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# Metabolic changes in the mammary gland of sheep in late pregnancy and lactation

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## ABSTRACT

Four sheep fitted with an indwelling catheter in the femoral artery and a transit time ultrasonic flow probe around the left pudendal artery (supplying the mammary gland) were used to quantitate changes in mammary blood flow (MBF) in late pregnancy and lactation, and to determine the extraction of glucose and oxygen by the mammary gland. Mammary vein blood samples were collected by venipuncture. Considerable variation existed in the pattern of change in MBF between individual sheep. In two animals, MBF through the pudic artery fitted with the flow probe doubled to 600 mL/min on the day of lambing ($D_L$), while in the other two sheep, MBF varied little in response to lambing. Possible explanations for this difference between individuals are discussed. Glucose extraction trebled on $D_L$ from 6.0 to 18.7% ($P<0.01$) and remained high during lactation in relation to milk output. Oxygen extraction by the mammary gland peaked during the last week of pregnancy (at 45.7%) and during lactation it was related linearly to milk output.

**Keywords:** lactating sheep; pregnancy; mammary gland; blood flow.

## INTRODUCTION

With the increase in the fecundity of sheep through selective breeding, there is a greater need to ensure high rates of milk production are achieved so that lamb growth rates are not held below potential. The supply of nutrients to the mammary gland is an important determinant of milk production, both indirectly by its affects on mammary development during pregnancy, and directly by affecting the supply of precursors for milk synthesis. Nutrient supply to the mammary gland is a function of both mammary blood flow (MBF) and the nutrient concentration in arterial blood supplying the gland. The aims of the experiment were to characterise the changes in mammary blood flow and extraction of glucose and oxygen during late pregnancy and lactation, and measure the relation–ship between MBF and milk production in early- and late-lactation.

## MATERIALS AND METHODS

### Animals

Four adult, multiparous twin-bearing Romney ewes were housed in metabolism crates from day 65 of pregnancy and fed a lucerne-pellet diet at a changing intake calculated to maintain maternal body weight throughout the experimental period (Williams and Butt, 1989). Food was offered at hourly intervals by an automatic feeder. On about day 100 of gestation, the sheep were fitted with catheters in the mesenteric (2 sites), portal and hepatic veins, and in the femoral artery as described by Lobley *et al*. (1995), for use in a larger experiment that investigated the partitioning of cysteine between the mammary gland and skin of pregnant and lactating sheep. At the same time that the catheters were inserted, a transit time ultrasonic flow probe (Transonics Inc., Ithaca, NY) was fitted around the external pudic artery for measurement of MBF. A jugular catheter was inserted on about day 125 of pregnancy for continuous infusion of either cysteine (2 g/day) or saline. The data presented here are pooled for the four animals as no differences were associated with the cysteine vs saline infusion. All ewes delivered and suckled twin lambs.

### Blood collection and analyses

Blood samples were collected to determine arterio-venous differences across the mammary gland for glucose and oxygen. Each sample of arterial blood was collected from the catheter by peristaltic pump over a period of 30-45 minutes into tubes on ice containing 0.1 mL saturated $Na_2EDTA$. An intravenous injection of 5000 IU of heparin ensured that the blood did not clot during the collection period. Venous blood was collected from the superficial epigastric vein by venipuncture. Some of the animals had distinct branching of the mammary veins, so blood was sampled as close to the gland as possible at a major vein. Blood plasma was harvested by centrifugation and stored at -85°C. The concentration of oxygen in whole blood was determined immediately after collection using a galvanic oxygen cell (Grub and Mills, 1981) against air standards, while plasma glucose was determined by an enzymatic Glucose PAP kit using a Cobas Fara II autoanalyser (Roche, Basle, Switzerland) according to the manufacturer's guidelines. The percentage extraction (%extraction) of glucose and oxygen by the mammary gland was calculated by (A-V)/A*100, where A and V are the arterial and venous concentrations of glucose or oxygen.

