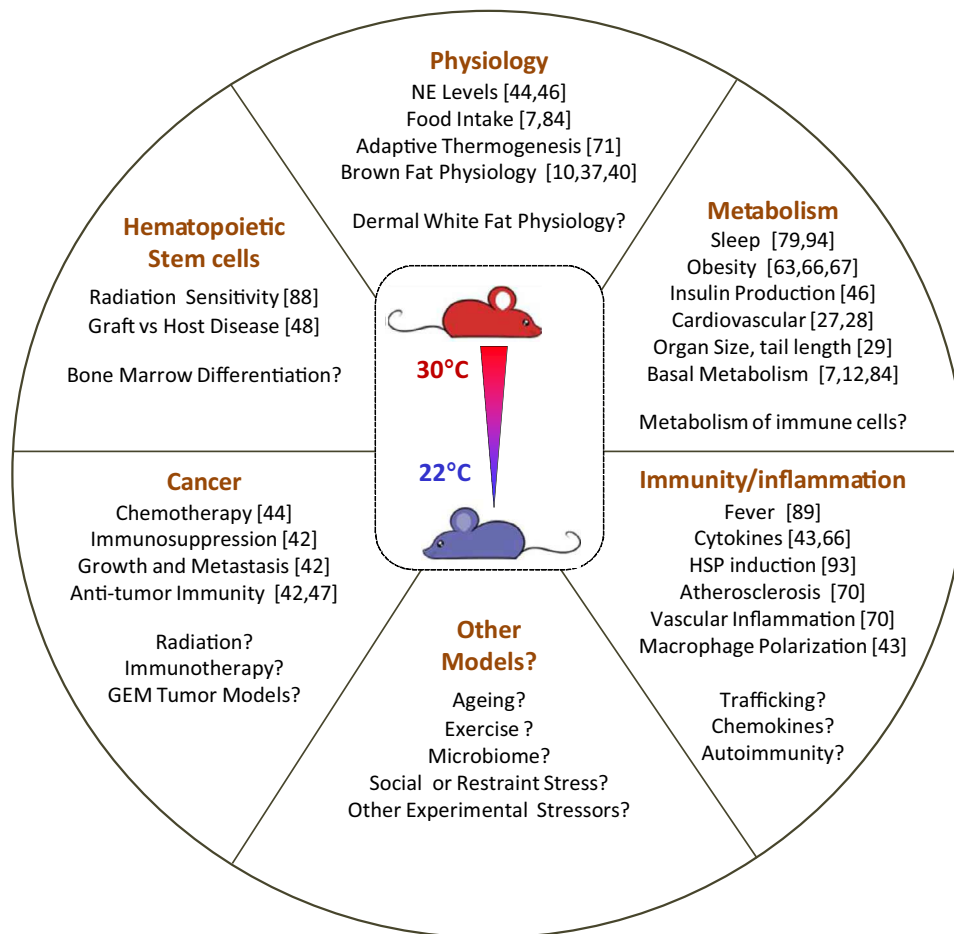
Would conducting experiments under more than one set of conditions improve translation of knowledge to the clinic and lead to improved patient care?

Are there biomarkers of ‘stress’ in mice (e.g., NE levels) that could be helpful for researchers to assess the degree of cold stress present in their particular mouse model? Could these biomarkers be helpful in predicting the responses of patients to immunotherapy, chemotherapy, and radiation? Is there an adjuvant role for β -AR antagonists or agonists in combination with other therapies?

Given the current configuration of animal facilities and caging systems, and the need for thermal comfort of animal care staff, what are practical approaches to carrying out experiments at ST and TT? Can new caging systems be developed to allow mice to naturally reduce cold stress levels?

How do variations in environmental factors between experiments, labs, and institutions affect the reproducibility of cancer research?

In a mechanisms-based, hypothesis-driven funding environment, what is the best way to support experiments which focus on determining differences in experimental outcomes caused by standard, IACUC regulated, environmental factors?



