Behavioral thermoregulation in obese and lean Zucker rats in a thermal gradient

MICHAEL MASKREY,¹ PAUL R. WIGGINS,² AND PETER B. FRAPPELL²
¹Discipline of Anatomy and Physiology, School of Medicine, University of Tasmania, Hobart, Tasmania 7001; and ²Department of Zoology, La Trobe University, Melbourne, Victoria 3086, Australia

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Maskrey, Michael, Paul R. Wiggins, and Peter B. Frappell. Behavioral thermoregulation in obese and lean Zucker rats in a thermal gradient. Am J Physiol Regulatory Integrative Comp Physiol 281: R1675–R1680, 2001.—Genetically obese Zucker (Z) rats have been reported to display a body core temperature (Tb) that is consistently below that of their lean littermates. We asked the question whether the lower Tb was a result of deficits in thermoregulation or a downward resetting of the set point for Tb. For a period of 45 consecutive hours, lean and obese Z rats were free to move within a thermal gradient with an ambient temperature (Ta) range of 15–35°C, while subjected to a 12:12-h light-dark cycle. Tb was measured using a miniature radio transmitter implanted within the peritoneal cavity. Oxygen consumption (V̇O₂) was measured using an open flow technique. Movements and most frequently occupied position in the gradient (preferred Ta) were recorded using a series of infrared phototransmitters. Obese Z rats were compared with lean Z rats matched for either age (A) or body mass (M). Our results show that obese Z rats have a lower Tb [37.1 ± 0.1°C (SD) vs. 37.3 ± 0.1°C, P < 0.001] and a lower V̇O₂ (25.3 ± 1.9 ml·kg⁻¹·h⁻¹) than lean controls [33.1 ± 3.7 (A) and 33.9 ± 3.9 (M) ml·kg⁻¹·h⁻¹, P < 0.001]. Also, the obese Z rats consistently chose to occupy a cooler Tb [20.9 ± 0.6°C vs. 22.7 ± 0.6°C (A) and 22.5 ± 0.7°C (M), P < 0.001] in the thermal gradient. This suggests a lower set point for Tb in the obese Z rat, as they refused the option to select a warmer Ta that might allow them to counteract any thermoregulatory deficiency that could lead to a low Tb. Although all rats followed a definite circadian rhythm for both Tb and V̇O₂, there was no discernible circadian pattern for preferred Ta in either obese or lean rats. Obese Z rats tended to show a far less definite light-dark activity cycle compared with lean rats.

body temperature; preferred ambient temperature; metabolic rate

Genetically obese Zucker (Z) rats have been reported to display a body core temperature (Tb) that is consistently below that of their lean littermates (1, 2, 20, 22, 24). A low Tb could be brought about either by a decreased rate of heat production, an increased rate of heat loss, or some combination of these two conditions. The reports regarding the relative rates of heat production or oxygen consumption (V̇O₂) in obese compared with lean Z rats are contradictory. Obese Z rats are variously claimed to show a higher V̇O₂ (1, 6), a lower V̇O₂ (17, 28), or a similar V̇O₂ (2, 22). Moreover, the situation is further complicated by effects of ambient temperature (Ta; Refs. 6, 17), prior temperature acclimatization (2, 17), or the actual units and exponents used to report the V̇O₂ measurements (2, 22, 28). Less information has been reported on possible differences in the rate of heat loss for obese compared with lean Z rats. Because obese Z rats have more body fat (28), it follows intuitively that they possess more body insulation. Against this are reports that obese Z rats display a higher minimal rate of heat loss than lean rats (6).

A possibly more important question is whether the lower Tb in obese Z rats is due to deficits in temperature regulation or the result of a controlled resetting of the set point for Tb. It appears that obese Z rats can certainly match their lean littermates in heat production if required to do so (1, 2, 6, 22, 34). It could be, however, that heat loss is much less well-regulated in obese Z rats. After all, it is known that obese Z rats show evidence of diabetes (8, 10, 21), which in turn is associated with deficits in autonomic innervation to peripheral blood vessels and a correspondingly poor control of peripheral blood flow. On the other hand, diabetes limits substrate supply to body cells. It might make sense to lower the basal levels of heat production required to maintain Tb and so to lower the set point around which Tb is controlled.

