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LIMITS TO SUSTAINED ENERGY INTAKE

III. EFFECTS OF CONCURRENT PREGNANCY AND LACTATION IN *MUS MUSCULUS*

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Summary

To determine whether mice were limited in their capacity to absorb energy during late lactation, we attempted to increase the energy burden experienced by a group of female mice during late lactation by mating them at the *postpartum* oestrus, hence combining the energy demands of pregnancy and lactation. These experimental mice were therefore concurrently pregnant and lactating in their first lactation, and were followed through a normal second lactation. In a control group, females also underwent two lactations but sequentially, with the second mating after the first litter had been weaned. Maternal mass and food intake were measured throughout the first lactation, second pregnancy and second lactation. Maternal resting metabolic rate (RMR) was measured prior to the first mating and then at the peak of both the first and second lactations. Litter size and litter mass were also measured throughout both lactations. In the first lactation, experimental mice had a lower mass-independent RMR ($F_{1,88}$=5.15, P=0.026) and raised significantly heavier pups (t=2.77, d.f.=32, P=0.0093) than the control mice. Experimental mice delayed implantation at the start of the second pregnancy. The extent of the delay was positively related to litter size during the first lactation ($F_{1,19}$=4.58, P=0.046) and negatively related to mean pup mass ($F_{1,19}$=5.78, P=0.027) in the first lactation. In the second lactation, the experimental mice gave birth to more (t=2.75, d.f.=38, P=0.0092) and lighter (t=–5.01, d.f.=38, P<0.0001) pups than did the controls in their second lactation. Maternal asymptotic daily food intake of control mice in the second lactation was significantly higher (t=–4.39, d.f.=37, P=0.0001) than that of the experimental mice and higher than that of controls during their first lactation. Despite the added burden on the experimental females during their first lactation, there was no increase in their food intake, which suggested that they might be limited by their capacity to absorb energy. However, control females appeared to be capable of increasing their asymptotic food intake beyond the supposed limits estimated previously, suggesting that the previously established limit was not a fixed central limitation on food intake. As RMR increased in parallel with the increase in food intake during the second lactation of control mice, the sustained energy intake remained at around 7.0×RMR.

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

Introduction

Late lactation is the most energetically demanding time for a female mammal (Millar, 1977; Gittleman and Thompson, 1988; Kenagy et al., 1990; Forsum et al., 1992; Thompson, 1992; Speakman and McQueenie, 1996). Sustained energy intake during late lactation has been suggested to be limited (Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond and Diamond, 1997; Hammond et al., 1996; Speakman and McQueenie, 1996) at around seven times resting metabolic rate (RMR) (Kirkwood, 1983; Peterson et al., 1990; Hammond and Diamond, 1997), which has consequences for the size and mass of litters produced. As litter size increases, there is a decrease in the mean mass of individual pups (Meyer et al., 1985; König et al., 1988; Hammond and Diamond, 1992; Kam and Degen, 1994; Rogowitz, 1996; Rogowitz, 1998), indicating that the female may be unable to supply larger litters with more energy. The MF1 strain of mouse has been shown to reach a plateau of food intake during late lactation and over litter sizes of 9–15 pups (Johnson et al., 2001a), suggesting that the females are not able to supply sufficient energy for pups of larger litters to wean at the same size as those from smaller litters, possibly because of limits in their capacity to ingest more food or to supply more milk.

Many rodents have a *postpartum* oestrus (Bateman, 1957; Asdell, 1964) and can therefore conceive on the day after parturition, but thereafter not until the litter is weaned. These animals become pregnant whilst lactating. An advantage of mating during a *postpartum* oestrus is that there is a shorter inter-

litter interval, resulting in more litters being produced during a short breeding season (Roy and Wynne-Edwards, 1995).

Pregnancy also involves a significant increase in expenditure above non-reproductive levels (Gittleman and Thompson, 1988; Garton et al., 1994; Speakman and McQueenie, 1996). Combining the demands of pregnancy and lactation would provide a natural opportunity to examine the limits to reproductive output under increased energy burdens. If the concurrently pregnant and lactating females increase their food intake during late lactation, it is unlikely that they will be limited centrally at the gut.

The relative durations of pregnancy and lactation are important in determining the extent of the increase in the energy burden in concurrently pregnant and lactating females. If the duration of pregnancy is long compared with the duration of lactation, then lactation will overlap only with the initial phase of pregnancy, which involves little, if any, increase in energy requirements above non-reproductive levels (Bateman, 1957; Gittleman and Thompson, 1988; Forsum et al., 1992; Speakman and McQueenie, 1996). However, if the durations of pregnancy and lactation are similar, then the peak demand of pregnancy will overlap with the peak demand of lactation, thus increasing the energy burden experienced. Laboratory mice have very similar gestation and lactation lengths (19 and 18 days, respectively) and are therefore likely to be under an increased energy burden during late lactation.

Previous studies of concurrent pregnancy and lactation have examined the effects on the mother and the offspring in the first lactation and on the number of offspring born in the second pregnancy, but have generally not followed the second lactation through until weaning (*Mus musculus*, Bateman, 1957; Norris and Adams, 1981; Knight and McLelland, 1988; *Rattus norvegicus*, Woodside et al., 1981; Woodside et al., 1987; Gilbert et al., 1983; Leon and Woodside, 1983; Oswald and McClure, 1987; *Homo sapiens*, Merchant et al., 1990; *Sigmodon hispidus* and *Neotoma floridana*, Oswald and McClure, 1990; *Phodopus campbelli*, Roy and Wynne-Edwards, 1995). These studies have all found that there are no detrimental effects of being concurrently pregnant to the suckling litter in terms of the number of pups raised or the mean mass of the pups.

