

Insulin resistance of hind-limb tissues *in vivo* in lactating sheep

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1. The effects of varying the plasma insulin concentration by infusion while maintaining euglycaemia by infusion of glucose on nutrient arterio-venous differences across the hind-limb and mammary gland in lactating and non-lactating sheep were investigated. 2. Insulin infusion increased the glucose arterio-venous difference across the hind-limb; this effect of insulin was decreased by lactation, suggesting that lactation induces insulin resistance in skeletal muscle. 3. Lactation increased but insulin infusion decreased the plasma concentrations of acetate, β -hydroxybutyrate and non-esterified fatty acids. 4. Insulin infusion decreased the arterio-venous differences of acetate and hydroxybutyrate across the hind-limb; this effect of insulin is probably indirect, resulting from the decrease in plasma concentrations of these metabolites. 5. Infusion of insulin had no effect on the glucose arterio-venous difference across the mammary gland, but did decrease the oxygen arterio-venous difference. 6. The results suggest that lactation results in insulin resistance in skeletal muscle, at least with respect to glucose utilization; this should facilitate the preferential utilization of glucose by the mammary gland.

INTRODUCTION

The glucose requirements of the mammary gland during lactation can equal or even exceed the glucose requirements of the rest of the body in both rats (Williamson, 1980; Girard *et al.*, 1987) and ruminants (Annison, 1983; Bauman & Elliot, 1983). In rats this increased demand for glucose is met by increasing the food intake (Williamson, 1980). Ruminant animals, however, derive little glucose from the diet due to fermentation of carbohydrates in the rumen and so have to synthesize most of their glucose requirements by gluconeogenesis (Leng, 1970; Bergman *et al.*, 1974). Lactation thus increases gluconeogenesis in ruminants markedly (Vernon, 1988). This need for extra gluconeogenesis is partly diminished by a decrease in glucose utilization by adipose tissue during lactation in ruminants (Vernon, 1988). Surprisingly, there is little (Pethick & Lindsay, 1982a) or no (Oddy *et al.*, 1985) change in glucose utilization by the hind-limb (mostly skeletal muscle) during lactation. In addition, the ability of insulin to stimulate glucose utilization by adipose tissue is diminished in sheep (Vernon & Taylor, 1988) and rats (Burnol *et al.*, 1983, 1986; Jones *et al.*, 1984; Kilgour & Vernon, 1987). Use of the euglycaemic-hyperinsulinaemic clamp technique (De Fronzo *et al.*, 1979) coupled with measurement of 2-deoxy[3 H]glucose uptake by individual tissues suggested that the ability of insulin to stimulate glucose utilization is impaired in epitrochlearis muscle but not in soleus or extensor digitorum longus muscles *in vivo* in lactating rats (Burnol *et al.*, 1987). In the present study we have used the euglycaemic-hyperinsulinaemic clamp to investigate the effects of lactation on the ability of insulin to increase glucose uptake by the whole hind-limb *in vivo* in sheep; evidence is presented for insulin resistance in the hind-limb during lactation.

EXPERIMENTAL

Sheep were 3–5-year-old Finn \times Dorset Horn cross-bred ewes. Control animals were neither pregnant nor lactating (all had lambed in previous years). Lactating ewes, suckling either two or three lambs, were used at about 18 days *post partum*. Sheep were fed on a diet of cereals and hay as described previously (Vernon *et al.*, 1981). Sheep were housed individually in metabolism crates for at least 7 days before the start of an experiment; lambs

remained with their mothers throughout this training period and during the experimental period.

In preliminary studies catheters were inserted into the femoral vein and artery under general anaesthesia (Hay *et al.*, 1984) at least 3 days before an experiment. In subsequent studies catheters were inserted into the femoral artery and vein via the saphenous artery and vein in the groin under local anaesthesia. This latter procedure was preferred as it was less stressful for the sheep (they did not miss a meal). These animals were used after a minimum of 24 h post-operation. The location of the catheter tips was checked by X-ray and infusion of an opaque marker (Omnipaque; Nycomed Ltd., Oslo, Norway). Two catheters were inserted into one jugular vein and a third catheter into the other jugular vein 24 h before an experiment. Arterial catheters were kept patent with heparin (10 units/ml in 0.15 M-NaCl) and venous catheters with 25 mM-citrate in 125 mM-NaCl.