Trends in Cancer

Figure 1. Mouse Models Used To Study Human Diseases Are Influenced by Housing Temperature. Depicted here is the wide variety of phenotypes that have been reported to differ when mice are housed under standard temperatures ($\sim 22^{\circ}\text{C}$) versus thermoneutral temperatures ($\sim 30^{\circ}\text{C}$) (followed by references [7,10,12,27–29,37,40,42–44,46,47,63,66,67,70,71,79,84,89,93,94]). Several other conditions or phenomena that are modeled in mice and are likely to be influenced by housing temperature, but for which there is little or no published information are also listed (followed by ?). Abbreviations: GEM, genetically engineered mice; HSP, heat shock proteins.

biomedical applications, and how this condition may influence decisions for translation to the clinic (see Outstanding Questions). It is likely that many, if not all, of the metabolic abnormalities noted previously in mouse models stem largely from chronic mild cold stress and the increase in adaptive thermogenesis. Given the great need to enhance the reliability of mouse models, we advocate recognition of this potential problem and routine reporting of the ambient temperature at which experiments are conducted. Given that the degree of cold stress experienced by mice can also be affected by caging density and by the presence or absence of nesting materials, this information should also be reported under housing conditions.

It would also be very beneficial if researchers could assess the degree of cold stress in their models by measuring biomarkers, such as the level of stress hormones, especially NE, such that differences in experimental outcome may be better quantified. Comparisons of BAT by MRI could also prove to be a valuable and relevant biomarker of the degree of cold-stress in experimental mice at different ambient temperatures [40].

In the long-term, addressing questions of how housing parameters influence the basic physiology of mice used to model human disease will require involvement and financial support from agencies such as the NIH, where funding is typically devoted to questions related to mechanism of disease and therapeutic efficacy, and the FDA, where mice are used to validate and regulate immunotherapies for cancer. Moreover, organizations such as the NRC and the AALAS (American Association for Laboratory Animal Science), as well as AAALAC, which oversees guidelines for animal research, must be involved in discussion with researchers in terms of feasible strategies for testing whether key determinants of the immune system or metabolic pathways are altered by housing guidelines.

Other challenges include determination of how experiments can be conducted which explore the role of ambient temperature. In our experiments, we currently place cages within incubators (Thermo Fisher Scientific) maintained at 22° or 30 °C [42,44,47,48]. We have also used rooms with the temperature set at 22° or 30 °C, but working at 30°C is indeed uncomfortable for the personnel. Nevertheless, research institutes should consider providing some space for selected comparative experiments. Alternatively, providing additional nesting material could also allow mice to construct a thermoneutral shelter for themselves which they could freely access as desired [25,26] and avoid overheating which is a possibility when using a constant thermoneutral temperature. However, there still could be considerable variability between laboratories using a variety of different beddings. Another possibility is alternative cage designs which allow mice to behaviorally thermoregulate, as developed by Gordon *et al.* [29]. Ultimately, new designs in standard caging systems that can minimize thermal stress may be needed.

In summary, we are not suggesting that there is a single housing temperature that will enable mouse tumor models to most accurately mimic human physiology; instead we are suggesting that conducting experiments only at ST, causing adrenergic stress (as evidenced by increased NE levels), and/or diverting large amounts of fuel toward heat production, can bias experimental outcomes. As evidenced by our finding that tumor-bearing mice prefer a much higher temperature (~37–38 °C) than normal mice (~30–32 °C) [42], the growth of tumors may create far greater cold stress in tumor-bearing mice than experienced by tumor-free mice. Therefore, by paying attention to the degree of cold stress, and by carrying out studies at ST and TT, we will likely obtain a much more accurate picture of how the range of responses that can be obtained correlates with stress levels. In regard to this point, Lutgendorf *et al.* found, in ovarian cancer patients, that higher NE levels in the tumors correlated with more advanced disease and the degree of social stress experienced by the patients [95]. Because patients are under varying degrees of different stresses (physical, emotional, psychological), understanding the range of outcomes that can be obtained in mouse models may allow us to better predict the range of responses which will be seen in patients.

