Brief Communication

MOLECULAR METABOLISM

What is the best housing temperature to translate mouse experiments to humans?

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ABSTRACT

Objectives: Ambient temperature impinges on energy metabolism in a body size dependent manner. This has implications for the housing temperature at which mice are best compared to humans. In 2013, we suggested that, for comparative studies, solitary mice are best housed at 23–25 °C, because this is 3–5 °C below the mouse thermoneutral zone and humans routinely live 3–5 °C below thermoneutrality, and because this generates a ratio of DEE to BMR of 1.6–1.9, mimicking the ratio found in free-living humans.
Methods: Recently, Fischer et al. (2017) challenged this estimate. By studying mice at 21 °C and at 30 °C (but notably not at 23–25 °C) they concluded that 30 °C is the optimal housing temperature. Here, we measured energy metabolism of C57BL/6 mice over a range of temperatures, between 21.4 °C and 30.2 °C.
Results: We observed a ratio of DEE to BMR of 1.7 at 27.6 °C and of 1.8 at 25.5 °C, suggesting that this is the best temperature range for housing C57BL/6 mice to mimic human thermal relations. We used a 24 min average to calculate the ratio, similar to that used in human studies, while the ratio calculated by Fisher et al. dependent on short, transient metabolic declines.
Conclusion: We concur with Fisher et al. and others that 21 °C is too cool, but we continue to suggest that 30 °C is too warm. We support this with other data. Finally, to mimic living environments of all humans, and not just those in controlled Western environments, mouse experimentation at various temperatures is likely required.

Keywords Housing temperature; Thermoneutrality; Comparative physiology; Basal metabolic rate; Human; Mouse

1. INTRODUCTION

Temperature is a key environmental variable that exerts various impacts on physiology and health of all animals, including humans. Mice are currently the most widely used animal model for human disease and fundamental biology. Yet they differ from humans, most notably by being about 3.5 orders of magnitude smaller in body mass. This difference has an impact on their thermal relations. Historically, the temperature at which animal facilities have been maintained is around 20–21 °C. The choice of this ambient temperature was not based on any objective evaluation of whether it best suits the animals in question. It was also not based on any evaluation of whether it promotes the most efficient translation of data from mouse to human. In recent years, this has raised some debate (e.g. [2–5]). Prior to 2013, this debate was framed largely as follows. The argument was made that humans generally live at thermoneutral temperatures, which minimises their energy demands, and maximises thermal comfort. It was then noted that 21 °C lies well below the thermoneutral zone of the mouse, and that their thermoneutral zone is around 30 °C. It was therefore postulated that mice should not be housed at 21 °C, but rather at 30 °C (e.g. [2,6]).

In 2013, we questioned the logic of this argument on several grounds [7]. First, we showed that humans routinely maintain their living environments about 3–5 °C below their thermoneutral zone, not as is widely suggested 'inside the thermoneutral zone', which was further confirmed by Kingma et al. [8]. Second, we showed that the lower critical temperature, which is the lower end of the thermoneutral zone, where animals experience maximal comfort when metabolising at basal metabolic rate, is dependent on the size and strain of the mouse under consideration. So, 30 °C only coincides with the lower critical temperature for certain smaller strains; for larger mice, it may be as low as 24 °C. For the most commonly used stain (C57BL/6), it is around 27–29 °C. A third and more salient issue, however, is that the lower critical temperature is only the most desirable to balance heat production in the basal state. If an animal expends energy above basal, then the temperature at which it exactly balances its heat budget will

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Abbreviations: DEE, Daily energy expenditure; Hilpda, hypoxia induced lipid droplet associated 2; PAL, physical activity level; RER, Respiratory exchange ratio; RMR, Resting metabolic rate

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be lower, unless it also modulates body temperature. Indeed, humans very seldom expend energy at basal levels, but instead expend energy at around 1.6–1.9x basal metabolic rate [9]. Hence, this explains why the preferred temperature for humans to balance their heat budget is several degrees below their lower critical temperature. We suggested then that for a standard sized mouse (e.g. C57BL/6) without a nest, housed solitarily, the optimum temperature for housing to provide maximum translatability to humans might be around 23–25 °C; also 3–5 °C below their lower critical temperature.

In a recent paper, Fischer et al. [1] challenge these arguments. Their argument starts from the premise (as did Maloney et al. [3]), that we recommended that mice should be housed at the standard temperature, which they state is 20 °C. This is incorrect; we only suggested that this housing temperature might be appropriate for group housed mice, that can huddle to keep warm, with lots of bedding and deep litter to serve as insulation. For single housed mice, as were studied by Fischer et al. [1], we recommended 23 °C–25 °C. They then, going back to the arguments pre-2013, compared the metabolic rates of mice measured at 21 °C (our supposed recommendation) with mice measured at 30 °C (the supposed mouse thermoneutral). They ultimately concluded that mice at 30 °C expend energy at around 1.8x basal levels, thereby closely mimicking the human level of energy expenditure, while mice at 21 °C expend energy at 3.1x basal and, hence, are chronically cold stressed. Regrettably, however, since the paper is framed as a direct rebuttal of our recommendations, they chose not to measure mice at the temperature we did recommend.

