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Control of Gluconeogenesis in the Lactating Sheep

C. B. Gow, G. H. McDowell and E. F. Annison

Dairy Research Unit, Department of Animal Husbandry,
University of Sydney, Camden, N.S.W. 2570

Abstract

Ewes which had been lactating for 3–4 weeks and which had been milked by hand from the day of parturition were subjected to food restriction for 4 days. One group of three ewes was fed *ad libitum* and a second group of four ewes was fed to meet calculated requirements for maintenance and milk production. Over 4 days food intake was reduced by 80% in both groups of ewes.

In response to food restriction, milk yields and body weight decreased. Blood amino acids, plasma glucose, glucose pool size, glucose irreversible loss, insulin, thyroxine and the insulin:glucagon molar ratio decreased. In contrast, plasma glucagon remained relatively unaffected and plasma free fatty acids and growth hormone increased. These changes were similar for both groups of ewes and were reversed when food intake was restored.

The results suggest that the hormonal control of gluconeogenesis in the ruminant is similar to that in the non-ruminant.

Introduction

Although the hormonal control of gluconeogenesis in non-ruminants has been broadly defined, the situation in ruminants remains relatively unresolved (see Bassett 1975, 1978). The demand of the lactating mammary gland for glucose (Annison and Linzell 1964; Bergman and Hogue 1967) is such that gluconeogenesis is greatly increased during lactation. On the other hand, the rate of gluconeogenesis is depressed during food restriction (Kronfeld and Simesen 1961; Steel and Leng 1968), suggesting that the lactating ewe subjected to feed restriction would serve as a particularly useful model for studying the control of gluconeogenesis in the ruminant. We have used this model to examine the extent to which body tissues may be mobilized to maintain glucose in circulation and have monitored the hormonal changes which occur in the lactating ewe in response to feed restriction.

Materials and Methods

Sheep

Multiparous crossbred ewes (Border Leicester × Merino) free of obvious mammary gland abnormalities were used. All ewes were accustomed to handling and had been milked by hand, twice daily, from the day of parturition (3–4 weeks before the experiment) when lambs were removed permanently from their dams. The ewes had been hand-milked throughout a number of previous lactations and were accustomed to the metabolism cages in which they were housed for 2 weeks before and during the period of the experiments.

Hormone Assays

In order to eliminate possible variability of radioimmunoassay measurements stemming from differences in experimental conditions, all of the assays of each particular hormone were carried out in a single batch. Radioimmunoassays were considered valid if the intra-assay coefficient of variation did not exceed 15%. The sensitivity of the assay procedure for each hormone was determined by the method of Burger *et al.* (1972).

Growth hormone

The talc radioimmunoassay described by Wallace and Bassett (1970) was used. Antiserum specific for ovine growth hormone was obtained from Mr A. L. C. Wallace, Division of Animal Production, CSIRO, Prospect, N.S.W. and concentrations of growth hormone were expressed in terms of the standard preparation NIH-G-SII. The sensitivity of the assay was 1·0 ng/ml.

Insulin

Levels of insulin were measured using the talc radioimmunoassay described by Rosselin *et al.* (1966). Antiserum specific for ovine insulin and the purified preparation of ovine insulin used as standard were obtained from Mr A. L. C. Wallace. The sensitivity of the assay was 0·10 ng/ml.

Thyroxine

Levels of thyroxine were determined using the charcoal radioimmunoassay described by Eastman *et al.* (1975). Antiserum specific for ovine thyroxine was obtained from Mr A. L. C. Wallace and concentrations of thyroxine were expressed in terms of a purified preparation of L-thyroxine (Sigma Chemical Co., St Louis, Mo.). The sensitivity of the assay was 0·17 ng/ml.

Glucagon

Levels of glucagon were measured using a radioimmunoassay kit obtained from the Novo Research Institute (Denmark). Supplied with the kit were highly purified porcine glucagon and an antiserum specific for porcine pancreatic glucagon. The assay procedure adopted was as described by the supplier and the sensitivity of the assay was 0·03 ng/ml.

