Effects of mammary engorgement and feed withdrawal on microvascular function in lactating goat mammary glands

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Farr, Vicki C., Colin G. Prosser, and Stephen R. Davis. Effects of mammary engorgement and feed withdrawal on microvascular function in lactating goat mammary glands. Am J Physiol Heart Circ Physiol 279: H1813–H1818, 2000.—The responses of the mammary microvasculature in lactating goats (n = 8) during feed withdrawal (18–20 h) and mammary engorgement (26–28 h of milk accumulation) were compared using an indicator-dilution technique with FITC-albumin and [14C]sucrose as the intravascular and diffusible indicators, respectively. Feed withdrawal and mammary engorgement caused a 50–60% decrease in mammary arterial blood flow (MBF) and milk yield (MY) (17). A large part of this relationship can be attributed to variation of udder mass. A larger mass of secretory tissue will not only produce more milk (16) but will also have a greater blood supply. However, in ruminants and nonruminants, variation in MBF can occur as part of the regulatory response to milking frequency, milk accumulation, or feed availability.

During established lactation in goats, frequent (every 1–2 h) milking with oxytocin increased MBF in conjunction with an increase in MY (22). Conversely, MY and MBF decreased during prolonged (>24 h) periods of milk accumulation in goats (8, 28) and rats (12) and in response to short-term feed withdrawal (FW) in ruminants (4, 15) and rats (14, 29).

The mechanisms leading to the reduction in blood flow in response to FW or milk accumulation have not been defined. The effects of FW are likely to be due to a systemic mechanism coordinating feed intake and milk output. This mechanism is associated with a reduction in cardiac output and a reduction in MBF as a proportion of cardiac output (1, 4). In contrast, the reduction in MBF in response to milk accumulation will likely involve a local (intramammary) pathway, which downregulates MBF in association with the decline in milk output. In ruminants, this occurs 16–20 h after milking (28). In the rat, the endocrine response to suckling also appears to be important to the maintenance of MBF (12).

Measurement of gross changes of flow in the mammary arteries is not necessarily informative to changes in mammary function or to changes occurring in the mammary capillary bed. Certainly, there were experimental situations where MBF was maintained during the blockade of milk secretion (13). Thus MBF and milk secretion can be “uncoupled.”

In experiments where MBF was deliberately increased by close-arterial infusion of a variety of vasodilators and there was no MY response, the result was impossible to interpret without knowledge of the associated changes in substrate delivery to the secretory epithelial cell at the level of the capillary (24). Thus, when blood flow was increased artificially, it was important to know whether “nutritive” flow to the udder was increased or whether any increment in flow represented increased throughput through nonnutritive channels. Much more information is required if an understanding is ever to be achieved as to how, and under what circumstances, MBF can regulate milk synthesis.

The lactating ruminant mammary gland is readily suited to the type of study described here, because there is a single accessible arterial inflow and a simple venous drainage, a major part of which is through the superficial epigastric vein across the abdominal surface (17). The benefit of this anatomy is that the indi-

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METHODS

Animal care and surgical preparation. This study was undertaken with the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand). A total of 10 lactating Saanen goats (8 goats in the first experiment, and 3 of these plus 2 new goats in the second experiment) in mid to late pregnancy were milked twice daily at 0730 and 1530 h. Goats were surgically prepared, as described in (23), with a blood flow probe (6 mm, Transonics Systems, Ithaca, NY) around the external pudic artery of one gland and an indwelling catheter. Briefly, anesthesia was induced with 5% Thiivet (C-Vet Veterinary Products, Lancashire, UK) injected via the jugular vein and maintained with a halothane-oxygen mixture via an endotracheal tube.

The external pudic artery was catheterized by inserting a length of polyvinyl tubing, 1.0 mm outer diameter (OD) and 0.5 mm inner diameter (ID), through a small arterial branch until the tip entered the lumen of the main artery, downstream of the blood flow probe. Intermittent arterial occlusion between the udder halves was achieved by tying off and cauterizing both the external pudic arteries. The catheter was exteriorized at the rear and held in a pouch above the udder attached to the animals by a harness. Surgery was performed at least 2 wk before experimentation.

