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LIMITS TO SUSTAINED ENERGY INTAKE

I. LACTATION IN THE LABORATORY MOUSE *MUS MUSCULUS*

M. S. JOHNSON, S. C. THOMSON AND J. R. SPEAKMAN*

Aberdeen Centre for Energy Regulation and Obesity (ACERO), Department of Zoology, University of Aberdeen, Aberdeen AB24 2TZ, Scotland, UK

*Author for correspondence (e-mail: j.speakman@abdn.ac.uk)

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Summary

Laboratory mice (strain MF1) were used to determine whether sustainable rates of energy intake are limited during lactation. Mice raising natural-sized litters (*N*=71) reached an asymptote in their daily food intake between days 13 and 16 of lactation at 23.1 g day^{-1} and also between litter sizes of 9 and 15 pups (22.8 g day^{-1}). A second group of 37 females had their litter sizes manipulated at birth to raise more or fewer offspring than they gave birth to. When the litter size was increased, females did not increase their food intake to match their new litter size. However, when litter size was decreased, females decreased their asymptotic daily food intake during late lactation in relation to the extent of reduction in litter size. Therefore, it appeared that females were limited during late lactation and with large litter sizes. The milk energy exported amounted to 44 % of the gross energy intake, and the estimated daily energy expenditure was therefore considerably lower than the sustained energy intake [8.0×RMR(gross), 6.6×RMR(assimilated)], and averaged 3.1×RMR, where RMR is resting metabolic rate. It was not possible to determine whether the apparent limit on sustained energy intake was acting centrally or peripherally because of the asymptotes in both food intake and milk energy output with increasing litter size.

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

Introduction

The maximal rates of energy expenditure by animals are of interest in several different contexts, perhaps most importantly because they define an upper boundary for features of animal performance that depend critically on energy, such as reproductive output (Thompson, 1992; Bryan and Bryant, 1999) and thermoregulatory capabilities, which may define the global distribution limits of endotherms (Root, 1988; Bozinovic and Rosenmann, 1989). It is widely believed that the maximal rate of energy expenditure that animals can sustain for protracted periods of days and weeks (termed sustainable metabolic rate, SusMR) is intrinsically limited by some aspect, or aspects, of physiology (Peterson et al., 1990; Daan et al., 1990; Weiner, 1992; Hammond and Diamond, 1997; but see Speakman, 2000).

A potential area of confusion is that some studies have measured energy expenditure directly to evaluate sustainable metabolic rates (for reviews, see Nagy et al., 1999; Speakman, 2000), but other studies have employed food intake (e.g. Hammond et al., 1994). When animals are at stable body mass, food intake provides an estimate of sustainable metabolic rate because the majority of ingested energy is metabolised, and demonstrated limits on food intake would translate to limits on expenditure. This link between food intake and energy expenditure, however, breaks down when there is substantial export of energy, such as occurs during lactation (see discussion in Speakman and McQueenie, 1996; Speakman, 2000). In this situation, limited food intake still reflects an absolute maximum potential level of expenditure, but actual expenditure will be substantially lower and may be limited at some lower threshold. In the present study, we have also measured food intake as an indicator of sustainable maximum metabolic rate, but use the term sustainable energy intake (SusEI) to emphasize the potential contrast to SusMR.

Limits on SusEI and SusMR are likely to be particularly important during peak lactation, which is the time of greatest energy demand on female mammals (Kenagy et al., 1990; Thompson, 1992). Limitations on SusEI at this time may determine the total investment that mammals can make in their offspring and may, thus, define maximum litter and offspring sizes. Lactating laboratory mice (*Mus musculus* L.) provide a convenient model animal in which to investigate limitations on SusEI and SusMR, and there have been many recent studies of this system (Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond et al., 1994; Hammond et al., 1996; Speakman and McQueenie, 1996). In addition, many earlier studies quantified food intake during lactation in this species

(Bateman, 1957; Myrcha et al., 1969; Studier, 1979; König et al., 1988), although not in the context of the theories of sustainable metabolic rates.

Several manipulations have been performed on lactating mice to investigate where limits occur in this system. Swiss Webster mice have been shown to raise a maximum of 14 pups (even though some litters were manipulated up to 26 pups), with the lactating females increasing their food intake throughout lactation and with increasing litter size (Hammond and Diamond, 1992). Hammond and Diamond (Hammond and Diamond, 1994) extended the duration and level of demands placed on the lactating mother by restricting the access of pups to food until they were 21 days old, and found that the mothers did not respond by elevating their food intake above that achieved by mothers raising 14 pups during a normal lactation. However, when lactating mice were also challenged with cold-exposure, they were able to increase their food intake further (Hammond et al., 1994). By surgically manipulating the number of teats on lactating female mice, Hammond et al. (Hammond et al., 1996) found that females with only two teats were unable to raise any pups, and that females with five and 10 teats with the same mammary pressure (pups per teat) raised pups that did not differ in their body masses, even though the mothers with five teats had only half the number of pups to raise.

Overall, these experiments suggest that lactating mice are limited peripherally at the mammary gland in their milk output and regulate their food intake to match this limit. This interpretation is supported by several previous studies of milk production in small mammals. These have shown that, although females with larger litters can produce more milk than those with smaller litters, this is generally insufficient to support the growth rates observed in small litters and, hence, that pups from larger litters are often lighter (Russell, 1980; Meyer et al., 1985; Knight et al., 1986; Fiorotto et al., 1991; Rogowitz and McClure, 1995; Rogowitz, 1996; Rogowitz, 1998).

There is some evidence that different strains of laboratory mice may respond differently to the sustainable limit. The Swiss Webster mice studied by Hammond and Diamond (Hammond and Diamond, 1992; Hammond and Diamond, 1994) and Hammond et al. (Hammond et al., 1994; Hammond et al., 1996) had a maximum food intake of 19 g in late lactation and raised a maximum of 14 pups in their first lactation (Hammond and Diamond, 1992). Yet MF1 mice eat up to 26 g and raise up to 16 pups in their first lactation (Speakman and McQueenie, 1996). Moreover, the relationship between food intake and litter size for this latter strain does not appear to reach an asymptote (Speakman and McQueenie, 1996) in the range of natural litter sizes, indicating that limits may be set at much higher levels in this strain. In the light of these strain differences, we aimed to investigate the limitations on food intake during lactation in the MF1 mouse and to evaluate how these limitations relate to milk output and the demands of the offspring. An asymptote in maternal food intake with increasing litter size would be consistent with the central limitation hypothesis, whereas an asymptote in milk energy output would be consistent with the peripheral limitation hypothesis.

