

LIMITS TO SUSTAINED ENERGY INTAKE

IV. EFFECT OF VARIATION IN FOOD QUALITY ON LACTATING MICE *MUS MUSCULUS*

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Summary

Observations were made on 30 MF1 mice with their litters. The animals were fed either normal pelleted mouse food (SDS BP Nutrition Ltd) containing 13.4 kJ g^{-1} digestible energy or a specially formulated diet that provided 25% less digestible energy (9.75 kJ g^{-1}) but equivalent amounts of protein and essential minerals and vitamins per gram as the normal diet. Half the animals were switched to the low-energy diet during early pregnancy and half after parturition. The food intake of the two groups increased enormously following parturition, reaching an asymptote over the last few days of lactation. In both groups, the asymptotic food intake exceeded that previously observed across 71 litters of this strain of mice fed the normal diet throughout pregnancy and lactation; the intake of the group fed the low-energy diet from early lactation significantly exceeded that of the mice switched to the low-energy diet after parturition. The increased intakes of the experimental groups were, however, insufficient to offset the lower digestible energy content of the food during lactation. The body mass of the mothers at the end of lactation did not differ between the two experimental groups and the controls. Offspring mass at weaning was inversely related to litter size, but also did not differ between the three groups; pup mortality did not

differ between the experimental and control groups. Behavioural observations showed that during both the dark and light phases the general activity of the mother declined enormously from early pregnancy to late lactation. In the dark phase, the time spent in general activity was replaced by time spent both feeding and resting (suckling young), but in the light phase it was replaced only by feeding. At peak lactation, the mice fed for 30–50% of the dark phase and for 30–40% of the light phase. The data indicate that a previously observed asymptote in food intake during peak lactation at 23 g day^{-1} is unlikely to be a limit mediated centrally by the alimentary tract. A higher central limit may exist, at 26.9 g day^{-1} , but this is unlikely to reflect the time available for feeding. The data are consistent with limits on sustainable daily energy intake being mediated by the performance of the mammary glands. Animals appeared to accommodate the demands for milk production within a constrained total energy budget by compensating their behaviour, most notably by reductions in the time spent in 'general activity'.

Key words: energetics, maximal metabolic rate, sustained metabolic rate, pregnancy, lactation, reproduction, mouse.

Introduction

Over the past two decades there has been considerable interest in the maximum sustainable rate at which animals can maintain their energy expenditures for protracted periods (Drent and Daan, 1980; Koteja, 1991; Peterson et al., 1990; Hammond and Diamond, 1992; Bryant and Tatner, 1991). This interest follows the seminal study of Drent and Daan (Drent and Daan, 1980), who suggested that the maximal daily energy expenditure of birds might be linked to their basal metabolic rate (BMR). Subsequent reviews have indicated that maximum sustainable metabolic rates of endotherms may indeed be limited at around 4–7 times the BMR (Peterson et al., 1990; Koteja, 1991; Bryant and Tatner, 1991; Hammond and Diamond, 1997), although other studies making more

appropriate phylogenetic corrections have questioned the existence of such a link (Ricklefs et al., 1996; Speakman, 2000).

The suggestion that maximal rates of energy expenditure might be limited intrinsically by aspects of an animal's physiology, rather than extrinsically by the supply of energy from the environment, is important because maximal rates of energy expenditure may define the limits at which animals are able to perform in their natural environment. Indeed, several studies have indicated that correlations exist between BMR and population variables such as the intrinsic rate of population increase (Henneman, 1983; McNab, 1980) and geographical distributional limits (Root, 1988; Bozinovic and Rosenmann,

1989), which are consistent with this framework of functional links between maximal daily energy expenditure and BMR. It should be noted that other studies have questioned the existence of relationships between BMR and such population-level characteristics at the species level (Harvey et al., 1991) and that studies aiming to establish such relationships within species have been similarly disappointing (Hayes et al., 1992; Derting and McClure, 1989).

The exact nature of the putative links between BMR and sustainable daily energy expenditure has been the matter of some debate. One idea, termed the central limitation hypothesis, suggests that daily energy expenditures are limited by the capacity of the alimentary tract and associated organs such as the liver to digest, absorb and process ingested energy. Since these organs also have very high metabolic rates (Krebs, 1950), animals that possess large or very metabolically active alimentary processing tissues have high potential maximal daily energy intakes and, hence, expenditures but pay for this capability by also having high BMRs. The alternative hypothesis, termed the peripheral limitation hypothesis, holds that maximal potential expenditures are set at the sites where the energy is utilized (for example, the skeletal muscles during exercise or the mammary glands during lactation) and that a link develops between daily energy expenditure (DEE) and BMR either because these sites also have high levels of expenditure or because the alimentary system responds to the level of the demands and the BMR is mostly a function of the alimentary tract size and activity and the associated energy-processing machinery.

