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Experimental Diabetes in Lactating Sheep: Effects of Alloxan on Plasma Insulin, Glucose, Glucose Kinetics and Milk Characteristics

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Abstract

After intravenous administration of alloxan (50 mg kg^{-1} liveweight) to lactating ewes, there were triphasic changes in plasma glucose and insulin. Almost immediately, plasma insulin decreased and hyperglycaemia occurred, then, between *c.* 5–12 h, insulin increased and ewes became hypoglycaemic. Thereafter, insulin decreased and glucose increased from *c.* 20 h after alloxan and the diabetic state was established. Changes in glucose production and utilization correlated with changes in plasma glucose.

Exogenous insulin was administered from 30 h after alloxan, and it took some 2 weeks to stabilize ewes. During this period, when mild hyperglycaemia persisted, milk yields and feed intakes were decreased but milk fat content was elevated. Once ewes were stabilized, plasma glucose, milk yield, feed intake and milk fat content returned to levels prior to alloxan. These observations are consistent with insulin playing a role in the aetiology of the 'low milk fat syndrome' in the ruminant.

It appears that the alloxan-treated, insulin-stabilized ewe would be a useful model for studying the role of insulin during lactation, but it is necessary to allow time for animals to overcome effects of administration of alloxan.

Extra keywords: glucose production and utilization, milk yield and fat content, feed intake.

Introduction

Results of recent studies at this laboratory have shown that alteration of the supply of metabolites to the mammary gland affects milk secretion in the ewe. Thus, milk synthesis is reduced in ewes submitted to feed restriction (Gow *et al.* 1981) and in ewes given prolonged intravenous infusions of adrenaline (Leenanuruksa and McDowell 1985).

Insulin is known to affect the utilization of several key nutrients, including glucose, in the body and so it might be expected that interference with insulin secretion would affect milk synthesis. The role of insulin in regulating metabolism in ruminants has been investigated by inducing diabetes with the drug alloxan (Nowak and Dzialoszynski 1967; Brockman and Bergman 1974; Hove 1978; Pethick *et al.* 1981; Prior and Smith 1983). The first object of the present studies was to monitor closely the effects of treatment of lactating ewes with alloxan on plasma glucose and insulin and on glucose kinetics during the onset of diabetes. Secondly, once diabetes had been induced and the ewes stabilized with exogenous insulin, an assessment was made of the role of insulin in maintenance of lactation. Thirdly, a long-term aim of the studies was the development of a model suitable for studying the interaction of insulin with other hormones in the lactating ewe.

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Materials and Methods

Sheep

Three multiparous crossbred ewes (Border Leicester × Merino) were used for the experiment; they were free of obvious abnormalities of the mammary glands and had been lactating for 50 days. They had been maintained in metabolism cages from the day of parturition when lambs were permanently removed from their dams. All ewes were accustomed to handling and were milked by hand twice daily at *c.* 0830 and 1630 h.

Water was provided *ad libitum* and 2·5 kg of a pelleted diet (9·6 MJ metabolizable energy and 218 g crude protein per kg dry matter, where crude protein was calculated as N × 6·25) containing 40% lucerne chaff, 14·5% wheaten chaff, 33% oats, 12% fishmeal and 0·5% minerals was supplied continuously using a belt feeder, to avoid postprandial changes in metabolites and hormones.

Both external jugular veins of each ewe were fitted with indwelling catheters (polyvinyl chloride, 1·0 mm internal diameter and 1·5 mm external diameter; Dural Plastics, Sydney) at least 24 h prior to commencing the experiment. Catheters were kept patent by flushing with minimum amounts of sterile heparinized saline (2×10^5 i.u. heparin and 9·0 g NaCl per litre of distilled water).

Experimental Procedures

The experiment was conducted over 19 days. After a control period of 4 days, 10% (w/v) alloxan monohydrate (Sigma Chemical Co., St Louis, Mo), freshly prepared in sterile saline, was injected intravenously through one indwelling catheter at the rate of 50 mg alloxan base per kilogram liveweight. Three hours prior to injecting alloxan, a priming dose of 613·7–674·9 kBq uniformly labelled ^{14}C-glucose (Amersham International plc, Amersham, U.K.) was infused rapidly into one jugular vein, then labelled glucose was infused continuously into the same jugular vein for 22 h at a rate of 5·11–5·62 kBq min^{-1}. Three blood samples (5 ml) were collected during the 30 min immediately before administration of alloxan; in total, 44 blood samples were collected at intervals over the 28 h after injection of alloxan.

