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Dietary Regulation of Mammary Lipogenesis in Lactating Rats

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The proportion of medium-chain fatty acids ($C_{8:0}$, $C_{10:0}$ and $C_{12:0}$) in rat milk increased significantly between day 4 and day 8 of lactation and for the remainder of lactation these acids comprised 40–50mol% of the total fatty acids. The milk fatty acid composition from day 8 was markedly dependent on the presence of dietary fat and altered to include the major fatty acids of the fats fed (peanut oil, coconut oil and linseed oil). The distribution of fatty acids made within the gland, however, was independent of dietary lipid and $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$ acids accounted for over 70% of the fatty acids made. The rates of lipogenesis in both the mammary gland and liver determined *in vivo* after the administration of ^3H_2O were affected by the presence of dietary lipid. In the mammary gland the rate for rats fed a diet containing peanut oil for 7 days was only one-fifth that for rats fed a fat-free diet. Coconut oil also suppressed lipogenesis. Both dietary fats also suppressed lipogenesis in the liver.

It has been known for some time that as well as the long-chain fatty acids found in animal tissues, the milk of several species including the rat and the rabbit contain significant proportions of medium-chain fatty acids (C_8–C_{12}) (Glass *et al.*, 1967). Studies with lactating rabbits have shown that these unusual fatty acids are synthesized within the mammary gland as a result of the activity of a specific medium-chain thioesterase, which prematurely terminates chain elongation by the fatty acid synthetase (Knudsen *et al.*, 1976; Chivers *et al.*, 1977). A similar enzyme has been found in the mammary gland of lactating rats (Libertini & Smith, 1978, 1979).

These experiments were initiated to investigate whether the fatty acid composition of rat milk, and specifically the proportion of medium-chain fatty acids, is altered by changes in the diet or by the stage of lactation. The distribution of fatty acids synthesized within the gland was determined under each of these experimental situations and compared with the fatty acid composition of the milk. The rates of lipogenesis in both the mammary gland and the livers of these rats were also determined and correlated to the dietary treatments.

Experimental

Pregnant rats of the Wistar strain were purchased from the University of Otago Animal Breeding Station. Litter sizes were between eight and 14 pups. Lactating rats were fed *ad libitum* either a commercial pelleted 'breeder' diet (A. E. Reeves, Dunedin, New Zealand) or one of five semi-synthetic diets. These diets consisted of (by wt.) 25% starch, 30% sucrose, 20% casein, 1% vitamin mix (Goulding & Malthus, 1969), 4% salt mix (Hubble *et al.*, 1937) and the remaining 20% comprised peanut oil, coconut oil, linseed oil or starch (fat-free diet). The three oils were chosen because of their widely different fatty acid compositions and were found to contain as major acids: peanut oil, $C_{18:1}$ (46%), $C_{18:2}$ (36%); coconut oil, $C_{12:0}$ (49%); linseed oil, $C_{18:3}$ (62%). The pelleted diet contained 10.2% lipid. After 4 days on any one diet, the mothers were separated from the pups and after 2h were treated with 20mg of a muscle relaxant, ketamine (Ketalar® Parke Davis, Caringbah, N.S.W., Australia) and 10 units of oxytocin and milked. To determine the distribution of fatty acids made within the gland rats were injected intraperitoneally with 25μCi of [U-^{14}C]glucose (sp. radioactivity 4mCi/mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.) 2h before milking.

The milk was diluted approx. 5-fold with water and equal volumes of methanol and chloroform were added. The lipids were recovered by evaporation of a chloroform layer and redissolved at 50mg/ml of chloroform. Lipid samples (5mg) were heated in capped tubes at 100°C for 20min in 2ml of 1.6M-HCl in methanol and the methylated fatty acids were extracted into hexane before separation by g.l.c. To avoid potential losses of the medium-chain fatty acid methyl esters the hexane extracts were not concentrated. An analytical column of 10% DEGS-PS on Chromosorb WAW (100–120) was used with the temperature programmed from 80 to

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190°C at 8°C/min. For preparative g.l.c. a column of 10% SP2300 on Chromosorb WAW (100–120) was used with a splitter (2:1) mounted between the column and the detector. Fractions were collected in Pasteur pipettes on chloroform-soaked glass wool. Fatty acids were washed into scintillation vials with toluene and after adding scintillant (Cooper & Grigor, 1978) the vials were counted for radioactivity in a Packard liquid-scintillation spectrometer. All g.l.c. materials were supplied by Supelco Inc., Bellefonte, PA, U.S.A.