Other Assays

Blood and plasma metabolites

Levels of amino acids were determined in blood deproteinized using sulfosalicylic acid (500 mg/100 ml blood) after addition of 0·5 ml 2-aminoethyl phosphoric acid (717·78 μmol/ml) for 3 ml supernatant. Amino acids were separated using a Durrum High Pressure Single Column System (Durrum Chemical Co., U.S.A.).

Concentrations of free fatty acids (FFA) and glucose in blood plasma were measured using the methods described by Dole (1956) and Huggett and Nixon (1957) respectively.

Milk lactose

The procedure of Cowie *et al.* (1969) was used for measurement of milk lactose.

Glucose biokinetics

Glucose biokinetics were determined by isotope dilution using the single-injection method of Katz *et al.* (1974).

Experimental Procedures

Two groups of ewes were used. Three days before each experiment, polyvinyl chloride cannulae (1·5 mm o.d. by 1·0 mm i.d.; Dural Plastics, Sydney) were fitted in both external jugular veins. Cannulae were kept patent by flushing twice daily with sterile heparinized saline (200 units heparin per millilitre; 9 g NaCl per litre).

Three ewes (group 1) were fed lucerne chaff (8·2 MJ/kg dry matter, 179 g crude protein/kg dry matter) *ad libitum* for 2 weeks. The average daily feed intake was 1·5–1·6 times the metabolizable energy requirement for maintenance plus milk production (M.A.F.F. 1975). A second group of four ewes (group 2) were fed a mixture of lucerne chaff and rolled barley (9·8 MJ/kg dry matter, 190 g crude protein/kg dry matter) in sufficient quantities to meet calculated energy requirements for maintenance plus milk production (M.A.F.F. 1975) for a similar period.

Feed intakes of both groups of ewes were restricted by 80% for 4 days then restored to the prerestriction level. Throughout the experiments feed was provided continuously using a belt-feeder to ensure that feed was consumed at an even rate.

Milk yields were recorded daily and the ewes were weighed before, and at 4 and 14 days after the onset of feed restriction. Glucose biokinetics studies were conducted 1–2 days before, 4 days after the onset of feed restriction, and again when feed intake had been restored to the level prior to feed restriction, using procedures described earlier (Gow *et al.* 1981). Blood samples were taken daily at 0900, 1200 and 1500 h for measurement of plasma FFA, insulin, growth hormone, glucagon and thyroxine. Aprotinin (Novo Research Unit, Denmark) was added to blood collected for analysis of glucagon (0·10 mg aprotinin/ml). Blood samples for amino acid assays were collected before, then 2 and 4 days after the onset of feed restriction.

Statistical Analysis

The significance of differences between values for ewes within and between groups, for the parameters measured, were evaluated using Student's *t*-test (Steel and Torrie 1960).

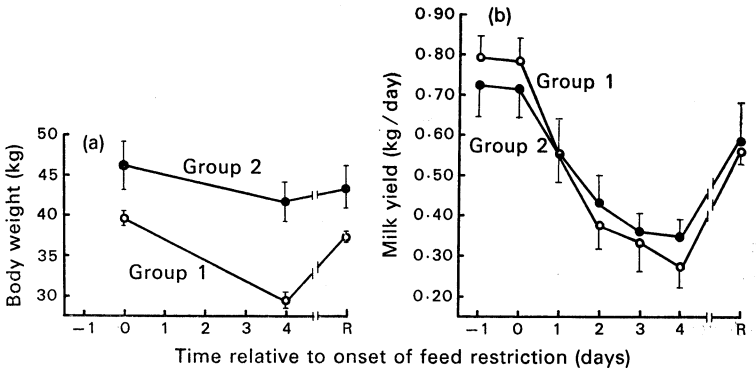

Fig. 1. Body weights (*a*) and milk yields (*b*) before feed restriction, during the period of feed restriction, then 10–14 days after restoring feed intake (R) for group 1 ewes ($n = 3$) initially fed *ad libitum* and for group 2 ewes initially fed to calculated requirements for metabolizable energy. In Figs 1–3 values presented are means and standard errors are shown as vertical bars.

