Dietary supplementation increases milk output in the rat

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1. The effects of dietary supplementation on milk output and maternal body composition were investigated in the lactating rat. The supplement was a cooked homogeneous mixture of eggs and maize oil, and had the same protein-energy:total energy value as the control diet.

2. During 2–12 d post partum rats were fed *ad lib.*, either on the control diet alone or on the control diet plus the supplement. Measurements were made of milk output using an isotope-dilution technique, milk composition, and dam and litter body-composition changes.

3. Compared with the dams receiving only the control diet, dams provided with the supplement consumed 19·7% more energy and protein and produced 31·2% more milk and mobilized less body fat.

4. By 12 d of age, pups in litters of dams receiving the supplement were significantly heavier than those in litters of dams receiving the control diet only, and they contained more protein and more fat.

5. It is concluded that dietary supplementation of lactating rats can enhance lactational performance.

The relation between energy intake and lactational performance in women has been the subject of considerable controversy. Some evidence indicates that low energy intake during lactation is associated with reduced breast milk output (Gopalan, 1958; Martinez & Chavez, 1971; Prentice, 1980; Van Steenbergen *et al.* 1981). However, several groups of workers have supplemented the energy intake of undernourished mothers, some for considerable periods of time, and yet failed to detect an increase in breast milk output (Gunther & Stanier, 1951; Chavez *et al.* 1975; Prentice *et al.* 1980; Whitehead *et al.* 1983).

Some information is available regarding the effects of dietary supplementation on milk output in animal species. Rolls and co-workers (1980, 1981, 1982, 1983) have reported that reductions in litter growth and milk output occur in rats when high-energy dietary supplements are provided during lactation alone or continuously from weaning onwards. However, the supplements provided in those studies were low in protein, having protein-energy:total energy (P:E) values of 0·04–0·08, compared with 0·18 in the control diet. The lactating rat has a high requirement for dietary protein, as a consequence of the high protein output in milk (Mueller & Cox, 1946; Godbole *et al.* 1981), and diets which have P:E values of less than 0·16 have been shown to reduce milk output when compared with diets having P:E values of 0·20 or more (Venkatachalam & Ramanathan, 1964; Menaker & Navia, 1973).

The present study was designed to provide information on the effects of a high-protein dietary supplement provided during lactation, on milk output, maternal body composition and litter growth in the rat.

EXPERIMENTAL
Animals and diets

Animals. Hooded rats from a colony maintained under specific pathogen-free conditions at the Dunn Nutritional Laboratory were used. They were raised in litters of eight and weaned when 3 weeks old. After weaning, the female rats were housed individually in plastic-sided wire-bottomed cages, in a room where the ambient temperature was maintained at 19–21°. Controlled lighting provided 12 h of light and 12 h of dark, with the light period from 07.00 to 19.00 hours. Water was freely available.

Diets. From weaning until the end of pregnancy, the rats were fed on a pelleted commercial diet *ad lib.* (Rodent Breeding Diet no. 1, Spratts Ltd, Barking, Essex). This diet contains approximately 17.2 kJ metabolizable energy (ME)/g, and its P:E value is 0.21. Throughout lactation, the control diet was the semi-synthetic powdered diet described by Lunn *et al.* (1976), which contains 16.5 kJ ME/g and has a P:E value of 0.20. A supplement was also offered to some of the rats. It was made by cooking a homogeneous mixture of eggs, maize oil and sodium chloride ($0.900:0.099:0.001$, by wt) to a solid consistency, and contained 10.5 kJ ME/g and had a P:E value of 0.20.

Procedure

The rats were mated when 12–14 weeks old and were inspected daily. A vaginal plug in the litter tray indicated the first day of pregnancy, when males were removed. At 2 d before the expected date of delivery, a tissue-paper nesting material was provided. The morning after delivery was designated 1 d post partum and at this time the diet was changed from the commercial diet to the control diet. Litter sizes were adjusted to eight pups.

Milk composition was determined in twelve rats, at 12 d post partum. The rats were paired on the basis of litter weight, to within 0.5 g/litter at 2 d post partum. One rat from each pair received the control diet (group A). The other dam from each pair received the control diet plus the supplement from 2 d post partum onwards (group B). The diets were fed *ad lib.* On the evening of 11 d post partum, litters were removed from their dams. After 8 h the litters were returned for a 20 min sucking period and were then killed with an overdose of diethyl ether vapour. Milk was subsequently collected from the stomachs of the pups, and the samples were pooled for each litter. After 2 h the dams were anaesthetized with an intraperitoneal injection of sodium pentobarbitone solution (Sagatal; May & Baker Ltd, Manchester), at a dose level of 1 ml/kg body-weight, and injected intraperitoneally with 4 units oxytocin (Sandoz Products Ltd, Feltham, Middlesex). Milk was then collected by gentle aspiration of each teat, using a soft plastic tube (internal diameter 3 mm) connected to a collection vessel and a bench water-pump. All milk samples were weighed for subsequent analysis and stored at $-20°$.