One of the most comprehensive studies examined concurrent pregnancy and lactation in cotton rats *Sigmodon hispidus* and wood rats *Neotoma floridana* (Oswald and McClure, 1990). The two species differed in the relative lengths of pregnancy and gestation, cotton rats having a 26–27 day gestation and a 12 day lactation and wood rats a 33–34 day gestation and a 24 day lactation. There was no difference between concurrently pregnant and lactating cotton rats and those solely lactating in any aspect of the first litter or in the number born in the second. There was also no difference between the two groups of wood rats in the first litter, but concurrently pregnant and lactating wood rats delayed implantation in the second pregnancy and gave birth to significantly fewer pups in the second litter.

In some species, the duration of the second pregnancy has been found to be longer than the first gestation: Wistar rats *Rattus norvegicus* (Woodside et al., 1981; Woodside et al., 1987; Gilbert et al., 1983; Oswald and McClure, 1987), laboratory mice *Mus musculus*, (Bateman, 1957; Norris and Adams, 1981) and Djungarian hamsters *Phodopus campbelli* (Roy and Wynne-Edwards, 1995) when concurrently pregnant and lactating. Delayed implantation (Bateman, 1957; Asdell, 1964) is presumed to be the cause of the extended gestation because the extension can be prevented by injections of oestradiol (Roy and Wynne-Edwards, 1995).

In the present study, we examined the effects of concurrent pregnancy and lactation on the energy budgets of laboratory mice *Mus musculus* L. As a consequence of the similar gestation and lactation lengths following a *postpartum* mating, females would potentially encounter an increased energy burden, particularly at peak lactation. We aimed to determine how the females coped with this potentially elevated demand by quantifying the body mass and food intake of the mothers and both the number and masses of the suckling and gestating pups in both the first and second lactations. By comparing the resting metabolic rate (RMR) of females prior to breeding and at peak lactation, we aimed to determine the extent of the increase in RMR of concurrently pregnant and lactating females compared with lactating females.

Materials and methods

Animals and housing

Virgin female white mice (outbred strain MF1), 9–10 weeks old, were housed individually in cages (44 cm×12 cm×13 cm) with sawdust and paper bedding. Food [Rodent chow: CRM(P), 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. An experimental group of 24 mice were paired with males, which remained with them throughout pregnancy until 5 days after parturition to ensure a *postpartum* mating. The maternal food intake and body mass and the number and mass of the litters in the first lactation were compared with values from a sub-set of 19 mice that were part of a control group of 71 mice undergoing lactation only (Johnson et al., 2001a). These 19 control mice were mated for a second time 1–2 weeks after weaning. For these repeated matings, females were paired with a male for 6 days, after which the male was removed. The second litters of the control mice provided a comparison for the second litters of the experimental mice, 21 of which had a second litter.

Body mass and food intake

Maternal body mass and food intake were measured (Sartorius top-pan balance, accurate to 0.01 g) daily (between 09:00 h and 11:00 h) throughout the first lactation, the second pregnancy (post-weaning in experimental females) and the second lactation. Food intake was determined by the amount of food eaten from the hopper each day. The bedding was checked daily for uneaten food, which was also weighed. In a separate experiment, only 1.7±0.41 % (mean ± S.E.M.) of the food missing

from the hopper was found in the bedding (Johnson et al., 2001a). Following parturition, the number of pups and the mass of the litters were also recorded daily in both lactations.

Resting metabolic rate (RMR)

Resting metabolic rate (RMR) was quantified as oxygen consumption, using an open-flow respirometry system (as described previously; Hayes et al., 1992; Speakman and McQueenie, 1996). Air was pumped (Charles Austin Pumps Ltd) through a sealed Perspex chamber within a constant-temperature incubator (INL-401N-010, Gallenkamp) set at 30 °C (within the thermoneutral zone: Speakman and Rossi, 1999). A flow rate of 500–700 ml min^{-1} was metered using an Alexander Wright flowmeter (DM3A) upstream of the chamber. A sample of air (approximately 150 ml) in the excurrent stream was dried (silica gel) and directed through a paramagnetic oxygen analyser (Servomex 1100A) (as described previously; Johnson et al., 2001a). We did not absorb CO_2 in the outflow stream prior to gas analysis as this minimised error in the conversion of oxygen consumption to energy expenditure when respiratory quotient is unknown (Koteja, 1996; Speakman, 2000). Each female was measured three times: prior to breeding, at the peak of the first lactation (day 18) and at the peak of the second lactation (day 18).

Statistical analyses

Repeated-measures analysis of variance (ANOVA) was used to detect changes in body mass and food intake through lactation. Two-sample *t*-tests were used to compare control and experimental results. Paired *t*-tests were employed to detect potential increases in RMR between pre-breeding and peak lactation. To compare body masses and food intakes on each day of lactation between the groups, two-sample *t*-tests were used, and the sequential Bonferroni technique was used to correct the *P* value for multiple comparisons (see Rice, 1989). The *P* values were ranked from most to least significant. The smallest (most significant) *P* value was then compared with the significance level divided by the number of comparisons (e.g. 0.05 divided by 18 becomes 0.0028). If the highest ranked *P* value was less than this value, the comparison was said to be significant at the 0.05 level (as the standard Bonferroni correction). The second *P* value was then compared with 0.05/(*n*–1), the third with 0.05/(*n*–2), etc., until the result was not significant. At this point, all remaining *P* values were considered non-significant. The significance level applied in 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). Values are presented as means ± S.E.M.

Results

First lactation

Body mass

Prior to breeding for the first time, the experimental females weighed on average 30.1±0.6 g (mean ± S.E.M.). For these experimental mice, maternal body mass increased significantly through the first lactation ($F_{17,360}$=90.07, P<0.001) from a mean of 39.8±0.9 g on the day after the first parturition to a mean of 52.4±1.4 g at the end of the first lactation. Prior to breeding, the experimental females were significantly heavier (on average by 2.2 g) than the control females (*t*=4.58, d.f.=21, *P*=0.0001), which weighed on average 27.9±0.2 g. Between days 0 and 13 of the first lactation, there was no difference in body mass between the experimental and control groups of mice (Table 1); however, on days 14–17, the experimental mice were significantly heavier than the control mice (Table 1). Over these days, there was no further increase in mass of the control mice, but the mass of the experimental females continued to increase because of the developing foetuses of the concurrently gestating litter.