Materials and methods

Animals and housing

Female mice (outbred MF1), 8–9 weeks old, were housed in individual cages (44 cm×12 cm×13 cm) with sawdust and paper bedding. Rodent chow [CRM(P), 18.36 kJ g^{-1} dry mass, 11.5 % water, 16.3 kJ g^{-1} wet mass, 14.5 % fibre, 13.3 % protein; Special Diet Services, BP Nutrition, UK] and water were available *ad libitum*. The environment was regulated at 21 °C on a 12 h:12 h L:D photoperiod.

One hundred and eight females were paired with males for 6 days, after which the males were removed. Pregnancy was detected by an increase in mass over the following 7 days. Following parturition (day 0), 71 of the females were allowed to raise a natural litter to peak lactation (day 18 of lactation). These females were termed the ‘control’ females. The remaining 37 females (manipulated) had their litter size manipulated by cross-fostering on day 0 so that they raised more or fewer offspring than they gave birth to. This extended the range of natural litter sizes, which varied from five to 15 pups (control) to between three and 18 pups (manipulated). Females readily accepted the fostered pups.

Female body mass and food intake were measured (using a Sartorius top-pan balance) prior to breeding and then daily throughout pregnancy and lactation. No measurements were made on the days when a male was present. Food intake was calculated as the mass of food missing from the hopper each day. The bedding was checked for bits of uneaten food, which were weighed and returned to the hopper. We fine sorted, by hand, the bedding of 17 of the lactating mice and found that only 1.7±0.41 % (mean ± S.E.M.) of the food missing from the hopper was left in the bedding. Following parturition, the number of pups and the total mass of the litter were also recorded each day. All masses were accurate to 0.01 g.

To determine the assimilation efficiency, faeces produced over a 5-day period were collected from nine females prior to breeding and from 16 lactating females between days 10 and 15 of lactation. The faeces were weighed, dried at 60 °C (Gallenkamp air-fan oven) for 14 days and reweighed. Total food intake over the same time period was also measured. Gross energy content was determined for faeces from non-breeding females (N=5) and lactating females (N=6) and for the food by adiabatic bomb calorimetry (Gallenkamp Autobomb, Rowett Research Institute Analytical Services, Aberdeen, UK). Assimilation efficiency was expressed as the total gross energy intake minus the energy in faeces divided by the total gross energy intake.

Energy expenditure of the litters

On day 13 of lactation, the resting metabolic rate of 10 entire litters was measured using an open-flow respirometry system connected to a paramagnetic oxygen analyser (Servomex

model 1100A), as described previously (Hayes et al., 1992; Speakman and McQueenie, 1996). Dry air was pumped (Charles Austin Pumps Ltd) through a sealed Perspex chamber housed inside a constant-temperature incubator (INL-401N-010, Gallenkamp) set at 21 °C (the temperature at which the mice were housed). A flow rate of 1000–1500 ml min^{-1} was metered continuously using an Alexander Wright flowmeter (DM3A) upstream of the chamber. A sample of the excurrent air leaving the chamber (approximately 150 ml min^{-1}) was dried (silica gel) and directed through the oxygen analyser. Carbon dioxide (CO_2) in the outflow was not absorbed prior to measurement of oxygen content, as this provides the most accurate method for measuring energy expenditure (Koteja, 1996; Speakman, 2000) in the absence of a known respiratory quotient (RQ). We calculated the rate of oxygen uptake, $\dot{V}_{O_2}$, from the product of the downstream oxygen content difference from ambient and the upstream flow rate. As the flow rate was measured upstream of the chamber, oxygen consumption was converted to energy expenditure assuming that RQ=1, using the equation of Weir (Weir, 1949; for calculation details, see Speakman, 2000).

Measurements of the difference in oxygen concentration between ambient and excurrent air were digitised approximately 80 times each second, and the mean was calculated every 30 s over a 1 h period and stored on a microcomputer (Viglen PC) interfaced with the oxygen analyser. Pups were observed to settle very quickly (within 15 min of entering the chamber), and a stable trace was always obtained within the hour. The lowest 5 min of oxygen consumption (corrected to STP) was taken as an estimate of the resting metabolic rate (RMR) (ml min^{-1}). This was converted to an estimated equivalent daily energy expenditure (DEE) (kJ day^{-1}), which excludes the costs of any activity.

The total energy requirement (TER) of the litters was assumed to equal the sum of the daily energy expended (DEE) on respiration and the energy diverted to growth, measured from the change in mass of the litters between day 13 and day 14. This was converted to energy (kJ day^{-1}) using the calorific value of pups (2.14 kcal g^{-1}=8.95 kJ g^{-1} wet mass; from Brisbin, 1970). Since the TER was based on our estimates of DEE, it also excluded any costs of activity in the litter.

Milk production

Milk production of lactating females was estimated from the difference between the total water turnover and the summed water loss in faeces, urine and by evaporation. This difference was taken to be the volume of water in the milk. Water turnover was measured in 21 lactating females (day 14–15 of lactation) by the isotope dilution method using tritiated water (HTO). A blood sample (100 µl) was obtained from the females (by taking a 1 mm scissor snip from the end of the tail) and flame-sealed in glass capillaries (Vitrex, Camlab Ltd) to determine a background activity of tritium. Females were dosed intraperitoneally with 0.2 ml of tritiated water (15.21 MBq ml^{-1}) on day 14 of lactation. The dose was determined by weighing the syringes before and after the injection (to 0.0001 g; Ohaus Analytical Plus). After the isotope had equilibrated with the body water (1 h) (Speakman, 1997), an initial blood sample was obtained. A final blood sample was obtained in the same way 24 h (±5 min) after the initial sample (Speakman and Racey, 1988).

Water was obtained from the blood samples by vacuum distillation (Nagy, 1983) prior to determination of the specific activity of tritium (liquid scintillation counter; Packard, model 1600TR). Samples of 10 µl of HTO were weighed (accurate to 0.0001 g; Ohaus Analytical Plus) and added to 2 ml of scintillation fluid (Ultima Gold XR), vortex-mixed and counted for 5 min. To correct for variations in the amount of HTO added, the activity of the samples was expressed per microgram of the original water. Samples were analysed in triplicate, and the mean of two separate counts on each vial was taken. All samples were corrected for background counts from vials containing only scintillation fluid. Specific activity was expressed as disintegrations per minute (disints min^{-1}), corrected for quenching.

The fractional turnover rate of tritium (k_{HTO}) over the 24 h was calculated using the following equation:

$$k_{HTO} = (\ln C_i - \ln C_f)/t \quad (1)$$

(Nagy, 1975), where C_i is the specific activity in the initial sample (disints min^{-1} μg^{-1}), C_f is the specific activity in the final sample (disints min^{-1} μg^{-1}) and t is the time between the initial and final samples (1 day).

