Unstressing intemperate models: how cold stress undermines mouse modeling

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Mus musculus **enjoys pride of place at the center of contemporary biomedical research. Despite being the current model system of choice for in vivo mechanistic analysis, mice have clear limitations. The literature is littered with examples of therapeutic approaches that showed promise in mouse models but failed in clinical trials. More generally, mice often provide poor mimics of the human diseases being modeled. Available data suggest that the cold stress to which laboratory mice are ubiquitously subjected profoundly affects mouse physiology in ways that impair the modeling of human homeostasis and disease. Experimental attention to this key, albeit largely ignored, environmental variable is likely to have a broad transformative effect on biomedical research.**

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The Journal of Experimental Medicine

Mice have triumphed as the dominant in vivo experimental model system in contemporary biomedical research for obvious reasons. Mouse biology often mirrors human biology, and the genetic tractability and generation time of mice provide clear practical benefits. Vast resources have been dedicated to the study of mice and, in turn, their experimental use has provided extraordinary insights into human physiology and disease. However, mouse models have clear limitations. Tuberculosis and atherosclerosis, diseases that cause an immense burden of morbidity and mortality in the world at large, provide instructive examples of these limitations. In the vast majority of the estimated 2 billion people infected with *Mycobacterium tuberculosis*, latency, i.e., mycobacterial persistence in the absence of clinical disease, is achieved (Dye and Williams, 2010). Such latency has not been modeled in mice (Barry et al., 2009). Mice are also quite resistant to atherosclerosis, and available models depend on rather blunt genetic deletion strategies that, even when combined with dietary manipulations, fail to model the full spectrum of human atherosclerotic disease (Libby et al., 2011). These difficulties are presumed to be caused by basic biological differences between species. However, available data suggest that the environmental conditions ubiquitously used in mouse husbandry lead to profound alterations in mouse physiology, impairing the utility of mice as models for these and other diseases.

Anthropocentricity in the mouse house

Humans tend to seek thermal comfort and prefer (and tend to reside in) temperatures within their thermoneutral zone, which is the range of ambient temperatures in which minimal metabolic energy is expended for warmth (or cooling). For reasons of human comfort and historical contingency, mice are systematically housed at temperatures comfortable for clothed humans, i.e., "room temperature," which is 19–22°C. However, the thermoneutral zone for most mouse strains (during the day when they are inactive) is 30–32°C (Gordon, 1993). Mice are thus normally subjected to cold stress, the extent of which is illustrated by the dramatic decrease in heart rate (200 beats/min from 550–600 to 350–400 beats/min) observed when mice are shifted from such temperatures to thermoneutrality (Swoap et al., 2008). Paralleling this, the "basal" metabolic rate of mice housed under standard conditions is elevated by 50–60% compared with that of mice housed at thermoneutrality (Feldmann et al., 2009; Cannon and Nedergaard, 2011). Lest it be thought that *Mus musculus* has some special affinity for human room temperature, thermal gradient experiments have demonstrated that

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The Rockefeller University Press $30.00
J. Exp. Med. Vol. 209 No. 6 1069-1074
www.jem.org/cgi/doi/10.1084/jem.20120988

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they prefer temperatures associated with their own metabolic thermoneutrality (Gordon, 1985). Furthermore, although wild house mice are able to forage over a wide range of temperatures, their nests tend to be within their thermoneutral zone (Jakobson, 1981).

The likely implications of inattention to this fundamental environmental variable are considerable and go far beyond, for example, the erroneous conclusion that the resting heart rate of the mouse—unlike that of the human—is under sympathetic rather than parasympathetic control (Swoap et al., 2008). Despite an impressive body of literature on thermoregulation in rodents (Gordon, 1993), the fact that laboratory mice are normally housed under relatively severe cold stress has not been appreciated outside a small group of thermoregulation cognoscenti. Those working in obesity and metabolism have led the way in increasing appreciation of these issues (Cannon and Nedergaard, 2011).