Apart from setting up a straw man, by claiming we had recommended 20–21 °C, and then showing that solitary mice at this temperature are cold stressed, there are a number of issues with the study by Fisher et al. [1]. To address this, we first present data on the oxygen consumption of C57BL/6 mice measured across the range of temperatures from 21.4 to 30.2 °C, and in the light of these new data discuss some problems with the previous report by Fisher et al. [1] and more generally with the idea that the best temperature to translate mouse to human is 30 °C.

2. MATERIALS AND METHODS

The experiments were approved by the Institutional Ethical Review Board of the Chinese Academy of Sciences, Institute of Genetics and Developmental Biology, Beijing, approval number AP2014011. Oxygen consumption of four to eight male C57BL/6J mice was measured at six different temperatures: 20, 22, 24, 26, 28, and 30 °C, using a standard open-flow indirect calorimetry system (TSE Phenomaster system, TSE Ltd, Bad-Homberg, Germany). We used 2 different systems. One had 16 chambers paired to 8 analysers, while the other had 6 chambers paired to 2 analysers. Mice had *ad libitum* access to a standard low fat diet (D12450B with 20% protein and 10% fat: Research Diets Inc, New Jersey, USA) and drinking water. The cages had a light covering of sawdust to absorb urine, but were without bedding. The photoperiod was fixed at 12 h:12 h with lights on at 0730 h. The same individual mice were not always measured at each temperature. On average, the mice were 10–12 weeks old and weighed 27–30 g when the measurements were made. The actual temperatures inside the cages during the measurements were measured and averaged 21.4 °C ($n = 8$), 22.0 °C ($n = 8$), 23.5 °C ($n = 4$), 26.8 °C ($n = 4$), 27.0 °C ($n = 6$) and 30.2 °C ($n = 6$), in the six conditions. Within any 24 h cycle, the temperature within a cage varied by ±0.5 °C. Since the nominal 26 and 28 °C groups ended up being at 26.8 °C and 27 °C, respectively, the data in these conditions were pooled ($n = 10$ mice), providing measurements at 5 different temperatures. Mice were placed in the chambers for 3 days. The first day's data were rejected, and the data for the next 2 days were retained. The cycle of measurements was 6 min in the larger system and 12 min in the smaller system. Hence, in each 24 h, a total of either 120 or 240 measurements was made, with 240 or 480 individual measurements over the two days. All data were recalculated using the known body weights as ml O_2/h, according to Tschöp [10]. For illustrative purposes, the average oxygen consumption was calculated across all mice over the 24 h cycle. These averaged data were plotted against time of day.

For each ambient temperature, the average oxygen consumption was calculated across all the measurements across each individual ($n = 31$ measurements). RMR at 30 °C was estimated in three different ways. First, the absolute lowest value in a single 12 min interval for each individual over each of the two days of measurement was taken and then this lowest value was averaged across the two days. Second, the running average oxygen consumption over 24 min was calculated. Then the lowest of these averaged data in each of the two measurement days was used, averaged across the two days. Finally, the running average over an hour was taken and the same daily minima in these hourly averages was calculated, then averaged across the two days of measurement for each individual. These RMRs are referred to as RMR_{lowest}, RMR_{24} and RMR_{60}, respectively. Several additional values were subsequently derived. The ratio of the average daily oxygen consumption of each individual mouse was calculated as the minimum resting oxygen consumption at 30 °C, using the three different estimates of RMR. This ratio is roughly equivalent to the calculated physical activity level or PAL in studies of humans (daily energy expenditure/basal energy expenditure: e.g. [11]). We plotted these individual ratios against the corresponding individual average temperature experienced by each mouse and fitted a least squares regression to the data. We then interpolated on this fitted curve the temperature corresponding to ratios of 1.8 and 1.7.

In a separate experiment using 7 male C57BL/6 mice, the impact of a diurnal cycle in temperature was explored, as suggested by Fisher et al. [1]. The aim was to get a cycle from 30 °C in the day to 25 °C at night, however the response time of the system generated a cycle that peaked at 30.1 °C and had a minimum of 26.4 °C (Figure 3B). All the metabolic parameters measured were the same as above.

3. RESULTS

The relationship between the average oxygen consumption and time of day at each of the 5 different ambient temperatures averaged across all individuals at each temperature are presented in Figure 1. In all conditions there was an evident diurnal cycle of oxygen consumption, with values being higher during the period of darkness (black bar), when the mice were most physically active. The points of lowest metabolism are indicated by small arrows and were invariably in the afternoon between 1230 and 1700 h. The total oxygen consumption and the resting oxygen consumption both increased as the temperature declined from 30.2 °C to 21.4 °C (Figure 2A). Fitting a line between the average mouse body temperature of 36.6 °C [12–14] and the data below 30.2 °C (a so-called 'Scholander plot') allowed us to estimate that the lower critical temperature was about 28 °C (Figure 2A), identical to our previous estimate [7]. Because both total and resting rates of oxygen consumption seemed to converge as temperature declined, the ratio of the two declined. Hence, at 30.2 °C the ratio of resting to the absolute minimum resting rate was 1.30. In comparison at 21.4 °C, the value of this ratio was 1.11.