Total body water (*TBW*) (g) was calculated using the following equation:

$$TBW = (M_i/C_i) \times (C_{inj} - C_i), \quad (2)$$

where M_i is the mass of the injectate (g) and C_{inj} is the activity of the injectate solution (disints min^{-1} μg^{-1}). The activity of the injectate solution was calculated from the mean of five dilution experiments in which a weighed amount of HTO was added to a known mass of water, and samples of the solution were counted. The activity of the original injectate solution was calculated to be 757,834 disints min^{-1} μg^{-1}.

To calculate the water turnover in the females (ml H_2O day^{-1}), the fractional turnover rate (k_{HTO}) was multiplied by the total body water (*TBW*). We assumed that 25 % of the water leaving the body was fractionated (Speakman, 1997). We applied a fractionation factor for tritium of 0.9179, assuming a ratio of 3:1 for the equilibrium and kinetic fractionation factors (0.9222 and 0.905, respectively; from Speakman, 1997). One datum was removed from the analysis because the female died during the experiment.

Evaporative water loss was determined by placing individual lactating females (N=10) in a small chamber (308 cm^3) with a continuous through-flow of air for 1 h. Air leaving the chamber was dried (silica gel). The increase in mass of the silica gel was an estimate of the evaporative water loss after correcting for the water content of the air by running the system without a mouse in the chamber for 1 h.

Total daily urine and faecal production were measured in five lactating mice. Each female and her litter were placed in

a metabolic chamber for 24 h with food and water. After 24 h, urine was collected and weighed (accurate to 0.0001 g; Ohaus Analytical Plus balance), dried to constant mass (60 °C, Gallenkamp oven) and reweighed. The estimate of urinary water loss was corrected for evaporation from the sides of the chamber. The water content of fresh faeces was measured by observing mice and collecting faeces within 5 s of them being produced. These were then weighed and dried (as above), and the water content was measured. This water content together with the daily production of faeces (dried from the metabolic chamber) were used to calculate daily faecal water loss. The mean total water loss by urinary, faecal and evaporative loss was 30.6±0.8 ml day^{-1} (mean ± S.E.M.). This was subtracted from the water turnover (estimated from tritiated water) to estimate the water diverted to milk production. From the analysis of the composition of milk, 1 g of water was equivalent to 1.72 g milk. This value was used to convert water in milk production values into total milk production.

Milk quality

Ten of the lactating females used to measure milk production were separated from their pups for a period of approximately 3 h on day 15 of lactation. After this separation, which was not long enough to affect milk production (Bateman, 1957; König et al., 1988), the females were injected with 0.25 ml of oxytocin to stimulate milk let-down. The teats were manually palpated, and the milk was collected in capillaries. Each teat that was milked was emptied as far as possible because it has been shown that the fat content is atypically low in the first part of the milk extracted (Oftedal, 1984). A total of 0.5 ml of milk was collected from each mouse and analysed for water content, fat F, lactose L and protein P content (Rowett Research Institute Analytical Services, Aberdeen, UK). The gross energy content E (kcal g^{-1}; 1 cal=4.184 J) of the milk was estimated from its composition using the formula developed by Perrin (Perrin, 1958) (cited in Derrickson et al., 1996):

$$E = 9.11F + 5.86P + 3.95L\,. \quad (3)$$

The units for fat, protein and sugar are grams per gram of whole milk.

Statistical analyses

Means are quoted ± the standard error of the mean (S.E.M.). Repeated-measures analysis of variance (ANOVA) was used to determine the significance of changes in body mass and food intake over time. Least-squares regression analysis was used to examine relationships between maternal food intake, litter size and litter mass. Multiple regression was used to examine the effects of maternal mass, increase in maternal mass and litter size on asymptotic food intake. Direct comparisons of the litter sizes of natural and manipulated females over the same period were made using two-sample t-tests. The significance level for all the above tests was 0.05. All statistical analyses were performed using commercially available software (Minitab versions 7.3 and 11; Ryan et al., 1985).

Results

Maternal body mass

Prior to breeding, there was no significant difference between the mass of the control female mice (27.1±0.25 g) and that of the manipulated mice (27.5±0.48 g) (P=0.48). Only 19 of the control females were measured during pregnancy. Body mass increased significantly during pregnancy in both control ($F_{13,252}$=436.06, P<0.001) and manipulated ($F_{13,350}$=696.15, P<0.001) females, reaching a peak of 58.7±1.49 g, and 57.1±1.20 g for control and manipulated females, respectively, just prior to parturition (Fig. 1A). The maternal body mass of the control mice increased significantly over the first 12 days of lactation ($F_{11,840}$=275.2, P<0.001) from 37.8±0.30 g on the day after parturition to 44.0±0.77 g on day 11. After day 12, there was no further increase in body mass (P=0.077), the animals weighing on average 43.8±0.04 g. During lactation, the pattern of change in maternal body mass of the manipulated females mirrored almost exactly the pattern observed in the control females, increasing significantly ($F_{17,648}$=161.28, P<0.001) from 38.3±0.53 g on the day after parturition to

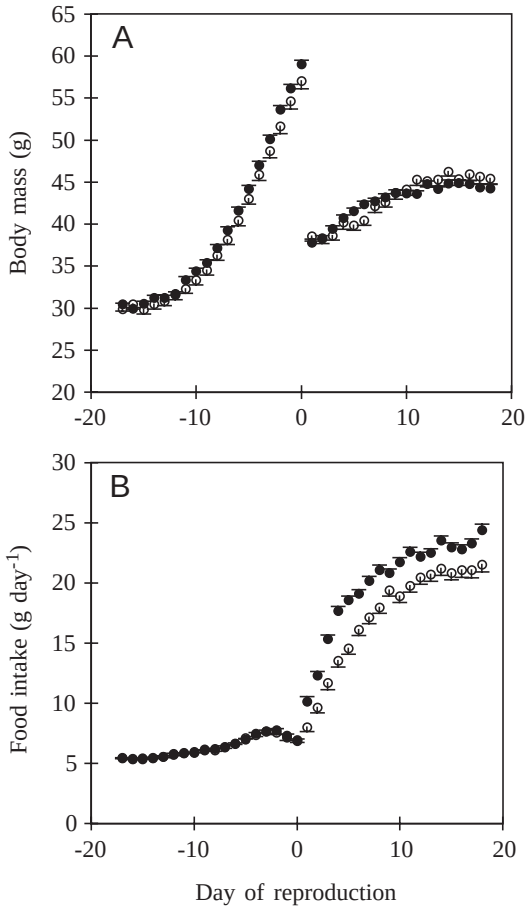

Fig. 1. (A) Mean female body mass and (B) mean daily food intake of female mice throughout pregnancy and lactation in mice raising natural litters (filled symbols, N=71) and manipulated litters (open symbols, N=37). Error bars represent ±1 S.E.M. Parturition is day 0.

