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Evolution of the mammary capillary network and carbonic anhydrase activity throughout lactation and during somatotropin treatment in goats

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During the normal course of lactation, mammary metabolic activity and blood flow are closely correlated. Six lactating goats were used in this experiment to test the hypothesis that the capillary network and the capillary enzyme, carbonic anhydrase (CA; EC 4.2.1.1) are important regulatory factors involved in the coordination of mammary blood flow (MBF) and metabolic activity. Milk vein blood velocity was determined as a measure of MBF, and fine needle mammary biopsies were obtained at different time points during lactation and by the end of a 14-d bovine somatotropin (BST) treatment initiated 3 months post partum. In mammary sections, CA activity was determined histochemically and alveolar and capillary structures by image analyses upon azure blue staining. In early lactation, alveoli were large and surrounded by many small capillaries with high CA activity. As lactation progressed, capillaries almost tripled in size, whereas number of capillaries surrounding each alveolus decreased by 1/3, and CA activity more than halved. BST treatment did not affect capillary traits but increased number of alveoli in mammary sections, and BST thus appeared to be targeted mostly towards the mammary epithelial cell. Milk vein blood velocity decreased over the course of lactation, when capillary area markedly increased, suggesting that control of mammary blood perfusion is not at the level of the capillary itself, but at pre- or post-capillary sites. We suggest that the observed changes in capillary diameter and CA activity with progressing lactation contributes to reduce efficiency of nutrient and waste product exchange across the capillary-mammary epithelial cell barrier, and this could be an important factor in regulation of mammary (epithelial cell) metabolic activity and lactation performance.

Keywords: Alveolus, milk vein blood velocity, somatotropin.

During the peripartum period, mammary parenchyma undergoes intensive growth and remodelling (Knight & Wilde, 1993) and the vascular system is expanded to form a dense network of capillaries around the secretory and myoepithelial cells (Cvek et al. 1998). As milk production ceases in late lactation, both secretory tissue (Anderson et al. 1981) and the capillary network regress (Tatarczuch et al. 1997; Cvek et al. 1998). Mammary blood flow increases markedly at the onset of lactation, and in ruminants mammary blood flow is closely correlated to milk yield during lactation and somatotropin treatment (Linzell, 1974; Nielsen et al. 1995). Milk production and hence metabolic activity of the mammary gland may therefore be closely related to development and functionality of the mammary microvascular system. Metabolic activity of the mammary epithelial cells is associated with release of CO_2 in proportion to milk production (Guinard-Flament et al. 2007). The local blood perfusion is important for provision of nutrients and for elimination of metabolic waste products, and it can be regulated in accordance with metabolic activity in body tissues through release of vasoactive substances (Prosser et al. 1996) such as vasodilatory CO_2. Nielsen et al. (1995) demonstrated that changes in mammary metabolic activity and CO_2 production during lactation in dairy goats were reflected in changes in HCO_3^- in the venous blood leaving the mammary gland. CO_2 is

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Table 1. Schedule for biopsy sampling, milk vein blood velocity (MBV) measurements, and bovine somatotropin (BST) treatment

Period†	Month of lactation	Time in relation to BST treatment
EL	~1	
ML1	~2½	Day before initiation of BST treatment (pre-treatment control)
BST	~3	1 d after cessation of the 14 d BST treatment period
ML2	~3½	2 weeks after cessation of BST treatment (post-treatment control)
LL	7	
D	9	

†EL: Early lactation, ML1: Mid lactation prior to BST treatment, BST: mid lactation by the end of a 14-d BST treatment period, ML2: mid lactation 14 d after cessation of BST treatment, LL: Late lactation, D: Late lactation just before drying-off

converted to HCO_3^- in a reversible reaction: $CO_2+H_2O\leftrightarrow H_2CO_3\leftrightarrow H^++HCO_3^-$, catalysed by the enzyme carbonic anhydrase (CA; EC 4.2.1.1).

Carbonic anhydrase is widely distributed in mammalian tissues, and is involved in local regulation of pH in body tissues and in the diffusion of CO_2 from cell to blood. There are 16 known isozymes of CA, of which some are soluble, some are membrane bound and some are secreted (Krishnamurthy et al. 2008; Supuran, 2008). In the lactating goat mammary gland, CA is located exclusively in the capillary membranes (Cvek et al. 1998) and is most likely identical to the membrane bound isozyme CA IV, as in capillaries of other organs (Ridderstråle, 1976, 1991). In lactating goats, CA can thus be used as a capillary marker (Cvek, 1997).

Cvek et al. (1998) followed goats in the peripartum period and found that when one mammary gland was dried off before parturition, CA activity gradually disappeared in the gland not milked, but remained unchanged in the milked gland. This observation confirms that CA activity in mammary gland is closely related to milk production. The number of mammary capillaries stained for CA changed during lactogenesis, and CA activity increased in parallel to the rise in milk production. The authors therefore suggested that CA is a prerequisite for milk secretory processes rather than for tissue growth, since CA staining was scarce during periods with extensive secretory tissue growth (i.e. during the dry period).

