

Substrate selection and oxygen uptake by the lactating mammary gland

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Sélection de substrats et consommation d'oxygène par la glande mammaire en lactation

RÉSUMÉ

Toutes les protéines et tout le lactose, et environ 50% des acides gras des lipides du lait sont synthétisés par les cellules épithéliales de la glande mammaire. Au pic de la lactation, il y a une demande énergétique élevée auprès de ces cellules pour fournir les substrats circulants (glucose, acides gras, corps cétoniques, triacylglycérols et acides aminés), pour les réactions de biosynthèse, et pour la sécrétion de lait. Le coût d'énergie individuelle le plus élevé est imputé à la synthèse des lactoprotéines. Cette énergie provient en grande partie de l'oxydation du glucose (chez les non-ruminants), et du glucose plus acétate (chez les ruminants). Comme on peut s'y attendre, les taux mesurés de l'extraction d'O₂ par la glande en lactation chez le rat *in vivo* sont plus élevés (d'environ 50%) que ceux requis pour les réactions purement de synthèse, et l'absorption d'O₂ reste importante lorsque la production de lait est faible (comme chez le rat à l'état de jeûne). Normalement, le principal précurseur de la lipogenèse est le glucose chez le non-ruminant, l'acétate et le 3-hydroxybutyrate chez le ruminant. Le glucose est aussi le précurseur du lactose et du glycéride-glycérol. Cependant, la consommation de substrats par le tissu mammaire est très sensible aux modifications de l'alimentation (un régime riche en glucides opposé à un régime riche en graisses, le jeûne), et aux hormones plasmiques (déficience en prolactine ou en insuline). Nous discuterons ici les facteurs susceptibles de contrôler la consommation et la sélection des substrats par la glande mammaire (y compris le flux sanguin du tissu).

The mammary gland is a unique tissue in that during lactation it has a high metabolic rate, yet its biosynthetic products are of no direct benefit to the lactating mother. Indeed, the mammary gland can be considered as a benign, highly regulated tumour (Williamson *et al.* 1988). Furthermore, because of the drain on the maternal energy supplies, the metabolism of the gland and that of other tissues must be carefully integrated (Bauman & Currie, 1980; Williamson, 1980). Although there are differences in the type and amounts of substrates utilized by mammary tissue of various species, particularly between ruminants and non-ruminants, the strategies employed in regulating their uptake and utilization are similar. Much of the pioneering work on defining the

substrates of the mammary gland was carried out over 20 years ago on the lactating goat by Linzell and his colleagues (for reviews, see Linzell, 1968, 1974). More recent work has been directed to the mechanisms that determine fuel selection in response to dietary and hormonal changes. The present contribution concentrates mainly on the mammary gland of the lactating rat, primarily because its biochemical profile is likely to be similar to that of the human and the majority of the recent advances in the regulation of mammary gland metabolism have been made in this species.

SUBSTRATE UTILIZATION

The uptake and utilization of a substrate by a tissue are governed by several factors, including availability in the circulation, mass of tissue and blood flow to it, transport systems of cells and organelles, activities of key enzymes and the hormonal milieu (Williamson, 1984).

Methodology

Much of the information on substrate utilization by the lactating mammary gland has been obtained by measurement of arterio-venous differences for the various substrates and determination of blood flow by distribution of $^3\text{H}_2\text{O}$ or $^{86}\text{RbCl}$ (Chatwin *et al.* 1969; Hanwell & Linzell, 1973) or radioactive microspheres (Jones & Williamson, 1984; Viña *et al.* 1987) in the gland. In larger animals simultaneous measurements of arterio-venous differences and blood flow can be made in unstressed, conscious animals, a technique not possible with small animals. In these species, such measurements must be interpreted with caution, because stress and anaesthesia may alter blood flow. To overcome these problems, alternative methods have been developed for the rat which allow assessment of mammary gland substrate utilization without knowledge of blood flow, and without anaesthesia. These methods depend on the accumulation of a non-metabolizable product within the gland over a period of time. (For example, deoxy[^3H]glucose-6-phosphate is used to monitor uptake of glucose (Threadgold & Kuhn, 1984; Burnol *et al.* 1987); α -amino[1- ^{14}C]isobutyrate, an analogue of alanine, for uptake of amino acids (Tedstone *et al.* 1990) or a product which is present in milk, e.g. ^{14}C -labelled lipid for utilization of ^{14}C -labelled triacylglycerols (Oller do Nascimento & Williamson, 1986).) In addition, conversion of substrates to fatty acids (lipogenesis) can be measured with $^3\text{H}_2\text{O}$ (Robinson *et al.* 1978).