44.3±0.67 g on day 10 of lactation, after which time there was no further change (P=0.077).

Food intake

The food intakes of the control and manipulated females were not significantly different prior to breeding (P=0.150) or during pregnancy (P=0.540), so only the data for the control females are presented for these periods. Prior to breeding, the control mice ate a mean of 5.2±0.09 g day^{-1}. This increased significantly during pregnancy ($F_{13,252}$=28.71, P<0.001), reaching a maximum of 7.7±0.23 g on day 16 of pregnancy, before decreasing over the next 2 days to 6.9±0.30 g on the day before parturition (Fig. 1B).

Food intake increased significantly during the first 13 days of lactation in the control females ($F_{12,910}$=276.04, P<0.001) from 9.7±0.34 g on the day after parturition to 22.4±0.34 g on day 12. Over the next 4 days (days 13–16), daily food intake remained stable (P=0.263) at an average of 23.1±0.36 g day^{-1} (gross intake 369.5 kJ day^{-1}). We termed the food intake averaged over these 4 days the asymptotic daily food intake. The food intake of the manipulated females also increased significantly ($F_{10,396}$=196.9, P<0.001) from 8.1±0.39 g on the day after parturition to 19.6±0.55 g on day 10 of lactation, thereafter reaching an asymptote until the end of lactation (P=0.608). The asymptotic daily food intake, calculated over the same period as the control mice (days 13–16), was 20.8±0.59 g (343.2 kJ gross energy intake equivalent to 273.3 kJ assimilated intake), which was significantly lower than that of the control mice (t=–3.71, d.f.=105, P=0.0005).

There was a significant positive relationship between asymptotic daily food intake I of both the control (r^2=0.097, $F_{1,69}$=7.44, P=0.008) and manipulated (r^2=0.205, $F_{1,35}$=9.00, P=0.005) females and maternal body mass M in late lactation. Daily food intake increased above the asymptotic level on day 18 of lactation (Fig. 1B), probably because the pups started to feed directly on the food. On average, the asymptotic daily food intake of the control females at peak lactation was 4.5 times the food intake prior to breeding. The resting metabolic rate of the adult mice at peak lactation was equivalent to 47.05 kJ day^{-1} (Johnson et al., 2001) and, hence, at the maximum, sustained gross energy intake (=asymptotic daily food intake multiplied by the energy content of the food) was 8.0×RMR.

Larger litters in the control group were associated with significantly increased asymptotic daily food intake ($F_{10,60}$=2.42, P=0.017) when litters had fewer than seven pups, but when litters had between seven and 13 pups there was no correlation. The asymptotic daily food intake of mice with 14 pups (26.0±0.70 g) was significantly higher than for those with 13 pups (23.5±0.53 g) ($F_{1,14}$=5.79, P=0.029). The asymptotic daily food intake (24.1 g) of the single female that raised a natural litter of 15 pups was not significantly different from the intakes of mothers raising either 13 (P=0.79) or 14 (P=0.288) pups. The asymptotic mean intake between litters of 9 and 15 was 22.8 g day^{-1}.

On average, the nature of our manipulations meant that there was an over-representation of small and large litters in the manipulated data set (Fig. 2). Asymptotic food intake was positively related to litter size for manipulated litters of between three and six pups ($F_{11,25}$=5.31, P<0.001), but for larger litters there was no further increase in daily food intake with increasing litter size (P=0.381). The difference in mean asymptotic daily food intake between the manipulated and control mice (Fig. 1B) therefore reflected in part the over-representation of smaller litters in the manipulated data set. However, this did not explain the entire effect. To establish how the manipulated mice altered their food intake in response to the manipulation, we calculated their expected daily food intake if they had raised the litter to which they had given birth (from the relationship established in the control mice). The difference between their expected and observed intake was calculated and compared with the extent of the manipulation. For those females that were given more pups, there was no significant correlation between the number of pups added and the difference between expected and observed asymptotic daily food intake (P=0.729) (Fig. 3). These mice ate significantly less food than expected from the number of pups they gave birth to (t=2.81, d.f.=14, P=0.014), despite the fact their litters were increased. This effect also contributed to the overall lower mean asymptotic daily food intakes of the manipulated individuals. For females that had had the size of their litters reduced, there was a significant positive relationship between the number of pups that had been removed and the difference between actual and expected intake (r^2=0.171, $F_{1,21}$=4.32, P=0.05) (Fig. 3). The more pups that were removed from the litter, the less the females ate relative to the amount expected from their litter size at birth. The mothers appeared to be able to downregulate their asymptotic food intake in response to reduction in their litter sizes, but were unable to increase it in response to enlargement of their litters.

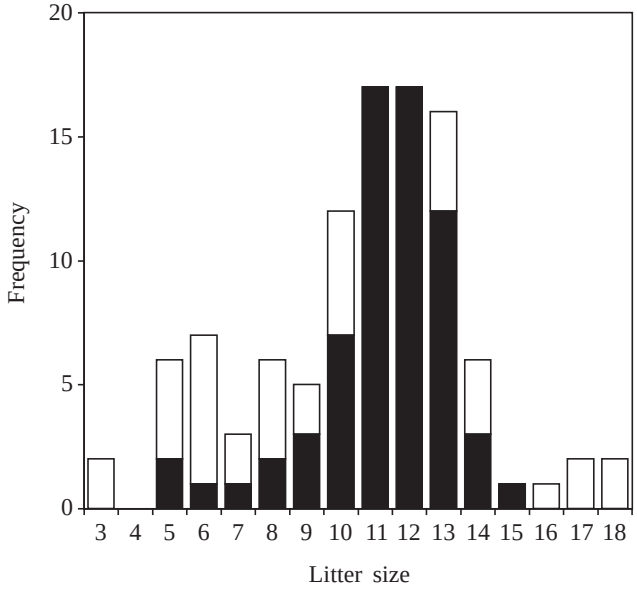

Fig. 2. Histogram of the litter sizes at weaning (day 18) of natural (filled columns) and manipulated (open columns) litters.

