Maternal protein reserves and their influence on lactational performance in rats 2. Effects of dietary protein restriction during gestation and lactation on tissue protein metabolism and Na^+,K^+-ATPase (*EC* 3.6.1.3) activity

BY A. P. PINE, N. S. JESSOP* AND G. F. ALLAN

Institute of Ecology and Resource Management, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG

AND J. D. OLDHAM

Genetics and Behavioural Science Department, Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG

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Changes in tissue protein synthesis and an associated membrane transport system in rats were investigated during lactation and under conditions of dietary protein restriction. Following mating, female Sprague-Dawley rats (second parity) were caged individually and offered a high-protein diet (H; 215 g crude protein (N × 6·25; CP)/kg dry matter (DM)) *ad lib.* until day 12 of gestation. Subsequently half continued to receive diet H, whilst the remainder were offered a low-protein diet (L; 65 g CP/kg DM) until parturition. On day 1 of lactation females were then allocated to either diet H or another low-protein diet (L_2; 90 g CP/kg DM) which were offered *ad lib.* until day 13 of lactation, giving four lactation groups HH, LH, HL_2 and LL_2. On days 1 and 13 of lactation groups of females were used in the estimation of tissue protein synthesis (flooding dose of [^{3}H] phenylalanine) and Na^+, K^+-ATPase (*EC* 3.6.1.3) activity (polarographically) in skeletal muscle, mammary gland, liver and duodenal mucosa. By day 1 of lactation diet L had reduced fractional and absolute synthesis rates (FSR and ASR) of muscle protein ($P < 0·05$) and the O_2 consumption associated with Na^+,K^+-ATPase, although not significantly ($P < 0·10$). Rates of protein synthesis in the other tissues studied were not affected on day 1 of lactation by the gestation dietary treatment. By day 13 of lactation the feeding of diet L_2 had reduced muscle FSR and ASR of group HL_2 to rates that were lower than those on day 1 ($P < 0·05$), comparable to those of group LL_2 and lower than those of groups HH and LH ($P < 0·05$). Diet H had allowed group LH to increase their muscle protein synthesis compared with that on day 1 ($P < 0·05$). Muscle Na^+,K^+-ATPase activity on day 13 of lactation was also lower in groups offered diet L_2 ($P < 0·05$). Mammary protein synthesis was increased during lactation with the feeding of diet H ($P < 0·05$), which was prevented by diet L_2 such that rates of groups HL_2 and LL_2 were lower than those of the two high-protein groups on day 13 ($P < 0·01$). Mammary respiration and in particular Na^+,K^+-ATPase activity was increased during lactation by the feeding of diet H ($P < 0·05$). Rates of protein synthesis and respiration in liver and duodenal mucosa were not significantly affected by the gestational or lactational dietary treatments. Calculated rates of muscle protein degradation suggest that whilst the loss of muscle protein in group HL_2 during lactation might have been promoted by the decline in synthesis, the increase in degradation may have been quantitatively more important.

Lactation: Protein synthesis: Na^+,K^+-ATPase: Rat

When dietary protein is limiting, the extent of maternal protein reserve repletion at parturition has a significant impact on a female's ability to sustain lactation, with more

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protein available for mobilization allowing an increased food intake and improved lactational performance (Mahan & Mangan, 1975; Pine *et al.* 1994). Clearly loss of body protein occurs when the rate of degradation exceeds the rate of synthesis although, from the literature, it is not clear whether this is a result of a decrease in synthesis, an increase in breakdown or both (Siebrits *et al.* 1985; Vincent & Lindsay, 1985; Sainz *et al.* 1986; Millican *et al.* 1987; Baracos *et al.* 1991).

In our earlier studies we did not make any measurements of tissue protein metabolism. A purpose of the work reported here was to explore the influence of feeding protein in gestation and in lactation on rates of protein synthesis in different tissues of the lactating rat. In the light of other work conducted in our laboratory (Friggens *et al* 1993) which described the relationships between dietary nutrient balance, food intake and lactational performance in rats, there was reason to think that a response to inadequate protein nutrition might be an elevation in metabolite energy expenditure by active tissues. As both protein synthesis and Na^+,K^+-ATPase (*EC* 3.6.1.3) activity are two major energy-requiring processes (Milligan & Summers, 1986), the protein synthesis measurements made here were augmented with studies of Na^+,K^+-ATPase activities.

MATERIALS AND METHODS

The experiment reported here adopted a similar experimental protocol to that reported in an earlier experiment (Pine *et al.* 1994) in which the effect on lactational performance of variations in the extent of maternal protein reserve repletion was investigated. The objective of the experimental protocol was to establish at parturition two groups of females that had distinct differences in the size of their maternal protein mass and, thus, reserves. The experimental protocol is described in detail below.

Experimental design

Forty-four, second parity, female Sprague-Dawley rats (Harlan and Olac UK Ltd., Shaws Farm, Bicester, Oxon.) weighing on average 302·3 (SE 2·1) g were caged individually in a room regulated at 22° and relative humidity from 40–60%, under a 12 h light–dark cycle with the light period from 08.00–20.00 hours. For mating the females were placed, at the appropriate time, individually in a wire-bottomed cage with a proven male breeder. The morning on which mating was confirmed, through the presence of vaginal plugs, was designated day 1 of gestation and the females were returned to solid-bottomed plastic cages for the remainder of the experiment.

Following mating the females were offered a high-protein diet (H, 215 g crude protein (N × 6·25; CP)/kg dry matter (DM) (Table 1) *ad lib.* until day 12 of gestation. Subsequently half of the females continued to receive the high-protein diet while the remainder were offered a low-protein diet (L, 65 g CP/kg DM) *ad lib.* until parturition. Immediately following parturition four groups of females were selected for slaughter, two groups to be used in carcass analysis (*n* 4) while the other two groups (*n* 6) were used in the analysis of tissue metabolism (protein synthesis and Na^+,K^+-ATPase activity).

