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EFFECTS OF DIETARY PROTEIN INTAKE ON LACTATION PERFORMANCE OF THE LABORATORY MOUSE, *Mus musculus*

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ABSTRACT

Laboratory mice (strain MF1) were used to investigate the effects of dietary protein content on lactation performance to test the heat dissipation limit hypothesis. The specific dynamic action (SDA) for high protein (HP) and high carbohydrate (HC) diets was measured using open-flow respirometry at 9.4% and 6.1%, respectively. The same two diets were fed *ad libitum* to mice during lactation. Mice fed on HP and HC diets at 21 ºC reached a plateau in their daily food intake at 12.3±0.2 g day^{-1} and 16.6±0.2 g day^{-1}, respectively between days 12-17 of lactation. HP-fed mice had a significantly higher daily energy expenditure (DEE) measured by doubly labelled water and higher water turnover than HC-fed mice but the energy they exported as milk was significantly lower than that of HC-fed mice and therefore resulted in poor growth rate of their offspring. The urea production of HP-fed mice from their daily protein intake of 7.1 g was estimated at 1994 mg which required 10.2 mls of water per day to be cleared. The mice increased their urine production by 14.4 mls probably to eliminate this urea. High protein diet had negative effects on lactation, indicating the growth of pups in previous studies was not protein limited. The negative effects of the HP diet were due to the high DEE that greatly reduced the energy available for milk production, rather than a toxicity effect of the urea production. The different DEE of the two diets suggests that other factors were involved in the delivery of energy to the offspring.

KEY WORDS: Laboratory mouse, dietary protein, specific dynamic action, and daily energy expenditure,

INTRODUCTION

The factors that limit the maximal rate of food intake are important because they determine an upper limit to the ability of animals to survive and reproduce (Weiner, 1992; Hammond and Diamond, 1997; Król and Speakman, 2003a, b; Król *et al*., 2007). One model system that has provided a rich avenue for studying the limits to sustained energy intake is the lactation energetics of small mammals (Kenagy *et al*., 1989; Hammond and Diamond, 1994; Hammond *et al*., 1996; Speakman and McQueenie, 1996; Rogowitz, 1998; Johnson and Speakman, 2001c; Johnson *et al*., 2001a, b; Król and Speakman, 2003a). Lactation is the most energetically demanding period encountered by small mammals (Thompson, 1992; Thompson and Nicol, 2002). This physiological state is characterised by a large energy demand for milk production that increases greatly the nutrient needs of the female animal. As a result, small mammals elevate their food intake dramatically during lactation when compared with non-reproductive animals (Flint and Vernon, 1998; Malabu *et al*., 1994; Brogan *et al*., 1999; Denis *et al*., 2003b), but they reach a limit during peak lactation beyond which they appear unable to increase their food intake (Johnson *et al*., 2001a, b, c) and this adversely affects lactation performance. As a result, this issue has attracted the attention of many reproductive physiologists in the past 20 or so years. It has been reported that small mammals lactating at 21 ºC would not elevate their food intake at peak lactation (Perrigo, 1987; Johnson *et al*., 2001a, b, c), whatever the additional demands placed on them because ingesting additional food would make them dangerously hyperthermic (Król and Speakman, 2003a, b). All the previous studies have fed diets high in carbohydrate but the extent to which lactating mice would respond to a diet high in protein is not resolved.

Diets with different macronutrient contents have different specific dynamic action (SDA). Protein has higher SDA than carbohydrate and fat (Kriss *et al*., 1934; Gawecki and Jeszka, 1978; Kagya-Agyemang, 2008). In this situation, it would be predicted that mice fed a high protein diet should be able to consume less food at peak lactation with negative consequences for milk production and growth of their offspring.

The present study aimed to assess the effects of feeding high protein on energy expenditure and lactation performance of the MF1 mouse. This work seeks to throw more light on the limits to protein intake during lactation in mice.

MATERIALS AND METHODS

Animals and experimental protocol

Forty virgin female mice (*Mus musculus* L.: out bred MF1) aged 9-10 weeks old were used in this study (Harlan UK Limited, Shaw's Farm, England). The

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animals were individually housed in cages (44 cm x 12 cm x 13 cm) with sawdust. Rat and mouse breeder and grower diet and water were supplied *ad libitum*. The environment was regulated at 21 ºC (± 1 ºC) on a 12 L: 12 D photoperiod with lights on at 07:00 h. After acclimation to the experimental conditions, baseline measurement of body mass was used to allocate mice into two experimental groups with 20 animals per group. Each female was paired with a male for 11 days. After the males had been removed, female body mass and food remaining in each hopper were weighed each morning between 08:00 h and 10:00 h, using a (Mettler Toledo, Switzerland) top-pan balance (± 0.01 g). Food intake was calculated from the difference between the amount of food provided and that left in the hopper.

