Physiological significance of altered insulin metabolism in the conscious rat during lactation

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1. Uptake of radioactively labelled insulin by the mammary gland of the rat increased 12-fold in lactation compared with non-lactating controls. 2. This uptake was decreased by the presence of unlabelled insulin, indicating that it occurred via insulin receptors. 3. The plasma half-life of insulin is decreased in lactation from 9.4 min to 4.8 min, and the metabolic clearance rate for insulin increased from 7.26 to 13.03 ml/kg body wt. per min. 4. The basal insulin and glucose concentrations in the plasma were decreased in lactation. 5. Infusion of insulin at a dose which led to a small physiological rise in plasma insulin concentration increased lipogenic rates in the mammary gland by 100% without causing marked hypoglycaemia. 6. It is concluded that the lactating mammary gland is a highly insulin-sensitive tissue and that the lower plasma insulin during lactation occurs primarily as a result of this sensitivity increasing extraction of glucose by the gland and thus producing a decrease in the plasma glucose concentration. 7. It is suggested that a secondary result of the fall in plasma insulin concentration is the preferential direction of substrates (glucose and non-esterified fatty acids) towards the lactating mammary gland and away from adipose tissue and the liver.

Glucose is the major substrate for synthesis of lactose and fatty acids by mammary gland of non-ruminants, but the importance of insulin in the regulation of metabolism in the lactating gland remains open. Low plasma concentrations of insulin are observed in several species during lactation (Robinson *et al.*, 1978; Flint *et al.*, 1979; Lomax *et al.*, 1979; Lenz *et al.*, 1981; Burnol *et al.*, 1983), and, indeed, an inverse relationship has been reported between plasma insulin concentrations and milk yields in cattle (Koprowski & Tucker, 1973).

In vitro and *in vivo* it has been difficult to demonstrate consistent stimulatory effects of insulin on mammary-gland metabolism at physiological concentrations (see Tucker, 1979; Williamson, 1980). However, acute insulin deficiency can depress mammary-gland metabolism (Robinson *et al.*, 1978), and the mammary gland has insulin receptors whose numbers vary in parallel with changes of metabolic activity (Flint, 1982a).

It has been suggested that insulin extraction and degradation by the mammary gland could accommodate the apparent paradox of lowered insulin concentrations (Robinson *et al.*, 1978), since, in the

rat, insulin concentrations vary inversely with the weight of the lactating mammary gland (Wilde & Kuhn, 1979) and in the cow measurements of the arteriovenous difference for insulin have shown that mammary tissue extracts this hormone (Beck & Tucker, 1978).

The following experiments were conducted to quantify the rate of insulin uptake by the lactating rat mammary gland *in vivo* in conscious unrestrained rats and to relate this to the short-term regulation of lipogenesis within the gland.

Methods

Lactating Wistar rats with nine or ten pups were used at peak lactation (10–16 days). Non-lactating controls were rats whose pups had been weaned 3–4 weeks previously. Under general anaesthesia polyethylene cannulae (internal diam. 0.63 mm, external diam. 0.96 mm) were inserted into the right carotid artery for blood sampling, and into the right external jugular vein for intravenous infusions. The cannulae were exteriorized and sutured to the skin over the scapulae, and the animals were allowed to recover for 48 h.

All experiments were conducted with animals in their home cage and with the pups still present. The cannulae were extended to reach outside the cage to facilitate blood sampling, and thus minimal disturbance was caused to the behaviour of the mother and to suckling by the pups.

Glucose was measured enzymically (Slein, 1963), insulin by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972), and rates of lipogenesis *in vivo* were measured by the incorporation of ^3H into non-esterified fatty acids as previously described (Robinson *et al.*, 1978). Iodinated insulin tracer ($50\ \mu\text{Ci}/\mu\text{g}$) was prepared from crystalline pig insulin (Novo, Copenhagen, Denmark) and was purified by gel electrophoresis (Linde *et al.*, 1980).

Low-dose insulin infusion

Conscious unrestrained lactating fed rats were infused through the indwelling venous cannula for 90 min with either 0.9% (w/v) NaCl containing 1% (w/v) bovine serum albumin or a solution of insulin (pig soluble; Actrapid MC, Novo), in 0.9% NaCl containing 1% bovine serum albumin. The insulin concentration was 60 units/ml and rats were infused at a rate equivalent to 120 munits/kg body wt. per h. At 30 min they were given an intraperitoneal injection of $^3\text{H}_2\text{O}$. Blood samples were taken at 0, 15, 30, 60 and 90 min to measure changes in plasma glucose and insulin concentrations.