Table 1. *Mean body mass of the concurrently pregnant and lactating mice (*N=*24) and the control lactating mice (*N=*19) on each day of the first lactation*

Day	Experimental (g)	Control (g)	*t*	*P*
0	39.8±0.9	37.8±0.3	2.16	0.0410
1	40.0±0.9	38.2±0.3	2.00	0.0570
2	40.5±0.9	39.3±0.3	1.22	0.2300
3	41.7±0.9	40.5±0.4	1.19	0.2500
4	42.6±1.0	41.4±0.4	1.14	0.2700
5	43.1±0.9	42.1±0.4	0.98	0.3400
6	43.5±0.9	42.7±0.4	0.82	0.4200
7	44.7±0.9	43.0±0.4	1.76	0.0900
8	44.6±0.9	43.4±0.4	1.13	0.2700
9	45.5±1.0	43.5±0.4	1.89	0.0700
10	45.9±1.0	43.8±0.4	2.01	0.0550
11	46.7±1.0	44.2±0.4	2.27	0.0320
12	47.2±1.1	44.2±0.4	2.62	0.0150
13	47.8±1.1	44.5±0.4	2.95	0.0066
14	49.3±1.2	44.9±0.4	3.61	0.0014*
15	50.3±1.3	44.7±0.4	4.16	0.0003*
16	51.3±1.4	44.6±0.4	4.70	0.0001*
17	52.4±1.4	44.2±0.4	5.58	<0.0001*

Values are means ± S.E.M.

The results from two-sample *t*-tests are also shown for each day.

Because of the large number of comparisons made, we applied the sequential Bonferroni correction to the significance level. Significant values at the 95 % confidence level are represented by an asterisk.

Food intake

There were no data for the food intake of the experimental mice over the first 5 days of lactation because males were still in the cages with the females. After this, there was a significant increase in food intake through the first lactation ($F_{11,240}$=6.8, P<0.001) from 19.4±0.7 g on day 6 to a maximum of 22.9±0.9 g on day 13 (Fig. 1A). The food intake of the experimental mice was not significantly different from that of the control mice on any day throughout the first lactation (Table 2).

However, the asymptotic daily food intake (mean daily

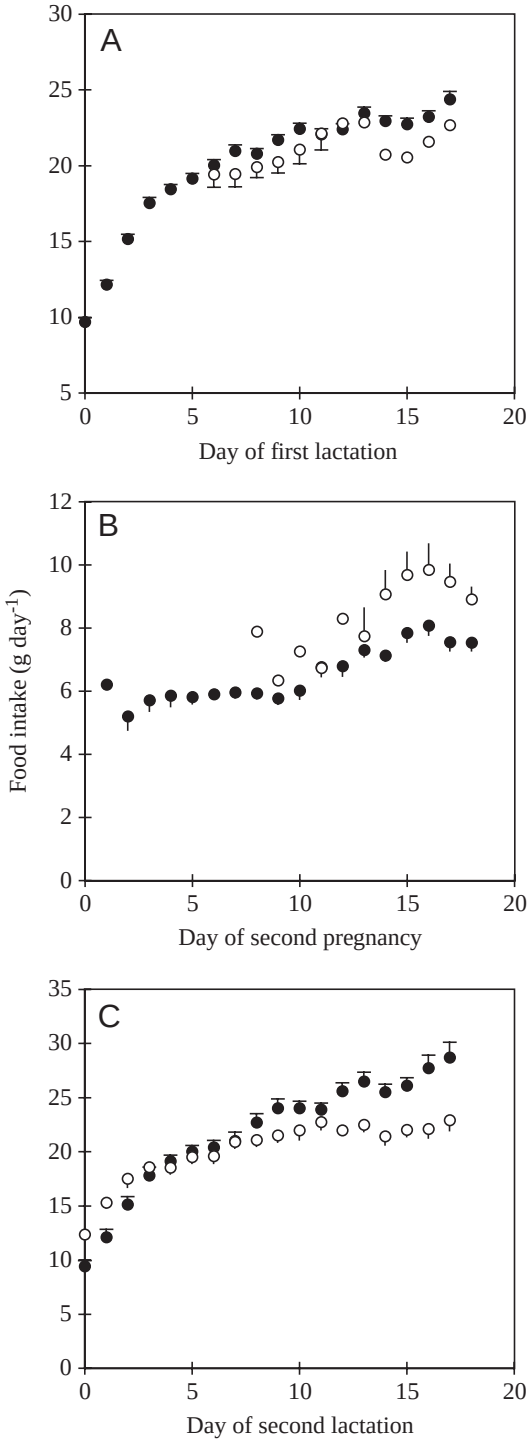

Fig. 1. Mean daily food intake of the control (filled circles) and experimental (open circles) mice throughout the first lactation (A), second pregnancy (B) and second lactation (C). In the experimental animals, the second pregnancy occurred concurrent with the first lactation; in the control animals, the second pregnancy occurred after completion of the first lactation. Values are means ± S.E.M. *N*=19 for the controls and *N*=24 for the experimental mice in A, and *N*=21 for experimental females in C. In B, the sample size for the experimental mice varied over time depending on when pregnancy was initiated relative to when the male was removed. Sample sizes for sequential points are 1, 1, 1, 3, 5, 8, 11, 16, 19, 21 and 21.