On day 1 of lactation the remaining females were allocated factorially to either diet H or another low-protein diet (L_2, 90 g CP/kg DM) which was then offered *ad lib.* for the remainder of the experiment. This dietary allocation produced four groups of females (HH, HL_2, LH and LL_2, the first letter representing the dietary treatment from day 12 of gestation and the second letter representing the lactation diet) that reached day 13 of lactation, on which all females and litters were slaughtered and measurements of maternal tissue metabolism were made.

All diets were formulated to provide approximately 21 MJ gross energy (GE)/kg DM with a constant carbohydrate : fat ratio of 2·4 : 1. All litters were standardized to twelve pups

Table 1. *Composition of high- and low-protein diets (g/kg dry matter (DM))*

Diet...	High-protein (H)	Low-protein (L)	Low-protein (L_2)
Casein*	215	65	90
Starch–sucrose†	439	542	525
Vegetable fat	196	243	235
Vitamin mix‡	50	50	50
Mineral mix‡	100	100	100
Diet analysis:			
Protein (g CP/kg DM)	212	63	88
GE (MJ/kg DM)	21·7	21·5	21·7
Antioxidant (butylated hydroxytoluene; g/kg fresh wt)	0·01	0·01	0·01

CP, crude protein (N × 6.25); GE, gross energy.
* Casein supplemented with DL-methionine (99:1, w/w).
† Starch–sucrose mixture (2:1, w/w).
‡ Vitamin and mineral mixes were formulated to meet requirements of the National Research Council (1978).

on day 1 of lactation to maximize the lactational stress imposed. This was achieved by removal or cross fostering of pups from one litter to another. In our experience females who had successfully raised a previous litter would accept additional pups whereas with primiparous females this was more difficult to achieve. Litter weights were recorded daily throughout lactation. Dam body weights and food intakes were recorded throughout the experiment. All females were given free access to drinking water.

Measurement of protein synthesis

On either day 1 or 13 of lactation, rates of total protein synthesis were measured *in vivo* in the mammary gland, liver, gastrocnemius muscle and duodenal mucosa using the flooding-dose technique of Garlick *et al.* (1980).

Between 09.00 and 13.00 hours dams were injected via a lateral tail vein with a solution containing 150 mmol L-phenylalanine/l and 1·85 MBq L-[2,6-^{3}H] phenylalanine/ml (Amersham International Ltd., Bucks) at 10 ml/kg body weight and returned to their litters. After 10 min dams were decapitated and samples of the left inguinal abdominal mammary gland, liver, gastrocnemius muscle and duodenal mucosa were quickly excised and plunged into liquid N_2. A sample of mucosa was obtained by opening a length of duodenum, washing with ice-cold saline (9 g NaCl/l) and scraping with a microscope slide. In the present study the gastrocnemius muscle was used because it is thought to be a good indicator of the response of the body's musculature to dietary treatment (Waterlow *et al.* 1978). Samples of tissue were used to measure fractional synthesis rate (FSR) of total tissue protein from the incorporation of [^{3}H] phenylalanine into tissue protein. Correction for the gradual linear decline of specific activity of tissue free phenylalanine during the 10 min incorporation period was ignored because of the previous observation that the rate of decline in mammary tissue, liver and muscle is slow and insignificant (Garlick *et al.* 1983; Sampson *et al.* 1986). Calculation of FSR (%/d) uses the formula:

$$\text{FSR} = \frac{S_B \times 100}{S_A \times t},$$

where S_B and S_A are specific activities of protein-bound and free phenylalanine respectively, and t is the time in days that elapsed between injection and rapid cooling of tissue. Absolute

synthesis rates (ASR) are calculated from FSR and tissue protein content. Tissue RNA concentration was measured as described by Munro & Fleck (1969), with muscle RNA calculated using the equation of Ashford & Pain (1986), and tissue protein concentration measured with the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Measurement of Na^+,K^+-ATPase activity

The tissue activity of the enzyme Na^+,K^+-APase on days 1 and 13 of lactation was estimated through measurements of the inhibition of tissue O_2 consumption caused by the addition of ouabain, a specific inhibitor of the Na^+,K^+-ATPase enzyme (Albers *et al.* 1968), to the incubation medium. Ouabain concentrations of 1 μmol/l or greater have been shown to give maximal inhibition of Na^+,K^+-ATPase-associated respiration (Gregg & Milligan 1982*a*).

O_2 consumption rates were measured polarographically using an oxygen electrode (Rank Bros., Cambridge). Weighed samples of liver snips, mammary gland slices (20 μm); (Bartley & Abraham, 1976), muscle fibre bundles (tied with sutures; Gregg & Miligan, 1982*b*) and duodenal mucosal scrapes were washed and placed in an oxygen electrode chamber in 3 ml Minimal Essential Medium (MEM; Sigma Chemical Co. Ltd, Poole, Dorset) containing 5 mmol Hepes/l at 37°, pH 7·4, and the O_2 consumption was recorded for 10–15 min. After this time ouabain was added to a final concentration of 10^{-4} mol/l and the O_2 consumption was recorded for a further 10 min. The difference between the initial O_2 consumption and that following ouabain treatment was termed the Na^+,K^+-ATPase-dependent respiration. The percentage inhibition of the original O_2 consumption associated with Na^+,K^+-ATPase activity was calculated using the ratio of Na^+, K^+-ATPase dependent respiration to the initial O_2 consumption. The size of the piece of tissue used was such that the reduction in the O_2 content of the incubation medium during the 20–25 min procedure was never greater than 25% of its initial value.