In the context of comparing mouse to human metabolic rates, the ratio of the average daily oxygen consumption to the resting rate measured

at thermoneutrality is of particular interest. Taking 30 °C as a thermoneutral temperature (see Figure 1), the ratios of average daily oxygen consumption for each individual mouse to the three different estimates of resting metabolism at 30.2 °C (RMR_{lowest}, RMR_{24} and RMR_{60}) are plotted against ambient temperature in Figure 2B–D. These data show that independent of the method for calculating RMR, the ratio increased as it became cooler. The least squares fit regression through these individual data had an r^2 of 0.53 ($n = 33$). However, the absolute values of the ratios differed depending on the RMR calculation method that was used. Using the absolute minimum resting metabolic rate (RMR_{lowest}) the ratio was 1.66 at 30 °C, and 2.13 at 21.4 °C. Using the lowest average over 24 min (RMR_{24}) the ratio was 1.58 at 30 °C and 2.02 at 21.4 °C, and finally using the lowest average over 60 min (RMR_{60}), the ratio was 1.46 at 30 °C and 1.86 at 21.4 °C. The average ratio of daily energy expenditure (DEE) to basal energy expenditure, called PAL, in free living humans living in Europe was 1.8 (range 1.6–1.9 [11] Figure 1B in that citation). If we interpolate the value of 1.8 on the fitted relationships in Figure 2B–D, then these suggest that using the RMR_{lowest} the equivalent temperature to generate human like measures of metabolic rate would be 27.2 °C, using the RMR_{30} the equivalent temperature is 25.5 °C and using the RMR_{60} gives an equivalent temperature of 22.3 °C. Using a PAL value of 1.7, which is more representative of humans living in North America [11], gives values of 29.1 °C, 27.6 °C and 24.6 °C, respectively.

We also explored the impact of a diurnal cycle in ambient temperature between 26.4 °C and 30.1 °C, on the metabolic rates of 7 mice, which is shown in Figure 3, along with the actual temperature cycle. This group of mice weighed 22–24 g and had lower metabolic rates overall than the groups represented in Figures 1 and 2. The average temperature throughout the 24 h was 28.1 °C. Based on the relationship in Figure 2C one would anticipate a ratio of DEE to RMR_{24} of 1.67. The actual ratio derived from the average metabolic rate plot was 2.07.

4. DISCUSSION

These data on the DEE to RMR ratio clearly contrast with the conclusions of Fisher et al. [1], who found a ratio of 1.8 at 30 °C and of 3.1 at 21 °C, and concluded on this basis that 30 °C is the best temperature at which to keep mice to mimic human metabolic responses. We discuss now the reasons for this discrepancy. The largest issue is how Fisher et al. [1] measured resting metabolism. They used 'high resolution' respirometry (a 5.6 L chamber measured every other minute) to measure the metabolism of mice. They claim that by using this system they are able to detect transient reductions in metabolic rate that are not detected by less sensitive systems. It is these transient reductions that they suggest are the 'true' basal metabolic rates of the mice. This is important, because if one uses these transient short reductions in reported oxygen consumption to characterise basal metabolism the result is substantially lower than if the metabolic rate surrounding these regions is used, and the resultant ratio is correspondingly elevated. The paper by Fischer et al. [1] is not the first to observe these transient reductions in oxygen consumption in metabolism traces. We previously showed, using an even greater resolution system (a 0.5 L chamber measured every 10 s), that such transient declines are common in mice when measured at 30 °C [15]. However, our previous interpretation of these short periods was not that these represent the true 'basal' metabolism, but are rather more likely apneic intervals when oxygen exchange transiently ceases. Alternatively, they may reflect periods of deep sleep and hence sub-basal levels of metabolism. Our interpretation in 2013 [7] was that the best representation of basal metabolic rate is not coincident with such transient drops, but is located in the surrounding region where the metabolic rate is low and most stable. This level would be adequately measured by lower resolution systems.

As we demonstrate here, the measurement of RMR depends on how wide the region is encompassing the lowest average metabolism. As previously shown by Hayes et al. [16] this relation to measurement duration is a consequence of statistically sampling a normal distribution of instantaneous metabolic rates, and not because the region includes a mix of resting and non-resting metabolism. As this region is made wider the estimated RMR increases, and the consequent ratios of average daily metabolism to resting metabolism become lower, and hence the derived optimum temperature mimicking human metabolism also becomes lowered. Using the lowest 12 min of metabolism we found the 'best' temperature was 27.2 °C–29.1 °C (depending on the human reference measure), but using the average over 24 min gave 25.5 °C–27.6 °C and the average over 1 h gave 22.3 °C–24.6 °C. The differences in how RMR is measured are crucial because using a high resolution system that is sensitive to the transient metabolic rate drops as Fischer et al. [1] do, leads to the conclusion that mice at 30 °C are routinely metabolising at around 1.8x basal metabolism, and hence that 30 °C is the optimum housing temperature. Clearly this is just a further extension of the series of optimum temperatures derived above.