Acknowledgments

The authors thank Dr Christopher Gordon for his advice and early recognition of the importance of murine thermal physiology in the assessment of disease models. We also thank Mark Bucsek, Dr Kathleen Kokolus, and Dr Yasmin Thanavala for comments on the manuscript. This work was supported by National Institute of Health Grant R03 AG049489, Harry J. Lloyd Charitable Trust, and The Roswell Park Cancer Institute Alliance Foundation.

References

1. Guyton, A. and Hall, J. (2006) Body temperature, temperature regulation and fever. In *Text Book of Medical Physiology* (11th edn), pp. 889–900, Elsevier
2. Hardy, J.D. and Bard, P. (1968) Body temperature regulation. In *Medical Physiology* (Vol. 2) (Mountcastle, V.B., ed.), pp. 1305–1342, C.V. Mosby
3. Schmidt-Nielsen, K. (1997) Temperature. In *Animal Physiology*, (5th edn), pp. 215–297, Cambridge University Press
4. Stitt, J.T. (2001) Central regulation of body temperature. In *Exercise, Heat, and Thermoregulation* (Gisolfi, C.V. *et al.*, eds), pp. 1–47, Cooper Publishing Group
5. Gordon, C.J. (1993) *Temperature Regulation in Laboratory Rodents*, Cambridge University Press
6. Gordon, C.J. (2012) Thermal physiology of laboratory mice: defining thermoneutrality. *J. Therm. Biol.* 37, 654–685