Carcass analysis

For the groups selected for carcass analysis on day 1 of lactation, dams were killed by decapitation and their carcass, liver, mammary gland and gastrointestinal tract were dissected and analysed for dry matter, protein, ash and fat. The procedures used for these analyses have been described elsewhere (Pine *et al.* 1994).

Statistical analysis

For statistical treatment of the results two-way analysis of variance and one-way analysis of variance were used and, where appropriate by calculation of least significant differences, *t*-test were used to compare sample means between diets and stages of lactation. To establish the impact that the gestation treatments had on the extent of maternal protein reserve repletion at parturition, the carcass composition values for dams slaughtered on day 1 of lactation were amalgamated with those for rats from a parallel experiment (Pine *et al.* 1994) and treated similarly during lactation.

RESULTS

Food intakes and body weight changes during gestation

The food intakes, body weight and carcass composition changes, mean pup birth weights and pups/litter of females offered diets H or L during gestation are shown in Table 2. Feeding low-protein diet during the second half of the gestation did not significantly affect food intake or maternal gestation weight gain compared with females offered the high-

Table 2. *Maternal body weight gain, carcass composition, food intakes and pup birth weight of rats given either high (H)- or low (L)-protein diets during gestation*†

Diet...	H (*n* 21)	L (*n* 21)	SD
Dam wt gain (g)	12·0	3·3	19·5
Food intake (g DM), days 1–11 of gestation	193·8	193·8	16·7
Food intake (g DM), day 11–parturition	182·3	186·8	24·8
Carcass composition‡, day 1 of lactation:			
Protein (g)	43·5	38·7**	3·6
Fat (g)	22·8	23·5	5·8
Abdominal Fat (g)	19·6	21·1	6·1
Mammary wt§ (g)	15·0	14·3	4·2
Protein (g)	1·6	1·4	0·3
Fat (g)	5·5	6·2	2·1
Liver wt§ (g)	11·2	10·7	1·4
Protein (g)	2·2	1·9	0·2
Fat (g)	0·9	1·1	0·3
Gastrointestinal tract wt§ (g)	7·9	8·2	1·6
Protein (g)	1·1	1·2	0·2
Fat (g)	1·0	1·3	0·7
Litter size (pups/litter)	12·8	11·9	3·2
Mean pup birth wt (g)	5·7	5·4	0·8

DM, dry matter.
Mean value was significantly different from that of the high-protein group: $^{**}P < 0{\cdot}01$.
† For details of diets and procedures, see Table 1 and pp. 182–184.
‡ Composition estimated using data from Pine *et al.* (1994): H, *n* 8; L, *n* 7.
§ H, *n* 4; L, *n* 4.

protein diet. The low-protein dietary treatment also did not impair fetal development, and the mean pup birth weight and litter size (pups/litter) did not differ between the two treatment groups.

In order to provide a stronger indication of the extent of maternal protein reserve repletion at parturition the effects of the gestation dietary treatments on the carcass composition of females slaughtered on day 1 of lactation were estimated using data from two parallel experiments. From Table 2 it can be seen that the feeding of the low-protein diet during the second half of gestation had reduced ($P < 0{\cdot}01$) the carcass protein content and thus protein reserves of group L, while having no significant effect on the size of their carcass and abdominal fat stores. The gestation dietary treatment had therefore the desired effect of ensuring that females at parturition had variations in the size of their protein reserve.

Dietary protein restriction during gestation also had no significant effect on the size and composition of the mammary gland, liver and gastrointestinal tract on day 1 of lactation.

Effects of the gestational and lactational dietary treatments on dam food intakes, body weight changes and litter weight gains during lactation

The results for the four lactation groups HH, LH, HL_2 and LL_2 are shown in Table 3. The changes in maternal body weight during lactation were significantly affected only by the lactational dietary treatment. All four groups lost weight, but the feeding of the low-protein diet resulted in a greater weight loss ($P < 0{\cdot}05$), although the weight losses by groups HL_2

Table 3. *Body weight loss, food intake and litter weight gain during days* 1–13 *of lactation of rats offered either high* (*H*)- *or low* (*L*)-*protein diets during gestation* (*Ge*) *and then H or a different low* (L_2)-*protein diet during lactation* (*La*)†

Diet sequence...	HH (*n* 6)	LH (*n* 5)	HL_2 (*n* 6)	LL_2 (*n* 6)	SD	Statistical significance of diet effects		
						Ge	La	Ge × La
Dam wt change (g/12 d)	−17·4	−6·6	−63·1	−51·6	28·0	NS	**	NS
Food intake (g DM/12d)	337	293	257	227	53·5	*	***	NS
Days 1–6 (g DM)	130	103	127	105	19·5	**	NS	NS
Days 7–13 (g DM)	207	190	130	122	45·6	NS	***	NS
Litter wt gain (g/12 d)	194	153	94	69	52·5	***	***	NS
Days 1–6	82	52	52	32	21·6	***	***	NS
Days 7–13	112	101	42	37	35·2	*	***	NS

DM, Dry matter; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see Table 1 and pp. 182–184.

and LL_2 were not significantly different and this reflects their similar weight gains during gestation. The weight losses of the two high-protein groups also did not differ.

