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RESEARCH ARTICLE

Limits to sustained energy intake. XVI. Body temperature and physical activity of female mice during pregnancy

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SUMMARY

Lactation is the most energy-demanding phase of mammalian reproduction, and lactation performance may be affected by events during pregnancy. For example, food intake may be limited in late pregnancy by competition for space in the abdomen between the alimentary tract and fetuses. Hence, females may need to compensate their energy budgets during pregnancy by reducing activity and lowering body temperature. We explored the relationships between energy intake, body mass, body temperature and physical activity throughout pregnancy in the MF1 mouse. Food intake and body mass of 26 females were recorded daily throughout pregnancy. Body temperature and physical activity were monitored every minute for 23 h a day by implanted transmitters. Body temperature and physical activity declined as pregnancy advanced, while energy intake and body mass increased. Compared with a pre-mating baseline period, mice increased energy intake by 56% in late pregnancy. Although body temperature declined as pregnancy progressed, this served mostly to reverse an increase between baseline and early pregnancy. Reduced physical activity may compensate the energy budget of pregnant mice but body temperature changes do not. Over the last 3 days of pregnancy, food intake declined. Individual variation in energy intake in the last phase of pregnancy was positively related to litter size at birth. As there was no association between the increase in body mass and the decline in intake, we suggest the decline was not caused by competition for abdominal space. These data suggest overall reproductive performance is probably not constrained by events during pregnancy.

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INTRODUCTION

Lactation is widely recognised to be the most energy-demanding phase of mammalian reproduction (Butte and King, 2005; Speakman, 2008; Wade and Schneider, 1992). Although the energy demands of pregnancy are considerably lower than those of lactation, important events during pregnancy prepare the female for lactation. These include the growth of the mammary tissue (Gjorevski and Nelson, 2011; Hovey and Aimo, 2010; Landskroner-Eiger et al., 2010; Sinha et al., 1970) and expansion in the size of the alimentary tract and liver (Campbell and Fell, 1964; Jolicoeur et al., 1981a; Jolicoeur et al., 1981b; Speakman and McQueenie, 1996; Uvnäs-Moberg, 1989). Mammary tissue development in pregnancy is stimulated by growth hormone, prolactin and placental lactogens that are produced in direct relation to the number of gestating fetuses (Bateman, 1957; Feldman et al., 1993; Forsyth, 1994; Horseman, 1999). In addition to this growth of somatic tissue, many species also deposit fat stores during pregnancy, which can be drawn from later in lactation. Rats (*Rattus norvegicus*), for example, increase adiposity in the last week of pregnancy (Asarian and Geary, 2006; Douglas et al., 2007; Gray and Wade, 1981; Shirley, 1984) and pregnant dairy cattle (*Bos taurus*) increase stored fat during early gestation before the development of the mammary glands starts (Uvnäs-Moberg, 1989). In this way the pregnant female may programme her performance to match anticipated demands in lactation (but see Duah et al., 2013).

Although energy demands in pregnancy are lower than those in lactation, several unique features of pregnancy may still mean that events in pregnancy affect, or even limit, overall reproductive performance (Speakman, 2008). In support of this view, some studies have suggested that milk production in lactation may depend in part on nutritional status during pregnancy in both dairy cattle (Blaxter, 1944; Campbell and Flux, 1948; Flux, 1950) and sheep (*Ovis aeries*) (Foot and Russel, 1979; Munro, 1955; Stern et al., 1978; Treacher, 1970; Wallace, 1948). To supply adequate nutrients to the fetuses during pregnancy, as well as supporting tissue growth and fat deposition, female mammals need to increase food intake during pregnancy. However, food intake may be limited, particularly in late pregnancy, because the alimentary tract, the developing fetuses and stored abdominal fat must compete for the limited space in the mother's abdomen (Bermudez et al., 1989; Forbes, 1968; Forbes, 1977). In accordance with this proposal, food intake is frequently observed to decline over a number of days prior to parturition in several species including mice (e.g. Cooper et al., 1994; French, 2006; Gordon and Tribe, 1951; Johnson et al., 2001a). This trade-off may potentially impose a limit on total reproductive investment, and more generally competition by different organs for abdominal

space has been envisaged as a constraining factor in evolution, particularly in herbivores (Clauss et al., 2003).

Faced with this potential constraint on energy intake, pregnant animals may enable compensatory mechanisms to conserve energy expenditure, thereby diverting additional resources into fetal growth. Reduction of spontaneous activity during late pregnancy has been reported in many different species. Pregnant women reduced daily activity by 26% as assessed by accelerometry and by 15% as assessed by self-report interview from the second to third trimester (Rousham et al., 2006). Dwarf hamsters (*Phodopus campbelli*) decreased activity during early pregnancy and wheel-running activity during late pregnancy (Scribner and Wynne-Edwards, 1994). A single Mongolian gerbil (*Meriones unguiculatus*) implanted with a telemetry device reduced activity levels to 85% of baseline over the last 10 days of gestation (Weinandy and Gattermann, 1995).

Changes in physical activity contribute to variation in body temperature in rodents (Refinetti, 1994; Weinert and Waterhouse, 1998). Previous studies have suggested that body temperature decreases during late gestation. Eliason and Fewell (Eliason and Fewell, 1997) showed that pregnant rats (*R. norvegicus*) reduced their core body temperature by 1°C in the last stage of gestation. Dwarf hamsters (*P. campbelli*) also decreased body temperature during late pregnancy (Scribner and Wynne-Edwards, 1994). Further, the decrease in body temperature at lower ambient temperatures (14 and 16°C) was significantly greater in late gestation rats than in non-pregnant and lactating rats, which did not alter body temperature at these ambient temperatures (Eliason and Fewell, 1997). These data suggest that body temperature in late pregnancy is reduced independently of ambient temperature. While this could be a direct consequence of reduced physical activity, reductions in body temperature may also underpin reductions in thermogenesis, which would further compensate energy requirements. Perhaps the most extreme examples of such responses are among bats where transient reductions in food supply in pregnancy may stimulate the animals to abandon thermoregulation completely and enter torpor (Racey, 1973). As fetal growth must also stop during torpor, this can lead to large variations in the duration of gestation (Racey, 1973; Racey and Swift, 1981).

Bhatia and colleagues (Bhatia et al., 1995) found that nest building was increased in Syrian hamsters (*Mesocricetus auratus*) during the last 4 days of gestation, suggesting these animals used additional behavioural strategies to conserve heat loss. In pregnant rats and mice, however, there were no major changes in the mitochondrial content of brown adipose tissue (BAT) which would be expected to be associated with changed thermogenesis (Andrews et al., 1986; Villarroya et al., 1986). In contrast, golden hamsters and Djugarian hamsters (*P. campbelli*) reduced BAT during pregnancy (Schneider and Wade, 1987; Wade et al., 1986). Responses therefore appear to vary among different species in terms of thermoregulation and energy intake during pregnancy.

We have previously studied the responses to reproduction in the outbred MF1 mouse, focusing in particular on sustained energy intake in lactation (Duarte et al., 2010; Johnson et al., 2001b; Johnson and Speakman, 2001; Król et al., 2007; Król and Speakman, 2003a; Speakman and Król, 2011). In the current paper, we used this same model system to explore the patterns of change in food intake, body mass, thermoregulation and physical activity throughout pregnancy to establish the extent to which energy budgets in pregnancy may be compensated by changes in body temperature and physical activity. Additionally, we aimed to explore the putative trade-off in performance due to competition between the alimentary tract and fetuses for abdominal space in late pregnancy.