We hypothesized that the capillary network and the capillary enzyme CA are important regulatory factors involved in coordinating mammary blood flow according to changes in mammary metabolic activity and hence milk yield during established lactation. The main objective of the present study was to study morphological changes in the capillary network and CA activity during lactation and after induction of changes in mammary metabolic activity by 14 d of bovine somatotropin (BST) treatment in mid-lactation, and to relate these to changes in mammary blood flow.

Materials and Methods

Experimental animals and feeding

All animal experimental procedures were conducted under protocols approved by the Danish Animal Experiments Inspectorate and complied with the Danish Ministry of Justice Law no. 382 (10 June 1987) and Acts 739 (6 December 1988) and 333 (19 May 1990) concerning animal experimentation and care of experimental animals. Six Danish Landrace goats (parity 3–5) were used. They were surgically prepared with both superficial epigastric caudal veins (milk veins) exteriorized in skin-covered loops. Goats were housed in individual pens, fed hay ad libitum and concentrate (barley, molasses and a commercial mix) according to Danish feeding standards and had free access to water. They were fed and milked twice daily at approx. 8.15 and 17.00, half the concentrate and hay ration being given at each milking.

Experimental procedures

The experiment was conducted during months 1–9 of lactation, and none of the goats were pregnant. As shown in Table 1, recordings and samplings were performed at six different time points during lactation: in early lactation (EL; between weeks 3 and 6 for individual goats), mid-lactation (ML1; between weeks 11 and 16 for individual goats; BST treatment was initiated the day after this biopsy sampling), by the end of a 14-d period of BST treatment (BST), 14 d after cessation of the BST treatment (ML2), late lactation (LL, between weeks 28 and 31 for individual goats) and by the end of lactation when milk yield dropped to <1 kg/d and they were about to be dried off (D; between weeks 37 and 39 for individual goats).

BST treatment consisted of daily subcutaneous injections for 14 d of 10 mg Somidobove® (Lot no. 505 ALO, Lilly Research Centre Ltd., Windlesham, Surrey, GU20 6PH, UK) dissolved in saline. Injections were given subcutaneously in the shoulder region at 9.30. BST treatment was initiated the day after recordings and samplings in period ML1.

Cross-sectional areas of milk veins remain constant during lactation in multiparous goats, and changes in milk vein blood velocity (MBV) were determined as a measure of changes in mammary blood flow (MBF) (Christensen et al. 1989). MBV was determined in both milk veins in the morning (approx. 09.00) and afternoon (approx. 14.00) by the ultrasound Doppler technique. Averages of these four measurements were used in the statistical analyses. Vena pudenda externa was manually clamped during

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MBV measurements to reduce risk of drainage of blood from non-mammary origin through the milk veins.

Two mammary biopsies were collected from each mammary gland the day after MBV measurements were performed. Biopsies were sampled from the same spot on the mammary gland, approx. 6–8 cm from the base of the udder and approx. 2–3 cm from the midline at the point where the gland protrudes the most caudally. Biopsies in the BST period were collected the day after cessation of BST treatment. Prior to biopsy sampling, the mammary gland was milked, the biopsy area was disinfected and locally anaesthetized by subcutaneous administration of 20 mg lidocainhydrochloride (Lidokain, Sygehus apotekerne i Danmark, Denmark). Fine-needle biopsies were sampled at a depth of 2–3 cm with a 17G biopsy needle using a biopsy gun (Biopty®, Radiplast, 750 07 Uppsala, Sweden). Each collected biopsy was approximately 10–15 mm^3. After biopsy sampling, the site of incision was disinfected and sealed with a wound spray, and any accumulated blood removed by milking. Biopsies were immediately frozen in liquid N_2 and stored at −80 °C pending analyses.

Level of milk production in each sampling period was expressed as average milk yield over the last 3 d prior to biopsy sampling.

Histological analyses

Biopsies were fixed in phosphate-buffered 2·5% glutaraldehyde (pH 7·2) for 6 h, subsequently rinsed in phosphate buffer (pH 7·2) and dehydrated through graded ethanols and embedded in water-soluble resin (Historesin®, Leica Instr., 69115–69126 Heidelberg, Germany). CA was localized using the cobalt precipitation method of resin embedded tissue described by Ridderstråle (1976, 1991), which results in a black precipitate at sites of enzyme activity, including all CA isozymes. From each biopsy, 2-μm thick sections were cut and subsequently incubated for 6 min floating on the surface of an incubating medium containing 157 mM-$NaHCO_3$, 3·5 mM-$CoSO_4$, 11·7 mM-KH_2PO_4, and 52·6 mM-H_2SO_4. After incubation, the sections were rinsed in 0·67 mM-phosphate buffer (pH 5·9) for 1 min, treated with 0·5% $(NH_4)_2S$ for 3 min, and finally rinsed in two successive baths of distilled water, 1 min in each. Controls were run with the specific CA inhibitor acetazolamide (10^{-5} M) in the incubation medium. Prior to mounting, some sections were weakly counter-stained with azure blue to stain alveoli for image analyses.

