The effect of an extended milking interval on mammary blood flow and tight junctions between mammary cells in goats

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ABSTRACT

The timing of changes in mammary epithelium cell tight junction integrity and mammary blood flow during a 36-h milk accumulation was studied in six lactating (2208 ± 216 g milk/d) Saanen goats. Tight junctions became disrupted following 21 h of milk accumulation, and mammary blood flow started to decline at 21 hr. The time both events occurred was not significantly different ($P > 0.10$) from the time milk secretion began to decline (19 h). The decline in MBF may be the result of a negative feed-back response to a reduced demand for metabolites due to a reduced rate of milk secretion. The fact that mammary tight junctions became disrupted when milk secretion declined, suggests that impairment of mammary tight junction integrity is associated with a decrease in milk secretion during an extended milking interval.

Keywords: goat, milking interval, plasma lactose, tight junction, blood-milk potential difference.

INTRODUCTION

It has been amply demonstrated that an extended milking interval (e.g. once-a-day milking) reduces milk yield in cows (Carruthers *et al.*, 1991) and goats (Wilde and Knight, 1990). The exact reasons for this loss of production are not known, but there are a number of factors that may play a role. Preliminary data by Knight and Dewhurst (1992) indicate that mammary gland cisternal capacity may be limiting in cows milked once daily. However, treatment of cows with bovine somatotropin during once-a-day milking increased milk yield by 13% (Carruthers *et al.*, 1991), suggesting that gland capacity was not limiting. Evidence is also accumulating that an autocrine inhibitor, present in the alveolar milk fraction, may be, at least partly, responsible for this loss of milk yield (Wilde and Peaker, 1990; Wilde *et al.*, 1990).

Maintaining the integrity of tight junctions between adjacent mammary epithelial cells may also be important in preventing milk yield losses associated with an extended milking interval. Tight junctions prevent paracellular leakage of blood serum components into the milk and milk components into the blood. They are also instrumental in maintaining a small transepithelial blood-milk potential difference. Neville and Peaker (1981) showed that induced disruption of mammary tight junctions depressed milk yield in goats. Fleet and Peaker (1978) studied daily changes in mammary blood flow (MBF) and mammary function following cessation of milking in goats. It is, however, not known how soon following milking changes in tight junction integrity and MBF occur.

The objective of the present study was therefore to investigate the timing of changes in tight junction integrity and MBF during intramammary milk accumulation during an extended milking interval in goats.

MATERIALS AND METHODS

Animals and management

Six multiparous lactating Saanen goats were used. At least 14 d prior to the beginning of the experiment all goats had a transit-time ultrasonic blood flow probe surgically implanted around one pudic artery. On the day of the experiment each goat was fitted with a mammary vein catheter, on the same side as the flow probe, as well as a jugular vein catheter.

The goats were housed indoors in individual stalls, and were fed twice daily a diet consisting of hay (ad libitum access) and concentrates (2 kg). The animals were machine-milked normally at 0700 and 1500 h and producing 2208 ± 216 g milk/d.

Measurements

All measurements were made on one mammary gland only, i.e. the gland on the same side where the blood flow probe was implanted. On the morning of the first day of the experiment the animals were milked as usual (Milking A). Each animal then received 200 mIU (i.v.) of oxytocin and was milked out by hand; this procedure was repeated two more times, respectively after 3 and 8 min. Following 36 h of milk accumulation all goats were milked as before (Milking B), after which twice daily milking resumed at normal milking times.

Mammary gland volume, determined by water displacement (Linzell, 1966), was measured at 0, 4, 8, 12, 16, 20, 22, 24, 26, 28, 30, 32, 34, and 36 h following Milking A, and used as an indicator of milk secretion. Blood-milk potential difference (PD) was measured in one mammary gland (test gland) as described by Peaker (1977). Briefly, two 10 to 15 cm long pieces of sterile polyethylene tubing, containing sterile saturated KCl in 3% (w/v) agar ("KCl-agar bridges"), were inserted inside the mammary vein catheter (blood side) and via

