INTEGRATION OF METABOLISM IN TISSUES OF THE LACTATING RAT

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1. Introduction

Lactation is characterized by a number of physiological changes including increased cardiac output [1], hypertrophy of liver [1], heart [1], intestine [2,3] and mammary gland [1], and an increase in dietary intake [2] (for details see table 1). In addition, there are profound alterations to the metabolism of the tissues of the lactating rat. The net result of these changes is that a high proportion of the substrates available in the circulation (glucose, triacylglycerols, non-esterified fatty acids and ketone bodies) are 'directed' [4] to the gland for the production of milk. The aim of this contribution is to describe some of the metabolic changes that are known to occur during lactation in the rat and to speculate on the 'signals' (hormones or substrates) which may be involved. Lactation in other species will not be dealt with except where the data are pertinent to the situation in the rat. This contribution is not intended to be a comprehensive review of the area or the literature and merely contains a selection of topics and questions which interest the writer.

Information on the integration of tissue metabolism in the lactating rat is not only of fundamental interest, but is also relevant to the study of dietary-induced obesity, because despite a large increase in dietary intake (table 1) the lactating rat does not

Table 1
The effects of lactation on dietary intake, cardiac output, organ weights and organ blood flow in rats

Measurement	Lactating		Non-lactating Day 12
	Day 1	Day 12	
Body weight (g)	252	315	285
Dietary intake (g/24 h)	18	50	16
Cardiac output (ml/min)	91	143	89
Mammary gland			
Wet weight (g)	13.2	24.2	5.7
Blood flow (ml . 100 g^{-1}. min^{-1})	33	62	12
Liver			
Wet weight (g)	12.8	16.3	11.7
Blood flow (ml . 100 g^{-1}. min^{-1})	38	74	50
Small intestine			
Wet weight (g)	12.2	21.5	10.7
Blood flow (ml . 100 g^{-1}. min^{-1})	66	94	80

Non-lactating rats refers to rats which had pups removed at birth. Data taken from [1] except dietary intake [2]

Dedicated to Professor Sir Hans Krebs, FRS, on his eightieth birthday

become obese, and the mass of white adipose tissue may actually decrease [5], although other workers suggest that the weight of the parametrial fat body does not change [6].

2. Alterations in tissue metabolism during lactation

In this section I intend to discuss briefly the overall changes in metabolism which occur in certain key tissues of the lactating rat (for a summary see table 2). The major emphasis will be on carbohydrate and lipid metabolism.

2.1. *Lactating mammary gland*

The key to an understanding of the alterations in whole body metabolism during lactation are the substrate requirements of the gland for the production

Table 2
Carbohydrate requirements of the mammary gland of the lactating rat

	Substrate	Flux rate (mmol/24 h)
Lactating rats (10–18 days)	Glucose	28.2
	Lactate	6.3
	Pyruvate	0.6
	Total C_3 units	63.3
Male rats (330 g)	Glucose turnover (whole body)	33.9

The values for substrate utilization by the gland refer to fed rats and were calculated from arteriovenous difference measurements across the gland [45] and blood flow rates and gland weights [1]. Whole body glucose turnover for non-lactating rats is from [93]

Table 3
Summary of metabolic changes during lactation in the rat

Tissue	Process	Change
Mammary gland	Glucose utilization	Increased
	Lactose synthesis	Increased
	Lipogenesis	Increased
	Fatty acid esterification	Increased
	Triacylglycerol uptake	Increased
	Amino acid utilization	Increased
	Protein synthesis	Increased
Liver	Glucose utilization (glycolysis)	Increased
	Lipogenesis	Increased
	Fatty acid esterification	Increased
	Ketogenesis	Decreased
	Triacylglycerol secretion	Decreased
	Amino acid metabolism	?
	Protein synthesis	?
White adipose tissue	Glucose utilization	Decreased
	Lipogenesis	Decreased
	Triacylglycerol uptake	Decreased
	Lipolysis	Increased
Brown adipose tissue	Glucose utilization	Decreased
	Lipogenesis	Decreased
	Fatty acid oxidation	?
Intestine	Glucose utilization	?
	Lipogenesis	?
	Fatty acid esterification	?
	Amino acid metabolism	?

? Indicates where information is not available

of the main components of milk: lactose, casein and lipid. On the normal laboratory diet (high in carbohydrate and low in fat) all the lactose and casein, and about 50% of the lipid are synthesized within the gland. It can be estimated [7] that at peak lactation the glucose uptake by the gland is about 30 mmol or 5.4 g/24 h of which about 7.0 mmol/24 h is used for lactose synthesis [8] and the rest for lipogenesis (table 3). Less important potential substrates for lipogenesis in the gland include lactate and pyruvate (table 3). It is not widely appreciated that mammary gland is the most active site of lipogenesis in vivo during lactation; the rate of lipogenesis on a g tissue basis being 5-fold higher than that of liver (table 7; [9]). The remainder of the milk lipid is derived from triacylglycerols and non-esterified fatty acids taken up from the blood [10]. This large requirement of the gland for glucose and triacylglycerols implies that the rate of production or entry of these substrates is increased and/or their rate of utilization by other tissues is decreased. Part of the increased requirement can be met by the higher dietary intake, but part must be met by changes in the metabolism of other tissues. In addition, the gland must be able to respond rapidly to changes in the availability of these substrates in the circulation.