The key question then is what is the most appropriate duration of RMR measurement to compare to humans. Human BMR is commonly measured using hood calorimetry which is equivalent to high resolution respirometry and the effective chamber size is very small. The usual procedure is to allow the person to settle down for around 30 min and then measure for 30 min using the average over the last 20 min of the measurement as the estimated RMR (e.g. [17,18]). On this basis, we consider that using the lowest 24 min is likely the most appropriate measure and from the measurements made here this leads to an optimum hosing temperature between 25.5 °C and 27.6 °C. Note that humans do not demonstrate the same transient drops in metabolism exhibited by mice ([5]; pers. obs.).

We have previously published 24 h metabolism profiles for mice using low resolution respirometry in their home cages with bedding, drink, and food, at both 22 °C and 29 °C [19]. Using the lowest average over 3 consecutive readings to represent RMR (equivalent to 24 min), these data showed that at 29 °C, the ratio for DEE_{29}/RMR_{29} was 1.38, while at 22 °C, the ratio DEE_{22}/RMR_{29} was 2.10, similar to the ratios derived here. This estimate is also consistent with the data from Abreu-Viera et al. [20], who performed a detailed analysis of energy expenditure in mice over a wide range of temperatures, from 4 °C to 33 °C, in which they determined basal metabolic rate, the thermic effect of food, physical activity energy expenditure, and cold induced thermogenesis. These authors observed that body temperature, the thermic effect of food, and physical activity energy expenditure were stable between 18 °C and 28 °C [20]. Their study shows that the ratio of basal metabolic rate plus cold induced thermogenesis over basal metabolic rate is 1.7 at approximately 24.5–25 °C. If we consider that both rest and a post-prandial condition are difficult to maintain in mice, their ratio of (basal metabolic rate plus the thermic effect of food, plus physical activity energy expenditure plus cold induced thermogenesis) over (basal metabolic rate plus the thermic effect of food) could be considered, which is 1.7 at approximately 24 °C [20].

Their established temperature generating 1.7 times basal metabolic rate (24.8 °C) is 4.5 °C below the lower critical temperature (29.3 °C) Abreu-Viera et al. found for mice on chow [20]. The estimate made here for the temperature generating 1.7 to 1.8x basal metabolism (averaging 25.5 °C) is also about 3 °C lower than the estimated lower critical

