

Control of Glucose Homeostasis in Lactating Ewes: Use of the Alloxan-diabetic/Insulin-stabilized Ewe to Study Effects of Insulin and Growth Hormone

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Abstract

Two separate experiments were conducted with alloxan-induced, diabetic ewes. In one study it was found that the diabetes induced by alloxan could be stabilized with exogenous insulin ($1.2\text{--}1.3\text{ U h}^{-1}$). Feed intake and milk yield were maintained at normal levels even though a mild hyperglycaemia persisted. Despite this, milk fat content tended to increase, an observation that is consistent with insulin being a key factor in the aetiology of the low-milk-fat syndrome in the ruminant.

Interruption of insulin infusion then resumption at 90% of the rate previously required to stabilize the diabetes was followed by marked changes in glucose kinetics. Initially, glucose production increased with little change in glucose utilization. This resulted in an increase in plasma glucose, which remained high even though both glucose production and utilization increased, to be similar on resumption of insulin infusions. It seems that the changed sensitivity to insulin reflects 'up-regulation' of insulin receptors.

In a second study, exogenous recombinant bovine growth hormone (rebGH) was administered to insulin-stabilized, diabetic ewes. Immediately after the first injection of rebGH, glucose production increased with little change in glucose utilization, which led to increased plasma glucose. This observation suggests that rebGH was glucogenic. Ultimately, it was necessary to increase the dose of insulin to stabilize plasma glucose and by the fourth day of injection of rebGH, the insulin infusion rate required to stabilize the ewes had doubled from *c.* 1.5 to *c.* 3 U h^{-1} . After cessation of injections of rebGH the dose of insulin required to stabilize the ewes decreased. These observations confirm the diabetogenic activity of growth hormone (GH) in the sheep.

Extra key words: glucose kinetics, milk yield and composition.

Introduction

The key role of insulin in regulating glucose homeostasis in most species is well recognized and characterized (Brockman and Laarveld 1986a; Weekes 1986). In ruminants, little or no glucose is absorbed from the alimentary tract and it has been suggested that the tissues of ruminants may be less sensitive to insulin than the tissues of non-ruminants. There is, however, evidence to suggest that the tissues of ruminants are just as sensitive to insulin as those of non-ruminants and that insulin is crucial for regulation of glucose production and disposal (see McDowell 1983).

It appears that GH is one of several hormones that act in concert with insulin to regulate availability of glucose, and other metabolites in the body. It seems likely that diabetogenic effects of GH (see Hart 1983) may, at least in part, explain the galactopoietic effects of GH, which are now well recognized (see reviews by McCutcheon and Bauman 1985; Johnsson and Hart 1986). Although the galactopoietic effects of GH are well known,

the mechanisms by which the hormone increases milk production have not been explained fully.

Recently, we described procedures for induction of diabetes in lactating ewes with alloxan and subsequent stabilization of the diabetic state with exogenous insulin (Leenanuruksa and McDowell 1988). In the studies reported here, alloxan-treated, lactating ewes stabilized with intravenous insulin were used with the view to gaining insights into the roles of insulin and GH in controlling glucose homeostasis.

Materials and Methods

Sheep

Multiparous lactating crossbred ewes (Border Leicester \times Merino) free from obvious abnormalities of the mammary glands were used in the experiments. The ewes had been lactating for c. 30 days and 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 twice daily at c. 0830 and 1630 hours.

Water was provided *ad libitum* and a pelleted diet containing 40% lucerne chaff, 14.5% wheaten chaff, 33% oats, 12% fishmeal and 0.5% minerals (9.6 MJ metabolizable energy and 218 g crude protein kg^{-1} dry matter) was fed continuously using a belt feeder. The daily allocation of the pellets was 2.5 kg and feed residues were measured daily.

Both external jugular veins of each ewe were fitted with indwelling polyvinyl chloride catheters (1.0 mm internal diameter by 1.5 mm external diameter; Dural Plastics, Sydney) at least 24 h prior to starting the experiment. Catheters were kept patent by flushing with minimum amounts of sterile heparinized saline (2×10^5 i.u. heparin and 9.0 NaCl l^{-1} of distilled water).

Experimental Procedures

Two separate experiments were performed using the same animals. In one study (Experiment 1) with alloxan-diabetic/insulin-stabilized ewes glucose kinetics were monitored after interruption of the insulin infusions required to stabilize the ewes. Effects of rebGH on plasma insulin and glucose were measured before and after induction of diabetes in the other study (Experiment 2). Glucose kinetics also were measured after injection of rebGH into the diabetic/insulin-stabilized ewes in the latter study. Details of the experiments were as follows.