Twenty-four rats were used for the measurement of milk output and body composition changes during lactation. Six of them (group C) and their litters were killed by suffocation, using diethyl ether vapour, at 1 d post partum, for carcass analysis. The remaining eighteen rats were matched in threes, on the basis of litter weight, at 2 d post partum. During the period 2–12 d post partum, one dam from each trio received the control diet *ad lib.* (group D), and one received the control diet plus the supplement *ad lib.* (group E). The third animal in each trio (group F) received both diets, but in the ratio consumed by the matched dam in group E, and intake was restricted to the amount consumed by the matched dam in group D. Milk outputs of dams in groups D and E were measured during 4–12 d post partum. Maternal food intakes, and dam and litter body-weights, were recorded for all animals daily. Animals in groups D and E were killed at 12 d post partum for carcass analysis.

METHODS

Milk composition. Fat content was measured in milk samples weighing 0.6–1.0 g by the gravimetric method of Hudson *et al.* (1979). For total nitrogen, duplicate samples of 0.15–0.20 g were digested with a selenium–sulphuric acid (5 g/l) mixture, and N content was determined by the automated microKjeldahl procedure of Weber (1973). Protein was calculated as $N \times 6.38$. Total carbohydrate was measured in duplicate samples of 0.1 g by the automated reducing-sugar method of Hudson *et al.* (1976). Total solid content was determined by freeze-drying 2–3 g samples of milk to constant weight. Milk solids obtained

Table 1. *The effect of dietary supplementation during lactation on fat, protein, carbohydrate, ash and total solid contents of rat milk (g/kg), collected either directly from dams (M1) or from the stomachs of pups (M2)*

(Mean values with their standard errors for six rats per group)

Diet during lactation*	Group		Fat		Protein		Carbohydrate		Ash†		Total solids	
			M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
Control	A	Mean	93·3	86·0	90·0	100·8	44·1	40·2	14·2	12·9	246·7	246·3
		SE	7·8	10·9	2·0	2·7	1·0	1·3	—	—	6·2	14·8
Control + supplement	B	Mean	116	104	82·8	93·1	40·6	36·2	13·8	12·6	257·4	251·3
		SE	13·1	1·48	2·0	4·2	4·3	1·3	—	—	12·1	114·8

* For details, see p. 2.
† Values were for pooled group samples.

in the total solid analysis were pooled for each group, and ash contents were determined by heating the samples to constant weight in a muffle furnace.

Milk output. A modification of the method of Coward *et al.* (1982) was used to measure the milk output of dams during four successive 2 d periods. On the day before the start of the measurement of milk output, six pups from each litter were injected subcutaneously with 15–20 μCi ^3H_2O in 0·02 ml isotonic saline (9 g NaCl/l). On the next day, and four subsequent alternate days, each pup was identified by its natural skin markings and weighed. Approximately 0·05 ml urine was collected and subsequently weighed into a vial and counted. To correct for the water recycling that occurs between dams and their litters (Baverstock & Green, 1975), the mean specific activity of tracer in the urine of non-injected pups was subtracted from that of the injected pups. The body water content of each pup was estimated from its body-weight using a regression equation derived from data on other pups (six litters per dietary group) that were killed at intervals between 4 and 12 d of age. Those pups were killed with an overdose of diethyl ether vapour, weighed and freeze-dried to determine body water content.

The equation given by Coward *et al.* (1982) was used to calculate total water intake of each injected pup, from the decline in tracer specific activity in urine and the increase in size of the body water pool. Milk intake was calculated from total water intake using the values for water and nutrient content of milk given in the results section, and values for the water yield of oxidation of protein, fat and carbohydrate reported by Bergmann *et al.* (1974). Milk output of dams was calculated as the mean milk intake by injected pups × 8.