2.2. *White adipose tissue*

In the non-lactating rat white adipose tissue is an important site of lipogenesis and therefore of glucose utilization. In addition, dietary fat, present in the circulation as chylomicrons, is taken up by adipose tissue for storage after hydrolysis to non-esterified fatty acids by lipoprotein lipase (EC 3.1.1.34) and subsequent re-esterification within the tissue. During lactation the rate of lipogenesis in white adipose tissue in vivo [9] and in vitro [5,11] is considerably decreased as are the activities of certain key lipogenic enzymes [11,12]. Similarly, the activity of lipoprotein lipase decreases rapidly at parturition and remains low throughout lactation [6,13]. In contrast, there is evidence in vitro for increased lipolysis in adipose tissue from lactating rats [14]. The net result of these changes is that, in lactation, less glucose and triacylglycerol is removed by adipose tissue and more non-esterified fatty acids and glycerol are released to the blood stream.

2.3. *Brown adipose tissue*

It has long been appreciated that brown adipose tissue has an important role in heat production in non-shivering thermogenesis, but recent experiments suggest that this tissue may be important in 'burning off' excess energy, i.e., dietary-induced thermogenesis [15,16]. In the non-lactating rat there is a 10-fold increase in lipogenesis in interscapular brown adipose tissue after an intragastric load of glucose [17], but this response does not occur in short-term (2 h) insulin deficiency [17]. If the rate of lipogenesis can be directly related to that of fat oxidation in this tissue then these findings provide further evidence for its role in dietary-induced thermogenesis. During lactation the weight of the interscapular brown adipose tissue is not increased despite the hyperphagia and the rate of lipogenesis in the tissue no longer increases in response to an intragastric load of glucose [17]. These changes in the metabolism of the tissue can be interpreted as aiding in the conservation of lipogenic substrates (mainly glucose) because it clearly would not make physiological sense to 'burn off' excess energy in a situation in which the animal's requirements for substrates for the mammary gland are greatly increased. It would be of interest to examine whether blood flow to brown adipose tissue is decreased in lactation because this would also aid in substrate conservation.

2.4. *Liver*

The liver increases by about 40% in weight during lactation and this hypertrophy is accompanied by changes in the activity of enzymes concerned in the metabolism of carbohydrate and lipid. The activity of glucokinase (EC 2.7.1.2) increases at peak lactation whereas the activities of key enzymes concerned in gluconeogenesis either remain constant (glucose 6-phosphatase, EC 3.1.3.9) or decrease (phosphoenolpyruvate carboxykinase, EC 4.1.1.32; pyruvate carboxylase, EC 6.4.1.1) and this has led to the suggestion that glycolysis may be increased in the liver of the lactating rat [18]. Evidence in support of this suggestion is the finding that hepatocytes from fed rats at peak lactation have a higher rate of glycolytic flux and a lower output of glucose compared to hepatocytes from virgin or pregnant rats [19]. One important role of hepatic glycolysis is to supply carbon for fatty acid synthesis. Two of the enzymes concerned in lipogenesis, namely ATP-citrate lyase (EC 4.1.3.8) and acetyl-CoA carboxylase (EC 6.4.1.2) are increased in activity in liver at peak lactation [18] and in vitro measurements of lipogenesis with ^3H_2O

have shown a 2-fold higher rate in hepatocytes from rats at peak lactation compared to that in liver cells from virgin rats [20]. Alterations to lipid metabolism in livers of lactating rats are not confined to the synthesis de novo of fatty acids. A higher proportion of long-chain fatty acid (oleate) taken up by isolated hepatocytes from fed lactating rats (10–14 days) is converted to esterified products (mainly triacylglycerols) and less to ketone bodies than in hepatocytes from virgin rats [19]. The net result of these changes in hepatic metabolism would be expected to be increased secretion of triacylglycerols, formed either by synthesis de novo of fatty acids (glycogen or glucose as substrate) or by esterification of long-chain fatty acids originating from adipose tissue (see section 2.2). Recent measurements in vivo, however, have not confirmed this prediction in that the secretion rate of triacylglycerols (expressed on a 100 g/body wt basis) is decreased in early lactation and at peak lactation is similar to that for virgin rats ([21]; see section 4.2).