On the day of an experiment, samples of femoral arterial and venous blood were taken simultaneously to provide pre-infusion values of metabolite arterio-venous (a-v) differences across the hind-limb; three pairs of 5 ml samples were taken for metabolite assays and two pairs of 1 ml samples for oxygen determination. Samples of jugular venous blood were also taken to provide a pre-infusion value for plasma glucose concentration. After this, a primed-constant rate infusion of insulin was begun with a concomitant variable rate of infusion of glucose at a rate necessary to maintain euglycaemia, using the initial glucose concentration of the jugular venous plasma as the reference point. This is the euglycaemic clamp technique of De Fronzo *et al.* (1979) as applied to sheep according to Hay *et al.* (1984). Samples of jugular venous blood were taken at 5 min intervals and the glucose concentration was determined with a glucose analyser (Analox Instruments Ltd., London, U.K.). This provided a plasma glucose concentration within 3 min of sampling, after which the glucose infusion rate was adjusted if necessary. Jugular venous blood is an acceptable alternative to arterial blood for monitoring blood glucose during a euglycaemic clamp (Andrews *et al.*, 1984). Steady-state conditions (i.e. plasma glucose concentration remained constant without further change in the rate of glucose infusion) were achieved after about 60 min. Once a steady state was reached, samples of femoral arterial and venous blood were taken simultaneously as described previously, and then 2 \times 10 ml samples of jugular venous blood were

Abbreviation used: a-v difference, arterio-venous difference.

Table 1. Effect of varying the plasma insulin concentration while maintaining euglycaemia on the plasma concentrations and a-v differences across the hind-limb of various metabolites

Non-lactating and lactating ewes were infused with insulin at rates of about 3.5 and 21 pmol/min per kg body weight (low and high rates respectively) while maintaining euglycaemia by infusing variable amounts of glucose. Plasma concentrations and a-v differences across the hind-limb were measured before and after insulin infusion as described in the text. Plasma concentrations of insulin, fatty acid, glycerol and cortisol are for jugular venous blood; all other plasma concentrations are for femoral arterial blood. Values are in $\mu\text{mol/ml}$, except for insulin and cortisol which are in pmol/ml. Results are for up to nine non-lactating and seven lactating sheep, and were analysed by analysis of variance (REML). Values quoted are observed means for pre-infusion and high infusion results, and best linear unbiased estimates of the mean for low infusion rate, as a full set of these values was not obtained. For each row of results, S.E.D.₁ is the standard error of the difference for comparison of mean values from animals in the same physiological state; S.E.D.₂ is the standard error of the difference for comparing mean values of non-lactating sheep with those for lactating sheep (i.e. S.E.D.₁ is for paired values, S.E.D.₂ for unpaired values). DF, degrees of freedom; ns, not significant. Negative values of a-v show a net output.

Variable	Insulin infusion rate...	Non-lactating			Lactating			S.E.D. ₁	S.E.D. ₂	Error DF	Effect	
		Pre-infusion	Low	High	Pre-infusion	Low	High				Lactation	Insulin
Insulin concn.		0.29	0.84	5.55	0.37	0.60	4.73	0.72	0.74	23	ns	$P < 0.001$
Glucose concn.		4.27	4.48	4.62	4.57	4.65	4.64	0.13	0.19	23	ns	ns
Glucose a-v		0.15	0.45	0.95	0.16	0.14	0.50	0.07	0.10	23	$P < 0.01$	$P < 0.001$
Acetate concn.		1.29	1.29	0.95	2.44	1.85	1.32	0.27	0.33	12	$P < 0.02$	$P < 0.001$
Acetate a-v		0.56	0.52	0.43	0.65	0.60	0.51	0.086	0.095	12	ns	$P < 0.05$
β -Hydroxybutyrate concn.		0.24	0.25	0.16	0.56	0.42	0.25	0.05	0.06	15	$P < 0.01$	$P < 0.001$
β -Hydroxybutyrate a-v		0.11	0.12	0.08	0.17	0.14	0.12	0.023	0.026	15	$P < 0.05$	$P < 0.05$
Lactate concn.		0.64	0.53	0.65	0.54	0.52	0.61	0.08	0.09	23	ns	ns
Lactate a-v		-0.09	-0.08	-0.13	-0.11	-0.12	-0.19	0.05	0.05	23	ns	ns
Oxygen concn.		5.74	5.89	6.02	5.66	5.63	5.84	0.20	0.31	22	ns	ns
Oxygen a-v		2.46	2.21	2.86	2.26	2.63	2.83	0.24	0.39	22	ns	ns
Fatty acid concn.		0.13	0.10	0.06	0.26	0.18	0.09	0.05	0.05	15	$P < 0.05$	$P < 0.01$
Glycerol concn.		0.13	0.12	0.12	0.15	0.14	0.14	0.008	0.015	22	ns	ns
Cortisol concn.		44.4	25.6	57.4	39.4	37.2	37.2	19.8	20.4	12	ns	ns