Experimental procedure. On the day before measurements were made, a catheter (1.1 mm ID, 1.7 mm OD; Braun Cavafix, Braun Melsungen, Melsungen, Germany) was inserted into a superficial epigastric (mammary) vein of eight of the prepared goats.

In the first experiment, treatments were applied in random order and consisted of control animals milked twice daily and fed; animals with 18–20 h of FW; and animals with 26–28 h of milk accumulation, which achieved a condition we termed mammary engorgement (ME). In reality, although the udder is full of milk after about 25 h (28), some milk synthesis and secretion is maintained.

In the second experiment, five goats were also studied post-ME within 40 min of milking (with oxytocin) after the ME treatment. MBF was monitored continuously throughout each experiment via a flowmeter (model T108, Transonics Systems).

Indicator-dilution technique. The dual indicator-dilution technique involved a single injection of a mixture of two indicators into the pudic artery while continuously sampling the superficial epigastric venous outflow for up to 1 min after the injection. The two indicators employed were FITC-albumin (1.5 mg; Sigma Chemical, St. Louis, MO), the nondiffusible indicator, and [14C]sucrose (1.5 μCi; Amersham, Buckinghamshire, UK), which is freely diffusible across the capillary endothelium. The FITC-albumin was washed with saline three times before injection through a 30-kDa microconcentrator (Amicon, Beverly, MA) to remove unconjugated FITC.

To ensure that the indicators were delivered as a single pulse, 0.1 ml of the indicator mixture (in isotonic saline) was preloaded in a length of polyvinyl catheter tubing (1 mm OD and 0.5 mm ID) attached to the indwelling external pudic artery catheter. At time 0, the bolus of indicators was flushed rapidly into the artery with 3 ml of saline. Simultaneously, venous blood was pumped from the mammary vein at 10 ml/min, and fractions were collected at 2-s intervals. Heparin (20 IU/ml plasma; Bomac Laboratories, Manukau City, New Zealand) was added continuously (1 ml/min) during blood collection. Plasma was prepared by centrifugation at 2,000 g for the determination of indicator concentrations, and corrections were made for dilution with the heparin solution.

Fluorescent intensity in the venous plasma was measured using a Bio-Tek PL5000 fluorescence microplate reader (Bio-Tek Instruments, Winooski, VT) at 480 and 520 nm excitation and emission wavelengths, respectively. The amount of [14C]sucrose recovered in the plasma was measured using a Wallac 1409 Scintillation Counter (Wallac Oy, Turku, Finland). The indicator concentration was expressed as a percentage of the initial dose into the external pudic artery.

Calculations. Extraction ratios [E(t)] were determined for each time point during the dual-indicator washout curve (3)

\[ E(t) = \frac{C_{AI}(t) - C_{Bl}(t)}{C_{AI}(t)} \] (1)

where \( C_A \) and \( C_B \) are the normalized concentrations of FITC-albumin and [14C]sucrose, respectively, in the mammary vein and \( t \) is mean transit time. Extraction \( E \) of the diffusible indicator, [14C]sucrose, was calculated from the mean of the extraction ratios on the upstroke of the dual-indicator washout curves, i.e., until \( t = 10 \) s postinjection (3, 26).

Mammary tissue weight was estimated from milk production in the week preceding the experiment on the basis that, on average in goats, 1 g of mammary tissue produces 1.7 ml of milk/day (16).

MBF was determined from the area under the FITC-albumin curve corrected for any recirculation of indicator by extrapolation of the semilog plot of the downslope (as described by Ref. 9). Where comparisons were made with transit-time flow probe measurements, average flow over the collection period was used.

The permeability-surface area product of the capillaries (PS; measured in ml·100 g tissue \(^{-1}\)·min\(^{-1}\)) was calculated from the equation (3)

\[ PS = -\frac{F}{E} \log_e (1 - E) \] (2)

E is the mean relative extraction of sucrose (see above) up to 10 s postinjection before back diffusion of sucrose (see Fig. 2), and \( F \) is the MBF (calculated from the FITC-albumin curve) (expressed as ml·100 g tissue \(^{-1}\)·min\(^{-1}\)).