The food intakes of the four treatment groups were influenced by both gestational and lactational dietary treatments. The total food intake (g DM/12 d) of diet H by group LH was less than that of group HH ($P < 0·05$) due to the lower intake of group LH during the first six days ($P < 0·05$). Both groups HH and LH showed a considerable increase in food intake between the two halves of lactation. The feeding of diet L_2 during lactation resulted in a suppression ($P < 0·05$) in total food intake of groups HL_2 and LL_2 when compared with group HH and was greatest for group LL_2; intake by group LL_2 being lower ($P < 0·05$) than that for group HL_2 during the first 6 d and the whole 12 d period. During the first half of lactation the DM intake of diet L_2 by group HL_2 was greater than that of group LH ($P < 0·05$) but not that of group HH. During the second half of lactation intakes of both high groups had increased sufficiently to be greater than that of HL_2 ($P < 0·05$).

Lactational performance was estimated by the weight gain of a standardized litter and was influenced by both gestation and lactation dietary treatments. The lactational performance of group LH was less ($P < 0·05$) than that of group HH, reflecting the difference in their intakes of diet H. For groups HH and LH, litter weight gain increased between the two halves of lactation, again reflecting the considerable increase in food intake. Dietary protein restriction significantly impaired the litter weight gain of groups HL_2 and LL_2 when compared with HH and LH. However, the lactational performance of the low-protein groups was influenced by their gestation dietary treatment, with group LL_2 having a lower litter weight gain (total and first 6 d) than group HL_2 ($P < 0·01$). The greater performance of Group HL_2 during the first half of lactation was supported by their greater food intake and maternal protein reserves, and allowed them to achieve a similar lactational performance to that of group LH. Despite this, group HL_2 was unable to maintain this greater performance and their rate of litter weight gain was reduced by 20% during the second half of lactation.

Effect of gestational dietary treatment on tissue protein synthesis on day 1 of lactation

Rates of protein synthesis and tissue composition for the gastrocnemius muscle, mammary gland, liver and duodenal mucosa for females offered diet H or L during the second half of gestation are shown in Tables 4–7.

The feeding of diet L resulted in a reduction in muscle weight ($P < 0.001$), protein content ($P < 0.01$) and RNA content ($P < 0.001$) by day 1 of lactation. The rates of muscle protein synthesis were also reduced ($P < 0.05$) by the low-protein dietary treatment, with muscle FSR (% per d) and ASR (mg protein/d) being reduced from 4·4% per d and 15·0 mg/d to 3·4% per d and 9·9 mg/d in groups H and L respectively. There was no significant difference in muscle RNA activity between groups H or L. The reduction in gastrocnemius muscle weight and protein content, by 18 and 16% respectively, of group L females reflects the loss of maternal protein reserves, which is also indicated by the reduced carcass protein content of such females (Table 2).

The rate of protein synthesis and tissue composition in the mammary gland, liver and duodenal mucosa on day 1 of lactation were not significantly affected by gestation dietary treatment (Tables 5–7), and this reflects the lack of effect of such dietary treatment on organ size (Table 2). The main effect of the low-protein dietary treatment on the depletion of maternal protein reserves was therefore reflected in muscle protein metabolism.

Effect of gestational dietary treatment on tissue O_2 consumption and Na^+,K^+-ATPase-dependent respiration

The O_2 consumption and associated Na^+,K^+-ATPase activity of the muscle, mammary gland, liver and duodenal mucosa on day 1 of lactation are shown in Tables 4–7.

Dietary protein restriction during the second half of gestation tended to reduce the total, Na^+,K^+-ATPase-dependent and independent respiration of the tissues studied by day 1 of lactation, although with the exception of the liver total O_2 consumption ($P < 0.05$) these reductions were not statistically significant at the 5% level.

In gastrocnemius muscle the proportion of O_2 consumption inhibition by ouabain (Na^+,K^+-ATPase-dependent respiration) tended to be lower ($P < 0.10$ for group L than for group H and ranged from 24·0–32·3%. This proportion in mammary gland, liver and duodenal mucosa ranged from 12·5–15·7, 18·9–21·1 and 17·6–20·3% respectively.

The effects of gestational and lactational dietary treatments on tissue protein synthesis and O_2 consumption on day 13 of lactation

Muscle. The rate of muscle protein synthesis, muscle composition and tissue respiration of the four lactation treatment groups HH, LH, HL_2 and LL_2 on day 13 of lactation are shown in Table 4.

The rate of muscle protein synthesis on day 13 of lactation was influenced solely by the lactational dietary treatment, with both FSR and ASR of groups HL_2 and LL_2 being lower ($P < 0.05$) than those of the two high-protein groups. The reduced rates of muscle protein synthesis, both FSR and ASR, of group HL_2 were also lower ($P < 0.05$) than those of group H on day 1, being reduced from 4·40 to 3·34% per d and 15·05 to 8·44 mg/d respectively. A similar effect was seen for group LH, where feeding of diet H increased ($P < 0.05$) muscle protein synthesis when compared with that of group L on day 1, increasing from 3·40 to 4·84% per d and 9·87 to 14·55 mg/d for FSR and ASR respectively. The rate of muscle protein synthesis in groups HH and LL_2 did not change during lactation.