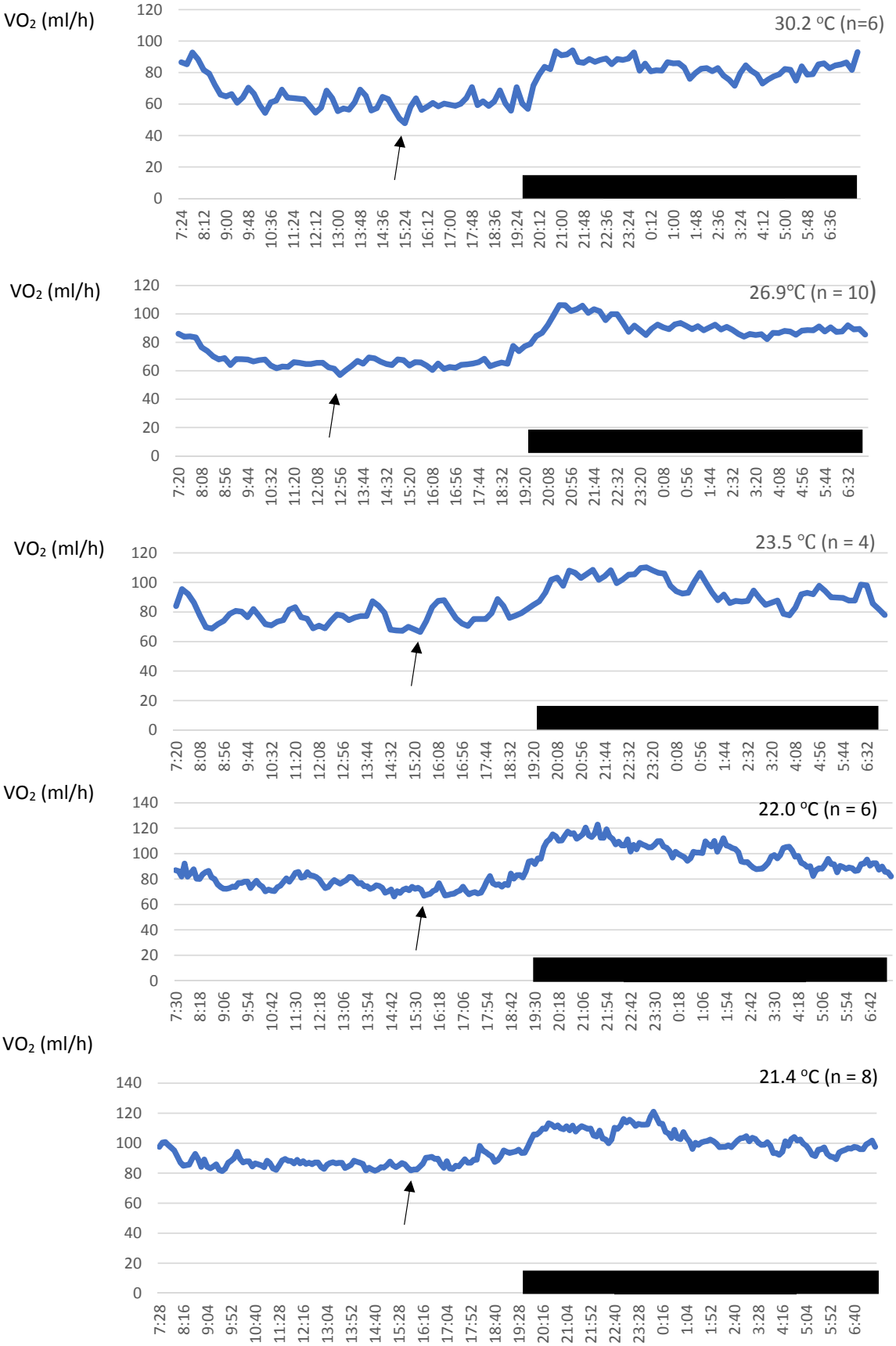

Figure 1: Oxygen consumption (ml/h) measured over the complete 24 h daily cycle averaged across 4 to 10 individual C57BL/6 mice measured over 2 days. Data are presented for 5 different ambient temperatures between 21.4 °C and 30.2 °C. The small arrows indicate the points of lowest metabolic rate. The black bar represents the period of darkness.