MATERIALS AND METHODS

Experiments were conducted across 3 years (2005, 2006 and 2007) using a total of 26 female laboratory mice (*Mus musculus*: outbred MF1; Harlan UK Ltd, Bicester, UK). Sample sizes (*N*) were eight, eight and 10 mice in 2005, 2006 and 2007, respectively. Female and male mice were about 15 and 13 weeks old, respectively, when they were mated. Female mice were individually housed in shoebox cages with sawdust bedding and, additionally, a cardboard tunnel in 2005 and 2006. Commercial rodent chow [Standard chow, CRM(P), Special Diets Services, BP Nutrition, Witham, UK] was supplied *ad libitum* in 2005 and 2006. In 2007, animals were individually housed in BioDaQ cages (Research Diet Inc., New Brunswick, NJ, USA). The BioDaQ equipment allows food consumption and time spent consuming food to be monitored. The BioDaQ cages had a square hole (4×4 cm) where a feeding hopper on a sensor connected to a computer was attached. High carbohydrate diet (D12450B, Research Diet Inc.) was fed to the mice in 2007 as the use of this diet in the BioDaQ cages resulted in less food spillage than with the standard chow. Nutrient and energy content were almost identical for the two different diets. Both food and water were available *ad libitum*. The mass of food in the hopper (plus any obvious uneaten pieces of food in the cage bedding) and body mass were measured using a top-pan balance (±0.01 g, Sartorius, Epsom, Surrey, UK) daily between 13:00 h and 14:00 h in 2005, and between 09:00 h and 10:00 h in 2006 and 2007. Routine husbandry was carried out in the same hour, during which measurements of body temperature and physical activity were not recorded. Food intake was calculated by subtracting the remaining food mass in the feeding hoppers from the mass the previous day. Previous studies suggested the error due to small items of food lost in the sawdust and bedding was about 2% (Johnson et al., 2001a). For the calculation of energy intake, dry mass content and apparent digestibility values of 94.4% and 74.9%, respectively, were used for the commercial rodent chow as measured previously (Król and Speakman, 2003b). For the high carbohydrate diet, energy content and apparent digestibility were 16.12 kJ g^{-1} (Research Diet, Inc.) and 92.2%, respectively (Kagya-Agyemang et al., 2009). For the CRM(P) diet, the energy content was 17.35 kJ g^{-1}. Food intake for mice in the BioDaQ cages was also monitored manually by weighing the food each day and reporting the amount missing from the hopper. As we did not disturb mice on the day they gave birth (day 0), we could not measure the food intake between day −1 and day 0 for those mice where the intake was recorded manually. However, we could measure intake on day 0 for the 10 individuals where the intake was recorded using the BioDaQ cages. Apart from the data generated on day −1 from the BioDaQ cages, all the data reported here are from manual measurements. The experimental room was maintained at 21±1°C and 50–60% relative humidity. The photoperiod was 12 h:12 h light:dark with lights on at 07:00 h.

All procedures concerning animal care and treatment were approved by the ethical committee for the use of experimental animals of the University of Aberdeen, and were licensed by the UK Home Office and performed under PPL 60/3705.

Physical activity and body temperature

Female mice were implanted intraperitoneally with passive transponders that monitored physical activity levels and core body temperature *via* a pad located underneath their cage (Vital view transponder, Mini Mitter, Bend, OR, USA). Once the mice had recovered from surgery (1 week), food intake and body mass were measured daily for a baseline period of 7 days. The females were mated with unique individual males until pregnancy was confirmed

by body mass gain. The mating duration (the period that males were left with the females) was a minimum of 8 days (N=11/26 mice) but was longer when the females did not initially show signs they were pregnant. The actual day of pregnancy was retrospectively counted backwards from the day of parturition (day 0) following the numbering system used previously (Johnson et al., 2001a). Body temperature and physical activity were monitored each day starting at 14:00 h in 2005 and at 10:00 h in 2006 and 2007. The data were recorded every second and averaged over each minute. The minute averages were saved for 23 continuous hours (i.e. 14:00 h until 13:00 h in 2005, and 10:00 h until 09:00 h in 2006 and 2007). Means were calculated on a daily basis throughout baseline, mating and pregnancy across all 26 mice.

It is well established that physical activity can cause elevated body temperature (Brown and Refinetti, 1996; De Castro, 1978; Franken et al., 1992; Kent et al., 1991; Refinetti, 1994; Refinetti, 2003; Refinetti, 2010; Weinert and Waterhouse, 1998). In addition to measuring the overall mean body temperature, we were interested in the temperature at which the mice were physically active and that when they were at rest. By simultaneously observing mice and the records obtained using the activity recorder, we found that mice that appeared to be inactive still generated a low level of counts (<10 counts per minute, c.p.m.), but mice that were moving around never generated fewer than 10 c.p.m. Accordingly, we set a criterion of 10 c.p.m. as a separation point between 'active' and 'inactive' mice. Detailed analysis of simultaneous traces of temperature and physical activity in our mice indicated that body temperature was indeed elevated by physical activity (Fig. 1); however, there was an inertia in the change of body temperature relative to the onset and offset of physical activity, as has been reported previously (Weinert and Waterhouse, 1998; Weinert and Waterhouse, 1999). Because of this inertia, the body temperature immediately after the onset of activity mostly reflected the prior inactivity, while the body temperature immediately after activity had ceased reflected the elevation due to the activity. Thus, it was inappropriate to simply split the data into 'active' and 'inactive' measurements by taking the body temperature records that were simultaneous to the physical activity record. To overcome this problem, we the measured 'active' and 'inactive' body temperatures that were generated when the physical activity and temperature traces were moved relative to each other. This process suggested that the difference between 'active' and 'inactive' body temperatures was maximised when the activity trace was displaced by 15 min relative to the body temperature trace; accordingly, to define 'active' and 'inactive' body temperatures we displaced the minute-by-minute records by 15 min.

Because we had relatively little data from the present study on food intake and body mass changes during the last few days of pregnancy, we supplemented the current data with unpublished observations and data collected in other published studies where the focus was not on late pregnancy (Król and Speakman, 2003a; Vaanholt et al., 2013). In total, we had observations of the food intake and body mass on days −1 and −3 of pregnancy for 99 MF1 mice and 74 mice from two strains selected for high (N=53) and low (N=21) food intake (Sharp et al., 1984), making a total of 173 individuals for which we were able to compare the change in intake between days −3 and −1 with the increase in body mass over the same period, to test the idea that competition for space constrains intake in late pregnancy.

Data analysis

All data are expressed as means ± s.d. Repeated measures ANOVA was used for assessing significant differences of energy intake, body mass, body temperature including active and inactive body temperature, and activity counts between baseline, mating and pregnancy days. Furthermore, all active, inactive and mean body temperatures and activity counts were analysed for their differences between the hours of a day, across the three different periods, and within each period by ANOVA. Tukey *post hoc* tests were conducted on all parameters among days or hours of baseline, mating and pregnancy when significant differences were found by ANOVA.