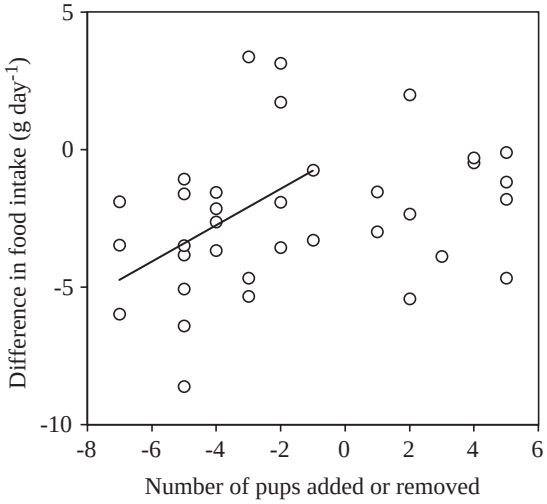

Fig. 3. The difference (observed minus expected) in food intake of mice raising manipulated litters in relation to the numbers of pups added to or subtracted from the natural litter sizes. The line represents the relationship fitted to the data for subtractions (y=0.66x–0.12). There was no significant relationship for additions.

Maternal asymptotic daily food intake per pup was negatively related to the number of pups raised in both the control (r^2=0.739, $F_{1,69}$=195.12, P<0.001) and manipulated (r^2=0.911, $F_{1,35}$=359.07, P<0.001) groups (Fig. 4).

Apparent assimilation efficiency

In non-breeding females, the mean apparent assimilation efficiency was 81.0±0.57 % and, despite the enormous increase in food intake (Fig. 1B), this did not change significantly during lactation (P=0.77), when it averaged 80.8±0.35 %. Gross energy content of the faeces did not differ between non-breeding and lactating females (P=0.140) and averaged 16.9±0.04 and 16.6±0.20 kJ g^{-1} dry mass for non-breeding and lactating females, respectively. The energy content of the faeces was significantly lower than that of the food (18.36 kJ g^{-1} dry mass) for both lactating and non-lactating females (t=11.25, P<0.001 and t=13.67, P<0.001, respectively). Within the lactating females, there was no significant correlation between litter size and gross energy content of the faeces (P=0.237). For every dry gram of food ingested by the females, they assimilated 15.17 kJ (=13.42 kJ g^{-1} wet mass). Hence, although the gross intake at peak lactation averaged 369.5 kJ day^{-1}, the assimilated energy averaged only 310.2 kJ day^{-1}, which was 6.6 times the RMR at peak lactation.

Litter size and mass

The control mice gave birth to an average of 11.7±0.26 pups (range 5–15) and weaned an average of 11.3±0.24 pups (range 5–15) (Fig. 2). The manipulated litters ranged from 3 to 19 pups at birth and from 3 to 18 pups at weaning (Fig. 2). Litter mass in the control group increased from 17.8±0.36 g at birth to 86.7±1.41 g at weaning. The mean mass of individual pups increased from 1.7±0.14 to 7.9±0.17 g over the same time period. Pups from larger litters were significantly smaller than pups from smaller litters in both the control (r^2=0.596, $F_{1,69}$=101.7, P<0.001) and manipulated (r^2=0.773,

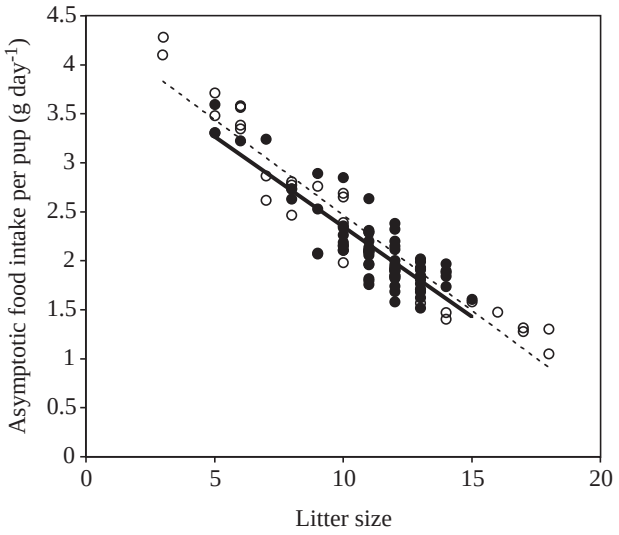

Fig. 4. Maternal asymptotic daily food intake per pup (food intake averaged over days 13–16 of lactation) in relation to litter size in females raising natural (filled symbols) and manipulated (open symbols) litters. Each point is one litter. The relationship is described by y=–0.18x+4.18 for the control females and by y=–0.19x+4.41 for those with manipulated litters.

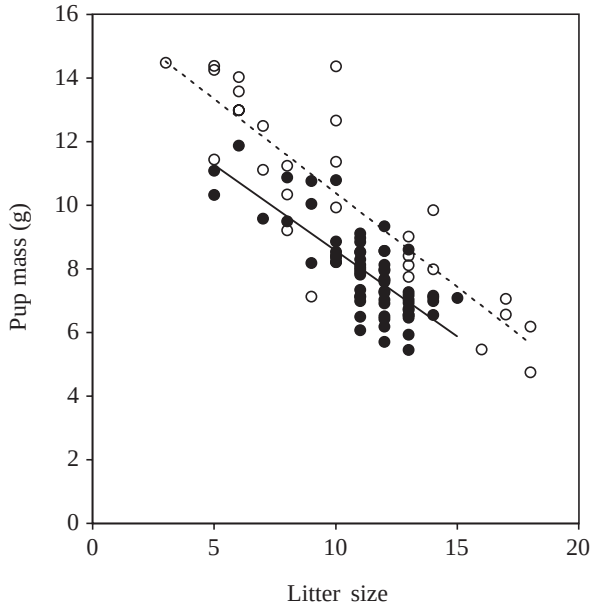

Fig. 5. Mean individual pup mass as a function of litter size in natural (filled symbols) and manipulated (open symbols) litters. The least-squares regression lines for the two sets of data are also shown. The relationships are described by the equations: y=–0.54x+13.97 for the natural, control litters and y=–0.59x +16.3 for the manipulated litters.

$F_{1,35}$=119.16, P<0.001) groups (Fig. 5). For every increase of one pup in manipulated litter sizes at weaning, there was a decrease in mean pup mass of 0.55 g. The same pattern was observed in the control litters, but the trends were not identical. For comparable litter sizes (5–15 pups), the pups from the manipulated litters were significantly heavier than those from the same-sized control litters (ANOVA $F_{1,90}$=35.02, P<0.001) (Fig. 5).