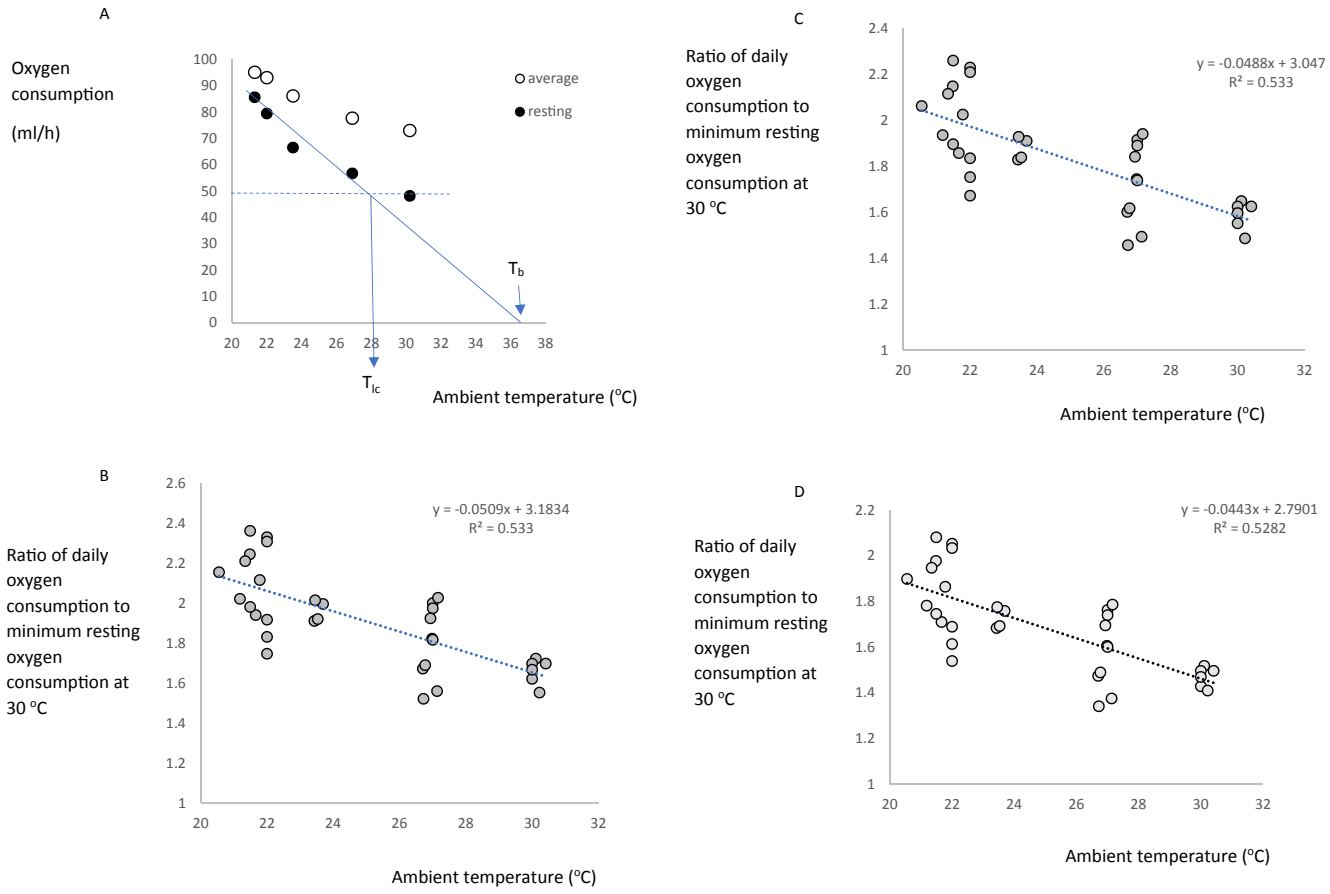

Figure 2: A: The average daily oxygen consumption (ml/h) averaged across individual mice at each ambient temperature between 30.2 and 21.4 °C, with the average resting oxygen consumption averaged across the same individuals. A: A line was fitted between the data below 30 °C extrapolating to the mouse body temperature (T_b) of 36.6 °C from the literature. This gave an estimated lower critical temperature of approximately 28 °C. Panels B, C and D: The ratio of the average daily oxygen consumption at various ambient temperatures to three different measures of resting oxygen consumption measured at 30.2 °C. B: The absolute lowest measurement averaged across n = 6 individuals. C: The average lowest over 24 min averaged across n = 6 individuals. D: The average lowest over 60 min averaged across n = 6 individuals.

temperature for these same mice. Following our previous arguments, this also matches the fact humans routinely occupy thermal environments about 3–5 °C below the human lower critical temperature.

4.1. Mice do not prefer to spend all their time at 30+°C

A second strand of the argument by Fischer et al. [1] relates to thermal preference of the mice. This critique of our recommendations has also been raised previously [21]. In a thermal preference test during daytime, the mice in their experiment routinely chose to rest at temperatures around 32 °C [22], well in excess of the suggested lower critical temperature. The reasons for this choice remain unclear. It is implicated that this observation refutes our housing recommendation, because, based on our arguments, the mice should prefer to select temperatures below thermoneutral. First, we used this argument for humans, that is to operate below thermoneutrality to be able to dissipate excess body heat, and projected this on mice. Second, our argument regarding the need to operate below thermoneutrality applies principally to the period when mice are operating at elevated metabolic rates, and hence need to dissipate their excess body heat above their basal production — i.e. at night time, when they are active. At night, other studies of mouse thermal preference indicate a preference for 26–29 °C [23–25], thus underscoring that mice prefer a substantially lower temperature when active.

4.2. 30 °C does not provide the best translation for other aspects of physiology

Fischer et al. [1] reference a large number of studies (References 1–18 in [1]) that show that environmental housing temperature affects physiological outcomes. A more extensive overview of effect of temperatures on physiological outcomes was recently published [26]. We fully agree that temperature affects physiological outcomes, but we do not agree with the subsequent conclusion that this implies that studies should be performed at thermoneutrality. We disagree for two reasons. First, for most effects, it is not clear which condition best represents the human condition; second, almost all experimental studies compare 21–22 °C to 29–30 °C, and only three studies that were cited by Fisher et al. [1], Yamauchi et al. [27], Wanner et al. [28], and Dudele et al. [29], include the intermediate range that we recommended. Furthermore, one cited study examined only 28 °C, 30 °C and above, concluding that 28 °C was below thermoneutral [30]. Closer inspection of the three cited studies that examined an intermediate range revealed for Yamauchi et al. [27] that these authors in fact concluded '*the temperature range of 20-26 degrees C to be optimal for laboratory mouse rooms*'. Similarly, Wanner et al. [28] do not advocate 30 °C. These authors performed their study in rats, not in mice, and observed clear differences in LPS response in the brain between 24 °C and 30 °C, and concluded that their control response at 24 °C agrees with