On the day of parturition (day 0 of lactation), no measurements were made on the lactating mothers and their pups to avoid any distress. From days 1 to 18 of lactation maternal body mass, litter size, litter mass and pup mortality were recorded. On days 2 to 3 of lactation, mothers from each experimental group were presented with either high protein or high carbohydrate diets while still supplied with standard rodent chow *ad libitum*. The diets used were high protein (HP) diet with 60% kcal from protein and high carbohydrate (HC) diet with 70% kcal from carbohydrate (both from Research Diets, New Brunswick, NJ, U.S.A). The composition of experimental diets is shown in Table 1. From day 4 of lactation onwards, the animals were switched from the chow diet to either HP or HC diet exclusively. Maternal food intake was recorded between days 5-18 of lactation. Food intake was not monitored when mixed diets were presented on days 1-4. All animals were maintained in accordance with the United Kingdom Home Office Animals (Scientific procedures) Act 1986.

Table 1: Composition of macronutrient diets

Diet code	D04080301	D12450B
Diet	High protein	High carbohydrate
%	kcal	kcal
Fat	10	10
Carbohydrate	30	70
Protein	60	20
Total	100	100
Ingredients (g/kg diet)		
Casein	600	200
L-cystine	9	3
Corn starch	112	315
Maltodextrin	35	35
Sucrose	147	350
Cellulose	50	50
Soya oil	25	25
Lard	20	20
Mineral mix	10	10
Dicalcium phosphate	13	13
Calcium carbonate	5.5	5.5
Potassium citrate	16.5	16.5
Vitamin mix	10	10
Choline bitartrate	2	2
Gross energy (kJ g^{-1})	19.88	19.89

Source: Research Diets, New Brunswick, NJ, U.S.A.

Doubly labelled water measurements

The doubly labelled water (DLW) method was used to measure daily energy expenditure (DEE) from the elimination rates of ^{2}H (deuterium) and ^{18}O in females during peak lactation. The total water turnover (rH_2O) from the elimination rates of ^{2}H was also calculated. Measures of DEE were made to determine the milk energy output (MEO) from the difference between metabolizable energy intake (MEI) and DEE (Król and Speakman, 2003b).

The DLW measurements were conducted on 29 lactating females (HP, N=11 and HC, N=18). On day 16 of lactation (between 8:00h and 11:00h) individual mice were weighed to ±0.01 g using a balance (Mettler Toledo, Switzerland) and labelled with an intra-peritoneal injection of approximately 0.2 g of water containing enriched ^{2}H (36.3 atoms%) and ^{18}O (59.9 atoms%). The syringe used to inject the DLW was weighed (±0.0001 g; Ohaus Analytical Plus, Brookylin, USA) immediately before and after the injection to provide an accurate measurement of the amount of the isotope injected. Mice were placed back in their cages during the 1 h equilibration period (Speakman, 1997). An initial 30-80 µl blood sample was collected by tail tipping 1 h after the injection, which was the time generally assumed to be required for the isotope to reach equilibrium (Król and Speakman, 1999). Blood samples were immediately flame-sealed into pre-calibrated 50 µl pipettes (Vitrex, Camlab Limited, Cambridge, UK) and stored at 4 °C until analysis. A final blood sample was collected approximately 48 h after the initial blood sample was collected to estimate isotope elimination rates.

Samples of blood in capillaries were vacuum-distilled (Nagy, 1983) and water from the resulting distillate was used to produce CO_2 (Speakman *et al*.,

1990) and H_2 (Speakman and Król, 2005b). Gas source isotope ratio mass spectrometry was used to analyse the isotope ratios of ^{18}O:^{16}O and ^{2}H:^{1}H. The samples were run alongside high enrichment standards that were used to correct the raw data to these standards.

For each lactating mouse, initial ^{2}H and ^{18}O dilution spaces were calculated by the intercept method (Coward and Prentice, 1985; Speakman and Król, 2005b) and then converted to mass assuming a molecular mass of body water of 18.020 and expressed as a percentage of body mass before injection. The intercept method was used since the actual body water pool estimated by desiccation using the intercept method was more accurate than the plateau method (Speakman and Król, 2005b). The final ^{2}H and ^{18}O dilution spaces were inferred from the final body mass, assuming the same percentage of body mass as measured for the initial dilution spaces. The isotope elimination rate (k) was calculated following Nagy (1975). For calculation of DEE based on CO_2 production, single pool model equation 7.17 (Speakman, 1997) was used. Energy equivalents of rates of CO_2 production were calculated using a conversion factor of 24.026 J ml^{-1} CO_2, derived from the Weir equation (Weir, 1949).