the teat sphincter inside the cistern of the corresponding mammary gland (milk side). The KCl-agar bridges were connected externally to the animal to silver-coated electrodes that were submerged in saturated KCl. Potential difference was measured every 30 min between 1 h before and 20 h after Milking A, and subsequently every 15 min for the next 16 h. Jugular vein blood samples were taken at -1, -0.5, 0, 4, 8, 16, 20 h following Milking A, and hourly between 20 and 36 h. Blood was collected in EDTA coated vacutainers, centrifuged for 15 min at 630 x g, and plasma was stored at -20 C until analyzed for lactose (Arthur *et al.*, 1989). Mammary blood flow was recorded continuously throughout the experiment, starting one hour prior to Milking A. The change in the rate of milk secretion (based on mammary volume) followed a curvilinear pattern. For the data on each animal a straight line was fitted through the linear ascending part of the curve and through the plateauing part of the same curve. The time at which the rate of milk secretion began to fall was then arbitrarily chosen at the intersection of the two straight lines. The individual PD, plasma lactose concentration, and MBF profile were of a more complex nature. Hence, fifth-order polynomials were fitted to smooth the data of each animal. The time points at which these variables began to change, were then arbitrarily chosen as the first data point one standard error beyond the first trough (PD, plasma lactose) or peak (MBF).

Functional mammary gland capacity (FMGC) is defined as the quantity of milk in the mammary gland, when, following a period of milk accumulation, the secretion rate is zero (Davis and Hughson, 1988). The time at which FMGC was reached was calculated for each goat by dividing its FMGC by its normal hourly rate of milk secretion. The latter was derived from the average 24-h milk secretion during the two days preceding the experiment.

Statistical analysis

Data are expressed as means and standard errors. Differences between means were evaluated by paired t-test analyses and were considered significant at $P < 0.05$.

RESULTS

At Milking B (i.e at 36 h) mammary gland volume had plateaued (Fig. 1), indicating that milk secretion had ceased. Hence, all goats had reached their FMGC (test gland: 1063 ± 102 g).

Pre-experiment blood-milk PD varied from -31 to -40 mV, with a mean value of -36.1 ± 1.4 mV (milk negative with respect to blood). The PD decreased 21.6 ± 3.0 % ($P < 0.001$) from a maximum of -31.6 ± 2.1 mV at 20.8 ± 2.4 h.

Plasma lactose concentration (Fig. 2) remained fairly constant until 21.2 ± 1.7 h when it started to increase rapidly. At 36 hours there was a significant 3-fold increase in plasma lactose.

Mammary blood flow was maintained between 90 and 100% of pre-experiment values during the first 12 hours of milk accumulation, but then increased briefly (probably due to feeding and eating related activity) before starting to decrease at 20.5 ± 2.3 h. At 36 h MBF had decreased by 44.0 ± 6.6% ($P < 0.01$) of MBF immediately before the experiment. Following the next normal morning milking MBF had still not (71.9 ± 4.8%, $P < 0.01$) returned to the pre-experiment level.

The rate of milk secretion (indicated by changes in mammary gland volume) and MBF began to decrease several hours before FMGC was reached (Table 1), whereas the time transepithelial PD and plasma lactose concentrations began

FIGURE 1: Mean mammary gland volume in goats during 36 h of milk accumulation.

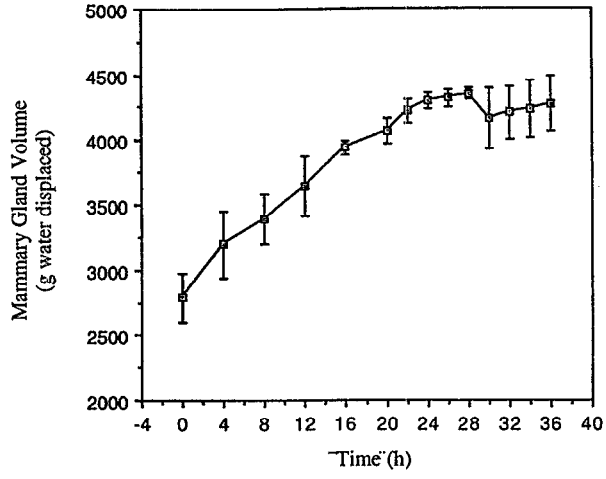

FIGURE 2: Mean plasma lactose concentration in goats during 36 h of milk accumulation.