One system that has attracted considerable attention in the context of sustainable limitations is the period of late lactation in mammals. During late lactation, females must ingest large quantities of energy to supply their offspring with milk, and it is widely agreed that this period imposes the greatest energy demands on most mammals (Loudon and Racey, 1987; Thompson, 1992). Using Swiss Webster mice as a model system, Hammond and Diamond (Hammond and Diamond, 1994) have shown that these mice appear to be limited in their capacity to take in food during this period, because when litter sizes were artificially enlarged or lactation was artificially prolonged they did not respond by increasing their food intake. These observations were initially considered as consistent with the central limitation hypothesis. However, more recent work with the same system has demonstrated that, under the combined loads of lactation and cold-exposure, Swiss Webster mice were able to elevate their food intake, indicating that the limit was actually imposed by the capacity of the mammary glands to synthesize milk (a peripheral limitation) (Hammond et al., 1994). Moreover, manipulations of teat number have confirmed that the limitations are imposed by the mammary glands (Hammond et al., 1996). Similar findings for cotton rats (*Sigmodon hispidus*) have been reported by Rogowitz (Rogowitz, 1998).

In a series of studies using a different strain of mice (MF1 strain), we have also investigated the limits to sustainable metabolic rates. A preliminary study (Speakman and

McQueenie, 1996) suggested that this strain may not be limited in its food intake during lactation. However, more recently, we have shown that the mice do have a limited intake of approximately 23 g day⁻¹ during late lactation and when raising large litters (Johnson et al., 2001a). Giving mice more offspring to raise, up to a total of 18 (Johnson et al., 2001a), or making them concurrently pregnant while lactating (Johnson et al., 2001b) did not force them to eat more food than the apparent 23 g limit. However, late in their second lactation, control females that were not concurrently pregnant and lactating did increase their asymptotic daily energy intake by approximately 13% above that in the first lactation. This latter observation calls into question the presence of a constant centrally mediated limit to food intake in this strain of mice. In the present study, we investigated the effects of diluting the energy content of the food (while maintaining the levels of protein and other nutrients). We predicted that if food intake were a central limit, mice would be unable to respond to the diluted energy content by increasing their food intake and would be forced to raise either smaller litters or smaller offspring. We considered that diluting the diet might expose different types of central limitation. First, the mice might already be allocating all their time to feeding behaviour and, thus, be incapable of allocating more time to food intake. Consequently, when the food was diluted, they would be unable to expand the duration of their feeding behaviour to take in more. Second, they might be limited by the physical capacities of their alimentary tracts. It has already been established in non-breeding small mammals that diluting the energy content of the diet results in hypertrophy of the alimentary tract (e.g. Hammond, 1993; Stark et al., 1996). Lactating animals also undergo hypertrophy of the alimentary tract (Speakman and McQueenie, 1996), but there may be limits to the extent to which the tract can grow. Lactation may induce maximal changes in gut capacity, and further expansion may not be possible. To explore these different possibilities, we made two types of manipulation and also made some supplementary observations. In particular, in addition to measurements of food intake, we also monitored the behaviour of the mice to determine whether time spent feeding was a constraint on their investment. Since previous studies have indicated that responses to diluted diets may take time to develop, we manipulated the animals at two different times in their reproductive cycle (early in pregnancy and at the start of lactation). Differences in the responses of these groups might then reveal whether time to respond to the diluted diet was a constraining factor.

Materials and methods

Animals and housing

Observations were made on 30 female MF1 mice *Mus musculus* L. aged approximately 10 weeks, with their litters. The data were compared with those collected previously for

71 litters of the same species at the same age (Johnson et al., 2001a). The mice were allocated to two groups of 15 individuals. All the females were paired with a male for 7 days. In one group (pregnancy-onset), the female mice were fed a standard diet (CRM pellets, SDS BP Nutrition Limited; gross energy content 18.36 kJ g^{-1} dry mass, digestible energy 13.4 kJ g^{-1}) while with the male; once the male had been removed, they were given a diet with a lower gross energy content. The diet was custom-prepared by SDS BP Nutrition Ltd and contained 25 % less digestible energy than the normal diet (9.75 kJ g^{-1}). The dilutant was cellulose. Other components of the diet were adjusted so that protein content per gram and the contents of all the other essential vitamins and minerals per gram were all equal to the normal diet (crude protein 22.3 %, vitamin A $20\,000 \text{ i.u. kg}^{-1}$, vitamin D3 $2900 \text{ i.u. kg}^{-1}$, vitamin E 100 i.u. kg^{-1}). Mice in the second group (lactation-onset) were fed the normal diet throughout pregnancy. They were switched onto the low-energy diet following parturition. All the mice were housed in individual shoebox cages and were provided with shredded paper bedding throughout the experiments. Room temperature was maintained at $22 \pm 1 \text{ }^\circ\text{C}$. A 12 h:12 h L:D photoperiod with lights on at 08:00 h was provided. Food and water were continuously available in substantial excess (approximately 10 times daily requirements). Between 10:00 h and 12:00 h each day throughout lactation (18 days), we measured the food intake (from the food missing from the hoppers), the mass of the female and the litter size. All masses were measured using a Sartorius top-pan balance ($\pm 0.01 \text{ g}$). Previous measurements have indicated that the loss of food into the bedding amounted to less than 2 % of intake (Johnson et al., 2001a). Although this previous quantitative estimate refers to mice feeding on a low-fibre diet, casual observations indicated that the mice did not differ in their propensity to grind the food between the diets. We also measured the masses of the offspring on days 2, 5, 8, 11, 14 and 17 of lactation.