2.5. *Small intestine*

The small intestine of the rat increases in weight and length during lactation (table 1; [2,3]). There is hyperplasia of the mucosal epithelium and an increase in total absorptive capacity of the gut. However, the absorptive capacity per unit area of intestine for both monosaccharides and amino acids actually decreases [3] which suggests that the enterocytes may be less efficient or more immature. There has been much speculation on the mechanism for the intestinal hyperplasia during lactation. It has been attributed to: (i) 'work hypertrophy', i.e., induced by the increased food intake; or (ii) hormonal induction. Experiments in which exogenous luminal nutrition, or bile and pancreatic secretions, were excluded from the jejunum did not diminish the hyperplasia [22] suggesting the influence of some systemic factor. Hormones that might be responsible for the hyperplasia include the obvious candidate, prolactin, and thyroxine and enteroglucagon. Prolactin administration does not cause mucosal hyperplasia in virgin mice [23] nor does hyperprolactinaemia induced by perphenazine in rats [22]. However, treatment of rats with bromocryptine, which inhibits prolactin secretion, decreases the jejunal wet weight only in lactating rats [24]. One criticism of this study is that the possibility of changes in food intake was not considered. Hyperthyroidism in non-lactating rats results in a 30–40% increase in intestinal weight [25] and there is evidence for raised concentrations of thyroid hormones during lactation [26].

In marked contrast, there have been comparatively few studies on the metabolic properties of the mucosal epithelium of lactating rats. The activities of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and isocitrate dehydrogenase (EC 1.1.1.42) increase in the mucosal epithelium, whereas those of lactate dehydrogenase (EC 1.1.1.27), succinate-tetrazolium reductase (EC 1.3.9.9) and sucrase (EC 3.2.1.26) do not change significantly [27]. Dipeptidases which are important for the final stages of protein digestion, are increased considerably in activity in the small intestine during lactation [28].

3. Regulation of glucose metabolism and lipogenesis in the lactating mammary gland

Glucose is the precursor for the synthesis of lactose and for a large proportion of milk fat, so that when its availability decreases (e.g., in starvation or on a low-carbohydrate diet) the gland's requirement for this substrate must be decreased otherwise hypoglycaemia might result because of the depletion of the animal's carbohydrate reserves. Any discussion of the control of glucose utilization by the gland also involves consideration of the regulation of lipogenesis, because only about 10% of the glucose utilized is completely oxidized in this tissue [29]. Thus in this section the overall pathway of glucose metabolism in the lactating gland is considered to be its conversion to lipid or to lactose.

3.1. *Potential regulatory enzymes of glucose metabolism*

One method of identifying regulatory enzymes is to compare the activities of the enzymes of the pathway with that of the flux rate of the substrate (i.e., glucose) of the pathway. On this basis the reactions catalysed by the following enzymes may be considered potential regulatory sites in mammary gland: hexokinase, phosphofructokinase, pyruvate dehydrogenase and acetyl-CoA carboxylase (table 4; [30]) and these enzymes have all been implicated as regulatory enzymes in other tissues [31]. Glucose metabolism requires hexokinase as the initiating enzyme, and this is present in the gland predominantly as the type I and II isoenzymes (K_m for glucose 0.010–0.10 mM [32]; the intracellular glucose is thought to be 0.29

Table 4
Relative activities of the enzymes of glucose metabolism in the lactating mammary gland of the rat

Pathways and enzymes	Relative activity
Glucose utilization	1
Glucose activation	
Hexokinase	3.4
Embden–Meyerhof pathway	
Phosphoglucose isomerase	162
Phosphofructokinase	1.4–5.4
Aldolase	11.2
Glyceraldehyde 3-phosphate dehydrogenase	38
Pyruvate kinase	32
Lactate dehydrogenase	44
Pentose phosphate pathway	
Glucose 6-phosphate dehydrogenase	62
Gluconate 6-phosphate dehydrogenase	14
Fatty acid synthesis	
Pyruvate dehydrogenase	1.5
Acetyl-CoA carboxylase	0.44
Fatty acid synthetase	13

The rate of glucose utilization (1.6 μmol . min^{-1} . g wet wt^{-1}) in isolated acini [45] has been set at unity and the enzyme activities related to this. The activities are from [30] and have been corrected to 37°C

mM [33]. The activity of phosphofructokinase is important in determining the glycolytic flux and the mammary gland enzyme like that from muscle, recently has been shown to be inhibited by citrate [34]. Pyruvate dehydrogenase determines the proportion of glucose carbon that is converted into lactate or lipid (acetyl-CoA) [35,36] and alterations in the activity of this enzyme play a key role in the regulation of glucose metabolism in the gland. Utilization of acetyl-CoA generated by cleavage of citrate in the cytosol is controlled by acetyl-CoA carboxylase, which is activated by citrate and Mg^{2+}, and inhibited by certain fatty acids or their acyl-CoA esters in mammary gland [37,38]. One role of the pentose phosphate pathway is to supply NADPH for fatty acid synthesis and this pathway is present in high activity in the lactating gland. It is controlled by the availability of $NADP^+$ and NADPH in the cytosol [39]; NADPH being a potent inhibitor of glucose 6-phosphate dehydrogenase [40]. Thus a decrease in the rate of NADPH utilization for lipogenesis will result in inhibition of the pentose phosphate pathway activity. Flux through the glycolytic and pentose phosphate pathways is closely 'coupled' in mammary gland and other lipogenic tissues, but the mechanism which brings this about is not clear [29,30,41]. The primary rate-limiting step in lactose synthesis appears to be galactosyltransferase (EC 2.4.1.22) [42].