Regulation of Tb is not solely due to changes in autonomic function; Tb is also controlled by behavioral means. Rodents have been shown consistently to select a particular Ta (preferred Ta) when given the opportunity to move along a thermal gradient (11). This preferred Ta can be altered by a number of factors, including the light-dark cycle (4, 12), experimentally induced fever (32), and exposure to hypoxia (7, 14). It also varies with the strain of rat studied (11, 31). The preferred Ta for Z rats has not been reported. Even the thermoneutral temperature (Tₜₙ) for Z rats is not definitely established, being variously quoted as 25°C (17)

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and 29°C (see Ref. 22). Even so, it is known that $T_{in}$ is no guide to the preferred $T_a$; rats normally selecting a $T_a$ somewhat below $T_{in}$ (11). Neither is $T_{in}$ necessarily the temperature to which the rat is best suited from the point of view of normal health (35). The circadian rhythms for $T_b$ and activity in Z rats have been reported by Murakami et al. (24), and circadian changes in hormone levels have been reported by Martin et al. (21). However, neither of these studies provides information on $V_O_2$ or preferred $T_a$.

The present study was conceived to investigate whether the lower $T_b$ in obese Z rats is due to either 1) a deficit in thermoregulation or 2) a controlled reduction in set point. We argue that if a deficit in thermoregulation were the case, then the obese rats would select a higher $T_a$ than their lean littermates and that the difference in $T_{in}$ between the two groups would decrease and perhaps vanish. On the other hand, if a controlled reduction in set point were the case, then the obese Z rats would select a lower $T_a$ and the difference in $T_b$ would persist. As controls, we used both age-matched and weight-matched lean Z rats to investigate possible contributions of age and mass to any differences observed.

**MATERIALS AND METHODS**

All experiments had the approval of the La Trobe University Animal Ethics Committee.

Obese ($n = 8$, age = 46 ± 3 days, mass = 172.1 ± 28.3 g), lean age-matched ($n = 8$, age = 45 ± 3 days, mass = 121.0 ± 15.5 g), and lean weight-matched ($n = 8$, mass = 172.6 ± 11.4 g) Z rats were housed at a $T_b$ of 22°C and a 12:12-h light-dark photoperiod with the onset of the light phase at 0700. Before experimentation, each rat was anesthetized with Fluothane, and a small abdominal incision was made. A calibrated (±0.1°C) single-stage miniature radio transmitter (mass ~1 g, Sirtrack, New Zealand) in which pulse frequency altered with temperature was inserted into the peritoneal cavity to record $T_b$, and the wound was closed with vicryl sutures. After a minimum recovery time of 48 h, rats were ready to be used for experimental purposes.

A preweighed rat was placed in a chamber (1,500 mm × 100 mm × 100 mm) with the base and walls constructed from 10-mm-thick aluminum and fitted with a plastic floor raised 10 mm above the base. A Plexiglas lid (5-mm thick) fitted with an inlet and outlet port for air was sealed with a film of grease and screwed down to ensure an airtight seal. Air flow through the chamber was 1,200 ml/min.

The chamber was preheated at one end by water at 43°C and cooled at the other end by water at 5°C, thereby establishing a thermal gradient that was approximately linear from 15°C to 35°C. The sides of the chamber were fitted with a series of infrared phototransistors spaced at 35-mm intervals; the photocell closest to the warm end of the gradient that was obscured by the rat recorded the position (i.e., preferred $T_a$) of the animal within the thermal gradient. An antenna placed alongside the thermal gradient and connected to a scanner (Uniden Scanner, UBC900XLT) obtained the pulse signal from the implanted transmitter, which in turn was transformed to a rate (pulses/min; in-house converter). Food and water were available ad libitum; the food was distributed along the gradient, and water was provided at three sites, centrally and one at each end of the chamber. A 12:12-h light-dark photoperiod was maintained with the onset of light at 0700. Experiments were started at 1500, and rats remained in the chamber for 45 h.