Female total water turnover (rH_2O) was calculated by multiplying the fractional turnover rate by the total body water (TBW). It was assumed that 25% of the water leaving the body was fractionated (Speakman, 1997). Therefore, a fractionation factor of 0.9366 was applied for deuterium (Speakman, 1997). This approach assumes that rates of water influx and efflux are constant, so rH_2O = total water influx = total water efflux (Nagy and Costa, 1980).

Milk energy output

Milk energy output (MEO) was evaluated as the difference between metabolisble energy intake (MEI) and daily energy expenditure of lactating females (Król and Speakman, 2003b).

Statistics

The differences in baseline and pregnancy data between the groups for body mass and food intake were analysed using repeated measures analysis of variance (ANOVA). The significance of changes in body mass and food intake over time was assessed by general linear modeling (GLM). Significant differences between days and diets were detected using the post-hoc Tukey test. Asymptotic food intake across the two dietary treatment groups in late lactation was compared using ANOVA. The asymptotic food intake in late lactation was defined as the period during which no significant differences in food intake between days were detected. Least squares regression analysis was used to examine the relationship between DEE and maternal body mass, total water turnover and maternal body mass, milk energy output (MEO) and litter size, and litter growth and MEO. Data are represented as means ± standard deviation (SD). Unless stated otherwise, N = number of animals. All statistical analyses were performed using Minitab for Windows (version 14; Minitab Inc., State College, PA, USA; Ryan *et al*., 1985). All statistical significance were determined at $P<0.05$.

RESULTS

Maternal body mass

Before mating, there was no significant difference between the body mass of HP females (29.51±1.52 g, N=20 and HC females (29.52±1.42 g, N=20; ANOVA: $F_{1, 38}=0.01$, P=0.997). Body mass increased significantly during days 12-21 of pregnancy in HP females (ANOVA: $F_{9, 140}=16.35$, P<0.001) and HC females (ANOVA: $F_{9, 170}=39.14$, P<0.001) reaching a peak of 53.55±7.60 g (N=15), and 56.28±5.24 g (N=19) for HP and HC females, respectively just prior to parturition (Figure 1).

Maternal body mass of HP (N= 11) and HC (N=18) females did not show any significant difference (ANOVA: $F_{1, 126}=0.85$, P=0.353) and averaged 41.57±4.41 g and 41.79±3.05 g on the day after parturition and increased to 42.08±3.77 g and 42.39±3.25 g on day 4, respectively for HP and HC mice. Between days 5-8 of lactation when the mice were fed macronutrient diets exclusively, the body mass of HP mothers was significantly lower than that of HC mothers (ANOVA: $F_{3, 114}=118.64$, P<0.001) and averaged 37.58±0.51 g and 43.75±0.71 g, respectively. There was no significant effect of day of lactation (GLM: $F_{13, 378}=1.24$, P=0.246) on maternal body mass. However, there was a highly significant effect of diet (GLM: $F_{13, 378}=194.00$, P<0.001). All Tukey pairwise comparisons among levels of diet showed that the HC-fed mothers were significantly heavier (P<0.05) than HP-fed mothers between days 5-18 of lactation. There was no significant interaction effect (GLM: $F_{13, 378}=4.42$, P=0.993).