Within 30 h after alloxan, diabetes was controlled by daily subcutaneous injections of variable amounts of long-acting crystalline insulin (20–100 units day^{-1}; Ultralente MC, CSL, Melbourne) and intravenous injection of regular insulin (1–20 units day $^{-1}$; CSL, Melbourne) as necessary. The amounts of insulin required per day were adjusted to achieve normal blood glucose as measured with a glucometer (Miles Laboratories, Elkhart, Ind.). Prior to each milking, blood samples (2 ml) were collected from the indwelling catheters for measurement of 'daily' values of glucose. A representative subsample of milk from each ewe was retained from the pooled evening and morning milk harvested each day and analysed for milk fat.

Analytical Methods

Plasma glucose was measured by the autoanalyser method of Bernt and Lachenicht (1974). The talc radioimmunoassay of Rosselin *et al.* (1966) was used to measure plasma insulin. The sensitivity of the assay was 2·4 $\mu U\ ml^{-1}$ and details of the assay were described previously by Gow *et al.* (1981). All samples to be compared were assayed simultaneously to avoid inter-assay variation.

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 before (Leenanuruksa and McDowell 1985).

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

Results

Immediate Responses to Alloxan

In two of the three ewes there were no obvious effects of treatment with alloxan, but in the third ewe obvious ill-effects of treatment were apparent. Within 4 min of administration of the drug to the third ewe, there was a noticeable increase in respiration rate and the ewe temporarily lost co-ordination, becoming recumbent for several minutes. For this ewe, changes in plasma metabolites and hormones followed trends similar to those in the other ewes but differences were apparent as outlined below.

Plasma glucose

Changes in plasma glucose are depicted in Fig.1. Before injection of alloxan, plasma glucose was stable at *c.* 3·6 mM. Within 10 min of injection of alloxan, plasma glucose had increased

dramatically, with concentrations fluctuating markedly over the first 30 min after alloxan. By 4 h after alloxan injections, plasma glucose peaked at *c.* 16 mM before decreasing to *c.* 4–5 mM some 8 h later. At this time (*c.* 12 h after alloxan), marked hypoglycaemia (*c.* 1·0–1·5 mM) was observed in two ewes; in the ewe which showed obvious ill-effects of alloxan, mild hyperglycaemia (*c.* 6–11 mM glucose) persisted. By 21 h after alloxan administration, plasma glucose had increased once again with all ewes displaying marked hyperglycaemia.

