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Comparison of Glucose Biokinetics in Parturient Ewes and Ewes Induced to Lactate Artificially

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Abstract

Glucose biokinetics of six normal pregnant/lactating ewes during the periparturient period were compared with those of five non-pregnant ewes induced to lactate artificially by treatment with oestrogen and progesterone, followed by a series of infusions of oxytocin. Normal ewes produced large amounts of milk (800–900 g/day) on day 1, and yields remained relatively constant until day 8 post partum. Milk production of induced ewes, however, was negligible on day 1 (30 g/day) but increased progressively until day 8 (540 g/day) after the start of milking. The glucose irreversible loss per minute 2–8 days before (6·4 *v.* 5·6 mg per kilogram body weight$^{0·75}$), and 2 days after the onset of lactation (9·1 *v.* 5·5 mg/min per kg$^{0·75}$) was significantly greater ($P < 0·05$) in normal pregnant/lactating ewes than in ewes induced to lactate artificially. By the eighth day of lactation rates of glucose irreversible loss per minute (7·4 *v.* 6·4 mg per kilogram body weight$^{0·75}$) were not significantly different ($P > 0·05$). The data were consistent with the hypothesis that glucose supply is rate limiting for milk production for several days after the initiation of lactation in non-pregnant, hormone-treated ewes.

In parturient ewes, the rate of glucose irreversible loss was significantly increased 1–2 days post partum.

Introduction

Previous studies have shown that essentially normal lactation may be induced by hormone treatment of non-pregnant ewes (Fulkerson and McDowell 1974, 1975), but during the first few days after the onset of lactation, milk yields remain lower than yields of normal parturient ewes. In view of the quantitative importance of glucose in mammary metabolism (Hardwick *et al.* 1963; Annison *et al.* 1974) it is possible that during the first few days after the onset of lactation in ewes induced to lactate artificially, glucose supply to the mammary gland is insufficient to meet the demands for the synthesis of milk constituents.

The present studies were conducted to test the hypothesis that the rate-limiting factor for milk production in early lactation is glucose supply, and that increased gluconeogenesis must occur before copious quantities of milk can be secreted. To this end, glucose biokinetics in normal pregnant/lactating ewes were compared with those of ewes induced to lactate artificially.

Materials and Methods

Sheep

Multiparous crossbred ewes (Border Leicester × Merino) from the same flock were used. All were accustomed to handling and were free of obvious abnormalities of the mammary glands.

Indwelling jugular catheters (1·0 mm i.d. by 1·5 mm o.d., Dural Plastics, Sydney) were fitted in both jugular veins of each ewe 7 days before the experiment. Ewes were housed in metabolism cages, and fed on good-quality lucerne chaff *ad libitum*. Feed intakes, which were recorded daily, were 1·4–1·6 times the metabolizable energy requirement for maintenance plus milk production.

Daily milk yields were recorded and samples of milk were stored at −16°C.

Hormones

Progesterone (Calbiochem, La Jolla, California) and oestradiol benzoate (β-oestradiol-3-benzoate, Sigma Chemical Co., St Louis, Missouri) were dissolved in a small volume of ethanol, then suspended in peanut oil to give a preparation containing oestradiol benzoate and progesterone at final concentrations of 120 μg/ml and 30 mg/ml respectively. Synthetic oxytocin (Syntocinon, Sandoz Ltd, Basel, Switzerland) was diluted in 0·9% (w/v) saline to give a concentration of 0·5 i.u./ml.

Experiments

Experiment 1

Six normal ewes (group 1) were mated after their synchronization of oestrus by the procedures of Moore and Holst (1967). The date of lambing was determined by assuming a gestation length of 148 days. A second group of five non-pregnant ewes (group 2) was induced to lactate artificially by using the hormone treatments described by Fulkerson and McDowell (1975). A series of 20 subcutaneous injections of 240 μg oestradiol benzoate plus 60 mg progesterone given at intervals of 3 days induced mammary gland development. Three days after the last injection, milk secretion was initiated by rapid intravenous infusion of 1 i.u. oxytocin given four times each day (0600, 1200, 1800 and 2400 h) for 5 days (trigger phase). Regular hand-milking started on the first day of the trigger phase. The experiment was designed to ensure that the day of lambing for ewes in group 1 coincided with the first day of milking for ewes in group 2.

