Diurnal variations in food intake and in lipogenesis in mammary gland and liver of lactating rats

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1. Despite the hyperphagia, the food intake of the lactating rat showed marked diurnal changes which paralleled those of virgin rats. The major difference was that lactating rats consumed a higher proportion (35%) of their diet during the light period than did virgin rats (14%). 2. The peak rate of lipogenesis in the lactating mammary gland occurred around midnight, and this decreased by 67% to reach a nadir around mid-afternoon; this corresponded with the period of lowest food intake. The diurnal variations in hepatic lipogenesis in lactating rats were much less marked. 3. The changes in hepatic glycogen over 24 h suggest that it acts to supply carbon for lipogenesis during the period of decreased food intake. 4. The activation state of acetyl-CoA carboxylase in mammary gland altered during 24 h, but the changes did not always correlate with alterations in the rate of lipogenesis. 5. The changes in plasma insulin concentration tended to parallel the food intake in the lactating rats, but they did not appear to be sufficient to explain the large alterations in lipogenic rate in the mammary gland.

At peak lactation (10–14 days) the mammary gland is the major site of lipogenesis in the rat (Robinson *et al.*, 1978). Previous work has shown that the rate of lipogenesis (measured with $^3\text{H}_2\text{O}$; Jungas, 1968) in the lactating gland is very sensitive to the composition and availability of the diet; for example, a high-fat diet decreases the rate of lipogenesis (Grigor & Warren, 1980; Agius *et al.*, 1980), as does an oral load of triacylglycerols (Agius & Williamson, 1980). Withdrawal of food for 24 h decreases the rate of lipogenesis by over 90%, and this can be restored by re-feeding for 2 h (Robinson *et al.*, 1978; Munday & Williamson, 1981).

There is a marked diurnal rhythm in the rate of lipogenesis *in vivo* in liver and adipose tissue of mice fed on a starch-based diet (Hems *et al.*, 1975; Cornish & Cawthorne, 1978), the peak rate of synthesis (measured with $^3\text{H}_2\text{O}$) occurring during the dark period, when mice consume most of their food. Increased rates of hepatic fatty acid synthesis (measured with ^{14}C acetate) during the dark period have also been found in the rat (Kimura *et al.*, 1970). The dietary intake of the rat is increased by some 300% at peak lactation (Fell *et al.*, 1963;

Cripps & Williams, 1975), and it therefore might be expected that this hyperphagia would alter the feeding pattern of the lactating rat. There is evidence that the percentage of the daily food intake consumed during the light period is greater in lactating rats than in virgin rats (Tachi *et al.*, 1981). Diurnal variations in the rate of lactose synthesis in mammary gland *in vivo* have been reported (Carrick & Kuhn, 1978), and this process appears to be sensitive to the amount of food consumed (Wilde & Kuhn, 1979). The present paper addresses the question whether there is a diurnal rhythm in the rate of lipogenesis in the lactating mammary gland of the rat and whether this is related to the food intake pattern. In addition, measurements are reported of the diurnal variations of hepatic glycogen, the activity of acetyl-CoA carboxylase in mammary gland and of plasma insulin.

Experimental

Materials

Rats of the Wistar strain (200–300 g) with between eight and ten pups were used after a lactation period of between 10 and 14 days. Virgin rats weighed 200–250 g. Rats were fed on PRM diet (E. Dixon and Sons Ltd., Ware, Herts., U.K.) *ad libitum* and were maintained on a constant lighting

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schedule (lights on 08:00–20:00h). Food intake was measured at 3–4 h intervals for 24 h.

All biochemicals were obtained from Boehringer Corp. (London).

$\text{NaH}^{14}\text{CO}_3$ and $^3\text{H}_2\text{O}$ was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

The rate of lipogenesis in mammary gland and liver *in vivo* was measured by the incorporation of $^3\text{H}_2\text{O}$ into saponified lipid essentially by the method of Stansbie *et al.* (1976), with the modifications described by Robinson *et al.* (1978).

Hepatic glycogen was determined by removal of a piece of liver (about 2 g) from an anaesthetized rat (60 mg of Nembutal/kg body wt.) and clamping it between light-alloy tongs cooled in liquid N_2 (Wollenberger *et al.*, 1960). The frozen tissue was then treated as described by Robinson & Williamson (1977), and the glycogen was determined enzymically (Keppler & Decker, 1974) in the neutralized extracts.

The activity of acetyl-CoA carboxylase in mammary gland was determined by the incorporation of $\text{NaH}^{14}\text{CO}_3$ into acid-stable materials (Majerus *et al.*, 1968; Halestrap & Denton, 1973) as described by Munday & Williamson (1982).