### Mammary volume and milk production

A daily measurement of mammary volume was obtained during the final 5 days before parturition. A glass container was filled with warm water and placed around the

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udder, and mammary volume was measured by displacement. Milk production was measured in early lactation (between day 11 and 15) and late lactation (between day 52 and 57) by hand-milking the sheep and injecting oxytocin intravenously to ensure complete milk removal. On the day of measurement, the lambs were removed from their mothers for about 8 hours and the ewes were milked at intervals of about 1.5 hours. The average milk production expressed per minute was multiplied by 1440 to estimate daily milk production.

### Statistical analyses

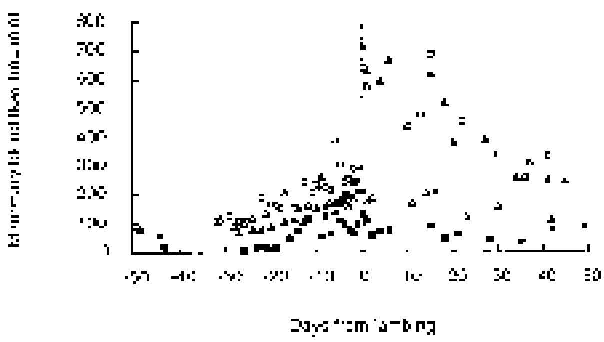
Data was subjected to ANOVA with time (i.e. stage of pregnancy and lactation) as the main factor, using the computer package SAS (1985).

## RESULTS

### Mammary blood flow

Mammary blood flow (MBF) increased steadily from 50 days before lambing until parturition ($D_L$) although, at any one time, there were differences of up to 3-fold in MBF between individual animals (Figure 1). In two of the sheep (no.'s 857 and 836), there was a large increase in MBF on $D_L$ from about 280 to over 600 mL/min. The remaining two sheep (no.'s 885 and 161) did not exhibit such a marked increase in MBF at $D_L$ and their maximum rate of flow was attained about 10 days before $D_L$. During lactation, the MBF of all sheep tended to remain above 80% of peak flow rate for about 15 days, before it declined steadily over the next 20 days.

**FIGURE 1:** Changes in blood flow to one half of the mammary gland during late pregnancy and lactation. Symbols used for individual sheep referred to in the text are: ▲ 857; □ 836; △ 885; ■ 161.



### Metabolic changes in the mammary gland during late pregnancy and lactation

The extraction of glucose by the mammary gland did not change during pregnancy (Table 1) but on $D_L$ it trebled from 6.0 ± 1.07 to 18.7 ± 2.74% (P<0.01). Glucose extraction remained at an elevated level during the second week of lactation (26.6 ± 3.60%) but declined to 16.7 ± 4.88% (P<0.05) by late lactation. Oxygen extraction doubled during late pregnancy, from 23.1 ± 7.98% at 2-3 weeks before lambing to an average of 42.2 ± 2.52% during the last 5 days of pregnancy (P<0.05). On $D_L$ oxygen extraction decreased by more than half to 17.6 ± 3.06% (P<0.05) before increasing (P<0.05) during lactation to reach 38.1 ± 7.61% in early lactation (Table 1).

**TABLE 1:** The proportion of arterial glucose and oxygen removed by the mammary gland (% extraction) during late pregnancy and lactation. Values are least squares means (n = 4). SED = standard error of difference between means. $D_L$ = day of lambing.

| | Days from lambing | Glucose % extraction | Oxygen % extraction |
|---|---|---|---|
| Late pregnancy | -14 to -22 | 4.6 | 23.1 |
| Pre-lambing | -5 | 7.7 | 40.4 |
| | -4 | 7.2 | 41.4 |
| | -3 | 3.8 | 43.0 |
| | -2 | 7.8 | 40.3 |
| | -1 | 6.0 | 45.7 |
| | $D_L$ | 18.7 | 17.6 |
| Early lactation | 11 to 15 | 26.6 | 38.1 |
| Late Lactation | 52 to 57 | 16.7 | 26.6 |
| SED | | 2.58 | 5.84 |