Table 2. *Mean food intake of the concurrently pregnant and lactating mice (*N*=24) and the control lactating mice (*N*=19) on each day of the first lactation*

Day	Experimental (g)	Control (g)	*t*	*P*
0	–	9.7±0.3	–	–
1	–	12.1±0.3	–	–
2	–	15.2±0.3	–	–
3	–	17.5±0.4	–	–
4	–	18.4±0.3	–	–
5	–	19.1±0.3	–	–
6	19.4±0.7	20.0±0.3	–0.81	0.4300
7	19.4±0.8	21.0±0.4	–1.76	0.0880
8	19.9±0.7	20.8±0.3	–1.10	0.2800
9	20.2±0.9	21.7±0.3	–1.55	0.1300
10	21.0±0.8	22.4±0.4	–1.56	0.1300
11	22.1±0.7	22.1±0.4	0.09	0.9300
12	22.8±0.9	22.4±0.4	0.44	0.6700
13	22.9±0.9	23.5±0.4	–0.65	0.5200
14	20.7±0.7	22.9±0.4	–2.78	0.0092
15	20.5±0.7	22.7±0.4	–2.60	0.0140
16	21.6±0.9	23.2±0.4	–1.65	0.1100
17	22.7±1.1	24.4±0.5	–1.41	0.1700

Values are means ± S.E.M.

Results from two-sample *t*-tests are also shown for each day.

Because of the large number of comparisons made, we applied the sequential Bonferroni correction to the significance level. There were no significant values at the 95 % confidence level.

intake over days 13–16, when there was no further increase in food intake) was 21.4±0.7 g in the experimental mice (equivalent to a gross energy intake of 347.7 kJ day^{-1} and an assimilated energy intake of 287.2 kJ day^{-1}), which was significantly lower (t=–2.22, d.f.=27, P=0.035) than the asymptotic food intake of the control mice (mean 23.1±0.4 g). At the peak of lactation, concurrently pregnant and lactating mice ate 7 % less than mice that were only lactating (Fig. 1A).

Litter size and mass

The experimental females gave birth to an average of 11.2±0.5 pups and weaned 10.5±0.5 pups in their first lactation. These litter sizes were not significantly different from those of the control mice at the first parturition (P=0.46) or at weaning (P=0.14) of their first lactation. The mean mass of the experimental first litters increased from 16.7±0.7 g at birth to 90.4±2.8 g at peak lactation. Mean pup mass in experimental first litters increased from 1.5±0.02 to 8.8±0.3 g over the same period (Fig. 2A). Litter masses of the experimental mice were not significantly different from those of the litters of control mice at birth (P=0.14) or at peak lactation (P=0.30). The mean masses of the pups of experimental and control mice in their first lactation were also not significantly different at birth (P=0.97); however, at peak lactation, the mean mass of the experimental pups was 11 % greater than that of the control pups (t=2.77, d.f.=32, P=0.0093) (Fig. 2A). Hence, although they ate significantly

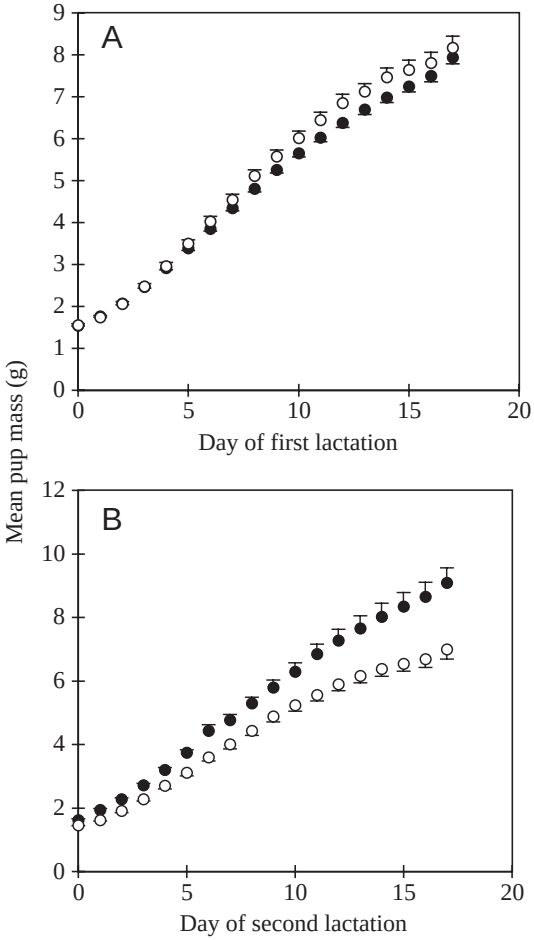

Fig. 2. Comparison of the mean pup mass of the litters of the control females (filled circles, *N*=19) and the experimental females (open circles, *N*=24 for lactation 1 and *N*=21 for lactation 2) during the first (A) and second (B) lactation. Values are means ± S.E.M.

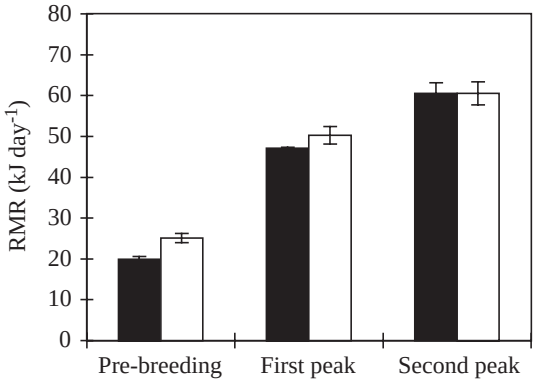

Fig. 3. Mean maternal resting metabolic rate (RMR, kJ day^{-1}) prior to breeding, at the peak of the first lactation and at the peak of the second lactation for the control (filled columns, *N*=19) and experimental (open columns, *N*=21) females. Values are means ± S.E.M.

less food at peak lactation and were also concurrently pregnant, the experimental mice did not reduce their litter size and produced significantly heavier pups in the first lactation.