Plasma insulin was measured by radioimmunoassay with charcoal phase separation as described by Albano *et al.* (1972). The plasma was obtained from an arterial blood sample collected into heparinized tubes from the abdominal aorta.

Results and discussion

Food intake

In general agreement with previous studies (Fell *et al.*, 1963; Cripps & Williams, 1975), the total daily intake of food by lactating rats at peak lactation was about 380% higher than that of virgin rats (Fig. 1). Only 14% of the daily intake was consumed by virgin rats during the light period (Fig. 1; Kimura *et al.*, 1970; Bruckdorfer *et al.*, 1974), whereas the lactating rats consumed 35% during the same period (Fig. 1; Tachi *et al.*, 1981). From 20:00 to 08:00h in the present study, virgin rats exhibited the bimodal feeding pattern previously reported in male rats (Siegel, 1961) and virgin females (Tachi *et al.*, 1981). This has been assumed to represent two active periods of feeding separated by a period of satiety. This pattern is gradually lost *post partum*, until at peak lactation it has become a single peak (Fig. 1; Tachi *et al.*, 1981). Nevertheless, the profiles of the food intake in virgin and lactating rats were similar, and it is noteworthy that the marked diurnal variation at peak lactation is not obscured by the hyperphagia.

Fig. 1. Food intake in virgin and lactating rats during 24 h

For details see the Experimental section. The results are mean values $\pm$ S.E.M. for six virgin and 19 lactating rats and are expressed as g of diet consumed/h.

Lipogenesis

The peak of mammary-gland lipogenesis was around midnight, and then it declined 3-fold to a nadir around 15:00h (Fig. 2), when food intake was considerably decreased (Fig. 1). It is noteworthy that lactose synthesis in the gland is lowest at 18:00h (Carrick & Kuhn, 1978), when lipogenesis is apparently increasing again in conjunction with food intake. Lactating rats spend more time with the litter during the light period than during the dark period (Grotta & Adler, 1969). This suggests that milk intake is increased during the light period; however, direct measurements of the ^3H in the saponified lipids of the gastric contents of the suckling pups indicate that the transfer of labelled lipid during the experiment (60 min) is less than 5% of that in the mammary gland (D. H. Williamson, unpublished work). The pattern of lipogenesis in the liver also tended to parallel food intake, but the diurnal changes were less striking than those for the rate of mammary-gland lipogenesis (Fig. 2) or those reported for hepatic lipogenesis in normal and *ob/ob* mice (Hems *et al.*, 1975). Diurnal variations in hepatic glycogen have previously been reported in rats and mice (Ågren *et al.*, 1931; Nowell, 1970), where the glycogen content was lowest in the light period and reached a peak in the dark period of the cycle. This was observed in the liver of the lactating rat, where glycogen content increased by 130% from nadir to peak (Table 1).

Interestingly, the peaks of both liver and mammary-gland lipogenesis (00:30h) preceded the peak content of hepatic glycogen (07:00h). Thus

between 00:30 h and 07:00 h, when food intake was still high and yet mammary-gland and hepatic lipogenesis were declining, there was a major replenishment (90% increase) of the hepatic glycogen stores. This glycogen was subsequently utilized during the light period, reaching its nadir at 19:00 h.

There is thus an indication that, during the hours of light, when food intake is relatively decreased, hepatic glycogen plays an important role in supplying the demand for carbohydrate. Perhaps, in part, it

Fig. 2. Rates of lipogenesis in liver (□) and mammary gland (■) and plasma concentration of insulin (○) in lactating rats during 24 h

For details see the Experimental section. The results are mean values $\pm$ S.E.M. for at least nine lactating rats.

acts as a precursor for hepatic (and possibly mammary-gland) lipogenesis, as predicted in mice by Hems and co-workers (Salmon *et al.*, 1974) and rats (Clark *et al.*, 1974) and shown experimentally in cycloheximide-treated rats (Roberts *et al.*, 1982).