withdrawn. Blood samples were taken with heparinized syringes, and plasma was prepared by centrifugation and stored at -20°C before assay of glucose, acetate, lactate (Vernon *et al.*, 1981) and β -hydroxybutyrate (Williamson *et al.*, 1962) in the samples of femoral arterial and venous plasma, and of insulin, glycerol (Vernon *et al.*, 1981), non-esterified fatty acids (Mabon *et al.*, 1982) and cortisol (Flint *et al.*, 1984) in the jugular venous plasma. Blood oxygen concentration was determined immediately after sampling using a blood gas analyser (Radiometer Co., Copenhagen, Denmark).

Two insulin infusion rates were used of approx. 3.5 and 21 pmol/min per kg body weight; insulin infusion solution was prepared as described by Hay *et al.* (1984). Initially sheep were infused at the lower rate first and then at the higher rate on the same day. In later experiments animals received one infusion on the first day and then the second no less than 24 h later.

A catheter was inserted into the caudal superficial epigastric (milk) vein (draining from the mammary gland) in three ewes at the time of placing the catheters in the femoral vessels. Samples of mammary venous blood were taken at the same time as femoral arterial and venous blood during the experiments.

In three preliminary experiments blood flow through the hind-limb was measured by dye dilution using Indocyanine Green (Bell *et al.*, 1974) before and after insulin infusion. Blood flow was measured after sampling of blood for metabolite assays.

Statistical analysis was performed using analysis of variance (REML) (Patterson & Thompson, 1975); values presented are true means or best linear unbiased estimates of the mean when there was an incomplete data set for all animals.

RESULTS

Plasma insulin concentrations before infusion were similar in

both lactating and non-lactating sheep (Table 1). Infusion of insulin at the lower rate approximately doubled the plasma insulin concentration, whereas infusion at the higher rate resulted in mean plasma insulin concentrations of about 5 nM (Table 1). Maximum stimulation of glucose turnover in whole sheep (Weekes *et al.*, 1983; Hay *et al.*, 1988) and goats (Debras *et al.*, 1989) using the euglycaemic clamp technique were achieved with insulin concentrations in excess of 3 nM (Weekes *et al.*, 1983). Thus the higher infusion rate should result in a maximum stimulation of metabolite utilization by the hind-limb and mammary gland. Lactation had no significant effect on insulin concentrations achieved by infusion (Table 1).

Plasma glucose concentrations in femoral arterial blood were the same before and at the end of the period of insulin infusion (Table 1), showing that successful euglycaemic clamps had been achieved. Lactation had no effect on the mean plasma glucose concentration (Table 1). The concentrations of oxygen and lactate in arterial blood and plasma and of glycerol in jugular venous plasma were unchanged by lactation (Table 1). In contrast, lactation increased the concentrations of acetate ($P < 0.02$) and β -hydroxybutyrate ($P < 0.01$) in arterial plasma and of non-esterified fatty acids ($P < 0.05$) in jugular venous plasma (Table 1). Insulin infusion decreased the plasma concentrations of acetate ($P < 0.001$), β -hydroxybutyrate ($P < 0.01$) and non-esterified fatty acids ($P < 0.01$), but had no effect on the plasma concentrations of the other variables measured (Table 1).

