Review

MOLECULAR METABOLISM

Not so hot: Optimal housing temperatures for mice to mimic the thermal environment of humans

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ABSTRACT

It has been argued that mice should be housed at 30 °C to best mimic the thermal conditions experienced by humans, and that the current practice of housing mice at 20–22 °C impairs the suitability of mice as a model for human physiology and disease. In the current paper we challenge this notion. First, we show that humans routinely occupy environments about 3 °C below their lower critical temperature (T_{lc}), which when lightly clothed is about 23 °C. Second, we review the data for the T_{lc} of mice. Mouse T_{lc} is dependent on body weight and about 26–28 °C for adult mice weighing > 25 g. The equivalent temperature to that normally experienced by humans for most single housed adult mice is therefore 23–25 °C. Group housing or providing the mice with bedding and nesting material might lower this to about 20–22 °C, close to current standard practice.

Keywords Mouse; Human; Lower critical temperature; Thermoneutral; Thermoregulation; Ambient temperature

1. INTRODUCTION

The mouse is the model of choice for understanding the genetic basis of human disease. Recommendations on the housing temperature cover a substantial margin, being between 20 and 26 °C [8], 20 and 24 °C [22] or 19 and 23 °C [7]. Most mouse facilities however are operated at an ambient temperature of 20–22 °C, which is set primarily to match the comfort requirements of animal husbandry staff [27]. Mice have a different thermoregulatory response curve from humans, and the argument has been made that this housing temperature is not optimal to provide the best model for human metabolism or disease. In particular, it is argued that humans normally live at thermoneutral temperatures, while the thermoneutral zone of the mouse is at 30 °C, so laboratory mice at 20–22 °C are routinely under mild to moderate cold stress, because they are 8–10 °C colder than the equivalent temperature in humans [4,5,11,36,47]. It has been suggested that this persistent cold stress profoundly affects mouse physiology in ways that impair its suitability as a model for human physiology and disease [27]. The recommendation has therefore been made that studies of mice should optimally be made at 30 °C, within the mouse thermoneutral zone, to best facilitate comparisons to humans ([4,5,11,31,36]—but see [49] for some practical problems with this recommendation).

Perhaps the best recent example, among metabolic studies, of the importance of ambient temperature in determining a significant outcome variable was the finding that the effects of genetic ablation of the uncoupling protein 1 (UCP-1) were strongly dependent on ambient temperature. Enerbäck et al. [10] showed that UCP-1 KO mice on a mixed genetic background of C57BL/6 and sv129 mice, when housed at 21 °C, were cold intolerant but did not become any more obese than wild type mice when fed a high fat diet. When these UCP-1 KO mice were back-crossed onto a pure B6 background, however, they were actually resistant to weight gain when raised on a high fat diet at 21 °C, relative to wild type mice that had intact UCP-1 [30]. At 27 °C this difference was abolished [30]. Moreover, when UCP-1 KO mice on a pure B6 background were observed at 30 °C, they became obese, even when fed chow, and substantially more obese than wild-type mice when fed a high fat diet [12]. Hence there was a complete spectrum of responses in these mice, from protection against high fat diet induced obesity at 21 °C, to no effect at 27 °C and finally susceptibility to obesity at 30 °C. A less publicised example was the observation that ovariectomised mice become obese [52] but this effect was attenuated at 30 °C [6]. However, there are many previous examples, notably from the fields of immunology and parasitology that show ambient temperature is a key variable influencing the ability of mice to fight off infections or mount a response to lipopolysaccharide injections (reviewed in [27]). Temperature also affects chemical toxicity. One example is the U shaped curve of the lethal dose of salicylate at different temperatures, with a minimum around 25 °C [2]. Various other compounds show either increased or decreased toxicity with increasing temperature. Moreover, core body temperature can be differently affected by, for example, ethanol, depending on the environmental temperature [13,32]. Indeed temperature affects many aspects of mouse physiology, reproduction and behaviour [44,53]. Clearly housing temperature affects the outcome of many experiments, and analysis at different ambient temperatures may reveal important aspects of mechanisms that studies at single temperatures cannot, as is exemplified by the different responses to a high fat diet of mice without UCP-1, at different ambient temperatures [10,12,30]. A key question, however, is which ambient temperature allows for the most optimal translation of mouse data to humans and hence