Behavioural observations

Observations were made of the females in both experimental groups at two times of day: during the dark phase between 04:00 h and 06:00 h, and during the light phase between 15:00 h and 17:00 h. Observations in the dark were made using a low-wattage (30 W) red light. Each mouse was observed for a period of 30 s, and its dominant behaviour over this interval was classified into one of four categories using an ethogram devised previously for this strain (Speakman and Rossi, 1999). Behaviour was classed as grooming, feeding, resting or general activity. The latter class included climbing on the cage bars, walking around the cage and rearing up. Grooming included self-grooming and grooming the pups during lactation. During pregnancy, resting involved being inactive in any location either in or out of the nest. When females 'rested' during lactation, they also generally suckled their offspring. Each mouse was observed in sequence, and a series of 15 cages (pregnancy-onset group or lactation-onset group) was therefore

observed every 7.5 min. After 30 min (four observations per mouse), the next batch of 15 cages was observed, and this sequence was alternated over the 2 h of observations. Thus, in total, each mouse was observed eight times during each session. The percentage times spent in each behaviour were calculated from the pooled data across all the experimental mice in each group. The total number of behavioural samples was therefore approximately $8 \times 15 (=120)$ in each session for each group, and the standard deviation for the percentage time spent performing each behaviour was approximately 2.5 %. Several females failed to conceive and were retrospectively eliminated from the sample.

Statistical analyses

We examined the relationship between food intake and time using a repeated-measures analysis of variance (ANOVA). Significant differences between days were detected using Tukey tests. The asymptotic food intake in late lactation was defined as the period during which no significant differences between days were detected. We compared asymptotic food intakes across the three groups (pregnancy-onset and lactation-onset groups and the controls) using ANOVA. The effects of litter size and treatment group on the mass of offspring at weaning were determined using analysis of covariance (ANCOVA) with litter size as a covariate. Time spent in behaviours was recorded as a percentage of the total observations across all individuals in each group. Data are presented as percentages, but analyses were performed on arcsine-square-root-transformed data to normalise the distributions. Trends in each component of the behaviour over time were established using least-squares regression. All statistical analyses were performed using commercially available software (Minitab versions 7.3 and 11; Ryan et al., 1985). Results are presented as means \pm S.E.M.

Results

Food intake

By the end of pregnancy, the experimental group fed on the low-energy diet from the time the males were removed had compensated their food intake almost exactly to account for the lower digestible energy content when compared with the control animals. On the first day of lactation, this group had a mean food intake of $14.4 \pm 0.7 \text{ g}$ ($N=15$) compared with $9.7 \pm 0.34 \text{ g}$ in the control group ($N=71$; means \pm S.E.M.) (Johnson et al., 2001a,c). The difference in food intake amounted to 4.7 g, which was 48.5 % greater than the mice fed the normal diet ($100 \times 4.7/9.7$). Since the experimental mice were fed a diet containing 25 % fewer digestible calories, their 48.5 % greater intake meant that the experimental group consumed greater amounts of digested energy (pregnancy-onset group $14.4 \times 9.75 \text{ kJ g}^{-1} = 140.4 \text{ kJ day}^{-1}$; control group $9.7 \times 13.4 \text{ kJ g}^{-1} = 130.0 \text{ kJ day}^{-1}$). In contrast, the experimental lactation-onset group ($N=13$), which were switched to the low-energy diet at parturition, compensated their food intake on the

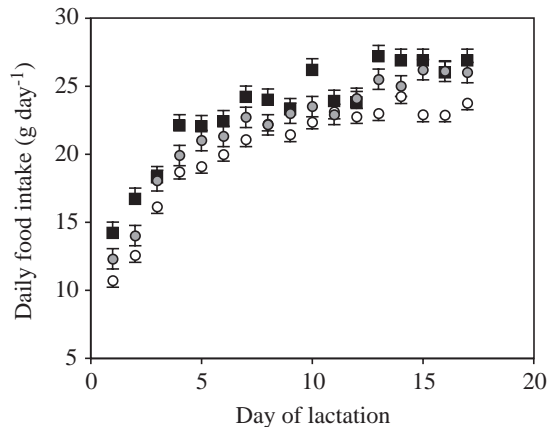


Fig. 1. Changes in daily food intake throughout lactation (day 0 is parturition) in experimental mice fed the low-energy diet from early pregnancy (black symbols, $N=15$), experimental mice fed the low-energy diet from the first day of lactation (stippled symbols, $N=13$) and control animals fed the standard diet (white symbols, $N=71$). Values are means \pm S.E.M. Some error bars are smaller than the point dimensions.

first day of lactation to account for the lower energy density almost exactly and ingested an average 12.7 ± 0.8 g of food (equivalent to 123.8 kJ).