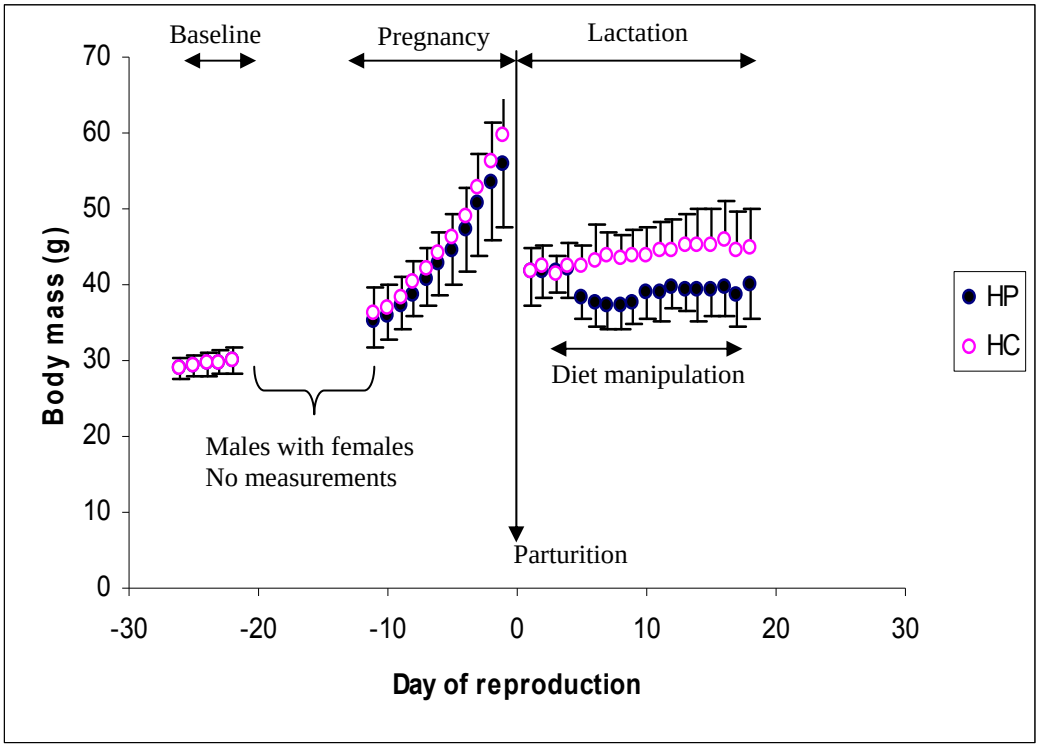

Figure 1: Mean body mass of female mice throughout baseline, pregnancy and lactation. Day 0 was parturition. Maternal body mass was measured between days 1-18 of lactation. Diet manipulation started on day 4 of lactation. High protein diet is denoted by HP while high carbohydrate diet is denoted by HC. Females were significantly lighter on the HP diet. Error bars represent 1 sd of the mean.

Food intake

The food intake of HP and HC females was not significantly different prior to mating (ANOVA: $F_{1, 6}$=0.01, P=0.928). The animals consumed a mean of 5.29±0. 31 g day $^{-1}$ (N=20) and 5.27±0.36 g day $^{-1}$ (N=20) for HP and HC females, respectively.

Food intake increased significantly during days 12-21 of pregnancy in HP (ANOVA: $F_{9, 140}$= 7.55, P<0.001) and HC females (ANOVA: $F_{9, 170}$=9.44, P<0.001) reaching a maximum of 7.81±1.37 g (N=15) and 8.49±1.09 g (N=19) before decreasing to 6.81± 1.09g and 7.20± 0.57g on the day before parturition (Figure 2).

Food intake was not measured on days 1-4 of lactation when animals were fed a mixed diet. There was a significant increase in food intake between days 5-11 of lactation in females fed both the HP diet (ANOVA: $F_{6, 70}$=8.67, P<0.001) and HC diet (ANOVA: $F_{6, 119}$=8.18, P<0.001) from 7.72±1.12g to 11.42±2.05 g (N=11) in HP and from 12.43±2.68 g to 16.33±1.88 g (N=18) in HC. Over the next 6 days (days 12-17), there was no further significant increase in daily food intake (ANOVA: P>0.05) and it averaged 12.29±0.26 g day $^{-1}$ and 16.57±0.26 g day $^{-1}$ for mice fed HP and HC diets, respectively. The food intake averaged over these 6 days was termed the asymptotic daily food intake. The corresponding asymptotic gross energy intakes were 244.95±5.22 kJ day $^{-1}$ and 294.98±4.64 kJ day $^{-1}$. These were equivalent to digestible energy intakes of 219.76±4.99kJ and 266.67±4.45 kJ for mice fed HP and HC diets, respectively, using the estimated assimilation efficiencies derived below.

On day 18 of lactation, the daily food intake increased above the asymptotic level (Figure 2) because the pups started feeding directly on the food in the hoppers on this day. Over all, there was a highly significant effect of day of lactation (GLM: $F_{13, 378}$=15.80, P<0.001) on maternal food intake. All Tukey pairwise comparisons among levels of food intake showed that the food intake of HC-fed mothers was significantly higher (P<0.05) than that of HP-fed mothers between days 5-18 of lactation (GLM: $F_{13, 378}$=487.16, P<0.001). There was no significant interaction (GLM: $F_{13, 378}$=1.27, P=0.227).