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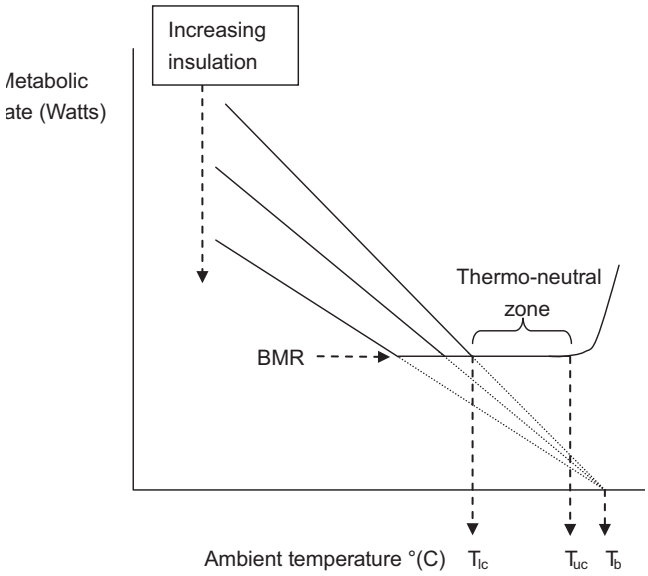

Figure 1: The classical Newtonian cooling model for an endotherm (after [40]).

maximises the suitability of the mouse model (see also discussion of this issue with respect to temperatures at which experiments should be performed on rats: [39]).

The suggestion that mice should be housed at 30 °C is based on two main arguments. First, humans normally live almost continuously at thermoneutral temperatures, and second 30 °C in mice is thermoneutral. We challenge here the idea that mice should optimally be held at 30 °C to best mimic human thermal physiology. We agree that attempting to mimic human thermal physiology in the mouse is a good idea to maximise its utility as a model for human physiology and disease. Our argument is based on the derivation of equivalent ambient temperatures, relative to the thermoregulatory curves for humans and mice, which suggests to us that the optimal temperature to achieve this aim is not 30 °C.

The classic thermoregulatory curve for an endotherm is shown in Figure 1 (after [40]). The thermoneutral zone is the region where basal energy expenditure generates more than enough heat to balance heat losses due to the difference between ambient (T_a) and body temperatures (T_b). At the lower margin of this region (called the lower critical temperature or T_{lc}) heat requirements to maintain T_b and basal metabolic rate are exactly matched. At higher temperatures basal metabolic rate provides too much heat and this needs to be dissipated. Generally this is achieved by elevating evaporative water loss. At some upper critical temperature (T_{uc}) however other mechanisms need to be recruited and these paradoxically require an elevation of metabolism. Below T_{lc} metabolic rates must be higher than basal levels to balance heat loss, hence metabolic rate increases linearly as temperature declines and the gradient of this relationship reflects the degree of external insulation. Greater levels of external insulation lower the gradient of this line and hence also lower T_{lc}. These curves extrapolate on the x-axis to body temperature.