The rates of lipogenesis were determined at day 14 of lactation after the animals had been on the test diet for 7 days. Rats were removed from their pups and immediately injected intraperitoneally with 4mCi of ^3H_2O (The Radiochemical Centre) in a volume of 1ml. After 1h the mothers were killed and duplicate samples of mammary gland (approx. 1g), whole livers and blood were taken. The livers were homogenized in water and samples of the homogenate equivalent to 1g of liver along with the mammary gland samples were digested in KOH (1g in 5ml of water/ethanol, 1:1, v/v) for at least 2h at 100°C in capped tubes. After cooling, 2ml of concentrated HCl were added and the lipid was extracted three times with 10ml of hexane. The combined hexane extracts were washed three times with 10ml of water and evaporated to dryness. Two samples of the lipid (equal to one-fifth the total lipid) were then counted for radioactivity in the presence of the scintillant. Plasma samples were diluted and also counted for radioactivity. From the ratio of the lipid activity per g of tissue to the plasma specific activity the rate of lipogenesis was calculated as μmol of water incorporated/g per h. This procedure requires the assumption that the sp. radioactivity of the plasma water is the same as that of the intracellular water. The duplicate samples from each gland gave similar results, showing that the sample size taken was sufficiently large to be representative of the gland. For selected samples g.l.c. analyses of the fatty acids of the digested mammary tissue were performed and these showed that there had been no loss of the medium-chain acids in the procedure.

Results

In our initial experiment the effect of the stages of lactation on the distribution of milk fatty acids was investigated. A marked and highly significant increase in the concentration of the medium-chain acids ($P<0.01$) was observed between the samples taken on day 4 and day 8 (Fig. 1). For the remainder of the lactation the composition tended to remain constant although a slight increase in the medium-chain acids occurred again at the end of the lactation period so that, by this stage, they represented over 50% of the total fatty acids in the milk. Compensating decreases in the proportion of long-chain acids, particularly oleic acid, occurred. No significant differences could be detected in the distribution of fatty acids made within the gland from [U-^{14}C]glucose at any stage of lactation and greater than 70% of the acids made within the gland had medium-chain lengths (Table 1).

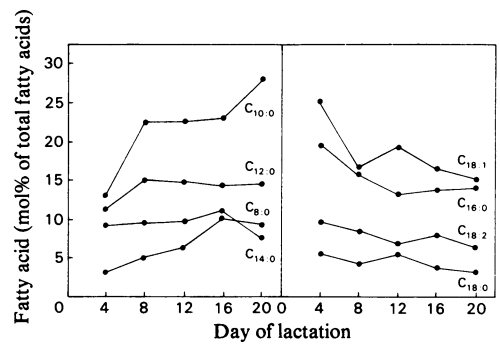

Fig. 1. *Effect of stage of lactation on milk fatty acid composition*

Rats were fed a pelleted diet and milked on the days indicated. The mol% of each fatty acid was calculated after g.l.c. analysis as described in the text. Points represent the means of analyses of individual milk samples from eight rats (day 4) and four rats (other days). For clarity standard-deviation bars have been omitted. The standard deviations amounted in some cases to over 30% of the mean value (see the Discussion section). The difference in the sum of the medium-chain acids ($C_{8:0}+C_{10:0}+C_{12:10}$) was statistically significant ($P<0.01$) between days 4 and 8, but not for any other interval. The result for $C_{16:1}$ fatty acid, which comprised approx. 2% in all the analyses, is not shown.