Experiment 1

Five ewes were rendered diabetic with intravenous alloxan (Leenanuruksa and McDowell 1988) then maintained on intravenous infusions of variable amounts of insulin (regular insulin; Commonwealth Serum Laboratories, Melbourne). Blood glucose was monitored frequently every day with a glucometer (Miles Laboratories, Elkhart, U.S.A.) and the insulin infusion rate was adjusted to maintain euglycaemia.

Milk yields and feed intakes were recorded over 4 days before and for 14 days after administration of alloxan. Blood samples (3 ml) were collected from the indwelling catheters, prior to each milking, for measurement of 'daily' values of plasma glucose, insulin and growth hormone. A representative subsample of milk from each ewe was retained from the pooled evening and morning milk harvested each day and analysed for milk protein and milk fat.

On day 14 after alloxan, glucose kinetics were measured in three ewes after interruption of the insulin infusion. Three hours prior to stopping the insulin infusion, a priming dose of $613.7\text{--}674.9 \text{ kBq U-}^{14}\text{C-glucose}$ (Amersham International plc, Amersham, U.K.) was infused rapidly into one jugular vein. Starting immediately thereafter, $\text{U-}^{14}\text{C-glucose}$ was infused continuously into the same jugular vein for 8 h at a rate of $5.11\text{--}5.62 \text{ kBq min}^{-1}$. The infusion of insulin was stopped for 30 min commencing 3 h after initiation of infusion of labelled glucose. Infusion of insulin was then resumed at the rate of 90% of the preceding infusion rate to test whether the sensitivity of tissues to insulin would be affected by interruption of insulin infusion. Three blood samples (5 ml) were collected during the 30 min immediately before stopping insulin infusion and another 16 blood samples were obtained over the next 5 h for determination of plasma concentrations of glucose, insulin and GH and of glucose specific radioactivity.

Experiment 2

Two weeks before beginning Experiment 1 (viz. induction of diabetes), the five ewes were given daily subcutaneous injections of bicarbonate buffer pH 9.0 (control) for 4 days then, for a further 4 days, injections of recombinant bovine growth hormone (rebGH, 0.1 mg kg⁻¹ liveweight; donated by American Cyanamid Company, New Jersey, U.S.A.) dissolved in bicarbonate buffer. One week after finishing Experiment 1, three ewes were selected and given control injections for 4 days then injections of rebGH for a further 4 days.

Glucose kinetics in non-steady state were measured in two diabetic ewes on the first day of GH injection as outlined above. Three blood samples (5 ml) were collected during the 30 min immediately before injection of GH and another 15 samples were collected over the next 6 h for determination of plasma glucose and glucose specific radioactivity.

Blood samples (5 ml) were collected from the indwelling catheters, prior to each milking for measurement of 'daily' values of plasma glucose, insulin and GH. A representative subsample of milk from each ewe was retained from the pooled evening and morning milk harvested each day and analysed for milk protein and milk fat.

Analytical Methods

Concentrations of fat and protein in milk were measured as described by Davis (1959). Plasma glucose was measured by the autoanalyser method of Bernt and Lachenicht (1974).

Concentrations of GH and insulin were measured using the talc radioimmunoassays described by Wallace and Bassett (1970) and Rosselin *et al.* (1966) respectively. Details of these assays were described previously by Gow *et al.* (1981). Sensitivities of the assays were 1.0 µg l⁻¹ and 2.4 mU l⁻¹ for GH and insulin respectively. For each hormone all samples to be compared were assayed simultaneously to avoid inter-assay variation, and assays had intra-assay coefficients of variation of <15%.

The method of Jones (1965) was used to isolate glucose as glucose pentaacetate for measurement of glucose specific activity. The method originally described by Steele (1959) and subsequently modified by Cowan and Hetenyi (1971) was used to measure glucose biokinetics in non-steady state as described previously (Leenanuruxsa and McDowell 1985).

The significance of differences between mean values for parameters measured were evaluated using the paired *t*-test (Steel and Torrie 1960).