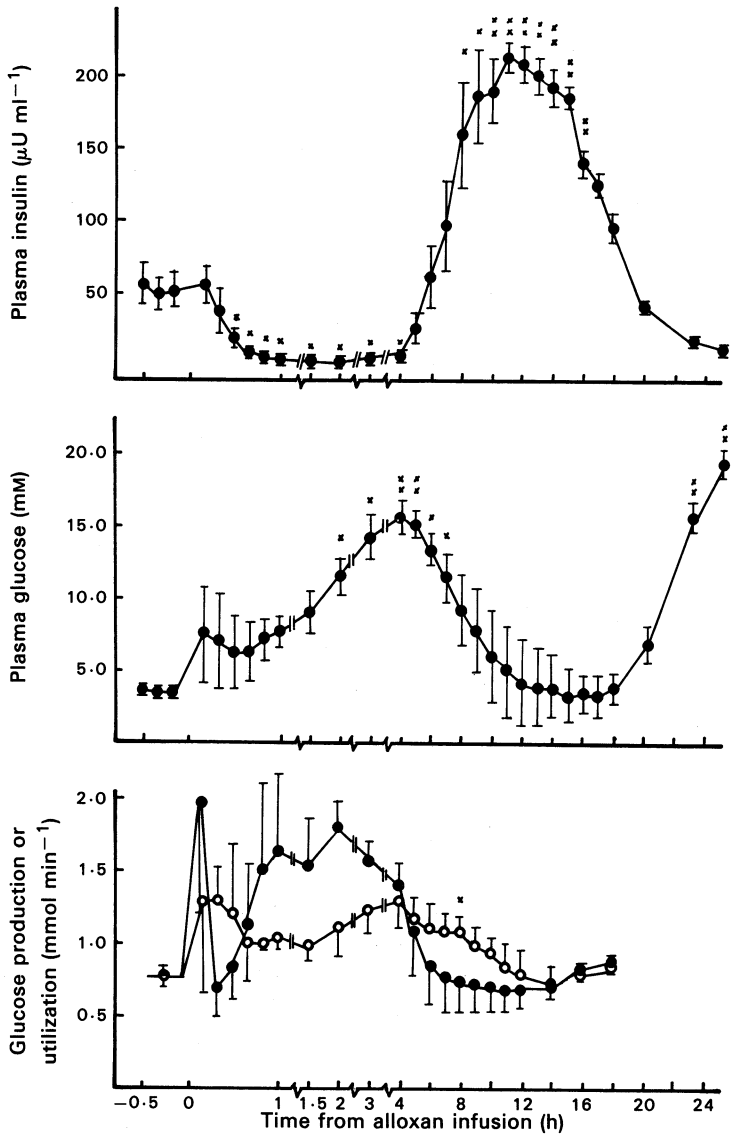

Fig. 1. Mean values during the first 30 h after alloxan treatment for (*top*) plasma insulin, (*middle*) plasma glucose, and (*bottom*) glucose production (●———●) and glucose utilization (○———○). Plotted points, mean values for three ewes. Vertical s.e. Values which differ significantly from the mean value bars, before alloxan are indicated. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

Plasma insulin

Concentrations of insulin during the first day after alloxan are depicted in Fig.1. Before injection of alloxan, stable concentrations of *c.* 50 μU ml^{-1} insulin were measured. Within 20 min of the injection, plasma insulin had decreased. Concentrations had further decreased to *c.* 10 μU ml^{-1} by 40 min. Low levels of plasma insulin were maintained until *c.* 4 h after alloxan treatment, then concentrations rose from *c.* 5 h to reach very high values (115–215 μU ml^{-1}) by 9 h after alloxan treatment.

High concentrations of insulin were observed for a further 8 h before concentrations decreased to very low levels (*c.* 5 μU ml^{-1}) by 27 h after alloxan administration.

Glucose production(R_a) and glucose utilization (R_d).

During the first 60 min after alloxan injection, abrupt changes occurred for both R_a and R_d (see Fig.1). By 30 min, both R_a and R_d were higher than before alloxan, and R_a exceeded R_d until *c.* 4 h. From 5–12 h, R_d exceeded R_a. Values for R_a and R_d were similar from 12-18 h after alloxan injection.

Responses of Alloxan-treated/Insulin-stabilized Ewes

From 30 h after injections of alloxan, exogenous insulin was administered. Doses of exogenous insulin were adjusted in an attempt to maintain stable plasma glucose.

Effects on feed intake

Before injection of alloxan, ewes consumed *c.* 2·2 kg day^{-1} feed. During the first days after alloxan, when ewes were being 'stabilized', feed intakes were suppressed. Thereafter, once ewes had been 'stabilized' with exogenous insulin, feed intake returned to normal levels before increasing to be significantly higher ($P<0{\cdot}05$) than before alloxan (see Table 1).

Table 1. Mean values (n = 3) ± s.e.m. for feed intake, plasma glucose, milk yield and milk fat 8–15 days after intravenous alloxan injection

For each parameter, values which differ significantly from the value before alloxan treatment are indicated thus: *P < 0·05; **P < 0·01

	Time from injection of alloxan (days)								
	0[A]	8	9	10	11	12	13	14	15
Plasma glucose	3·8	11·6**	8·4*	8·9*	8·6*	6·1	3·5	3·9	3·8
(mM)	±0·1	±0·9	±0·5	±0·6	±1·1	±1·5	±0·6	±1·1	±1·7
Feed intake	2·20	1·70*	2·10	2·20	2·50	2·50	2·48*	2·45*	2·42
(kg day^{-1} air dry)	±0·1	±0·03	±0·2	±0·1	±0·03	±0	±0·02	±0·03	±0·08
Milk yield	948	513	953	920	904	913	817	963	781
(g day^{-1})	±300	±303	±340	±358	±371	±264	±235	±249	±183
Milk fat	79	101*	92	92	97*	91	79	73	78
(g kg^{-1})	±10	±8	±12	±11	±7	±3	±8	±10	±15

[A]Average for 3 days for each ewe.

Plasma glucose

Initially, plasma concentrations of glucose were significantly raised as shown in Table 1. By 13–15 days after alloxan, relatively stable concentrations of plasma gulcose were attained by administration of exogenous insulin.

