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Insulin Affects Glucose Uptake by Muscle and Mammary Tissues of Lactating Ewes

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Abstract

Effects of insulin on exchanges of glucose across skeletal muscle and mammary tissue were measured in short-term studies in lactating ewes. Insulin secretion was suppressed by a primed/continuous infusion of somatostatin, then insulin was administered by continuous intravenous infusion of doses that were increased, in a step-wise manner, from 0 to 2 U h^{-1}. Plasma glucose was maintained essentially constant by frequent monitoring and intravenous administration of exogenous glucose.

Somatostatin suppressed but did not completely inhibit insulin secretion as shown by maintenance of plasma concentration of C-peptide. As plasma insulin was increased, while arterial glucose was maintained stable, uptake of glucose by skeletal muscle increased and glucose uptake by the mammary gland decreased. These observations confirm the role of insulin in regulating glucose uptake by skeletal muscle and raise the possibility that insulin also regulates glucose uptake by the mammary gland.

Extra key words: somatostatin, insulin, C-peptide, plasma glucose.

Introduction

Glucose is an indispensable nutrient for the mammary gland and in ruminants accounts for some 50–80% of the glucose entry rate into the body (Annison 1983). Although the key role of insulin in regulating glucose disposal in the ruminant is now recognized (see Brockman and Laarveld 1986; Weekes 1986), there are indications from results of *in vitro* (Folley and McNaught 1958; Bauman *et al.* 1973; Baldwin and Louis 1975) and *in vivo* (Hove 1978*b*; Chaiyabutr *et al.* 1983; Laarveld *et al.* 1985) studies that the ruminant mammary gland is insensitive to insulin. However, it is possible that results of the several previous studies on effects of insulin on glucose uptake by the mammary gland were confounded by failure to maintain stable concentrations of glucose and/or hormones including insulin. Accordingly, in the present study the role of insulin in controlling glucose uptake by skeletal muscle and mammary tissue has been studied in lactating ewes in which plasma concentrations of glucose and insulin were controlled closely.

Materials and Methods

Sheep

Four multiparous crossbred ewes (Border Leicester × Merino), free of obvious abnormalities of the mammary glands, which had been lactating for 3 weeks, were used for the experiment. The ewes had been maintained in metabolism cages from the day of parturition, when lambs were removed permanently from their dams. All ewes were accustomed to handling and were milked by hand without restraint and obvious distress. Milking occurred at *c.* 0800 and 1630 hours each day. The ewes produced 1045 ± 54·1 g day^{-1} (mean ± s.e.m.) of milk during the week over which the experiment was performed.

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Water was provided *ad libitum* and a good-quality ration containing rolled barley : chopped licerne (50 : 50 air dry; 9·6 MJ metabolizable energy and 218 g crude protein kg^{-1} dry matter) was fed continuously, using belt feeders, to avoid *post-prandial* changes in metabolites and hormones. Amounts of feed offered were sufficient to satisfy requirements for metabolizable energy for maintenance plus milk production (Anon. 1975).

Ewes were surgically prepared as described previously by Oddy *et al.* (1981) and Teleni and Annison (1986). Approximately 10 days before measurements were made, polyvinyl chloride catheters (0·86 mm internal diameter by 1·27 mm external diameter; Dural Plastics, Dural, N.S.W., Australia) were inserted into a deep femoral artery (A). Three to five days before experiments, additional polyvinyl chloride catheters (1·00 mm internal diameter by 1·5 mm external diameter) were inserted in both external jugular veins, a medial subcutaneous mammary vein (MV) and a deep femoral vein (LV), via the lateral saphenous vein. The tips of the catheters in LV and MV were located so as to collect blood draining predominantly leg muscle and mammary tissues respectively. All catheters were kept patent by flushing with minimal amounts of sterile heparinized saline (2×10^5 i.u. heparin and 9·0 g NaCl l^{-1} of distilled water).