Dietary protein restriction during lactation reduced ($P < 0.05$) muscle weight, protein and RNA content of groups HL_2 and LL_2 when compared with those of groups HH and LH. These reduced muscle protein contents were also lower ($P < 0.05$) than those of groups H and L on day 1 of lactation, with muscle protein losses of approximately 93 and 45 mg between days 1 and 13 for HL_2 and LL_2 respectively. Muscle weight and RNA content of group HL_2 were also lower ($P < 0.05$) than those of group H on day 1 of lactation. The muscle weight and protein content of group LH were lower ($P < 0.05$) than those of group HH on day 13 of lactation and were not significantly greater than values for group L on

Table 4. *Muscle composition, protein synthesis, and O_2 consumption (nmol/g tissue per min) on days 1 (groups H and L) and 13 (groups HH, LH, HL_2 and LL_2) of lactation of rats offered either a high (H)- or a low (L)-protein diet during gestation (Ge) and then either H or a different low (L_2)-protein diet during lactation (La)*†

	Gestation				Lactation					
Dietary group...	H	L		Ge × diet	HH	LH	HL_2	LL_2		La × diet
n...	5	4	SD	effect	5	5	4	5	SD	effect
Wet wt (g)	1·70	1·40	0·2	***	1·60	1·43	1·26	1·28	0·2	***
Protein (mg)	345	291	48·0	**	331	301	252	245	40·8	***
RNA (mg)	2·21	1·75	0·3	***	1·99	1·89	1·41	1·54	0·3	***
FSR (% per d)	4·4	3·4	0·8	*	4·9	4·8	3·3	3·9	0·8	**
ASR (mg protein/d)	15·0	9·9	3·4	*	16·0	14·5	8·4	9·7	3·6	***
RNA activity	6·84	5·70	1·4	NS	8·13	7·83	5·99	6·30	1·5	**
O_2 consumption:										
Total respiration	312	254	108·8	NS	264	272	234	244	52·4	NS
Ouabain-insensitive	219	197	102·4	NS	165	178	166	174	32·4	NS
Ouabain-sensitive	93	57	32·9	*	99	94	68	70	30·2	*
% Inhibition	32·3	24·0	10·2	*	36·7	33·6	29·3	28·4	6·4	*

FSR, fractional synthesis rate; ASR, absolute synthesis rate; RNA activity, mg protein/mg RNA; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see Table 1 and pp. 182–184.

Table 5. *Mammary protein synthesis, composition and O_2 consumption (nmol/g tissue per min) on days 1 (groups H and L) and 13 (groups HH, LH, HL_2 and LL_2) of lactation of rats offered either a high (H)- or a low (L)-protein diet during gestation (Ge) and then either H or a different low (L_2)-protein diet during lactation (La)*†

	Gestation				Lactation					
Dietary group...	H	L		Ge × diet	HH	LH	HL_2	LL_2		La × diet
n...	5	4	SD	effect	5	5	4	5	SD	effect
FSR (% per d)	58·9	60·8	10·9	NS	91·6	82·4	58·9	59·3	20·0	**
ASR (mg/g tissue per d)	52·4	47·4	14·5	NS	118·2	113·5	74·1	64·7	28·6	**
Protein (mg/g)	89	77	13·8	NS	130	139	124	110	16·2	**
RNA (mg/g)	4·66	3·75	0·9	NS	11·4	10·4	8·62	7·92	2·0	**
RNA activity	11·9	12·5	3·6	NS	10·7	10·9	8·58	8·09	2·0	**
O_2 consumption:										
Total respiration	518	346	179·9	NS	594	642	516	361	169·3	**
Ouabain-insensitive	445	303	147·4	NS	467	500	408	286	147·4	**
Ouabain-sensitive	72	43	24·7	NS	127	142	108	75	44·7	*
% Inhibition	15·7	12·5	7·4	NS	22·3	22·8	20·0	21·9	7·4	NS

FSR, fractional synthesis rate; ASR, absolute synthesis rate; RNA activity, mg protein/mg RNA; NS, not significant.
* $P < 0·05$, ** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 182–184.

day 1. Group LH were thus unable to replenish their depleted muscle protein when offered the high-protein diet despite the fact that their protein synthetic rate and RNA activity were increased compared with day 1 of lactation. Group HH showed no change in their muscle protein metabolism or composition compared with group H on day 1.

On day 13 of lactation, muscle energy expenditure, represented by total O_2 consumption, showed no significant effect of gestation or lactation dietary treatments, although the O_2 consumption of the groups offered diet L_2 tended to be lower than that of the two high-protein groups (Table 4). This lower muscle O_2 consumption by HL_2 and LL_2 was reflected in a reduced ($P = 0.062$) Na^+,K^+-ATPase-dependent respiration compared with that of the groups offered diet H. When these results are presented as the proportion of total O_2 consumption (% inhibition), the proportion associated with Na^+,K^+-ATPase activity was lower ($P < 0.05$) in groups HL_2 and LL_2 when compared with that of group HH. On day 13 of lactation Na^+,K^+-ATPase activity accounted for between 28 and 37% of muscle respiration. There were no significant changes in total muscle respiration between days 1 and 13 of lactation although it can be seen that dietary protein restriction reduced the proportion associated with Na^+,K^+-ATPase (Table 4).

Mammary tissue. The rates of mammary protein synthesis, mammary composition and tissue respiration for the four treatment groups HH, LH, HL_2 and LL_2 on day 13 of lactation are shown in Table 5.

Feeding diet H during lactation promoted an increase ($P < 0.05$) in mammary protein synthesis when compared with that on day 1 of lactation, with FSR increasing from around 59% per d on day 1 to 92 and 82% per d on day 13 for groups HH and LH respectively. Dietary protein restriction during lactation prevented any substantial increase in mammary protein synthesis of groups HL_2 and LL_2, except for the ASR of HL_2 ($P < 0.05$), and resulted in the two low-protein groups having lower ($P < 0.01$) rates of mammary protein synthesis, both FSR and ASR (mg protein/g tissue per d), when compared with those of groups HH and LH.

Lactation resulted in a change in mammary composition, with the protein and RNA contents (mg/g tissue) increasing ($P < 0.05$) in all groups when compared with day 1, although protein restriction of group LL_2 prevented as great an increase as that of groups HH and LH (Table 5). On day 13 of lactation the protein content, RNA content and activity of the groups offered diet L_2 were significantly lower than in those offered the high-protein diet during lactation. The RNA activity of the four treatment groups tended to be lower than that of groups H and L on day 1, with the reduction in RNA activity of the LL_2 group being significant ($P < 0.05$; Table 5).