Energy expenditure of the litters

Heavier litters had a greater daily energy expenditure (DEE) estimated by extrapolation from respirometry (r^2=0.455, $F_{1,19}$=15.88, P=0.001) (Fig. 6A). The DEE of a 90 g litter was 82.8 % higher than the DEE of a 60 g litter. The relationship with litter size, however, failed to reach significance (P=0.211). The absence of a relationship with litter size was probably due to the large variation in litter mass at any given litter size. Even though the litters were only away from their mothers for a period of 1 h, they had a significantly lower growth rate than litters that were not measured ($F_{1,85}$=29.14, P<0.001). We therefore used the equation relating DEE with litter mass to predict the DEE of the litters of the control females on day 13 (between nine and 15 pups) and combined this predicted energy expenditure for respiration with the calculated energy devoted to growth between days 13 and 14 to estimate the total energy requirement (TER) of the litters. Both predicted DEE and TER were significantly positively related to litter size during late lactation (for predicted DEE, r^2=0.161, $F_{1,63}$=12.08, P=0.001, Fig. 6B; for TER, r^2=0.061, $F_{1,63}$=4.11, P=0.047, Fig. 6C). As the litter size increased from nine to 15 pups, the predicted total energy requirement of the litters increased from 103 to 121 kJ day^{-1}.

Milk production

There was a significant positive curvilinear relationship between maternal milk production and litter mass (r^2=0.56, $F_{1,18}$=13.54, P=0.002) (Fig. 7A), but not between milk production and litter size (P=0.106) (Fig. 7B). Milk production and maternal body mass were not significantly correlated (P=0.719).

Milk quality

Milk in late lactation comprised on average 26.4±0.95 % fat, 11.3±0.35 % crude protein, 1.7±0.09 % lactose and 59.1±1.39 % water. Females raising larger litters (mass) produced milk with a significantly lower fat content (r^2=0.741, $F_{1,8}$=22.94, P=0.001) (Fig. 8A), but the relationship between

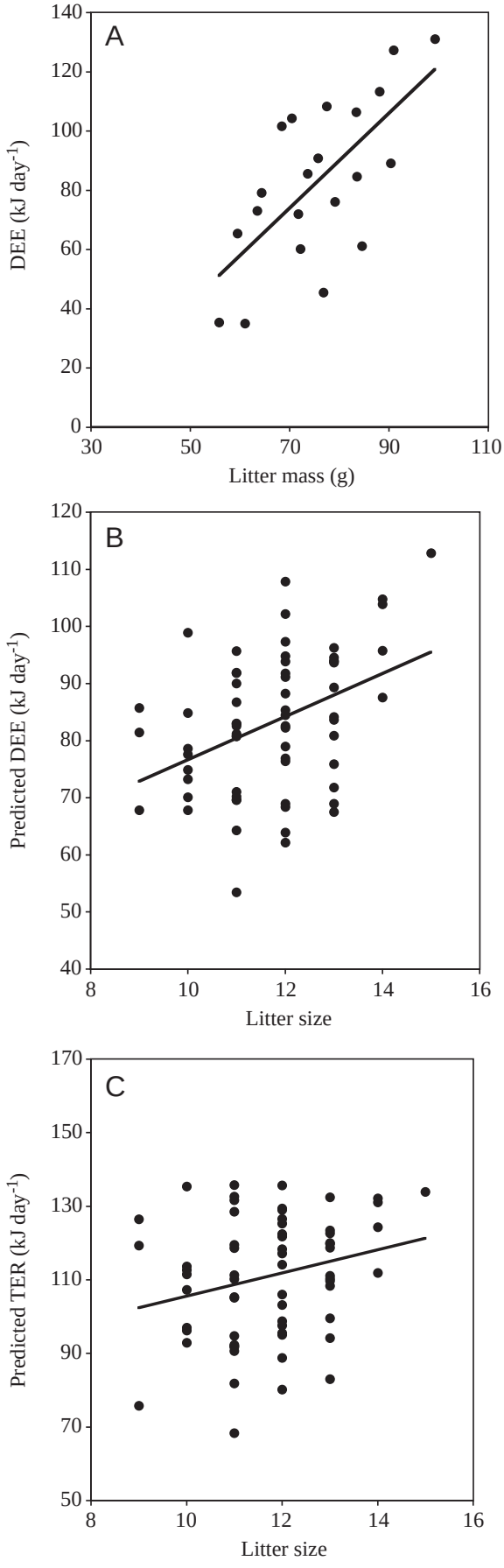

Fig. 6. (A) Daily energy expenditure (DEE) of mouse litters as a function of litter mass. The least-squares relationship explained 45.5 % of the variation in DEE and was described by y=1.6x –38.0. (B) Predicted DEE and (C) predicted total energy requirement (TER) for natural litters in late lactation. The relationships between predicted DEE (B) and predicted TER (C) and litter size were described by y=3.77x+38.9 and y=3.15x +74.03 respectively.

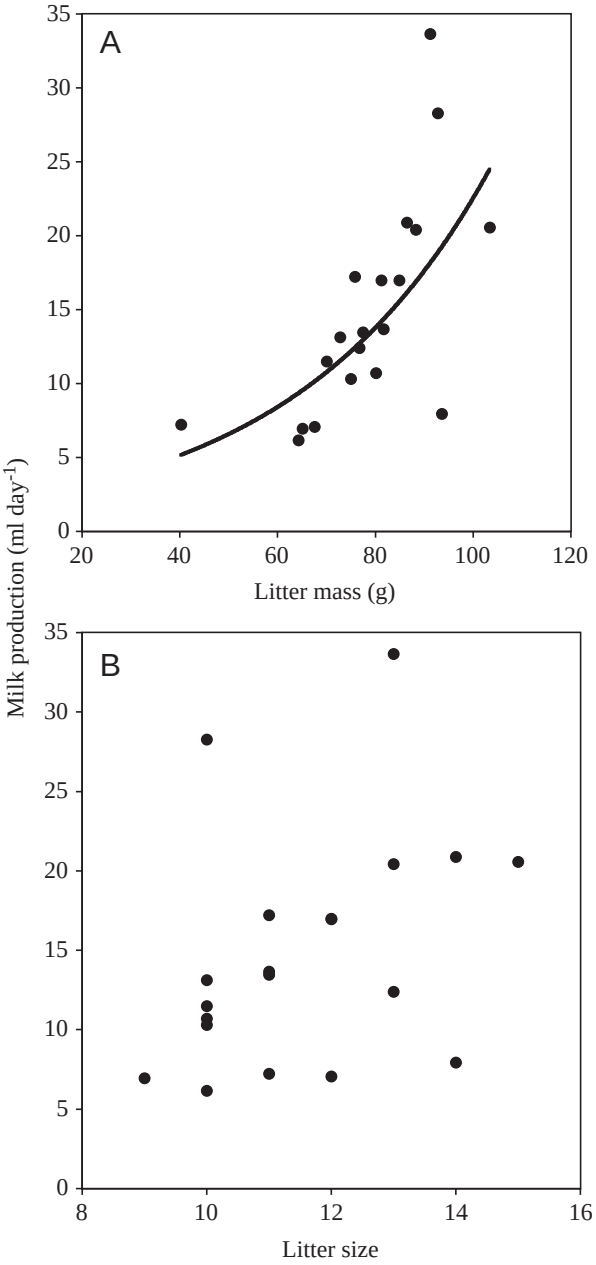

Fig. 7. Maternal milk production in relation to (A) litter mass and (B) litter size. The line represents the best-fit curvilinear relationship between milk production and litter mass, described by $y=1.91e^{0.025x}$.