Food intake in both the experimental groups increased rapidly over the early phase of lactation (Fig. 1). Variation with day of lactation was highly significant in both groups (repeated-measures ANOVA: pregnancy-onset group, $F_{17,252}=442.8$, $P<0.0001$; lactation-onset group, $F_{17,216}=394.1$, $P<0.0001$). The pattern of variation in food intake with day of lactation was similar to that observed in the control group (Johnson et al., 2001a). In both experimental groups, the asymptote in food intake was reached on day 13, as was the case for the control group. Between days 13 and 16, the pregnancy-onset group reached an asymptotic food intake of 26.9 ± 1.2 g day $^{-1}$ ($N=15$) and the lactation-onset group reached an asymptotic intake of 25.8 ± 0.8 g day $^{-1}$ ($N=13$; means \pm S.E.M.). The difference between the two groups was significant (ANOVA: $F_{1,26}=29.03$, $P<0.01$; Tukey test: $P<0.01$), and both groups had greater asymptotic food intakes than in the control group, which averaged 23.1 ± 0.4 g day $^{-1}$ ($N=71$; mean \pm S.E.M.; Tukey test: $P<0.01$ in both cases). The asymptotic intake of the pregnancy-onset group was 3.8 g higher than that of the control group. This elevation was not significantly different from that observed on the first day of lactation. Indeed, the extent by which food intake remained elevated above that of the control group was remarkably constant throughout the whole of lactation for the pregnancy-onset group (Fig. 2). In contrast, the lactation-onset group, which were only switched to the low-energy food on the day of parturition, changed the extent of the elevation of their intake relative to the controls over the period of lactation (Fig. 2). This increase was significant (least-squares regression: $r^2=0.10$, $P<0.05$).

Since food intake was increased enormously over the same

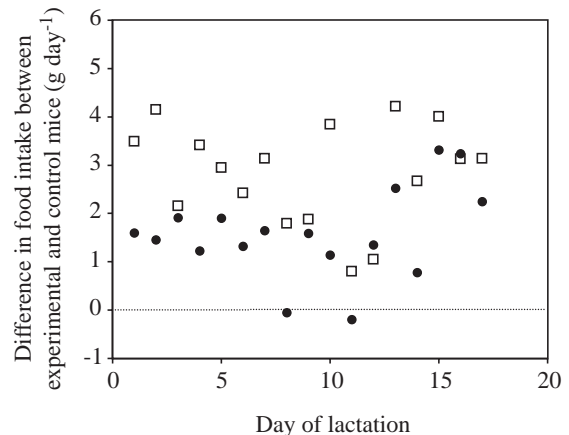


Fig. 2. The difference in food intake between experimental mice fed the low-energy diet from early pregnancy (open squares) or from parturition (filled circles) and control mice throughout lactation. There was a significant increase over time in the difference relative to control mice in the group switched to the low-energy diet at parturition but no change over time in the group switched in early pregnancy.

period (Fig. 1), the proportional extent to which the intake was higher in the experimental groups became relatively smaller as lactation progressed. Thus, on day 1, the pregnancy-onset and lactation-onset groups ingested 48.5 and 30.9% more than the control animals, respectively; by day 16 of lactation, these elevations had declined to 16.5 and 11.7% greater than the control intake, respectively. These elevations of intake were both insufficient to offset the lower digestible energy content of the low-energy food. Thus, control mice at the asymptotic intake were absorbing on average 23.1×13.4 kJ = 310.2 kJ day $^{-1}$, while the pregnancy-onset group absorbed on average 26.9×9.75 kJ day $^{-1}$ = 262.3 kJ day $^{-1}$, which is 15.4% lower than the controls $100(310.2 - 262.3)/310.2$. The lactation-onset group absorbed on average 25.8×9.75 kJ day $^{-1}$ = 251.6 kJ day $^{-1}$, which was 18.9% lower than the controls.

Female body mass

Female mice in all three groups increased in body mass throughout lactation. There was a highly significant effect of day of lactation (repeated-measures ANOVA: $F=127.34$, $P<0.001$), but there were no significant differences between the groups (repeated-measures ANOVA: $F=0.25$, $P>0.05$).

Offspring mass

Offspring mass on day 2 did not differ significantly between the three groups and averaged (means \pm S.E.M.) 1.73 ± 0.15 g, 1.85 ± 0.15 g and 1.7 ± 0.15 g for the pregnancy-onset, lactation-onset and control groups, respectively. By day 16, the offspring mass was significantly inversely and linearly related to the litter size in both experimental groups (ANCOVA: $F=42.2$, $P<0.001$). The gradient of the effect did not differ significantly between the groups or from that previously established in the control animals (ANCOVA: interaction $F=1.97$, $P=0.15$), and there were no significant differences either between the two

experimental groups or between these two groups and the controls (ANCOVA: group effect $F=1.98$, $P=0.15$).