The effect of diet on milk fatty acid composition was then investigated. The animals were milked between day 8 and day 20 of lactation after 4 days on any one diet. Because no trends were apparent with time for this stage of lactation, these results have been averaged for the rats on each diet. Marked differences were, however, noted between the milk fatty acids from the rats on the different diets (Table 2). In each case the fatty acid composition appears to be a combination of that of the dietary fat fed and the milk of a rat receiving the fat-free diet. However, the distribution of glucose label incorporated into fatty acids did not change with dietary treatment and was similar to that for the rats fed pellets (Table 1).

Significant differences were observed in the rates of lipogenesis in both the mammary gland and the liver of the rats receiving the different diets (Table 3). The rates were significantly higher in both tissues from the rats receiving the fat-free diet. Of the fats

Table 1. *Distribution of fatty acids made within mammary gland from [U-^{14}C]glucose*

The results are expressed as mol% of acids of each carbon number calculated from the total radioactivity found in each fraction. The values are the means with standard deviations shown in parentheses for *n* rats on each diet, except for pellets, where results are means for 19 estimations made on samples from eight rats. Although experiments were performed at different stages of lactation no differences were apparent in the distributions observed for the stage of lactation. Only the difference in C_8 acid between pellets and the fat-free diet was statistically significant ($P < 0.02$).

Fatty acid chain length	Diet ...	Fatty acid distribution				
		Pellets	Fat-free	Peanut oil	Coconut oil	Linseed oil
C_8		11 (4)	4 (2)	11 (4)	7 (2)	14 (7)
C_{10}		37 (7)	32 (10)	34 (6)	39 (5)	31 (4)
C_{12}		22 (4)	24 (2)	22 (2)	24 (2)	18 (7)
C_{14}		13 (5)	19 (4)	15 (5)	15 (2)	13 (3)
C_{16}		10 (6)	16 (8)	13 (3)	10 (4)	16 (5)
C_{18}		5 (4)	4 (1)	5 (2)	3 (1)	6 (2)
Number of rats (*n*)		8	6	4	3	4

Table 2. *Effect of dietary lipid on fatty acid composition of rat milk*

Values represent the mol% of each fatty acid with standard deviations shown in parentheses for samples from *n* rats in each group milked between days 8 and 20 of lactation. No marked trends were detected within any group over this period of lactation.

Fatty acid	No. of rats ...	Fatty acid composition			
		Fat-free	Peanut oil	Coconut oil	Linseed oil
		6	7	7	4
$C_{8:0}$		5 (3)	7 (6)	4 (4)	4 (2)
$C_{10:0}$		19 (7)	13 (5)	14 (5)	12 (2)
$C_{12:0}$		19 (3)	7 (1)**	34 (5)**	6 (2)**
$C_{14:0}$		12 (3)	4 (1)**	15 (2)	6 (3)
$C_{16:0}$		20 (3)	15 (4)	16 (3)	14 (4)
$C_{16:1}$		3 (1)	1 (1)**	2 (1)	1 (–)**
$C_{18:0}$		2 (1)	3 (1)	2 (1)	3 (1)
$C_{18:1}$		18 (4)	29 (5)**	10 (4)	17 (4)
$C_{18:2}$		2 (1)	21 (3)**	1 (1)	11 (2)**
$C_{18:3}$		–*	–	–	25 (8)**

* Less than 1%.

** Difference from value for rats fed on the fat-free diet was statistically significant at $P < 0.01$.

Table 3. *Effect of diet on rates of lipogenesis in mammary gland and liver and milk-fat secretion*

Rates of lipogenesis were determined *in vivo* after injection of ^3H_2O as described in the text. The milk fat secreted was determined as twice the product of the weight gain of the pups and the fat content of the milk samples. Values are means ± S.D. for the numbers of animals in parentheses. Differences from values for rats fed a fat-free diet were statistically significant by *t* test at: *$P < 0.05$; **$P < 0.02$; ***$P < 0.01$.

