Mice as experimental models for human physiology: when several degrees in housing temperature matter

Some 'species differences' between mice and humans can be diminished simply by housing mice at warmer temperatures. Failure to strategically turn up the thermostat may undermine the translation of findings in mice to insights into human metabolic diseases.

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For the convenience of human experimentalists and caretakers, and to decrease costs, mice are generally housed at environmental temperatures below their thermoneutrality. Our comfort and fiscal frugality come at costs that may include inappropriate interpretation of findings and even compromised translatability of experimental findings to human physiology and disease. For practical and scientific reasons, mice have become the model organism of choice in a vast swath of biomedical research. Mice thrive and breed well in laboratory environments and can be housed economically, and powerful experimental approaches are now widely available to alter gene expression and to specifically evaluate molecular, cellular, tissue or systems biology. Importantly, basic metabolic and physiological mechanisms are generally conserved between these species. For example, mice are omnivores that eat in discrete bouts, and the mouse gastrointestinal tract largely develops and functions similarly to that of humans.

Despite these clear advantages, several barriers exist in using mice to understand and treat human disease. We often think of these barriers as species differences, that is, differences relating to the genomes of mice because they evolved on a different path from that of humans[1]. For example, mice produce a heparin-binding epidermal growth factor–like growth factor that, unlike that in humans, does not bind diphtheria toxin. This genomic difference renders mice insensitive to the toxic effects of diphtheria toxin and makes them a poor model for understanding the effects of diphtheria toxin in humans. However, these differences do allow for selective ablation of cells in mice through the expression and activation of human heparin-binding epidermal growth factor–like growth factor in specific cell types[2]. Thus, when used cleverly, differences between species can be exploited to gain insight into the similarities.

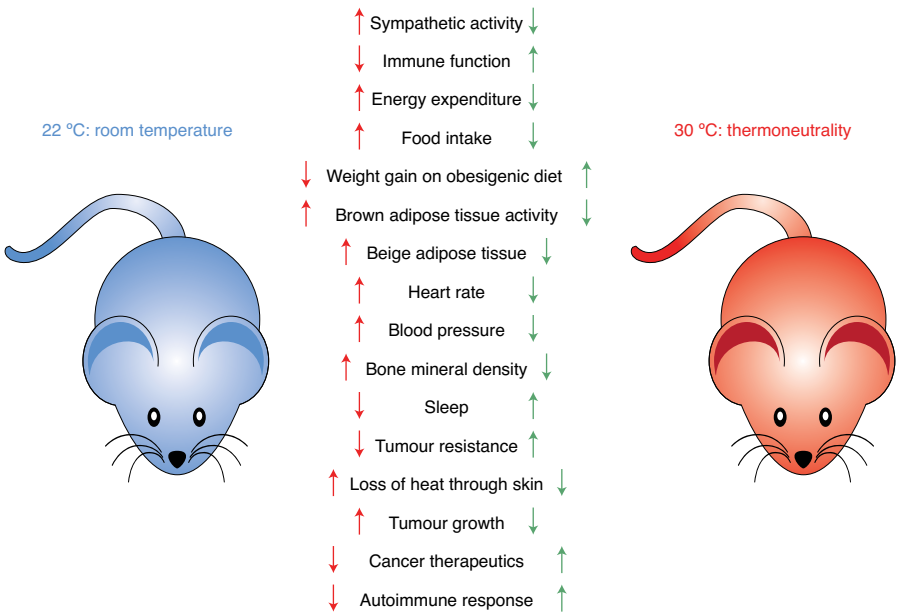

Fig. 1 | Room temperature versus thermoneutrality in mice. A comparison of a variety of physiological systems in mice housed under typical room-temperature conditions or close to the thermoneutral temperature. The green arrows at right indicate that the parameter appears to be closer to that in humans when mice are housed under thermoneutral conditions. Thus, for many experiments, housing mice at thermoneutral conditions would appear to increase the translatability of mouse experimental findings to humans.

Unlike humans, most laboratory mice are mildly cold stressed

Although we clearly should consider genetic divergence between species, we should also be aware that disparities in results can arise from how experiments are performed. One factor deserving further scrutiny is environmental temperature—in the modern world, humans spend much of their time at temperatures close to thermoneutrality, whereas mice are generally housed well below their thermoneutral zone. Thermoneutrality is the ambient temperature range for which energy is expended only to maintain the basal metabolic rate. When the environmental temperature is cooler than thermoneutrality, warm-blooded organisms burn additional energy to maintain their core body temperatures. At temperatures above thermoneutrality, organisms expend additional energy to cool themselves.

Mice and humans both maintain core body temperatures of approximately 37 °C; however, the peripheral tissues and appendages of both species are cooler and are highly influenced by environmental

temperatures. Although mice and humans share many behavioural and physiological mechanisms for regulating heat loss, such as vasodilation or constriction of blood vessels in the skin, a true species difference comes from the tails of mice, which account for approximately 5–8% of total body heat loss[3]. Homeothermic animals can adapt to a wide range of temperatures. Indeed, mice survive well at 20 °C as long as they have food, nesting materials and time to adapt[4]. However, mice and humans prefer to live in temperatures at or slightly below thermoneutrality[5]. Factors that influence thermoneutrality include body size, shape and composition; age; sex; clothing or fur; temperature acclimation; and energy expenditure[6]. Because of these factors, thermoneutrality for mice is generally higher (~29–32 °C) than that for humans (~22 °C). Because mice are typically housed in rooms kept at temperatures ideal for human staff (20–22 °C), mice are almost always at temperatures below their thermoneutrality. Consequently, mice use considerably more energy to maintain their core body temperature than humans.