Milk yield and composition

During the first days after alloxan treatment, and prior to attaining stable plasma glucose, milk yields decreased. By 13–15 days after alloxan, when stable glucose concentrations were attained, milk yields had returned to values prior to alloxan treatment. Milk fat content was significantly higher ($P<0{\cdot}05$) on days 8 and 11 and tended to be higher until 12 days after alloxan, even though mild hyperglycaemia occurred. Once euglycaemia was achieved between

days 13–15, milk fat content returned to control values.

Discussion

Changes in plasma concentrations of glucose during the first 24 h after alloxan followed the triphasic pattern previously described by Jarrett (1946) and Rerup (1970). The results of our study show that the changes in plasma glucose correlate closely with changes in glucose production and utilization during the first hours after alloxan (see Fig.1).

It has been reported that alloxan exerts specific, irreversible effects on the β-cells of the pancreas (see Rerup 1970) leading to complete inhibition of the biosynthesis of pro-insulin (Yamamoto *et al.* 1981). The early decrease in plasma insulin and the maintenance of low plasma concentrations for *c.* 4 h after alloxan is consistent with cessation of insulin secretion in the pancreas as described by Borg *et al.* (1979) and Borg (1981).

The subsequent hyperinsulinaemia measured between *c.* 5 and 9 h after alloxan can be attributed to uncontrolled leakage of insulin from the secretory granules of damaged β-cells as suggested originally by Hughes *et al.* (1944). Ultimately, depletion of stored insulin and inability to synthesize further insulin (or pro-insulin) would have led to the extremely low plasma concentrations measured some 24–27 h after alloxan treatment. By this time alloxan-induced diabetes was fully developed.

Although the pattern of changes in plasma insulin and glucose were broadly similar, changes for one ewe differed from those in the other two ewes. This ewe showed obvious ill-effects of injection of alloxan shortly after injection of the drug. Furthermore, in this ewe, hyperglycaemia persisted beyond the initial 4 h after injection of alloxan. In this ewe, release of adrenaline in response to alloxan injection may have been responsible for the changes observed (see Hughes *et al.* 1944; Rerup 1970). We have shown previously that exogenous adrenaline promotes glucose production and suppresses glucose utilization (Leenanuruksa and McDowell 1985). Prolonged release of adrenaline in the one sheep would explain maintenance of the hyperglycaemia following alloxan treatment. It is likely that the sharp increase in plasma glucose during the first minutes after alloxan treatment in all sheep was due to promotion of glucose production by adrenaline rather than to effects on plasma insulin at this time. Indeed, plasma insulin concentrations were unchanged by alloxan during the first minutes after alloxan injections.

During the two weeks or so after injection of alloxan, when plasma glucose concentrations remained elevated, feed intakes and milk yields were suppressed. This may have been attributable to the mild hyperglycaemia occurring during this period when doses of exogenous insulin were being adjusted to control plasma glucose. Although this possibility can not be discounted, similar observations have been made in sheep where good control of plasma glucose has been achieved after alloxan (Leenanuruksa, unpublished observations). Accordingly, it is suggested that the suppressions in feed intake and milk yield were attributable to reversible effects of alloxan on the kidney. Indeed, others have recorded temporary renal lesions in alloxan-diabetic sheep and non-ruminants (Jarrett 1946; Reid *et al.* 1963; Rerup 1970). Recovery from the adverse effects of alloxan had presumably occurred by 13–15 days when feed intakes and milk yields had recovered (see Table 1).

The content of fat in milk was increased when mild hyperglycaemia occurred, between days 8–12 after alloxan treatment. In normal lactating ruminants, hyperglycaemia induced by diet (Annison *et al.* 1974) or intravenous infusion of glucose (McClymont and Vallance 1962; Leenanuruksa 1982) is associated with reduced synthesis of milk fat. This has been interpreted as evidence that insulin is a key factor in the aetiology of the 'low milk fat syndrome' in the lactating ruminant (McClymont 1951; see also Annison and McDowell 1980). The present results are consistent with this being the case. The content of milk fat was decreased when sufficient insulin to establish euglycaemia was infused 13–15 days after alloxan.

In conclusion, the present results show that the changes in plasma glucose concentrations after treatment of sheep with alloxan are attributable to effects of alloxan on insulin and thus glucose production and/or utilization. Furthermore, the present results indicate that the insulin-stabilized, alloxan-diabetic sheep should serve as a suitable model for studying effects of insulin on metabolism. However, it is evident that, for some 2 weeks after treatment with alloxan,

the sheep is unstable even though insulin is infused to replace endogenous hormone.

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