Air from the outlet port in the chamber lid was subsampled (100 ml/min) and passed through a drying column (Drierite) and CO₂ scrub (Ascarite) before passing through an oxygen analyzer (S3A-I, Applied Electrochemistry). The fractional concentration of oxygen ($F_O_2$) was measured continuously. Baseline values of $F_O_2$ were checked every 3 h using a solenoid that switched between incumbent and excurrent gas. Any drift in the recorded signal was assumed to be linear. The rate of $V_O_2$ was determined from the product of the air flow through the chamber and the difference between inflowing and outflowing $F_O_2$ ($F_O_2$, and $F_O_{20}$, respectively), taking into account respiratory quotient-related errors (see Ref. 9), as

$$V_O_2 = \text{flow} \times (F_O_{20} - F_O_2)/(1 - F_O_{20})$$

where prime indicates dry CO₂-free gas.

**Data collection and analysis.** The outputs from the oxygen analyzer, the photocells, and the rate of the implanted temperature transmitter were recorded every 5 min using an analog-to-digital converter (PowerLab/800, AD Instruments, Sydney, Australia). For $V_O_2$, $T_b$, and $T_a$ (calculated from photocell position), the data were averaged for each hour. Data were statistically analyzed by repeated-measures ANOVA, with post hoc Bonferroni modified t-tests for the comparisons of interest. In all cases, significance was defined at $P < 0.05$.

**RESULTS**

Obese rats displayed a significantly greater mass than lean Z rats of the same age (Table 1). Over a 24-h period, the mean values for $T_b$, $V_O_2$, and preferred $T_a$ in age-matched lean Z rats were identical to those recorded from weight-matched lean Z rats (Table 1).

The mean $T_b$ of the obese Z rats was significantly lower ($P < 0.001$) for both lean age and weight groups (Table 1). Likewise, the mean $V_O_2$ of the obese Z rats was significantly lower ($P < 0.001$) than that of their lean counterparts. These differences persisted throughout the light-dark cycle (Fig. 1), with the zenith occurring during the dark phase and the nadir during the light phase for both $T_b$ and $V_O_2$ in both lean and obese rats.

**Table 1.** Rate of $V_O_2$, $T_b$, and preferred $T_a$ for obese and lean (age and weight) matched Zucker rats in a thermal gradient

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Lean (Age)</th>
<th>Lean (Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass, g</td>
<td>172.1±28.3</td>
<td>128.5±20.3*</td>
<td>172.6±11.4</td>
</tr>
<tr>
<td>Age, days</td>
<td>46±3</td>
<td>45±4</td>
<td>54±6*</td>
</tr>
<tr>
<td>$V_O_2$, ml/min -1kg-1</td>
<td>25.3±1.9</td>
<td>33.1±3.7</td>
<td>33.9±3.9</td>
</tr>
<tr>
<td>$T_b$, °C</td>
<td>37.1±0.11</td>
<td>37.3±0.1</td>
<td>37.3±0.1</td>
</tr>
<tr>
<td>Preferred $T_a$, °C</td>
<td>20.86±0.58*</td>
<td>22.72±0.61</td>
<td>22.47±0.68</td>
</tr>
</tbody>
</table>

Values are means ± SD for a 24-h period after an initial 18-h period of familiarization in the thermal gradient; values in parentheses are averages for corresponding light and dark periods, respectively. $V_O_2$; oxygen consumption; $T_b$, body temperature; $T_a$, ambient temperature. *Significantly different from obese rats; †significantly different from lean (age and weight) rats. No difference existed between lean cohorts.
Obese rats tended to show a far less definite light-dark activity cycle compared with lean rats (Fig. 3). Lean rats displayed little movement during the light phase and a great deal of movement during the dark phase, which is to be expected for a nocturnal animal and is in line with the circadian rhythm found for Tb and \( \dot{V}O_2 \). Obese rats, on the other hand, displayed considerable activity during both the light and dark phases and during activity tended not to move as far (note the number of times the rats moved 10 or more positions).