Carcass analysis. After killing, both dams and pups (pooled in litters) were put in tin-foil trays, weighed and then stored at −20°. Subsequently, the trays were covered and autoclaved for 1 h at 124 kN/m². Covers were removed before cooling and the carcasses were freeze-dried to constant weight. The freeze-dried carcasses were weighed, ground to a homogeneous mixture using a domestic food processor and stored in sealed containers. Fat content was measured by a modification of the procedure of Woodward (1978). Duplicate extracts of fat were made for each dam and each litter by mixing 7·5 g carcass powder with 40 ml tetrachloroethylene; the mixtures were kept in sealed containers for 2 h while shaken periodically and then filtered. Filtrates collected in the first 2 min (approximately 30% of the total filtrate) were used for indirect estimation of fat content. The specific gravity of each solution was measured in a Fosslet 15310 instrument (Foss Electric Ltd, York), and
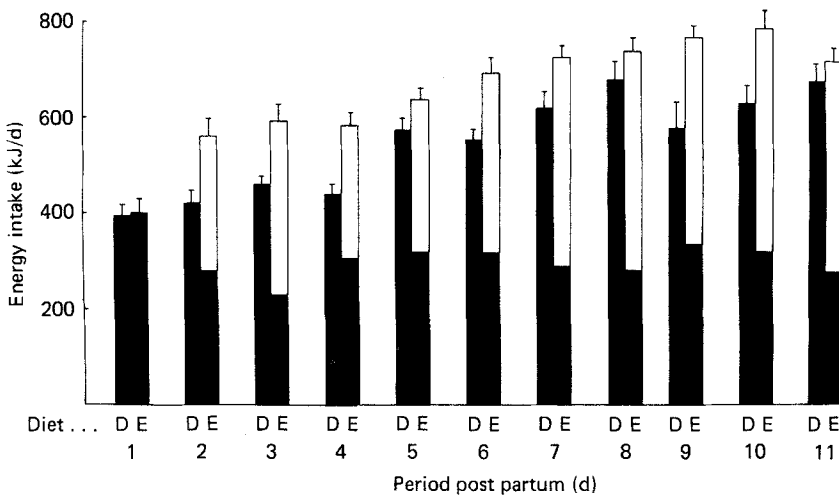
Fig. 1. Energy intake (kJ/d), from control diet (■) and a supplement (□) having the same protein-energy:total energy value (0·20) as the control diet, in lactating rats fed *ad lib.* either on the control diet alone (group D) or on the control diet plus the supplement (group E). Values are means, with their standard errors represented by vertical bars (*n* 6).

the value was converted to fat concentration using the tables provided by the manufacturer. N was measured using the method described for milk. Protein was calculated as N × 6·25.

Statistical analysis

Differences between groups were evaluated statistically using Student's *t* test for unpaired values. Differences in mean values were considered significant when $P < 0.05$.

RESULTS

Milk composition

Table 1 shows the composition of milk collected either directly from dams or following a standardized routine of suckling or from the stomachs of their pups. With respect to the concentrations of constituents measured (fat, protein, carbohydrate and ash), there were no substantial differences between the milk collected in the two ways. Milk collected directly from dams receiving the supplement (group B) contained 24% more fat than milk collected directly from dams receiving only the control diet (group A), but 8% less protein and 8% less carbohydrate; however, these differences were not statistically significant.

Energy intake, milk output and maternal and litter body composition

The energy intakes of the dams fed on the control diet alone (group D) and those fed on the control diet plus the supplement (group E) are shown in Fig. 1. Normal lactation-induced hyperphagia was observed in dams in group D, and at 11 d post partum intakes were 77·3% greater than on day 1. The energy intake of dams in group E was significantly greater than that of group D, by 19·7% ($P < 0.01$). This increase was distributed evenly over the period of study. The average percentages of energy from the control diet and the supplement were 48 and 52 respectively. Milk outputs of animals in the two groups are shown in Fig. 2. Dietary supplementation increased milk output over the whole of the measurement period,
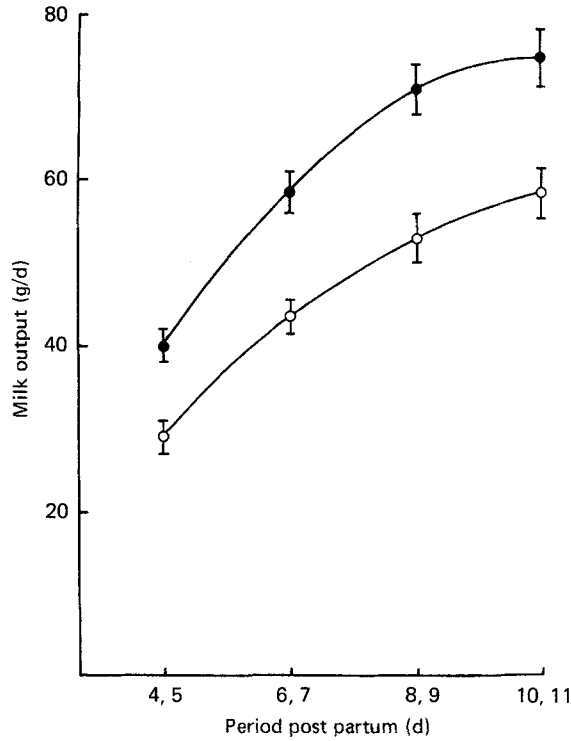
Fig. 2. Milk output (g/d) of lactating rats fed *ad lib.* either on the control diet (group D, O—O) or on the control diet plus a supplement having the same protein-energy:total energy value (0·20) as the control diet (group E, ●—●). Values are means with their standard errors represented by vertical bars (*n* 6).