Relative insulin uptake

Relative uptake of insulin by various tissues was assessed by measuring the distribution of injected iodinated insulin. A tracer dose of ^{125}I -insulin (2 ng/rat; approx. 100 000 c.p.m./rat) was injected intravenously in 0.3 ml of 0.9% NaCl from a pre-

Thus:

Relative concentration index =

$$\frac{\text{Total tissue specific radioactivity}}{\text{Plasma specific radioactivity}} \times 5$$

To confirm that ^{125}I -insulin was extracted intact, samples of liver and mammary gland were also homogenized in ice-cold 1 M-acetic acid containing 7 M-urea and centrifuged at 3000 g for 30 min. The supernatant (1 ml) was chromatographed on a Sephadex G-50 (fine grade) column ($35\text{ cm} \times 1.2\text{ cm}$) with the same solvent at a flow rate of 0.6 ml/min.

In one group of lactating rats, excess unlabelled insulin (6 units) was injected with the labelled insulin. $^3\text{H}_2\text{O}$ (10 mCi/ml) was injected into the peritoneum 30 min before the injection of ^{125}I -insulin.

Insulin half-life

The half-life of plasma insulin was estimated from the rate of decrease of endogenous plasma insulin during the early phase of inhibition of insulin release by an infusion of somatostatin. Baseline blood samples were obtained over a 15 min period. Glucose (200 mg/kg; 20%, w/v) was then injected intravenously to elevate plasma insulin concentrations moderately, and after 5 min an infusion of somatostatin (100 $\mu\text{g}/\text{kg}$ body wt. per h) was started. Blood samples were taken at 2, 4, 6, 8, 10, 15 and 20 min from the start of the infusion. The plasma insulin half-life was estimated by least-squares analysis of the log-linear plot of insulin concentration against time. An estimate of insulin metabolic clearance rate (MCR) was then derived by using the following formula, assuming an insulin space of 85 ml/kg (Halban *et al.*, 1979):

$$\text{MCR (ml/min per kg)} = \text{insulin space (ml/kg)} \times \frac{\ln 2}{\text{Insulin half-life (min)}}$$

weighed syringe. The rats were killed 3 min later by intra-cardiac injection of sodium pentobarbitone. The tissues were immediately dissected from the carcass, the total weights of mammary gland, liver, kidney and heart measured, and weighed samples of each tissue counted for radioactivity in a gamma counter. Results are expressed as the percentage dose of radioactivity taken up by each of the tissues and as a relative concentration index compared with label in the extracellular space. It was assumed that plasma specific radioactivity represented the specific radioactivity of labelled insulin in the extracellular space and that the average extracellular volume of the tissues studied was 20% (Guyton, 1976).

Statistics

Data are presented as means $\pm$ S.E.M. Statistical comparisons were made by Student's *t* test.

Results

Insulin infusions

Infusion of low-dose insulin (120 munits/kg per h) caused a small increase in plasma insulin concentrations (30 munits/l) and a fall in plasma glucose concentrations of about 20% (decrease of 1 mmol/l). A significant increase in the rate of mammary-gland lipogenesis was observed [saline-infused, 73 ± 19 ($n = 14$) μmol of $^3\text{H}_2\text{O}$ incorpor-

ated into saponified lipid/h per g; insulin-infused, $146 \pm 22 \mu\text{mol/h per g}$; $n = 5$; $P < 0.05$]. There was no difference in the lipogenic rates in liver, white adipose tissue or brown adipose tissue (results not shown).

¹²⁵I-insulin uptake

Sephadex G-50 chromatography indicated that most of the radioactivity in all the tissues studied was eluted as intact insulin (results not shown). Consistent with previous published reports, within 3 min of injection the liver accumulated approx. 25% of the labelled insulin in both lactating and non-lactating animals (Table 1) (Sodoyez *et al.*, 1983). In the presence of excess unlabelled insulin, the hepatic extraction was decreased to 7% (Table 1), consistent with uptake of label being mediated via insulin-specific receptors.

Uptake by mammary gland accounted for only 0.6% of the total dose in non-lactating animals, but this increased to 6% in lactation. Competition by unlabelled insulin did not decrease this total uptake, in contrast with the liver. The kidney concentrates label principally by glomerular filtration, and the increase in the fraction of label extracted by this tissue when unlabelled insulin was injected reflects the lower uptake by the liver.