Hormone Solutions and Infusates

Cyclic somatostatin, obtained from Peptide Technology Limited (Dee Why, N.S.W., Australia), was dissolved in sterile 0·9% (w/v) NaCl. Recombinant bovine growth hormone (rebGH), obtained from American Cyanamid Company (Princeton, U.S.A.), was dissolved initially in bicarbonate buffer (pH 9·5). An infusate containing both somatostatin and rebGH was prepared by mixing appropriate amounts of each solution. Insulin infusates contained crystalline bovine insulin (regular insulin; Commonwealth Serum Laboratories, Parkville, Victoria, Australia), which was diluted appropriately with a solution of bovine serum albumin (1 g l^{-1}; Commonwealth Serum Laboratories). All infusates were sterilized by filtration (0·22 μm; Millipore Corporation, Massachusetts, U.S.A.) prior to use.

Experimental Procedures

The experiment was conducted over 4 days such that measurements were made on only one ewe per day. Starting 30 min after morning milking (time zero) the ewe was given a rapid infusion of 160 μg somatostatin via the catheter in one jugular vein. Immediately afterwards the mixed infusate containing somatostatin and rebGH was administered at rates of 80 μg h^{-1} and 20·8 μg h^{-1} respectively over a period of 10 h. Over successive periods of 2 h insulin was infused at the rate of either 0, 0·15, 0·5, 1·0 or 2·0 U h^{-1}. The doses of somatostatin used have been reported to suppress insulin secretion in sheep with similar liveweights (Brockman and Greer 1980; Brockman and Halvorson 1981). Since somatostatin also suppresses secretion of growth hormone (Brockman and Laarveld 1986), rebGH was infused to maintain plasma concentrations of growth hormone (GH).

Throughout the 10 h of the experiment on each ewe, variable amounts of glucose (50% w/v; Abbott Australia Pty Ltd) were infused via the second jugular catheter so as to maintain blood glucose level as close as possible to the level prior to time zero. Concentrations of blood glucose in A were monitored at intervals of *c.* 5 min, by the one operator using a glucometer (Miles Laboratories, Elkhart, U.S.A.).

Three sets of blood samples (2 ml per vessel) were collected simultaneously from A, LV and MV over the 10 min before time zero, then over the 10 min at the end of each period of 2 h thereafter. In addition, a further 11 samples of 2 ml were collected from A, at intervals of *c.* 10 min, during the first 2 h of the experiment, during which time no insulin was infused concomitantly with somatostatin/rebGH. All blood samples were collected into ice-chilled tubes containing heparin as anti-coagulant. Plasma was prepared within 15 min of collection of blood. An aliquot of plasma was stored as such at −16°C and a further aliquot was deproteinized with 0·44 M $HClO_4$ prior to storage at −16°C.

Analyses

Blood glucose was monitored with the glucometer as outlined by the supplier (Miles Laboratories) whereas plasma glucose was measured in deproteinized samples using the autoanalyser method of Bernt and Lachenicht (1974).

Plasma concentrations of GH and insulin were measured as outlined previously by Gow *et al.* (1981) with the exception that polyethylene glycol was used to separate 'free' and 'antibody-bound' insulin as described by Desbuquois and Aurbach (1971). Both assays measure bovine and ovine hormones with similar avidity (Leenanuruksa and McDowell, unpublished observations). Plasma concentrations of

C-peptide were measured, within a week of collection of samples, by the method described by Leenanuruksa and McDowell (1988).

To avoid interassay variation, for particular hormones measurements were made on single batches of samples that were to be compared. Assays had intra-assay coefficients of variation of <15% and the sensitivities of the assays were 1·0 μg l^{-1}, 2·4 mU l^{-1} and 0·13 nM for GH, insulin and C-peptide, respectively.

The significance of differences between mean values for parameters measured during the various periods of the experiment were assessed using paired *t*-tests.