The thermoregulatory curve for naked humans has a lower critical temperature averaging about 28 °C (Table 1). Providing light clothing increases external insulation, lowers the gradient of the thermal response line and lowers the lower critical temperature. Light clothing such as would typically be worn indoors consisting of a long sleeved shirt or blouse (Clo = 0.2) and light trousers (Clo = 0.25) provide together 0.45 Clo units of insulation [54]. The effect of such clothing would be to lower the lower critical temperature by 5.1 °C (using the correction equation for clothing on T_{lc} in [28]). This results in a lower critical temperature of 22.9 °C. Humans, however, seldom operate at basal metabolic rate. Studies using doubly labelled water show that routinely our energy expenditure is about 1.6–1.8 × basal requirements [3,46]. If we lived at our lower critical temperature defined from basal metabolism, we would be under continuous mild heat stress, so we normally seek out cooler temperatures than thermoneutral, where our routine heat production is balanced by a thermal gradient that generates an equivalent heat loss. This is why buildings are regulated at 19–21 °C (about 3 °C below the lower critical temperature) rather than within the thermoneutral zone (which for a lightly clothed individual is 23–27 °C). The UK Workplace (Health, Safety and Welfare) regulations 1992, for example stipulate that rooms should ideally be maintained at 19–21 °C to provide a comfortable working environment. Although it is routinely stated that in mice 30 °C is thermoneutral (after [18]). Figure 1 emphasises that 'being thermoneutral' is not the same as being at the lower critical temperature. In fact Gordon [18] cites the thermoneutral zone as spanning from 26 to 34 °C in mice. A review of some estimates of T_{lc} in mice is shown in Table 2. There is a strong negative relationship between T_{lc} and body weight (Figure 2). For adult mice weighing > 25 g the T_{lc} is about 28 °C. The predicted T_{lc} for mice weighing 40 g is 25.7 °C. Consequently, if we want to keep mice at an equivalent temperature to that routinely occupied by free-living humans (3 °C lower than T_{lc}), this equates to an ambient temperature of 23–25 °C.

While this calculation is arithmetically equivalent it assumes that the thermal responses of mice and humans are proportionally the same.

Reference	T_{lc} (°C)
[9]	27
[21]	30
[51]	29
[41]	27
[15]	27
[38]	27
[28]	28.6
Average	28

Table 1: Some estimates of lower critical temperature (T_{lc}) in naked humans.

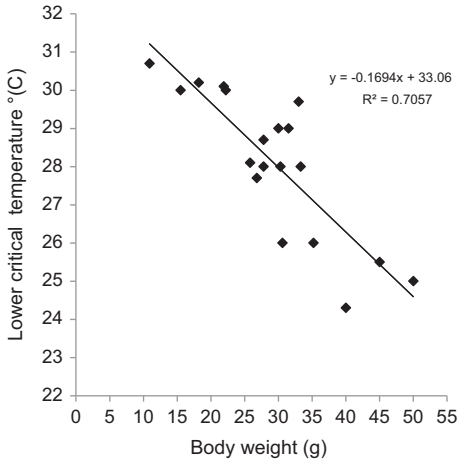

Figure 2: Lower critical temperatures of some mouse strains plotted against body weight. There was a strong and significant negative relationship (for data sources see Table 1).

Strain	BM (g)	T_{lc} (°C)	Reference
–	–	28.5	[48]
Hairless	–	29	[1]
–	–	25	[24]
Hairless	32.8	32	[35]
LACA	31.5	29	[37]
Wild	15.5	30.0	[26]
FVB	33.0	29.4	[29]
DTA-UCP	54.7	29.7	[29][a]
MF1	33.3	28.0	[45]
MH	30.3	28.0	[42]
ML	27.8	28.0	[42]
C3HeB/FeJ	27.8	28.7	[34]
Sma1 (+/−)	18.2	30.2	[34]
Sma1 (−/−)	10.9	30.7	[34]
TR-KO	22.2	30.0	[17]
Mixed	30.6	26.0	[17]
C57BL/6	30.0	29.0	[25][b]
Ob/Ob (6)[c]	~45	25.5	[25][b]
Ob/Ob (10)[c]	~50	25.0	[25][b]
Db/Db	40.0	24.3	[25][b]
Trpv1 KO	45.0	32.5	[14][d]
WT	40.0	31.5	[14][d]
C57BL/6[e]	21.9	30.1	[33]
C57Bl/6	26.8	27.7	[33]
C57BL/6[f]	25.8	28.1	[33]
MF1	35.2	26.0	[43]

Table 2: Some estimates of lower critical temperatures (T_{lc}) in different mouse strains. [a] UCP diphtheria toxin A mice with impaired thermoregulatory function. [b] Precise weights not given. [c] (6) and (10) refer to weeks old. [d] Measured using the thermal camera method. Not comparable to other data. [e] Under caloric restriction. [f] Fed rapamycin.