Image analysis

Photomicrographs were taken of the azure blue counter-stained sections using the Nikon Microphot-FXA imaging system, and image analysis performed using the TEMA image analysis data software (Bio-Rad Scan Beam A/S, 9560 Hadsund, Denmark). The images analysed were placed successively to cover the entire biopsy but avoiding the disrupted structures at the edges of the biopsy. Azure blue stains the different structures in the mammary gland. Thereby the alveoli could be outlined in the computer. The blackening of the capillaries and the azure blue staining were used to outline the capillaries for calculation of capillary area. The capillaries were manually marked with an individual click of the computer mouse on each capillary. The image analysis program then calculated the number of capillaries and alveoli, capillaries per alveolus and mean alveolar and capillary area (μm^2). The mean capillary area was calculated by the program as total area covered by capillaries in the image divided by number of capillaries. At least 40 alveoli were analysed from each section, except in a few biopsies containing less secretory tissue. The procedure is described in detail by Cvek et al. (1998). Capillary membranes were detected by the black staining of CA. Blackened areas were assigned a pixel value between 0 and 256, which increased with decreasing level of blackening, i.e. the higher the pixel value, the lower the CA activity. To avoid any bias in the analysis of the sections, the identities of the individual slides were not disclosed until all the sections had been analysed.

Calculations and statistical analyses

Total number of alveoli and capillaries are standardized to (and will in the following refer to) a number per 100 000 μm^2. Alveolar and capillary density (μm^2) refer to the area covered by alveoli or stained capillaries, respectively, per 100 000 μm^2 mammary section. The alveolar diameter is expressed as the mean Feret diameter, which is the separation between pairs of parallel lines that just enclose an object. The mean Feret diameter was calculated as the mean of the two diameters in parallel to the x- and y-axis.

Statistical analyses were performed using the MIXED procedure in SAS® (SAS Institute Inc., Cary 27512 NC, USA). All data were tested for normal distribution. Milk yield, MBV, total number of alveoli, alveolar density, mean Feret alveolar diameter, total number of capillaries, capillary density, capillary-to-alveolar density, number of capillaries per alveolus, total capillary pixel value and pixel value per capillary were analysed for fixed effect of period (EL, ML1, BST, ML2, LL, D) and random effect of goat with period as the repeated measure. Covariance structures CS and AR(1) were fitted as described by Littell et al. (1998). Effect of BST treatment was evaluated by comparing BST period against ML1 and ML2 periods, using them as pre- and post-treatment controls, respectively. Pearson correlation coefficients were calculated for selected traits. All results are presented as LSMEANS ± SEM.

Results

Milk vein blood velocity and milk yield

Recordings of MBV in early lactation (EL) were unfortunately lost. MBV (Fig. 1) was fairly stable during the

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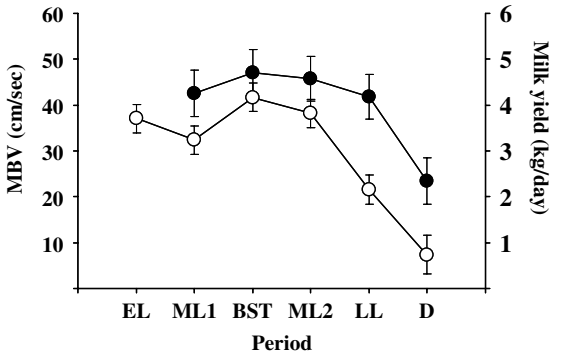

Fig. 1. Milk vein blood velocity (MBV, cm/sec; ●) and milk yield (kg/d; ○) of experimental goats. EL, ML1, BST, ML2, LL and D: see footnote to Table 1.

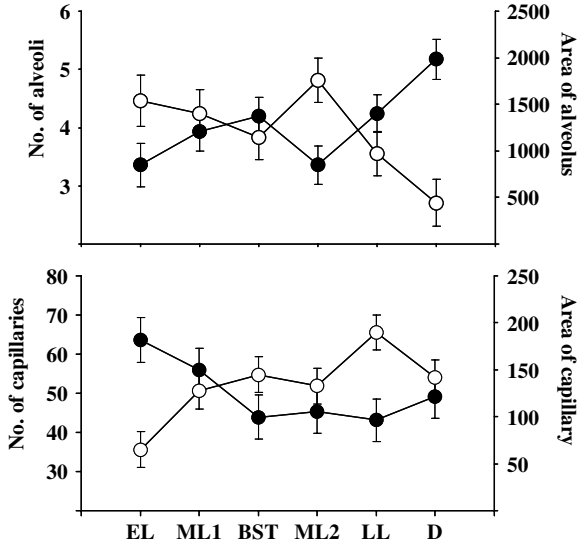

Fig. 2. Upper panel: Total number of alveoli per 100 000 μm^2 mammary section (●) and area of the individual alveolus (μm^2) (○). Lower panel: Total number of capillaries per 100 000 μm^2 mammary section (●) and area of the individual capillary (○). EL, ML1, BST, LL and D: see footnote to Table 1.