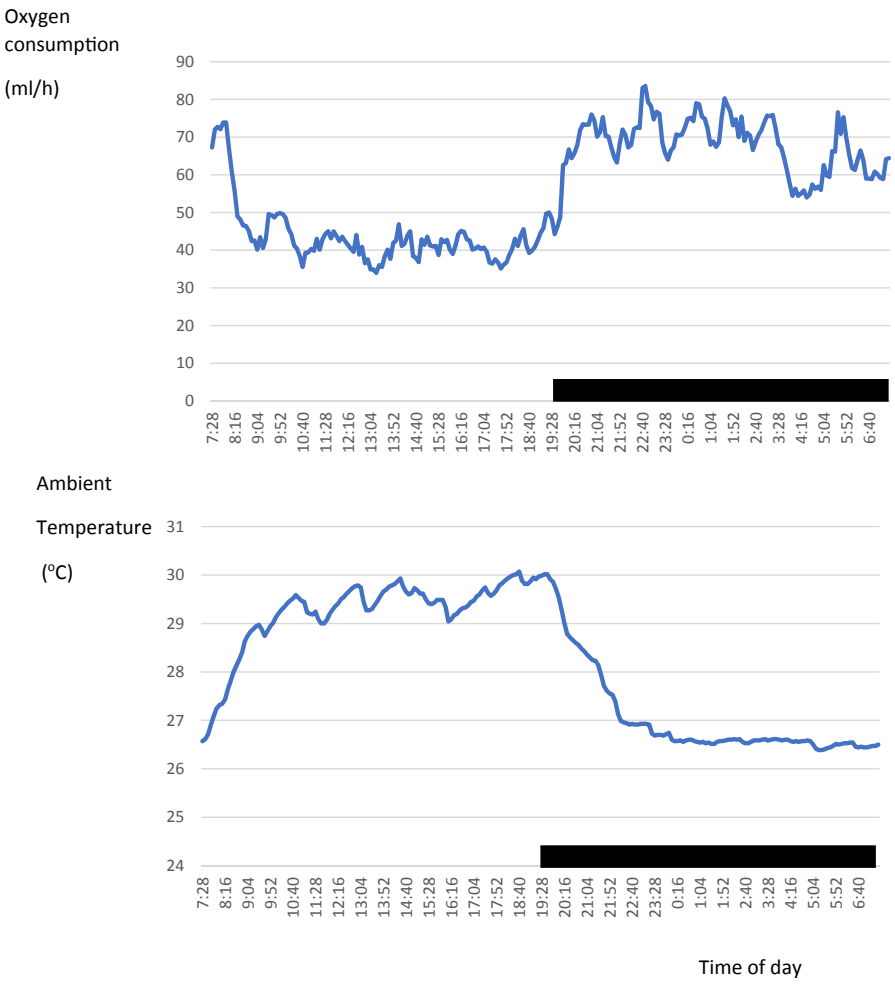

Figure 3: Responses of 7 C57BL/6 mice to a diurnal cycle in ambient temperature. A: The oxygen consumption averaged over 24 h. B: The simultaneous average temperatures across the seven cages. The minimum temperature was 26.4 °C and the maximum 30.1 °C.

existing knowledge on the function of the neurons that were examined. Next, Dudele et al. [29] show an identical pattern of fasting insulin levels plotted against body mass at 15 °C, 20 °C, and 25 °C. At 30 °C, this pattern was different with much higher fasting insulin levels, while glucose tolerance was diminished in diet induced obese compared to control mice. The authors stated that 30 °C masks responses, but then surprisingly concluded that studies should be done at 30 °C, because otherwise effects may be seen. We say surprisingly, because it seems that 30 °C is the outlier, and most different from the response as is observed in humans. Uncited by Fischer et al. [1], we note that studies in multiple mouse strains confirm that housing mice at 30 °C is severely detrimental to their reproductive performance compared to those housed at cooler temperatures [31–35].

Focussing on three other studies cited by Fischer et al. [1] in which the effects may be interpreted as translatable to humans, such as insulin and glucose responses in diet induced obesity, there are a number of considerations to be made. First, Giles et al. [36] found that many diet-induced differences in physiological effects, including fatty liver, indeed were more pronounced at 30 °C compared to 22 °C degree. However, other effects, e.g. glucose tolerance after antibiotics, were more pronounced at 22 °C. Moreover, the interpretation of the findings in this study are difficult, because an unrefined chow was compared to an undescribed high fat diet (usually semi-purified); both likely being composed of very different ingredients [36]. Since different ingredients may induce physiological effects on their own (such as specific fatty acids, e.g. [37]), this may work out differently at different temperatures. Rather than being a case for performing studies at thermoneutrality, this study highlights the importance of using defined diets with identical ingredients in the control and experimental conditions to assess effects of housing temperature. In another study by Giles et al. [38], the adverse cardiovascular and metabolic effects of a Western diet were found to be more pronounced at 30 °C compared to 22 °C. Also, differences between the control and the high fat high cholesterol Western style diet were more pronounced at 30 °C. This argues for 30 °C rather than 22 °C, but, again, the intermediate temperature that we recommended was not examined. Furthermore, these differences were associated with the extent of obesity that was induced under the various conditions [38]. This highlights the need to take possible confounding variables such as the duration of a study and the rate at which the mice become obese into account. In another study [39], thermoneutrality worsened inflammation, but importantly not glucose tolerance and insulin resistance. The latter would thus argue for 22 °C

rather than 30 °C. So, while Fisher et al. cites these studies as evidence for thermoneutrality as the best comparative temperature, we consider that this is not necessarily the case.

Glucose tolerance and insulin resistance are key parameters of metabolic health, that usually differ between lean and obese humans. In mice kept at 22 °C, there is a clear difference between mice fed a low fat diet and mice fed a high fat diet in glucose tolerance and (markers for) insulin resistance ([29,40,41], and many others), in lipid accumulation in the liver [42], and in white adipose tissue inflammation [43]. The diet dependent differences in these biomarkers disappeared at 30 °C [44]. This argues for comparative studies at a temperature below thermoneutrality, especially because many confounding factors were controlled for in [41–44]. These studies used the same C57BL/6j substrain, individual housing, and exactly the same diets, with the low fat and the high fat diets being composed of the same ingredients, only differing in fat to carbohydrate ratio.