The mean transit time of the intravascular and diffusible indicators was calculated as described by Goresky (9). The intravascular distribution of the indicator was calculated as the product of flow and the intravascular transit time, and the extravascular (sucrose-accessible space) distribution of sucrose was calculated as the product of flow and the difference between intravascular and diffusible indicator transit times, respectively (9).

Statistical analyses. Data are presented as means ± SE or means ± SE of the difference (SED). Comparisons among
treatments were made by ANOVA, fitting the treatment and goat effects.

RESULTS

ME and FW reduced MBF by about half relative to the control treatment (Table 1). Peak concentrations of the intravascular indicator were attained 10–12 s after injection in control and ME goats but later in FW goats, at 15–20 s (Fig. 1). Extraction of the diffusible indicator was constant for about the first 10 s after indicator injection but declined thereafter for all treatments, although more slowly for the FW treatment (Fig. 2). Extraction of the diffusible indicator was lower \((P < 0.05)\) with ME treatment relative to both FW and control goats (Table 1 and Fig. 2) up to the time of peak concentration of the intravascular indicator. There was a net positive reentry of diffusible indicator \(\text{[when } E(t) \text{ becomes negative]}\) after 25.3 and 29 s for control and ME treatments, respectively \((\text{SED } = 4.17, P > 0.05)\), whereas this did not occur until after 49 s for the FW treatment \((\text{SED } = 4.17, P < 0.01; \text{Fig. 2})\).

The effects of recirculation of indicators on the respective decay curves were very small. This is based on an independent estimation of recirculation time after injection of indicators into the mammary vein, which indicated that recirculation times were 20–25 s in control and ME goats but >45 s in FW goats (data not shown). No perturbations in decay curves suggestive of significant recirculation of indicator were obvious at these time points.

Mean transit time did not differ between ME and control treatments but was almost doubled after FW treatment (Table 1; \(P < 0.01\)). Changes in \(PS\) values were similar to those seen with MBF, i.e., \(PS\) values for ME and FW treatments were approximately half those of the control treatment (Table 1).

After five of the goats were milked out after the ME treatment, mammary arterial flow, sucrose extraction, sucrose-accessible space, and \(PS\) values were not significantly different from those during ME (data not shown). However, there was a small but significant increase in mean transit time during post-ME from 19.5 to 22.5 s \((\text{SED}; P < 0.05)\).

MBF measured using transit time blood flow probes gave a blood flow-to-MY ratio of 277 ± 36 (SE) for the eight goats during control conditions. Calculation of MBF from the initial dose of FITC-albumin and the area under the FITC-albumin concentration curve (cor-

Table 1. Effects of mammary engorgement and feed withdrawal in lactating goats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Mammary Engorgement</th>
<th>Feed Withdrawal</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary arterial flow, ml·100 g tissue⁻¹·min⁻¹</td>
<td>50.3*</td>
<td>32.0†</td>
<td>26.8†</td>
<td>7.6</td>
</tr>
<tr>
<td>Mean transit time, s</td>
<td>17.3*</td>
<td>20.7†</td>
<td>30.9†</td>
<td>2.2</td>
</tr>
<tr>
<td>Vascular volume, ml</td>
<td>92*</td>
<td>44†</td>
<td>77*</td>
<td>13</td>
</tr>
<tr>
<td>Vascular volume, ml</td>
<td>100</td>
<td>78</td>
<td>76</td>
<td>13</td>
</tr>
<tr>
<td>Vascular volume, ml</td>
<td>0.63*</td>
<td>0.51†</td>
<td>0.65*</td>
<td>0.05</td>
</tr>
<tr>
<td>Permeability-surface area product, ml·100 g tissue⁻¹·min⁻¹</td>
<td>50.4*</td>
<td>25.3†</td>
<td>27.9†</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Values are means ± SE and means ± SE of the difference (SED); \(n = 8\). The effects of mammary engorgement and feed withdrawal in lactating goats on parameters of the mammary microvasculature were determined using the indicator-dilution technique. Arterial blood flow was calculated from the area under the FITC-albumin curve, and extraction of the diffusible indicator was calculated from the mean of the extraction ratios up to 10 s postinjection. 

