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Thermoregulatory responses of two mouse *Mus musculus* strains selectively bred for high and low food intake

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Abstract We examined the thermoregulatory responses of male and female mice *Mus musculus* that had been divergently selected on voluntary food intake, corrected for body mass, to produce a high-intake and a low-intake strain. Resting metabolic rate was determined by indirect calorimetry (at 30°C, 25°C, 15°C and 5°C). Body temperature responses were measured in a separate group of mice in a parallel protocol. High-intake mice had significantly elevated body masses compared to low-intake mice in both sexes. Lower critical temperature in both strains appeared to be around 28°C. At 30°C there was a significant strain effect on resting metabolic rate, with high strain mice having greater metabolism than low strain mice. Sex and body mass were not significant main effects on resting metabolic rate and there were no significant interactions. Body temperature measured at 30°C, 25°C, 15°C and 5°C differed significantly between sexes (females higher) and there was a significant sex×body mass interaction effect, but there was no difference between strains. Thermal conductance was significantly related to strain and sex, mice from the high strain and males having greater thermal conductances than mice from the low strain and females. Artificial selection has resulted in high-intake mice having greater body masses and greater thermal conductances, which together account for up to 45% of the elevated daily energy demands that underpin the increase in food intake. The greater levels of food intake were also associated with higher resting metabolic rates at 30°C.

Keywords Resting metabolic rate · Thermal conductance · Body temperature · Food intake · Artificial selection

Abbreviations *BM* body mass · *DEE* daily energy expenditure · *GLM* generalized linear model · *NST* non-shivering thermogenesis · *RMR* resting metabolic rate · RMR_{30} resting metabolic rate at 30°C · T_a ambient temperature · T_b body temperature · T_{lc} lower critical temperature

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Introduction

The allocation of energy between growth, reproduction, maintenance, activity and thermoregulation has been studied widely, particularly in small mammals (Hammond and Diamond 1992; Hayes et al. 1992a; Hammond et al. 1994; McDevitt and Speakman 1996; Koteja 1996a; Ricklefs et al. 1996; Koteja et al. 1999; Speakman 2000; Speakman and Johnson 2000). It is well established that an important component of the energy budget of small endothermic animals is the energy demand associated with the maintenance of body temperature (T_b), and it has been suggested that in many situations supplying such demands must take priority over other functions (Wunder 1984).

Within the thermoneutral zone, by definition, animals metabolise energy at a minimal rate with no thermoregulatory demands (Kleiber 1961). Below this thermoneutral zone, however, animals must increasingly rely on additional sources of heat to maintain a stable Tb (Jansky 1973). Such additional sources of heat include shivering thermogenesis, non-shivering thermogenesis (NST), and locomotor activity (Jansky 1973;

Bartholomew 1982; Richardson et al. 1994), with animals also exhibiting a wide repertoire of behavioural responses to conserve body heat (Contreras 1984; Canals et al. 1989; Hayes et al. 1992b; Redman et al. 1999). The process of NST liberates heat without shivering (Jansky 1973; Bartholomew 1982), and two types of NST, basal and regulatory, have been described (Jansky 1973; Richardson et al. 1994). Basal NST is generated in the thermoneutral zone (basal metabolic rate) and regulatory NST is the additional heat produced at temperatures below thermoneutrality. Therefore, differences in maximal NST (regulatory + basal) between animals can arise from an increase in either, or both, regulatory and basal NST.

The process of artificial selection has proved invaluable in helping to identify the links between various physiological parameters and energy expenditure (e.g. Konarzewski and Diamond 1995; Jackson and Diamond 1996). In the present study, we examined the thermoregulatory responses of two mouse *Mus musculus* strains, divergently selected for high and low food intake, corrected for body weight, over 38 generations. In previous studies (Hastings et al. 1997; Bünger et al. 1998), these have been referred to as the M-lines (MH and ML). The high-intake strain have food intake rates some 47% higher (corrected for body mass, BM) than low-intake individuals (Hastings et al. 1997; Bünger et al. 1998), and daily energy expenditures (DEE, corrected for BM at 22°C) 35% higher than low-intake mice (Bünger et al. 1998).

There are several alternative mechanisms by which selection may have resulted in different DEEs of the two strains. The time budgets of small mammals may be separated into periods when the animals are active and when they are resting. Selection may have altered the relative contribution of these components of the daily time budget or elevated the intensity (metabolic costs per unit time) of the activity. The high-intake strain has been reported previously to be 'more active' than the low-intake strain although this increase in activity was not quantified (Bünger et al. 1998). Locomotion itself, however, may have a very small effect on an animal's total energy budget (Garland 1983; Koteja et al. 1999) and locomotory activity may actually have a detrimental effect on other heat production mechanisms at low temperatures (Richardson et al. 1994). Alternatively (or additionally) selection may have acted on the resting metabolism of the animals. As the animals were selected at room temperature (22 ± 1°C), which is below the normal thermoneutral zone of mice (Mount 1971), this elevation of resting metabolism could occur by a combination of potential effects on resting metabolic rate (RMR) and thermal conductance.