Substrate availability

A striking feature of lactation in the rat is the considerable increase (up to 300%) in food intake (Fell *et al.* 1963; Cripps & Williams, 1975) accompanied by a doubling in weight of the intestinal tract (Craft, 1970; Cripps & Williams, 1975). There is also hypertrophy of the mammary gland and liver. A comparison of a range of substrate concentrations in the arterial blood of two non-ruminants (rat and pig) and two ruminants (goat and sheep) shows that the major differences are in the values for acetate which are approximately 10-fold higher in the latter (Table 1). Delivery of available substrates (and hormones) to the lactating gland is assured by the increase in the cardiac output and in the proportion of blood flow received by the gland (Hanwell & Linzell, 1973).

Table 1. *Substrate availability in arterial plasma during lactation*

Species	Substrate (mmol/l)								References
	Glucose	Lactate	Acetate	3-Hydroxybutyrate	Acetoacetate	Triacylglycerols	NEFA	Amino acids	
Rat	4.59*	0.93*	0.25*†	0.14*	0.20*	0.91	0.61	4.0*‡	Hawkins & Williamson (1972)
Pig	6.79	1.30	0.22	0.19	0.06	0.44	0.33§	3.5§	Spincer <i>et al.</i> (1969)
Goat	2.52	0.75	1.48	0.56	<0.01	0.29	0.44	3.1	Linzell (1968)
Sheep	4.57	0.54	2.44	0.56	nd	nd	0.26	nd	Vernon <i>et al.</i> (1990)

nd, not determined; NEFA, non-esterified fatty acids.

* Measurements on whole blood.

† Buckley & Williamson (1977).

‡ Viña *et al.* (1987).

§ Linzell *et al.* (1969).

If arterio-venous differences and blood flow to the gland are known, the uptake of circulating substrates can be expressed as the amount extracted per unit time. However, for many purposes, the relative uptake of substrates calculated on the basis of C atoms (Table 2) is more informative, and does not require knowledge of the blood flow. On this basis two important substrates of mammary gland, in addition to amino acids, are glucose and triacylglycerols in both ruminants and non-ruminants (Table 2). The main difference is that in the rat glucose is a precursor for lipid and lactose synthesis, whereas in the goat it is either converted to lactose or oxidized (Linzell, 1968). Ruminants lack citrate lyase (*EC* 4.1.3.8) so precluding conversion of glucose to fatty acids; acetate is the main lipogenic precursor in the ruminant. The low (rat) or apparently absent (goat and pig) contribution of NEFA (non-esterified fatty acids) to mammary gland substrate supply (Table 2) must be interpreted with caution because NEFA liberated by the action of lipoprotein lipase (*EC* 3.1.1.34) on triacylglycerols (chylomicrons or VLDL) may be released to the venous plasma and, thus, result in artefactually-low arterio-venous

Table 2. *Substrate utilization by lactating mammary gland*

Substrate	Relative uptake by mammary gland (%)*		
	Rat	Pig	Goat
Glucose	20	70	31
Lactate	1.1	0	4.3
Acetate	–	1.1	11.0
3-Hydroxybutyrate	0.7	0.4	8.6
Acetoacetate	1.1	0	0
NEFA	11.7	0	0
Triacylglycerols	66	28	45

NEFA, non-esterified fatty acids.

* Expressed as percentages of the total substrate uptake calculated on the basis of C atoms (e.g. glucose 6, NEFA 18, triacylglycerols 54).

differences for NEFA (Annison *et al.* 1967). Nevertheless, there is no doubt that triacylglycerols in the form of lipoproteins (chylomicrons and VLDL) are the more important lipid substrate for the mammary gland.

There is comparatively little information on the transport of substrates into mammary gland epithelial cells, but where it exists (e.g. amino acid transport; Neville *et al.* 1980) the mechanisms involved are similar to those in other tissues. Burnol *et al.* (1990) have shown that the glucose transporter in the lactating mammary gland is GLUT1 rather than the insulin-sensitive GLUT4. This suggests that glucose entry is not directly regulated by insulin in this tissue, although administration of the hormone *in vivo* relieves the depressed transport of 3-O-methylglucose into mammary glands of starved lactating rats (Threadgold & Kuhn, 1984).