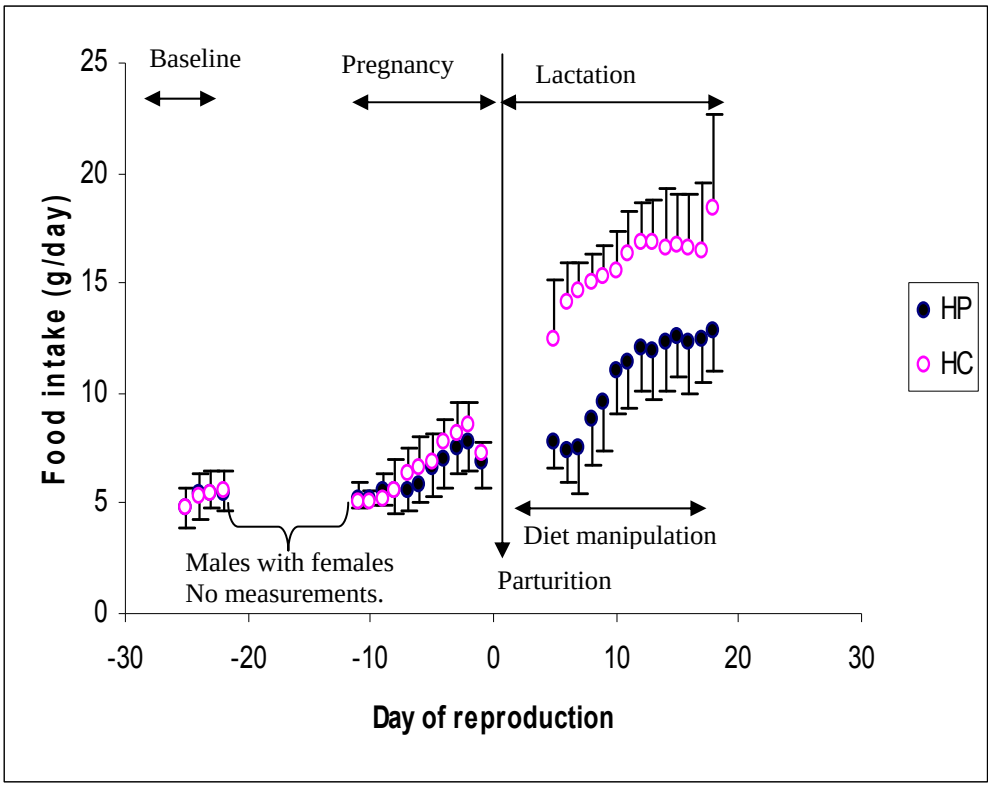

Figure 2: Mean daily food intake of female mice throughout baseline, pregnancy and lactation. Day 0 was parturition. Diet manipulation started on day 4 of lactation. High protein diet is denoted by HP while high carbohydrate diet is denoted by HC. Maternal food intake was measured between days 5-18 of lactation. Error bars represent 1 sd of the mean.

Doubly labelled water measurements: daily energy expenditure and total water turnover of lactating mice

The DEE measured on day 16 of lactation was significantly different (ANOVA: $F_{1, 27}$=48.77, P<0.001) between lactating mice fed on HP and HC diets and averaged 133.42±4.82 kJ day^{-1} (range 127.06-141.47 $kJday^{-1}$, N=11) and 102.11±14.29 kJ day (range 75.44-127.90 $kJday^{-1}$, N=18), respectively (Table 2).

The total water turnover (rH_2O) of HP-fed lactating females and HC-fed controls averaged 41.15±6.28 g day^{-1} (range 31.66-47.84 g day^{-1}, N=11) and 26.76±6.58 g day^{-1} (range 17.15-46.31 g day^{-1}, N=18), respectively (Table 2). When body mass was used as a covariate, analysis of variance showed that the effect of body mass was not significant (GLM: $F_{1, 26}$=0.53, P=0.473) but there was a highly significant group effect (GLM: $F_{1, 26}$=36.20, P<0.001).

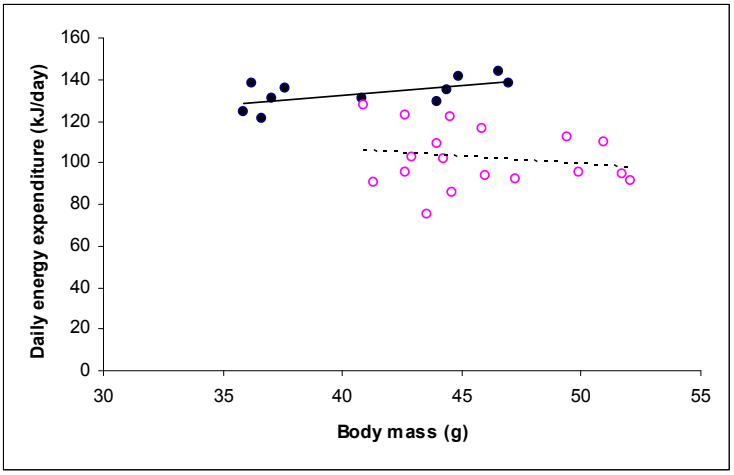

Figure 3: Daily energy expenditure as a function of body mass for lactating females fed on HP diet (filled circles, y=1.26x +81.41, r^2= 0.65, N=11) and lactating females fed on HC diet (open circles, y=-0.74x +136.12, r^2=0.03, N=18).