**DISCUSSION**

*Body temperature difference between obese and lean Z rats.* Both lean and obese Z rats display a \( T_b \) oscillation of \(-0.7^\circ C\), which is greater than that previously reported in Z rats (24) and slightly lower than that reported in Sprague-Dawley rats (23). The mean \( T_b \) of the obese Z rats (37.1°C) was significantly lower than that of the lean rats (37.3°C). This finding confirms previous studies in which a difference in \( T_b \) was reported (1, 10, 20, 22, 24). Variations in \( T_b \) between obese and lean rats appear to be independent of age, gender, or prior acclimatization temperature (see Ref. 22 for discussion). Our mean values for \( T_b \) are very similar to those reported by Murakami et al. (24). The difference in \( T_b \) of 0.2°C, however, is much smaller than that reported from other studies (1, 10, 22), in which the difference was on the order of 1.0–1.2°C. The common features of the present study and that of Murakami et al. (24) include that in both studies 1) \( T_b \) was measured using an implanted miniature radio transmitter, 2) the measurements were recorded continuously over an extended period, and 3) the animals were able to exhibit normal behavior. In the studies of Armitage et al. (1) and Godbole et al. (10), \( T_b \) was obtained daily as a single value by using a rectal probe. Maskrey et al. (22) also used a rectal probe, but this was kept in place throughout the experiment. How-
ever, because this last study included measurements of pulmonary ventilation, the animals were required to remain closely confined so that their body movements were limited.

The relevance of these differences in protocol lies in the fact that rats commonly increase \( T_b \) when stressed, whether the stress is due to handling (5), a novel environment (33), or immobilization (25). The important factor in all of this is that lean \( Z \) rats show these stress-induced increases in \( T_b \), whereas obese \( Z \) rats generally do not (29). The reason for this is that the increase in \( T_b \) is mainly brought about by an increased sympathetic outflow to brown adipose tissue (19). Obese \( Z \) rats appear to activate this pathway less readily (26, 27, 30, 37). Therefore, eliminating stressors such as handling, novelty, and close confinement reduces brown fat thermogenesis in lean \( Z \) rats, allowing the measured \( T_b \) to fall. Nevertheless, a significant difference in \( T_b \) between the lean and obese \( Z \) rats is still apparent. It is the cause of this difference that forms the main subject of the remainder of this report.

**Metabolic rate difference between obese and lean \( Z \) rats.** Metabolic rate measured in obese \( Z \) rats was significantly lower than that measured from lean rats. This appears to be a general finding when lean and obese rats are compared simply on a body weight basis (2, 17, 28). However, when metabolic rate is recalculated to take into account body surface area (2) or effective body mass (28), then the difference between obese and lean age-matched values disappears, although the difference between obese and weight-matched animals remains. This reflects the problem of standardization, and the finding in the present study of no difference between the two lean cohorts when standardized per unit body mass supports the argument that metabolic rate is low in obese \( Z \) rats when compared with lean rats.

Armitage et al. (1) reported that metabolic rate was higher in obese than in lean \( Z \) rats. No absolute body weight data were provided in their study. However, taking into account the statement that the obese rats weighed an extra 230 g, and judging by studies carried out on \( Z \) rats of a similar age (22, 28), we anticipate that the obese animals must have been of a body mass at least 35% greater than their lean counterparts. This weight difference would certainly be enough to eliminate the claimed difference in metabolic rates. By contrast, Demes et al. (6) report that, in their hands, obese rats definitely do show a higher metabolic rate than lean rats, whatever the units used to express this measurement.