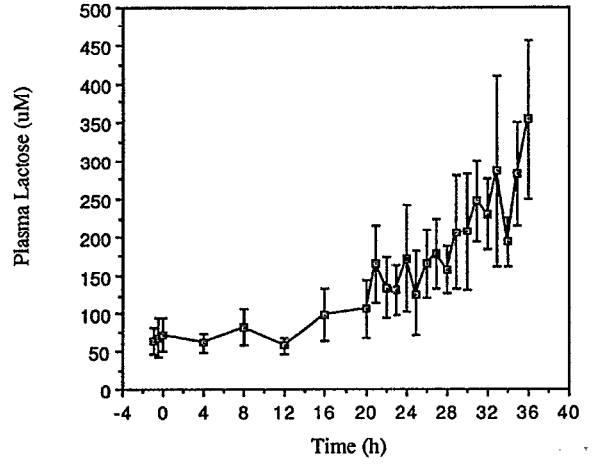

TABLE 1: Time when functional mammary gland capacity (FMGC) was reached, and when changes in the rate of milk secretion[1], transepithelial potential difference (PD), mammary blood flow (MBF), and plasma lactose concentrations occurred during a 36-h period of mild accumulation in goats.

Variable	Time (h)
FMGC reached	25.0 ± 1.9[a]
Rate of milk secretion starts to decrease	18.7 ± 1.2[b]
PD starts to decrease	20.8 ± 2.4[ab]
Plasma lactose starts to increase	21.2 ± 1.7[ab]
MBF starts to decresase	20.5 ± 2.3[b]

[1] Based on changes in mammary gland volume

[a,b] Means with different superscripts differ significantly ($P < 0.05$)

to change did not differ significantly from that when FMGC was reached.

DISCUSSION

This experiment describes the timing of changes in mammary tight junction integrity and MBF during an extended period of milk accumulation.

Since milk is the only source of plasma lactose in cows and goats (Kuhn and Linzell, 1970), the sharp increase in plasma lactose concentrations clearly demonstrated the occurrence of paracellular leakage. These data taken together with a significant decrease in transepithelial PD indicate that loss of tight junction integrity occurred after approximately 20 h of milk accumulation. Furthermore, the fact that paracellular leakage occurred at the same time that mammary gland volume started to decline, suggests that impairment of tight junction integrity is associated with a decrease in milk secretion. What causes the impairment of mammary tight junctions is not clear. Fleet and Peaker (1978) showed that following cessation of milking intramammary pressure increases for three days, and then decreases due to loss of epithelial cell integrity. However, the fact that in the present study tight junctions became already impaired after 20 h, i.e long before intramammary pressure reached its maximum (Fleet and Peaker, 1978) suggest that factors other than intramammary pressure cause loss of tight junction integrity.

Loss of tight junction integrity may alter cytoskeletal activity in epithelial cells (Schneeberger and Lynch, 1992) and, because microtubule components of the cytoskeleton are involved in the process of milk secretion (Loizzi, 1987), this may constitute a mechanism through which milk yield is reduced during periods of extended milk accumulation.

Detailed time course data on MBF during extended periods of milk accumulation are not available. Fleet and Peaker (1978) showed that in goats 24 hours after cessation of milking MBF had not changed, but that at 48 h MBF had stabilized at approximately 50 % of the initial MBF. Continuous MBF recording in the present experiment showed that MBF was already reduced by 50 % after 36 h of milk accumulation and that the decline started after 21 h, i.e., at approximately the same time that the rate of milk secretion started to decline, and before FMGC had been reached. However, it is not clear if the decline in MBF is, at least in part, responsible for decreased milk secretion. The decline in MBF may be the result of a negative feed-back response to a reduced demand for metabolites resulting from a reduced rate of milk secretion, which as discussed previously, may have been caused by disruption of tight junctions between the mammary cells.

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