Diet	Rate of lipogenesis in mammary gland (μmol of water/g per h)	Rate of lipogenesis in liver (μmol of water/g per h)	Liver wt. (g)	Rate of lipogenesis in liver (μmol of water/liver per h)	Wt. gain of pups (g/day)	Milk fat (mg/g)	Milk fat secreted (g/day)
Pellets	70 ± 15**	14 ± 7***	14.4 ± 2.6	220 ± 150***	12.6 ± 2.4	150 ± 15	4.2 ± 1.0
	(5)	(6)	(6)	(6)	(5)	(3)	(3)
Fat-free	148 ± 58	57 ± 20	17.3 ± 2.1	1005 ± 410	11.7 ± 4.3	158 ± 53	3.6 ± 1.5
	(5)	(5)	(6)	(5)	(6)	(6)	(6)
Peanut oil	33 ± 18***	21 ± 9***	12.7 ± 3.1**	255 ± 90***	18.7 ± 6.3**	146 ± 33	5.2 ± 1.7
	(6)	(6)	(6)	(6)	(7)	(7)	(7)
Coconut oil	68 ± 38*	25 ± 8***	13.2 ± 1.6***	320 ± 120***	12.4 ± 2.4	188 ± 42	4.7 ± 1.6
	(6)	(5)	(6)	(5)	(7)	(7)	(7)

fed, peanut oil caused the greatest suppression of lipogenic rate in the mammary gland, although no differences for the various fats were detectable in the liver. An unexpected observation was the increased size of the livers of the rats receiving the fat-free diet. This meant that the differences in total lipogenic capacity of these livers were even greater than those per unit mass.

The diet fed appeared to alter the growth rate of the litters (Table 3). In particular the pups of the rats fed the peanut oil gained weight more rapidly than those from the rats fed the other diets. The daily milk production was estimated as twice the growth rate of the litters (Cowie, 1969) and from this the amount of milk fat secreted per day was calculated. It would appear that the rats fed the peanut oil diet secreted more milk fat per day than those fed the fat-free diet, although with the number of animals used this difference was not statistically significant.

Discussion

The fatty acids found in milk have two sources. They may be derived from the circulation or be the product of lipogenesis in the mammary gland itself. The incorporation of dietary fatty acids into milk lipids has been described previously in studies with a number of species including humans (Insull *et al.*, 1959; Beare *et al.*, 1961; De Man & Bowland, 1963; Tollerz & Lindberg, 1965; Smith *et al.*, 1969; Scott *et al.*, 1971; Mellies *et al.*, 1979). Because of the presence of significant proportions of medium-chain fatty acids in the milk of the rat, this species was considered eminently suitable for further studies of the regulation of milk-fat composition. A number of reports have shown that the medium-chain acids found in the milks of the rat, rabbit and mouse are synthesized within the gland (Dils & Popjak, 1962; Smith *et al.*, 1969; Strong & Dils, 1972; Libertini & Smith, 1978) and this is confirmed by our experiments with the incorporation of labelled glucose into the milk fatty acids.

It was noteworthy, however, that the distribution of the acids made from the glucose was independent of both the stage of lactation and the diet fed. Of the test fats, coconut oil was chosen because it contains over 45% lauric acid ($C_{12:0}$), but even this fat failed to alter the distribution of the fatty acids made. This observation suggests that in the rat, at least, the thioesterase responsible for the synthesis of the medium-chain acids is not regulated independently of fatty acid synthesis.

With the gland making predominantly the medium-chain acids, the long-chain acids, in particular the C_{18} acids, of the milk fat must come from the circulation and in most cases will originate in the diet. However, for the rats fed the fat-free diet, the liver is the most probable source of these acids. Evidence for this came from a preliminary experiment where milk was taken from rats 24 and 48h after injection of [U-^{14}C]glucose and the C_{18} acids were found to be the most strongly labelled.

The changes in milk fatty acid composition with the stage of lactation reported here are similar to those that occur in the rabbit in the early part of lactation (Hall, 1971). The increase in the proportion of the medium-chain acids is likely to be a consequence of an increase in the proportion of the fatty acids made in the gland relative to those from the circulation. Thus, as the total amount of milk fat secreted increases, a greater proportion would be synthesized within the gland. The gland, however, does not have the ability to determine these proportions precisely. It was noted that in a group of rats fed the peanut oil diet the fatty acid composition varied considerably for each rat for several milkings over a period of 3 days. The fatty acid distributions of the medium-chain acids considered alone or the long-chain acids considered alone were always very similar and the differences were in the relative amounts of the medium- to long-chain acids. These differences did not appear to be related to the time of day and are the cause of the large standard deviations found for any one fatty acid in the compositions reported in the present paper (Fig. 1, Table 2).