7. Overton, J. (2010) Phenotyping small animals as models for the human metabolic syndrome: thermoneutrality matters. *Int. J. Obes.* 34 (Suppl. 2), S53–S58
8. Hart, J.S. (1971) Rodents. In *Comparative Physiology of Thermoregulation* (Vol. 2) (Whitrow, G.C., ed.), pp. 1–149, Academic Press
9. Abreu-Vieira, G. (2015) Integration of body temperature into the analysis of energy expenditure in the mouse. *Mol. Metab.* 4, 461–470
10. Cannon, B. and Nedergaard, J. (2004) Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359
11. Cannon, B. and Nedergaard, J. (2009) Thermogenesis challenges the adipostat hypothesis for body-weight control. *Proc. Nutr. Soc.* 68, 401–407
12. Cannon, B. and Nedergaard, J. (2011) Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J. Exp. Biol.* 214, 242–253
13. Herrington, L.P. (1940) The heat regulation of small laboratory animals at various environmental temperatures. *Am. J. Physiol.* 129, 123–139
14. Hart, J.S. (1953) Rate of gain and loss of cold resistance in mice. *Can. J. Zool.* 31, 112–116
15. Kaplan, H.M. et al. (1983) Physiology. In *The Mouse in Biomedical Research* (Vol. 111) (Foster, H.L. et al., eds), pp. 248–292, Academic Press
16. Ribeiro, M.O. et al. (2010) Expression of uncoupling protein 1 in mouse brown adipose tissue is thyroid hormone receptor-beta isoform specific and required for adaptive thermogenesis. *Endocrinology* 151, 432–440
17. Silva, J.E. (2006) Thermogenic mechanisms and their hormonal regulation. *Physiol. Rev.* 86, 435–464
18. Schmidt-Nielsen, K. (1984) Metabolic rate and body size. In *Scaling: Why is Animal Size So Important?*, pp. 56–74, Cambridge University Press
19. Golozubova, V. (2004) Depressed thermogenesis but competent brown adipose tissue recruitment in mice devoid of all hormone-binding thyroid hormone receptors. *Mol. Endocrinol.* 18, 384–401
20. Martin, B. (2010) 'Control' laboratory rodents are metabolically morbid: why it matters. *Proc. Natl. Acad. Sci. U.S.A.* 107, 6127–6133
21. Karp, C.L. (2012) Unstressing interperate models: how cold stress undermines mouse modeling. *J. Exp. Med.* 209, 1069–1074
22. Maloney, S.K. et al. (2014) Translating animal model research: does it matter that our rodents are cold? *Physiology* 29, 413–420
23. National Research Council of the National Academies (2011) *Guide for the Care and Use of Laboratory Animals*. (8th edn), National Academies Press
24. Helppi, J. et al. (2015) Mouse reproductive fitness is maintained up to an ambient temperature of 28 when housed in individually-ventilated cages. *Lab. Anim.* Published online October 13, 2015. <http://dx.doi.org/10.1177/0023677215611564>
25. Gaskill, B.N. et al. (2012) Heat or insulation: behavioral titration of mouse preference for warmth or access to a nest. *PLoS ONE* 7, e32799
26. Gaskill, B.N. et al. (2013) Impact of nesting material on mouse body temperature and physiology. *Physiol. Behav.* 110–111, 87–95
27. Swoap, S.J. et al. (2008) Vagal tone dominates autonomic control of mouse heart rate at thermoneutrality. *Am. J. Physiol. Heart Circ. Physiol.* 294, H1581–H1588
28. Swoap, S.J. et al. (2004) Effect of ambient temperature on cardiovascular parameters in rats and mice: a comparative approach. *Am. J. Physiol. Regul. Integr. Compar. Physiol.* 287, R391–R396
29. Gordon, C.J. et al. (2014) Behaviorally mediated, warm adaptation: a physiological strategy when mice behaviorally thermoregulate. *J. Therm. Biol.* 44, 41–46
30. Jun, J.C. et al. (2013) Thermoneutrality modifies the impact of hypoxia on lipid metabolism. *Am. J. Physiol. Endocrinol. Metab.* 304, E424–E435
31. Morgan, J.L. et al. (2014) Effects of housing density in five inbred strains of mice. *PLoS ONE* 9, e90012