Resting metabolic rate (RMR)

The mean RMR of the experimental females increased from 25.06±1.1 kJ day^{-1} prior to breeding to 50.25±2.2 kJ day^{-1} at the peak of the first lactation (Fig. 3). The mean RMR of the experimental mice was not significantly different from that of the control mice, either prior to breeding (t=1.67, d.f.=40, P=0.10) or at peak lactation (t=1.19, d.f.=40, P=0.24). When the difference in body mass of the two groups was accounted for in the analysis, RMR at the peak of the first lactation was significantly positively related to mass ($F_{1,88}$=18.00, P<0.001) and was significantly lower in the experimental mice than in the control mice ($F_{1,88}$=5.15, P=0.026) (Fig. 4). There was also a significant interaction between the effects of body mass and treatment group ($F_{1,88}$=6.5, P=0.013) (Fig. 4). The RMR at the peak of the first lactation was 50.25 kJ day^{-1} and, combined with a gross energy intake of 347.7 kJ day^{-1} and assimilated energy intake of 287.2 kJ day^{-1}, resulted in a sustained energy intake at peak lactation in the experimental mice equivalent to 6.8×RMR if gross energy intake was considered or 5.7×RMR if assimilated energy intake was used in the calculation. Repeating this for the control mice, with an RMR of 47.05 kJ day^{-1} (Johnson et al., 2001b) and gross and assimilated energy intakes of 369.5 kJ day^{-1} and 310.2 kJ day^{-1}, respectively, resulted in sustained energy intakes of 8.0×RMR and 6.6×RMR.

Second pregnancy

Because of the overlap between the first lactation and the second pregnancy in the experimental mice, we have no data on food intake for the entire duration of the second pregnancy. Different females overlapped by different amounts. Between days 8 and 11, we have results from only one female, but by

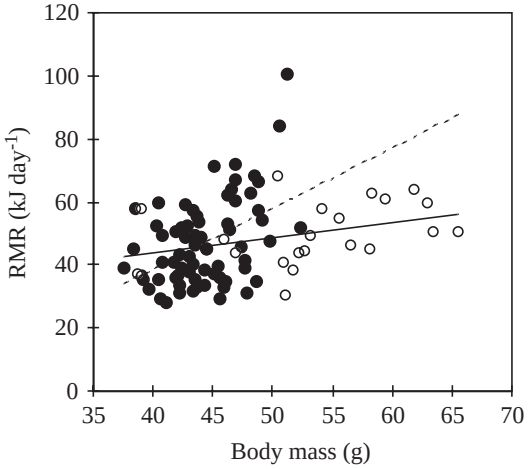

Fig. 4. Relationship between maternal resting metabolic rate (RMR) and body mass at the peak of the first lactation in the control (filled circles) and experimental (open circles) females. The relationships are described by y=1.92x–38.0 and y=0.48x+24.4 for the control and experimental females, respectively.

day 17 we have data from all the experimental females that became pregnant a second time (N=21). The sample sizes at each time point are given in the legend to Fig. 1.

Body mass

The body mass of the experimental females increased during the second pregnancy to 65.6±1.4 g (N=21) on the day before parturition. The control females increased their mean mass to a maximum of 66.5±1.4 g (N=19) on the day before the second parturition.

Food intake

Food intake of the experimental mice increased over the second pregnancy to a maximum of 9.8±0.4 g (N=21) on day 16, before decreasing over the next 2 days to 8.9±0.6 g (N=21) (Fig. 1B). The food intake of the control mice increased to a maximum of 8.1±0.3 g (N=19) on day 16, before decreasing to a mean of 7.5±0.3 g on the day before parturition (Fig. 1B).

Duration of the second pregnancy

The control mice had a normal gestation length of 19 days, but the experimental mice had second gestations that lasted between 21 and 30 days (Fig. 5), having delayed implantation for between 2 and 11 days. The extent of the delay in implantation was significantly positively related to the number of pups weaned in the first lactation (r^2=0.194, $F_{1,19}$=4.58, P=0.046) (Fig. 6A) and negatively related to the mean mass of the pups at weaning in the first lactation (r^2=0.233, $F_{1,19}$=5.78, P=0.027) (Fig. 6B). Experimental mice raising more pups delayed implantation of the concurrent litter longer than those raising fewer pups.

With reference to the second lactation of the experimental mice, the delay in implantation was not significantly related to the number of pups subsequently born in the second litter (P=0.96), the mass of these litters (P=0.994) or the mean mass of the pups (P=0.774). However, there was a significant negative relationship between the extent of the delay and the asymptotic daily food intake at the peak of the second lactation (r^2=0.213, $F_{1,19}$=5.13, P=0.035) (Fig. 6C). Females that delayed implantation for longer ate significantly less at the

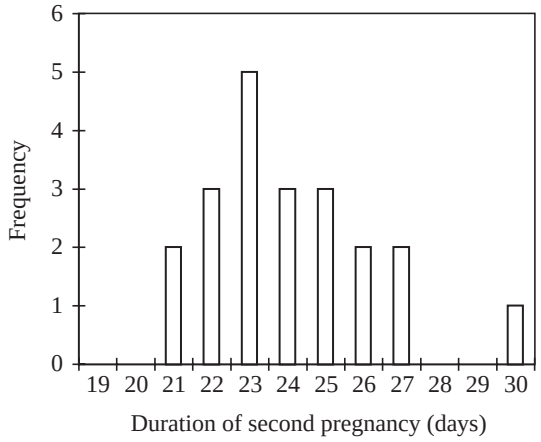

Fig. 5. Frequency distribution of the length of the second pregnancy of the experimental (concurrently pregnant and lactating) females. The normal gestation length of females that go through pregnancy when not lactating is 19 days.