Fig. 1. Outflow indicator-dilution curves for FITC-albumin and \([^{14}C]\)sucrose after injection into the external pudic arterial inflow of lactating goat udders. Curves are averages for 8 goats under control conditions (fed and milked twice daily) (A), after mammary engorgement (ME, 26–28 h of milk accumulation) (B), and after feed withdrawal (FW, 18–20 h) (C). Bars represent means ± SE.
were applied in random order to each of 8 goats. Bars are pooled SE resulted in:

In a pilot study with three goats, we induced a functional hyperemia (doubling blood flow) in the udder that this in itself results in impaired milk production.

mammary gland is flow limited at low flow rates and more, the synthetic ability of the udder does not appear to be impaired by short-term FW because the in vitro production of lactose by rodent and ruminant mammary cells after short-term FW was similar to those of cells from fed animals (5, 30).

Blood flow to the udder is coordinated with feed intake, and it is well established that short-term (24 h) FW results in MBF being reduced by ~50% in ruminants (4, 15) and, similarly, after ~8 h of FW in rodents (14). In ruminants, this reduction in MBF is associated with a reduction in cardiac output but no change in blood pressure (1, 4). The MBF response was similar in denervated and intact glands, suggesting that the regulation is humorally mediated (1). Furthermore, the synthetic ability of the udder does not appear to be impaired by short-term FW because the in vitro production of lactose by rodent and ruminant mammary cells after short-term FW was similar to those of cells from fed animals (5, 30).

It is likely that the uptake of substrates by the mammary gland is flow limited at low flow rates and that this in itself results in impaired milk production. In a pilot study with three goats, we induced a functional hyperemia (doubling blood flow) in the udder after epinephrine infusion. We could find no evidence that this change in blood flow altered sucrose extraction (unpublished data).

Part of the pathway coordinating MBF with FW involves increased vascular resistance within the udder effected via the humoral mechanism, because MBF not only falls but also becomes a smaller proportion of cardiac output (4). Because our observations suggest that the mammary vascular bed remains open during FW, it is possible that the humoral effect is directly on the arteriolar resistance vessels. However, the nature of the effector remains to be determined; it is possible that a gut peptide sensitive to nutrient flow in the intestine is involved. To our knowledge there have been no systematic examinations of the vasoactive actions of gut peptides in the mammary gland.

There are significant differences between rodents and ruminants in the response of the mammary gland to FW. FW in rodents, in contrast to ruminants, almost abolishes mammary glucose extraction. Refeeding rats leads to a rapid (15–30 min) recovery in mammary glucose extraction, mediated, in part, by insulin (14). MBF recovers more slowly, over a period of 3–5 h (14). The ruminant mammary gland shows little change in glucose extraction after FW (15), and the MBF response to refeeding is relatively slow, taking 10–12 h (4).

During milk accumulation in goats, the udder becomes full of milk at ~25 h postmilking, whereas MBF begins to decline at 16–20 h, along with the rate of milk secretion (28).

Despite decreased arterial blood flow associated with the ME treatment, mean transit time was not different from that of control goats, suggesting that at the lower flow rates associated with ME a smaller number of capillaries were perfused. Hence, the decrease in PS product values with this treatment was attributable to a fall in the capillary surface area available for exchange. Thus, whereas overall flow through the udder was substantially reduced, the velocity of plasma flow in capillaries would be maintained, albeit in a smaller number of patent capillaries. Because the surface area is largely a reflection of the number of capillaries available for perfusion, the most likely explanation for the observed results is that ME results in capillary derecruitment within the mammary gland. The observed change in sucrose extraction with ME was probably the result of change in the proportion of nutritive to nonnutritive flow in the udder. Thus, with closure of the capillary bed in the secretory tissue, flow in less metabolically active tissues, such as the skin, may assume a greater proportion of total MBF.