SHORT-TERM REGULATION

Short-term regulation of substrate utilization by the mammary gland is necessary because the normal daily substrate consumption by the tissue is enormous (Table 3). On a high-carbohydrate diet, lipogenesis from glucose accounts for approximately 50% of milk fat and for the synthesis of lactose. When the availability of glucose decreases (e.g. in starvation or on a low-carbohydrate high-fat diet) the gland's requirement must be decreased, otherwise hypoglycaemia might result because of depletion of the animal's carbohydrate reserves. Thus, in short-term starvation or after an oral load of fat the rate of lipogenesis and lactose synthesis in the gland decreases and these effects are rapidly reversed on refeeding carbohydrate (Bussmann *et al.* 1984; Williamson *et al.* 1984). Similar considerations apply to amino acids (Viña *et al.* 1983) and triacylglycerols (Oller do Nascimento & Williamson, 1988) during starvation. The uptake of all substrates is decreased by at least 90% after 20 h starvation, with the notable exception of ketone bodies where the decrease is only 65%; these changes are reversed on refeeding (Table 3).

Sites of regulation

Starvation is accompanied by a large decrease (70%) in blood flow to the gland (Jones & Williamson, 1984; Viña *et al.* 1987) and this in part explains the decrease in substrate

Table 3. *Effects of starvation and refeeding on substrate extraction by lactating mammary gland of the rat*

(The results are mean values calculated from the arterio-venous differences and blood flow; for details, see Williamson, 1990)

State of rats	Substrate extracted (mmol/24 h per total tissue)				
	Glucose	Amino acids	Triacyl-glycerols	NEFA	Ketone bodies
Fed	41.6	22.3	3.6	2.0	3.5
Starved (6 h)	9.7	2.5	—	—	—
Starved (18–20 h)	1.5	0.6	0.24	0.22	1.2
Refed (2–2.5 h)	23.4	9.1	—	—	—

NEFA, non-esterified fatty acids.

utilization, but in addition there are metabolic changes. Apart from transport into the epithelial cells, the regulatory sites for glucose conversion to lipid are hexokinase (*EC* 2.7.1.1), phosphofructokinase (*EC* 2.7.1.11), pyruvate dehydrogenase (*EC* 1.2.4.1) and acetyl-CoA carboxylase (*EC* 6.4.1.2). In addition, the pentose phosphate pathway and the malate cycle are important for the generation of reducing equivalents (NADPH). In general, the mechanisms involved appear to be the same as those for other lipogenic tissues (liver and adipose tissue). Specifically, the activation state of the pyruvate dehydrogenase complex and acetyl-CoA carboxylase decreases on starvation or fat-feeding (Baxter & Coore, 1978; McNeillie & Zammit, 1982; Munday & Williamson, 1982) and there is evidence for inhibition of phosphofructokinase and hexokinase (Burnol *et al.* 1988; Mercer & Williamson, 1988). In addition, the conversion of glucose to lactose is highly sensitive to food withdrawal (Carrick & Kuhn, 1978). The main site of regulation of triacylglycerol utilization is the activity of lipoprotein lipase which decreases in starvation, but not on fat-feeding (Oller do Nascimento & Williamson, 1988). The uptake of 2-amino-[1-¹⁴C]isobutyrate (a non-metabolizable amino acid analogue) by isolated secretory cells from mammary glands is decreased on starvation of the rat (Viña *et al.* 1987; Tedstone *et al.* 1990) suggesting a change in the activity of the Na⁺-dependent transporter system for amino acids. The majority of these changes are rapidly reversed on refeeding chow to starved lactating rats (Table 3).

Regulatory signals

The large and relatively rapid changes in substrate utilization in response to changes in nutritional state (Table 3) must be mediated by extracellular signals that are related to the quantity and composition of the diet. An obvious candidate is plasma insulin, which is high during intake of carbohydrate and decreases in starvation or on a high-fat diet. Interestingly, there is relative hypoinsulinaemia during lactation in the rat (Robinson *et al.* 1978; Flint *et al.* 1979; Marynissen *et al.* 1983). This is probably due to a combination of a lower blood glucose (increased consumption by gland; Robinson *et al.* 1978), a decreased pancreatic β -cell mass (Marynissen *et al.* 1983) and increased removal of the hormone by the mammary tissue insulin receptors (Jones *et al.* 1984). However, plasma insulin shows the expected transient increase when a chow meal is fed to a lactating rat (Mercer & Williamson, 1986; Page & Kuhn, 1986). There is now strong evidence that insulin stimulates glucose conversion to lipid at a number of sites (Table 4). In addition, recent work indicates that it also positively regulates lipoprotein lipase activity in mammary tissue and, thus, promotes uptake of plasma triacylglycerols (Table 4; Da Costa & Williamson, 1994). As yet there is no experimental evidence that insulin controls amino acid uptake into the lactating mammary gland of the rat. Prolactin deficiency, however, does lead to decreased amino acid uptake (Viña *et al.* 1981). The role of prolactin in the regulation of substrate utilization appears to be that of an auxiliary hormone in that it maintains insulin receptors on mammary gland plasma membranes (Flint, 1982). No hormonal antagonist to insulin has yet been described for this tissue. There are no glucagon receptors on mammary gland plasma membranes (Robson *et al.* 1984) and, although β -adrenergic receptors are present, adrenaline does not antagonize insulin action. However, adrenaline is a powerful inhibitor of mammary blood flow in the goat (Linzell, 1960).