Pup mortality

Litter sizes at birth did not differ significantly between the three groups (ANOVA: $F=1.4$, $P=0.27$). In the control group, 29 offspring died prior to weaning from 830 that were born. In the experimental groups (pooled), 13 offspring died prior to weaning from 321 that were born. The difference was not significant ($\chi^2=0.189$, $P>0.05$, d.f.=1) and, on average, amounted to only 0.46 pups per litter. Consequently, the resultant mean litter size during the asymptotic phase of lactation was not significantly lower in the experimental groups than in the control group (ANOVA: $F=1.71$, $P=0.191$).

Behavioural observations

General activity

In both the group fed the low-energy diet throughout reproduction (pregnancy-onset group) and the group fed this diet from parturition (lactation-onset group), there was more general activity at night than during the day (Fig. 3; pregnancy-onset group paired t -test, $t=7.2$, d.f.=29, $P<0.0001$; lactation-onset group, $t=8.91$, $P<0.0001$). On average, for all readings, general activity accounted for $43.6\pm 3.1\%$ and $37.8\pm 2.8\%$ of records during the dark phase for pregnancy- and lactation-onset groups, respectively, and for $24.8\pm 2.7\%$ and $17.6\pm 1.7\%$ (means \pm S.E.M.), respectively, of records during the light phase. There was a strong decline in the time spent engaged in general activity at night as pregnancy progressed, and this continued during lactation. At the start of pregnancy, between 60 and 80% of the observation period in the dark was spent in general activity; by day 16 of lactation, this had declined to between 20 and 30%. The pattern was the same in both experimental groups and was highly significant (regression: pregnancy-onset group $r^2=0.757$, $F_{1,27}=84.07$, $P<0.0001$; lactation-onset group $r^2=0.604$, $F_{1,27}=41.1$, $P<0.0001$). During the light phase, there was also a decline as pregnancy and lactation progressed but the effect was less marked, involving a decline from 20–40% in early pregnancy to 5–15% by late lactation (Fig. 3) (regression: pregnancy-onset group $r^2=0.460$, $F_{1,27}=22.96$, $P<0.001$; lactation-onset group $r^2=0.538$, $F_{1,27}=31.4$, $P<0.001$).

Grooming

Grooming behaviour occupied a relatively minor part of the time budget, comprising between 0 and 20% of the observation periods in the different groups during the day and night (Fig. 4). There was no difference in the amount of grooming between day and night in either group (pregnancy-onset group paired t -test, $t=0.86$, d.f.=29, $P=0.4$; lactation onset-group $t=1.8$, d.f.=29, $P=0.08$). There were no significant trends in the amount of grooming throughout reproduction in either group during the day. However, in the lactation-onset group during the night, the amount of grooming declined significantly throughout the period of reproduction (regression: $r^2=0.323$, $F_{1,27}=12.88$, $P<0.001$; Fig. 4B).

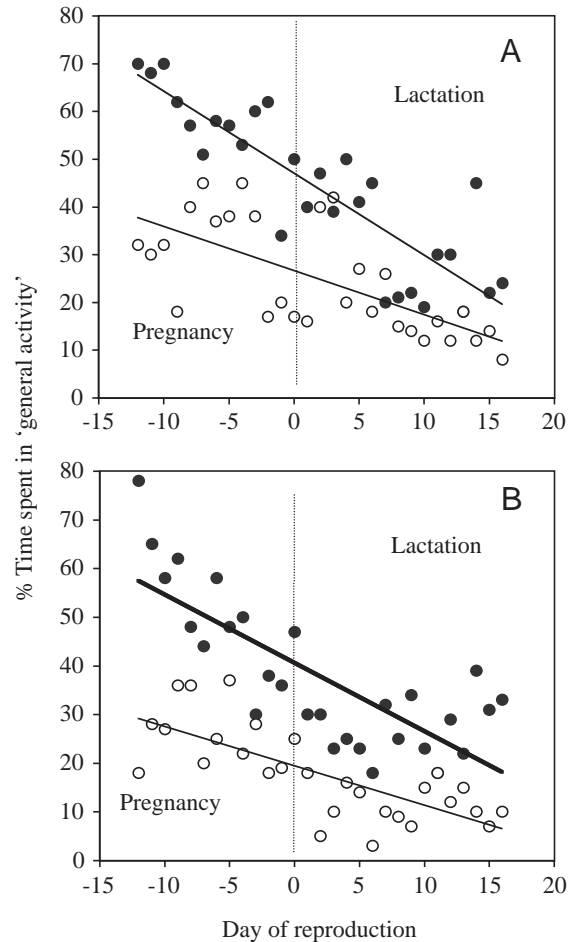


Fig. 3. Changes in the 'general activity' behaviour of mice fed the low-energy diet (A) from early pregnancy ($N=15$) and (B) from parturition ($N=13$). Observations made at night are identified by filled symbols; observations during the day are shown by open symbols. During both times of day, in both groups, the behaviour declined between early pregnancy and late lactation. Each point represents the mean percentage time spent in the behaviour across all mice. Parturition is day 0.