mid-lactation period with a small but non-significant increase during BST treatment, and then decreased ($P<0·001$) by approximately 50% from late lactation till the time of drying-off (D: 23·4±5·06 cm/sec). Milk yield (Fig. 1) peaked at 4·17±0·31 kg/d and like MBV declined during lactation ($P<0·001$). Milk yield decreased from EL to ML1 ($P<0·05$), increased to the highest levels in BST ($P<0·001$) and then decreased through LL to reach minimum levels in D ($P<0·001$). MBV and milk yield were positively correlated ($r=0·47$; $P<0·05$).

Alveolar number, area and density

Total number of alveoli per 100 000 μm^2 (Fig. 2, upper panel) increased by 54% from EL to D ($P<0·01$). It tended to increase in BST compared with EL ($P=0·055$) and was

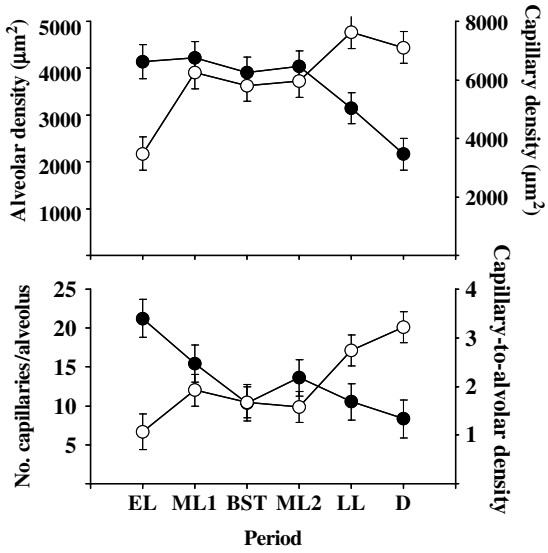

Fig. 3. Upper panel: Alveolar (●) and capillary (○) density (area in μm^2 covered by alveoli and capillaries per 100 000 μm^2 mammary section, respectively). Lower panel: Number of capillaries per alveolus (●) and capillary-to-alveolar density ratio in mammary sections (○). EL, ML1, BST, LL and D: see footnote to Table 1.

reduced after cessation of BST treatment in ML2 ($P<0·05$), whereafter it increased to reach the highest levels in D ($P<0·001$).

Alveolar density (area covered by alveoli in a 100 000 μm^2 section; Fig. 3, upper panel) decreased by 48% over lactation ($P<0·001$), and this could be ascribed to a decrease occurring in the last part of lactation (LL and D), whilst no significant changes occurred over the early and mid-lactation periods, including BST. Alveolar density and total number of alveoli were negatively correlated ($r=-0·57$; $P<0·001$).

Mean Feret alveolar diameter (results not shown) had a maximum value of 110·5±5·07 μm in EL and a minimum value of 77·6±4·78 μm in D, and was unaffected by BST treatment. It was negatively correlated with total number of alveoli per 100 000 μm^2 ($r=-0·61$; $P<0·001$), but strongly and positively correlated with alveolar density ($r=0·95$; $P<0·001$).

Area of the individual alveolus (Fig. 2, upper panel) was stable through EL to ML1 and BST, increased to the highest levels in ML2 (1760±241 μm^2; $P<0·05$) and decreased thereafter to reach the lowest level in D (441±253 μm^2; $P<0·001$), which was 75% lower than in EL. Area of the individual alveolus was negatively correlated to total number of alveoli per 100 000 μm^2 ($r=-0·60$; $P<0·001$), but positively correlated to alveolar density ($r=0·73$; $P<0·001$) and mean Feret alveolar diameter ($r=0·67$; $P<0·001$).

Alveolar density ($r=0·48$; $P<0·01$) and Feret alveolar diameter ($r=0·49$; $P<0·01$) were positively correlated to MBV.