Table 1. Intake of air dry feed, milk yield and contents of milk protein and fat before alloxan (B) then on days 11–14 after stabilizing ewes with intravenous insulin

Values are means ± s.e.m. of five ewes and the values for each parameter before alloxan are means for 4 days

	B	Days from infusion of alloxan			
		11	12	13	14
Feed intake (kg d ⁻¹)	2.45 ± 0.031	2.23 ± 0.117	2.35 ± 0.100	2.19 ± 0.154	2.17 ± 0.098
Milk yield (g d ⁻¹)	1080 ± 105.1	1007 ± 49.1	1018 ± 60.7	955 ± 58.0	994 ± 71.7
Milk protein (g kg ⁻¹)	55 ± 0.2	58 ± 2.3	59 ± 2.7	58 ± 2.0	57 ± 1.5
Milk fat (g kg ⁻¹)	65 ± 1.1	70 ± 5.0	72 ± 5.0	71 ± 5.4	69 ± 4.0

Results

Experiment 1

Feed intake, milk yield and milk composition

During the first week after treatment with alloxan, feed intake and both milk yield and composition were variable while the infusion rate of insulin was adjusted. By 11 days after induction of the diabetic state, ewes had been stabilized and values for each parameter were stable and similar (*P* > 0.05) to those before treatment with alloxan (see Table 1).

Plasma glucose

Prior to treatment with alloxan, plasma concentrations of glucose were *c.* 3.3 mM and between 11 and 14 days after alloxan, concentrations had increased, but not significantly ($P > 0.05$) except on day 11, to between *c.* 4.0 and 4.8 mM (Fig. 1a). In response to the interruption of insulin infusion for 30 min on day 14 after alloxan, plasma concentration of glucose increased significantly ($P < 0.05$) from *c.* 4.8 mM to *c.* 6.5 mM. Concentrations remained elevated for several hours even though infusions of insulin were resumed (see Fig. 2c).

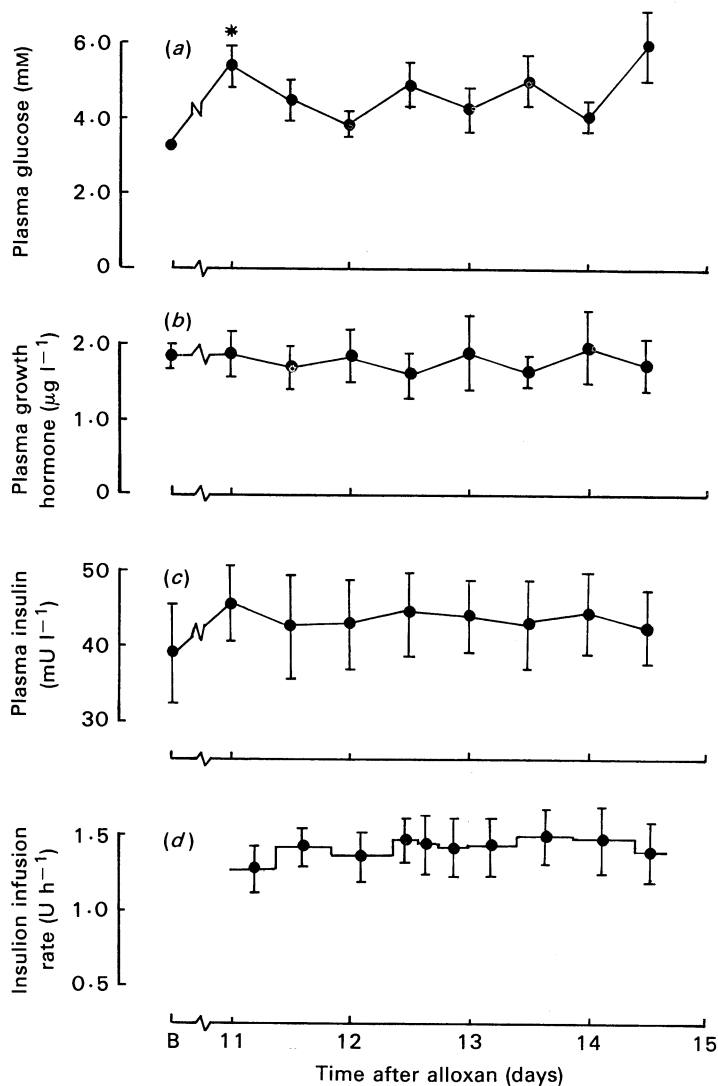


Fig. 1. Plasma concentrations of (a) glucose, (b) growth hormone and (c) insulin before induction (B) and after stabilization of alloxan-induced diabetes. Insulin infusion rates required to stabilize the diabetes are shown (d). The value shown before induction of diabetes represents the mean for 4 days. Plotted points are means for five ewes and s.e.m. are shown as vertical bars. Values for individual parameters that differ significantly from the value before alloxan treatment are indicated thus: $*P < 0.05$.

Plasma insulin and insulin infusion rate

Before treatment with alloxan plasma concentrations of insulin were relatively stable at 40–50 mU l⁻¹ (Fig. 1c). It was found that infusion rates between 1.1 and 1.3 U insulin h⁻¹ (c. 15–25 mU kg⁻¹ liveweight h⁻¹) (see Fig. 1d) maintained similar and quite stable concentrations in the ewes once diabetes had been induced, and between 11 and 14 days after infusion of alloxan concentrations of plasma insulin were within the above range (Fig. 1c).