First, selection may have resulted in an upward shift in the entire relationship between metabolism and ambient temperature (T_a)for the high-intake strain, including an increase in RMR (Fig. 1a). A second explanation for the higher DEE in the high-intake strain is that selection for high food intake has not altered RMR (basal NST) or the thermal conductance, but rather has affected the position of the lower critical limit of the thermoneutral zone, and hence also the Tb (Fig. 1b). A third possibility is that selection has resulted in changes in thermal conductance of the animals, requiring a compensatory increase in the regulatory component of NST, but RMR has remained unaffected (Fig. 1c). Existing empirical evidence, however, suggests that a relationship exists in mammals and birds between basal (at rest in thermoneutral zone) metabolic rate and DEE (Drent and Daan 1980; Peterson et al. 1990; Weiner 1992; Koteja 1996a; Hammond and Diamond 1997; but see also Ricklefs et al. 1996 and Speakman 2000). The fourth possibility therefore is that selection has acted both on thermal conductance and RMR (Fig. 1d). In combination, the differences in these two traits acting in concert would need to be smaller than if the difference at the selection temperature was a consequence of either mechanism acting alone. A final possibility (Fig. 1e) is that changes have occurred in thermal conductance, RMR and the lower critical temperatures (T_{lc}s). In the current study we aimed to distinguish between these alternative scenarios by measuring the thermal conductance, RMR and T_{lc}s of individual male and female mice drawn from the high and low strains.

Materials and methods

Study animals

The strains of mice *Mus musculus* used during this experiment were divergently selected for high and low food intake corrected for BM, at the Institute of Cell, Animal and Population Biology, University of Edinburgh, UK and described in detail elsewhere (Hastings et al. 1997; Bünger et al. 1998). In brief, the M(aintenance needs) line was derived from a common background population that was generated by a three-way cross: two inbred (CBA, JU) and one outbred line (CFLP) (Sharp et al. 1984). The mouse strains were developed by divergent selection for 38 generations on voluntary food intake between 8 weeks and 10 weeks of age, and corrected by phenotypic regression on BM to minimise correlated changes in BM. After generation 38, selection was suspended and at the start of generation 43 full sib matings were used to reproduce the lines to develop resources for future mapping studies. Mice used here were from generation 47.

We used a total of 22 male (11 high- and 11 low-intake) and 23 female (11 high- and 12 low-intake) mice for measurements of metabolic rate and a separate group of 37 mice (28 males: 13 high- and 15 low-intake and 9 females: 4 high- and 5 low-intake) for measurements of T_b. Mice were transported to the University of Aberdeen, Aberdeen, UK and individually housed (cage type M3: 48 cm×15 cm×13 cm), at 22 ± 3 C and under a 14 light:10 dark photoperiod (lights on 0700 hours). All individuals had ad libitum access to water and food (Rat and Mouse Number 1 Maintenance, Special Diets Services, BP Nutrition, UK), with sawdust provided for bedding.

Resting metabolic rate

All experimental procedures were carried out on mice between 8 weeks and 10 weeks of age. Immediately before a determination of RMR, BM was recorded (Sartorius, 0.01 g) and T_b measured using a rectal probe (Digitron) inserted 3–4 cm into the rectum. Prior to RMR measurements, individual mice were not denied