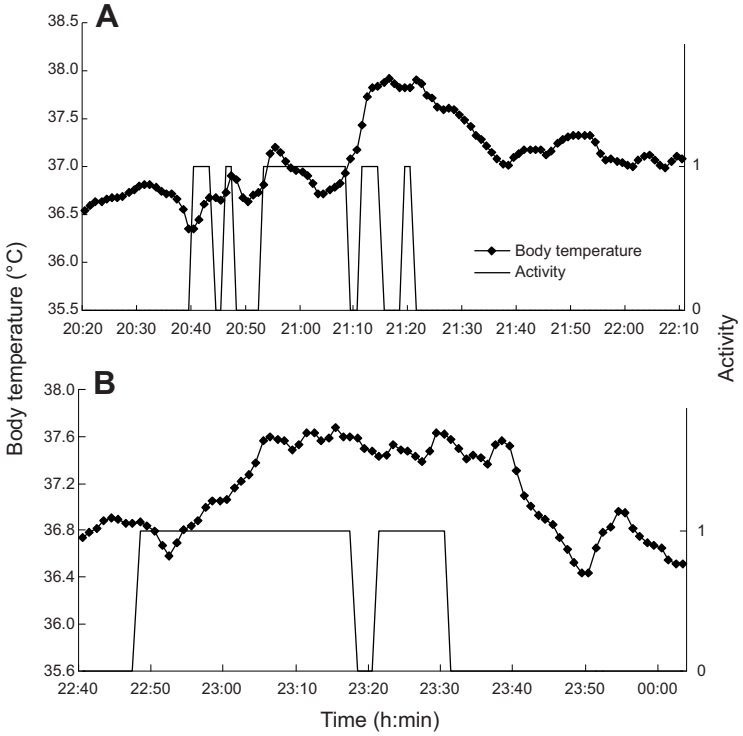

Fig. 1. Two typical time courses of simultaneous minute-by-minute records of physical activity (coded as 1=active and 0=inactive) and body temperature of female MF1 mice. There was a ~15 min lag in the onset of the temperature rise due to activity and a 15 min lag in the fall in body temperature once activity had ceased.

Relationships between energy intake, body mass, body temperature and activity counts were assessed by linear regression analysis. All statistical analyses were carried out using the R program (R Development Core Team, 2007).

RESULTS

Energy intake

Mean daily energy intake throughout baseline and pregnancy is shown in Fig. 2A (data are given in supplementary material Table S1). During the baseline period there was no significant difference in average intake between days. Mean intake across all the individuals (N=26) and days (N=7) was 69.9 kJ day^{-1} (s.d.=3.83 kJ day^{-1} across N=7 days). During pregnancy, food intake increased to a maximum of 106.5±23.4 kJ day^{-1} on day −3 (56.9% higher than during baseline), but thereafter declined so that on the day before the animals gave birth (day −1) the intake was only 69.65±18.7 kJ day^{-1}, which was almost identical to the baseline intake (paired t-test, t=–0.05, P=0.922). These data for day −1 were generated by the BioDaQ automatic monitoring of intake, while all the other data were generated manually. However, we are confident in these values because on days when both automatic BioDaQ and manual monitoring were used there was no significant difference (P>0.05) between the two measurements. Because males were left in the cages with the females for different periods, during which we could not measure the females' food intake, the number of females contributing to the data described above varied with the day of pregnancy(for details of the number each day, see supplementary material Table S1). We therefore also calculated mean daily intake using only the 11 individuals where males were removed after 8 days. The patterns of change in food intake were almost identical in the two data sets. At baseline, these 11 mice consumed on average 72.5±5.8 kJ day^{-1} and there were no significant differences among the 7 days of baseline. During pregnancy, energy intake varied significantly with day of pregnancy (one-way ANOVA, $F_{11,170}$=4.70, P<0.001). Compared with the mean energy consumed during baseline, pregnant mice consumed significantly more food on days −6 to −2 of pregnancy (Tukey pairwise comparisons, P<0.05). Daily energy intake reached a peak of 114.6±26.2 kJ day^{-1} on day −3 of pregnancy, which was 57.9% higher than that for the same mice during baseline.

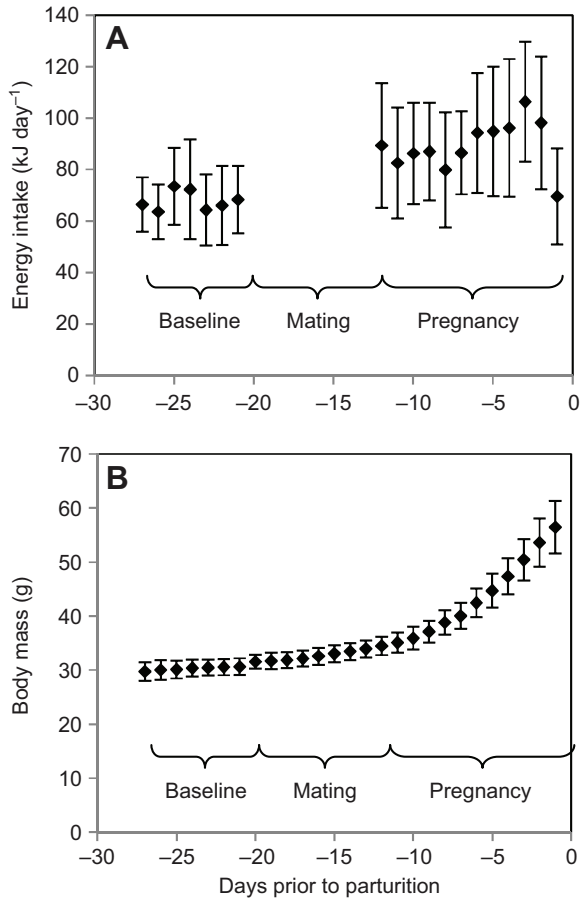

Fig. 2. (A) Mean energy intake of MF1 mice during baseline and pregnancy. The data are expressed as means ± s.d. (for sample sizes refer to supplementary material Table S1). (B) Mean body mass during baseline, mating and pregnancy. The data are expressed as means ± s.d. Sample sizes were N=23 on day −20 and N=26 for the other days.

There were no significant differences in body mass between days during the baseline period (one-way ANOVA, $F_{6,175}$=1.08, P=0.373). Mean body mass progressively increased as pregnancy advanced (one-way ANOVA, $F_{19,500}$=241.02, P<0.001) and reached a maximum of 56.5±4.87 g on day −1 (the day before parturition) (Fig. 2B). Mean body mass during pregnancy was significantly greater than that during baseline from day −15 onwards (Tukey pairwise comparisons, P<0.05).