Obesity results from an imbalance between energy intake and expenditure. In addition to obligatory thermogenesis (basal metabolic rate and the thermic effect of food), obligatory energy expenditure for growth and reproduction, and physical activity, energy expenditure also occurs through adaptive thermogenesis—the regulated production of heat in response to cold exposure or caloric excess. Adaptive thermogenesis covers three processes: cold-induced shivering thermogenesis, cold-induced nonshivering thermogenesis, and diet-induced thermogenesis (Tseng et al., 2010). The latter two processes occur via brown adipocytes, cells specialized for the dissipation of food energy into heat via uncoupling of mitochondrial respiration from ATP synthesis in a process dependent on uncoupling protein 1 (UCP1). When UCP1$^{-/-}$ mice were generated, they were found—as expected—to be highly susceptible to cold and dependent on shivering for thermoregulatory thermogenesis (Enerbäck et al., 1997; Golozoubova et al., 2001, 2004). In initial studies, however, these mice did not become obese when fed a standard or high-fat diet (Enerbäck et al., 1997), a finding that cast doubt on the role of UCP1 and brown fat in diet-induced thermogenesis. But later studies revealed that this result was confounded by the cold stress of standard mouse housing. When housed under thermoneutral conditions, UCP1$^{-/-}$ mice became obese on control diets and exhibited augmented obesity on high-fat diets (Feldmann et al., 2009). Further, diet-induced thermogenesis was formally demonstrable in wild-type, but not UCP1$^{-/-}$, mice housed at thermoneutrality (Feldmann et al., 2009).

Ambient temperature is thus a critical variable in metabolic studies; the cold stress of standard housing has the potential to generate both false-positive and false-negative results (Cannon and Nedergaard, 2011). Although some investigators working at the intersection of immunology and metabolism have taken fruitful note of these issues (Nguyen et al., 2011), they are outliers. This is problematic, as the known effects of cold stress on mouse physiology in general, and on immune responsiveness in particular, suggest that unreflexive attention to human comfort has hindered the optimal development of mice as models of human disease.

Cold and dampening of the immune system

Adaptation to cold stress involves, among other things, glucocorticoid production and sustained activation of the sympathetic nervous system (Leduc, 1961; Shum et al., 1969; Yahata et al., 1987; Kuroshima et al., 1988; Ohno et al., 1990; Gordon,

1993; Harper and Austad, 2000; Cannon and Nedergaard, 2004, 2011; Feldmann et al., 2009; Baccan et al., 2010). The immunosuppressive effects of glucocorticoids are widely appreciated, and activation of the sympathetic nervous system has broad effects beyond driving adaptive thermogenesis and supporting processes such as heart rate and cardiac output. For example, stimulation of β adrenergic receptors on myeloid cells suppresses their function, including down-regulation of proinflammatory (and up-regulation of anti-inflammatory) cytokines and chemokines by dendritic cells and macrophages, as well as inhibition of IFN-γ–mediated activation of macrophages and neutrophils (Elenkov et al., 2000). The immunobiological relevance of the increased β-adrenergic activity associated with standard mouse housing is suggested by the fact that β-adrenergic blockade under such conditions results in robust up-regulation of in vivo myeloid cell responsiveness (Elenkov et al., 2000).

Given the critical role of myeloid cells both as immune effectors and as central regulators of innate and adaptive immune responses, the blunting of myeloid cell responsiveness by cold stress is likely to have negative effects on the modeling of human immune-mediated diseases in mice. It should be noted that corticosteroids and catecholamines also exert potent effects on nonmyeloid immune cells and that peripheral lymphoid tissues are highly innervated by the sympathetic nervous system (Elenkov et al., 2000). Furthermore, the effects of cold stress clearly involve more than just activation of the sympathetic nervous system and the hypothalamic–pituitary–adrenal axis. For example, recently published data indicate that cold stress up-regulates the alternative activation of tissue macrophages (Nguyen et al., 2011), which would be expected to provide an additional brake on inflammatory responses. The full extent of the complex, primary, and secondary biological responses to the chronic cold stress universally imposed on mice is likely to be quite extensive, reaching beyond increased metabolic rate and decreased immune responsiveness to include (at least) cardiovascular stress and oxidative stress.

Infection, immunity, and thermoneutrality

Studies dating back at least to the 1940s indicate that ambient temperature profoundly alters the course of infection in diverse rodent models. In models of bacterial (*Salmonella typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Rickettsia typhi*), viral (influenza virus, herpes simplex virus, and rabies virus), and protozoal (*Trypanosoma cruzi*) infection, ambient temperature directly correlates with host responsiveness—lower temperatures leading to impaired immune responses (Moragues and Pinkerton, 1944; Miraglia and Berry, 1962; Previte and Berry, 1962; Underwood et al., 1966; Amrein, 1967; Baetjer, 1968; Previte et al., 1970; Won and Ross, 1971; Bell and Moore, 1974; Jiang et al., 2000; Rice et al., 2005). These effects can be dramatic: in experimental murine typhus, weather-associated changes in ambient laboratory temperature (from 29.4–36.6 to 18.3–22.8°C) shifted mortality rates from 9 to 100% (Moragues and Pinkerton, 1944). Notably, this literature is marked by a general lack of recognition that standard mouse housing involves cold stress.