The degree to which mice experience cool temperatures also depends on their housing conditions. Mice housed in groups huddle together to preserve warmth. Mice are avid nest builders, partly to provide protection against cold temperatures[7]. Finally, ventilated cage racks, which limit contamination between cages, increase convective heat loss, thus effectively lowering the temperatures experienced by caged mice. Unfortunately, considerable variation undoubtedly exists in the environmental temperatures experienced by mice across research settings, but this variation is generally not quantified or included in research methods.

Environmental temperature influences mouse physiology and pathophysiology

Important differences exist between the physiology of mice housed at thermoneutrality and those housed at room temperature. For example, mice have much higher heart rates than humans, but the extent of this difference is highly dependent on housing temperature. Mice at thermoneutrality have a heart rate of ~375 beats per minute, which increases to ~575 beats per minute 22 °C (ref. [8]). Hence, the prominent differences in heart rates between humans and mice are partly due to housing conditions rather than a species difference. Importantly, heart rate is highly correlated with blood pressure, which is also elevated at cooler temperatures[8]. A key mechanism for cool adaptation in mice is the elevation of sympathetic drive, which increases the heart rate, and is easily observed as higher norepinephrine content and turnover in adipose depots[9]. Although the vagus nerve is the predominant regulator of heart rate in humans, the importance of vagal tone in mice is revealed only when mice are maintained at thermoneutrality[10]. Thus, what may seem to be a species difference at room temperature disappears when the species are compared at thermoneutral temperatures (Fig. 1).

The activation of sympathetic drive has profound effects on other aspects of mouse physiology. Activation of the sympathetic nervous system results in suppression of the immune system as energy is directed from the immune system to heat generation. Although this response is adaptive to short-term cold exposure, chronic activation of sympathetic drive results in an immune system that operates differently under these conditions than at thermoneutrality. Immune cell metabolism, fever and response to autoimmune disease all increase at thermoneutrality[11,12]. This observation has affected the cancer field, in which thermoneutrality is well known to confer resistance to the growth of a wide variety of cancers and to increase the efficacy of cancer therapies that rely on immune cell function[12,13].

Housing temperature affects not only the development of metabolic diseases but also the potential to assess treatment strategies. Greater heat loss, owing to the greater ratio of surface area to volume in mice than in humans, causes mice to burn proportionally more energy to maintain core body temperature[14]. This difference is exacerbated when mice are housed at temperatures below thermoneutrality, and an elevated metabolic rate and the activation of brown adipose tissue affect their body weight and composition[15]. Growing appreciation for the role of the immune system in metabolic disease has spawned a field termed immunometabolism. However, as in immuno-oncology, the ability to translate findings in mice to humans is greatly limited by studying mice with chronically activated sympathetic nervous systems. For example, mice on a high-fat diet housed at thermoneutrality gain more adipose tissue, accumulate more liver lipids, and have elevated glucose intolerance and more adipose tissue inflammation than mice housed at room temperature[16]. It is simply difficult to imagine that these differences do not colour our view of metabolic disease progression and limit our ability to apply these lessons to human disease.

The bottom line is that housing temperature is the most prominent example of a species difference that is produced by the nature of the experiments rather than genetic divergence. Even as production of ever more sophisticated genetic mouse models improves the ability to 'humanize' mice, the most important step in making mice more similar to humans is to house them closer to thermoneutrality. However, comprehensively and consistently addressing issues of housing temperature comes at a high cost. Housing mice in thermoregulated chambers is expensive, considerably adds to the labour necessary for even simple experiments and renders some complicated physiological experiments exceptionally difficult. Raising the temperatures of mouse housing rooms is often not possible, given that the heating, ventilation and air-conditioning systems are designed to maintain mandated levels of air changes. Working in a room set for mouse thermoneutrality (~30 °C) is considerably challenging to people wearing appropriate personal protective equipment and can even be dangerous under some conditions. Less clear is under which circumstances a 30 °C temperature would be necessary or whether smaller increases in housing temperature might be sufficient.

When is housing mice at thermoneutrality warranted?

The ability of appetite suppressants to cause weight loss in humans was predicted on the basis of findings in obese mice under standard housing conditions, thus implying that mechanisms for regulating food intake are not uniformly disrupted to the point of misinforming human interventions. Nonetheless, ambient temperature can have rapid and profound effects on daily food intake in mice[17]. However, for mice with changes in body composition that are independent of food intake, evaluating these mice at thermoneutrality in addition to room temperature is important.

Consider the situation in which a genetic or pharmacologic perturbation results in increased whole-body metabolism, protection against obesity, and elevated beige and brown adipose tissue thermogenesis. Although the treatment might directly stimulate adaptive thermogenesis, it could also decrease the insulative properties of the skin and thus indirectly stimulate beige and brown fat activity secondarily to heat loss. In this case, if the treatment also protects against obesity when mice are housed at thermoneutrality, the results are more likely to be translatable to humans than if protection against obesity is observed only at room temperature. In addition, manipulations of the immune system that are found to alter metabolic function and are affected by increased sympathetic tone

must be tested at thermoneutrality to assess whether they could plausibly be linked to metabolic regulation in humans.

Many species differences between mice and humans can be addressed experimentally. A failure to do so in a systematic manner has the potential to undermine the translation of findings in mice to insights into human disease and treatment for no other reason than a failure to change the thermostat settings. ❐

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