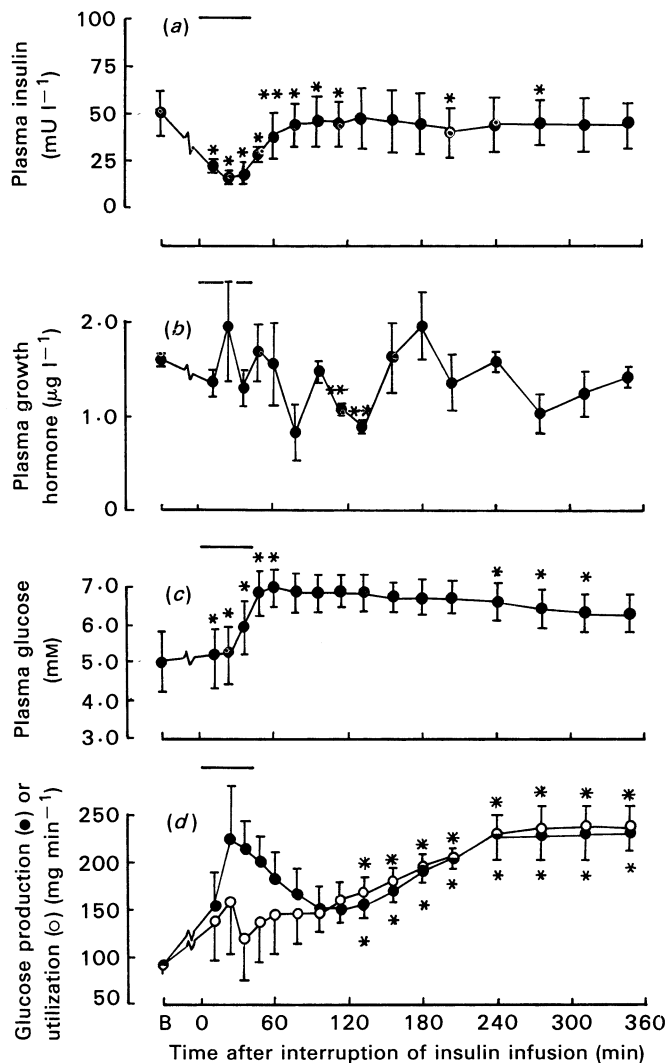


Fig. 2. Plasma concentrations of (a) insulin, (b) growth hormone and (c) glucose together with (d) rates of production and utilization of glucose before alloxan (B) and after interruption of infusion of insulin for 30 min then resumption of the infusion at 90% of the previous rate. The period of interruption of the infusion is marked by the horizontal bar. The value shown for each parameter before interruption of the insulin infusion represents the mean of three measurements made over 30 min for each ewe. Plotted points are means for three ewes and standard errors are shown as vertical bars. Values for individual parameters that differ significantly from the value before interruption of insulin infusion are indicated thus: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Interruption of insulin infusions for 30 min on day 14 resulted in a dramatic decrease in plasma concentrations from c. 50 mU l⁻¹ to c. 15 mU l⁻¹ by some 20 min after cessation of infusions (see Fig. 2a). After resumption of insulin infusions, at 90% of the previous rate, plasma concentrations of insulin increased to approach those before interruption of infusions. However, concentrations were lower than before interruption of infusion and differences were significant ($P < 0.05$) at 100, 110, 200 and 260 min after interruption of infusions (see Fig. 2a).

Plasma GH

Prior to induction of the diabetic state and after stabilization with infused insulin, plasma concentrations of GH were stable and varied from c. 1.7–2.0 µg l⁻¹ (Fig. 1b). After interruption of insulin infusions, then resumption of infusions at 90% of the previous rate, plasma concentrations of GH fluctuated markedly from c. 0.8–1.9 µg l⁻¹ for at least 3–4 h (see Fig. 2b).

Glucose production and glucose utilization

Immediately after interruption of the insulin infusions on day 14 after alloxan both glucose production and glucose utilization increased. Values for both parameters were not significantly ($P > 0.05$) higher than before interruption of infusion. Even so glucose production exceeded glucose utilization during most of the 30 min after interruption of infusions and for a further 30 min after resumption of infusions. Thereafter values for glucose production and glucose utilization were similar and significantly higher ($P < 0.05$) than before interruption of insulin infusions (see Fig. 2a).