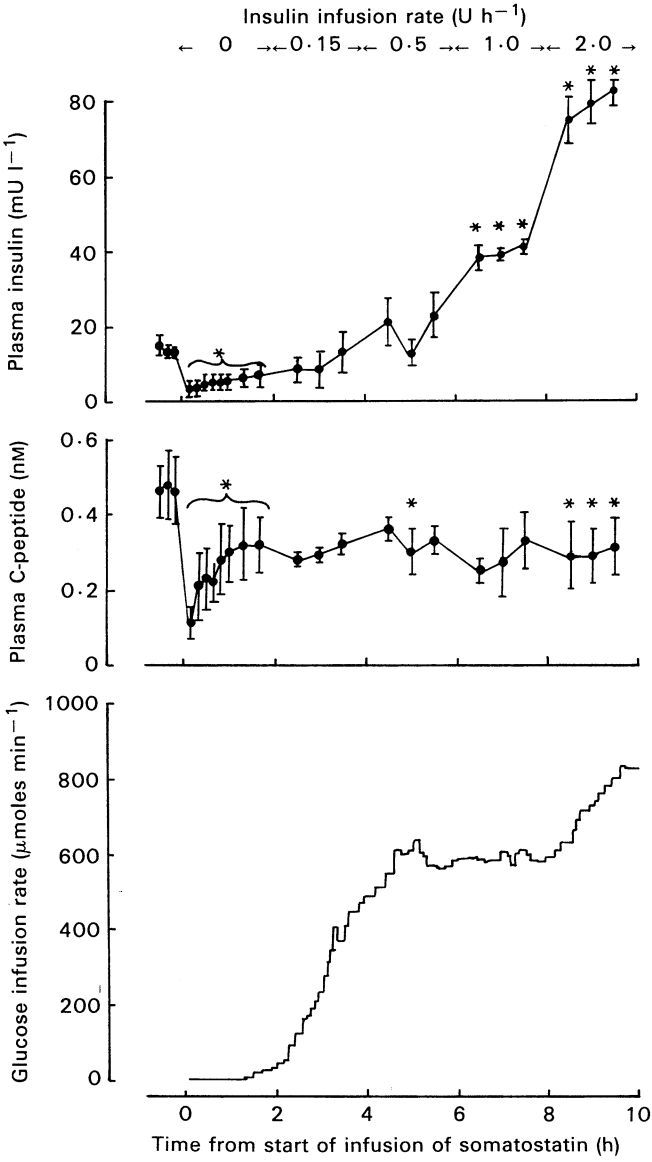

Fig. 1. Arterial plasma concentrations of insulin and C-peptide together with rates of infusion of glucose before and during infusion of somatostatin. Plotted points and histograms represent mean values for four ewes and standard errors are shown as vertical bars. Values that differ significantly from those before start of somatostatin infusion are indicated thus: $*P < 0{\cdot}05$.

Results

Plasma Insulin

Changes in plasma concentrations of insulin are depicted in Fig. 1. Before infusion of somatostatin plasma insulin concentration was stable at *c.* 14 mU l^{-1}. Within 5 min of the infusion plasma insulin concentration had decreased significantly ($P < 0 \cdot 01$) to *c.* 5·3 mU l^{-1} and had further decreased ($P < 0 \cdot 001$) to *c.* 3·5 mU l^{-1} by 15 min. Thereafter, during the first 2 h of the experiment, concentrations of insulin gradually increased but remained significantly lower ($P < 0 \cdot 05$) than before infusion of somatostatin.

In response to infusion of 0·15 U h^{-1} of insulin, plasma insulin concentration increased and was not significantly different ($P > 0 \cdot 05$) from that before infusion of somatostatin. When the insulin infusion rate was increased to 0·5 U h^{-1} in the third period, plasma insulin concentration increased, to be higher, but not significantly so ($P > 0 \cdot 05$), than the pre-infusion values. In the last two periods of insulin infusion (viz. 1·0 and 2·0 U h^{-1}), plasma insulin concentration increased progressively and significantly to *c.* 40 mU l^{-1} ($P < 0 \cdot 01$) and *c.* 80 mU l^{-1} ($P < 0 \cdot 001$) respectively.

Plasma C-peptide

Changes in concentrations of plasma C-peptide are shown in Fig. 1. Before infusion of somatostatin, plasma concentrations of C-peptide of *c.* 0·45 nM were measured. Within 10 min of the infusion, plasma C-peptide concentration had decreased significantly ($P < 0 \cdot 01$) to *c.* 0·11 nM. Thereafter, concentration increased to *c.* 0·22 nM ($<0 \cdot 01$) by 20–40 min and, at the end of the first period of the experiment, plasma C-peptide levels remained significantly reduced at *c.* 0·32 nM ($P < 0 \cdot 05$). For the remaining 8 h of the experiment and at all rates of infusion of insulin, plasma concentrations of C-peptide (*c.* 0·25–0·36 nM) were consistently lower than during the pre-infusion period. Even so, differences were significant ($P < 0 \cdot 05$) at only 5 h and between 8 and 10 h after time zero.