On day 13 of lactation the mammary total, Na^+,K^+-ATPase-dependent and -independent respiration rates were not significantly different for groups HH, LH and HL_2, although diet L_2 resulted in groups LL_2 having lower ($P < 0.05$) levels of respiration compared with the two high-protein groups. Both low-protein groups showed no significant increase in mammary respiration during lactation. However, the feeding of the high-protein diet during lactation resulted in an increased mammary O_2 consumption (Table 5). The increase in total and Na^+,K^+-ATPase-independent respiration was significant only for group LH ($P < 0.05$), while the Na^+,K^+-ATPase-dependent respiration was significantly increased in both HH and LH groups ($P < 0.05$). By day 13 of lactation the proportion of total respiration associated with Na^+,K^+-ATPase activity was increased ($P < 0.05$) in both LH and LL_2 groups compared with that on day 1, accounting for between 20 and 23% of total mammary respiration.

Liver. On day 13 of lactation the gestational and lactational dietary treatments had no significant effect on hepatic protein synthesis, with both liver FSR and ASR (mg protein/g tissue per d) not different for the four lactation treatment groups. These rates were also not significantly different from those on day 1 of lactation (Table 6).

Table 6. *Liver protein synthesis, composition and O_2 consumption (μmol/g tissue per min) on days 1 (groups H and L) and 13 (groups HH, LH, HL_2 and LL_2) of lactation of rats offered either a high (H)- or a low (L)-protein diet during gestation (Ge) and then either H or a different low (L_2)-protein diet during lactation (La)*†

	Gestation				Lactation					
Dietary group...	H	L		Ge × diet	HH	LH	HL_2	LL_2		La × diet
n...	5	4	SD	effect	5	5	4	5	SD	effect
FSR (% per d)	96·5	103·0	18·1	NS	92·9	85·5	98·2	103·5	16·5	NS
ASR (mg/g tissue per d)	190	190	24·5	NS	183	165	162	159	30·8	NS
Protein (mg/g)	198	189	20·9	NS	197	194	164	151	21·7	**
RNA (mg/g)	12·7	11·5	1·0	NS	8·37	8·51	8·66	7·59	0·8	NS
RNA activity	15·4	16·4	2·0	NS	22·0	19·4	18·5	21·0	3·2	NS
O_2 consumption:										
Total respiration	1·41	1·06	0·308	*	1·09	1·01	0·872	0·891	0·218	NS
Ouabain-insensitive	1·10	0·860	0·192	NS	0·866	0·821	0·696	0·739	0·179	NS
Ouabain-sensitive	0·316	0·202	0·145	NS	0·221	0·187	0·176	0·152	0·068	NS
% Inhibition	21·1	18·9	6·5	NS	20·6	18·4	20·0	16·9	4·9	NS

FSR, fractional synthesis rate; ASR, absolute synthesis rate; RNA activity, mg protein/mg RNA; NS, not significant.
* $P < 0·05$, ** $P < 0·01$.
† For details of diets and procedures, see Table 1 and pp. 182–184.

Table 7. *Duodenal mucosal protein synthesis, composition and O_2 consumption (nmol/mg protein per min) on days 1 (groups H and L) and 13 (groups HH, LH, HL_2 and LL_2) of lactation of rats offered either a high (H)- or a low (L)-protein diet during gestation (Ge) and then either H or a different low (L_2)-protein diet during lactation (La)*†

	Gestation				Lactation					
Dietary group...	H	L		Ge × diet	HH	LH	HL_2	LL_2		La × diet
n...	5	4	SD	effect	5	5	4	5	SD	effect
FSR (% per d)	116	132	22·0	NS	150	127	140	157	31·5	NS
ASR (mg/g tissue per d)	119	128	25·9	NS	170	137	151	156	31·9	NS
Protein (mg/g)	102	97	12·9	NS	113	109	108	100	11·8	NS
RNA (mg/g)	6·71	5·73	1·4	NS	6·72	6·63	6·71	6·33	0·8	NS
RNA activity	18·0	23·0	3·9	NS	25·9	20·7	22·2	24·6	4·6	NS
O_2 consumption:										
Total respiration	3·6	3·1	1·6	NS	5·2	3·6	4·0	4·0	1·4	NS
Ouabain-insensitive	3·0	2·5	1·4	NS	4·1	2·8	3·2	3·4	1·1	NS
Ouabain-sensitive	0·6	0·6	0·3	NS	1·1	0·8	0·8	0·6	0·8	NS
% Inhibition	17·6	20·3	6·9	NS	19·5	21·5	18·7	15·5	6·3	NS

FSR, fractional synthesis rate; ASR, absolute synthesis rate; RNA activity, mg protein/mg RNA; NS, not significant.
† For details of diets and procedures, see Table 1 and pp. 182–184.

Dietary protein restriction resulted in a loss of liver protein during lactation such that the hepatic protein contents (mg/g tissue) of groups HL_2 and LL_2 were lower ($P < 0{\cdot}05$) than both that on day 1 of lactation (Table 6) and those of groups HH and LH. The hepatic RNA contents (mg RNA/g tissue) for all lactation groups were lower ($P < 0{\cdot}05$) than those on day 1 of lactation, although they were not different from each other. Hepatic RNA activities were also not significantly different between the four lactation groups, although the RNA activity of the HH group was increased compared with day 1 ($P < 0{\cdot}05$; Table 6).

Hepatic energy expenditure on day 13 of lactation did not differ between the groups and was also not different from that at the start of lactation.