Table 2. Milk yields and contents of fat and protein in milk before (B) and during the 4 days of injection of growth hormone

Measurements were made before (normal, $n = 5$) and after (diabetic, $n = 3$) induction of alloxan diabetes. Values are means \pm s.e.m. and the values shown for each parameter before start of growth hormone treatment are means for 4 days. For individual parameters, values that differ significantly from the value before growth hormone treatment are indicated thus: * $P < 0.05$, ** $P < 0.01$

	B	Days after start of growth hormone treatment			
		1	2	3	4
Normal ewes					
Milk yield (g d ⁻¹)	1070 \pm 186.6	1178 \pm 186.7*	1276 \pm 175.2*	1390 \pm 157.6**	1434 \pm 156.5**
Milk protein (g kg ⁻¹)	56 \pm 0.9	53 \pm 1.3*	53 \pm 0.7	52 \pm 1.0**	52 \pm 1.0**
Milk fat (g kg ⁻¹)	66 \pm 4.4	72 \pm 4.2**	79 \pm 2.7*	78 \pm 2.0	77 \pm 2.4
Diabetic ewes					
Milk yield (g d ⁻¹)	852 \pm 146.4	848 \pm 189.0	794 \pm 111.6	840 \pm 111.1	844 \pm 102.7
Milk protein (g kg ⁻¹)	54 \pm 1.7	49 \pm 1.7	52 \pm 4.5	54 \pm 4.8	49 \pm 1.7
Milk fat (g kg ⁻¹)	75 \pm 6.3	75 \pm 3.7	89 \pm 3.5	89 \pm 1.2	95 \pm 3.5

Experiment 2

Milk yield and composition

Milk yields and contents of milk protein and fat are shown in Table 2 for the period before and during daily injections of GH in the ewes before and after induction of the diabetic state with alloxan.

Before induction of diabetes, daily injections of rebGH significantly increased ($P < 0.05$) milk yield and milk fat content and significantly decreased ($P < 0.05$) milk protein content. After induction of the diabetic state, daily injections of rebGH did not affect milk yield and milk protein content. There was a tendency, though not significant ($P > 0.05$), for milk fat content to increase during injection of rebGH in the diabetic ewes.

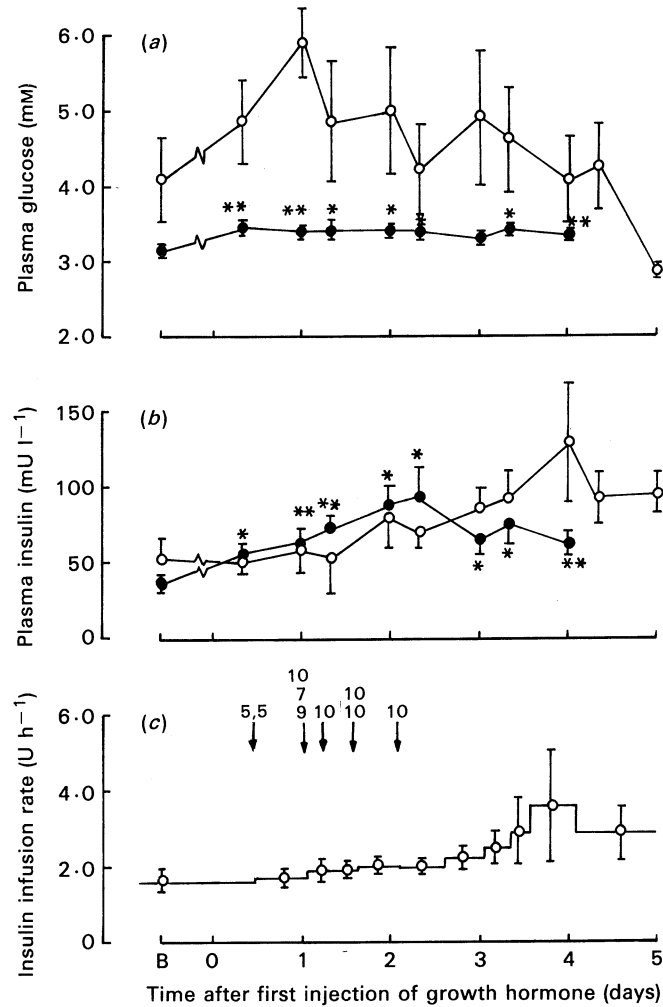


Fig. 3. Plasma concentrations of (a) glucose and (b) insulin before (B) and immediately after first injection of GH in normal (●—●, $n = 5$) and alloxan-diabetic/insulin-stabilized (○—○, $n = 3$) lactating ewes. Rates of infusion of insulin are indicated and doses of additional insulin injected at times indicated by arrows are shown (c). Values shown before first injection of GH are means for the control period of 4 days. Plotted points represent mean values and s.e.m. are shown as vertical bars. Values for individual parameters for normal ewes that differ significantly from the value before injection of GH are indicated thus: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Plasma glucose