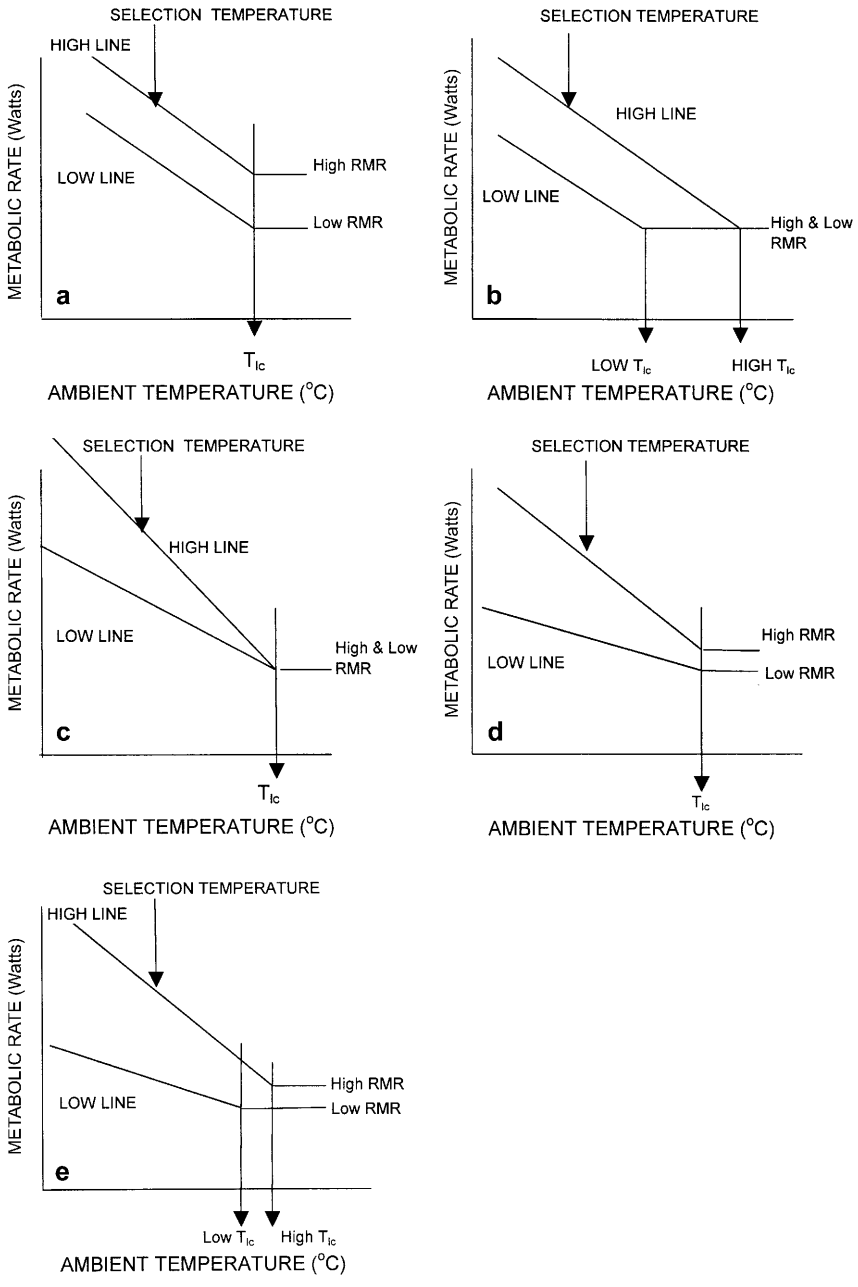

Fig. 1a–e Theoretical interrelationships between resting metabolic rate (RMR) and temperature leading to differences in metabolic rate at the selection temperature (22°C). In **a** the thermal conductances and lower critical temperatures (T_{lc}s) do not differ between the strains, but the RMR in thermoneutrality does. In **b** the thermal conductances and RMR do not differ but T_{lc} and body temperatures (T_bs) do. In **c** the thermal conductances differ but and RMRs at thermoneutrality and T_{lc}s do not, and in **d** thermal conductances and RMRs in thermoneutrality differ but T_{lc}s are the same. Finally, in **e** all three traits (thermal conductance, RMR in thermoneutrality and T_{lc}s) differ between the two strains

access to either food or water. However, most (>90%) food intake in these mice occurs nocturnally (C.C. Velten and L. Bünger, unpublished observations) and 'lights on' was normally 4–5 h prior to the initiation of the respirometry measurements. Mice were probably therefore post-prandial by the time measurements started. RMR was measured during the light phase, using an open-flow respirometry system (Servomex, Crowburgh, UK) employing a protocol previously described (Speakman and McQueenie 1996). In outline, mice were placed individually into a sealed Perspex chamber (total volume 1.02 l) contained within an incubator (Gallenkamp), and air, dried using silica gel (BDH, UK), was drawn through the system at a rate of 600–800 ml min^{-1}. Excurrent air was again dried and a subsample of 150 ml was passed through the oxygen analyser. To achieve maximal accuracy in the derived estimates of energy expenditure, carbon dioxide was not absorbed prior to oxygen analysis (Koteja 1996b; Speakman 2000). The measurements derived from the oxygen analyser were then recorded directly on a microcomputer at 30-s intervals. The ten lowest consecutive readings, (equivalent to 5 min within the respirometry chamber), were corrected for temperature and pressure, employing the appropriate equation from Hill (1972), and then used to estimate RMR.