Two other examples suggest that 30 °C is not the best comparative temperature. In humans, obesity and insulin resistance are associated with a decrease in the level and function of mitochondria in white adipose tissue, reflecting an impaired adipose tissue function [45]. Mice with adipose specific fumarate hydratase gene silencing, showing aberrant mitochondrial morphology and ATP depletion in white and brown adipose tissue, can be considered a model of dysfunctional adipose tissue mitochondria. In line with expectations, these mice develop glucose and insulin intolerance. However, the differences observed between wild type and adipose fumarate hydratase silenced mice in these key metabolic health parameters were observed at 22 °C, but were absent (glucose tolerance, insulin tolerance) or significantly smaller (liver mass, liver triglycerides) at 30 °C [46]. In yet another study, high fat diet induced defects in glucose and insulin tolerance were clearly observed at 22 °C in mice with white adipose tissue specific gene silencing of hypoxia induced lipid droplet associated 2 (*Hilpda*, also known as *Hig2*), but were diminished at 30 °C [47]. Similarly, clear effects were observed by brown adipose tissue specific *Hilpda* gene silencing at 22 °C, but no differences were seen at 30 °C [47]. Together these studies show the pronounced effects of temperature on physiological parameters and suggest for a variety of metabolic parameters, and particularly for glucose intolerance and insulin resistance, that 30 °C is not the best temperature to compare mice to humans.

5. CONCLUSIONS

We conclude that our original recommendation is robust to the suggestions of Fischer et al. [1] and that 30 °C remains an undesirably warm housing temperature, because it does not lead to a daily energy demand that mimics normal human daily life. Similarly, we continue to recommend, as we did previously [7], and concur with Fischer et al. [1], that 21 °C is also not ideal for solitary housed mice, because it is too cold. Given the observed ratio of DEE to BMR of 1.7 at 27.6 °C and 1.8 at 25.5 °C, we suggest that this is the best temperature range for housing C57BL/6 mice to mimic human thermal relations.

Another area where we concur with Fischer et al. [1] is that there is a strong diurnal cycle in mouse metabolism and hence heat production. Logically the temperature at which this is optimally dissipated will be different between day and night, cooler during the night when they are active and warmer in the day when inactive. Fischer et al. [1] indicate that this might be mimicked by exposing mice to a temperature cycle in their housing. We tested this idea by exposing mice to a cycle of temperature between 26.4 and 30.1 °C (Figure 3), and this did indeed produce an enhanced ratio of the total to resting metabolic rate compared to that predicted for the same stable average temperature. This enhanced ratio occurs because the mice can settle to a lower resting rate in the day time when it is warmer and then have a much higher metabolic rate at night when they are active and the chamber is cooler. This leads to a much more exaggerated diurnal cycle of metabolic rate than occurs when the temperature is stable, as predicted by Fisher et al. [1]. There are, however, a multitude of potential options here with respect to cycle amplitude and average temperature. Controlling the cycle to be the same across different laboratories may prove difficult. Another option then is to keep the temperature constant at the mid value recommended here (26.5 °C) and to provide mice with nesting material to build nests, into which they can retreat and create a locally heated microclimate during their quiescent periods [16], much as humans do during the night when they retire to bed. This in line with recommendations of the National Research Council [48] that says that animal rooms should be set below the animals' lower critical temperature to avoid heat stress, which, in turn, means that animals should be provided with adequate resources for thermoregulation (nesting material, shelter) to avoid cold stress.

Humans at 30 °C do not need to create a warm microclimate inside a bed, in the same way that mice housed at 30 °C do not extensively use or build substantial nests [49,50]. This raises a much wider issue, that all human populations are not equivalent in the thermal environments they experience, and the debate thus far has largely concerned whether mouse experiments mimic the thermal environment that is experienced by humans occupying controlled office and home thermal environments in the Western world. Large sectors of the global world population do not have access to environmental temperature controls. This suggests that mice housed at 30 °C may be a useful model for humans living in tropical regions without access to equipment to regulate their environmental temperatures. Similarly, mice housed at 21 °C may be a better representation of humans living in colder regions that also lack environmental temperature controls. It is important to recognise that spatial ambient temperature variations are strongly linked to the spatial variation in human metabolic disease risk. For example, 13% of the variation in prevalence of diabetes in the USA is linked to variation in average ambient temperature [51]. Hence the question of what temperature best mimics the situation in humans, depends to a large extent also on what human population one is considering. Indeed, even in the West there are differences in average PAL in different regions (noted above) which lead to differences of 2 °C in the predicted optimum housing temperature to mimic human thermal relations.

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CONFLICTS OF INTEREST

None.

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