When the data are presented as relative concentration index (Table 2), it is evident that both

mammary gland and liver concentrate label relative to the extracellular space. In both cases the concentrating effect is blocked by excess unlabelled insulin. Hence mammary gland also extracts label via a specific insulin-receptor-mediated process. Because of the lower extracellular water space of adipose tissue, the calculated relative concentration of insulin by this tissue (Table 2) may be an underestimate. However, comparisons between the different groups of animals would not be affected by assuming a different value for extracellular volume.

Somatostatin infusions

The changes in plasma glucose and insulin during the experiment are shown in Fig. 1. Basal plasma insulin and glucose concentrations were significantly lower in lactating rats, as previously described in anaesthetized or otherwise stressed animals (Robinson *et al.*, 1978; Flint *et al.*, 1979; Burnol *et al.*, 1983). Injection of glucose induced an immediate rise in plasma glucose and insulin of comparable size in both groups of animals.

Infusion of somatostatin produced a prompt fall in insulin to concentrations well below basal in both groups. A small decrease in glucose was also observed, as previously described (Chideckel *et al.*, 1975). This was greater in lactating animals and probably reflects the greater rate of peripheral glu-

Table 1. Uptake of ¹²⁵I-insulin by mammary gland, liver, kidney and heart in conscious lactating and non-lactating rats. For experimental details see the text. Results are expressed as the percentage of the total ¹²⁵I label injected that was extracted by each tissue. Values significantly different from those for non-lactating controls are shown: * $P < 0.0001$. Values significantly different from those for lactating animals are shown: † $P < 0.005$; †† $P < 0.0001$.

Tissue	n ...	Non-lactating 4	Lactating 14	Lactating, with excess unlabelled insulin 8
Mammary gland		0.57 ± 0.11	$6.22 \pm 0.88^*$	6.62 ± 1.03
Liver		22.80 ± 4.92	22.46 ± 1.66	$7.08 \pm 0.94^{\dagger\dagger}$
Kidney		9.65 ± 1.64	9.32 ± 0.86	$15.89 \pm 1.67^{\dagger}$
Heart		0.35 ± 0.83	0.35 ± 0.03	0.32 ± 0.02

Table 2. Relative concentration of ¹²⁵I-insulin by mammary gland, liver, kidney, heart and white adipose tissue in conscious lactating and non-lactating rats

Results are expressed as the ratio of the concentration of label within each tissue compared with the concentration of label in the extracellular space. Values significantly different from lactating rats are shown: * $P < 0.02$; ** $P < 0.001$; *** $P < 0.0001$.

Tissue	n ...	Non-lactating 4	Lactating 14	Lactating + unlabelled insulin 8
Mammary gland		2.93 ± 0.65	3.49 ± 0.36	$1.24 \pm 0.19^{***}$
Liver		19.51 ± 1.12	12.55 ± 1.10	$1.16 \pm 0.11^{***}$
Kidney		40.73 ± 2.87	36.15 ± 5.02	$20.29 \pm 3.33^*$
Heart		3.61 ± 0.22	2.96 ± 0.41	$0.92 \pm 0.05^{**}$
White adipose tissue		1.17 ± 0.40	1.11 ± 0.15	0.64 ± 0.13

Table 3. Comparison of basal insulin and glucose concentrations and of insulin metabolism in conscious lactating and non-lactating rats

Values significantly different from those in non-lactating controls are shown: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

	Non-lactating n ... 5	Lactating 6
Plasma insulin (munits/l)	41.3 ± 4.3	$13.9 \pm 3.3^{***}$
Blood glucose (mmol/l)	6.60 ± 0.44	$4.68 \pm 0.33^{**}$
Insulin half-life (min)	9.49 ± 1.63	$4.77 \pm 0.48^*$
Insulin clearance (ml/g per min)	7.26 ± 1.62	$13.03 \pm 1.37^*$

Fig. 1. Changes in plasma insulin and glucose concentrations before and during an infusion of somatostatin in lactating (—) and non-lactating (----) rats

cose consumption and the importance of glucose supplied from the liver under the influence of glucagon in maintaining normoglycaemia.

The changes in plasma insulin concentration when analysed on a log-linear basis demonstrated a more rapid disappearance of insulin in the lactating group. Plasma insulin half-life was significantly decreased ($P < 0.05$) in lactation, and clearance increased (Table 3).