It has also long been known that, compared with humans, mice (housed at standard room temperature) are very resistant to LPS challenge. This has been interpreted as a seminal difference between species, and one that undermines modeling in mice. However, this in vivo difference in sensitivity is not caused by intrinsic differences in the in vitro sensitivity of myeloid cells (monocytes isolated from the blood of mice and humans exhibit similar in vitro responsiveness to activation by LPS, other bacterial products, and whole bacteria; Munford, 2010; Warren et al., 2010), suggesting that the protection in mice may instead result from the in vivo dampening effect of cold stress on myeloid cell activation. The physiological consequences of cold stress in mice are further underscored by the observation that, unlike humans, mice housed under standard ambient temperatures fail to develop fevers after challenge with microbes or microbial products (in fact, they become hypothermic; Hasday et al., 2000; Rudaya et al., 2005). In contrast, LPS challenge and bacterial infection induce febrile responses in mice housed in the thermoneutral zone (Leon et al., 1999; Rudaya et al., 2005). Interestingly, aging (2 yr old) mice develop fevers in response to LPS under standard housing conditions (Habicht, 1981), which is the reverse of the diminished febrile response observed in aged humans. This observation may well be caused by increased size, decreased surface/volume ratio, and hence decreased cold stress in aging mice.

There is a history of using mice to define if and how the febrile response is helpful during infection that is also relevant to these issues. Important studies in the modern era have modeled the effect of fever on infection with *K. pneumonia,* a Gram-negative bacillus that causes virulent localized and systemic infections in mice and humans (Jiang et al., 2000; Rice et al., 2005). As mice housed at standard ambient temperature fail to develop fevers in response to challenge with *K. pneumonia*, exposure to higher temperatures (34–36°C) was used to drive febrile range hyperthermia (Jiang et al., 2000; Rice et al., 2005). Such exposure was associated with more vigorous immune responses and dramatically better control of bacterial burden in both *K. pneumonia* peritonitis and pneumonia; however, it was associated with increased lethality in the pneumonia model, as increasing the vigor of the immune response can also contribute to mortality, depending on the pathogen, dose, and route of infection (Jiang et al., 2000; Rice et al., 2005). This has been interpreted to demonstrate the amplifying effects of fever on immune responses, but the data may point just as cogently to (obviation of) the immunosuppressive effects of cold stress.

Although we model many infections fruitfully in mice, others have been more problematic. Tuberculosis is another case in point. Studies of latency eradication and natural reactivation have been hindered by the fact that this process does not occur in murine tuberculosis (Barry et al., 2009). After acute infection, mice fail to significantly reduce bacterial burdens and go on to develop a slowly progressive, ultimately fatal chronic inflammatory disease, the immunopathology of

which bears little resemblance to the variety of lesions seen in latently infected humans. In chronically infected mice, these inflammatory, granulomatous lesions are poorly organized and lack encapsulating fibrosis, hypoxia, necrosis, calcification, and giant cell accumulation. Notably, myeloid cell functions—including dendritic cell activation and IFN-γ–mediated macrophage activation and effector functions—are critical for host control of *M. tuberculosis* infection. Latent tuberculosis may well be achievable in mice if myeloid cells (and the immune system in general) are disinhibited by relieving cold stress.

Beyond infection

These considerations reach beyond infectious diseases. The centrality of the immune system to the pathogenesis and expression of most diseases (Karp and Murray, 2012) suggests that experimental attention to ambient temperature may improve mouse models of a broad spectrum of human diseases. Autoimmune, autoinflammatory, and allergic disease models are obvious candidates for exploration at thermoneutrality, as are studies investigating fundamental mechanisms of immune homeostasis and counter-regulation. But relieving our mice from cold stress (at the cost of subjecting vivarium workers to heat stress) has even broader potential benefits.