RMR was determined at four different T_as (30°C, 25°C, 15°C and 5±0.5°C). RMR was measured at 1-h intervals at each incubator (Gallenkamp) temperature, with a minimum period of 30 min between each temperature change, to enable the mice to adjust to the new temperature (see Redman et al. 1999). The data collected during periods of chamber temperature adjustment were not included in the subsequent RMR analyses, and the total time an individual resided in the metabolic chamber never exceeded 6 h. The chamber temperature at which a measurement run commenced was alternated daily between 5°C and 30°C, therefore some animals were measured at 30°C immediately (at around 1000 hours) following entry to the respiratory chamber, and others spent over 7 h in the chamber before being measured at 30°C. In previous studies we have found that this protocol eliminates any detectable effect of the heat increment of feeding on RMR determinations in mice since there are no differences in RMR measured at the same temperature

in the morning and afternoon sessions (Speakman and Rossi 1999). This is probably because even the mice measured in the morning were already 4–5 h post-prandial by the time the measurements started, combined with the fact the diet of these animals was predominately carbohydrate based with a low protein content (22%), and that the dominant dietary feature precipitating heat increment of feeding is protein.

We selected these temperatures because our previous studies of adult mice indicated that T_{lc}s typically lie somewhere in the range 25–30°C (Speakman and Rossi 1999). Measurements at 30°C should therefore always be within the thermoneutral zone, while the pattern of variation in metabolic rates in response to lower temperature should allow derivation of the T_{lc}s and thermal conductance. To further characterize the location of the T_{lc}s we measured the RMRs on a separate occasion in a sub-group of the animals ($n = 11$ high- and 11 low-intake individuals) at 28°C and 30°C.

Body temperature

The design of our respirometry chambers meant that we could not rapidly remove animals during the course of the respirometry runs to assess their T_bs. Accordingly we measured T_bs of a separate group of mice by placing them into the incubators using exactly the same protocol as the respirometry measures but housing the animals individually in small 'shoebox' cages (without food or water) from which they could be rapidly removed. Mice were exposed to each T_a for 1-h intervals and followed the RMR experiment timescale exactly. After 1 h at each temperature mice were rapidly removed from the chamber and their T_bs measured using a rectal probe (Digitron) inserted 3–4 cm into the rectum.

Because T_bs were highly consistent across individuals in any given condition and because we did not use the same individuals for the measurements of T_b and metabolism, we calculated thermal conductance at 25°C, 15°C and 5°C using the mean T_b of the mice at that T_a combined with the individual measurements of oxygen consumption. This calculation means that the variance within each group is a consequence only of the variation between individuals in their oxygen consumption, while the differences between groups combine the individual oxygen consumption effects with the mean differences in T_b between groups.

Statistical analyses

All values reported are mean ± SD, except where indicated. Data were analyzed using MINITAB Version 11 (Pennsylvania State University, USA). Generalized linear models (GLM) were used with sex and strain as factors and BM as a covariate. Results were considered statistically significant at P values < 0.05. When significant factors were detected, post-hoc Tukey-Kramer tests were employed to assess minimum significant differences between groups. Repeated measures tests were used where appropriate.

Results

The mean (± SD) BM (g) in the mice used for estimation of oxygen consumption was 33.5(± 1.5) and 31.0(± 2.1) for high- and low-intake males respectively, and 27.2(± 1.7) and 24.6(± 2.2) for high- and low-intake females, respectively. Both sex and strain were significant factors influencing the variation in BM (GLM: strain effect, $F_{1,42} = 20.8$, $P < 0.001$; sex effect $F_{1,42} = 127.3$, $P < 0.001$). The interaction between sex and strain was not significant. Post hoc comparisons (Tukey-Kramer method) revealed that all four sex-by-strain groups differed significantly from one another ($P < 0.05$).

A typical resting metabolic rate (RMR) recording for a mouse during this experiment is shown in Fig. 2. At 15°C and 5°C the trace becomes less stable because the mice became more active at these lower T_as. The lowest ten consecutive readings at each temperature (30°C, 25°C, 15°C and 5°C, equivalent to 5 min in the respirometry chamber) were used to determine RMR. Despite the greater activity at lower temperatures, there were sufficient periods of inactivity during which 5-min measurements of resting metabolism could be made. Figure 3 illustrates patterns of variation in mean RMR, as a function of T_a, for male and female mice from the two strains. Because the interval between temperatures was relatively large it was difficult to define exactly from these plots the location of a T_{lc} for either strain. To further characterize the location of the T_{lc}s we compared the metabolic rates measured at 30°C with those measured at 28°C using paired t-tests. These data indicated that there was no significant difference in the metabolic rates of either the high-intake strain (paired $t_{10} = 1.97$, $P > 0.05$), or in the low-intake strain (paired t-test, $t_8 = 1.59$, $P > 0.05$) of mice between 28°C and 30°C. This analysis suggested that T_{lc} was probably around 28°C in both the high and low lines. To test between the alternative models illustrated in Fig. 1, we analysed three separate traits – RMR at 30°C which, given the apparent location of the T_{lc}, we interpreted to equal RMR in the thermoneutral zone, T_b and thermal conductance at 25°C, 15°C and 5°C.