Feeding

Mice spent more time feeding during the dark phase than they did during the light phase in both experimental groups (Fig. 5; pregnancy-onset group paired t -test, $t=5.63$, d.f.=29, $P<0.0001$; lactation-onset group $t=5.31$, d.f.=29, $P<0.0001$). Although the mice in the pregnancy-onset group were fed food with a 25% lower energy content than the food provided to the lactation-onset group during pregnancy, they did not spend more time feeding either at night (paired t -test, $t=0.36$, d.f.=13, $P=0.76$) or during the day (paired t -test, $t=0.83$, d.f.=13, $P=0.54$). There was an increase in the time spent feeding throughout reproduction both during the night and during the day in both groups (Fig. 5; regression pregnancy-onset group, night, $r^2=0.597$, $F_{1,27}=39.9$, $P<0.0001$; regression pregnancy-onset group, day, $r^2=0.725$, $F_{1,27}=71.0$, $P<0.0001$; regression lactation-onset group, night, $r^2=0.470$, $F_{1,27}=23.9$, $P<0.0001$;

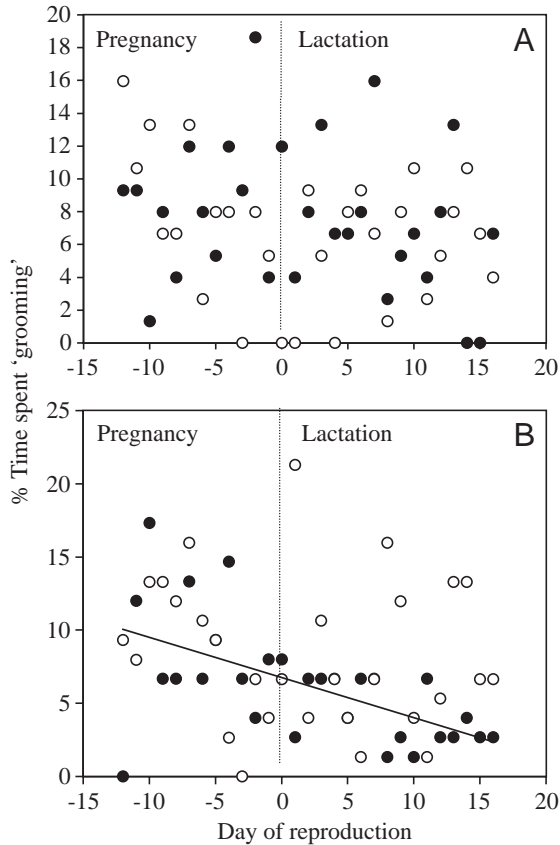


Fig. 4. Changes in 'grooming' behaviour of mice fed the low-energy diet (A) from early pregnancy ($N=15$) and (B) from parturition ($N=13$). Observations made at night are identified by filled symbols; observations during the day are shown by open symbols. A significant trend was apparent only for the lactation-onset group at night. Each point represents the mean percentage time spent in the behaviour across all mice. Parturition is day 0.

regression lactation-onset group, day, $r^2=0.659$, $F_{1,27}=62.3$, $P<0.0001$). By the end of lactation, feeding behaviour was occupying 30–50% of the time budget at night and 30–40% of the daytime budget.

Resting

The mice in both groups spent considerably more time at rest during the day than they did at night (Fig. 6: pregnancy-onset group paired t -test, $t=6.13$, $d.f.=29$, $P<0.0001$, lactation-onset group, paired t -test, $t=8.54$, $d.f.=29$, $P<0.0001$). The time spent resting during the day did not change significantly over the entire period of reproduction in either group (regression pregnancy-onset group, day, $r^2=0$, $F_{1,27}=0.8$, $P=0.964$; regression lactation-onset group, day, $r^2=0.02$, $F_{1,27}=1.01$, $P=0.95$), averaging 41.9 ± 2.0 and $52.0\pm 2.5\%$ of the sampled time in the pregnancy- and lactation-onset groups, respectively. In contrast, the time spent at rest at night increased significantly in both groups as reproduction progressed (Fig. 6; regression pregnancy-onset group, night, $r^2=0.543$, $F_{1,27}=32.1$, $P<0.0001$; regression