Mean energy intake during late pregnancy and body mass were compared with litter size recorded on day 1 of lactation (assumed to equal the fetal litter size at parturition the day before). Litter size on day +1 ranged from 3 to 15 across the 26 litters. Mean energy intake between day −5 and day −2 was significantly positively related to litter size on day +1 (y=8.53x+69.1, R^2=0.250, $F_{1,24}$=8.02, P=0.009; Fig. 3A). Body mass in late pregnancy (day −1) was also strongly correlated with the number of fetuses (y=0.65x+42.5, R^2=0.238, $F_{1,24}$=7.48, P=0.012; Fig. 3B). To explore the association between changes in food intake and changes in body mass in late pregnancy in more detail, we examined the relationship between the change in body mass between days −3 and −1 and the change in energy intake between the same 2 days across a total of 173 mice, including those in the present study that were monitored by the BioDAQ system (N=10) and from other published and unpublished studies (see Materials and methods for details). Day −3 was the point at which on average intake started to decline. There was a significant positive relationship between the increase in body mass between days −3 and −1 and the change in food intake over the same period (regression $F_{1,168}$=25.66, P=0.002, N=173; Fig. 3C), but there was no significant effect of strain (P=0.704) or any significant strain by body mass interaction (P=0.458).

Physical activity

The minute-by-minute activity data were averaged on a daily basis for each animal. The daily activity data across all animals were then averaged for each day in each period (supplementary material Table S2). Mean activity was 22.3±8.69 c.p.m. over the 7 days of baseline (Fig. 4). No significant differences were found in daily activity counts between days during baseline (one-way ANOVA, $F_{6,175}$=0.15, P=0.99). Activity counts varied significantly between days during mating (one-way ANOVA, $F_{4,125}$=4.56, P=0.002). On the first day of mating, activity increased to 30.00±9.26 c.p.m., which was significantly higher than that during both baseline and the subsequent days of mating (Tukey pairwise comparison, P<0.05). Activity levels also varied significantly with days of pregnancy (one-way ANOVA, $F_{19,497}$=24.99, P<0.001). Average daily activity counts decreased from 22.7±6.15 c.p.m. on day −15 to

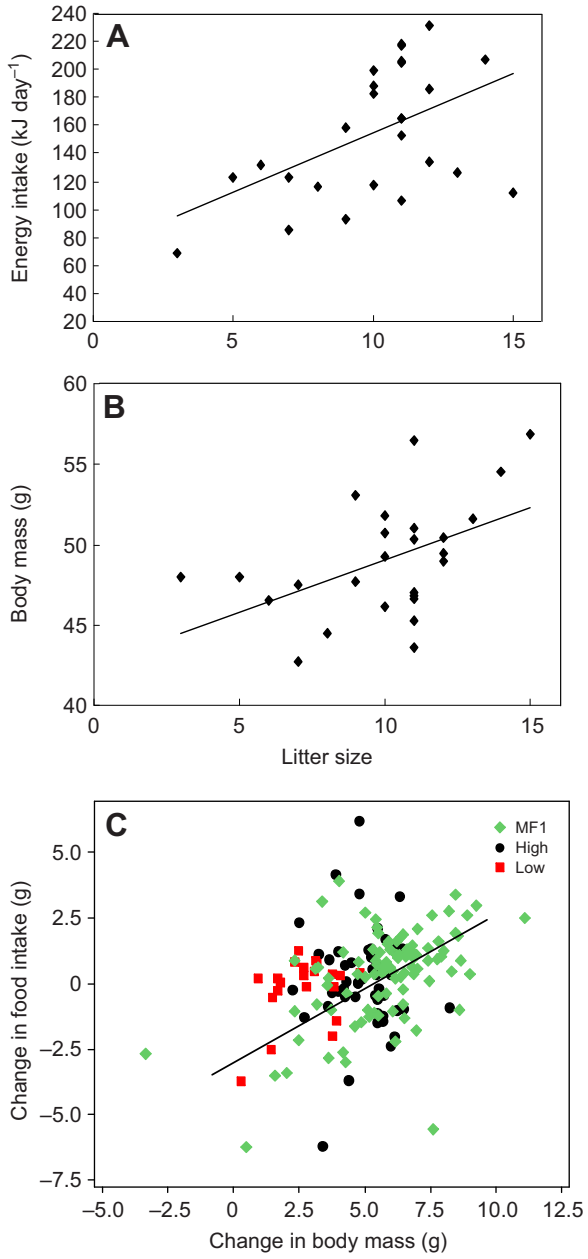

Fig. 3. The effect of litter size at birth on (A) energy intake and (B) body mass in late pregnancy. The data for litter size were collected from the day after parturition. Mean energy intake and body mass were calculated over 4 days between day −5 and day −2 in pregnancy. The regression lines are expressed by y=8.53x+69.1 for A and y=0.65x+42.5 for B. (C) The change in food intake between day −3 and day −1 plotted against the change in body mass over the same period for 99 MF1, 53 high food intake and 21 low food intake mice (refer to Materials and methods for strain details). There was a significant positive relationship represented by the regression line: y=–1.42+0.319x.

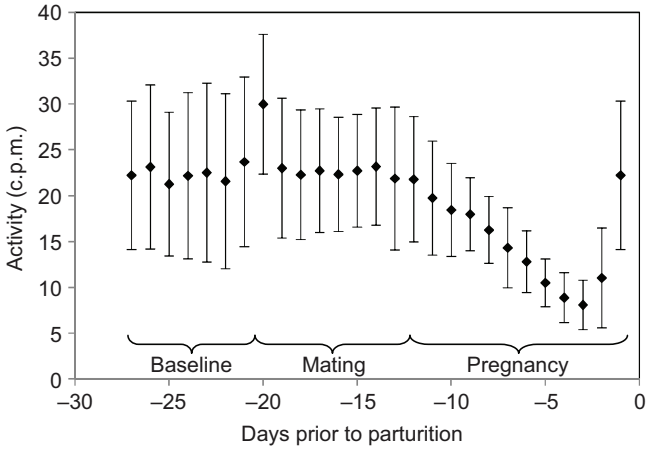

Fig. 4. Mean daily activity (counts per minute, c.p.m.) during baseline, mating and pregnancy. The data are expressed as means ± s.d.

8.1±2.72 c.p.m. on day −3. After day −3, however, the activity started to increase again and by day −1 it was almost identical to the baseline levels (at 22.4±8.10 c.p.m.). Compared with baseline, pregnant mice had significantly reduced physical activity levels from day −5 to day −2 (Tukey pairwise comparison, P<0.01).

Activity changes with time of day were compared among baseline, mating and late pregnancy periods (Fig. 5; data are given in supplementary material Table S3) using the minute-by-minute data averaged over each hour. Activity levels changed with time of day and the different periods (two-way ANOVA; time of day, $F_{22,1725}$=57.16, P<0.001; period, $F_{2,1725}$=146.63, P<0.001; interaction of time of day and period, $F_{44,1725}$=6.57, P<0.001). During the baseline period, there were significant differences in activity level between the different hours of the day (one-way ANOVA, $F_{22,575}$=25.42, P<0.001), but not during the light phase between 07:00 h and 18:00 h (Tukey pairwise comparisons, P>0.05) when counts were uniformly low. This indicates the mice predominantly rested throughout the light phase. The lowest value during baseline was 5.14±2.57 c.p.m. recorded at 15:00 h. Onset of activity was clearly synchronised with onset of darkness, so that mice became more active after 19:00 h. Activity counts in baseline reached a maximum of 58.5±33.5 c.p.m. at 20:00 h, about 10 times greater than the nadir in mid-afternoon. Mice were highly active between 19:00 h and 02:00 h, after which activity declined gradually throughout the night to the same level as that in the light phase. The lights going off seemed to stimulate activity, but activity levels started to decline much earlier than the lights coming on.