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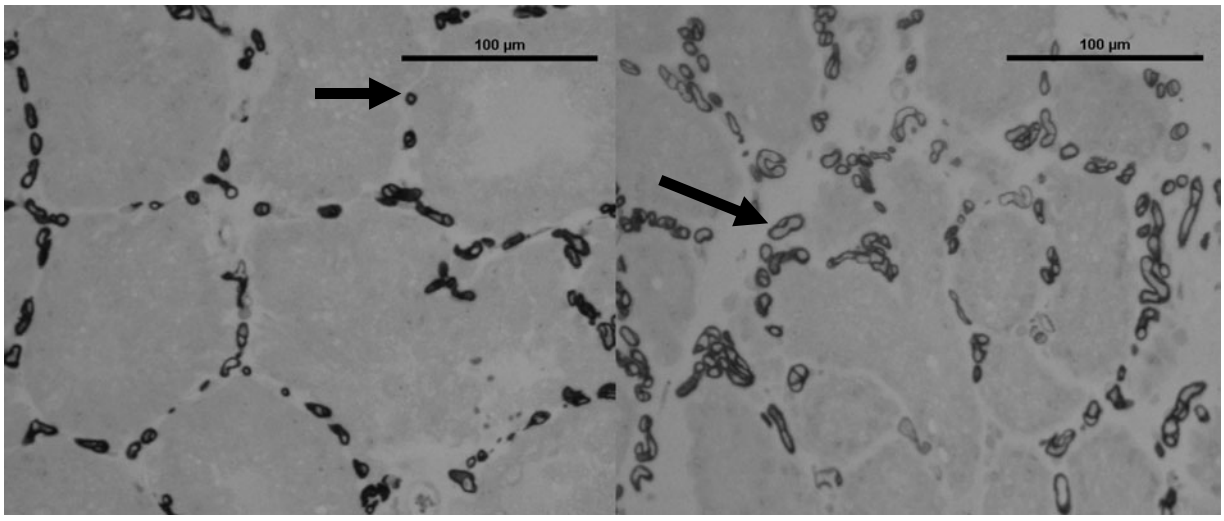

Fig. 4. Sections of azure blue counter stained lactating mammary tissue from the same goat, illustrating large alveoli surrounded by high numbers of heavily stained (high CA activity) small capillaries (arrow) in early lactation (left panel), and fewer but larger and less stained (lower CA activity) capillaries (arrow) surrounding smaller alveoli in late lactation (right panel).

Capillary density, number and area

Total number of capillaries per 100 000 µm^2 (Fig. 2, lower panel) was highest in EL (63·6±5·8), decreased significantly from ML1 to BST (P<0·05), where it stabilized at around 45 throughout the rest of the experimental period at a value 32% lower in LL compared with EL (P<0·01).

Area of the individual capillary was smallest (Fig. 2, lower panel and Fig. 4) in EL (65±19 µm^2); capillaries underwent dilatation from EL to ML1 (P<0·001), and dilated further in LL (189±19 µm^2) (P<0·001), at which point they had almost tripled in size (291%) compared with EL. From LL to D they decreased in size again (P<0·01).

Capillary density (Fig. 3, upper panel) was lowest in EL (3477±575 µm^2), increased from EL to ML1 (P<0·001), remained at a constant level through ML1, BST and ML2, and subsequently increased to maximum levels (7619±536 µm^2) in LL (P<0·01); more than two-fold higher (219%) compared with EL. A fairly strong positive correlation was found between capillary density and area of the individual capillary (r=0·76; P<0·001), whereas capillary density was negatively correlated to total number of capillaries (r=–0·48; P<0·001). MBV and milk yield were not correlated with either total number of capillaries or capillary density.

Total number of capillaries per alveolus (Fig. 3, lower panel) decreased across lactation by 61% from EL (21·24±2·45) to the lowest values in D (P<0·001) and was unaffected by BST treatment.

The capillary-to-alveolar density ratio (Fig. 3, lower panel) tripled from minimum values in EL to maximum levels in D (300%) (P<0·001) and was not affected by BST treatment. Thus high numbers of very small capillaries surrounded large alveoli in early lactation, and fewer but increasingly larger capillaries surrounded diminishing alveoli in the late lactating mammary gland.

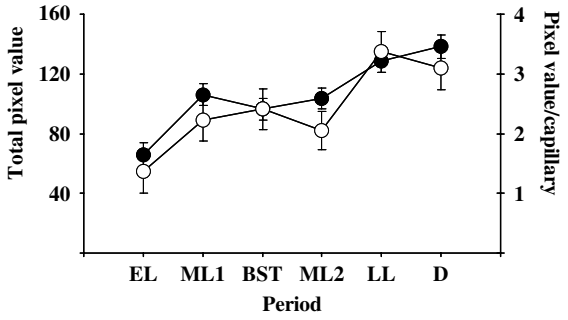

Fig. 5. Total capillary pixel value per 100 000 µm^2 mammary section (●) and pixel value per individual capillary (○). EL, ML1, BST, LL and D: see footnote to Table 1.