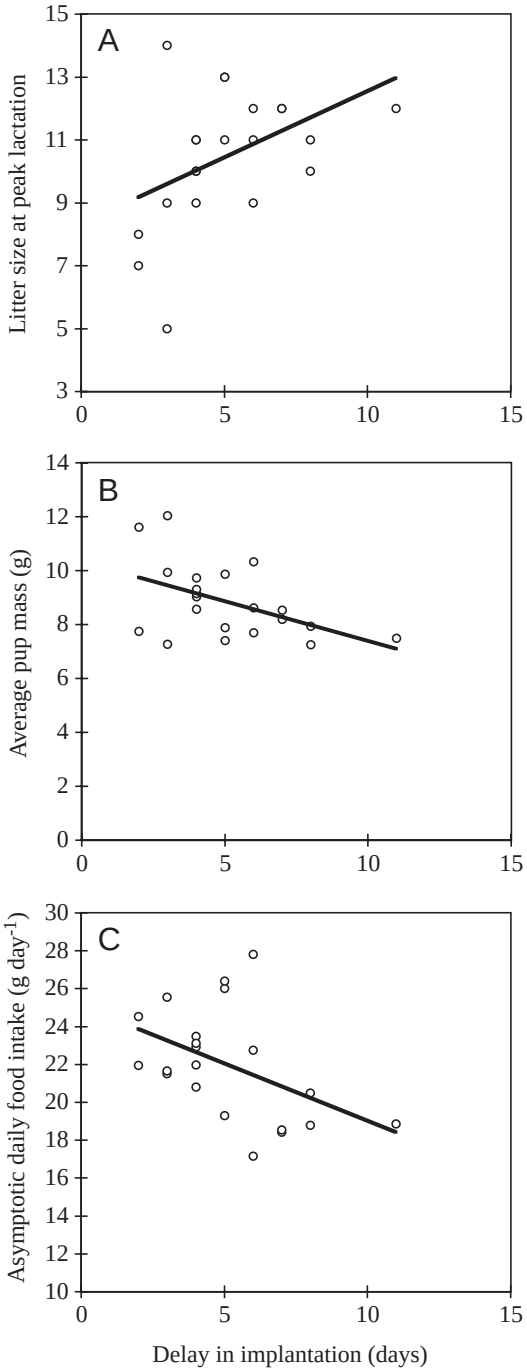

Fig. 6. Relationship between the delay in implantation at the start of the second pregnancy in the experimental females and (A) the litter size at the peak of the first lactation (y=0.42x+8.3), (B) the mean pup mass at the end of the first lactation (y=–0.3x+10.3) and (C) the asymptotic daily food intake at the peak of the subsequent (second) lactation (y=–0.6x+25.1).

peak of the second lactation than those that only delayed implantation for a short time.

Second lactation

Body mass

There was a significant increase in body mass of the experimental mice during the second lactation ($F_{17,360}$=35.57, P<0.001) from a mean of 42.5±0.9 g (N=21) on the day after parturition to a maximum mean mass of 48.6±0.9 g (N=21) on day 16. The body mass of the experimental females reached an asymptote between days 11 and 17, during which time there was no significant change in body mass (P=0.382).

There was also a significant increase in maternal body mass of the control females ($F_{17,324}$=66.51, P<0.001) during the second lactation from 45.4±0.9 g (N=19) on day 0 to a maximum of 52.8±1.1 g on day 16 of the second lactation. An asymptote was reached between days 12 and 17, during which time there was no further significant change in body mass (P=0.08). Throughout the second lactation, there was no significant difference in body mass between the two groups except on day 15, when the control mice (mean 52.9±1.1 g) were significantly heavier than the experimental mice (mean 48.0±1.0 g; Table 3).

Food intake

The food intake of the experimental mice changed significantly over the second lactation ($F_{17,359}$=36.06, P<0.001), increasing from a mean of 12.4±0.4 g (N=21) on day 0 to a maximum of 22.9±1.0 g (N=21) on day 17 (Fig. 1C). Food intake reached an asymptote between days 7 and 17, over which time there was no further significant increase (P=0.093).

There was also a significant increase in the food intake of the control mice over the second lactation ($F_{17,312}$=93.36, P<0.001) from a mean of 9.4±0.5 g (N=19) on the day after parturition to a maximum of 28.7±1.4 g on day 17 (Fig. 1C). Food intake of the control mice also reached an asymptote, but between days 12 and 16 (P=0.105). During the second lactation, the control females ate significantly more than the experimental females on days 12–16 (Table 4). There was a significant difference in the asymptotic food intake (days 13–16) (t=–4.39, d.f.=37, P=0.0001), with the control females eating on average 26.1±0.7 g (equivalent to 424.1 kJ day^{-1} gross energy intake and 350.26 kJ day^{-1} assimilated intake), which was 4.1 g or 18.6 % more than the experimental females, which ate 22.0±0.6 g (N=21) (equivalent to 357.5 kJ day^{-1} gross intake and 295.24 kJ day^{-1} assimilated intake). There was no difference in the asymptotic food intake of the experimental mice between their first and second lactations (2.8 %) (P=0.62), whereas the control mice ate significantly more (on average 13 %) during their second lactation (paired t=–4.01, d.f.=18, P=0.0001) than during their first.

Litter size and mass

The experimental females (N=21) gave birth to an average

Table 3. *Results of two-sample* t*-tests comparing the body mass of the concurrently pregnant and lactating females (experimental* N*=21) and that of the control lactating females (*N*=19) in the second lactation*

Day	Experimental (g)	Control (g)	t	P
0	42.5±0.9	45.4±0.9	–2.46	0.0190
1	44.1±0.9	45.9±0.9	–1.45	0.1500
2	45.1±0.9	46.0±0.9	–0.77	0.4500
3	45.7±0.9	46.8±0.8	–0.83	0.4100
4	46.4±1.0	47.9±0.9	–1.16	0.2500
5	46.5±1.0	48.4±0.9	–1.45	0.1500
6	46.4±1.0	48.5±0.7	–1.63	0.1100
7	47.1±1.0	49.7±1.0	–1.83	0.0760
8	47.3±1.1	50.4±0.9	–2.23	0.0320
9	47.0±1.1	50.5±0.9	–2.49	0.0180
10	47.3±1.1	51.1±1.0	–2.5	0.0170
11	47.9±1.1	51.5±1.1	–2.21	0.0330
12	48.6±1.1	51.7±1.1	–1.94	0.0590
13	48.6±1.1	52.2±1.0	–2.54	0.0150
14	48.5±1.0	52.9±1.0	–3.13	0.0034
15	48.0±1.0	52.9±1.1	–3.25	0.0025*
16	48.6±0.9	52.8±1.1	–2.91	0.0062
17	48.0±1.0	52.4±1.1	–3.01	0.0047

Daily means ± S.E.M. are also shown.