Experiment 2

Studies were made on a third group of four pregnant ewes (group 3) in which oestrus had been synchronized. The time of lambing of these ewes was later than for ewes in group 1. Lambs were removed from the ewes in groups 1 and 3 immediately after birth, and all ewes were hand-milked daily at 0800 and 1700 h.

Glucose Biokinetics

In normal pregnant/lactating ewes, glucose biokinetics were measured 2–8 days before and then 2 and 8 days after parturition (group 1), or 1–2 days before and then 1, 2 and 4 days after parturition (group 3). For ewes induced to lactate artificially (group 2), measurements were made 2–8 days before and then 2 and 8 days after commencing infusions of oxytocin (i.e. commencement of milking). Thus, for ewes in group 2, measurements were made before, during and after the period when oxytocin infusions were given.

Glucose biokinetics were measured by isotope dilution using the single-injection procedure of Katz *et al.* (1974). D-[U-^{14}C]glucose (*c.* 120 μCi, 5 mg), obtained from Radiochemical Co., G.B., was dissolved in 5 ml sterile saline (9% w/v) and injected into one jugular vein. Blood samples (5–10 ml) were withdrawn from the other jugular vein catheter 5 min before and 10, 15, 20, 25, 30, 35, 40, 50, 60, 90, 120, 150, 180, 240 and 300 min after the injection of labelled glucose, using heparin as anticoagulant. Blood samples were immediately cooled in ice and centrifuged for the preparation of plasma, which was stored at −16°C. Carrier glucose (100 mg) was added to plasma (1·0 ml) immediately after measurement of glucose content (Huggett and Nixon 1957), and the glucose isolated as pentaacetate (Jones 1965) for the measurement of glucose specific radioactivity.

Milk Lactose

Milk lactose was assayed by the method of Cowie *et al.* (1969).

Statistical Analyses

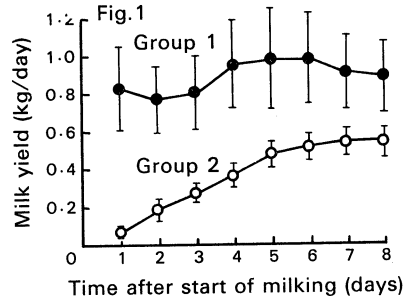
The significance of differences between mean values for parameters measured were computed using Student's *t*-test (Steel and Torrie 1960).

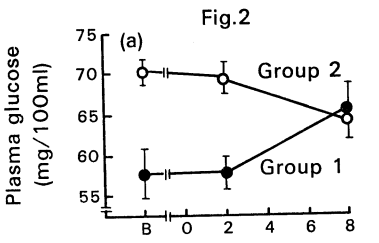
Results

Experiment 1

Yield and lactose content of milk

Ewes which had lambed (group 1) produced copious supplies of milk 24 h after parturition, and milk yield was maintained throughout the 8-day period of the experiment (Fig. 1). In contrast, yields of the ewes induced to lactate artificially (group 2) were very low on the first and second days of lactation, but increased progressively during the 8-day period (Fig. 1).





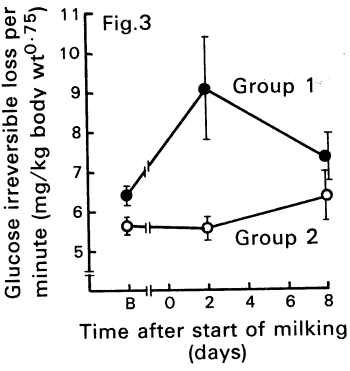


Fig. 1. Daily milk yield for ewes lactating after normal pregnancy (●, group 1), and non-pregnant ewes induced to lactate artificially (○, group 2). In Figs 1–4 plotted points represent mean values and standard errors are indicated by vertical bars.