### Milk production and volume of mammary gland

During early lactation (days 11-15) the mean rate of milk production was 2060 ± 174 g/day, ranging from 1750 to 2470 g/day. Milk production of all sheep declined by 33-87% in late lactation (days 52-57). Using data for all sheep pooled across early and late lactation, milk production was linearly related to both glucose and oxygen uptake, according to the following equations:

milk production (g/day) = -54.8 + (67.4 * glucose % extraction)
$R^2 = 0.64$ … (1)

milk production (g/day) = 123.6 + (39.5 * oxygen % extraction)
$R^2 = 0.55$ … (2)

During the final 5 days of pregnancy, the volume of the mammary gland increased steadily (P<0.05) at a rate of 122 mL/day, reaching an average volume of 2160 mL on $D_L$ (Figure 2).

**FIGURE 2:** The mean (± SE) volume of the mammary gland of sheep (n = 4) during the final 5 days of pregnancy and on the day of lambing.

## DISCUSSION

The pattern of change in mammary blood flow (MBF) differed considerably between animals in this study. There were two distinct patterns measured; either a 2-fold increase on the day of lambing ($D_L$) for two of the sheep (no.'s 857 and 836), or virtually no increase associated with parturition in the other two sheep (no.'s 885 and 161). An increase in MBF of similar magnitude to that attained for the two sheep that showed the marked response on $D_L$ is consistent with that found in goats (Davis *et al.*, 1979; Nielsen *et al.*, 1995). This raises a question of the validity of MBF measurements made on the two sheep that did not show a marked response in MBF on $D_L$. The blood flow probes were positioned around the left external pudic artery as the vessel entered the mammary gland, and post-mortem examination showed that each probe had remained around a functional vessel. However, the orientation of the probe relative to the vessel may have altered with time, hence altering the transit time dynamics to give underestimates of true flow. If flow was compromised by the probe, blood flow to the entire gland may have been supported by the development of a collateral arterial supply. Arterial flow may also have been perturbed by taking mammary venous samples by venipuncture, although the acceptable values for glucose and oxygen extraction (see below) would suggest that this was not a major problem.

While accepting that the probe values may be underestimates of true MBF for all sheep, the ratios of MBF: milk production do not eliminate the lower MBF measurements as biologically unreasonable. In early lactation, MBF : milk production ranged from 138:1 to 310:1 which is below the generally accepted 300-400:1 for lactating ruminants (Linzell, 1974). Nielsen *et al.* (1990), however, found that the ratio in goats varied from 123:1 to 680:1. MBF : milk production for all sheep increased by 2 to 4 times by late lactation (range 249:1 to 748:1), supporting the finding of Nielsen *et al.* (1990) that the ratio increases as milk yield declines.

The marked increase in glucose extraction that occurred on $D_L$ agrees with the pattern of change measured by Davis *et al.* (1979) in the goat. A sudden increase in glucose extraction reflects the onset of the second stage in lactogenesis (Fleet *et al.*, 1975) that occurs very close to parturition. The increase in volume of the mammary gland (Figure 1) during the last 5 days of pregnancy occurred before the marked increase in glucose uptake and probably reflects both the accumulation of colostrum, which was evident in all sheep at least 5 days before lambing, and the proliferation of mammary tissue. The high extraction of oxygen during the week prior to lambing may have reflected the energy required for the proliferation of mammary tissue and the relatively low MBF.

In conclusion, the results showed that in the final week of pregnancy, gland volume increased steadily and significant metabolic changes occurred in the mammary gland as indicated by a 2-fold increase in oxygen extraction. The marked increase in glucose extraction by the gland occurred later than the increase in oxygen extraction, i.e. at $D_L$, and was accompanied by up to a 2-3 fold increase in MBF. Milk production was closely correlated with both glucose and oxygen uptake by the gland in both early and late lactation.

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