Preparatory experiments showed that insulin had no effect on blood flow through the hind-limb (results not shown), so this measurement was discontinued in further experiments. Prior *et al.* (1984) found that insulin infusion had no effect on blood flow through the hind-limb of steers. Also, Oddy *et al.* (1985) found no effect of lactation on blood flow through the hind-limb in sheep. Thus changes in a-v difference found in the present

Table 2. Effects of various plasma insulin concentrations on metabolite a-v differences across the mammary gland in lactating sheep

Plasma insulin concentrations were increased by infusing insulin while maintaining euglycaemia as described in the legend to Table 1 and in the text. Mammary a-v differences were measured before and after insulin infusion. Results are means of three observations and were analysed by analysis of variance. S.E.D. is standard error of the difference. Negative values show a net output rather than a net uptake. ns, not significant.

Metabolite	Insulin infusion rate...	A-v difference ($\mu\text{mol/ml}$)			S.E.D.	Insulin effect
		Pre-infusion	Low	High		
Glucose		1.23	1.23	1.11	0.07	ns
Lactate		-0.03	-0.02	-0.02	0.06	ns
Acetate		1.93	1.46	0.96	0.29	$P < 0.1$
β -Hydroxybutyrate		0.36	0.23	0.16	0.07	$P < 0.1$
Oxygen		2.40	1.98	1.80	0.13	$P < 0.05$

study in response to insulin or lactation reflect changes in net uptake or output of the metabolite.

Insulin infusion increased ($P < 0.001$) the glucose a-v difference across the hind-limb (Table 1). Lactation had no effect on the glucose a-v difference before insulin infusion, but decreased significantly ($P < 0.01$) the increase in a-v difference in response to insulin infusion. Lactation appears to diminish both the response and the sensitivity of the hind-limb to insulin, for not only was the glucose a-v difference in the presence of a maximum insulin concentration decreased ($P < 0.01$), but in addition insulin infusion at a rate of about 3.5 pmol/min per kg body weight had no effect on glucose a-v difference in lactating sheep (Table 1). The β -hydroxybutyrate a-v difference across the hind-limb was increased by lactation ($P < 0.05$) and decreased by insulin infusion ($P < 0.05$) (Table 1). Insulin infusion also decreased the acetate a-v difference across the hind-limb ($P < 0.05$), but, in contrast with β -hydroxybutyrate, the a-v difference was not increased by lactation (Table 1). Neither lactation nor insulin infusion altered the a-v difference across the hind-limb for oxygen or lactate (Table 1).

Insulin infusion had no effect on either the glucose or the lactate a-v difference across the mammary gland (Table 2). The a-v difference for both acetate and β -hydroxybutyrate tended to decrease on insulin infusion ($P < 0.1$) (Table 2), and the oxygen a-v difference across the gland was significantly decreased ($P < 0.05$) by insulin.

DISCUSSION

The most important finding of this study is that lactation results in a decreased ability of insulin to stimulate glucose utilization by the hind-limb. This is most probably due to altered responsiveness (response and sensitivity) of skeletal muscle to insulin. The study also shows that, although there may be differences in the way individual muscles adapt to lactation, as shown in the rat (Burnol *et al.*, 1987), in the sheep at least the overall effect for a major mass of muscles is a diminished responsiveness to insulin. The ability of insulin to stimulate some aspects of adipose tissue metabolism is also diminished during lactation (Vernon & Taylor, 1988). Thus any increase in plasma insulin concentration, for example following a meal of cereals, is likely to result in little or no stimulation of glucose utilization by muscle and adipose tissue, thus favouring glucose utilization by the mammary gland.

The mechanism responsible for the diminished responsiveness to insulin during lactation is unknown. The ability of insulin to bind to receptors in membranes from skeletal muscle (Metcalf *et al.*, 1987) and to adipocytes (Vernon & Taylor, 1988) appears to be unchanged by lactation in sheep, suggesting a post-receptor impairment.