Carbonic anhydrase activity

Total capillary pixel value per 100 000 µm^2 (Fig. 5) increased 110% from lowest values in EL (65·9±7·9) to highest levels in D (138·2±10; P<0·001) (P<0·001) reflecting decreased CA activity in capillaries across the lactation period. Through ML1 to ML2 (including BST period) total capillary pixel values remained stable. A negative correlation was found between total capillary pixel value and milk yield (r=–0·39; P<0·05).

Pixel value per individual capillary (Fig. 4) increased 146% from lowest levels in EL (1·4±0·36) to peak value (3·4±0·34) in LL (P<0·01). Pixel value per capillary was stable through ML1 to ML2 and was not affected by BST treatment. Pixel value per capillary was negatively correlated with milk yield (r=–0·39; P<0·05) and total number

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of capillaries ($r=-0{\cdot}66$; $P<0{\cdot}001$) but positively correlated with capillary density ($r=0{\cdot}47$; $P<0{\cdot}01$) and area of the individual capillary ($r=0{\cdot}82$; $P<0{\cdot}001$) but not correlated to MBV.

Discussion

Changes in the mammary alveoli and capillaries during lactation and BST treatment

Parenchymal volume in the mammary gland (Knight & Wilde, 1993) and number of mammary epithelial cells (Capuco et al. 2001) have previously been reported to follow changes in milk production over the course of lactation. Our study supports this, as cross-sectional area of individual alveoli and the overall alveolar density in mammary sections decreased as lactation progressed. The gross morphological changes in the mammary capillary system were however more dramatic than the changes at the level of the alveoli, which is interesting since so little attention has been paid to functional changes in the mammary microvasculature in dairy animals, and the role in regulation of mammary function during lactation.

In the mouse it has been demonstrated by Matsumoto et al. (1992) that the capillary network in the developed mammary gland forms basket-like structures around the alveoli. When the mammary gland is lactating, the capillary endothelial cells develop numerous folds and microvillous processes, which is particularly prominent in thin-walled capillaries lying adjacent to secretory epithelial cells with well-developed basal infoldings. This establishes a close contact between the capillary endothelium and the secretory epithelium, whereby diffusion distances are reduced and the surface area available for blood-tissue exchange is increased. Along this line of thought, small diameter capillaries would therefore be expected to favour a more efficient blood-tissue exchange of nutrients and waste products, even if perfused by the same volume of blood, because diffusion distances obviously would be smaller than in large diameter capillaries. But in addition to that, a small capillary could potentially also create closer proximity to the mammary epithelial cell and shorten diffusion distances even further by fitting better into the epithelial cell infoldings compared with a larger capillary. The substantial reduction (61%) we observed in our study in number of capillaries per alveolus with progressing lactation, in combination with an almost threefold increase in capillary diameter, could therefore contribute to explaining why extraction of most nutrients across the goat mammary gland becomes less efficient from early to late lactation (Madsen et al. 2005) so that a higher mammary blood flow is required to sustain synthesis of 1 kg of milk in late compared with early lactation (Linzell, 1974).

To our knowledge, adaptations in the mammary capillary system during the course of lactation have not previously been reported in dairy or other animals. Adaptation to exercise and metabolic activity in skeletal muscle of rats and man involves an increase in numbers of capillaries per muscle fibre (Kano et al. 2004; Terzis et al. 2008). Likewise in the goat mammary gland, it appears that high metabolic activity (milk yield) in EL is associated with a higher number of capillaries per alveolus compared with LL, when metabolic activity (milk yield) has decreased. However, in skeletal muscle capillary luminal area is also increased in response to increased metabolic activity, and this was not the case in the mammary gland, where the smallest capillary areas were found in EL, when the mammary gland was most metabolically active. The reason for this discrepancy is not known.

Increased capillary area (diameter) with progressing lactation should theoretically be associated with reduced peripheral vascular resistance, and hence would favour a high rate of mammary blood flow. However, MBV declined across lactation despite the substantial increase in capillary diameter, confirming that MBF generally follows changes in milk production (Prosser et al. 1996). In the rat, mammary capillary blood flow is discontinuous, and occurs in stop-and-go movements (Davis et al. 1993), demonstrating that peripheral resistance and capillary perfusion in the murine mammary gland (and vascular beds of other tissues) is not determined simply by the number of capillaries, but can be regulated at pre- or post-capillary sites (Fujiwara & Uehara, 1984; reviewed by Prosser et al. 1996). Our results support the view that the capillary in itself may not be the major site that determines MBF in goats. However, the capillary changes over the course of lactation may be important determinants for the efficiency of nutrient and waste product exchange across the capillary-mammary epithelial cell membrane barrier. This could in turn determine the metabolic capacity of the mammary gland with advancing lactation, in addition to the metabolic and cell turnover events occurring at the level of the epithelial cell (e.g. Capuco et al. 2001).

Future studies are needed to elucidate the regulatory mechanisms underlying remodelling of the microvascular system during lactation, and implications for mammary epithelial cell function and overall lactation performance.