The sequential Bonferroni correction was applied to the significance level, and significant values are represented by an asterisk.

Table 4. *Two-sample* t*-tests comparing the food intake of the concurrently pregnant and lactating females (experimental* N*=21) with that of the control lactating females (*N*=19) in the second lactation*

Day	Experimental (g)	Control (g)	t	P
0	12.4±0.4	9.4±0.5	4.29	0.0001*
1	15.3±0.5	12.1±0.7	3.56	0.0011*
2	17.5±0.8	15.1±0.7	2.17	0.0370
3	18.6±0.6	17.8±0.8	0.83	0.4100
4	18.5±0.6	19.1±0.6	–0.75	0.4600
5	19.5±0.6	20.0±0.6	–0.63	0.5300
6	19.6±0.7	20.4±0.6	–0.84	0.4100
7	20.9±0.6	21.0±0.8	–0.10	0.9200
8	21.1±0.6	22.7±0.8	–1.65	0.1100
9	21.5±0.6	24.0±0.9	–2.34	0.0250
10	22.0±0.9	24.0±0.6	–1.81	0.0780
11	22.8±0.7	23.9±0.6	–1.26	0.2200
12	22.0±0.5	25.6±0.8	–4.07	0.0003*
13	22.5±0.7	26.5±0.9	–3.77	0.0006*
14	21.4±0.8	25.5±0.7	–3.80	0.0005*
15	22.0±0.7	26.1±0.7	–4.13	0.0002*
16	22.1±0.9	27.7±1.1	–3.49	0.0018*
17	22.9±1.0	28.7±1.4	–2.86	0.0110

Daily means ± S.E.M. are also shown.

The sequential Bonferroni correction was applied to the significance level, and significant values are represented by an asterisk.

of 14.8±0.7 pups in their second lactation (range 8–20) and weaned an average of 12.7±0.5 pups (range 8–16). The mass of the litters increased from a mean of 21.3±0.9 g at birth to 93.4±3.0 g at peak lactation, and mean pup mass increased from 1.5±0.4 to 7.5±0.4 g over the same period (Fig. 2B).

The control females (N=19) gave birth to a mean of 11.9±0.8 pups in the second lactation (range 5–17) and raised 11.4±0.7 pups (range 5–16). Litter mass increased from a mean of 20.3±1.2 g at birth to 102.9±3.4 g at peak lactation. Mean pup mass increased from 1.7±0.1 g at birth to 9.5±0.5 g over the same time (Fig. 2B). Pups of control females were significantly heavier than pups raised during the first lactation by an average of 1.4 g (18 %) despite coming from litters of the same mean size.

The experimental females gave birth to significantly more pups (t=2.75, d.f.=38, P=0.0092) in their second lactation than the control females; however, by peak lactation, there was no significant difference between the two groups (P=0.15). The mean litter mass at birth did not differ significantly between the two groups (P=0.56), but at peak lactation the control litters were 10.2 % heavier than the experimental litters (t=–2.04, d.f.=38, P=0.048). The mean mass of the experimental pups was 12 % less than that of the control pups at birth (t=–5.01, d.f.=38, P<0.0001) and 21 % less at peak lactation (t=–3.24, d.f.=38, P=0.0029). The mean mass of the pups at peak lactation was significantly related to litter size at peak lactation ($F_{1,29}$=11.26, P<0.001) and was also significantly different between the control and experimental mice ($F_{1,29}$=10.96, P=0.002).

Resting metabolic rate (RMR)

The RMR of the experimental females was 60.48±2.8 kJ day^{-1} and that of the control females at the peak of the second lactation was 60.48±2.6 kJ day^{-1} (Fig. 3). There was no significant difference between the two groups (P=0.49). There was a 17 % increase in RMR between the first and second lactations in the experimental mice ($F_{1,86}$=5.71, P=0.019) and a 40 % increase in the control mice ($F_{1,39}$=10.64, P=0.002). The RMR of the control mice at the peak of their second lactation was equivalent to a daily energy expenditure of 60.5 kJ day^{-1}. Although the asymptotic food intake at the peak of the second lactation exceeded the asymptotic daily food intake at the peak of the first lactation, the extent of the increase was lower than the parallel change in RMR. Hence, the ratio of sustained energy intake to RMR declined to 7.0×RMR for gross intake and to 5.8×RMR for assimilated energy intake.

Discussion

We will discuss first the effects of concurrent pregnancy and lactation on the mothers and then the effects on the offspring in the suckling and gestating litters. The body mass of the experimental females did not differ from that of the control females until towards the end of the first lactation, when their mass increased significantly, presumably because of the developing embryos of the concurrently gestating litter. Despite this difference in mass and the increased energy demand on experimental mice, they ate significantly less food than the control mice. In addition, the experimental females had a lower RMR, independent of body mass, at the peak of the first lactation. This is in contrast to previous studies on wood rats *Neotoma floridana* and cotton rats *Sigmodon hispidus*, which increased their mass-specific RMR when concurrently pregnant and lactating above values for lactation alone (Oswald and McClure, 1990). Even though RMR and food intake have been shown to increase during pregnancy (Forsum et al., 1992; Garton et al., 1994), including previous studies of this species (Speakman and McQueenie, 1996), there was no additional intake of food in the experimental mice above that attributable to lactation. Although the increase in food intake during pregnancy was small (2–3 g) compared with the increase during lactation, it was still large enough to be measured had the mice increased their intake at late lactation by this amount. Therefore, it appeared that during concurrent pregnancy and lactation the mice were limited by the capacity of the gut to provide energy for the tissues. Hypertrophy of the gut has been shown to occur in response to prolonged cold-exposure in laboratory mice (*Mus* sp., Toloza et al., 1991; Konarzewski and Diamond, 1994; Hammond and Wunder, 1995) and also between virgin and lactating females of this strain (Speakman and McQueenie, 1996). The mass of the intestine has been shown to increase by as much as 115 % (Speakman and McQueenie, 1996) to supply the increasing demands during lactation.