Duodenal mucosa. The rates of protein synthesis and tissue respiration of the duodenal mucosa for the four lactation treatment groups are shown in Table 7.

On day 13 of lactation the rate of mucosal protein synthesis and mucosal protein and RNA contents (mg/g tissue) were not significantly affected by dietary protein level and thus food intake during lactation. Mucosal protein synthesis and composition was also, in general, not significantly altered between days 1 and 13 of lactation (Table 7), although the ASR (mg protein/g tissue per d) of group HH was increased ($P < 0{\cdot}05$). The RNA activity on day 13 was not significantly affected by dietary protein content during lactation, although again the RNA activity of group HH was increased compared with day 1 ($P < 0{\cdot}05$).

In a similar way to mucosal protein synthesis, tissue respiration (nmol O_2/mg protein per min) on day 13 of lactation was not affected by dietary protein content and did not differ between days 1 and 13 (Table 7). On day 13 of lactation mucosal Na^+,K^+-ATPase activity accounted for between 15·5 and 21·5% of total mucosal energy expenditure, comparable with that of mammary gland and liver (Tables 5 and 6).

DISCUSSION

Data from this and our earlier study (Pine *et al.* 1994) have shown that dietary manipulation during gestation leads to differences at parturition in carcass protein content but not in the size of adipose tissue stores, organ weights and composition nor in pup size. Patterns of response in terms of food intake, maternal and litter performance in the present study were similar to those of the earlier study so that extension of the results of these studies to the performance data obtained previously seems reasonable.

The dietary treatments imposed during gestation would appear not to have compromised the ability of females to lactate. In general, rates of protein synthesis, O_2 consumption and protein synthetic capacity in mammary tissue, liver and duodenal mucosa were not affected. This is in contrast to the finding of Rosso *et al.* (1981) who showed that combined protein and energy restriction from the end of the first trimester of gestation in rats resulted in a significant reduction in mammary gland hypertrophy and hyperplasia by day 1 of lactation. In the present study, because of the isoenergetic nature of the diets used, feeding of diet L during gestation resulted in a restriction in protein supply only, and by day 1 of lactation there was no significant effect on mammary size, composition or rates of protein synthesis and respiration.

During gestation the increase in loss of muscle protein in those animals given diet L was probably aided by the reduction in muscle protein synthesis (FSR, ASR) that was recorded on day 1 of lactation. However, rates of muscle protein degradation cannot be calculated for day 1, therefore the possibility that degradation was enhanced cannot be ruled out. Other workers have suggested that such a decline in muscle protein synthesis during the catabolic phase of gestation in protein-restricted female rats may spare amino acids for the developing feto-placental unit (Mayel-Afshar & Grimble, 1983). Although muscle

respiration on day 1 was not significantly affected by the gestation treatment, feeding of diet L with the consequent reduction in amino acid intake tended to be associated with reduced Na^+,K^+-ATPase activity ($P < 0.10$), possibly reflecting the reduced requirement for amino acids by this tissue (Adeola *et al.* 1989).

The rate of liver protein synthesis (% per d) is not particularly sensitive to reductions in protein quantity and/or quality during lactation (Sampson & Jansen, 1984*b*; Sampson *et al.* 1986) and the absolute rate (ASR, mg/d) is only reduced following a reduction in liver weight and protein content (Jansen & Hunsaker, 1986). A similar lack of sensitivity in hepatic protein synthesis to dietary protein restriction during lactation is reported here, with the rate of protein synthesis on day 13 being unchanged from that on day 1.

Under conditions of adequate nutrition, lactation is normally associated with hypertrophy of the intestines, liver and mammary gland and with an elevation of food intake (Williamson, 1980; Canas *et al.* 1982). In our previous study gut and liver hypertrophy were seen in rats adequately nourished with a protein-rich food but not when dietary protein content (and food intake) were low (Pine *et al.* 1994). Applying the liver weights (in parentheses) and protein content, for females slaughtered on day 13 of lactation and offered similar dietary treatments from the earlier study (Pine *et al.* 1994), rates of liver ASR (mg/d) would have been approximately 4150 (22·7 g), 3737 (22·6 g). 2272 (14·1 g) and 2146 mg/d (13·5 g) for groups HH, LH, HL_2 and LL_2 respectively. From these data it is clear that during lactation hypertrophy of the liver would exaggerate the difference in absolute hepatic protein synthesis between the high- and low-protein groups. The loss of liver protein during lactation in response to a reduced dietary protein supply observed here is consistent with previous studies (Jansen & Hunsaker, 1986; Sainz *et al.* 1986).

It is generally accepted that the gastrointestinal tract makes a greater contribution to body protein turnover than the liver. In the present study fractional rates of protein synthesis in the duodenal mucosa were between 1·27 and 1·57 % per d and these are close to previously reported values for rat intestinal mucosa (McNurlan *et al.* 1979). On day 13 of lactation, rates of mucosal protein synthesis were unaffected by dietary treatment. However, the increase in intake of the high-protein diet during lactation would be associated with an increase in the size of intestinal mucosa (Lichtenberger & Trier, 1979), providing an increased surface area and digestive capacity of the gastrointestinal tract. Total, rather than fractional, protein synthesis associated with the intestinal mucosa would therefore be expected to be considerably increased during lactation, with a sizeable difference between females offered the high- or low-protein diets.

