Mammary Blood Flow Does Not Limit Milk Yield in Lactating Goats¹

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ABSTRACT

There is a close relationship between mammary blood flow (MBF) and milk production, but whether MBF is limiting milk yield has not been determined. Five lactating goats received close arterial (external pudic) infusion of PBS or the nitric oxide donor diethylamine NONOate (0.5 mg/h; NONate) for 6 h, according to a crossover design. Goats were hand milked (with oxytocin) every 2 h starting 2 h before and ending 6 h after the end of the infusion. In one goat, a transit time flow probe was implanted around the infused and noninfused artery, whilst in another goat a flow probe was implanted around the infused artery only. Infusion of PBS did not affect MBF or milk production. As with previous results (Lacasse et al., 1996), NONate induced a rapid increase (up to 250% of preinfusion level) in MBF in the infused gland only. Mammary blood flow was still above the preinfusion level at the end of the infusion period. Despite this increase in MBF, NONate did not affect milk production. Milk yield ratio (infused/noninfused gland) averaged 1.20, 1.12, and 1.17 for the preinfusion, infusion and post infusion periods, respectively. Similarly, protein, fat and lactose yields were not affected by PBS or NONate infusion. These results provide no support to the contention that increasing MBF can enhance milk production.

(Key words: mammary blood flow, nitric oxide, dairy goats)

Abbreviation key: MBF = mammary blood flow, NONate = diethylamine NONOate.

INTRODUCTION

The lactating mammary gland depends upon an adequate supply of nutrients and hormonal stimuli from blood to sustain milk synthesis. Mammary blood flow (MBF) is a major determinant of the rate of substrate

supply for milk synthesis (Davis and Collier, 1985). Within individual animals of a number of species, variation in milk yield is usually accompanied by a similar variation in MBF (Linzell, 1974). However, whether the change in MBF is a compliant response to a change in metabolic activity of the mammary gland (Prosser et al., 1996) or a major driving mechanism of milk synthesis (Bequette et al., 2001; Hanigan et al., 2001) is still a matter of debate.

We reported previously (Lacasse et al., 1996) that nitric oxide (NO) is a potent vasorelaxant for the mammary gland. This molecule has a very short half-life and intraarterial infusion of a NO donor directly into the mammary gland of lactating goats increases MBF in the infused gland only. This enabled study of the interdependence of MBF and milk yield more rigorously by perturbing MBF unilaterally.

MATERIALS AND METHODS

Five lactating Saanen goats (50 to 70 kg BW), at least 20 wk postpartum, were housed individually inside and fed a mixture of chopped hay and 2.5 kg of concentrates twice a day. In all goats, the external pudic artery supplying one gland was cannulated with polyvinyl tubing (1.0 mm o.d., 0.5 mm i.d.) according to the method described by Fleet and Mephram (1983). At the same time, a transit-time ultrasonic flow probe (Transonics Inc, Ithaca, NY) was implanted around the artery, upstream of the catheter, in two goats. In one goat, the external pudic artery of the contralateral gland was also implanted with a flow probe. The goats were allowed to recover from surgery for at least five weeks before infusion commenced. All animal manipulations were conducted in compliance with the Code of Ethical Conduct for Animal Experimentation and approval from Ruakura Animal Ethics Committee.

Five lactating goats received close arterial (external pudic) infusion of PBS (0.05 M phosphate, pH 9 and 0.15 M NaCl) or the nitric oxide donor diethylamine NONOate (**NONate**; Cayman Chemical Co, Ann Arbor, MI) for 6 h. NONate was dissolved in PBS to give 25 µg/ml and infused via the pudic artery catheter of the goats at 20 ml/h, giving an infusion rate of 0.5 mg/h. This molecule is unstable at blood pH (half life ~ 2.1

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Figure 1. Effects of close arterial (external pudic) infusion of 0.5 mg/h of the nitric oxide donor diethylamine NONOate for 6 h on blood flow of infused (closed symbols) and noninfused (open symbol) mammary glands of individual goats.

Figure 2. Effects of close arterial (external pudic) infusion of 0.5 mg/h of the nitric oxide donor diethylamine NONOate for 6 h on milk production of infused (■), noninfused (□) mammary glands and ratio of milk production of infused over noninfused gland (●). SEM = 8.8 ml/h and 0.18 for milk production and production ratio, respectively.

min) and dissociates to produce the free amine and NO. The dose was determined according to a previous study (Lacasse et al., 1996). All goats received each infusion, according to a crossover design, at least one week apart. Three goats received PBS for the first infusion and NONate for the second and two goats received NONate first and PBS second. Goats were hand milked (with oxytocin) every 2 h starting 2 h before and ending 6 h after the end of the infusion. A sample of milk of each gland was harvested at each milking for milk composition determination.

Each MBF data point represents the mean of 10 min of recording (5 min before to 5 min after). Average milk production data of preinfusion, infusion and postinfusion periods were analyzed as a repeated measurement design using the GLM procedure of SAS (1985). The model included treatments, periods and goats and assumed no interaction between goats and treatments.