Although the rate of lipogenesis in the livers of rodents has been known for some time to be affected by dietary fats (Romsos & Leveille, 1974), the experiments of Smith *et al.* (1969) suggest that the rate of lipogenesis in the mouse mammary gland as determined *in vitro* is not sensitive to dietary lipid. Recently Romsos *et al.* (1978) have shown that the rate of lipogenesis in the mouse gland determined *in vivo* using ^3H_2O is sensitive to the presence of dietary lipid and our results with a similar assay show that the rat gland is equally as sensitive as the liver in its response to dietary lipid. In the liver such regulation is affected largely through changes in the rates of synthesis and consequently the amounts of key lipogenic enzymes rather than the short-term regulation of their activity (Craig *et al.*, 1972; Volpe *et al.*, 1973). In the experiments carried out by Smith *et al.* (1969), the test diets were fed for only 3 days and the activities of several lipogenic enzymes were observed to be similar for all dietary treatments. In contrast, the activities of several lipogenic enzymes of the liver were considerably modified by the dietary treatment. Recently, Robinson *et al.* (1978) have used the ^3H_2O assay to investigate the effect of starvation on mammary lipogenesis in lactating rats *in vivo*. They observed that starvation for 24h markedly suppressed the rate of lipogenesis, but the rate was restored to its normal value within 2h of refeeding. The rapid change after refeeding sug-

gested that the suppression caused by starvation was a result of enzyme inhibition rather than changes in enzyme amounts. Taken together these results suggest the rates of synthesis and degradation of the important lipogenic enzymes are much less sensitive to dietary treatment in the mammary gland than in the liver. The longer feeding period used by Romsos *et al.* (1978) and in our experiments (respectively 5 and 7 days) make it likely that the changes in rates of lipogenesis that we have observed are consequences of changes in the amounts of the key lipogenic enzymes in the mammary gland. A very extensive literature on the short-term regulation of mammary lipogenesis has been reviewed by Bartley & Abraham (1976).

In summary, the combined effect of the incorporation of the long-chain fatty acids from the diet and the suppression of mammary lipogenesis is to cause major changes in the relative amounts of medium-chain and long-chain acids in the milk fat. This is likely to cause changes in the physical properties of the milk fat, since it is the proportions of dodecanoic acid and tetradecanoic acid rather than decanoic acid and octanoic acid that change the most.

This raises the question as to what function these medium-chain acids might have in milk. Although they are found mainly in the milk of herbivores (Glass *et al.*, 1967), there are a number of such species that do not produce them. Among closely related species there is some evidence that their presence is related to the level of immaturity of the young at birth. For example, among the rodents, rat milk may contain up to 50mol%, whereas guinea-pig milk contains no medium-chain acids. Also among the leporidae, hare milk contains much lower amounts of the medium-chain acids than does rabbit milk (Demarne *et al.*, 1978). However, several lines of evidence would argue against the medium-chain acids having an essential role in the nutrition of very immature animals. Marsupial milk is notably devoid of these acids (Glass *et al.*, 1967; Griffiths *et al.*, 1972; Grigor, 1980), whereas high concentrations are found in both mare's milk and elephant milk (Glass *et al.*, 1967; McCullach *et al.*, 1969). Furthermore, the observation that the proportion of these acids increases rather than decreases during the period of lactation in both the rat and rabbit would argue against such a hypothesis.

Alternatively it may be that the gland has evolved to synthesize these acids as a means of sparing glucose carbon, although producing the same molar amount of lipid. This would allow more of the glucose to be used for lactose synthesis or oxidized within the mammary cells. However, no correlation is observed between the lactose content of the milk and the content of medium-chain fatty acids (Jenness, 1974).

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