Discussion

Do these results explain the paradoxically low insulin concentration in lactation? We have demonstrated in conscious unrestrained animals that the mammary-gland lipogenic rates may be stimulated by small physiological increments in plasma insulin concentration. This indicates that the gland is highly insulin-sensitive. Failure to demonstrate such sensitivity in the past may be because pharmacological doses of insulin were used, which may have triggered counter-regulatory responses through, for example, hypoglycaemia (see, e.g., Munday & Williamson, 1981). Similarly, the report (Burnol *et al.*, 1983) of euglycaemic glucose clamping in the rat showed only moderate stimulation of lipogenesis in the mammary gland by a 100-fold increase in plasma insulin, but here the animals were under anaesthesia, which may have had additional effects.

The increased metabolic clearance rate of insulin in lactation reported here is consistent with increased insulin-sensitivity. Biological activity of modified or iodinated insulins varies directly with their metabolic clearance rates, an effect explained by insulin binding to its receptor being the initiating step in its action and in the degradation process (Izzo *et al.*, 1972; Jones *et al.*, 1976; Papachristodoulou *et al.*, 1977). In humans, a case of acanthosis nigrans, a condition associated with insulin resistance at the receptor level, has been reported in which insulin metabolic clearance rate was found to be low but where it returned towards normal when insulin resistance was decreased by treatment of the underlying condition (Ferrannini *et al.*, 1982). Lactation may therefore be a physiological state of increased sensitivity where an increase in the metabolic clearance rate of insulin is observed.

The demonstration of an insulin-specific insulin-uptake process indicates that mammary gland binds and degrades insulin via specific receptors, like other insulin-sensitive tissues. The quantitative increase in extraction by the mammary gland is large, parallels the increase in mammary-gland size in lactation (Chatwin *et al.*, 1969), and the increase in mammary-gland insulin-receptor numbers

(Flint, 1982a,b), and could account for the rise in insulin metabolic clearance rate in lactation.

Does uptake by the gland explain the low insulin

Fig. 2 Schematic representation of alterations in insulin physiology in lactation (a) compared with non-lactating controls (b)

The primary increase in mammary-gland insulin-sensitivity in lactation causes an increased flux of glucose towards the mammary gland and a fall in plasma glucose concentration. This leads to a fall in plasma insulin concentration, which decreases (i) insulin stimulation of glucose utilization by adipose tissue and (ii) insulin inhibition of adipose-tissue lipolysis and hepatic glycogenolysis. The net result is a co-ordinated redirection of substrate towards the mammary gland. Relative degrees of insulin stimulation and inhibition are indicated by the signs '+' and '-'. Abbreviation: NEFA, non-esterified fatty acid.

concentration in lactation, and what is the significance to the integration of metabolism in the whole animal? High insulin-sensitivity in the lactating mammary gland would lead to the high observed rate of glucose utilization by this tissue (Williamson, 1980). In turn this would lead to a fall in plasma glucose concentrations, assuming that substrate supply were limiting, and thereby to a secondary fall in plasma insulin if β -cell sensitivity to glucose is unchanged in the lactating rat, as in the human (Lenz *et al.*, 1981), or lowered, as in the cow (Lomax *et al.*, 1979). The net result of these changes would be the direction of substrates towards the mammary gland, since the fall in glucose would increase hepatic glycogenolysis and the lowered plasma insulin concentration would favour lipolysis in adipose tissue. These concepts are shown diagrammatically in Fig. 2. The fact that glucagon concentrations are unchanged in lactation and the demonstration that glucagon does not inhibit glucose metabolism in isolated acini from lactating mammary glands (Wilde & Kuhn, 1981; Williamson *et al.*, 1983; Robson *et al.*, 1984) would augment these effects, because of the fall in the insulin/glucagon ratio (Robinson *et al.*, 1978).

Thus an increase in mammary-gland insulin-sensitivity in lactation would lead to a co-ordinated redirection of substrates towards the mammary gland. The cause of the change in sensitivity and its mechanism of expression are unknown. Prolactin is known to be involved in the reciprocal regulation of the concentration of lipoprotein lipase in adipose tissue (Zinder *et al.*, 1974) and mammary gland during lactation and is a possible causal agent. Weaning, which inhibits prolactin secretion, leads to an acute rise in plasma insulin and glucose concentrations, which would be consistent with a sharp decrease in mammary-gland insulin-sensitivity (Agius *et al.*, 1979; Burnol *et al.*, 1983). Evaluation of the exact mechanism requires further experimentation.

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