RMR at 30°C

Because there were differences in BM both between strains and also between sexes, we explored the effects of strain, sex and BM on RMR at 30°C (RMR_{30}) using GLM. BM and sex did not emerge as significant main

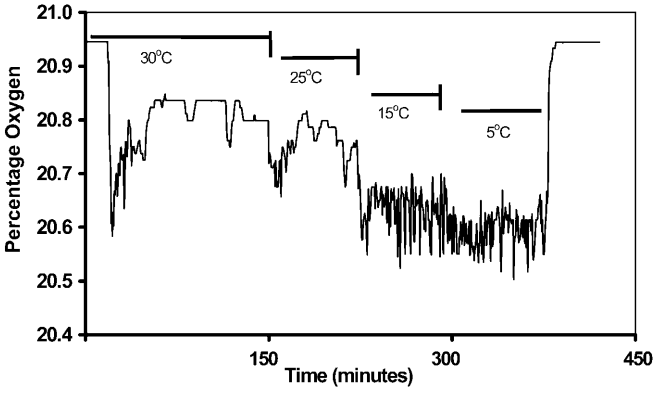

Fig. 2 A typical RMR plot for a mouse undergoing the experimental protocol employed during this experiment (see Materials and methods). The trace becomes less stable at lower temperatures (15°C and 5°C) due to an increase in activity by the mouse in the respirometry chamber. The lowest 5 min were used to determine RMR at each temperature, and the data collected during periods of chamber temperature adjustment were omitted from the subsequent RMR analyses