Results

Body Weight and Milk Yield

Body weights of both groups of ewes were significantly reduced ($P < 0·01$) after 4 days of food restriction and 10 days after restoring feed intakes body weights had increased to approach the weights before food was restricted (Fig. 1*a*). The body weights of ewes fed to calculated requirements (group 2), however, were still significantly lower ($P < 0·05$) at this time. Although ewes in group 1 tended to have lower body weights than ewes in group 2 throughout the period of the experiment, the two groups differed significantly ($P < 0·01$) only at 4 days after the onset of feed restriction (see Fig. 1*a*).

Milk yields of both groups of ewes were not significantly different ($P > 0{\cdot}05$) throughout the period of the experiment (Fig. 1*b*). Milk yields decreased significantly ($P < 0{\cdot}05$) from about 750 g/day before to about 300 g/day 4 days after feed restriction. Yields increased to about 600 g/day by 10 days after restoring feed intake but these yields were significantly lower ($P < 0{\cdot}05$) than prerestriction yields. Throughout the experiment, the lactose content of milk remained at 4–5% for both groups of ewes.

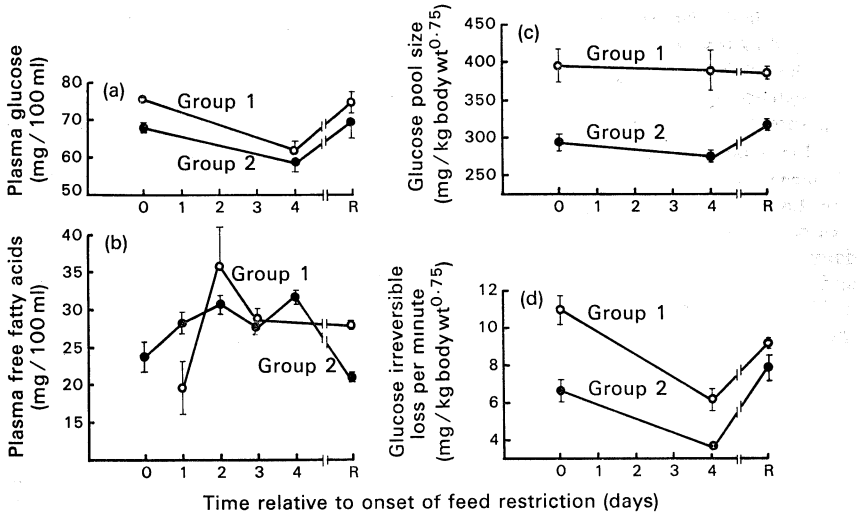

Fig. 2. Concentration of glucose (*a*) and free fatty acids (*b*) in plasma and glucose pool size (*c*) and rates of glucose irreversible loss (*d*) before feed restriction, during the period of feed restriction [4 days after restricting feed intake in (*a*), (*c*) and (*d*)], then 10–14 days after restoring feed intake (R) for group 1 ewes ($n = 3$) initially fed *ad libitum* and for group 2 ewes ($n = 4$) initially fed to calculated requirements for metabolizable energy.

Blood Metabolites

Plasma glucose

Concentrations of glucose in plasma decreased during the period of feed restriction in both groups of ewes but a significant decrease ($P < 0{\cdot}01$) occurred only in group 1 (fed *ad libitum*). After restoring feed intakes, concentrations of plasma glucose increased to prerestriction values. Concentrations of plasma glucose were always lower for group 2 (fed to requirements) than for group 1 (fed *ad libitum*) ewes, but differences between groups were significant ($P < 0{\cdot}01$) only before feed restriction (Fig. 2*a*).

Plasma FFA

Concentrations of FFA in plasma rose significantly ($P < 0{\cdot}05$) during feed restriction, and fell after restoration of feed intake in both groups of ewes (Fig. 2*b*). Changes for group 1 ewes (fed *ad libitum*) were greater than for group 2 ewes.