The maximum response to insulin could be limited by the capacity of the tissue to transport or utilize glucose. Hexokinase activity does not change with lactation in sheep muscle (Vernon *et al.*, 1987), although it may limit the maximum response to insulin in non-lactating sheep: estimated maximum insulin-stimulated rate of glucose utilization in non-lactating sheep is about 95 $\mu\text{mol/min}$ per kg in this study, compared with a hexokinase activity of skeletal muscle of about 80 $\mu\text{mol/min}$ per kg (Vernon *et al.*, 1987). Also, there is little reason to suspect a limitation at the level of glycolysis: activities of glycolytic enzymes exceeded greatly hexokinase activity in sheep muscle (Vernon *et al.*, 1987), oxygen consumption was unchanged by insulin and lactation, and lactate output was unchanged by insulin or lactation (present study). It is arguable that there may have been an increase in glucose oxidation to compensate for a diminished uptake of acetate and β -hydroxybutyrate during insulin infusion, but this would account for less than 10% of glucose uptake and would be expected to be greater in lactating sheep. It would seem then that there is probably a defect in the ability of insulin to stimulate either glucose transport or glycogen synthesis during lactation. Glycogen is the most probable fate of the additional glucose utilized during insulin infusion, and glycogen synthase of muscle is activated by insulin in sheep as in rats (Sasaki, 1989).

In addition to its effects on glucose utilization, insulin also decreased the uptake of both acetate and β -hydroxybutyrate by the hind-limb. This effect however is most probably indirect, as uptake of both of these metabolites by the hind-limb is proportional to their concentration in the blood (Pethick & Lindsay, 1982a,b). The higher β -hydroxybutyrate uptake by the hind-limb in lactating ewes is also probably due to the elevated plasma concentration. In contrast, despite the plasma acetate concentration being greater in lactating than in non-lactating ewes, acetate uptake by the hind-limb was not increased. This is consistent with the report of Pethick & Lindsay (1982a), that whereas uptake of acetate by the hind-limb is proportional to concentration in both lactating and non-lactating sheep, uptake at a given concentration is lower in lactating ewes. Thus the ability of the hind-limb to utilize acetate is also impaired by lactation.

Raising the plasma insulin concentration had no effect on the glucose a-v difference across the mammary gland, as found previously in studies with goats (Hove, 1978) and cattle (Laarveld *et al.*, 1981, 1985). The recent report that insulin infusion can decrease mammary glucose a-v in sheep (Leenanuruksa *et al.*, 1988) was not confirmed in the present study. Leenanuruksa *et al.* (1988) were also infusing somatostatin into their animals and the decrease in mammary glucose a-v was seen after about 6 h of insulin infusion; hence, several factors could account for the different findings.

There was a tendency ($P < 0.10$) for both acetate and β -hydroxybutyrate a-v differences across the mammary gland to decrease as plasma insulin was raised, but this was probably due to the concomitant fall in plasma concentrations of these metabolites. This view is supported by studies with cows in which insulin infusion failed to change the mammary a-v difference of either acetate or β -hydroxybutyrate, but on this occasion there was no change in the plasma concentrations of the two metabolites (Laarveld *et al.*, 1985).

An unexpected finding was the fall in oxygen a-v difference across the mammary gland during insulin infusion. The reason

for this is not certain. One possibility is an increase in mammary blood flow during insulin infusion, but this seems unlikely, as raising serum insulin had no effect on mammary blood flow in two previous studies with goats (Linzell, 1967; Chaiyabutr *et al.*, 1983). Hove (1978) did find an increase in mammary blood flow during a prolonged infusion of insulin into lactating goats, but this was accompanied by a decrease in glucose $a-v$ difference across the mammary gland so that glucose uptake was unaltered. An increase in mammary blood flow during insulin infusion in the present study would mean an increase in glucose uptake in response to insulin, which is at variance with other studies (see above). Alternatively a decrease in the oxygen $a-v$ difference may reflect a decrease in mammary metabolism arising from the fall in plasma concentration, and hence uptake by the mammary gland, of substances such as acetate, β -hydroxybutyrate and perhaps non-esterified fatty acids.

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