32. Paigen, B. et al. (2012) Physiological effects of housing density on C57BL/6J mice over a 9-month period. *J. Anim. Sci.* 90, 5182–5192
33. Toth, L.A. et al. (2015) Interactions between housing density and ambient temperature in the cage environment: effects on mouse physiology and behavior. *J. Am. Assoc. Lab. Anim. Sci.* 54, 708–717
34. Dicker, A. et al. (1995) Cold acclimation-recruited nonshivering thermogenesis: the Syrian hamster is not an exception. *Am. J. Physiol.* 269, R767–R774
35. Hanssen, M.J. et al. (2015) Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. *Nat. Med.* 21, 863–865
36. Hanssen, M.J. et al. (2015) Short-term cold acclimation recruits brown adipose tissue in obese humans. *Diabetes* pii: db151372
37. David, J.M. et al. (2013) The hidden cost of housing practices: using noninvasive imaging to quantify the metabolic demands of chronic cold stress of laboratory mice. *Comp. Med.* 63, 386–391
38. Barbatelli, G. et al. (2010) The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am. J. Physiol. Endocrinol. Metab.* 298, E1244–E1253
39. Harms, M. and Seale, P. (2013) Brown and beige fat: development, function and therapeutic potential. *Nat. Med.* 19, 1252–1263
40. Smith, D.L., Jr et al. (2013) Measurement of interscapular brown adipose tissue of mice in differentially housed temperatures by chemical-shift-encoded water-fat MRI. *J. Magn. Reson. Imaging* 38, 1425–1433
41. Alexander, C.M. et al. (2015) Dermal white adipose tissue: a new component of the thermogenic response. *J. Lipid Res.* 56, 2061–2069
42. Kokolus, K.M. et al. (2013) Baseline tumor growth and immune control in laboratory mice are significantly influenced by subthermoneutral housing temperature. *Proc. Natl. Acad. Sci. U.S.A.* 110, 20176–20181
43. Nguyen, K.D. et al. (2011) Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature* 480, 104–108
44. Eng, J.W. et al. (2015) Housing temperature-induced stress drives therapeutic resistance in murine tumour models through beta-adrenergic receptor activation. *Nat. Commun.* 6, 6426
45. Straub, R.H. et al. (2010) Energy regulation and neuroendocrine-immune control in chronic inflammatory diseases. *J. Int. Med.* 267, 543–560
46. Uchida, K. et al. (2010) Metabolic adaptation of mice in a cool environment. *Pflügers Arch* 459, 765–774
47. Kokolus, K.M. et al. (2014) Stressful presentations: mild cold stress in laboratory mice influences phenotype of dendritic cells in naive and tumor-bearing mice. *Front. Immunol.* 5, 23
48. Leigh, N.D. et al. (2015) Housing temperature-induced stress is suppressing murine graft-versus-host disease through beta2-adrenergic receptor signaling. *J. Immunol.* 195, 5045–5054
49. Cole, S.W. et al. (2015) Sympathetic nervous system regulation of the tumour microenvironment. *Nat. Rev. Cancer* 15, 563–572
50. Cole, S.W. and Sood, A.K. (2012) Molecular pathways: beta-adrenergic signaling in cancer. *Clin. Cancer Res.* 18, 1201–1206
51. Meads, M.B. et al. (2009) Environment-mediated drug resistance: a major contributor to minimal residual disease. *Nat. Rev. Cancer* 9, 665–674
52. Pasquier, E. et al. (2011) Propranolol potentiates the anti-angiogenic effects and anti-tumor efficacy of chemotherapy agents: implication in breast cancer treatment. *Oncotarget* 2, 797–809
53. Pasquier, E. et al. (2013) Beta-blockers increase response to chemotherapy via direct antitumour and anti-angiogenic mechanisms in neuroblastoma. *Br. J. Cancer* 108, 2485–2494
54. Bracci, L. et al. (2014) Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. *Cell Death Differ.* 21, 15–25
55. Galluzzi, L. et al. (2015) Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell* 28, 690–714
56. Zitvogel, L. et al. (2013) Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. *Immunity* 39, 74–88