Is it possible that the inconsistencies in the reports comparing metabolic rate in lean and obese \( Z \) rats are not entirely due to the units and exponents used to express the data? A clue to an answer to this question lies with the study of Kaplan (17), who reported that the higher metabolic rate seen in lean \( Z \) rats compared with obese rats when exposed acutely to a \( T_a \) of 25 or 30°C disappeared when rats were acclimatized for 48 h to a \( T_a \) of 30°C. Indeed, all studies claiming that metabolic rate in obese \( Z \) rats either matched or exceeded that of the lean rats were carried out at a \( T_a \) of 25 or 30°C (1, 6, 22). On the other hand, those studies that report a higher metabolic rate in lean \( Z \) rats were carried out at the lower \( T_a \) of 25°C (2, 28). In the present study, the \( T_a \) was not fixed; instead, the animals were able to select their preferred \( T_a \) within a thermal gradient. The mean \( T_a \) selected by both lean and obese rats was below 23°C (see below). We suggest that a \( T_a \) significantly above 25°C might produce a heat stress to obese \( Z \) rats and a consequent increase in metabolic rate. When \( Z \) rats are free to behave normally in a thermal environment of their choosing, obese rats settle on a rate of metabolism below that of lean rats of the same age or same body weight.

**Preferred \( T_a \) difference between obese and lean \( Z \) rats.** During the second day in the temperature gradient, the obese \( Z \) rats elected to spend the majority of their time at a \( T_a \) significantly below that chosen by lean rats. We believe that this constitutes evidence that the lower \( T_b \) in obese \( Z \) rats is due to a lower set point compared with their lean counterparts. This argument

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*Fig. 3. Activity in the various cohorts of \( Z \) rats. The activity index is determined by the rat moving at least a defined number of positions (2, 5, or 10) between subsequent recordings (5 min). Shaded area represents dark period. Values are means of all rats in each cohort.*

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*Downloaded from [http://ajpregu.physiology.org/](http://ajpregu.physiology.org/) by 10.220.32.246 on October 30, 2017*
has been used to demonstrate the association between preferred $T_a$ and set point $T_b$ during fever (e.g., Ref. 32) and has been used by Shido et al. (31) to explain the difference in preferred $T_a$ in two different strains of rat.

An alternative explanation for a lower preferred $T_a$ among the obese Z rats might have been that both rats have a similar rate of metabolism at thermoneutrality but that at a lower $T_a$ the lean rats require a higher rate of thermogenesis because of their greater surface area-to-volume ratio; hence they attempt to offset this by choosing a slightly warmer $T_a$. The counterargument to this suggestion is that lean rats of the same size as the obese rats (weight-matched controls) also select a warmer $T_a$. This reinforces the point already made that, because both lean cohorts are similar in all parameters measured, differences between lean and obese Z rats cannot be due to the effects of size.

The results of the present study may be compared with a study conducted on rats rendered hypothyroid after treatment with propylthiouracil (36). The treated rats showed deficits in thermogenesis evident as reduced metabolic rate and reduced $T_b$. Unlike the Z rats in the present study, however, they selected a $T_a$ higher than the untreated controls. Despite this, $T_b$ was still maintained below control levels. This study suggests that even when a thermoregulatory deficit clearly exists, changes in set point may still be relevant.

The absolute values for the mean preferred $T_a$ reported here for the Z rat of 22.5–22.7°C (lean) and 21°C (obese) lie below the values reported for other rat strains. Gordon (11) reported that for Sprague-Dawley, Fischer-344, and Long-Evans rats subjected to brief exposure periods, the preferred $T_a$ lay approximately between 20 and 25°C. However, in a later study conducted over a longer exposure period, preferred $T_a$ in the Long-Evans rat was reported to be as much as 9°C higher than that measured short term (13). Shido et al. (31) compared FOK, WKAH, and Donryu rats and reported mean daily preferred $T_a$ values between approximately 22 and 27°C. On the other hand, comparisons between different strains across separate studies may not be entirely valid. For instance, different thermoclines may provide differing microclimates due to the heat-radiating and -conducting properties of various materials used in their construction.