Activity levels also varied with hours of the day during mating (one-way ANOVA, $F_{22,575}$=21.62, P<0.001). The pattern during mating was almost identical to that during baseline. Activity during mating did not vary significantly between 10:00 h and 18:00 h (Tukey pairwise comparisons, P>0.05) and reached a minimum at 8.25±4.17 c.p.m. at 15:00 h. After the lights went off, activity increased and reached a maximum of 53.68±21.83 c.p.m. at 20:00 h. Females during the mating period started reducing their activity from 03:00 h. Although physical activity in late pregnancy was much lower than that in baseline and mating, there were still significant differences in the daily activity pattern in late pregnancy with time of day (one-way ANOVA, $F_{22,575}$=18.47, P<0.001). Activity counts reached the minimal level of 5.15±2.17 c.p.m. at 12:00 h. Compared with this minimal level, pregnant mice had increased activity between 17:00 h and 02:00 h (Tukey pairwise comparisons, P<0.05).

Comparing the daily activity patterns between baseline, mating and late pregnancy, there were no significant differences across the three periods between 10:00 h and 18:00 h. At 19:00 h when the light was turned off, mice became considerably more active in baseline and mating but much less so in late pregnancy (Tukey pairwise comparisons, P<0.01). After 19:00 h, no difference was found in

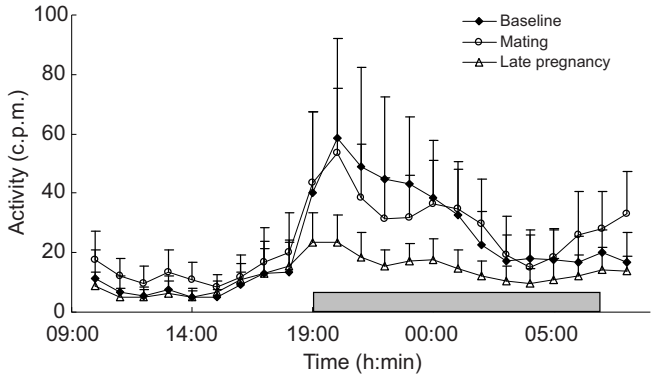

Fig. 5. Activity patterns throughout the day during baseline, mating and late pregnancy. All data are expressed as means ± s.d. (*N*=26). The grey bar indicates the dark phase.

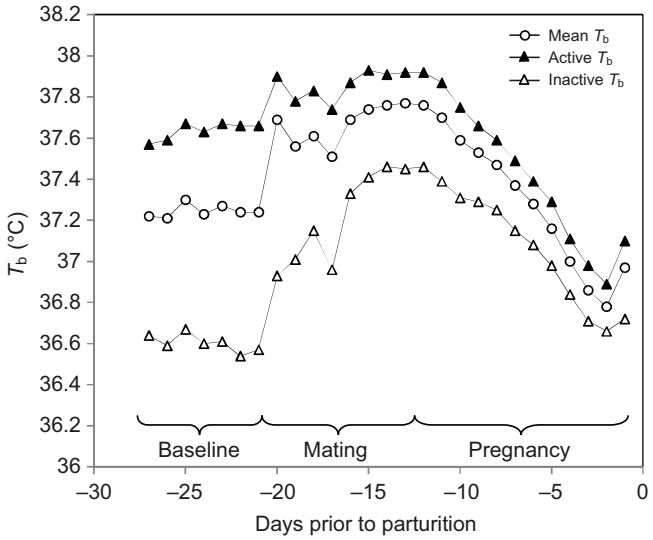

Fig. 6. Daily mean body temperature (T_b), active T_b and inactive T_b throughout baseline, mating and pregnancy. The data are means across all hours and all individuals on each day (*N*=26). Error bars are not shown, for clarity; s.d. is presented in supplementary material Table S2.

activity levels between baseline and mating until 06:00 h (Tukey pairwise comparisons, $P<0.01$). Physical activity in late pregnancy remained considerably and significantly lower from 19:00 h until 02:00 h compared with both baseline (Tukey pairwise comparisons, $P<0.01$) and mating (Tukey pairwise comparisons, $P<0.01$).

Body temperature

Body temperature (mean T_b) was averaged on a daily basis across all 26 females, as for activity counts, and the daily means of active and inactive T_b were also calculated. Fig. 6 shows changes in the mean, active and inactive T_b from baseline to day −1 of pregnancy. All three measurements of T_b changed significantly with day of measurement across the periods of baseline, mating and pregnancy (one-way ANOVA; mean T_b, $F_{26,672}=41.50$, $P<0.001$; active T_b, $F_{26,663}=46.00$, $P<0.001$; inactive T_b, $F_{26,672}=32.27$, $P<0.001$). Furthermore, the three measurements differed significantly from each other, and their differences varied between days (two-way ANOVA; T_b types, $F_{2,2007}=1064.01$, $P<0.001$; days, $F_{26,2007}=95.21$, $P<0.001$; interaction of T_b types and days, $F_{52,2007}=10.08$, $P<0.001$).

Daily mean T_b during baseline was 37.25±0.27°C and did not vary significantly over the 7 days (Tukey pairwise comparison, $P>0.05$). On the first mating day (day −20), mean T_b increased considerably to 37.69±0.25°C. Throughout the 5 days of mating, mean T_b remained as high as that on the first mating day and averaged 37.61±0.23°C. Except for day −17, mean T_b during mating was significantly higher than baseline (Tukey pairwise comparison, $P<0.05$). Between day −15 and −11 of pregnancy, mean T_b stayed at higher levels than that of all days of baseline (Tukey pairwise comparison, $P<0.05$). Mean T_b decreased to its minimal level at 36.78±0.21°C on day −2, which was significantly lower than that in baseline (Tukey pairwise comparison, $P<0.001$). On day −1 there was a slight increase in mean T_b. Mean active T_b was 37.63±0.23°C during baseline, and there were no differences in active T_b among the days of baseline (one-way ANOVA, $F_{6,175}=0.37$, $P=0.90$). Active T_b increased to 37.82±0.22°C on average during mating and varied significantly with the days of mating (one-way ANOVA, $F_{4,125}=3.58$, $P=0.008$). The significant difference found in active T_b during the mating days was due to a lower active T_b on day −17. In early pregnancy (day −15 to −11), active T_b was 37.91±0.20°C and was similar to that during mating. Active T_b declined during pregnancy from day −11 onwards until day −2, followed by a slight increase on day −1. The changing pattern in active T_b paralleled mean T_b throughout the whole experiment from baseline to pregnancy. Significant differences between mean T_b and active T_b were only found in baseline. The differences between these two measures of body temperature were relatively small during mating and pregnancy.

Mean inactive T_b followed a slightly different pattern to the daily changes in mean T_b and active T_b. The lowest values of inactive T_b were observed during baseline, while the lowest values of mean T_b and active T_b were observed in late pregnancy. There were no differences in inactive T_b between days at baseline, and it averaged 36.30±0.35°C (Tukey pairwise comparison, $P>0.05$). On the first day of mating, inactive T_b rose to 36.96±0.41°C, which was significantly higher than that during baseline (Tukey pairwise comparison, $P<0.01$). Inactive T_b continued to increase over the days of mating and reached a high point of 37.33±0.29°C on day −16. During pregnancy, inactive T_b remained at a high level until day −11 and then started declining, although it was still significantly higher than that in baseline at this stage (Tukey pairwise comparison, $P<0.01$). Finally, inactive T_b reached a level on day −4 not significantly different to that measured in baseline. Inactive T_b in the last 4 days of pregnancy averaged 36.73±0.28°C, not significantly different to baseline. More detailed analyses of the hourly changes in T_b during the three different periods are presented in the Appendix and supplementary material Tables S4–6 and Fig. S1.