For a comprehensive review of the enzymes concerned in glucose metabolism in the lactating mammary gland see [30,32].

3.2. *Effects of starvation*

In starvation the lactating rat is faced with two choices, either to continue to produce milk at a normal rate with the consequent danger of complete depletion of its carbohydrate and fat reserves or alternatively to decrease lactation and possibly lose the young. It appears that survival of the mother rather than the pups is more important and given the rat's high fertility this is a sensible choice. For the biochemist the question is how is the inhibition of lactation achieved?

Starvation of lactating rats with their pups for 24 h results in a dramatic decrease in the glucose uptake by the mammary gland (as measured by arteriovenous differences) without any significant change in the arterial glucose concentration (table 5). This change in glucose utilization is accompanied by output of lactate and pyruvate by the gland, whereas in the fed state these substrates are taken up (table 5). Mammary gland blood flow may decrease on starvation so that the decrease in the rate of removal of glucose by the gland is likely to be even greater than that indicated by the arteriovenous differences. The lower rate of removal of glucose is accompanied by the expected large decrease in the rate of lipogenesis in vivo in the gland as measured with ^3H_2O ([9] ; table 6). All these changes in mammary gland metabolism are returned to normal by refeeding the starved (24 h) lactating rat the normal chow diet for 2 h [9,43], which indicates that the metabolic alterations are reversible and are due to short-term regulation.

In order to identify the sites at which regulation is exerted it is necessary to carry out experiments in vitro. Much of the early work on mammary gland metabolism was performed with slices of the tissue, but recently preparations of isolated acini (secretory cells) have been described [44]. Acini have one

Table 5
Effects of starvation (24 h) and refeeding (2 h) on utilization of blood metabolites by lactating mammary gland

Metabolite	Arterial concentration			Arteriovenous differences		
	Fed	Starved (24 h)	Refed (2 h)	Fed	Starved (24 h)	Refed (2 h)
Glucose	4.74	4.80	4.94	−1.43	−0.58	−2.35
Lactate	1.75	0.98	2.66	−0.32	+0.42	−0.26
Pyruvate	0.18	0.12	0.15	−0.03	+0.06	+0.08
Ketone bodies	0.14	0.54	0.17	−0.005	−0.16	−0.07

Values are taken from [43,45] and are expressed as μmol/ml whole blood

important advantage over tissue slices in that they consist of a single cell type rather than a mixture of secretory cells and adipocytes. Their metabolism appears to parallel that which occurs in vivo in a variety of situations [45]. Thus acini from starved (24 h) lactating rats have a decreased rate of glucose utilization and the proportion of glucose utilized (expressed as C_3 units) which accumulates in the medium as lactate and pyruvate is 64% compared to 16% with acini from fed lactating rats [45]. The experiments in vivo and in vitro suggest that glucose metabolism in starvation is inhibited at the stage of pyruvate oxidation and this is confirmed by the finding that pyruvate dehydrogenase in glands from starved rats is inactivated [46,47].

The rate of lactose synthesis in vivo as measured with [U-^{14}C]glucose is very sensitive to food withdrawal and the decreased rate occurs without any change in the activity of galactosyltransferase [8].

It does not appear to be known whether the composition of milk alters on short-term food withdrawal, but it might be expected that the fat content would increase and that a higher proportion of this would be directly derived from lipid supplied by the blood.

To summarize, in short-term starvation glucose utilization by the mammary gland is decreased both for lipogenesis and for lactose synthesis, and of that glucose which is still metabolized a high proportion leaves the gland as lactate and pyruvate to be converted in the liver back to glucose. How the gland may sense the alteration in the nutritional state is discussed in section 3.6.

3.3. *Effects of a high fat diet*

Transfer of the lactating rat from the normal laboratory diet (chow; high in carbohydrate, low in fat) to one high in fat might be expected to have a marked effect on mammary gland metabolism, because of the decreased availability of glucose and increased supply of lipid. Lactating rats fed a high fat (20%) diet for 7–20 days show no change in the percentage of fat in the milk but the composition of the milk fat resembles that of the dietary fat [48,49]. In addition, the gain in weight of the pups is not changed on feeding

Table 6
Effect of diet on lipogenesis in vivo in mammary gland and liver of lactating rats

Tissue	Dietary status						Ref.
	Fat-free	Chow	Starved (24 h)	Refed chow (2 h)	Peanut oil	Coconut oil	
Mammary gland	–	121	2.1	148	–	–	[9]
	70	148	–	–	33	68	[49]
Liver	–	23	2.1	9	–	–	[9]
	57	14	–	–	21	25	[49]