Fig. 3. Body-weight (proportion of body-weight at 1 d post partum) of lactating rats fed *ad lib.* either on the control diet (group D, O—O) or on the control diet plus a supplement having the same protein-energy:total energy value (0·20) as the control diet (group E, ●—●). Values are means with their standard errors represented by vertical bars (*n* 6).
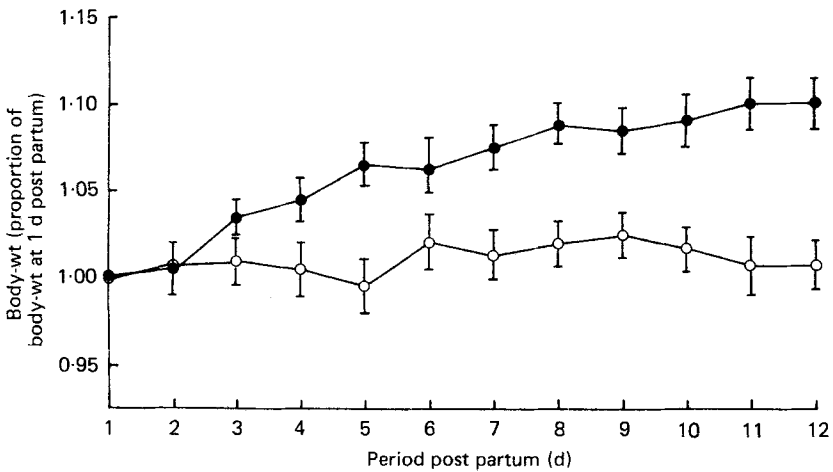
Table 2. *Body-weight and protein* (*TBP*) *and fat* (*TBF*) *contents of lactating rats at 1 d post partum, and at 12 d post partum after feeding* ad lib. *on the control diet or the control diet plus a supplement*

(Mean values with their standard errors for six rats per group)

Diet during lactation†	Group	Period post partum (d)	Body-wt (g) Mean	SE	TBP (g) Mean	SE	TBF (g) Mean	SE
—	C	1	249·7	4·2	40·3	1·4	33·1	1·4
Control	D	12	252·0	4·9	41·2	1·1	27·4*	1·6
Control + supplement	E	12	260·4	2·6	40·1	0·9	30·3	2·5

Value was statistically significantly different from that for group C: *P < 0·05.
† For details, see p. 2.

Table 3. *Body-weight and protein* (*TBP*) *and fat* (*TBF*) *contents of rat litters†at 1 d of age, and at 12 d of age after dams were fed* ad lib. *during lactation, either on the control diet or on the control diet plus a supplement*

(Mean values with their standard errors for six rats per group)

Diet of dam during lactation‡	Group	Age (d)	Body-wt (g) Mean	SE	TBP (g) Mean	SE	TBF (g) Mean	SE
—	C	1	47·0***	2·3	4·5	0·8	0·6***	0·1
Control	D	12	210·6	5·4	25·1	1·0	23·2	1·1
Control + supplement	E	12	246·2**	6·1	27·0	0·9	39·2***	1·4

Values were significantly different from those for group D: **P < 0·01; ***P < 0·001.
† All litters contained eight pups.
‡ For details, see p. 2.

4–12 d post partum, by 31·2% on average, which was a significant amount ($P < 0·01$). Supplementation also influenced the pattern of maternal body-weight change. During 1–12 d post partum a mean increase in body-weight of 9·6% occurred in dams in group E, compared with weight maintenance in dams in group D (Fig. 3). This difference was reflected in reduced mobilization of body fat during lactation, as judged by carcass fat content (Table 2). At 12 d post partum, dams in group E did not contain a significantly different amount of fat from that of dams analysed at 1 d post partum (group C), whereas dams in group D contained 5·7 g less fat ($P < 0·05$). There was no significant difference in body protein content between groups C, D and E (Table 2).