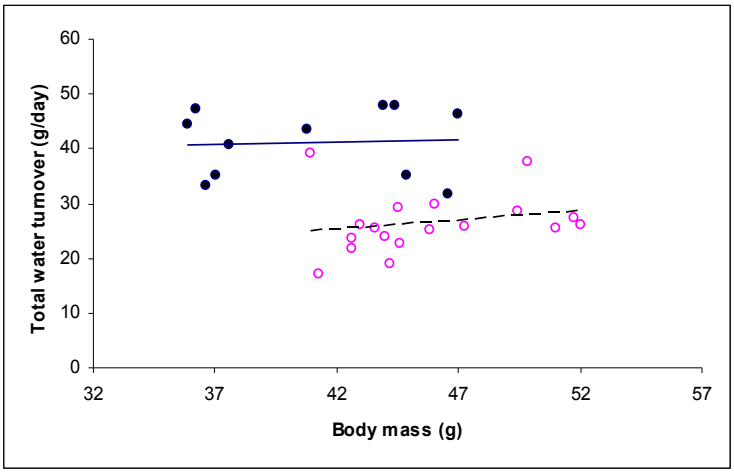

Figure 4: Total water turnover (rH_2O) as a function of body mass for lactating females fed on HP diet (filled circles, y=0.06x +38.66, r^2=0.002, N=11) and lactating females fed on HC diet (open circles, y=0.34x +10.81, r^2=0.049, N=18).

Table 2: Results of the DLW measurements of energy expenditure and water turnover in lactating high protein (HP) females (N=11) and lactating controls fed on high carbohydrate (HC) diet (N=18).

Trait	Lactating mice		ANOVA	
	HP	HC	$F_{1,27}$	*P*
BM (g)[a]	40.99± 4.44	45.80±3.59	10.21	0.004
k_d (h^{-1})[b]	0.059±0.008[a]	0.035±0.009[b]	45.91	<0.001
k_o (h^{-1})[c]	0.069±0.008[a]	0.047±0.009[b]	37.14	<0.001
k_o/k_d	1.177±0.024[a]	1.364±0.067[b]	77.31	<0.001
N_d (% of BM)[d]	73.896±3.600[a]	71.336±2.593[b]	4.95	0.035
N_o (% of BM)[d]	70.838±3.482[a]	68.269±2.333[b]	5.69	0.024
N_d/N_o	1.043±0.015	1.045±0.011	0.11	0.747
DEE (kJ day^{-1})[e]	133.42±4.82[a]	102.11±14.29[b]	48.77	<0.001
rH_2O (g day^{-1})[f]	41.15±6.28[a]	26.76±6.58[b]	33.73	<0.001

Values are means ± S.D.

[a]Body mass before injection; [b]deuterium elimination rate; [c]^{18}O elimination rate; deuterium (N_d) and ^{18}O (N_o) dilution spaces (moles) were converted to g assuming a molecular mass of body water 18.02 and were expressed as percentage of body mass before injection; [e]daily energy expenditure; [f]total water turnover.

Milk energy output

The milk energy output (MEO) calculated on day 16 of lactation was significantly different (ANOVA: $F_{1, 27}$=39.26, P<0.001) between lactating females fed on HP and HC diets and averaged 84.22±31.47 kJ day^{-1} (range 47.16-144.24 kJday^{-1}, N=11) and 164±30.59 kJ day (range 101.77-215.59 kJday^{-1}, N=18), respectively. MEO of HP and HC females was related to litter size on day 16 of lactation (ANOVA: $F_{1, 27}$=7.96, P=0.009) (Figure 5).

The relationship between growth of litters from both HP and HC females and MEO showed a highly significant effect of MEO on litter growth (GLM: $F_{1, 26}$=16.25, P<0.001). MEO also showed a highly significant group effect (GLM: $F_{1, 26}$=21.02, P<0.001) (Figure 6).

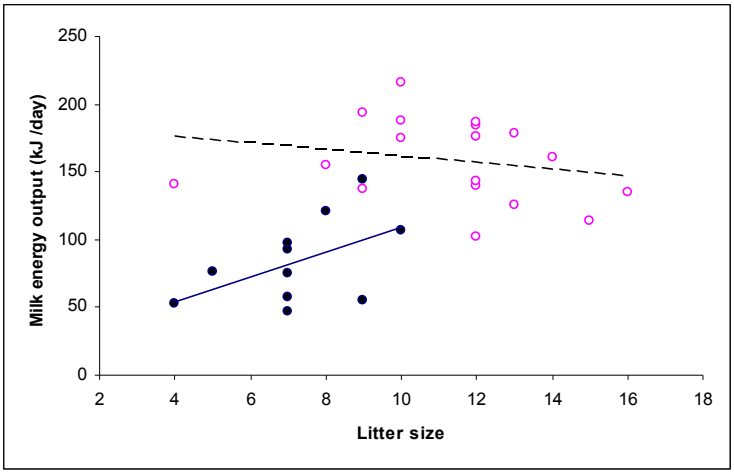

Figure 5: Milk energy output as a function of litter size for lactating females fed on HP diet (filled circles, y=9.21x +17.23, r^2=0.26, N=11) and lactating females fed on HC diet (open circles, y=-2.48x +186.43, r^2=0.05, N=18).