RESULTS

The MBF response to NONate and PBS infusion in two goats are depicted in Figure 1. As only one of these goats had a flow probe on the contralateral gland, MBF data from the noninfused gland is shown for this goat only. As for our previous results (Lacasse et al., 1996), infusion with PBS alone had no effect, but NONate induced an immediate increase in MBF in the infused gland. Although the MBF response declined slightly during infusion of NONate, MBF was still 200% of the preinfusion level at the end of the infusion period. Once the infusion of NONate was stopped, MBF dropped below preinfusion values and remained depressed up to 60 min postinfusion. Also in agreement with our previous

study (Lacasse et al., 1996) the MBF in the contralateral gland was not altered by PBS or NONate infusions.

Despite this increase in MBF, NONate did not affect milk production ($P > 0.1$; Figure 2). Milk yield ratio (infused/noninfused gland) averaged 1.20, 1.12, and 1.17 for the preinfusion, infusion and post infusion periods, respectively. Infusion of PBS did not affect milk production ($P > 0.1$; Figure 3).

Milk content and yield of protein, fat and lactose were not affected by the infusions PBS or NONate ($P > 0.1$; data not shown). Accordingly, ratios of component yield of infused over noninfused gland were not affected by NONate ($P > 0.1$; Table 1) or PBS ($P > 0.1$; data not shown).

Figure 3. Effects of close arterial (external pudic) infusion of PBS for 6 h on milk production of infused (■), noninfused (□) mammary glands and ratio of milk production of infused over noninfused gland (●). SEM = 11.5 ml/h and 0.28 for milk production and production ratio, respectively.

Table 1. Effects of infusion of the nitric oxide donor diethylamine NONOate (0.5 mg/h) for 6 h on milk components yield. Data are presented as the ratio of yield of infused over noninfused glands.

Milk component	Preinfusion	Infusion	Post-infusion	SEM	<i>P</i>
Protein	1.28 ¹	1.14	1.22	0.06	NS
Fat	1.20	1.15	1.22	0.11	NS
Lactose	1.23	1.16	1.18	0.06	NS

¹Least square means.

DISCUSSION

Homeorhesis is defined as the coordinated changes in metabolism of body tissues necessary to support a physiological state (Bauman and Currie, 1980). Accordingly, lactation results in increased cardiac output (Linzell, 1974) but blood flow does not increase proportionally to all organs. There is an increased flux to the liver and gut (Lomax and Baird, 1983), thus facilitating digestion and absorption of nutrients, and also to the mammary gland (Davis and Collier, 1985) therefore favoring a preferential use of nutrients for milk synthesis. There is a close relationship between MBF and milk production. Accordingly, variation in milk yield is usually accompanied by similar variation in MBF.

The galactopoietic effect of GH is well known and is associated with an increase in MBF and IGF-I (Mephram et al., 1984; Prosser et al., 1989; Lacasse et al., 1994). After a GH injection to lactating cows, MBF profile parallels with plasma and mammary afferent lymph concentrations of IGF-1 but not with serum concentration of GH (Lacasse, 1993; Lacasse et al., 1994). Using a protocol similar to the one used in this study, close arterial infusion of IGF-I increased MBF and milk production in the infused gland only (Prosser et al., 1990). There are two theories as to the mode of action by which this is accomplished. The first theory, as reviewed by Forsyth (1996), is that IGF-I increases mammary blood flow directly, which then increases mammary gland metabolism and, therefore, milk component yield. The second theory is that IGF-I increases metabolism within the mammary gland, thus leading to increased production of milk components. The increase in metabolism by the mammary gland leads to an increase in mammary blood flow.

In agreement with our earlier study (Lacasse et al., 1996), close arterial infusion of the vasorelaxant diethylamine NONOate induced a sharp increase in mammary blood flow only in the infused gland. The present findings further show that although this effect is maintained for the duration of the infusion, it is without any changes in milk yield and milk yield ratio (infused to noninfused gland). Other experiments using close arterial infusion of glucagon (Davis and Collier, 1985) parathyroid hormone-related protein (Prosser et al., 1994)

and isoprenaline showed similar lack of change in milk yield even though mammary blood flow was increased (Prosser et al., 1996) giving no support to the "push" theory. Moreover, in contrast to these vasodilators, IGF-1 infusion induces a gradual increase in mammary blood flow (Prosser and Davis, 1992) which is more compatible with an effect on mammary metabolism than a direct effect on the vasculature itself. In agreement with that, GH administration to nonlactating cows increases plasma and mammary afferent lymph concentrations of IGF-1 but not MBF (Lacasse, 1993).