The high milk output rate of dams in group E was associated with a fast rate of growth in their litters (Table 3). By 12 d of age, litters of group E dams were, on average, 36 g heavier than those of group D dams and contained 16 g more fat and 1·9 g more protein.

Table 4 shows the litter weights and maternal weight change of dams fed on the control diet *ad lib.* (group D) and of those receiving the same amount of energy and protein as dams in group D, but from a combination of control and supplementary diets (group F). There

Table 4. *The weights of litters* at 1 and 12 d of age, and maternal weight change during 2–12 d post partum, in lactating rats fed on the control diet* ad lib. *during lactation or pair-fed the same amount of energy and protein from a combination (0·96:1·04, w/w) of control diet and a supplement*

(Mean values with their standard errors for six rats per group)

Diet of dam during lactation†	Group		Litter wt (g)		Material wt change during 2–12 d post partum (g)
			1 d of age	12 d of age	
Control	D	Mean	43·7	210·6	+1·6
		SE	1·3	5·4	2·5
Pair-fed					
Control + supplement	F	Mean	45·7	214·9	+4·2
		SE	1·4	6·1	4·6

* All litters contained eight pups.
† For details, see p. 2.

were no significant differences in litter weights at 12 d of age or in maternal weight change during 2–12 d post partum between the two groups.

DISCUSSION

In terms of energy intake, milk output and body-weight change, the lactating rats which received the control diet were comparable to the lactating rats described by many other workers (see, for example, Ota & Yokoyama, 1967; Kametaka *et al.* 1974; Cripps & Williams, 1975). Food intake increased during 1–12 d post partum by 77·3%, milk output doubled in the 8 d measurement period and body-weight was maintained.

A supplement was formulated, following a series of preliminary dietary trials, that indicated a general preference of virgin rats for eggs rather than other protein-rich foods; those foods tested included cooked meat, nuts and cheese. The supplement consisted of homogenized eggs, maize oil and salt cooked to a solid consistency, and had the same P:E value as the control diet (0·20). When the supplement was provided during lactation, energy and protein intakes increased by 19·7%, on average. This increase was distributed evenly over the period of study and thus the normal pattern of lactation-induced hyperphagia was observed, although at a higher level than normal. The rats consuming this high intake produced significantly more milk than did those consuming the control diet alone, and this was reflected in faster rates of growth and fat and protein deposition in the litters. The concentrations of fat, protein and carbohydrate in milk were not significantly affected by dietary supplementation.

Rolls *et al.* (1980) have reported that rats provided with a high-energy supplement during lactation are hyperphagic compared with dams consuming a standard diet, but that litter growth rate is reduced, which indicates a reduction in milk output. The food supplement used in the present study was shown to have no effect on lactation when provided together with the control diet at the same level of energy intake as that consumed by dams fed *ad lib.* with the control diet alone. Therefore, it seems likely that the difference in results between the present study and that of Rolls *et al.* (1980) may be due mainly to the low protein content (P:E value 0·04–0·08) of the supplementary foods which they used. This suggestion is consistent with the results of studies on lactational performance in relation

to dietary protein concentration in rats, which have shown that the use of diets with P:E values less than 0·16 is associated with low milk output (Venkatachalam & Ramanathan, 1964; Menaker & Navia, 1973).

One explanation for the finding that lactating rats provided with a supplement produced more milk than did rats receiving only the control diet, is that the rats given only the control diet may have been in a state of relative energy deficiency, therefore producing less milk than they were capable of producing and not satisfying the milk requirements of the pups. This would be possible if the control diet became less palatable when consumed in large amounts, with the result that there was a partial restriction in food intake. However, there were no indications that this change occurred. An alternative explanation of the results is that the amount of energy and nutrients voluntarily consumed by lactating rats may have some direct influence on milk synthesis rate.

Dietary supplementation during lactation did not significantly affect body protein content, but reduced the extent of fat mobilization normally associated with lactation (Steingrimsdottir *et al.* 1980; Naismith *et al.* 1982). Additionally, dams given the supplement gained weight unlike dams fed on the control diet alone, which maintained weight. In view of the finding that supplementation did not cause body nutrient deposition, it is likely that the weight gain reflected an increase in tissue hydration (which in any case is high in lactation; see Spray, 1950) or an increase in gut water content. However, these possibilities were not tested experimentally.

In conclusion, we have found that lactating rats fed *ad lib.* respond to dietary supplementation by increasing their food intake and their milk output. This result is in contrast to reports that dietary supplementation does not increase breast milk output in women.

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