The relationship between activity and energy intake and body mass during baseline and pregnancy

There was no significant relationship between activity level and energy intake during baseline (least squares regression, LSR: $F_{1,24}=1.01$, $P=0.325$) early pregnancy (day −12 to day −9) ($F_{1,24}=0.93$, $P=0.361$) or late pregnancy (day −5 to day −2) ($F_{1,24}=2.63$, $P=0.118$). Excluding one female, with a very small litter, that ate 56% less than the average across all 26 mice during late pregnancy, mean activity and energy intake were significantly negatively related during late pregnancy (LSR: $y=-6.4706x+224$, $R^2=0.203$, $F_{1,23}=5.85$, $P=0.024$). Thus, there was a trend that activity levels were lower when pregnant mice ate more food. Physical activity had no relationship to body mass of individual

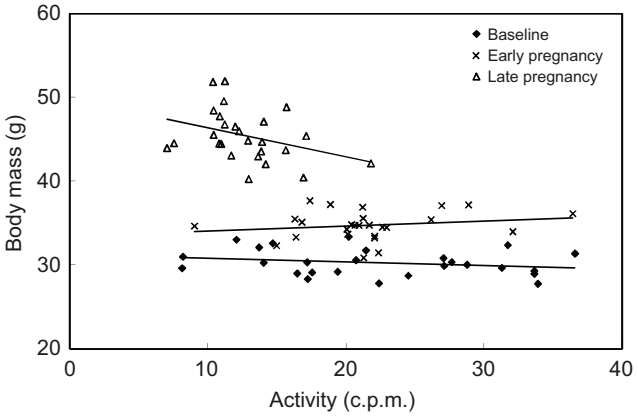

Fig. 7. The relationship between activity level and body mass during baseline, early pregnancy and late pregnancy. Lines express the best-fit linear regressions (see Materials and methods for details).

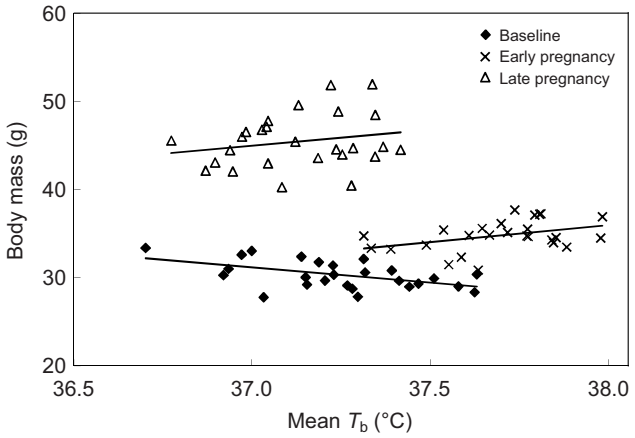

Fig. 8. The relationship between mean T_b and body mass during baseline, early pregnancy and late pregnancy. Lines express the best-fit linear regressions (see Materials and methods for details).

mice in baseline (LSR: $F_{1,24}$=1.34, P=0.259), early pregnancy (LSR: $F_{1,24}$=0.85, P=0.367) or late pregnancy (LSR: $F_{1,24}$=3.36, P=0.079) (Fig. 7). When the same female that ate less than the others was removed from this analysis, the relationship between body mass and physical activity reached significance in late pregnancy (LSR: y=–0.541x+54.619, R^2=0.198, $F_{1,23}$=5.675, P=0.026). Heavier individuals were on average less physically active in late pregnancy.

The relationship between T_b, energy intake and body mass during baseline and pregnancy

There was no significant relationship between mean T_b and energy intake during baseline (LSR: $F_{1,24}$=1.9, P=0.181) or in early pregnancy (day −12 to day −9) (LSR: $F_{1,24}$=3.17, P=0.109). However, in late pregnancy (day −5 to day −2), there was a significant relationship between mean T_b and energy intake (LSR: y=–110.8x+4248.4, R^2=0.203, $F_{1,24}$=6.12, P=0.021). When the female who had the exceptionally low intake was removed from the analysis, the result remained significant (LSR: y=–96.493x+3722.5, R^2=0.173, $F_{1,23}$=4.82, P=0.039). The more energy the pregnant mice consumed, the lower the body temperature they had. Mean T_b was also negatively related to body mass so that smaller animals had higher T_b during baseline (LSR: y=–3.4822x+159.99, R^2=0.2669, $F_{1,24}$=8.74, P=0.007; Fig. 8). In contrast, there was a significant positive relationship between mean T_b and body mass in early pregnancy (LSR: y=3.824x−109.41, R^2=0.158, $F_{1,24}$=4.5, P=0.044); thus, heavier animals tended to have higher T_b. During late pregnancy, the relationship was not significant (LSR: $F_{1,24}$=1.15, P=0.294).

DISCUSSION

Activity patterns and T_b during baseline and mating

Although the data reported here only covered 7 days, there was no indication of cyclic patterns of T_b and physical activity during the baseline period that might be linked to oestrus cycles. This was apparent when examining individual traces as well as the overall mean where individual differences might cancel out if the oestrus cycles were not synchronised. Although such cycles in activity and T_b have often been reported in rats (e.g. Anantharaman-Barr and Decombaz, 1989; Brobeck et al., 1947; Kent et al., 1991; McLean and Coleman, 1971; Refinetti, 2010; Slonaker, 1924; Wang, 1923), our data are consistent with previous data in three inbred mouse strains, which found no significant differences in T_b or locomotor activity in relation to oestrus state (Koehl et al., 2003).

As found almost 90 years ago in rats (Slonaker, 1924; Wang, 1923), female mice demonstrated a peak of activity on the first day of mating, although no increase in activity level was observed on the other mating days. Direct observations indicated that the male mice continuously harassed the females when they were first put together and the females spent much of their time running away from the male. Despite no changes in physical activity after the first day, female mice had a higher T_b throughout the mating period than in the baseline period, mostly due to an increase in their T_b when inactive. Because the mice were housed at 21°C, which is below their lower critical temperature (Speakman and Rossi, 1999; Speakman and Keijer, 2013; Speakman, 2013) the higher T_b maintained during the whole of mating probably occurred because the mice were able to huddle together to conserve heat loss (Contreras, 1984; Hayes et al., 1992; Vickery and Millar, 1984). Direct (but unsystematic) observations of the mice indicated that when not active the pairs always huddled together after the first day. This would also explain why the increase was mostly observed in the inactive T_b rather than in the active T_b.