Fig. 2. Plasma glucose levels (*a*) and glucose pool size (*b*) 2–8 days before (B) and then after the onset of lactation for normal pregnant/lactating ewes (●, group 1), and non-pregnant ewes induced to lactate artificially (○, group 2).

Fig. 3. Rates of glucose irreversible loss 2–8 days before (B) and then after the onset of lactation for normal pregnant/lactating ewes (●, group 1), and non-pregnant ewes induced to lactate artificially (○, group 2).

Milk lactose levels were not significantly different throughout the period of the experiment. On day 8 of lactation, milk lactose contents were $4{\cdot}3 \pm 0{\cdot}29$ and $4{\cdot}5 \pm 0{\cdot}32$ g/100 ml (mean $\pm$ s.e.) for ewes in groups 1 and 2 respectively.

Glucose biokinetics

Plasma glucose levels of ewes in group 1 were significantly lower ($P < 0.05$) than in group 2 before (57·9 ± 3·10 *v.* 70·3 ± 1·60 mg/100 ml, mean ± s.e.) and 2 days after (57·9 ± 2·00 *v.* 69·5 ± 2·00 mg/100 ml) the onset of lactation (Fig. 2). By the eighth day of lactation, levels of plasma glucose for both groups of ewes were similar (*c.* 65 mg/100 ml). Although values for glucose pool size, expressed in terms of metabolic body weight, for the two groups of ewes were not significantly different ($P > 0.05$) before lactation, significant differences ($P < 0.05$) between the values for the two groups of ewes were detected 2 and 8 days after the onset of lactation (Fig. 2).

Changes in the rate of irreversible loss of glucose are shown in Fig. 3. Before and 2 days after the onset of lactation values for ewes in group 1 were significantly higher ($P < 0.05$) than corresponding values for ewes in group 2. By day 8 of lactation, the rates of glucose irreversible loss for both groups of ewes were similar. For ewes in group 1, the glucose irreversible loss per minute on day 2 of lactation was significantly higher ($P < 0.05$) than before and 8 days after the onset of lactation (9·1 *v.* 6·4 and 7·2 mg per kilogram body weight$^{0.75}$, respectively).

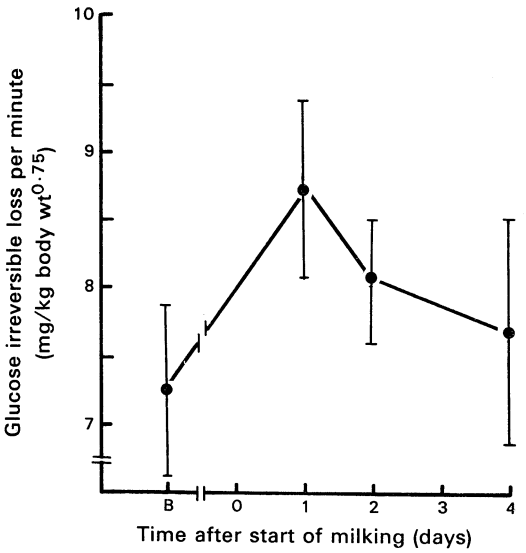


Fig. 4. Rates of glucose irreversible loss 1–2 days before (B) and then after the onset of lactation for normal pregnant/lactating ewes (group 3).

Experiment 2

Yield and lactose content of milk

Ewes in group 3 produced copious quantities of milk from the first day of lactation. Yields were 1·21 ± 0·190, 1·29 ± 0·230 and 1·34 ± 0·288 kg/day (mean ± s.e.) on days 1, 2 and 4 of lactation respectively. Throughout this period, the lactose content of milk (*c.* 4·5 g/100 ml) was within the range for normal ovine milk.