Plasma-GH

During the infusion of somatostatin concentrations of GH ranged from 1·3–1·9 μg l^{-1} and were not significantly different from the mean values (three observations for each ewe) measured during the period before infusion of somatostatin (1·4 μg l^{-1}). There was no evidence of pulsatile secretion of GH during the 10 min before or over the 10 h after the start of infusion of somatostatin.

Glucose Infusion Rate

The rates of infusion of glucose required to maintain 'normal' plasma glucose are depicted in Fig. 1. As early as 5 min after starting the infusion of somatostatin some ewes required a glucose infusion to maintain euglycaemia. Rates of infusion of glucose increased to *c.* 30 μmol min^{-1} by the end of the first period when no insulin was infused, then increased sharply when insulin was infused at the rate of 0·15 U h^{-1}. By the end of the second period of the experiment (viz. 4 h) glucose was infused at the rate of *c.* 490 μmol min^{-1} and the rate was increased further to *c.* 600 μmol min^{-1} when the insulin infusion rate was changed to 0·5 U h^{-1}. By the end of this third period and throughout the fourth period during which insulin was infused at the rate of 1·0 U h^{-1}, the rates of glucose infusion changed very little. A sharp increase in glucose infusion rate was required during the last period when insulin was infused at the rate of 2·0 U h^{-1}. The rate of glucose infusion was increased to *c.* 830 μmol min^{-1} at the end of this period.

Plasma Glucose

Changes in concentrations of glucose in arterial plasma are depicted in Fig. 2. Before infusion of somatostatin stable concentrations of *c.* 3·9 mM glucose were measured. By

10 min after somatostatin infusion, a significant decrease ($P < 0 \cdot 05$) was observed. Thereafter, plasma glucose concentrations were not significantly different ($P > 0 \cdot 05$) from the values for the pre-infusion period.

The arterio–venous difference for glucose across leg muscles (A–LV) was similar before and at the end of the first period of the experiment. Thereafter, as the rate of infusion of insulin increased, the value for A–LV for glucose increased and was significantly higher ($P < 0 \cdot 05$) than before time zero except during the last period of the experiment when insulin was infused at the rate of 2 U h^{-1} (see Fig. 2).

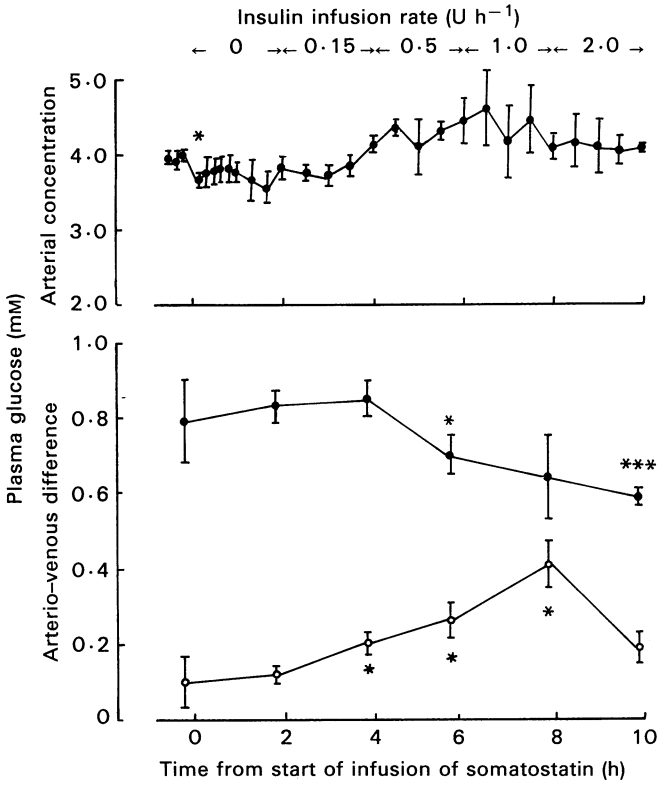

Fig. 2. Concentrations of glucose in arterial plasma and arterio–venous differences of plasma glucose across mammary (●—●) and leg muscle (○—○) tissues before and during infusion of somatostatin. Plotted points represent mean values for four ewes and standard errors are shown as vertical bars. The values for arterio–venous differences for individual ewes were means of three measurements. The single value for arterial glucose that differs significantly ($P < 0 \cdot 05$) from the pre-infusion value is indicated by the single asterisk. Values for arterio–venous differences of glucose that differ significantly from the value 2 h after the start of infusions are indicated thus: $*P < 0 \cdot 05$, $***P < 0 \cdot 001$.