The units are μmol ^3H_2O incorp. into saponified lipid . h^{-1}. g fresh wt tissue^{-1}

the high fat diet [49]. More significant in the context of the present discussion is the finding that the rate of lipogenesis in vivo (measured with ^3H_2O) is considerably decreased on the high fat diet (table 6; [49]) indicating decreased utilization of glucose by the gland; a similar finding has been reported in the lactating mouse [50]. Work from this laboratory with a cafeteria-diet (palatable supermarket foods with a high fat content) which induces hyperphagia, has shown a similar decrease in mammary gland lipogenesis in vivo [51]. In this case, acini isolated from the glands have a depressed rate of glucose utilization and lipogenesis in vitro, and this can be restored to normal by the addition of insulin [51]. All these studies have involved feeding a high fat diet for several days and the question arises whether the metabolism of the gland can respond acutely to increased availability of lipid. We have examined the effect of an intragastric load of long or medium-chain triacylglycerols on lipogenesis in lactating mammary gland ([52]; table 7). Both long and medium-chain triacylglycerols produce a dramatic inhibition in the rate of mammary gland lipogenesis and this can be partially relieved either by an intragastric load of glucose or by administration of insulin [52]. This decrease of lipogenesis in the gland after triacylglycerol loading is an obvious physiological advantage in that it spares lipogenic substrates (mainly glucose) when there is increased triacylglycerol available in the blood stream. Interestingly,

Table 7
Acute effects of an intragastric triacylglycerol load on lipogenesis in vivo in mammary gland and liver of lactating rats

Treatment of rats	Lipogenesis	
	Mammary gland	Liver
Saline	92	15.4
Long-chain triacylglycerol	9.9	8.4
Long-chain triacylglycerol +glucose	37	9.7
Long-chain triacylglycerol +insulin	57	31
Medium-chain triacylglycerol	17	31
Medium-chain triacylglycerol +glucose	70	29
Medium-chain triacylglycerol +insulin	67	33

The units are μmol ^3H_2O incorporated into saponified lipid . h^{-1} . g fresh wt tissue^{-1}. The rate of lipogenesis was measured 2 h after the load [52]

acini isolated from mammary glands of rats given the load of triacylglycerols do not show any significant alteration in glucose utilization or in lipogenesis and this contrasts with results of experiments with cafetaria feeding [51] or starvation [45]. It is unlikely that the availability of long-chain fatty acids per se is the signal for decreased lipogenesis in the gland in vivo, because oleate has only small inhibitory effects on glucose utilization and lipogenesis in isolated acini [53].

3.4. *Effects of short-term insulin deficiency*

The lactating mammary gland of the rat is considered to be an insulin sensitive tissue [41,54], although as yet the precise sites where insulin may act have not been elucidated. Glucose oxidation and its incorporation into lipid are decreased in mammary gland preparations (slices or cells) from lactating rats made insulin deficient with alloxan (2–3 days) [55,56] or anti-insulin serum (1 h) [55] and this impairment in glucose metabolism can be reversed by addition of insulin in vitro. But it must be emphasized that the effects of insulin on glucose metabolism in vitro in mammary gland are not as impressive as those of the hormone on adipose tissue. In addition, long-term insulin deficiency results in lower activities of key enzymes concerned in glucose metabolism [55] and this complicates the interpretation of the effects of insulin on the gland. However, it has been concluded from indirect evidence that an important action of insulin is to increase glucose transport into the mammary gland cell [30]. Acute insulin-deficiency (2 h) caused by streptozotocin administration results in a marked inhibition of mammary gland lipogenesis in vivo [9], output of pyruvate from the gland and slight depression of glucose uptake [45]. Surprisingly, in view of the conclusion that insulin promotes glucose transport [30], glucose utilization in acini isolated from streptozotocin-treated rats is not depressed, but its oxidation via the pentose phosphate pathway and its conversion to lipid are decreased [45]. In addition, a higher proportion of the glucose utilized accumulates in the medium as lactate and pyruvate, suggesting inhibition of pyruvate dehydrogenase. Although addition of insulin can partially reverse the decrease in the conversion of glucose to lipid it has little effect on the accumulation of lactate. This is analogous to the situation with acini from starved lactating rats ([45], see section 3.2). Evidence for a role of insulin in the regulation of pyruvate dehydrogenase activity in mammary gland in vivo has been provided by mea-

surements of the activation state of the enzyme after short-term (3 h) streptozotocin-treatment to decrease circulating insulin. The percentage of active enzyme is decreased to <10% without any change in the total activity and this inactivation is reversed by administration of insulin [57]. Starvation is also associated with marked inactivation of mammary gland pyruvate dehydrogenase in vivo [46,47] and this can be reversed by administration of glucose and insulin [47].

In marked contrast to the clear evidence that insulin is involved in the short-term regulation of glucose metabolism and lipogenesis in the lactating gland, there is little support for an effect of the hormone on lactose synthesis [8,58]. There appears to be no consistent relationship between rates of lactose synthesis in vivo and plasma insulin concentrations and injection of insulin does not restore lactose synthesis depressed by short-term (6 h) food withdrawal [8]. Furthermore, rats treated with alloxan during onset of lactogenesis show no decrease in the lactose content of the gland despite low plasma insulin concentrations [59]. Longer-term alloxan-diabetes does result in impaired pup growth [55,56] but in these experiments lactose synthesis was not specifically measured and in any case changes in enzyme concentration within the gland may have occurred. Acini isolated from malnourished rats do not show impaired lactose synthesis [58] which strongly suggests that a systemic factor is involved in the depression of lactose synthesis in vivo.