The resistance of mice to the development of atherosclerosis has been attributed in part to fundamental differences in lipoprotein metabolism between species—mice have high plasma HDL and low plasma VLDL and LDL levels. The generation of mouse models of atherosclerosis has thus required gross perturbation of lipoprotein metabolism, leading to hypercholesterolemia that greatly exceeds that normally seen in patients with atherosclerosis. Even on "Western" diets, the most susceptible wild-type (C57BL/6) mice only develop early lesions. Diverse transgenic and knockout approaches have thus been taken, but all have drawbacks (Libby et al., 2011). For example, $Ldlr^{-/-}$ mice provide a model of familial hypercholesterolemia—a rare disease despite the fact that LDL-driven atherosclerosis is very common in humans. These mice develop progressive atherosclerosis on a "Western" diet but exhibit levels of hypercholesterolemia far exceeding that seen in most patients with atherosclerosis. $Apoe^{-/-}$ mice also exhibit markedly different lipid profiles from humans (dramatically elevated cholesterol levels with a predominance of VLDL as opposed to LDL) and spontaneously develop lesions that evolve from early fatty streaks into complex lesions. Notably, spontaneous plaque rupture—a critical pathogenic feature of atherosclerosis-associated myocardial infarction and stroke in humans—is extremely rare in these models. Serum lipoprotein patterns become closer to those of humans when mice are housed under thermoneutral conditions (Cannon and Nedergaard, 2011), in part, perhaps, because cold exposure plays a major role in up-regulating plasma clearance of triglyceride-rich lipoproteins via uptake by brown adipose tissue (Bartelt et al., 2011). This fact, combined with the potent immunosuppressive effects of cold stress on monocytes and macrophages—cells integral to the pathogenesis of atherosclerosis—suggest that obviating cold stress may allow for the development of better, more humanized models of atherosclerosis.

Mice also provide poor models of cystic fibrosis (CF) lung disease—the main cause of morbidity and mortality in this most common of lethal autosomal recessive disorders in individuals of European ancestry. In CF, dysregulated neutrophilic inflammation and chronic infection lead to progressive destruction of the airways. CF patients also develop gastrointestinal disease. Experimental focus on (dys)regulation of epithelial ion transport by the responsible gene, *CFTR*, has provided a compelling account of the pathogenesis of gastrointestinal disease in CF. However, studies focused on altered ion and water transport have not yielded a similarly compelling explanation for pathogenesis in the lung, something that has hindered therapeutic advances. Although transgenic porcine and ferret CF models that appear to recapitulate human airway disease have recently been reported (Stoltz et al., 2010; Sun et al., 2010), the lack of a robust mouse model of CF lung disease remains a major problem in the field. Mice lacking *Cftr* recapitulate the gut disease, but their pulmonary phenotypes are very subtle. Whether this relates to different lung architecture, the effect of modifier genes, or stronger baseline counter-regulation in the mouse lung is unclear. Notably, residence in geographic areas with warmer annual ambient temperatures correlated with worse CF lung disease in four independent cohorts of patients on two continents (Collaco et al., 2011), underscoring the possibility that cold stress–driven inhibition of myeloid cell reactivity may play a major role in undermining the modeling of CF in mice. Cold stress is also associated with dramatically increased sympathetic activity and respiratory rate, both of which can facilitate mucociliary clearance (Delavoie et al., 2009), and this may also hinder effective modeling at the ambient temperatures common in vivaria.

Beyond effects on the modeling of human homeostasis and disease, the increased metabolic rate associated with cold stress, along with the profound effects of glucocorticoids on drug metabolism, suggest that standard housing has also impaired our ability to model human drug metabolism in mice. In this regard, it should be noted that the thermoneutral zone of rats is considerably closer to standard vivarium temperatures (and that rats, unlike mice, actually prefer temperatures slightly cooler than their thermoneutral zone; Gordon, 1993). Before the development of practical genetic tools for mice, drug development was largely done in rats. Is it possible that the comparatively greater success rates of the pharmaceutical industry early on stemmed in part from preferential use of rats, which provided a better model for thermoneutral humans?

Finally, it should be noted that these issues have important implications for the study of behavior as well. Social isolation is a commonly used stressor in murine behavioral research. As solitary mice cannot huddle for warmth, such isolation increases the cold stress associated with standard housing, thus introducing an important confounding variable. More broadly, our own experience in setting up a vivarium room to house mice at thermoneutrality has made it clear

that ambient temperature has a profound effect on mouse behavior—the aggressive nippiness of male C57BL/6 mice evaporates under such conditions.

Concluding remarks

It has become somewhat fashionable to decry the utility of mouse models and their predictive value for the preclinical development of novel therapeutic approaches to human disease. Indeed, mice are not humans (Mestas and Hughes, 2004). However, considerable data suggest that the cold stress endured by laboratory mice—a practical paradigm followed for nonscientific reasons—profoundly affects mouse (patho)physiology in ways that directly impair the modeling of human homeostasis and disease in mice. More predictive mouse models may well be available at the turn of a thermostat.

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