Silver (27) found evidence of capillary derecruitment during ME in rodents, suggesting that the mammary gland is capable of intrinsic control of the delivery of substrates to its secretory cells, probably by changes in the balance of locally produced vasoactive compounds. Certainly, extended periods of milk accumulation (in contrast to FW) can result in reduced rates of lactose synthesis of ruminant mammary tissue in vitro (20), implying that the metabolic capability of the udder is...
impaired by ME but not FW. In support of this, periods of milk accumulation up to 24 h also result in a reduction of the activities of key mammary enzymes (7, 11). Again, it appears that there are differences in regulation between rodents and ruminants in that MBF in rodents appears to be sensitive to the suckling stimulus rather than milk removal per se, at least in the first 24 h (12). The removal of pups from lactating rats also results in a significant reduction in cardiac output (12).

When milk was removed from the engorged gland, MBF, PS values, sucrose extraction, and sucrose-accessible space did not change from those measured during ME, suggesting that the local effects of milk accumulating in the mammary gland for extended periods were not reversed immediately. This observation is consistent with data in both goats (21) and rats (12), which showed that milk accumulation (and the associated changes in intramammary pressure) does not result in any physical restriction to MBF. However, there was a statistically significant, albeit small, increase in mean transit time, suggestive of a partial recruitment of capillaries, when the milk was removed. Nevertheless, the nature of the local regulatory pathway for capillary recruitment (and derecruitment) within the mammary gland remains to be defined but may be linked to changes in metabolic capability of the mammary tissue linked to local production/clearance of vasoactive agents. The mammary gland produces a variety of vasoactive agents, including parathyroid hormone-related protein, prostacyclin, and nitric oxide (see Ref. 24 for review), the output of which could be linked to mammary metabolism.

ME also reduced the sucrose-accessible space by about half, an observation that may be due to alveolar distension into the extracellular space, although the sucrose-accessible space did not recover in the period immediately after milking. The sucrose-accessible space was closely and positively correlated to MBF among the goats during control conditions ($r^2 = 0.61$, $n = 10$).

The microvascular architecture of the mammary gland has been investigated in pregnant and lactating mice. The capillary type is continuous, with some fenestrations (19), suggesting the possibility of significant variation among capillaries in solute transfer because solute movement across capillaries is restricted in continuous versus fenestrated capillaries (25). Indeed, differences in mammary capillary permeability to ferritin have been noted to occur with changes in physiological state (18), such changes being linked to the prevalence of pinocytotic vesicles in the endothelium (31). Many questions remain as to whether and how mammary function can be regulated by the endothelial barrier. For example, the barrier may be a significant impediment to the uptake of key hormones regulating mammary function. Certainly, insulin resistance may be associated, in part, with decreased insulin delivery across the vascular endothelium (2).

The values for MBF calculated in this study were similar, relative to milk production, to those determined by Linzell (17), who used a thermodilution method to measure blood flow-to-MY ratios of 500:1 at peak lactation, which rose slowly as lactation progressed. However, there was a substantial difference between this value and the value calculated from the flow probe measurements (277:1). The reason for the discrepancy was probably systematic error in the probe measurement because of misalignment of the probe with the external pudic artery. Perfect alignment of the probe (perpendicular to the artery) on the external pudic artery is difficult to achieve (and maintain), and deviations in alignment usually result in reduced flow values (see Ref. 10). However, this error has little bearing on the results or the interpretation of the results, given that comparisons were made among treatments within goats.

In conclusion, two physiological manipulations (FW and ME), which resulted in MBF to the udder being approximately halved, caused contrasting responses in the mammary capillary bed. FW appears to be associated with slowing of flow through the capillaries, whereas ME resulted in capillary derecruitment. The lactating goat provides an excellent model for study of systemic and local control mechanisms regulating flow in the capillary bed, although the nature of these mechanisms remains to be defined.

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Preliminary and partial data from this study were presented previously in Ref. 6.

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