Energy intake and T_b during pregnancy

The increase in energy intake in the mice during late pregnancy (*ca*. 57%) in the current study was similar to the 48% increase in food intake in late pregnancy reported previously in the same strain at the same ambient temperature (Johnson et al., 2001a). Consistent with other reports across a range of species (French, 2006; Johnstone and Higuchi, 2001; Morgan and Winick, 1981), energy intake decreased on the days immediately before parturition. This might have occurred because of space competition between the alimentary tract and enlarging fetuses in the abdomen (Bermudez et al., 1989; Forbes, 1968). If space competition was the cause of the decline in intake, one might anticipate that energy intake in late lactation would be negatively related to the number of developing fetuses. However, in contrast to this prediction the number of pups was positively correlated with energy intake in late pregnancy. Moreover, we found no relationship between the increase in body mass over the last few days of pregnancy and the decline in food intake. These data suggest space competition in the abdomen did not cause the decrease in the

food intake at the end of gestation. The cause of this decline in food intake therefore remains unclear. In addition, this also implies that space competition in the abdomen in late lactation cannot be a process whereby pregnancy exerts a controlling influence over performance in lactation *via* limits on litter size or litter mass at birth (see also Duah et al., 2013).

Activity levels and T_b during pregnancy

Physical activity declined progressively through gestation until 2 days before parturition. The decline in activity was consistent with a gradual decrease in wheel-running activity observed in dwarf hamsters (*P. campbelli*) as gestation progressed (Scribner and Wynne-Edwards, 1994). However, activity in dwarf hamsters decreased mostly in early gestation, while the mice in our study maintained activity in early pregnancy but reduced it later. Physical activity during late pregnancy in the mice studied here was only 36.4% of that during baseline. A reduction in activity level during pregnancy also agreed with the findings of a study on a single Mongolian gerbil; however, the pregnant gerbil reduced her activity level by 85% compared with that during pre-breeding (Weinandy and Gattermann, 1995). Consistent with wheel-running activity in dwarf hamsters (Scribner and Wynne-Edwards, 1994), the decrease in activity level mainly occurred during the dark phase. It appeared that only locomotor activity was removed from the scheduled behaviours in late pregnancy, while feeding behaviour was unchanged or even increased. This was shown by the fact that pregnant females consumed substantially more energy even though they were less active at night during late pregnancy.

Daily increases in body mass paralleled the reduction in physical activity as pregnancy advanced. Moreover, heavier individuals had lower physical activity in late pregnancy. This has several potential explanations. Perhaps the mice conserved energy by reducing activity, which could then be channelled into growth. Alternatively, the mice may have found it more difficult to move around as they got larger; hence, the activity reduction may have been caused by the mass change and not the reverse. Finally, the reduction of activity in relation to body mass may have been an adaptive response reflecting the possibility that in the wild heavily pregnant individuals might be more prone to predation risks. Minimising the time spent active as the individuals got bigger might then mitigate this elevated predation risk. The significant increase in body mass during pregnancy was detected from day −15, which was earlier than the time when mice became significantly less active (day −6). This might suggest the reduction in physical activity was a consequence of the increasing body mass (e.g. Vaanholt et al., 2010). However, on the last day of pregnancy when the mice were at their heaviest they increased their activity back up to the level observed at baseline, indicating the reduced activity was not because the mice were progressively less able to move around as a result of their increasing body mass. This suggests the reduction in activity was more likely to be regulated to conserve energy that might be channelled into growth, or to reduce predation risk. Reduced activity may therefore be a significant energy conservation strategy during pregnancy in mice.

Similar to physical activity, daily mean T_b gradually declined over the period of pregnancy. This was consistent with findings in several other rodent species including the Mongolian gerbil (*M. unguiculatus*) (Weinandy and Gattermann, 1995) and Sprague–Dawley rats (*R. norvegicus*) (Fewell, 1995). However, dwarf hamsters (*P. campbelli*) did not have a progressive decrease in T_b with days of gestation (Scribner and Wynne-Edwards, 1994). In addition, weekly mean T_b also did not show this gradual decrease in Long–Evans rats (*R. norvegicus*) as gestation advanced (Kittrell and Satinoff, 1988). The minimum body temperature in pregnant mice reported here was 36.8°C, which was lower than that of Sprague–Dawley rats (*R. norvegicus*) (Fewell, 1995) and the Mongolian gerbil (*M. unguiculatus*) (Weinandy and Gattermann, 1995). The minimal T_b was observed on the day before parturition in our mice, consistent with previous data for both mice and rats (Fewell, 1995), whereas the Mongolian gerbil decreased T_b to a minimum 3 days before parturition (Weinandy and Gattermann, 1995). This difference was probably because only one pregnant Mongolian gerbil was monitored (Weinandy and Gattermann, 1995), and individuals may differ in their responses of T_b to pregnancy. Alternatively, Mongolian gerbils might have different thermoregulation near the end of parturition from mice and rats, as Mongolian gerbils showed a different rhythmicity in physical activity over the time of day compared with that of mice and rats (Refinetti, 2006).

The decrease in mean T_b during late pregnancy was caused by a lower T_b in the dark phase, which was at least partially explained by the lower levels of physical activity at this time. In addition to the lower intensity of activity in late pregnancy in the dark phase, the heat generated by this activity would be distributed into the greater body mass, which would also contribute to the lower elevation of T_b when the mice were active. This attenuation of the diurnal cycle in T_b was similar to that observed in Sprague–Dawley rats (*R. norvegicus*) (Fewell, 1995). However, our mice maintained a circadian rhythm in T_b during late pregnancy (day −8 to day −2), while a circadian rhythm in mean T_b disappeared during the last 5 days of pregnancy in rats (*R. norvegicus*) (Fewell, 1995). This difference between rats and mice may in part be a methodological artefact as mean T_b was compared on an hourly basis in the current study while means of 3 h periods were compared in the rat study (Fewell, 1995). Inconsistent with the pattern for mice and rats, dwarf hamsters (*P. campbelli*) decreased the amplitude of the daily T_b rhythm by increasing T_b in the light phase (Scribner and Wynne-Edwards, 1994). This suggests that hamsters may have different strategies in thermoregulation as well as energy balance during pregnancy from those of mice and rats.

Eliason and Fewell suggested T_b was downregulated in late pregnancy in rats, and that this might be an additional energy conservation strategy (Eliason and Fewell, 1997). However, until day 19 of gestation, no changes were found in the activity of BAT (Andrews et al., 1986), although BAT activity did decrease at the very end of gestation (Trayhurn and Richard, 1985). Although there was a progressive decline in T_b throughout gestation in the mice studied here, this decline only reversed the increase that had occurred between baseline and early pregnancy. Hence, inactive T_b was not significantly different in late pregnancy from that in the baseline period prior to mating. The results from this study therefore do not support the hypothesis that thermogenesis was suppressed in late pregnancy in mice relative to baseline; rather, the lowered temperature was due to a reduced intensity of activity and a greater mass in which the generated heat was distributed. This was further reinforced by the fact that across individuals mean T_b in late pregnancy was unrelated to mean body mass, suggesting that those mice that maintained lower T_b derived no benefits in increased growth.