Table 4. *Summary of regulation of substrate utilization in lactating mammary gland of the rat*

Substrate	Regulated process	Extracellular signals			References
		Insulin	Ketone bodies	Intracellular signals	
Glucose	Transport	Activation	-	-	Threadgold & Kuhn (1984)
	Hexokinase (EC 2.7.1.1)	Activation	Inhibition	Glucose-6-phosphate (inhibitor)	Mercer & Williamson (1988), Burnol <i>et al.</i> (1988)
	Phosphofructokinase (EC 2.7.1.11)	Activation	Inhibition	Citrate (inhibitor), fructose-2,6-bisphosphate (activator)	Zammit (1979), Ward & Kuhn (1985), Mercer & Williamson (1988)
	Pyruvate dehydrogenase (EC 1.2.4.1)	Activation	Inhibition	Acetyl-CoA (inhibitor)	Baxter & Coore (1978), Munday & Williamson (1981), Kilgour & Vernon (1987)
	Acetyl-CoA carboxylase (EC 6.4.1.2)	Activation	-	-	Munday & Williamson (1987), Burnol <i>et al.</i> (1988)
Triacylglycerols	Lipoprotein lipase (EC 3.1.1.34)	Activation	-	-	Da Costa & Williamson (1994)
Amino acids	Uptake	-	Inhibition	-	Viña <i>et al.</i> (1983)

The only physiological inhibitory signals so far identified are plasma ketone bodies (Table 4) and NEFA, which both increase in starvation or on fat-feeding. Ketone bodies, and particularly acetoacetate *in vitro*, can inhibit glucose utilization and this is reversed by insulin (see Robinson & Williamson, 1980). Administration of ketone bodies decreases glucose (Robinson & Williamson, 1980) and amino acid (Viña *et al.* 1983) utilization *in vivo*. Feeding triacylglycerols rapidly decreases glucose utilization by the lactating gland (Agius & Williamson, 1980), but it is not certain whether this effect is due to chylomicrons *per se* or the accompanying increase in plasma NEFA and/or ketone bodies. Again, this inhibition is partially reversed by insulin.

ENERGETICS OF MILK MACRONUTRIENT SYNTHESIS

Although it has long been established that the lactating mammary gland is a highly active tissue, little attention has been paid to its oxidative metabolism. Notable exceptions are the studies of Linzell and his colleagues (see Linzell, 1968) on the lactating goat.

Oxygen uptake

Measurements of O₂ uptake in conscious lactating goats gave mean values of 0.73 µmol/min per g (Linzell, 1960) and 0.51 µmol/min per g (Reynolds, 1967). The O₂

Table 5. *Oxygen uptake by lactating mammary gland of the rat*

(Values are means with their standard errors)

Mammary gland preparation	Arterio-venous difference ($\mu\text{mol/ml}$)		O_2 uptake [†] ($\mu\text{mol/min per g}$)		References
	Mean	SEM	Mean	SEM	
Slices	–	–	0.69	–	Folley & French (1949)
Perfused	–	–	0.53	–	Clegg & Calvert (1988)
<i>In vivo</i> : Fed (<i>n</i> 6)	3.43	0.17	2.06	0.10	Present work
Starved 24 h (<i>n</i> 4)	2.61	0.38	0.52***	0.076	

Mean value was significantly different from that for *in vivo* fed rats: *** $P < 0.001$.