Acetyl-CoA carboxylase

Changes in the activity of acetyl-CoA carboxylase have been implicated in the regulation of lipogenesis in mammary gland and other lipogenic tissues. Acute changes in the initial activity of the enzyme in response to different hormonal treatments occur *in vivo* and *in vitro* in liver (Stansbie *et al.*, 1976; Geelen *et al.*, 1978; Witters *et al.*, 1979), adipocytes (Brownsey *et al.*, 1979) and lactating mammary gland (McNeillie & Zammit, 1982; Munday & Williamson, 1982). In all these tissues insulin has been shown to increase rapidly the initial activity of acetyl-CoA carboxylase. The total activity of the enzyme and its activation state decrease in mammary gland on starvation for 24 h, and it has been suggested that these changes play a role in the decrease in lipogenesis in this situation (McNeillie & Zammit, 1982; Munday & Williamson, 1982). Although the initial activity of the enzyme (measured in the presence or absence of 20 mM-citrate) was higher at the period of maximum food intake, changes in activation state during 24 h did not always correlate with the rate of lipogenesis (10:30 h), and do not appear sufficient (less than 50%; Table 2) to account for the large diurnal changes in lipogenesis (Fig. 2).

Table 1. Hepatic glycogen concentration in lactating rats during 24 h

Hepatic glycogen was determined as described in the Experimental section. The results are mean values $\pm$ S.E.M., with the numbers of observations in parentheses.

Time of day (h)	Glycogen content (μmol/g wet wt.)
00:30	147 ± 9.2 (9)
07:00	239 ± 12.2 (8)
10:30	206 ± 24.5 (8)
15:00	148 ± 21.9 (8)
19:00	103 ± 8.2 (7)

Table 2. Acetyl-CoA carboxylase activity in mammary gland during 24 h

Acetyl-CoA carboxylase activity was measured as described in the Experimental section. Where indicated, the activity was measured in the presence of 20 mM-citrate. Results are mean values $\pm$ S.D. for six observations.

Time of day (h)	Initial activity (% of total)		Total activity	
	0 mM-Citrate	20 mM-Citrate	(munits/min per mg of protein)	(units/g wet wt. of tissue)
00:30	28.6 ± 1.6	78.4 ± 1.6	50.9 ± 3.1	3.63 ± 0.20
07:00	19.8 ± 2.0	70.2 ± 2.8	41.2 ± 3.8	2.73 ± 0.45
10:30	32.0 ± 1.7	77.4 ± 1.6	45.7 ± 3.8	4.63 ± 0.47
15:00	16.9 ± 2.3	61.2 ± 5.0	39.8 ± 4.7	3.32 ± 0.46
19:00	19.1 ± 1.1	66.9 ± 1.6	55.9 ± 5.7	3.72 ± 0.49

Plasma insulin

Carrick & Kuhn (1978) have reported a diurnal variation in plasma insulin, with minimum values occurring during daylight hours, and a similar pattern has been found for non-lactating rats (Kaul & Berdanier, 1972; Bellinger *et al.*, 1975). In the present study the highest values for plasma insulin correspond to the peak of food intake (Fig. 2), but, like the previous findings (Carrick & Kuhn, 1978), the decrease in the concentration during the light period was not more than 50%. No appreciable diurnal changes in plasma glucose have been observed at this stage of lactation (Carrick & Kuhn, 1978). The absence of any large changes of plasma insulin or glucose at peak lactation, despite the hyperphagia and diurnal changes in food intake, is one of the remarkable features of the lactating rat.

Conclusions

The present studies indicate that there are marked diurnal changes in the rate of lipogenesis in the lactating mammary gland of the rat and that the magnitude of the changes is similar to those observed in liver, white and brown adipose tissue of the mouse (Hems *et al.*, 1975; Cornish & Cawthorne, 1978). The rate of lipogenesis in the gland correlates well with the food intake during the previous 4 h ($r = 0.69$; $n = 24$), and this suggests that there is a 'signal' which informs the gland of the dietary status of the lactating rat (see also Carrick & Kuhn, 1978). Although plasma insulin would seem a prime candidate for such a signal, the relatively small changes in concentration of this hormone and the difficulty in correlating these changes with changes in the rate of lipogenesis suggest the existence of other signals which act either synergistically with insulin or antagonistically to it. A similar conclusion has been reached with regard to the large decrease in lipogenesis in the lactating gland on short-term (6 h) withdrawal of food (Williamson *et al.*, 1983). One such alternative signal is the concentration of acetoacetate, which inhibits glucose uptake and lipogenesis in the lactating mammary gland of the rat (see Williamson, 1980). However, it is unlikely that ketone bodies reach sufficiently high concentrations in the blood of the normally fed lactating rat to affect the uptake of glucose by the gland. It is of course possible that the plasma concentration of insulin does not accurately represent the insulin status of the gland, either because of acute changes in blood flow and therefore in rate of delivery of hormone or because of alterations in the amount of hormone bound to its receptors in mammary tissue.

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