Further evidence against the "push theory" is provided by responses of MBF after experimental manipulation. An increase of MBF above normal levels follows even transient reduction in MBF after manual occlusion of mammary artery (Linzell, 1974) or short- or long-term infusion of adrenaline (McBride and Christopherson, 1986; Bernabe et al., 1988). Conversely, a decrease in MBF follows prolonged intramammary infusion of vasorelaxants (Lacasse et al., 1996; Prosser et al., 1996). These responses are consistent with the accumulation of vasoactive substances within mammary tissue. Several metabolic end products, such as lactate, CO₂, adenosine and low pH, induce vasodilatation in many body tissues. For the mammary gland, adenosine infusion has been shown to increase MBF (Prosser et al., 1996) while low arterial pCO₂ or cold (25°C) blood reduced blood flow in mammary glands perfused in vitro (Linzell, 1974).

In conclusion, it appears much more likely that rates of mammary metabolism control mammary blood flow than vice-versa. This is an important concept to fully understand and model milk synthesis.

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REFERENCES

- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514–1529.

- Bequette, B. J., C. E. Kyle, L. A. Crompton, V. Buchan, and M. D. Hanigan. 2001. Insulin regulates milk production and mammary gland and hind-leg amino acid fluxes and blood flow in lactating goats. *J. Dairy Sci.* 84:241–255.
- Bernabe, J., H. Rulquin, J. P. Caudal, and J. Duvere. 1988. Estimation du debit sanguine mammaire chez la vache laitière par la thermomodulation [Determination of mammary blood flow of dairy cows by thermomodulation]. *Reprod. Nutr. Dev.* 28:205–206.
- Davis, S. R., and R. J. Collier. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. *J. Dairy Sci.* 68:1041.
- Fleet, I. R., and T. B. Mepham. 1983. Physiological methods used in the study of mammary gland substrate utilisation in ruminants. Page 469 in *Biochemistry of Lactation*. T. B. Mepham, ed. Elsevier, Amsterdam, The Netherlands.
- Forsyth, I. A. 1996. The insulin-like growth factor and epidermal growth factor families in mammary cell growth in ruminants: action and interaction with hormones. *J. Dairy Sci.* 79:1085–1096.
- Hanigan, M. D., L. A. Crompton, J. A. Metcalf, and J. France. 2001. Modelling mammary metabolism in the dairy cow to predict milk constituent yield, with emphasis on amino acid metabolism and milk protein production: model construction. *J. Theor. Biol.* 213:223–239.
- Lacasse, P., V. C. Farr, S. R. Davis, and C. G. Prosser. 1996. Local secretion of nitric oxide and the control of mammary blood flow. *J. Dairy Sci.* 79:1369–1374.
- Lacasse, P., E. Block, Y. Couture, and D. Petitclerc. 1994. Bovine growth hormone and the local production of prostacyclin I_2 on mammary blood flow in dairy cows. *Proceeding of the New Zealand Society of Animal Production*. 54:111–114.
- Lacasse, P. 1993. Influence of plane of nutrition, growth hormone and growth factors on mammary gland development and function. Ph.D. Diss., McGill University, Montreal.
- Linzell, J. L. 1974. Mammary blood flow and methods of identifying and measuring precursors of milk. Page 143 in *Lactation*, vol. 1. B. L. Larson, V. R. Smith, ed. Academic Press, New York, NY.
- Lomax, M. A., and G. D. Baird. 1983. Blood flow and nutrient exchange across the liver and gut of the dairy cows. *Br. J. Nutr.* 49:481–496.
- McBride, G. E., and R. J. Christopherson. 1986. Effects of adrenaline, oxytocin and 2-br- α -ergocriptine on mammary blood flow in the lactating ewe. *Can. J. Anim. Sci.* 66:983–993.
- Mepham, T. B., S. E. Lawrence, A. R. Peters, and I. C. Hart. 1984. Effects of exogenous growth hormone on mammary function in lactating goats. *Horm. Metab. Res.* 16:248–253.
- Prosser, C. G., and S. R. Davis. 1992. Milking frequency alters milk yield and mammary blood flow response to intramammary infusion of insulin-like growth factor-1 in the goat. *J. Endocrinol.* 135:311–316.
- Prosser, C. G., S. R. Davis, V. C. Farr, and P. Lacasse. 1996. Regulation of blood flow in the mammary vasculature. *J. Dairy Sci.* 79:1184–1197.
- Prosser, C. G., V. C. Farr, and S. R. Davis. 1994. Increased mammary blood flow in the lactating goat induced by parathyroid hormone—related protein. *Exp. Physiol.* 79:565.
- Prosser, C. G., I. R. Fleet, and A. N. Corps. 1989. Increased secretion of insulinlike growth factor-I into milk of cows treated with recombinantly-derived bovin growth hormone. *J. Dairy Res.* 56:17–26.
- Prosser, C. G., I. R. Fleet, A. N. Corps, E. R. Froesch, and R. B. Heap. 1990. Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor-I into the mammary gland of the goat. *J. Endocrinol.* 126:437–443.
- SAS User's Guide: Statistics. Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.