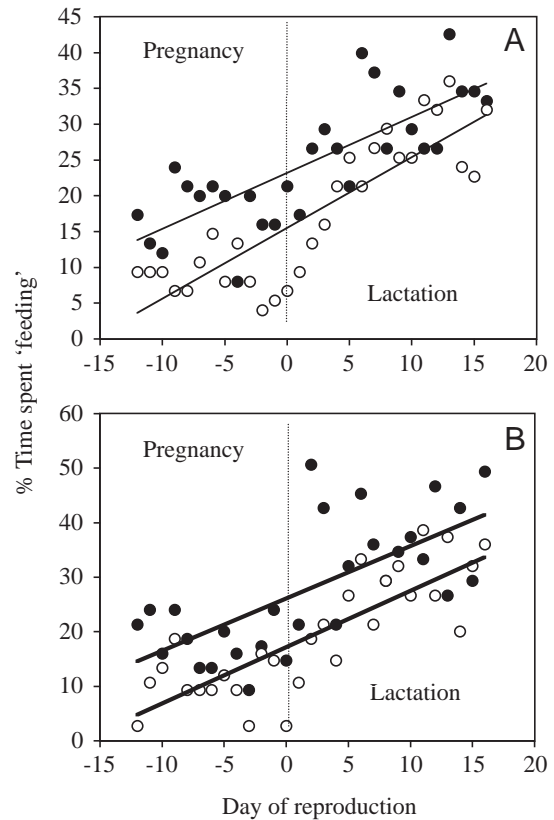


Fig. 5. Changes in the 'feeding' behaviour of mice fed the low-energy diet (A) from early pregnancy ($N=15$) and (B) from parturition ($N=13$). Observations made at night are identified by filled symbols; observations during the day are shown by open symbols. During both phases, and in both groups, the mice increased the amount of feeding behaviour as reproduction progressed. Each point represents the mean percentage time spent in the behaviour across all mice. Parturition is day 0.

lactation-onset group, night, $r^2=0.292$, $F_{1,27}=11.1$, $P<0.0001$).

Overall behaviour patterns

The most dramatic change in behaviour was the reduced levels of general activity observed as pregnancy and lactation progressed (Fig. 3). At night, this decline in time spent active was replaced by time spent either resting (Fig. 6) or feeding (Fig. 5) in approximately equal measure. During the day, however, the decline in activity was taken up only by increased feeding behaviour (Fig. 5).

Discussion

The MF1 mice we studied previously did not respond to artificially elevated litter sizes (Johnson et al., 2001a) or concurrent pregnancy and lactation (Johnson et al., 2001b) by increasing their food intake beyond an asymptotic intake of approximately 23 g during late lactation. This apparent asymptote in food intake may have reflected a central limit on sustainable

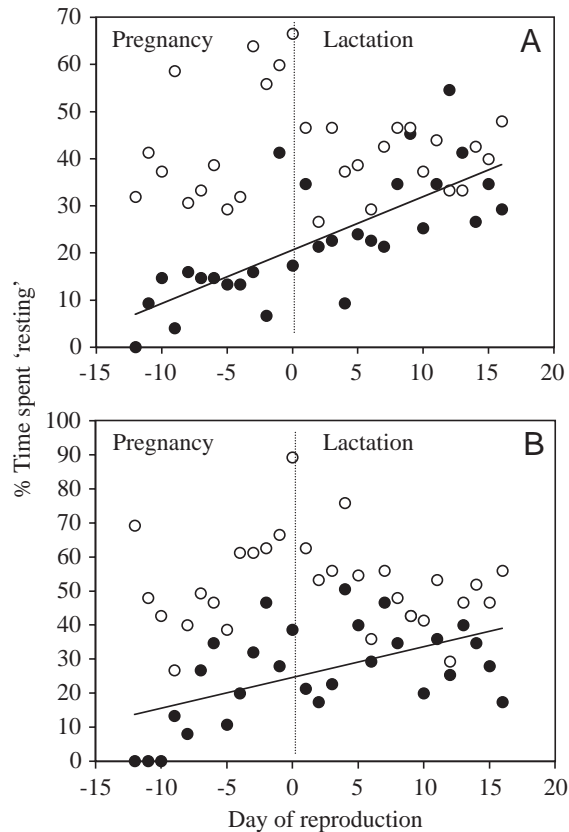


Fig. 6. Changes in the 'resting' behaviour of mice fed the low-energy diet (A) from early pregnancy ($N=15$) and (B) from parturition ($N=13$). Observations made at night are identified by filled symbols; observations during the day are shown by open symbols. During the day, 'resting' time was unchanged, but in both groups at night 'resting' increased as reproduction progressed. During lactation, 'resting' was also generally accompanied by suckling behaviour in the pups. Each point represents the mean percentage time spent in the behaviour across all mice. Parturition is day 0.

metabolic rates. In control mice undergoing two sequential pregnancies and lactations, however, asymptotic daily food intake at peak lactation was increased during the second lactation (Johnson et al., 2001b). Supporting these latter observations, the mice in the present study increased their food intake beyond the supposed 23 g limit when they were given food that had a lower digestible energy content. These data suggest, therefore, that the 23 g limit on food intake is not a centrally mediated limit on sustainable performance. The mice did increase their food intake, even though all the other constituents of the diet were available at the same level as in the diet fed to control mice, so a further implication of these data is that energy is the most important limiting factor during late lactation. Other key nutrients exported to offspring such as calcium and protein, which have previously been suggested to be important potential constraints on litter size in other groups (e.g. Barclay, 1994), appear to be less important in the present system.