Unlike lactose and lipid production (Williamson *et al.* 1984), protein synthesis in the mammary gland is not subject to diurnal variation (Sampson & Jansen 1984*a*). The timing of the protein synthesis measurement would therefore not have had a dramatic effect on the values obtained in this study. The quantity of dietary protein offered during lactation had a significant impact on the rate of mammary protein synthesis, with both mammary FSR and ASR (mg/g tissue per d) in groups HH and LH being significantly greater than that of the two low-protein groups and also compared with that on day 1 of lactation. Previous studies have shown a significant effect of protein quantity on mammary protein synthesis in rats, while improvements in protein quality at a similar level of protein quantity have a more dramatic effect (Jansen & Hunsaker, 1986; Sampson *et al.* 1986). Using mammary weights (in parentheses) from a previous experiment (Pine *et al.* 1994), for females slaughtered on day 13 of lactation in groups HH and LH the total rate of protein synthesis would have been 2960 (25·0 g) and 3178 mg/d (28·0 g) respectively. For all groups, mammary ASR was strongly correlated with daily litter weight gain on day 12 of lactation (r^2 0·61, $P < 0.001$).

The maintenance of tissue protein turnover is supported by a variety of cellular

mechanisms, one of which is the membrane transport system Na^+,K^+-ATPase which plays an essential role in the transport of amino acids and sugars across cell membranes. Na^+,K^+-ATPase activity accounts for a major part of cellular energy expenditure and on day 13 of lactation in the present study represented 28–37, 20–23, 17–21 and 16–22% of tissue respiration in the gastrocnemius muscle, mammary gland, liver and duodenal mucosa respectively.

The proportion of tissue energy expenditure associated with Na^+,K^+-ATPase activity has been reported to be significantly increased during lactation in the intestinal mucosa of cows from 35% (dry) to 54% (peak) and liver of sheep from 37% (dry) to 45% (peak yield; McBride & Milligan, 1984, 1985*a*). The results of the present study do not support this, with tissue respiration and the proportion associated with Na^+,K^+-ATPase activity in the liver and mucosa on the whole remaining unchanged between days 1 and 13 of lactation. It should be noted that although the rates of tissue respiration per gram were unchanged, the organ hypertrophy associated with lactation would result in a considerable increase in total and Na^+,K^+-ATPase-dependent energy expenditure in tissues such as the liver and gut. The Na^+,K^+-ATPase activity in the mammary gland was significantly increased during lactation in the groups offered diet H, and this reflects the increased metabolic activity and protein synthesis in this tissue.

The proportions of tissue respiration associated with Na^+,K^+-ATPase activity in liver and duodenal mucosa (approximately 20%) were considerably lower than other published data for the liver (37–45%) and mucosa (28–61%); McBride & Milligan 1985*a*,*b*). The lower contribution of Na^+,K^+-ATPase activity to tissue respiration reported here may be attributed to differences in the incubation medium used between these studies (Milligan & Summers, 1986; Jessop, 1988).

Dietary protein restriction during gestation and lactation significantly reduced the rate of muscle protein synthesis of days 1 and 13 of lactation and ultimately promoted a loss in muscle weight and protein content. Rates of muscle protein synthesis have been closely linked with the activity of Na^+,K^+-ATPase (Vandenburgh & Kaufman, 1981; Adeola *et al.* 1989) and protein restriction during lactation significantly reduced the proportion of tissue respiration associated with this enzyme. Muscle protein synthesis (mg/d) and Na^+,K^+-ATPase activity (nmol O_2/d) were highly correlated (r^2 0·48; $P < 0·001$; n 29), with no evidence to suggest a departure from this correlation in animals given the low-protein diets.

Estimations of the contribution that Na^+,K^+-ATPase activity makes to muscle total respiration vary between studies, being 22–25% in growing pigs (Adeola *et al.* 1989), 40% in growing calves (Gregg & Milligan, 1982*b*) and 42–46% in dry and lactating ewes respectively (Gregg & Milligan 1982*c*). These compare with 24–32% and 28–37% on days 1 and 13 of lactation respectively in the present study and further reflect the importance of this enzyme in muscle energy expenditure.

Assuming that both FSR changes and the loss of muscle protein during lactation occur at a steady, linear rate between days 1 and 13 of lactation, then the average rate of protein degradation (FDR) during lactation was 5·01, 3·81, 6·45 and 4·76% per d for groups HH, LH, HL_2, and LL_2 respectively. Whether these assumptions are realistic or not cannot be tested from these data but they seem to be reasonable. The calculated rates of protein degradation for the different treatments would suggest that both changes in muscle protein synthesis and degradation play a part in regulating the use of maternal protein, an observation which contrasts somewhat with the results from other studies concerning lactating animals in which increases solely in rates of degradation (Bryant & Smith, 1982) or decreases solely in rates of synthesis (Swick & Benevenga, 1977) have been reported.

It is possible that this calculation of FDR may have underestimated the true degradation rate since from earlier studies it has been suggested that the mobilization of maternal

protein occurs predominantly during the first half of lactation (Pine *et al.* 1994). The measurements made in the present study cannot detect such changes in protein turnover occurring during early lactation and thus further work on protein loss during this period is required.

Results of a previous study suggested that during lactation improvements in dietary protein supply to females previously protein-depleted allowed them to achieve a lactational performance similar to that of other females offered the same diet, while at the same time attempting to replenish their depleted reserves (Pine *et al.* 1994). In this current study the LH group showed a significantly increased rate of muscle protein synthesis (3·4–4·8% per d) and RNA activity during lactation, and this could represent part of the mechanism involved in protein replenishment.

In summary, the results of these studies show that the reduced milk secretion consequent on protein undernutrition is reflected in an impaired mammary protein metabolism, although other tissues appear to be less sensitive to reductions in protein supply during lactation. The mobilization of maternal protein reserves during lactation involves changes in both muscle protein synthesis and degradation. The reduction in muscle protein synthesis is also associated with reduced activity of the Na^+,K^+-ATPase enzyme.

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