Glucogenic amino acids in blood

Blood concentrations of glucogenic amino acids measured in group 2 ewes before, 2 and 4 days after the onset of food restriction showed that significant decreases

occurred over the period of food restriction, with the exception of glutamine and glycine. The level of glycine was significantly increased ($P < 0{\cdot}05$) after 4 days of feed restriction, but no significant changes were measured for glutamine (see Table 1).

Table 1. Levels of glucogenic amino acids (nmoles/ml) in blood of ewes fed initially to calculated requirements (group 2) before, then 2 and 4 days after feed intake was reduced by 80%

Values presented are means for four ewes. Asterisks denote values which differ significantly from the corresponding value before feed restriction as follows: $*P < 0{\cdot}10$; $**P < 0{\cdot}05$

Amino acid	Blood sample obtained: Before food restriction	2 days after food restriction	4 days after food restriction
Glycine	1062·1	1165·8	1671·6**
Alanine	295·8	186·0*	233·8
Serine	128·1	82·5**	93·2**
Asparagine	100·7	58·9**	58·6**
Aspartic acid	51·6	39·7	37·1*
Glutamine	204·8	154·3	204·5
Glutamic acid	256·4	169·0**	193·4*
Cysteine	48·9	40·6	39·1*
Valine	298·4	174·0**	157·8**
Ornithine	130·3	69·7**	83·1*
Arginine	122·1	84·2*	96·1

Glucose Biokinetics

Changes in glucose pool size and rates of irreversible loss are shown in Fig. 2*c*. Glucose pool size (g/kg body $wt^{0{\cdot}75}$) for group 1 ewes was significantly higher ($P < 0{\cdot}05$) than for group 2 ewes throughout the experiment. In both groups, total glucose pool size decreased significantly ($P < 0{\cdot}05$) during feed restriction and had returned to prerestriction values 10 days after restoring feed intakes. When glucose pool size was corrected for metabolic body weight, values for group 1 ewes remained constant, but for group 2 ewes glucose pool size decreased significantly ($P < 0{\cdot}05$) during feed restriction and increased significantly ($P < 0{\cdot}01$) after restoring feed intake, relative to the value before feed restriction (Fig. 2*c*).

Rates of glucose irreversible loss per minute (mg/kg body $wt^{0{\cdot}75}$) were significantly higher ($P < 0{\cdot}01$) for group 1 than group 2 ewes before and during the period of food restriction (Fig. 2*d*). After restoring feed intakes the difference between groups was not significant ($P < 0{\cdot}05$). Four days after the onset of food restriction the rates of irreversible loss of glucose for both groups of ewes were significantly lower ($P < 0{\cdot}01$) than before or after the period of food restriction.

Plasma Hormones

Growth hormone

Plasma growth hormone concentrations in group 1 ewes (fed *ad libitum*) were not significantly increased during feed restriction, but after feed restriction for 4 days, growth hormone concentrations were significantly raised ($P < 0{\cdot}05$) in group 2 ewes (Fig. 3*a*).

Thyroxine

Concentrations of thyroxine were only measured for ewes in group 2. During feed restriction concentrations were significantly lower ($P < 0{\cdot}01$) than before or after this period (see Fig. 3*b*).