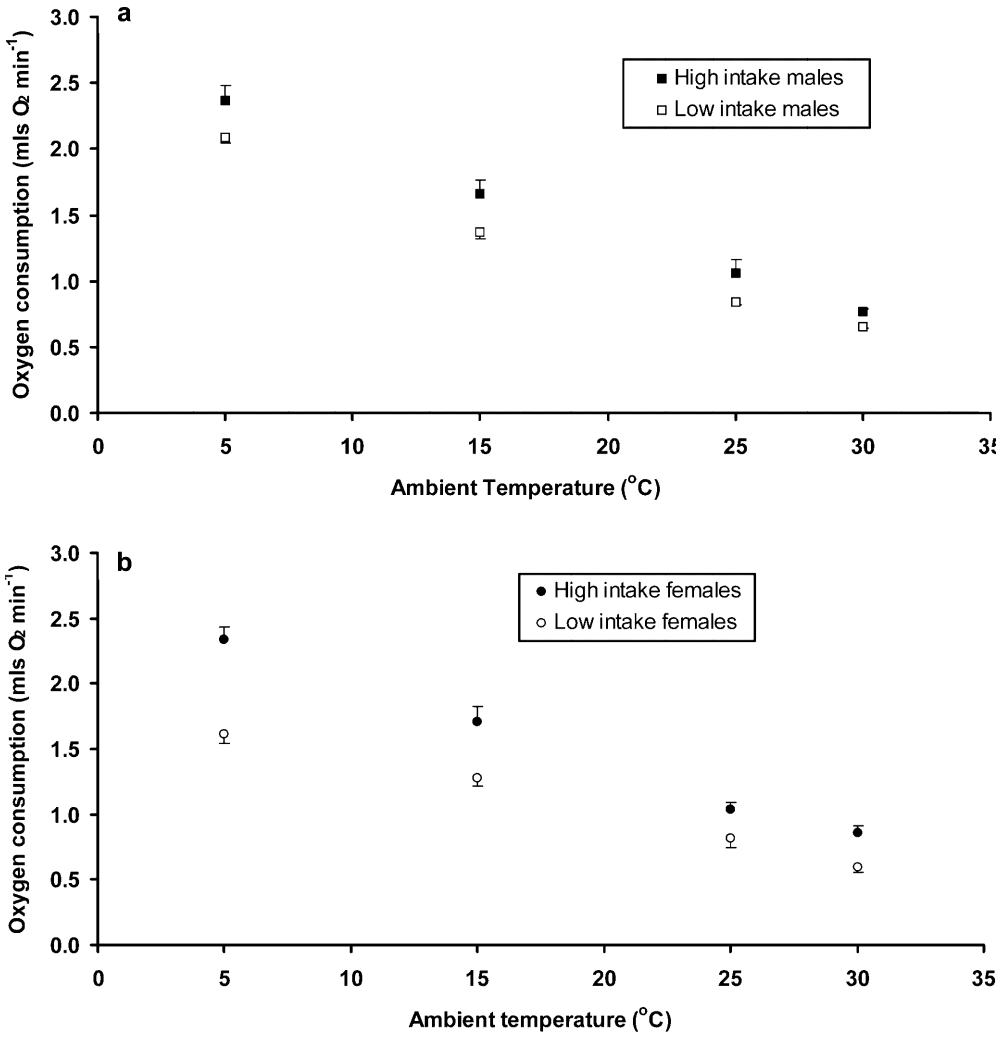

Fig. 3 Effect of ambient temperature (T_a) on RMR (mean ± SE) for high-intake and low-intake male (**a**) and female (**b**) mice

effects in this analysis ($P > 0.05$), and none of the interactions between factors were significant either ($P > 0.05$). The only significant factor affecting the RMR_{30} was strain (Fig. 4). On average the high-strain mice had RMRs at 30°C averaging 0.81 ml O_2 min^{-1} (SD = 0.14, $n = 22$) while the low-strain mice averaged only 0.63 ml O_2 min^{-1} (SD = 0.12, $n = 23$).

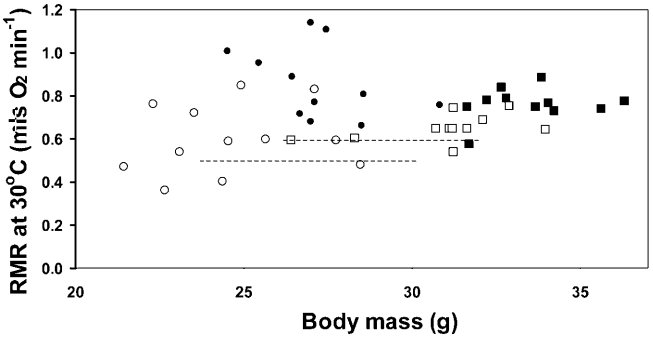

Fig. 4 Effects of body mass on RMR measured at 30°C for male (*squares*) and female (*circles*) mice from two strains selected for high food intake (*closed symbols*) and low food intake (*open symbols*). In a generalised linear model only strain emerged as a significant factor and mean levels of the two strains are indicated

Body temperature

Mean T_bs of the mice varied between 35.2°C and 36.5°C (Fig. 5). Within each group T_bs were highly consistent between individuals (SD averaged 0.3 across all groups). There were no significant effects of T_a ($F = 0.71$, $P = 0.549$) or BM ($F = 1.96$, $P = 0.164$) on the T_b. None of the two-way sex, strain, temperature interactions or the three-way interaction of these variables was significant. There was, however, a highly significant difference between the two sexes ($F = 10.97$, $P = 0.001$) with females at 35.71°C averaging 0.37°C warmer than the males at 35.34°C. Although BM differed significantly between the sexes, the main effect of BM on T_b was not significant. However, there was a significant sex×BM interaction effect ($F = 10.7$, $P = 0.001$). There was also a marginally significant strain effect ($F = 4.14$, $P = 0.044$) although the average difference between strains amounted to only 0.03°C.