Changes in arterio–venous difference for glucose across the mammary gland (A–MV) also are depicted in Fig. 2. The values for A–MV for glucose were similar before time zero and when insulin was infused at 0 and 0·15 U h^{-1}. When the rate of infusion of insulin was increased to 0·5 U h^{-1} and beyond, A–MV decreased and was significantly reduced during infusion of insulin at the rate of 0·5 U h^{-1} ($P < 0 \cdot 05$) and 2·0 U h^{-1} ($P < 0 \cdot 01$).

Discussion

Plasma concentrations of insulin were reduced substantially by administration of somatostatin, with the most marked effects occurring shortly after infusion of the 'priming' dose

of somatostatin (see Fig. 1). The depression of plasma insulin was maintained during the first 2 h of the experiment when no exogenous insulin was administered. These observations are consistent with previous reports that somatostatin suppresses secretion of insulin from the pancreatic β-cells (Koerker *et al.* 1974; Brockman and Greer 1980).

The data for plasma concentrations of C-peptide are of interest in connection with the above. Measurement of plasma C-peptide, the moiety joining the A and B chains of the pro-insulin molecule, allows assessment of insulin secretion rate (see Polonsky and Rubenstein 1984; Radziuk and Morishima 1985; Leenanuruksa and McDowell 1988). The sharp decrease in plasma C-peptide shortly after infusion of the 'priming' dose of somatostatin indicates a rapid and marked effect of somatostatin on insulin secretion. Moreover, the observation that plasma C-peptide remained lower than before somatostatin administration is consistent with suppression of insulin secretion by somatostatin. Infusion of insulin from the end of the second hour of the experiment did not increase plasma C-peptide, confirming the purity of insulin that was infused.

Even though plasma concentrations of both insulin and C-peptide were decreased during administration of somatostatin, it is apparent that the pancreatic β-cells continued to secrete insulin. Secretion of insulin throughout the first 2 h of the experiment was indicated by the maintenance of plasma concentrations of insulin at the lower end of the normal physiological range (see McAtee and Trenkle 1971). Further evidence that insulin secretion from the β-cells continued throughout the experiment is provided by the observation that C-peptide was measurable in plasma throughout the experiment.

The initial decrease in plasma glucose following administration of somatostatin might be explained by reduced glucose production resulting from effects of somatostatin in inhibiting glucagon (Brockman and Greer 1980). It is apparent from the data in Fig. 2 that the infusions of glucose compensated for effects of somatostatin on glucose production. Indeed, plasma concentrations of glucose were maintained essentially stable at *c.* 4 mM by closely monitoring plasma glucose and adjusting the rate of administration of glucose frequently (see Fig. 1). Overall, the amount of exogenous glucose required to maintain euglycaemia increased as the rate of insulin infusion increased. Even so, between 6 and 8 h after the start of infusion of somatostatin, when insulin was infused at the rate of 1 U h^{-1}, the requirement for exogenous glucose was similar to that required during the previous period when insulin was infused at 0·5 U h^{-1}.

It is evident from Fig. 2 that there were marked effects of insulin on arterio–venous differences of glucose across both mammary and muscle tissues. Although tissue blood flow rates were not measured in the present study it seems reasonable to assume that tissue blood flow rates would not have been affected by the procedures imposed. In this connection, Chaiyabutr *et al.* (1983) found no change in mammary blood flow in goats in which plasma insulin concentration was doubled by infusion of glucose. Similarly, Prior *et al.* (1984) measured no changes in blood flow to the hind-quarters of young cattle given short-term infusions of either insulin or glucose. Accordingly, it appears reasonable to suggest that differences in arterio–venous differences measured in the present experiment reflected glucose uptakes across leg muscle and mammary tissues.

The observed effects of insulin on uptake of glucose by leg muscle tissue were consistent with previous reports that insulin promotes glucose uptake by muscle in the ruminant (Prior *et al.* 1984; Brockman and Laarveld 1986; Weekes 1986), except at 10 h after the infusion of somatostatin began.