Before induction of diabetes with alloxan, injections of rebGH increased plasma concentrations of glucose significantly ($P < 0.05$) from c. 3.3 mM to c. 3.8 mM (see Fig. 3a). Once diabetes was induced and ewes stabilized with exogenous insulin, daily injections of

rebGH increased plasma concentrations of glucose from *c.* 5.0 mM to values that varied between 6.0 and 8.0 mM, but the changes were not significant ($P > 0.05$) (see Fig. 3*a*).

On the first day that injections of rebGH were given to the diabetic ewes concentrations of glucose remained stable for *c.* 120 min after injection. Thereafter plasma concentrations increased to *c.* 8.0 mM by 6 h after injection of rebGH by which time concentrations appeared to plateau (see Fig. 4*a*).

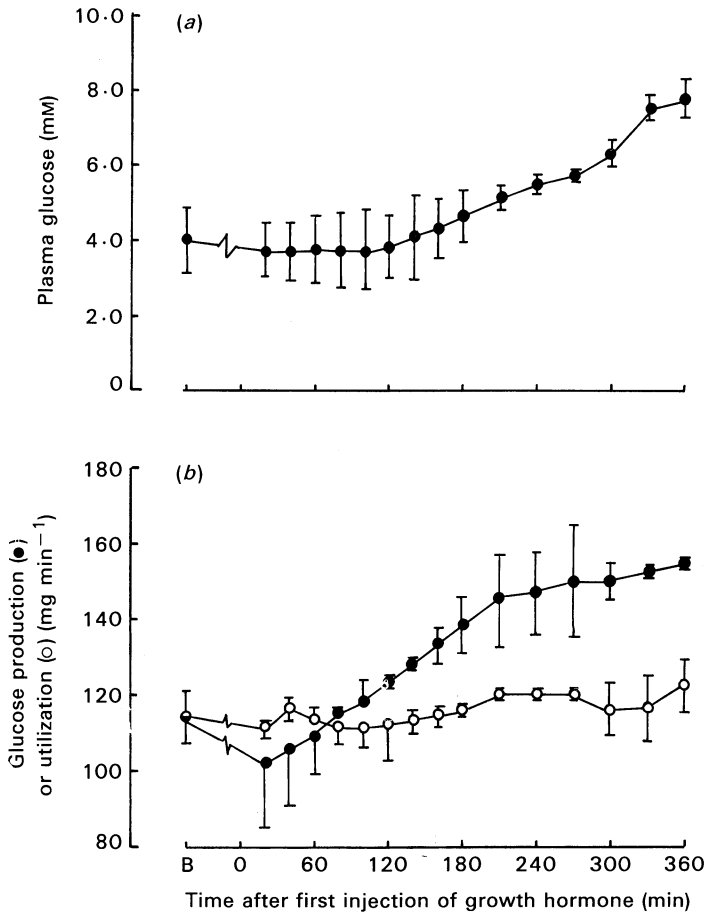


Fig. 4. Plasma concentrations of (a) glucose together with (b) rates of production and utilization of glucose after the first injection of growth hormone was given to alloxan-diabetic/insulin-stabilized ewes. Plotted points represent mean values for two ewes and s.e.m. are shown as vertical bars.

Plasma GH

Prior to injection of rebGH plasma concentrations of the GH were stable at between 1.5 and 2.0 $\mu\text{g l}^{-1}$ in ewes before and after induction of diabetes. Injections of rebGH increased plasma concentrations of GH during the hours immediately following each daily injection. At 7–8 h after each daily injection plasma concentrations of GH were significantly increased ($P < 0.01$) to *c.* 7 $\mu\text{g l}^{-1}$ and by 24 h after injection concentrations had returned to basal values of *c.* 2 $\mu\text{g l}^{-1}$. These changes were observed in ewes before and after treatment with alloxan.

Plasma insulin and insulin infusion rate

Before induction of diabetes, daily injections of rebGH significantly increased ($P < 0.05$) plasma insulin from c. 40 mU l^{-1} to between 50 and 80 mU l^{-1} (see Fig. 3b). Once the diabetic state had been induced, concentrations of plasma insulin increased gradually from c. 60 mU l^{-1} on the first day to c. 120 mU l^{-1} on the fourth day of injection of rebGH. This increase was associated with a marked increase in the rate of infusion of exogenous insulin over the 4 days of injection of rebGH (see Fig. 3c). The rate of infusion of insulin was increased, in an attempt to maintain euglycaemia, from c. 1.5 U h^{-1} to c. 3.0 U h^{-1} on the fourth day of injection of rebGH. Intravenous injections of insulin, as shown in Fig. 3c, were administered as well in an attempt to maintain euglycaemia and it is clear that it was difficult to maintain stable concentrations of plasma glucose.