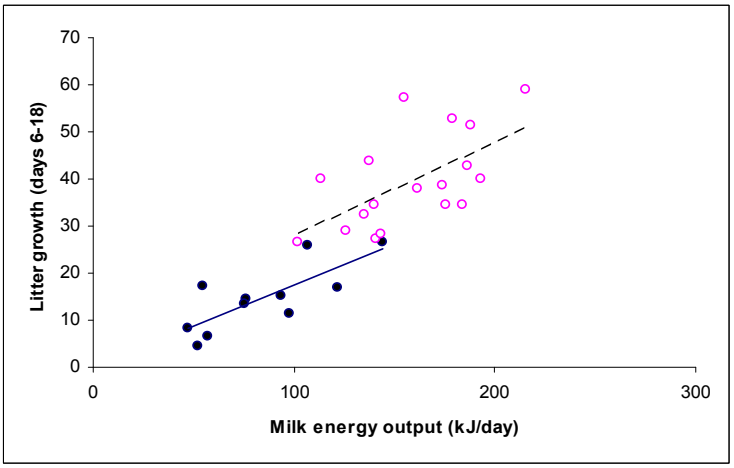

Figure 6: Litter growth as a function of milk energy output for lactating females fed on HP diet (filled circles, y=0.17x -0.05, r^2=0.60, N=11) and lactating females fed on HC diet (open circles, y=0.19x +8.14, r^2=0.36, N=18).

DISCUSSION

The body masses of female mice increased significantly during pregnancy. There was loss of body mass of HP-fed mice during early lactation as soon as they were fed HP diet exclusively. The loss of body mass of the mice was associated with lower food intake than the HC-fed mice. Milk production also declined as body reserves were mobilised to support milk production. Protein intake at an elevated ambient temperature resulted in a marked decrease in food intake and consequently losses in body mass and back fat depth correlated with a decline in milk production in sows (Renaudeau *et al*., 2001; Renaudeau and Noblet, 2001; Renaudeau *et al*., 2002). Contrary to some previous studies (Hammond and Diamond, 1994; Speakman and McQueenie, 1996), the MF1 mice in the present study did not continue to increase their food intake until the end of lactation, but instead reached a plateau between days 12 to 17 of lactation (see also Johnson *et al*., 2001a). The energy demand of the pups was still increasing at this time because they were growing but there was no corresponding increase in maternal food and energy intake, indicating that they were limited.

In previous studies, the asymptotic daily food and energy intakes of MF1 mice at 21 °C were 23.1 g day^{-1} and 301 kJ day^{-1} (Johnson *et al*., 200a) and 20 g day^{-1} and 266.3 kJ day^{-1} (Krol *et al*., 2007). In this study, the asymptotic daily food and energy intakes were 16.6 g day^{-1} and 266.7 kJ day^{-1}, indicating that peak lactation energy intake varies with ambient temperature. The SDA for HP and HC diets was measured using open-flow respirometry at 9.4% and 6.1% (Kagya-Agyemang, 2008). The effects of feeding HP to lactating mice were far greater than anticipated from the 3.3% difference in SDA. It appears that other factors must be involved in energy delivery to the offspring.

Król and Speakman (2003a, b) suggested that the limits to energy intake during peak lactation are imposed by the capacity of the animal to dissipate body heat generated as a by-product of processing food and producing milk. In present study, the heat production consequent of digestion and milk production of HP-fed mice was elevated as evidenced by the higher DEE, suggesting that the HP-fed mice were heat stressed. This therefore affected lactation performance in the HP-fed mice.

There are several potential explanations to account for the large negative effects of the HP diet. Protein probably involves other sources of heat production than SDA that might include urea synthesis (ureagenesis) and gluconeogenesis. The cost of urea synthesis in the liver is a major energy-consuming

process (McBride and Kelly, 1990). Various estimates for the energy cost of urea synthesis have been derived assuming a stoichiometry of 4 adenosine triphosphate (ATP) per mole of urea (McBride and Kelly, 1990). Gluconeogenesis has also been shown to generate heat during the conversion of amino acids into glucose for eventual export in milk as lactose (McDonald *et al*., 2002).