[†] Blood O_2 was measured with a co-oximeter. Mammary gland blood flows used for the calculations of uptake were means of published values (Chatwin *et al.* 1969; Hanwell & Linzell, 1973; Jones & Williamson, 1984; Viña *et al.* 1987).

uptake is approximately 50% lower in non-lactating goats. For comparison we have measured O_2 uptake by mammary glands of anaesthetized rats (Table 5). The uptake on a per g tissue basis is considerably higher than those in the goat or in preparations of rat mammary gland *in vitro* and decreases by 75% in the starved lactating rat (Table 5) where milk production has virtually ceased and substrate extraction is greatly decreased (Table 3). The difference in O_2 uptake between the mammary glands of fed and starved lactating rats ($1.54 \mu\text{mol O}_2/\text{min per g}$ tissue; Table 5) presumably represents the O_2 required for the processes associated with substrate transport, synthesis of milk components, transport of the ionic components (Ca^{++} , Na^+ , K^+) and milk secretion in the fed state.

ATP requirement

It is instructive to calculate the theoretical amount of ATP required within the gland for the daily synthesis of milk (50 ml) of typical composition (g/l; lactose 44, protein 90, lipid 92) from a chow-fed lactating rat (Table 6). Protein synthesis has by far the major requirement for ATP, exceeding lactose synthesis and uptake of exogenous lipid by nearly an order of magnitude (Table 6). The point of interest is that lipogenesis from glucose is an energy-yielding process (Flatt, 1970). The amount of ATP gained depends on the relative flow of glucose-C through the pentose phosphate pathway and the malate cycle and ranges from one to five ATP equivalents per acetyl-CoA from glucose incorporated into fatty acid; we have used a value of three ATP equivalents (Table 6). There is net utilization of ATP equivalents (in the form of reduced nicotinamide adenine nucleotide) for lipogenesis from quantitatively less important substrates (e.g. lactate, acetoacetate, leucine). If the secretory pool of triacylglycerol (in the mammary gland) undergoes lipolysis and re-esterification, as is the case in liver (Wiggins & Gibbons, 1992), then there would be an additional requirement of seven ATP per mol triacylglycerol reformed (Elia *et al.* 1987). However, present evidence suggests this process proceeds at a slow rate in mammary tissue (D. H. Williamson, unpublished results). When glucose-C provides 50% of the milk lipid as in Table 6, example A (high-carbohydrate diet), the ATP produced virtually balances that required for protein

Table 6. *Energetics of synthesis of milk macronutrients in the lactating mammary gland of the rat*

(Example A is a typical composition and yield of milk from a rat on a high-carbohydrate (chow) diet (Roberts & Coward, 1985); example B is for a high-fat diet. The daily requirement for ATP for synthesis has been calculated as follows: three ATP per lactose (Kuhn & White, 1977); four ATP per peptide bond; two ATP per non-esterified fatty acid activated. It is assumed that lipogenesis from glucose produces three ATP per acetyl group incorporated into a C₁₀ medium-chain fatty acid (see p. 171). The values for lipogenesis have been corrected by subtraction of ATP required for activation of the medium-chain fatty acid)

	Example A		Example B	
	Synthesis (g/24 h)	ATP (mmol/24 h)	Synthesis (g/24 h)	ATP (mmol/24 h)
Lactose	2.2	-18	2.2	-18
Protein	4.5	-150	4.5	-150
Lipid: Lipogenesis	2.3	+139	0.2	+14
Exogenous uptake	2.3	-16	4.4	-31
Balance		-45		-185

synthesis. However, in example B (a high-fat diet), where glucose provides only 4% of the milk lipid, the production of ATP is only 10% that required for protein synthesis. In this situation other substrates must be oxidized and likely candidates are NEFA, ketone bodies and certain amino acids (alanine, threonine and glutamine) which are taken up by the gland in excess of the proportion of those amino acids required for casein synthesis. Thus, the O₂ extraction by the lactating mammary gland depends on a number of factors including blood flow, amount of glucose utilized for lipogenesis compared with other substrates, and rate of milk secretion. As one might expect from the previous discussion, there is a correlation in the goat between O₂ uptake and milk secretion (Linzell, 1960; Reynolds, 1967).

CONCLUSION

This is a brief review of the regulation of substrate utilization by the mammary gland at peak lactation. While the pathways of synthesis of the major components of milk are broadly understood, many aspects of lactation and its regulation need clarification, for example, how blood flow to the gland is controlled and whether changes in blood flow precede alterations in substrate utilization or *vice versa*; the contribution of substrate cycling to the high O₂ consumption of the gland; the processes that determine the relative proportions of lipid, lactose and protein in milk. Clearly, this metabolically versatile tissue does not deserve its Cinderella image.

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