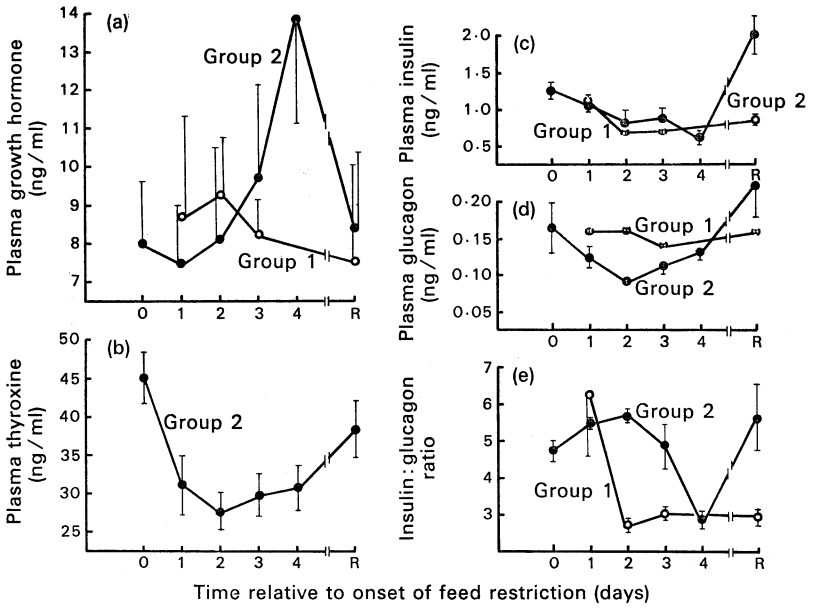

Fig. 3. Levels of growth hormone (*a*), thyroxine (*b*), insulin (*c*), glucagon (*d*), and the molar ratio of insulin to glucagon (*e*) before feed restriction, during the period of feed restriction, then 10–14 days after restoring feed intake (R) for group 1 ewes ($n = 3$) initially fed *ad libitum* and for group 2 ewes ($n = 4$) initially fed to calculated requirements for metabolizable energy. For each ewe a pooled plasma sample was prepared using samples obtained throughout each day.

Insulin and glucagon

Concentrations of insulin in plasma decreased significantly ($P < 0{\cdot}05$) in both groups of ewes during the period of food restriction and increased after feed intake was restored (Fig. 3*c*). Concentrations of glucagon were constant for group 1 ewes (fed *ad libitum*) and decreased, but not significantly ($P > 0{\cdot}05$), for ewes in group 2 (Fig. 3*d*).

The molar ratio of insulin:glucagon had decreased by 50% 2–4 days after the onset of feed restriction. The value of the insulin:glucagon ratio for ewes in group 1 was significantly ($P < 0{\cdot}10$) lower than the prerestriction value 3 days after the onset of feed restriction. A similar response occurred in group 2 ewes in which the insulin:glucagon ratio fell significantly ($P < 0{\cdot}05$) 4 days after the onset of feed restriction. After feed intake was restored, the molar ratio of insulin:glucagon increased to prerestriction values for group 2 ewes, but remained low for group 1 ewes (Fig. 3*e*).

Discussion

The rate of gluconeogenesis in ruminants is directly related to the availability of glucose precursors absorbed from the digestive tract (Judson and Leng 1973*a*, 1973*b*;

Steel and Leng 1973*a*, 1973*b*). In the fed animal, absorbed propionate and amino acids are the major precursors of glucose but during feed deprivation, the reduced availability of these substrates leads to the mobilization of tissue reserves for glucose synthesis. If the extent of feed restriction is significant, gluconeogenesis declines and less glucose is available for maintenance and productive functions, as exemplified by the present studies. In both groups of ewes, feed restriction resulted in substantial and rapid decreases in milk output, losses of body weight and significant decreases in the rates of glucose irreversible loss and glucose pool size.

The changes in plasma FFA and blood amino acid levels during feed deprivation were consistent with increased mobilization of body tissues for gluconeogenesis and energy supply. Although adipose tissue is mobilized during feed restriction largely to supply energy in the form of FFA (see Annison 1976), the concommitant release of glycerol, a glucose precursor, may make a significant contribution to glucose supply. Glucogenic amino acids released from tissue protein constitute the most important precursors of glucose during feed deprivation (see Bergman 1973), and the decline in the plasma concentrations of these substrates in the present studies was consistent with earlier reports (see Ballard *et al.* 1976). Blood concentrations of metabolites are of little quantitative significance, but the progressive decline in blood amino acid concentrations presumably reflected increased uptake of amino acids by the liver and kidney (Lindsay *et al.* 1977).

The interpretation of total body weight changes in ruminants subjected to feed deprivation is made difficult by uncertainty concerning effects on gut fill. The higher loss of body weight during feed restriction of the ewes fed *ad libitum* (group 1) relative to those fed to requirement (group 2) was probably due to differences in the nature of the diets, and the amounts consumed. The lucerne chaff fed to group 1 ewes would be expected to have a longer residence time in the alimentary tract than the lucerne chaff/grain mixture fed to the second group of ewes. Moreover, group 1 ewes consumed much more dry matter (*c.* 2·2 kg/day) than group 2 ewes (*c.* 1·3 kg/day).