There are two hypotheses potentially explaining why the experimental mice did not increase their food intake. The first is that the females had reached a limit in the processing and absorption of energy, even after the dramatic increase in gut mass. The second hypothesis concerns the space available for hypertrophy in the abdomen. During concurrent pregnancy and lactation, the abdomen is filled with the developing foetuses, which progressively occupy more space. It is therefore possible that, in this situation (concurrent pregnancy and lactation), hypertrophy of the gut was limited by space. However, as the morphology of the gut was not measured in concurrently pregnant and lactating females, it was not possible to distinguish between these two hypotheses. The females may have been limited centrally by the capacity of the gut and, hence, the experimental females had to bear the burden of both the suckling litter and the developing pups whilst only having the energy intake associated with lactation alone. We might anticipate that this situation would affect the suckling pups, the gestating pups or both. We found no evidence of the experimental mice compromising the suckling pups in favour of the gestating litter and neither have previous studies (Bateman, 1957; Woodside et al., 1987; Merchant et al., 1990; Oswald and McClure, 1990; Roy and Wynne-Edwards, 1995). Indeed, at the end of the first lactation, the pups from the experimental mothers were significantly heavier than those from the control mothers. Rather than withdrawing energy from the suckling pups, the females actually appeared to

provide more for these pups, as measured by their greater mass at weaning. Pups from concurrently pregnant and lactating female Norway rats *Rattus norvegicus* were also found to be heavier than pups from lactating controls (Leon and Woodside, 1983). This suggests that females are more likely to support the present litter over a future one when they are unable to increase their food intake to match the energy demands of both. Female Norway rats have been shown to sacrifice the gestating litter when the pressure was increased by food deprivation (Woodside et al., 1981; Woodside et al. 1987) or in response to injections of oestrogen to stimulate implantation so that the peak demands of pregnancy and lactation coincide (Oswald and McClure, 1987).

Previous studies have found a reduction in the number of pups born in the second litter (Oswald and McClure, 1990), more pups stillborn (Oswald and McClure, 1987) or no difference in the litter sizes (Gilbert et al., 1983; Oswald and McClure, 1990). Although we might have expected the experimental mothers to sacrifice something from the second litter, they gave birth to significantly more pups than the control females in the second lactation. However, as a result of greater mortality, there was no difference in litter size by weaning. As a consequence of the large litter sizes at birth, the pups of the experimental females were significantly lighter at birth and were also lighter at weaning than the pups from the control mice. Because investment by the mothers was not measured, it was not possible to determine whether the experimental pups were lighter at peak lactation as a result of lower investment by the mothers or as a consequence of being smaller at birth. When the mean masses of the pups from the two groups were compared (Fig. 2B), the control pups were always heavier than the experimental pups; however, the magnitude of this difference increased towards the end of lactation, possibly as a result of lower investment by the experimental mothers.

The only significant effects of combining the energy burdens of pregnancy and lactation on the experimental mice were an increased mean pup mass in the pups from the first lactation and a greater litter size in the second lactation. Both these effects were opposite to those anticipated *a priori* under the assumption of limited energy budgets. The absence of negative effects was probably because the mice avoided increased demands by delaying implantation and thus increased the length of the second gestation. By delaying implantation, the females ensured that the peak demand of pregnancy occurred after the suckling litter had been weaned, thereby avoiding the peak demands of pregnancy and lactation occurring simultaneously. The experimental females under the greatest energy demands delayed the start of their second pregnancy for longer (Woodside et al., 1981; Oswald and McClure, 1987). If females were raising small litters and the energy demands were not so high, then they delayed implantation just long enough to ensure that the first litter was weaned before the second was born. Females with larger litters becoming pregnant at a *postpartum* mating appeared to need longer between the weaning of the first litter and the birth of the next.

Experimental females with the shortest gestations were able to increase their food intake during the second lactation above that of those females with longer gestations, but this was still less than the food intake of the control females. The energy burden during the first lactation appears to be reflected in the food intake in the subsequent lactation. Although there were no discernible effects on the litters of the mother being concurrently pregnant and lactating, there appears to be a stress effect on the females, in that they are unable to increase their food intake to a value as high as that of the controls during the second lactation. The food intake of the control mice during the second lactation was significantly elevated relative to the asymptotic level observed during the first lactation (Johnson et al., 2001a). This elevation strongly suggests that the limits observed in the first lactation were not mediated centrally since the mice were subsequently capable of increasing their food intake beyond this supposed limit. However, pups weaned from these second litters were also significantly heavier than pups produced from the first litters (at the same litter sizes). This could point to increases in the efficiency of milk energy utilisation by the pups in the second lactation (see also Kunkele and Kenagy, 1997), but could also reflect increased milk energy production by the females during the second lactation. The change in RMR that paralleled the increased food intake meant that the sustained energy intake remained at around 7.0×RMR, equal to the maximum sustainable limit proposed by Peterson et al. (Peterson et al., 1990) and Hammond and Diamond (Hammond and Diamond, 1997).

In summary, although we anticipated some trade-offs between the investment in the two litters when mice were concurrently pregnant and lactating, we failed to find any. During the first lactation, there was no difference in maternal food intake, RMR, litter size or litter mass between the experimental and control mice. The only differences observed in the first lactation were the increased mean pup mass in the experimental litters and the reduced food intake at peak lactation. However, experimental mice did respond to the increased energy burden by delaying implantation at the start of the second pregnancy. The presence of a delay appears to support the central limitation hypothesis in that the females appear to be unable to meet the costs of both suckling and gestating litters simultaneously. The length of this delay was related to the number of pups suckling and, consequently, the mean mass of these pups, with females of large litters (and hence smaller pups) delaying implantation for longer. Experimental females gave birth to more, smaller pups than control females in the second lactation. By weaning, there was no significant difference between the litter sizes, but the pups from experimental litters remained smaller and experimental mothers ate less than control mothers at the peak of the second lactation.

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