The preferred $T_a$ is below the $T_m$ of the Z rat, variously quoted to be 25°C (17) or 29°C (see Ref. 22). This is in agreement with the report of Gordon (11), who found that in all the rat strains he tested, the preferred $T_a$ was significantly below the lower critical temperature. Gordon in fact observed that his rats very rarely selected a $T_a$ of 30°C or above, even though this is where $V_{O2}$ was lowest.

The $T_a$ selected by Z rats did not vary between the light and dark cycles. This finding contrasts with other studies (4, 12) where a circadian rhythm of preferred $T_a$ was evident, although this rhythm often took as long as 4 days to become established (12), whereas our study extended for a 2-day period only.

The fact that when first placed in the thermal gradient all rats showed a distinct preference for the cooler end has been observed previously (12). It is of interest in this regard to note the differences in time taken for the Z rats to settle on their preferred $T_a$. Whereas lean rats took 6–12 h to achieve this, it took obese rats as long as 18–24 h. The reason for this is not obvious. It may be that, in addition to having a lower set point for $T_b$, obese rats may also display a less precise feedback mechanism for matching peripheral temperature sensory inputs with the preferred $T_a$.

Activity differences between obese and lean Z rats. The activity index used for the present study shows that although overall activity levels appear similar between obese and lean Z rats, the pattern of activity over the light-dark cycle is quite different. Whereas the lean rats display a great deal of activity during the dark phase and considerably less in the light, the obese rats show fewer large dark-phase excursions and many more light-phase movements.

A close relationship between the light-dark activity cycle and the $T_b$ cycle is well established (e.g., Ref. 12). However, the degree to which activity determines $T_b$ is not yet fully understood (15, 16). Yet, in the Z rat, where we have shown a significantly lower $T_b$ and $V_{O2}$ in obese compared with lean animals, this question is particularly pertinent.

Keesey et al. (18) report that the level of activity, as well as the circadian activity pattern, is similar for lean and obese Z rats. Murakami et al. (24), on the other hand, report that obese Z rats show much less overall activity and also a depressed amplitude of circadian rhythm for activity. The present study agrees with different aspects of each of these studies. We find that overall activity is similar in the two genotypes but that the circadian rhythm of activity is much less apparent in obese rats. This last fact is especially evident in that obese rats appear much more active during the light phase than do lean rats. This may reflect a higher incidence of feeding behavior during the light phase. In this regard, Bertin et al. (2) report that food ingestion during the daytime is twice as great in obese rats compared with lean rats.

Conclusions. The main conclusion of the present study is that the genetically obese Z rat maintains a lower set point for $T_b$. This is able to achieve by maintaining a lower level of metabolic heat production and, if available, selecting a lower $T_a$. Evidence also suggests that obese Z rats may display a less precise feedback mechanism for matching peripheral temperature sensory inputs with their preferred $T_a$.

The present study is unable to determine whether heat loss is also changed in the obese Z rat compared with lean littermates.

Perspectives

Since the introduction of the term “set point” to describe a controlled end point for $T_b$ into the literature on temperature regulation, much of our thinking has been driven by this concept. Another engineering anal-
ology that has proven useful is “load error,” the degree to which actual $T_d$ deviates from set point. In behavioral thermoregulation, preferred $T_a$ may be considered an adjunct to set point in that it describes the external thermal conditions to which an animal aspires. It is interesting to note the longer time interval taken by the obese Z rat to reach its preferred $T_a$ goal. Does this delay denote a poorer ability to detect a load error or a lesser urgency to correct it (i.e., a greater tolerance)? It may prove profitable to pursue this question, not just in the Z rat but in other models of thermoregulatory behavior. The present study also raises once again the unresolved question as to whether the obese Z rat is best regarded as a collection of endocrine, metabolic, and regulatory defects or rather as a unique assemblage of well-integrated, although unusual, physiological adaptations.

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