The pattern of variation in food intake by the experimental pregnancy-onset group, which were fed the low-energy-

content food from early pregnancy, was remarkably similar to the pattern observed in Swiss Webster mice forced to lactate in cold conditions (Hammond et al., 1994). The extent of elevation of food intake above the 'normal' lactation condition remained constant throughout the lactation period. This suggests that, although the alimentary system of the experimental mice had a greater capacity to process food than that of the control group, the extent of this elevation was somehow fixed. In contrast, the mice that were switched to the low-energy diet at parturition (lactation-onset group) continuously expanded the extent of elevation of their intake above that of the controls, suggesting that their alimentary systems were in the process of adjusting to the lower energy density of the food. The extent of this adjustment, however, never reached the level achieved by the group fed the low-energy diet from early pregnancy onwards.

Although mice can modify their alimentary tracts to digest greater quantities of food than 23 g day^{-1} , the different responses of the two experimental groups indicate that the rapidity with which their tracts respond may be restricted. The fact that even those mice that had been fed the low-energy diet from early pregnancy did not fully compensate their food intake to account for the lower energy content of the diet during late lactation also suggests that the extent of the response may be limited.

The failure to compensate fully during late lactation for the lower energy content of the low-energy-density food by increasing food intake resulted in a progressive shortfall of energy intake throughout lactation relative to the control animals, which was most marked in late lactation. There are several potential ways in which the female mice may have responded to this shortfall in energy intake. First, they could withdraw fat stores. During the last 5 days of lactation, the shortfall amounted to approximately 190 kJ in the pregnancy-onset group and 243 kJ in the lactation-onset group. If the mice were using fat to make up this shortfall, they would need to withdraw between 4.8 and 6.2 g more fat from their body stores than the control mice. Body composition analyses of lactating controls, however, suggest that they have already almost completely depleted their fat reserves by this phase of lactation (M. S. Johnson and J. R. Speakman, unpublished observations) and do not have this amount of stored fat available to utilize. Moreover, the body mass profiles of experimental and control animals were almost identical, lending support to the suggestion that fat withdrawal does not make up the loss.

There was also no indication that the experimental female mice responded to the shortfall in their energy intake by reducing their supply of milk to their offspring. The offspring were weaned at the same masses observed in the control litters, and there was no detectable increase in mortality of the litters from experimental animals. It seems most likely, therefore, that the female mice were compensating their energy budgets during lactation. We have no direct evidence to support this suggestion. However, there was a precipitous reduction in general activity in these animals, which would be consistent with such a trend. Whether this was greater in the

present experimental mice than in the control mice fed a normal food, however, remains to be demonstrated. Previous studies of energy budgets of lactating brown long-eared bats *Plecotus auritus* have indicated that they too compensate their energy budgets during lactation (McLean and Speakman, 1999) and that at least part of the savings may accrue because the animals reduce the amount of grooming they perform (McLean and Speakman, 1997) when lactating. We found a significant decline in levels of grooming only in the lactation-onset group at night. However, baseline levels of grooming were much higher in the bats we studied previously, which may reflect their wild ancestry and ectoparasite burdens compared with the absence of ectoparasites in the laboratory mice studied here.

The overall patterns observed here suggest that a food intake of 23 g day⁻¹ is not a central limit in these mice. The failure of the experimental mice to compensate completely for the reduced energy content of their food during late lactation indicates, however, that a central limit may exist at a higher level of approximately 26.9 g day⁻¹. This is similar to the asymptotic daily food intake of 26.1 g day⁻¹ reported for the second lactation of the same strain of mice (Johnson et al., 2001b). The nature of this putative central limit is unclear at present. However, despite eating low-energy food, the mice in the present study managed to ingest their total daily intake of 26.9 g by feeding for approximately 45% of the dark-phase and 35% of the light-phase observation periods. Although our observations did not cover the entire dark and light phases, and there may be significant variation in the percentage time spent feeding at different times of the day and night, these data suggest that there would be substantial further scope to expand the amount of time spent feeding if the animals needed to. If a central limit does exist at 26.9 g, therefore, it is unlikely to be mediated *via* the available time for feeding. The mice did not respond to this putative limit by cutting back the supply of milk to their offspring (as judged by the size of the offspring at weaning) or by reducing the size of their litters. Compensation of the energy budgets by behavioural changes (perhaps involving reduced activity) therefore seems a likely explanation for how the mice coped with the shortfall in supply from their dietary intake at peak lactation (although we cannot rule out physiological adjustments as well). The data are consistent with the suggestion from studies of other strains of mouse (Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond et al., 1996) and cotton rats (*Sigmodon hispidus*; Rogowitz, 1996; Rogowitz, 1998) that limits on maximal energy budgets in late lactation are imposed peripherally by the secretory activity of the mammary glands rather than centrally by the digestive capacity of the alimentary tract.

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