Feed restriction resulted in a rapid and substantial decrease in milk yield (Fig. 1*b*), but the identity of the rate-limiting nutrient(s) for milk synthesis during feed restriction remains unknown. Glucose would seem to be the most likely metabolite, since in addition to its role as a precursor of lactose, the oxidation of glucose provides much of the energy for the synthesis of the major constituents of milk (see Annison 1971). Davis and Bickerstaffe (1978) have shown in the lactating ewe that the ratio of glucose uptake by the mammary gland to lactose output in milk is about 2. If we use this ratio to calculate mammary uptakes of glucose during feed restriction, falls in lactose output from 36 to 12 g/day in group 1 ewes and from 33 to 16 g/day in group 2 ewes correspond to reductions in the mammary uptakes of glucose of 48 and 34 g/day respectively. These amounts were much less than the falls in glucose supply measured as irreversible loss over the same period, which were 140 and 82 g/day in group 1 and group 2 ewes respectively. The magnitude of the changes in glucose irreversible loss (Fig. 2*d*) was consistent with the hypothesis that glucose is the rate-limiting nutrient for milk synthesis during feed restriction. After restoration of feed intake, blood glucose concentrations returned to prerestriction values and milk production increased to stable levels of *c.* 0·60 kg/day in both groups of ewes by about 10 days after restoring feed intake. Failure of milk yields to return to prerestriction values may have been the result of senescence of portions of the mammary parenchyma (see Swan 1979).

Insulin and glucagon are crucial for metabolic regulation in ruminants. Insulin stimulates uptake and utilization of glucose, inhibits proteolysis and lipolysis and promotes protein and fat synthesis in most tissues. Glucagon is involved principally in regulation of hepatic metabolism, increasing glucose output by promoting gluconeogenesis from amino acids and possibly propionate, and by promoting glycogenolysis. The interrelation between insulin and glucagon is such that it is the insulin:glucagon molar ratio which is of major importance (Bassett 1975, 1978; Trenkle 1978). The present data were consistent with the known actions of insulin and glucagon. Food deprivation reduced glucose supply, and concentrations of insulin decreased. Enhanced gluconeogenesis from amino acids was presumably regulated by glucagon, since the concentrations of this hormone remained relatively unchanged, resulting in a substantial decrease in the molar ratio of insulin:glucagon from about 6 to 3.

Changes in the concentrations of growth hormone in plasma from group 2 ewes were similar to those reported by other workers (Bassett and Madill 1974; Bassett 1975; Hart *et al.* 1978; Trenkle 1978). Inadvertent loss of samples from group 1 ewes prevented measurement of concentrations of growth hormone before feed restriction for these ewes. In spite of this, it appeared that concentrations of growth hormone increased in response to food restriction. Overall, in response to feed restriction, concentrations of growth hormone increased resulting in a negative correlation between levels of growth hormone and insulin. These changes are consistent with the need to reduce glucose utilization by peripheral tissues during feed restriction.

Concentrations of plasma thyroxine in ewes in group 2 fell substantially during the period of feed restriction, and increased on refeeding. A negative correlation between level of thyroxine in plasma and milk production of lactating cows and rats has been reported (see Shaw *et al.* 1975). In the present studies, plasma thyroxine decreased as milk yield declined, perhaps reflecting a reduction in metabolic rate in response to feed restriction.

In conclusion, the results of the present studies are consistent with and extend the results of previous studies on metabolic regulation in the ruminant. Changes in concentrations of insulin, glucagon and growth hormone occurred in response to feed restriction in a manner consistent with attempts to maintain blood glucose supply in the face of a dramatic reduction of food intake. The data are consistent with results of previous studies with ruminants (see Bassett 1975, 1978; Trenkle 1978), and suggest that the hormonal control of gluconeogenesis in the ruminant is similar to that in the non-ruminant (see Bassett 1975).

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