Once daily injections of rebGH ceased, the rate of infusion of insulin required to maintain euglycaemia decreased (see Fig. 3c).

Glucose production and glucose utilization

Immediately after the first injection of rebGH was given to the diabetic ewes, glucose production decreased. However, by 60 min after the injection of rebGH glucose production began to increase before reaching a plateau some 40% higher than before rebGH by 240 min. Throughout the first 360 min after first injection of rebGH in the diabetic ewes, glucose utilization remained relatively stable (see Fig. 4b).

Discussion

The observations made in Experiment 1 that feed intake, milk yield and milk composition were maintained after induction of the diabetic state are similar to results of our recent studies in which injections of long-acting insulin were used to replace endogenous insulin (Leenanuruxsa and McDowell 1988). It is interesting to note that the infusion rates of insulin required once the ewes had been stabilized on exogenous insulin (viz. $15\text{--}25 \text{ mU kg}^{-1} \text{ liveweight h}^{-1}$) were similar to the insulin secretion rate of $15.4 \text{ mU kg}^{-1} \text{ liveweight h}^{-1}$ calculated by Trenkle (1971). These rates are, however, higher than the rate of $7.8 \text{ mU kg}^{-1} \text{ liveweight h}^{-1}$ reported by Brockman and Bergman (1975) for fed sheep or 5 and $14 \text{ mU kg}^{-1} \text{ liveweight h}^{-1}$ reported by McCann and Reimers (1985) for lean and obese heifers respectively.

Although the route of administration of exogenous insulin used in the present study (i.e. via the jugular vein) differs from the delivery of endogenous insulin from the pancreas, it appears that the amount required to maintain euglycaemia would have approximated endogenous production rate. In this connection, Rizza *et al.* (1981) showed that the amounts of insulin required to stabilize alloxan-induced diabetes in dogs, and peripheral concentrations of insulin in these dogs, were similar whether exogenous insulin was administered via the portal vein or via a peripheral vein.

It is evident from the results of Experiment 1 that stable concentrations of insulin and GH were achieved between 11 and 14 days after alloxan. The results of our previous studies (Leenanuruxsa and McDowell 1988) indicated that a period of approximately 10 days was required to achieve a stable state after administration of alloxan, then maintenance doses of exogenous insulin. Even though plasma concentrations of hormones were similar before and 11–14 days after alloxan, plasma glucose concentrations were higher than before induction of diabetes.

Despite the mild hyperglycaemia that prevailed after ewes had been stabilized with exogenous insulin, the concentration of fat in milk tended to be higher between days 11 and 14 after alloxan than before alloxan (see Table 1). This observation is consistent with insulin playing a key role in the aetiology of the 'low-milk-fat syndrome' as discussed previously by Leenanuruxsa and McDowell (1988).

It has been proposed that ruminants are less sensitive to insulin than non-ruminants

(Brockman and Laarveld 1986a; Weekes 1986) and that this lowering of sensitivity is reflected by lowered rates of glucose disposal and reduced glucose production by the liver. There is evidence that the ruminant is just as sensitive to insulin as the non-ruminant (Bassett 1975) and that the apparent lack of sensitivity to insulin reflects the relative importance of acetate as an energy substrate.

The latter is supported by several observations on relationships between circulating insulin and the number of receptors for insulin in ruminant tissues. Gill and Hart (1981) showed an inverse relationship between insulin concentration and insulin binding to goat hepatocytes. Others have reported similar relationships for purified liver cell membranes from growing lambs (Grizard and Szczygiel 1983) and bovine adipocytes (Vernon *et al.* 1985). These observations suggest both 'up-regulation' and 'down-regulation' of insulin receptors in the ruminant as in other species (Gavin *et al.* 1974).

The results of Experiment 1 are consistent with changed sensitivity of the tissues to insulin, dependent on circulating concentrations of insulin. Interruption of insulin infusions for as short a time as 30 min then resumption of infusions at 90% of the previous rate had marked effects. The rate of glucose utilization virtually doubled within 2–3 h after resumption of insulin infusions at the reduced rate. This change is consistent with 'up-regulation' of insulin receptors due to increased receptor number and/or increased affinity of receptors (Gavin *et al.* 1974; Roth and Grunfeld 1985).