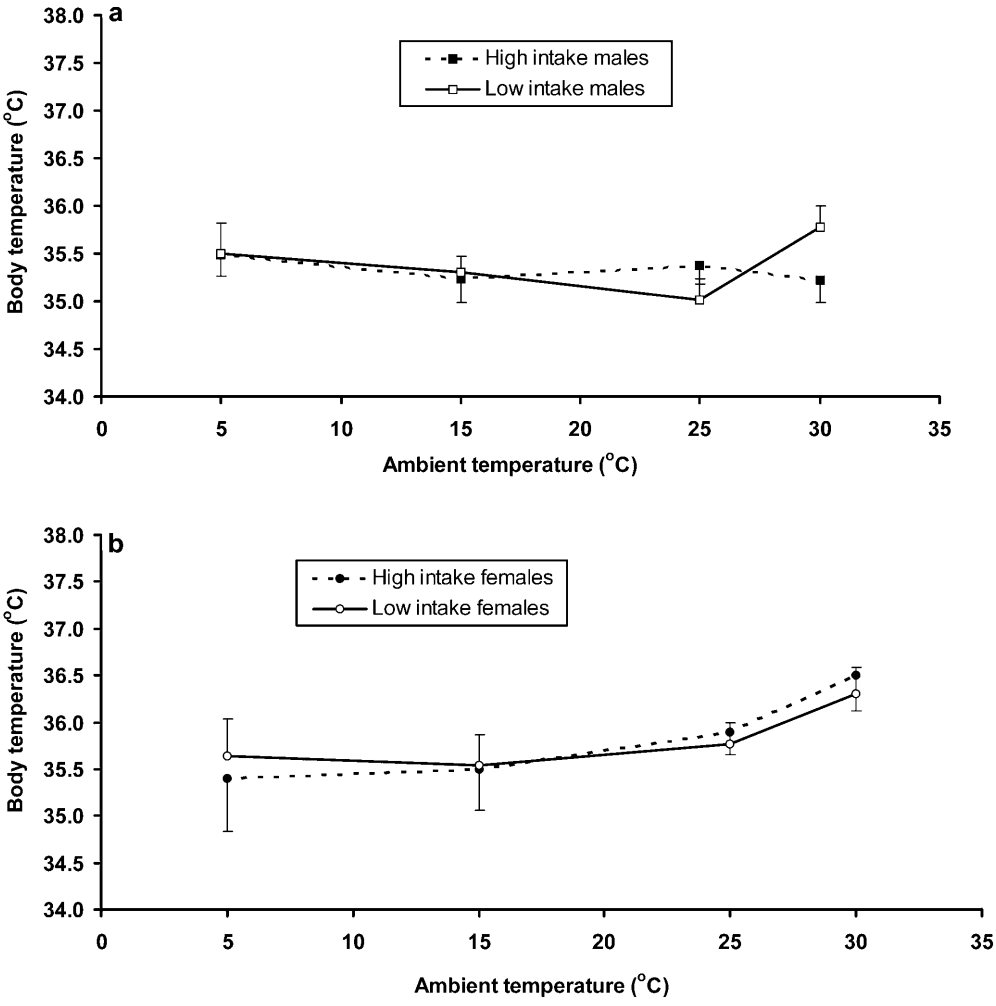

Fig. 5 Effects of T_a on the T_bs (mean ± SD) of high- and low-intake male (**a**) and female (**b**) mice. In a generalised linear model only sex emerged as a significant main effect, although there was also a significant sex×body mass interaction effect

Thermal conductance

We examined the effect of sex, strain, BM and T_a on the calculated values of thermal conductance which combine the individual estimates of oxygen consumption with the mean effects of T_b using GLM. None of the three-way interactions of the factors were significant. The largest effect was that of strain ($F = 12.14$, $P = 0.001$) with the high line having greater thermal conductance at all T_as (Table 1). Sex was also a significant factor ($F = 5.82$, $P = 0.018$) with males having greater conductance than females. Although BM was not a significant main effect ($F = 2.96$, $P = 0.089$) there was a significant two-way interaction effect of BM×sex ($F = 7.64$, $P = 0.007$). The effect of T_a was not significant ($F = 0.51$, $P = 0.602$) and none of the other two-way interactions were significant.

Discussion

The T_{lc}s for the mice in the present study (probably around 28°C for both the high- and low-intake strain) were consistent with previous studies which indicate that T_{lc}s in haired mice generally lie between 25°C and 30°C (Lacy and Lynch 1979; Speakman and Rossi 1999). The lower critical limits are higher in hairless and nude mice

Table 1 Mean (± SD) thermal conductance (ml O_2 min^{-1} $°C^{-1}$), at 25°C, 15°C and 5°C, in high (*H*)- and low (*L*)-food-intake male and female mice. *n* is the number of individuals measured to estimate body temperature (T_b). Thermal conductance was calculated from the average T_bs and the individual resting metabolic rate (RMR) measurements

Strain and sex	*n*	Thermal conductance at 5°C (ml O_2 min^{-1} $°C^{-1}$)	Thermal conductance at 15°C (ml O_2 min^{-1} $°C^{-1}$)	Thermal conductance at 25°C (ml O_2 min^{-1} $°C^{-1}$)
H males	13	0.078 ± 0.002	0.082 ± 0.004	0.103 ± 0.007
L males	15	0.069 ± 0.003	0.068 ± 0.002	0.084 ± 0.007
H females	4	0.077 ± 0.003	0.084 ± 0.004	0.096 ± 0.005
L females	5	0.053 ± 0.002	0.062 ± 0.002	0.076 ± 0.004

(Mount 1971) as might be expected from the low levels of external insulation they carry. The T_b patterns as a function of T_a were also consistent with a T_{lc} at around 28°C as there was no significant elevation of T_b at a T_a of 30°C and the absence of any decline in T_b with declining T_a indicated the mice were not becoming hypometabolic at the lower T_as. The significant effect of sex on T_b, with females having T_bs approximately 0.35°C higher than males, was consistent with our previous observations on another mouse strain (MF1: J.R. Speakman, E. Krol and T.K. Korhonen) but is not mentioned in the comprehensive review by Gordon (1993).