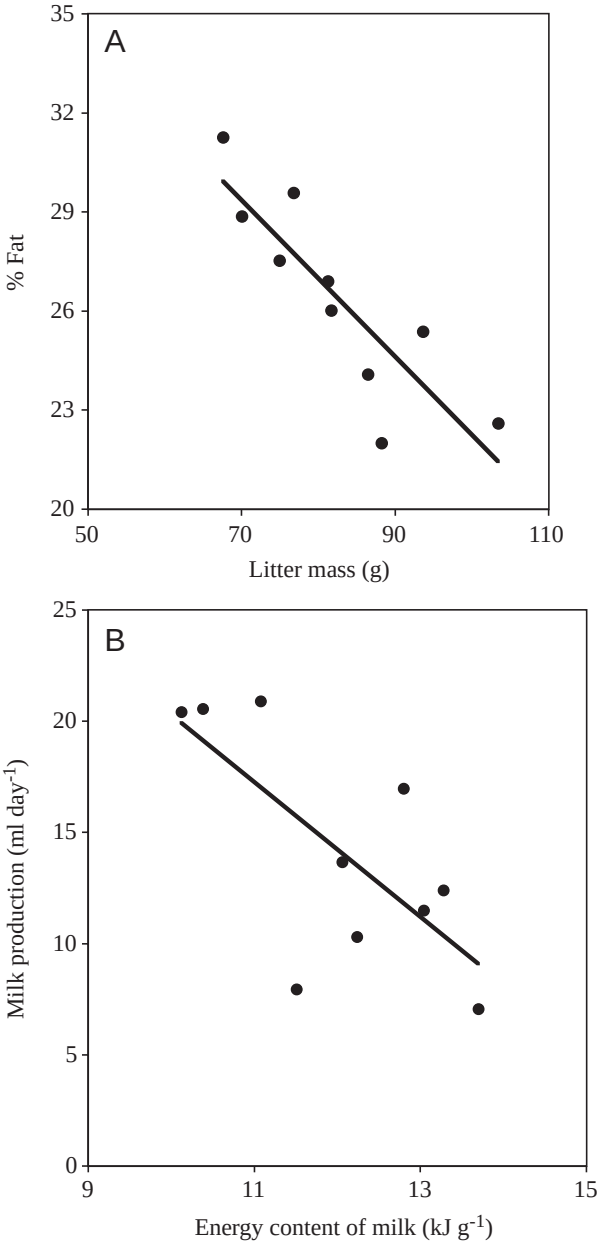

Fig. 8. (A) Relationship between litter mass and the percentage of fat in the milk. (B) Energy content of milk in relation to the volume of milk produced. Lines in both cases are the least-squares regressions and are described by (A) $y=-0.24x+45.95$ and (B) $y=-3.03x+50.59$.

fat content and litter size marginally failed to reach significance (P=0.089). The other components of the milk did not vary significantly with differences in the litter mass or litter size: crude protein (P=0.098 for litter mass and P=0.227 for litter size), lactose (P=0.073 for litter mass and P=0.669 for litter size) and total dry matter (P=0.938 for litter mass and P=0.410 for litter size).

Females that produced large volumes of milk also produced milk that had a lower energy content (kJ g^{-1}) (r^2=0.503, $F_{1,8}$=8.09, P=0.021: Fig. 8B). Total milk energy output, calculated as the product of the energy content of the milk and the volume produced, was not significantly related to either litter size (P=0.316) or litter mass (P=0.217) and averaged 164.6 kJ day^{-1}. The energy exported as milk accounted for on average 43.9±3.73 % of the gross energy obtained from food intake by the mother. On average, the estimated total energy requirement of the litter (TER) was 63±6.03 % of the energy exported as milk. Since the TER increased significantly with increases in litter size, but energy transfer in milk was independent of litter size, this percentage was significantly positively related to litter size.

Discussion

During lactation, the body mass of female MF1 mice either increased or was constant, indicating that they were probably in energy balance and not withdrawing body stores. The asymptotic daily food intake was much higher in this study than the maximum intake recorded for lactating Swiss Webster mice (Hammond and Diamond, 1992; Hammond and Diamond, 1994). The unmanipulated MF1 mice averaged a food intake of 23.1 g day^{-1} at peak lactation across all litter sizes, which was 21 % higher than the maximum intake of Swiss Webster mice (19 g day^{-1}) raising the largest litter size (14 pups). This appears to be a consequence of the different strains used. MF1 mice were larger and raised larger litters than Swiss Webster mice. Contrary to previous studies (Hammond and Diamond, 1992; Hammond and Diamond, 1994; Speakman and McQueenie, 1996), the mice in the present study did not continue to increase their food intake until the end of lactation, but instead reached a plateau after the first 13 days. The energy demand of the pups was probably still increasing at this time because they were still increasing in body mass, but there was no corresponding increase in maternal food intake, indicating that it might have been limited. This putative limit occurred at 4.5 times the pre-breeding intake and was approximately 8.0 times the RMR during late lactation. This multiple is substantially higher than the suggested limitations on sustained metabolic rate (SusMR) at around 4×RMR (Drent and Daan, 1980) or 7×RMR (Peterson et al., 1990; Hammond and Diamond, 1997), although if the assimilated energy is considered rather than the gross intake the multiple drops to 6.6×RMR, which is much closer to the limit postulated by Hammond and Diamond (Hammond and Diamond, 1997). However, because the mice were exporting approximately 44 % of their gross energy intake as milk, their actual daily energy expenditures were substantially lower and averaged only 3.1×RMR, which is close to the limit postulated by Drent and Daan (Drent and Daan, 1980). These differences highlight the potential confusion that arises by equating food energy intake with energy expenditure when there is substantial energy export (see also discussions in Speakman and McQueenie, 1996; Speakman, 2000). The present data indicate that a limit may exist in sustained energy intake at around 6.6–8.0×RMR depending on whether gross or assimilated energy is considered. This may provide an upper bound for expenditure, but actual expenditure in our mice was substantially lower and did not exceed the limit of 4×RMR proposed by Drent and Daan (Drent and Daan, 1980).