57. Shiao, S.L. and Coussens, L.M. (2010) The tumor-immune micro-environment and response to radiation therapy. *J. Mammary Gland Biol. Neoplasia* 15, 411–421
58. Gollnick, S.O. and Brackett, C.M. (2010) Enhancement of anti-tumor immunity by photodynamic therapy. *Immunol. Res.* 46, 216–226
59. Allott, E.H. and Hursting, S.D. (2015) Obesity and cancer: mechanistic insights from transdisciplinary studies. *Endocr. Relat. cancer* 22, R365–R386
60. Enerback, S. *et al.* (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387, 90–94
61. Liu, X. *et al.* (2003) Paradoxical resistance to diet-induced obesity in UCP1-deficient mice. *J. Clin. Invest.* 111, 399–407
62. Anunciado-Koza, R. *et al.* (2008) Inactivation of UCP1 and the glycerol phosphate cycle synergistically increases energy expenditure to resist diet-induced obesity. *J. Biol. Chem.* 283, 27688–27697
63. Feldmann, H.M. *et al.* (2009) UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* 9, 203–209
64. Kozak, L.P. (2010) Brown fat and the myth of diet-induced thermogenesis. *Cell Metab.* 11, 263–267
65. Goldhof, M. *et al.* (2014) The chemical uncoupler 2,4-dinitrophenol (DNP) protects against diet-induced obesity and improves energy homeostasis in mice at thermoneutrality. *J. Biol. Chem.* 289, 19341–19350
66. Stemmer, K. *et al.* (2015) Thermoneutral housing is a critical factor for immune function and diet-induced obesity in C57BL/6 nude mice. *Int. J. Obes.* 39, 791–797
67. Xiao, C. *et al.* (2015) Anti-obesity and metabolic efficacy of the beta3-adrenergic agonist, CL316243, in mice at thermoneutrality compared to 22 degrees C. *Obesity* 23, 1450–1459
68. Koizumi, A. *et al.* (1996) A tumor preventive effect of dietary restriction is antagonized by a high housing temperature through deprivation of torpor. *Mech. Ageing Dev.* 92, 67–82
69. Shalpour, S. and Karin, M. (2015) Immunity, inflammation, and cancer: an eternal fight between good and evil. *J. Clin. Invest.* 125, 3347–3355
70. Tian Xiao, Y. *et al.* (2016) Thermoneutral housing accelerates metabolic inflammation to potentiate atherosclerosis but not insulin resistance. *Cell Metab.* 23, 165–178
71. Ravussin, Y. (2015) Temperature matters with rodent metabolic studies. *Obesity* 23, 1330
72. Gilon, P. and Henquin, J.C. (2001) Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr. Rev.* 22, 565–604
73. Elenkov, I.J. (2002) Systemic stress-induced Th2 shift and its clinical implications. *Int. Rev. Neurobiol.* 52, 163–186
74. Elenkov, I.J. and Chrousos, G.P. (1999) Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends Endocrinol. Metab.* 10, 359–368
75. Hou, N. *et al.* (2013) A novel chronic stress-induced shift in the Th1 to Th2 response promotes colon cancer growth. *Biochem. Biophys. Res. Commun.* 439, 471–476
76. Balkwill, F.R. and Mantovani, A. (2012) Cancer-related inflammation: common themes and therapeutic opportunities. *Semin. Cancer Biol.* 22, 33–40
77. Grivennikov, S.I. *et al.* (2010) Immunity, inflammation, and cancer. *Cell* 140, 883–899
78. Trinchieri, G. (2012) Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu. Rev. Immunol.* 30, 677–706
79. Jhaveri, K.A. *et al.* (2007) Effect of environmental temperature on sleep, locomotor activity, core body temperature and immune responses of C57BL/6J mice. *Brain Behav. Immun.* 21, 975–987
80. Freedman, L. and Gibson, M. (2015) The impact of preclinical irreproducibility on drug development. *Clin. Pharmacol. Ther.* 97, 16–18
81. Prinz, F. *et al.* (2011) Believe it or not: how much can we rely on published data on potential drug targets? *Nat. Rev. Drug Discov.* 10, 712
82. Schein, P.S. and Scheffler, B. (2006) Barriers to efficient development of cancer therapeutics. *Clin. Cancer Res.* 12, 3243–3248
83. Talmadge, J.E. *et al.* (2007) Murine models to evaluate novel and conventional therapeutic strategies for cancer. *Am. J. Pathol.* 170, 793–804
84. Toth, L.A. (2015) The influence of the cage environment on rodent physiology and behavior: Implications for reproducibility of pre-clinical rodent research. *Exp. Neurol.* 270, 72–77
85. Begley, C.G. and Ellis, L.M. (2012) Drug development: raise standards for preclinical cancer research. *Nature* 483, 531–533
86. Landis, S.C. *et al.* (2012) A call for transparent reporting to optimize the predictive value of preclinical research. *Nature* 490, 187–191
87. Editorial (2009) Troublesome variability in mouse studies. *Nature Neurosci.* 12, 1075
88. Povinelli, B.J. *et al.* (2015) Standard sub-thermoneutral caging temperature influences radiosensitivity of hematopoietic stem and progenitor cells. *PLoS ONE* 10, e0120078
89. Rudaya, A.Y. *et al.* (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
90. Ravussin, Y. *et al.* (2012) Effects of ambient temperature on adaptive thermogenesis during maintenance of reduced body weight in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 303, R438–R448
91. Romanovsky, A.A. *et al.* (1998) Methodology of fever research: why are polyphasic fevers often thought to be biphasic? *Am. J. Physiol.* 275, R332–R338
92. Hasday, J.D. *et al.* (2000) The role of fever in the infected host. *Microbes Infect.* 2, 1891–1904
93. Eng, J.W. *et al.* (2014) Housing temperature influences the pattern of heat shock protein induction in mice following mild whole body hyperthermia. *Int. J. Hyperthermia* 30, 540–546
94. Murray, N.M. *et al.* (2015) Insomnia caused by serotonin depletion is due to hypothermia. *Sleep* 38, 1985–1993
95. Lutgendorf, S.K. *et al.* (2011) Social isolation is associated with elevated tumor norepinephrine in ovarian carcinoma patients. *Brain Behav. Immun.* 25, 250–255