[1]		6.0 °C
[48]		5.0 °C
[24]		8.0 °C
[37]		9.5 °C
[29]		4.4 °C
[34]		6.0 °C
[17]		7.0 °C
[26]		7.5 °C
[42]	high	6.1 °C
	low	5.5 °C
[43]		4.0 °C
Mean		6.27 °C
Sd		1.62 °C

Table 3: Approximate reductions in ambient temperature below the lower critical temperature that would be necessary to achieve an increase in metabolism from basal to 1.7 × basal in various studies of mice. For details of studies and strains refer to Table 2.

That is it assumes a reduction of temperature by 3 °C has the same impact on a mouse as a human. Another way to approach this calculation is to calculate how much of a reduction in ambient temperature below the lower critical temperature in the mouse would be necessary to increase the metabolic rate from basal to 1.7 × basal (the average level of energy expenditure in free living humans: [3,46]). Using the published thermoregulation curves in some of the papers cited in Table 2 we have made this calculation and the results are shown in Table 3. These data suggest that to increase metabolic rate to 1.7 × BMR the temperature would need to be on average about 6.3 °C below the lower critical temperature. Consequently, this might suggest it would be most appropriate to keep mice weighing 25–40 g at ambient temperatures of 19.4–21.7 °C. However, this is probably an overestimate because the value of 1.7 × BMR for humans is based on our total daily energy demands, including periods when we are physically active and outdoors, while the temperature we regulate our buildings at (3 °C below lower critical) is geared towards balancing heat production during light activities such as sitting, computer use, preparing and having lunch and visiting the rest-room etc. If we use a value of 1.3–1.4 × BMR for these activities [50] then the equivalent reduction in ambient temperature below lower critical temperature, to generate a 1.3–1.4 fold increase in metabolism, is a reduction by 2.7–3.6 °C. This suggests the estimate of 23–25 °C as an equivalent temperature at which solitary mice should be housed to mimic humans is probably appropriate.

The estimates of T_{lc} for mice are based on respirometry measurements of solitary mice in respirometry chambers. Two common aspects of housing that are normally absent in such measures might further lower this estimate of the optimal housing temperature to mimic human physiology. First, mice that are group housed can huddle together to lower their thermoregulatory requirements [19,20]. This effectively lowers the T_{lc}, in part because of the reduced combined surface area [23]. Similarly, providing mice with bedding or nesting material acts as additional insulation which shifts the position of the thermoregulatory response line (see Figure 1) also lowering the T_{lc} [20]. This might further lower the optimal temperature. However, it is difficult to estimate to what extent, since mice will adapt their behaviour to the environmental temperature. Depending on the temperature, they will or will not bury themselves in bedding and nesting material or huddle together [16,20]. Consequently, this may suggest that the optimal housing temperature for comparison to humans of 3 °C below T_{lc} (23–25 °C) may be further decreased to as low as 20–22 °C with the availability of deep bedding, nesting material and/or group housing.

2. SUMMARY

The argument that to best mimic human physiology in mouse studies we should set the thermoregulatory conditions so that the metabolism of the two species is well matched makes a lot of sense. However, comparing the thermoregulatory curves of humans and mice, combined with the temperatures routinely selected by humans, suggests that the optimal temperature to achieve this is in the range from 23 to 25 °C for single housed mice, and around 20–22 °C for group housed mice. Keeping mice at 30 °C as has been recently advocated probably does not mimic well the situation in humans.

CONFLICT OF INTEREST

None declared.

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