Interestingly, after interruption of insulin infusions, plasma concentrations of glucose increased and remained elevated even though insulin infusions were resumed. Although glucose production and clearance had increased to be similar by *c.* 90 min after interruption of insulin infusion, glucose production was elevated immediately following interruption of insulin. Presumably, the glucose pool size was increased during the early period after interruption of insulin, resulting in maintenance of high plasma glucose.

The responses of the ewes in Experiment 2, before induction of diabetes, to exogenous rebGH were similar to responses previously reported. Increased milk yield and fat content of milk have been shown previously in lactating sheep (McDowell *et al.* 1988) and cows (Johnsson and Hart 1986; McDowell *et al.* 1987a, 1987b) given exogenous GH. Similarly, increased plasma concentrations of insulin and glucose have been reported previously in lactating ruminants injected with GH (McDowell *et al.* 1987b, 1988). The latter changes are in conformity with development of insulin resistance and it is pertinent that diabetogenic effects of exogenous GH have been described in the ruminant (see Hart 1983; Hart *et al.* 1984).

After induction of the diabetic state, the responses of the ewes to rebGH were different from those of normal ewes. Milk yield did not increase in response to the rebGH even though milk fat content tended to increase. Failure to measure a change in milk yield possibly was due to the insulin infusions and injections used in an attempt to maintain plasma glucose at stable levels.

It is apparent that it was not possible to maintain stable plasma glucose. The most pronounced change after injection of rebGH in diabetic ewes was the marked increase in plasma glucose commencing *c.* 2 h after injection of rebGH. This was due to a substantial increase in glucose production but no change in glucose utilization (see Fig. 4b). It appears reasonable to suggest that these observations provide direct evidence that GH is glucogenic in the ruminant. Substrates for gluconeogenesis may have been glycerol released as a result of the lipolytic effects or peptide amino acids, concentrations of which increase dramatically during injections of GH or rebGH (M. Jois, G. H. McDowell and E. F. Annison, unpublished data).

Although in the present study, and in others from our laboratory (see McDowell *et al.* 1987a, 1988), exogenous GH increased plasma glucose, this increase in plasma glucose has not always been recorded following injections of GH. Failure to observe the increase in plasma glucose has been explained by the compensatory increase in insulin secretion, at least in humans (Metcalfe *et al.* 1981; Press *et al.* 1984). Certainly, plasma insulin concentrations

increased in the present studies after injection of rebGH prior to induction of diabetes (see Fig. 3*b*). Increased secretion of insulin would suppress hepatic glucose production (Brockman and Laarveld 1986*b*) and promote glucose utilization by peripheral tissues (Harrison and Flier 1980; Rizza *et al.* 1982).

The dramatic increase in dose of insulin required to 'control' plasma glucose after injections of rebGH once diabetes had been induced provides good evidence that GH exerts diabetogenic effects. In this respect the results of Experiment 2 are consistent with results of several previous studies. Christiansen *et al.* (1982) reported that the daily insulin requirement for maintenance of euglycaemia in C-peptide-negative, diabetic human subjects was increased by *c.* 75% after administration of physiological doses of GH. Others have shown impairment of hepatic and extra-hepatic responses to insulin in acute studies in humans given exogenous GH (Bratusch-Marrain *et al.* 1982; Rizza *et al.* 1982) and in chronic excess of GH such as in acromegaly (Hansen *et al.* 1986).

Hart (1983) and Hart *et al.* (1984) showed that exogenous GH impaired glucose clearance in goats and sheep, suggesting lowered sensitivity to insulin following exogenous GH. In contrast, Laarveld *et al.* (1986) failed to measure changes in glucose metabolism and clearance of injected glucose in sheep infused with GH and somatostatin. It is possible that Laarveld *et al.* (1986) did not observe effects of GH because fasted sheep were used. In this connection, Bassett and Wallace (1966) showed that the hyperglycaemic effect of exogenous GH was greater in sheep fed generously than in sheep fed maintenance rations and negligible in sheep fasted before administration of GH.

In conclusion, the results of the present studies with alloxan-treated/insulin-stabilized ewes show the usefulness of this model for studying the role of hormones in maintenance of glucose homeostasis in the ruminant. It is apparent that ruminants are sensitive to insulin and that the sensitivity of the tissues to insulin probably is related to the number and activity of insulin receptors. The results of Experiment 1 suggest that in the presence of lowered plasma insulin 'up-regulation' of receptors occurs. Exogenous GH was found to exert diabetogenic effects and/or promote gluconeogenesis. This effect may be masked in animals with an intact pancreas where exogenous GH increases insulin secretion which in turn prevents demonstration of effects of growth hormone on glucose production.

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