BST stimulation of milk yield in our study coincided with increases in number and density of alveoli in mammary sections, but without changing the size of the individual alveolus. BST has been reported to stimulate mammary epithelial cell renewal in dairy cows (Capuco et al. 2001) and to increase mammary glandular weight in dairy goats without however increasing alveolar size or number of epithelial cells within alvoli (Boutinaud et al. 2003). BST therefore appears to exert its galactopoietic effect by expanding the mammary epithelial cell population through formation of new alveoli rather than by increasing the cell number and size of already existing alveoli. Our results do not suggest, however, that BST is a major player in regulation of microvascular remodelling within the mammary gland during lactation, since none of the capillary measures were impacted by the BST treatment. This interpretation must however be taken with

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some caution. In several previous studies BST has been reported to stimulate MBF as part of its galactopoietic effect (Davis & Collier, 1985; McDowell et al. 1988; Mepham et al. 1984). But for some unknown reason the treatment failed to do so significantly in the present experiment, although we did observe a modest numerical increase in MBV.

Carbonic anhydrase and mammary blood flow regulation and function

Our study confirmed that CA activity in the lactating goat mammary gland was associated exclusively with the capillary endothelium. This is in contrast to the rat, where mammary CA has been found both within the mammary epithelial cell cytoplasma as well as in the capillary endothelium (Cvek, 1997). In dairy cows, the only studies on CA we are aware of have focused specifically on the secretory form of the enzyme (CA VI), which is located mainly in epithelial cells and secreted into milk, with highest concentrations immediately post partum, and rapidly declining over the first 70 d of lactation (Keitaro et al. 2003; Nishita et al. 2007). Although the goat has been a much used model for dairy cows in lactation physiology, species differences do exist, such as different distributions of CA within the mammary gland and expression of different isoform(s) of CA. The implications of these differences are not known.

The decrease in mammary capillary CA activity in goats with decreasing milk production shows that there is coordinated adaptation also of the capillary network function as mammary metabolic activity decreases during lactation. Whether it is the changes in mammary metabolic activity that govern the changes in capillary morphology and CA or the other way around is not known. Although CA is a capillary enzyme in the caprine mammary gland, it may be coupled less to regulation of MBF than to metabolic activity and milk synthesis in mammary epithelial cells, by facilitating removal of metabolic CO_2 and buffering of intracellular pH. Indications in support of this view are 1) the positive correlation observed between milk production versus CA activity (Cvek et al. 1998; present study) and mammary CO_2 production (Nielsen et al. 1995) and 2) the ability of the mammary gland to regulate conversion of CO_2 to HCO_3^- over the course of lactation, whereby partial pressure of CO_2 in blood leaving the mammary gland is kept constant (Nielsen et al. 1995).

BST treatment did not impact CA activity in line with our previous observations (Nielsen et al. 1995) where increased mammary metabolic activity (i.e. CO_2 production) during BST treatment affected venous blood pCO_2, but not HCO_3^-, indicating that the conversion of CO_2 to HCO_3^- by CA activity is not regulated by BST.

In conclusion, the early lactating mammary gland was characterized by large lactating alveoli surrounded by high numbers of small capillaries with high CA activity. As lactation progressed, capillaries almost tripled in size and CA activity more than halved. BST did not affect capillary traits, but increased number of alveoli and appeared to be targeted mostly towards the mammary epithelial cell. Capillary diameter and CA are apparently not major determinants of MBF, but the changes in capillary diameter and CA activity with progressing lactation could contribute to reduce efficiency of nutrient and waste product exchange across the capillary-mammary epithelial cell membrane barrier. Future studies are needed to improve our limited knowledge of mammary microvascular remodelling, and to assess whether this could be as important a factor in regulation of lactation performance as the mammary epithelial cell itself.

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References

Anderson RR, Harness JR, Snead AF & Salah MS 1981 Mammary growth pattern in goats during pregnancy and lactation. *Journal of Dairy Science* **64** 427–432

Boutinaud M, Rousseau C, Keisler DH & Jammes H 2003 Growth hormone and milking frequency act differently on goat mammary gland in late lactation. *Journal of Dairy Science* **86** 509–520

Capuco AV, Wood DL, Baldwin R, McLeod K & Paape MJ 2001 Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of BST. *Journal of Dairy Science* **84** 2177–2187

Christensen K, Nielsen MO, Bauer R & Hilden K 1989 Evaluation of mammary blood flow measurements in lactating goats using the ultrasound Doppler principle. *Comparative Biochemistry & Physiology* **92A** 385–392

Cvek K, Ridderstråle Y & Dahlborn K 1998 Localization of carbonic anhydrase in the goat mammary gland during involution and lactogenesis. *Journal of Dairy Research* **65** 43–54

Cvek K 1997 *Mammary gland function with special reference to vascularization and atrial natriuretic peptide*. PhD thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden

Davis SR & Collier RJ 1985 Mammary blood flow and regulation of substrate supply for milk synthesis. *Journal of Dairy Science* **68** 1041–1058

Davis SR, Farr VC, Prosser CG & Thompson JG 1993 The nature of the microcirculation in the mammary gland of the lactating rat. *Proceedings of the New Zealand Society of Animal Production* **53** 171–172

Fujiwara T & Uehara Y 1984 The cytoarchitecture of the wall and the innervation pattern of the microvessels in the rat mammary gland: a scanning electron microscope observation. *American Journal of Anatomy* **170** 39–54

Guinard-Flament J, Delamaire E, Lamberton P & Peyraud JL 2007 Adaptions of mammary uptake and nutrient use to once-daily milking and feed restriction in dairy cows. *Journal of Dairy Science* **90** 5062–5072

Kano Y, Sampei K & Matsudo H 2004 Time course of capillary structure changes in rat skeletal muscle following strenuous eccentric exercise. *Acta Physiologica Scandinavica* **180** 291–299

Keitaro K, Nishita T, Yamato M, Sakamoto K, Hagino A, Katoh K & Obara Y 2003 Expression and localization of carbonic anhydrase in bovine mammary gland and secretion in milk. *Comparative Biochemistry & Physiology* **134A** 349–354

Knight CH & Wilde CJ 1993 Mammary cell changes during pregnancy and lactation. *Livestock Production Science* **35** 3–19

Krishnamurthy VM, Kaufman GK, Urbach AR, Gitlin I, Gudiksen KL, Weibel DB & Whitesides GM 2008 Carbonic anhydrase as a model for biophysical and physical-organic studies of proteins and protein-ligand binding. *Chemical Reviews* **108** 946–1051

Linzell JL 1974 Mammary blood flow and methods of identifying and measuring precursors of milk. In *Lactation: A Comprehensive Treatise. The mammary gland/Development and maintenance. Volume 1* (Eds BL Larson & VR Smith) pp. 143–225. London, UK: Academic Press

Littell RC, Henry PR & Ammerman CB 1998 Statistical analysis of repeated measures data using SAS® procedures. *Journal of Animal Science* **76** 1216–1231

Madsen TG, Nielsen L & Nielsen MO 2005 Mammary nutrient uptake in response to dietary supplementation of rumen protected lysine and methionine in late and early lactating dairy goats. *Small Ruminant Research* **56** 151–164

Matsumoto M, Nishinakagawa H, Kurohmaru M, Hayashi Y & Otsuka J 1992 Pregnancy and lactation affect the microvasculature of the mammary gland in mice. *Journal of Veterinary Medical Science* **54** 937–943

McDowell GH, Leenanuruksa D, Niumsup P, Gooden JM, van der Walt JG & Smithard R 1988 Short term effects of exogenous growth hormone: effects on milk production and utilization of nutrients in muscle and mammary tissues of lactating ewes. *Australian Journal of Biological Sciences* **41** 279–288

Mepham TB, Lawrence SE, Peters AR & Hart IC 1984 Effects of exogenous growth hormone on mammary function in lactating goats. *Hormone and Metabolic Research* **16** 248–253

Nielsen MO, Schleisner C, Jakobsen K & Andersen PH 1995 The effect of mammary O_2 uptake, CO_2 and H^+ production on mammary blood flow during pregnancy, lactation and somatotropin treatment in goats. *Comparative Biochemistry & Physiology* **112A** 591–599

Nishita T, Tanaka Y, Wada Y, Murakami M, Kasuya T, Ichihara N, Matsui K & Asari M 2007 Measurement of carbonic anhydrase isozyme VI (CA-VI) in bovine sera, saliva, milk and tissues. *Veterinary Research Communications* **31** 83–92

Prosser CG, Davis SR, Farr VC & Lacasse P 1996 Regulation of blood flow in the mammary microvasculature. *Journal of Dairy Science* **79** 1184–1197

Ridderstråle Y 1976 Intracellular localization of carbonic anhydrase in the frog nephron. *Acta Physiologica Scandinavica* **98** 465–469

Ridderstråle Y 1991 Localization of carbonic anhydrase by chemical reactions. In *The Carbonic Anhydrases: Cellular Physiology and Molecular Genetics* (Eds SJ Dodgson, RE Tashian, G Gros & N Carter) N0 p. 133. New York, USA: Plenum Press

Supuran CT 2008 Carbonic anhydrases—an overview. *Current Pharmaceutical Design* **14** 603–614

Tatarczuch L, Philip C & Lee CS 1997 Involution of the sheep mammary gland. *Journal of Anatomy* **190** 405–416

Terzis G, Spengos K, Manta P, Sarris N & Georgiadis G 2008 Fiber type composition and capillary density in relation to submaximal number of repetitions in resistance exercise. *Journal of Strength and Conditioning Research* **22** 845–850