Protein catabolism generates several waste products, including nitrogen (Yang and Bankir, 2005). In mammals, most of the nitrogenous wastes are excreted in the form of urea (Yang and Bankir, 2005). The amount of protein consumed by HP-fed mice at peak lactation was 7.1 g per day. Protein contains 16% nitrogen so when a HP-fed mouse consumed 7.1 g of protein per day it ingested 7.1x 0.16 g of nitrogen. This gives 1.136 g of nitrogen. Milk contains 10% of protein (Jenness, 1979) so if the mouse produced 12.9 g of milk per day, then the amount of protein in the milk was 1.29 g. The amount of nitrogen exported in the milk was estimated by multiplying the amount of protein in milk by 16% to get 0.2064. Therefore, the net nitrogen intake of HP-fed mouse was calculated by subtracting 0.21 g from 1.14g to give 0.93 g of nitrogen. Based on this, the urea produced per mouse per day was calculated by dividing 0.9296g by 0.4662% (i.e. % of urea that is nitrogen) to get 1993.9 mg.

The 7.1g of protein per day consumed by HP-fed mice generated 1.99g of urea (0.033 mols). The ATP requirement for this synthesis was 0.132 mols and the resulting heat liberation was 4.10 $kJday^{-1}$ (assuming 31 $kJ\ mol^{-1}$ ATP; Mathew *et al*., 2000). However, the HC-fed mice were also generating urea consequent of their protein intake requiring 1.86 $kJ\ day^{-1}$, so the extra heat associated with the HP diet was 4.10 $kJ\ day^{-1}$.

Some of the HP-fed mice killed and consumed some of their offspring. The infanticide was probably due to the low food and energy intakes coupled with the high DEE of the HP-fed mice that greatly reduced their milk production. Under inhospitable conditions, mothers can optimize reproductive success by either killing a portion of their offspring so as to utilize body reserves and intake to ensure their own survival and that of their remaining offspring, or terminating an entire litter, thereby delaying all reproductive effort until conditions are more favourable (Hardy, 1979; Hardy and Hausfater, 1984). In small mammals, short-term food deprivation or restriction during lactation increases cannibalism in rats (Grosvenor and Mena, 1983; Kanarek *et al*., 1986), mice (Zamiri, 1978; Bronson and Marstellar, 1985), and hamsters (Schneider and Wade, 1992).

Overall, the growth of offspring from HP-fed mothers was poor when compared with that of HC-fed mice. This shows that offspring growth did not improve on the HP diet. The finding of the present study contrasts the findings of Hitchcock (1927) and Goettsch (1960)) who reported improvement in growth of rat and mice offspring from mothers fed high protein diets during lactation. It seems that the 60% protein in the HP diet was too high for the lactating mothers and therefore resulted in reduced food and energy intakes, reduced litter size and litter mass, and a consequent high DEE that greatly reduced the energy available for milk export to pups. This idea is supported by the fact that lactational performance varies with the total calories consumed by the mother and also macronutrient ratios (Wade and Schneider, 1992).

The reduction in body mass and food intake at 21°C could be explained by the action of protein diet suppression of food intake centrally. It is believed that protein suppression of appetite is through the mammalian target of rapamycin (mTOR) protein (Cota *et al*., 2006; Woods *et al*., 2008). In the rat, mTOR signalling is controlled by energy status in specific regions of the hypothalamus and co-localizes with neuropeptide y (NPY) and pro-opiomelanocortin (POMC) neurons in the arcuate nucleus (ARC) of the hypothalamus (Cota *et al*., 2006). Central administration of leucine increases hypothalamic mTOR signalling and decreases food intake and body weight (Cota *et al*., 2006). Thus mTOR is an intracellular pathway that works as fuel sensor to regulate food intake and body weight (Cota et al., 2006; Woods *et al*., 2008). Leucine and high protein diet increased mTOR activity in the hypothalamus, leading to inhibition of NPY and stimulation of POMC expression to control food intake (Ropelle *et al*., 2007).

CONCLUSION

In conclusion, we have shown that MF1 mice fed HP diet at 21 ºC and the HC-fed mice reached plateaus in their daily food intake at 12.29 $g\ day^{-1}$ and 16.57 $g\ day^{-1}$ between days 12-17 of lactation. HP diet had negative effects on lactation at 21 ºC, suggesting that the growth of pups was not protein limited at peak lactation. Evidence suggests that the negative effects of the HP diet were due to the high DEE that greatly reduced the energy available for milk production, rather than a toxicity effect of the urea production. This study has shown that high protein intake has negative effects on lactation performance since it affects the maximal rate of food intake in lactating mice.

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