Glucose biokinetics

Although the level of plasma glucose fell from 67 ± 3·2 mg/100 ml (mean ± s.e.) before parturition to 61 ± 6·0 mg/100 ml on day 1 then to 58 ± 2·7 mg/100 ml by day 4 of lactation, the changes were not statistically significant ($P > 0.05$). In agreement with the results of experiment 1, the rate of glucose irreversible loss

increased in the post-partum period and on day 1 of lactation glucose irreversible loss appeared to be higher, although not significantly ($P > 0{\cdot}05$) higher than it was immediately before or on days 2 and 4 of lactation (Fig. 4).

Discussion

In previous studies it was observed that milk yields of ewes induced to lactate artificially approached those of ewes lactating after normal pregnancy (see Fulkerson and McDowell 1974). Indeed, in the present study (expt 1), yields of group 2 ewes rose to approach those of group 1 ewes by 8 days after the onset of lactation. A striking difference in the lactation patterns of the normal and hormonally induced ewes was the rapid onset of copious milk secretion in normal ewes, and the very low milk outputs on days 1 and 2 of lactation in the induced group. The latter observation was in agreement with earlier studies on ewes induced to lactate by hormone treatment (Fulkerson and McDowell 1974, 1975).

Plasma glucose level and pool size were considerably higher in the non-pregnant ewes (group 2) than in the pregnant ewes immediately prior to the initiation of lactation (Fig. 2). Glucose irreversible loss, however, was unrelated to plasma glucose level in the two groups of ewes because it was significantly higher in pregnant ewes (Fig. 3). This finding was in agreement with the earlier studies of Oddy (1979) who examined the relationships between plasma glucose concentration and glucose irreversible loss in pregnant and non-pregnant ewes over a range of feed intakes. At any concentration of plasma glucose, glucose irreversible loss was significantly higher in pregnant ewes, presumably reflecting increased glucose utilization by the uterus during pregnancy (see Oddy and Annison 1979). The high glucose requirement of the ovine foetus in late pregnancy, reported to be 32–40 g/day (Huggett *et al.* 1951; Kronfeld 1958), implies the development of an enhanced capacity for gluconeogenesis during pregnancy. At parturition, the demands of the uterus for glucose are succeeded by those of the mammary glands, and the copious production of milk on day 1 of lactation is evidence of the maintenance of glucose supply.

The sharp increase in glucose irreversible loss in the normal lactating ewes (groups 1 and 3) on days 1–2 of lactation was consistent with high milk yields at that time. The magnitude of glucose irreversible loss on days 1–2 of lactation, which exceeded those a few days later (see Figs. 3 and 4) in spite of relatively constant milk production over that period, was unexpected. This may have been due to the stimulation of glucose production in response to the raised circulating levels of glucocorticoids (Liggins and Grieves 1971) and growth hormone (Bassett *et al.* 1970) which occur at parturition. Thus, it appears that in the normal pregnant ewe, the metabolic adjustments which occur during late pregnancy in response to the requirements of the foetus and placenta for glucose allow the ewe to meet the glucose demands of the mammary glands immediately after parturition.

In contrast, the unchanged glucose metabolism at day 2 of lactation in ewes induced to lactate artificially was consistent with low milk yields at that time. It seems that oxytocin had no direct effect on glucose metabolism in group 2 ewes, since glucose irreversible losses before and 2 days after commencement of oxytocin infusions were constant (see Fig. 3). Instead, it seems likely that the slow increase in milk yield over 7–8 days in the induced ewes reflected the time-course of the development of enhanced gluconeogenic capacity.

In conclusion, the present studies provide further evidence that the procedures developed by Fulkerson and McDowell (1974, 1975) initiate essentially normal lactation in the ewe. Certainly, by 8 days after the onset of lactation, milk yields and rates of glucose irreversible loss for ewes in groups 1 and 2 were not significantly different.

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