3.5. *Effects of prolactin deficiency*

Although it is accepted that prolactin is required for the differentiation of the mammary gland and for the maintenance of lactation, comparatively little is known about the metabolic effects of deficiency of prolactin. The availability of bromocryptine, an inhibitor of prolactin secretion from the pituitary [60], has provided a valuable tool to study the metabolic roles of prolactin during lactation.

Prolactin deficiency (24 h) does not affect the removal of glucose by the gland as measured by arteriovenous difference measurements [45], but it decreases the rate of lipogenesis in vivo by about 40% [61]. In acini isolated from prolactin-deficient lactating rats the utilization of glucose is slightly decreased (20%) as is lipogenesis and a high proportion of the glucose removed accumulates as lactate and pyruvate [45]. Bromocryptine treatment for 24 h results in the inactivation of pyruvate dehydrogenase in vivo [57] and the results of the experiments with acini suggest that the decreased activity is retained in vitro. Short-term prolactin deficiency (3 h) also results in inactivation of pyruvate dehydrogenase, but to a lesser extent [57]. Another indication that the metabolic effects of prolactin deficiency are more long-term is the finding that deficiency of the hormone (3 h) does not prevent the stimulation of lipogenesis after refeeding (2 h) starved lactating rats, whereas deficiency of insulin does [9]. In general, the metabolic effects of prolactin deficiency are not so dramatic as those of insulin lack, but further work is required to define the acute action of prolactin on the gland.

3.6. *Potential 'signals' to the gland*

It is clear from the preceding sections that glucose metabolism in the lactating gland can be radically altered by changes in the hormonal and/or nutritional state of the animal (see table 8 for a summary of the changes). The gland must be able to respond to the changes and therefore presumably it 'recognizes' alterations in the concentration of blood borne 'signals'. These signals may be hormones or metabolites and it is unlikely because of the complexity of the metabolic effects that there is a single signal.

3.6.1. Glycerol

The action of lipoprotein lipase on the capillary epithelium of the mammary gland results in the liberation of non esterified fatty acids and glycerol from blood triacylglycerol [62]. A proportion of the glycerol moiety of the triacylglycerol appears in milk lipid but it is not known whether this enters the gland as free glycerol. Nevertheless, it might be expected that increased availability of triacylglycerol to the gland would result in a rise in the local concentration of free glycerol and thus this metabolite could act as a signal to decrease glucose uptake. Glycerol at physiological concentrations inhibits glucose utilization and lipogenesis in isolated acini and this inhibition is accompanied by a large increase in the concentration of glycerol 3-phosphate and the lactate/pyruvate ratio [63]. Acute elevation of the blood glycerol concentration to values around 3 mM (about 30-fold higher than normal) does not alter the rate of lipogenesis in the mammary gland (L. Agius, unpublished) so that it would appear that glycerol is not a signal in vivo.

Table 8
Summary of changes in glucose metabolism of lactating mammary gland in response to alterations in diet or hormonal balance

Situation	In vivo		In vitro			Ref.
	Lipogenesis	Pyruvate dehydrogenase activity	Glucose utilization	Lactate accumulation	Lipogenesis	
Starvation (24 h)	Decreased	Decreased	Decreased	Increased	Decreased	[9,45,47]
High-fat diet	Decreased	?	?	?	?	[48,49]
Cafeteria-diet	Decreased	?	Decreased	Slight increase	Decreased	[51]
Insulin-deficiency	Decreased	Decreased	Unchanged	Increased	Decreased	[9,45,57]
Prolactin-deficiency	Decreased	Decreased	Decreased	Increased	Decreased	[45,57,61]
Triacylglycerol load	Decreased	?	Unchanged	Unchanged	Unchanged	[52]