König et al. (König et al., 1988) found that milk production in BALB-c mice, *Mus musculus*, increased at the start of lactation and then reached a maximum between days 9 and 16, at the same time that we observed a plateau in food intake. Asymptotic daily food intake in the present study was related to the number of pups that were raised up to a litter size of seven, after which there was no further increase in food intake with increasing litter size up to 15 in the unmanipulated litters and up to 18 offspring in the manipulated litters. In contrast, Speakman and McQueenie (Speakman and McQueenie, 1996) found no such limit. However, the absence of an asymptote in this latter study was due to one mouse with a litter size of 13 that ate approximately 40 g day^{-1} in late lactation. This was possibly a mouse that ground its food into the bedding, but was not identified as such in the previous study. Removing this point brings the asymptotic daily food intake down to a value very similar to that observed in the present study.

The apparent asymptote in daily food intake combined with increasing demands of the offspring resulted in the mean mass of pups decreasing with increasing litter size (Fig. 5). It is possible that there is a minimum size that a pup must be to survive after weaning which, in combination with the limit on food intake, might place an upper limit on the maximum number of pups raised. There was some evidence supporting the notion that there is a minimum viable pup size since the pups raised in manipulated litters of 13–18 were not significantly smaller than the pups from natural litters of 10–15 and were heavier than the mass that might be anticipated by extrapolation of the curve relating mean pup mass to litter size in the unmanipulated mice (Fig. 5). However, there was no evidence to support the suggestion that limits on sustainable energy intake might set a limit on the maximum number of such minimally sized offspring that a female could raise, since the manipulated females managed successfully to raise these enlarged litters eating slightly, but significantly, less food at peak lactation than their unmanipulated counterparts.

There are several possible reasons why females do not successfully raise more than 15 pups in their first litters, despite being physically capable of successfully raising at least 18 pups. The most likely explanations, however, relate to the impact that performance during the first lactation has on subsequent reproductive events. If these mice are selected to maximise lifetime reproductive output, then we might not necessarily expect them to maximise performance in early breeding attempts if this was detrimental to their subsequent attempts and to overall productivity.

Bateman (Bateman, 1957) calculated an index of regulation (R) that indicates the extent to which females regulate their energy input to the pups:

$$R = S(W - w)/W(S - s) \quad (4),$$

where S is the pup mass in large litters, s is the pup mass in small litters, W is the litter mass in large litters and w is the litter mass in small litters. If R=0, then there is no regulation and the mass of litters will be the same regardless of how many pups they contain. If R=1, then there is complete regulation and the individual mass of the pups will be the same regardless of the size of the litter. In the present study, R=0.69 for litters between five and 15, which indicates incomplete regulation, in which the females are investing more to large litters but not sufficient for the pups to be of the same size. This supports the idea that a limit in the system restricts the level of maternal investment. That a limit is present was reinforced by the results from the manipulated litters. Females given more pups to raise did not increase their food intake to accommodate this

increased demand. Taking pups away from females resulted in a decrease in their food intake. The more pups that were removed, the greater the decrease. Hence, it appeared that the mice were able to downregulate their food intake, but not to upregulate it. This was also the case in mice of small and large litters made to suckle each other's litters towards the end of lactation (Bateman, 1957). The females that originally had large litters rapidly decreased their milk production to the level of small litters, but the mice with small litters failed to increase their milk production when made to suckle a large litter (Bateman, 1957). Although females with reduced litters downregulated their asymptotic daily food intake in late lactation to match that of mothers raising natural litters of the same size, the pups they produced were larger than those in natural litters of the same size (Fig. 5). This was probably because it took some time for females to adjust to their smaller litters and, during this phase of adjustment, the small litters were being nourished at the level appropriate for the much larger litters to which the female had given birth.

Although the volume of milk produced was greater in females with heavier litters (Fig. 7A), there was a reduction in energy content with increasing volume (Fig. 8B). Across the range of 9–15 pups, the energy provided by the females in milk was not significantly different. Therefore, in addition to there being no increase in maternal food intake across this range of litter size, there was also no increase in milk energy output for the litters. This failure of females to increase production for large litters has been shown previously in mice *Mus musculus* (Knight et al., 1986; König et al., 1988), rats *Rattus norvegicus* (Russell, 1980; Fiorotto et al., 1991), cotton rats *Sigmodon hispidus* (Rogowitz, 1996; Rogowitz, 1998) and dogs *Canis familiaris* (Meyer et al., 1985). The calculated total daily energy requirement of the litters (expenditure and growth) averaged 63 % of the calculated energy supplied to them in milk. This discrepancy reflects the fact that our measurements of respiratory energy expenditure extrapolated up to 24 h to yield DEE did not include the costs of activity, and that the milk energy is not completely digested. Since the TER increased in larger litters, but energy transfer in milk to the larger litters was constant, this suggests that larger litters were more efficient in their use of milk energy. The nature of this altered efficiency remains unclear but, given the mean disparity between the input and the estimated requirements, which excluded activity, there was substantial scope for variations in activity between litters of different sizes that would contribute to the efficiency difference.

Although this study indicates that a limit is present in late lactation, it is not clear exactly where this limit is imposed. When females raised large litters, they did not increase their food intake in late lactation to match the increased demand, which suggests that the gut could be limiting. This is supported by the observed asymptote in food intake towards the end of lactation even though the TER of the pups was still increasing at this time. However, the asymptotic food intake could be a consequence of the mammary tissue being limited in its capacity to produce milk, and females may therefore have adjusted their food intake to match milk energy production. Milk output by the mother did not increase with increasing litter size, but rather larger litters received approximately the same as smaller litters. The females appeared to be capable of altering the energy content of the milk by changing its composition, yet when raising more pups they did not increase the energy content.

There are two possible explanations for this failure to adjust the energy content of milk when raising larger litters. The first is that the mammary glands were working at their limit and, although the females appeared to be capable of altering the energy content, they may have been operating at their maximum capacity and were only able to alter the total energy content by decreasing the water content. A second possibility is that the mammary glands were not working at their limit, but were responding to the suckling stimulus received from the pups. Female mice have 10 teats and, assuming that whenever the litter is suckling all the teats are occupied, then the females would receive the same stimulus from a litter of 10 as they would from a litter of 15. This also assumes that litters differing in size were also suckling for the same total duration, but this was not measured. Instead of being limited by the capacity of the mammary glands themselves, the mothers may be limited by the action of hormones such as prolactin and growth hormone. These are released from the anterior pituitary in response to the suckling stimulus and act by stimulating milk synthesis in the mammary glands (Mepham, 1976; Flint and Gardner, 1994; Shand et al., 1995; Travers et al., 1996).

In summary, lactating MF1 mice reached a plateau in food intake at around 23.1 g day^{-1} between days 13 and 16 and with litter sizes of 9–15. When litter sizes were manipulated, females receiving fewer pups decreased their food intake; however, when pups were added, there was no increase in maternal food intake. Females with larger litters produced more milk but of lower energy content and, thus, milk energy output was not related to litter size.

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