The very high level of ‘inactive’ T_b in early pregnancy remains a mystery. During mating, high T_b was probably enabled by the mice huddling together. In early pregnancy, males were also present with most of the females, possibly contributing to their high T_b. Yet, the mice sustained considerably higher ‘inactive’ T_b during early pregnancy than they had immediately after the males were

introduced, suggesting additional factors were also at play. This higher temperature cannot reflect an elevated heat increment of feeding because food intake levels were higher later in pregnancy when the mice maintained cooler T_b. Moreover, in late pregnancy it was actually those mice that ate most food that were coolest. A potential reason for the high temperatures in early pregnancy relates to hormonal changes during this period, although the present experiment was not designed for monitoring hormone secretions so we could not correlate the observed changes with hormone titres in our animals. Nevertheless, progesterone secretion was elevated in pregnancy in Djungarian hamsters (*P. campbelli*) (Edwards et al., 1994) and rats (Asarian and Geary, 2006; Hervey et al., 1967). Adels and Leon (Adels and Leon, 1986) found an increase in T_b with progesterone administration in female brown rats (*R. norvegicus*). Clearly, changes in progesterone levels may have affected T_b in early pregnancy; however, why such effects were not continued into late pregnancy is unclear.

This study suggests that lowered T_b (reflecting lowered resting metabolism) does not contribute to energy savings in pregnancy in the mouse, as the mean T_b was higher during the majority of pregnancy than during baseline. However, the profound reduction in physical activity particularly from day −11 onwards may be an important part of the energy budget of pregnant mice, enabling them to divert ingested energy preferentially into fetal growth. The decline in food intake during the last 3 days of pregnancy did not appear to be caused by space competition between the alimentary tract and expanding fetal mass in the abdomen, and its cause remains unclear. However, the absence of such a trade-off suggests that pregnancy is unlikely to be a constraining period on the process of reproduction in these mice (see also Duah et al., 2013). The causes of the elevated T_b in early pregnancy remain unresolved.

APPENDIX

Variations of mean T_b, active T_b and inactive T_b with time of day

Changes of mean T_b throughout the day were compared among the different periods of baseline, mating and late pregnancy (supplementary material Fig. S1A). Mean T_b varied considerably with time of day and also varied significantly between the different periods (two-way ANOVA, time, F=60.22, P<0.001; period, F=364.96, P<0.001; interaction of time and period, F=8.89, P<0.001). Mice during baseline had a clear circadian rhythm in mean T_b, ranging from a minimal level of 36.55±0.29°C at 12:00 h to a maximal level of 38.11±0.55°C at 20:00 h. Mean T_b during the dark (19:00 to 07:00 h) was always higher than the lowest value in the light phase during the baseline period (Tukey pairwise comparisons, P<0.01), although it started decreasing significantly from 01:00 h compared with the maximal value at 20:00 h (Tukey pairwise comparisons, P<0.01). The changing pattern of mean T_b with time of day was very similar to the diurnal pattern of changes in physical activity during baseline (Fig. 5), suggesting that hourly mean T_b was strongly associated with physical activity changes. During mating, the lowest value of mean T_b was 37.20±0.24°C at 15:00 h. This was 0.65°C higher than the lowest value found during baseline. There was no significant difference in mean T_b between 11:00 and 18:00 h (Tukey pairwise comparisons, P>0.05). Compared with this period, mice had significantly higher body temperatures between 19:00 and 00:00 h. Maximal mean T_b was 38.11±0.38°C at 20:00 h. Similar to the hourly changes in physical activity during mating (Fig. 5), mean T_b increased again and became significantly higher from 05:00 to 08:00 h compared with the lowest T_b (Tukey pairwise comparisons, P<0.01). Mean T_b in late pregnancy varied from 36.93±0.19°C at 12:00 h to 37.37±0.30°C at 20:00 h. The amplitude of mean T_b was much smaller in late pregnancy than in either the baseline or mating periods. This meant during the day the pregnant mice had higher levels of mean T_b than at baseline (significant at 12:00 and 15:00 h, Tukey pairwise comparisons, P<0.01), but at night they had much lower levels (significant between 20:00 and 01:00 h, Tukey pairwise comparisons P<0.01). The mean T_b in late pregnancy was always lower than that during the mating period.

The pattern of change in active T_b (supplementary material Fig. S1B) was almost identical to that reported in mean T_b. Active T_b differed significantly with time of day in the different periods (two-way ANOVA, time, F=47.53, P<0.001; period, F=443.11, P<0.001; interaction of time and period, F=9.79, P<0.001). Active T_b in the baseline period varied from 36.75±0.38°C at 12:00 h to 38.20±0.44°C at 20:00 h. The active T_b at 12:00 h was significantly lower than that during all hours during darkness (Tukey pairwise comparisons, P<0.01). Active T_b remained at the maximal level (20:00 h) between 21:00 and 01:00 h (Tukey pairwise comparisons, P>0.05) and declined from 02:00 h onwards. There was no significant difference in active T_b between 10:00 and 18:00 h during mating (Tukey pairwise comparisons, P>0.05). The lowest and highest T_b values were 37.38±0.32 and 38.15±0.32°C, recorded at 15:00 and 20:00 h, respectively (Tukey pairwise comparisons, P<0.01). In late pregnancy, the lowest level of active T_b was 37.02±0.24°C at 15:00 h. There were no significant differences in active T_b between 11:00 and 18:00 h (Tukey pairwise comparisons, P>0.05). Active T_b in late pregnancy was maximised at 37.39±0.19°C at 19:00 h, which was significantly higher than values between 11:00 and 16:00 h (Tukey pairwise comparisons, P<0.05).

The differences in active T_b among baseline, mating and late pregnancy were similar to those in mean T_b among the three periods. In the light phase, between 11:00 and 16:00 h, active T_b during mating was higher than that of baseline (Tukey pairwise comparisons, P<0.01). It was also higher than that of late pregnancy between 11:00 and 14:00 h (Tukey pairwise comparisons, P<0.01). There were no differences in active T_b between baseline and late pregnancy during the daytime. After 19:00 h and until 04:00 h the following morning, active T_b was significantly lower in late pregnancy than during baseline and mating (Tukey pairwise comparisons, P<0.01), suggesting that not only were the mice less active in late pregnancy but they also elevated their body temperature less during this activity.

Inactive T_b differed with time of day and among the different periods (two-way ANOVA, times, F=11.56, P<0.001; periods, F=452.33, P<0.001; interaction of times and periods, F=1.62, P=0.001). Inactive T_b during baseline varied from 36.42± 0.27°C at 12:00 h to 37.13±0.68°C at 20:00 h. Inactive T_b was low between 11:00 and 18:00 h during daytime, but compared with those hours inactive T_b increased significantly between 19:00 and 23:00 h (Tukey pairwise comparisons, P<0.05). During mating, the highest inactive T_b (37.47±0.26°C) was recorded at 21:00 h, which was an hour later than that of mean T_b and active T_b. Inactive T_b was higher during mating than that during baseline. The lowest inactive T_b during mating was 37.00±0.33°C at 12:00 h.

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AUTHOR CONTRIBUTIONS

Y.G. co-conceived and co-designed the work and contributed the majority of effort to the data collection, performed the data analyses and co-wrote the paper. A.B. contributed to the data collection. S.E.M. and C.H. contributed to the data

collection and curation. C.H., A.A.J., L.M.V. and E.K. contributed data to the analysis shown in Fig. 3. J.R.S. co-conceived and co-designed the work, performed the final data analysis and co-wrote the paper. C.H., L.M.V., S.E.M. and E.K. revised and rewrote the paper.

COMPETING INTERESTS

No competing interests declared.

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