3.6.2. Ketone bodies

The physiological roles of ketone bodies (acetoacetate and 3-hydroxybutyrate) as substrates and signals for peripheral tissues is well established (reviewed [64]). Lactating mammary gland has high activity of the enzymes necessary to utilize ketone bodies [65] and this fact, together with the high blood flow, makes the gland a potential major site of ketone body metabolism. Ketone bodies are taken up and oxidized to CO_2 or incorporated into lipid by rat mammary gland both in vivo [10,45,66] and in vitro [63,67, 68,69]. Moreover, the presence of ketone bodies in the medium depresses glucose utilization by isolated preparations of mammary gland [66,67,69] but does not affect lactose synthesis (N. J. Kuhn, personal communication). The inhibition of glucose utilization is accompanied by increased concentrations of citrate and glucose 6-phosphate and a decreased concentration of glycerol 3-phosphate [66,69]. The mechanism for the inhibition of glucose uptake is presumably analogous to that postulated for heart muscle [70]. Metabolism of ketone bodies in the mitochondria generates acetyl-CoA resulting in increased formation of citrate that is transported to the cytosol, where it inhibits phosphofructokinase activity. Consequently, fructose 6-phosphate concentrations rise, and the concomitant increase in glucose 6-phosphate concentration inhibits hexokinase activity, thus decreasing glucose uptake. In addition the increased concentration of acetyl-CoA may decrease the activity of pyruvate dehydrogenase [71]. The concentration of ketone bodies in the blood increases in starvation and therefore it has been suggested that they may act as a signal to depress the metabolism of glucose by mammary gland when food is withdrawn [69]. Administration of acetoacetate to fed lactating rats to increase acutely the blood ketone body concentrations decreases the arteriovenous difference for glucose across the mammary gland and causes an output of pyruvate, but not lactate [72]. These experiments provide some support for the postulate that ketone bodies can regulate glucose metabolism in mammary gland in a physiological manner. A novel finding is that insulin can relieve the inhibition of glucose utilization brought about by acetoacetate in vitro [66,69]. Thus in starvation the low insulin concentrations [9] may potentiate the inhibition of glucose utilization and lipogenesis by ketone bodies. It should be mentioned that the ketone body concentrations in the blood of 24 h starved lactating rats are lower than those found in starved virgin rats [10]; this may be due to increased ketone body removal by the gland or to decreased production by the liver [19].

3.6.3. Insulin

It is clear from the preceding discussion (in particular section 3.4) that insulin plays a key role in the short-term regulation of mammary gland metabolism. It is therefore somewhat surprising to find that the plasma insulin concentration is lower during lactation than in virgin or non-lactating rats [5,9]. The blood glucose concentration is also about 1.5 mmol/l lower at peak lactation and recent experiments have shown that the plasma insulin is appropriate to the existing level of glycaemia (P. Ferré and J. R. Girard, personal communication). It may be that the high rate of glucose utilization by the gland is the reason

for the lower blood glucose during lactation, and this in turn might be responsible for the relative hypoinsulinaemia. As mammary gland is an insulin-sensitive tissue [41,54] how can one reconcile this apparent anomaly of a low plasma insulin being correlated with a high rate of glucose metabolism in the mammary gland? One possibility is that the lactating gland acts as a 'sink' for insulin, in that the tissue is likely to contain a large number of insulin receptors and the hormone is delivered to the gland at a fast rate due to the high blood flow. Recent experiments with thelectomized glands (removal of teats) have indicated the importance of the rate of delivery of hormones, rather than the blood concentrations, in the regulation of tissue receptor levels for prolactin and oestrogen [73]. Further support for the postulate that the lactating gland is a major site of insulin (and glucose) uptake is the finding that on removal of the pups (24 h) when the blood flow to the gland is halved [74] the blood glucose increases by 0.6 mmol/l and the plasma insulin rises 3-fold [61]. The decrease in plasma insulin on starvation and the rapid increase on refeeding [10] are in agreement with the postulated role of insulin as a signal of carbohydrate availability to the gland. It is not known whether mammary gland blood flow changes in these situations.

Assuming that the rate of insulin delivery is an important signal for the regulation of metabolism in the gland the question is the site(s) at which it may act. Apart from the postulated effect of insulin on membrane transport of glucose [30], an important site action of the hormone appears to be on pyruvate dehydrogenase activity. Attempts to study the mechanism whereby insulin may alter the activation state of this enzyme have been frustrated by the inability to demonstrate an effect of the hormone on the activity in vitro [36,45]. Indirect evidence suggests that insulin may act via modulation of the activity of the pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase responsible for the interconversion of the active and inactive forms of the pyruvate dehydrogenase complex [75–77]. However, pyruvate dehydrogenase may not be the only intracellular site at which insulin acts. Insulin causes only a slight increase in lipogenesis in acini prepared from glands of starved lactating rats [45], but if pyruvate dehydrogenase is activated by inclusion of dichloroacetate in the medium [78] insulin causes a considerably greater stimulation of lipogenesis (M. R. Munday and D. H. Williamson, unpublished). This suggests that insulin can activate a step which is after pyruvate dehydrogenase in the pathway of lipogenesis. A potential candidate is acetyl-CoA carboxylase, which is known to be a regulatory protein and to undergo phosphorylation and inactivation in response to adrenaline in adipose tissue [78,79,81] and to glucagon in liver cells [80]. Purified acetyl-CoA carboxylase from rabbit mammary gland can be phosphorylated by both cyclic AMP-dependent and -independent protein kinases [82] though as yet no hormone has been shown to inactivate the mammary gland enzyme or even inhibit lipogenesis in an in vitro preparation of the gland. How insulin might regulate the activity of acetyl-CoA carboxylase in mammary gland is still an open question.

4. Integration of mammary gland metabolism with that of other tissues

If, as seems likely, the availability of insulin is a key factor in regulating the metabolism of the lactating mammary gland of the rat, this raises a problem in that insulin also controls anabolic processes in adipose tissue and liver of non-lactating rats. In lactation there must therefore be mechanisms whereby the influence of insulin on tissues other than mammary gland is depressed. This could be achieved either by:

(i) 'Directing' the hormone to the gland (as postulated in section 3.6.3.) and presenting the other tissues with a lower plasma concentration of insulin; or
(ii) Decreasing the number of insulin receptors; or
(iii) Increasing the concentration of a hormone which acts antagonistically to insulin except in mammary tissue.