Glucose transport across the plasma membranes of insulin-sensitive and non-sensitive tissues occurs by a facilitated diffusion process through a glucose transporter (Baldwin and Lienhard 1981). In insulin-sensitive tissues, insulin stimulates glucose transport by translocation of glucose transporters to the plasma membrane from a large intracellular pool (see Cushman and Simpson 1985). The reduction in glucose uptake by hind limb muscle in the last period of infusion of insulin can be explained by mechanisms suggested by Kubo and Foley (1986). These workers considered that at physiological concentrations of glucose, in the presence of insulin concentrations that stimulate maximally, the rate-limiting step for

insulin-mediated uptake of glucose and for glucose metabolism in muscle shifts from glucose transport to some step beyond transport.

A suppressive effect of insulin at high concentration (while euglycaemia is maintained) on mammary glucose uptake has not been reported before. Kronfeld *et al.* (1963) showed that direct administration of insulin produced systemic hypoglycaemia and decreased milk yield in cattle. In other studies, in which glucose was infused concomitantly with insulin to prevent hypoglycaemia (Hove 1978*b*; Laarveld *et al.* 1981, 1985) or in which glucose was infused alone resulting in increased plasma insulin (Chaiyabutr *et al.* 1983), there were no obvious changes in mammary glucose uptake.

It is clear from Fig. 2 that at low levels of plasma insulin (over the first 4 h of somatostatin infusion) there was no obvious change in glucose uptake by the mammary gland. However, when plasma insulin was raised to concentrations *c.* two, three and six times higher than during the pre-infusion period, mammary uptake of glucose decreased to 84, 77 and 70% respectively of that measured in the first period of somatostatin infusion when no insulin was infused.

The present data suggest that, at low concentrations of insulin, glucose uptake by the mammary gland is 'permitted' and that a suppressive effect occurs at high concentrations of insulin. It is interesting to note that after 6 and 8 h of somatostatin infusion (insulin infusion rates 0·5 and 1·0 U h^{-1} respectively) glucose uptake by the mammary gland decreased whereas a stimulatory effect of insulin on glucose uptake by hind limb muscle was apparent.

Most investigations on effects of insulin on glucose transport and metabolism in the ruminant mammary gland *in vivo* have been designed to measure the effects of increased concentrations of insulin (Hove 1978*b*, Laarveld *et al.* 1981, 1985). There is a possibility that the mechanism of glucose transport in mammary tissue of ruminants is an insulin-mediated process and that saturation of the mammary transport system occurs at lower plasma concentrations of insulin than in insulin-sensitive tissues like muscle. Interestingly, the suppressive effect of insulin at high concentrations on glucose uptake by the mammary gland at 6, 8 and 10 h of somatostatin infusion resembled that in hind-limb muscle at 10 h.

Specific insulin receptors have been characterized in bovine mammary tissue (Oscar *et al.* 1986) and the number of microsomal receptors is altered by stage of pregnancy and lactation (Campbell *et al.* 1987). It appears that only a fraction of the total receptor number need be occupied to elicit a maximal cellular response (Olefsky and Ciaraldi 1981). Recently, Smith *et al.* (1986) suggested that if 10% receptor occupancy were to be accepted as the degree of receptor loading required to elicit a maximal intracellular response, a plasma insulin concentration as low as 4·8 mU l^{-1} would be sufficient to promote maximal uptake of glucose in bovine mammary tissue. This low concentration of insulin of 4·8 mU l^{-1} is within the physiological range reported for ruminants (Horino *et al.* 1968; McAtee and Trenkle 1971) and is lower than the concentration considered by Hove (1978*a*) to indicate insulin deficiency. At the end of the first 2 h of infusion of somatostatin in the present study, plasma insulin exceeded this value (see Fig. 1).

In conclusion, the data from this study show that low concentrations of plasma insulin favour glucose uptake by the mammary gland in lactating sheep but at high plasma concentrations insulin suppresses mammary glucose uptake even when glucose availability is apparently not limited. It is possible that the mechanism of glucose transport in ruminant mammary tissue is an insulin-mediated process and that the transport system is saturated at very low concentrations of plasma insulin. The present observations suggest the need to re-evaluate the role of insulin in controlling glucose uptake by the ruminant mammary gland.

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