Despite attempts to control for BM differences during selection, there were slight (ca. 10%), but significant, differences in BM between strains (see also Bünger et al. 1998). Previous studies (Hastings et al. 1997; Bünger et al. 1998) have indicated that individuals of the high-intake strain are both heavier, and also leaner, than individuals of the low-intake strain. Therefore, the elevated RMR_{30} in mice from the high-intake strain could potentially have reflected their greater total and lean BMs. However, when the effects of mass were controlled there was a strong and highly significant effect of strain on resting metabolic rate. This suggests that the effect of selection on voluntary food intake in the high-intake strain has involved changes in metabolism not only due to increased BM, but also due to a correlated response in the basal component of NST. Indeed, in this data set we could not detect any contribution of BM differences to the observed difference in metabolic rate.

In addition to the correlated effect of selection on RMR in the thermoneutral zone, we also detected a significant difference between the strains in their thermal conductance, suggesting that some of the selection precipitating their higher food intake was due to an increased thermoregulatory requirement driven presumably by a reduction in pelage insulation. Morphological analyses of these mice suggests that there were significant strain effects on the mass of the pelage (Selman et al. 2001), with low-strain mice having heavier pelages than the high-strain mice, consistent with the lower thermal conductance observed in this strain. However, the morphological analysis revealed that pelage mass differed between sexes, with males having heavier pelages than females, and also as a function of BM (Selman et al. 2001). In the present study, however, BM had no effect on thermal conductance, and the effect of sex was the opposite to that anticipated by the sex effect on pelage mass, indicating that thermal conductance is a more complex trait than can be attributed to pelage differences alone.

The difference in RMR at 30°C between strains was consistent with an effect of DEE on RMR (Drent and Daan 1980; Peterson et al. 1990; Weiner 1992; Speakman and McQueenie 1996; Hammond and Diamond 1997). A common assumption underpinning this frequently postulated association is that high levels of DEE must be fuelled by greater food intake, necessitating an increased mass of the alimentary tract and associated organs, which have high rates of tissue metabolism (Field et al. 1939; Krebs 1950). Any increase in mass of these metabolically active tissues is thought, in turn, to lead to reflect an elevated RMR. Morphological studies confirm that the high strain of mice have larger livers than the low strain, but gut masses were not significantly different (Selman et al. 2001).

Interpolating the effect of T_a on resting metabolism to estimate the difference in metabolism between the high and low strains at 22°C (the selection temperature) suggests the difference in RMR between the strains is about 24% in the males and 30% in the females (Table 1). Using values of 81% for the apparent absorption efficiency (Johnson et al. 2001) and an RQ of 0.95 for this high carbohydrate diet (Speakman and Rossi 1999) both measured in another mouse strain (MF1), this difference in RMR would translate to a difference in food intake of 8.9 kJ day^{-1} in males and 10.4 kJ day^{-1} in females. The reported difference in food intake between these strains is 47% (Hastings et al. 1997; Bünger et al. 1998), but this estimate refers to intake when feeding on a different diet to the mice in the current study. When the strains were fed the same diet as the mice used in the present investigation, their gross energy intakes differed by on average 20.1 kJ day^{-1} in males and 33.7 kJ day^{-1} in females (C. Hambly, K. Rance, J. M. Fustin, L. Bünger, W.G. Hill and J.R. Speakman, unpublished observations). Hence, the effects of selection on resting metabolism can account for about 44% of the food intake effect in males and 31% of the food intake effect in females. The balance of the effect probably reflects differences in the spontaneous activity levels and activity intensity differences between the strains. Resolving and quantifying these effects will be the subject of further study.

Selection on increased voluntary food intake at 22°C (Bünger et al. 1998) appears to have resulted from a combination of different effects. Selection for greater food intake resulted in a correlated effect on BM because there was insufficient selection against BM. This effect of BM was combined with an independent effect on thermal conductance, partly due to lowered pelage insulation in the high-intake strain, which together led to an effect on the resting energy requirements at 22°C. These effects on RMR may account for up to half of the total increase in food intake and were probably complemented by selection effects on activity. An increased food intake would require increases in the metabolic processing machinery, fuelling some of the thermoregulatory demand, but also leading to an elevation in RMR at thermoneutral levels (30°C). The thermoregulatory pattern underpinning the difference at the selection temperature was therefore most consistent with the theoretical effect illustrated in Fig. 1d.

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