These changes might in turn bring about alterations in the concentrations or activities of key enzymes, e.g. [12]. The number of insulin receptors is not altered in hepatocytes [83] or adipocytes [5] during lactation, whereas the receptor numbers are increased in these tissues during pregnancy. Prolactin would seem an excellent candidate to act as antagonist to insulin in liver and adipose tissue because its secretion is directly coupled to the suckling reflex [84] and therefore its plasma concentration signals the lactational drive. This section is concerned with integration of lipid metabolism between mammary gland, liver and adipose tissue and the possible role of prolactin in this process.

4.1. *Lipoprotein lipase activity and triacylglycerol uptake*

The key mechanism for regulating the inter-organ flux of triacylglycerol during lactation is the reciprocal changes in the activity of lipoprotein lipase in adipose tissue and mammary gland [6,13]. The activity of the enzyme decreases in adipose tissue just before parturition and remains low throughout lactation, whereas the activity in mammary gland rapidly increases at the end of pregnancy to reach high levels at peak lactation; the net result of these changes is that triacylglycerol available in the circulation is 'directed' to the mammary gland rather than to adipose tissue [4]. There is good evidence that the high plasma concentrations of prolactin during lactation are responsible for maintaining this different pattern of lipoprotein lipase activity between the two tissues [85]. Thus removal of the pups [6,86] or hypophysectomy of the lactating mother [85], both of which lower the plasma prolactin, result in a rapid increase in lipoprotein lipase activity in adipose tissue and a decrease in the mammary gland. The signals that trigger the changes in lipoprotein lipase activity around parturition are more complex [87]. It appears that prostaglandin $F_{2\alpha}$ initiates the alterations in lipoprotein lipase activity, whereas progesterone acts as a repressor [87]; the latter may also suppress lactogenesis [88].

4.2. *Lipogenesis*

The primary substrate for the synthesis de novo of lipid is glucose (or glycogen) and in order to conserve carbohydrate for the mammary gland the rate of lipogenesis must be decreased in adipose tissue and, to a lesser extent, in liver. There is ample evidence in vitro [5,11] and in vivo [9,61] that the rate of lipogenesis in adipose tissue is decreased during lactation. In liver the rate of lipogenesis in vitro is higher than in the non-lactating rat [19,89] but the rate in vivo is not increased, suggesting that a systemic factor may depress hepatic lipogenesis in vivo. If pups are removed from the mother for 24 h lipogenesis increases 4-fold in maternal adipose tissue and doubles in liver and these increases can be prevented by administration of prolactin [61]. Treatment of lactating rats in the presence of the pups with bromocryptine (24 h) increases lipogenesis in both adipose tissue and liver and again this does not occur of prolactin is administered simultaneously [61]. Removal of pups for 48 h increases the number of insulin receptors on parametrial adipose tissue and the rate of fatty acid synthesis in vitro [90]; these changes cannot be reversed by prolactin which indicates that the hormone's effects may be transient. These experiments suggest that alteration of the plasma prolactin concentration causes a reciprocal change in the rate of lipogenesis in both liver and adipose tissue. The advantage of this regulatory role for prolactin is that an elevation of the hormone in response to the suckling stimulus would depress lipogenesis in adipose tissue and liver so that glucose and lactate, the two main lipogenic precursors [44], are made available for the lactating gland. The fall in prolactin concentration on weaning allows replenishment of the depleted lipid stores in adipose tissue. It appears that the changes in lipoprotein lipase activity and lipogenesis in adipose tissue during lactation are synchronized [5,90] and that prolactin is the likely signal. Whether prolactin is responsible for the changes in hepatic lipid metabolism [18,19,61] is less certain. Rat liver does have prolactin receptors [91] although their role is not known. In addition, prolactin has been shown to depress hepatic lipogenesis in a teleost [92]. One problem in ascribing a primary role to prolactin in the regulation of lipogenesis is that, at least in the short-term, changes in plasma prolactin concentration are accompanied by reciprocal changes in plasma insulin [61]. It has been suggested that these effects of prolactin on lipogenesis require a functioning mammary gland [90] and this would explain why long-term hyperprolactinaemia in non-lactating rats does not bring about the same changes (L. Agius and D. H. Williamson, unpublished).

5. The future

The work reviewed here indicates that the metabolism of the tissues of the lactating rat is closely integrated so that the substrate and energy demands of lactation are met. Some progress has been made in our understanding of how the integration is achieved but much remains to be discovered, particularly in the areas of amino acid metabolism, regulation of enzyme turnover, and the role of the gut hormones. Apart from the acquisition of new knowledge further advances in this field may one day allow us to intervene experimentally to obtain the alterations in tissue metabolism and inter-organ substrate fluxes similar to those that the rat experiences during successive preg-

nancies and lactation. The eventual prize might be a novel means of controlling obesity.

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