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Inhibition of local blood flow control systems in the mammary glands of lactating cows affects uptakes of energy metabolites from blood

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ABSTRACT

To test the effect of mammary blood flow on net uptakes of milk precursors by the mammary glands, inhibitors of nitric oxide synthase (NOS) and cyclooxygenase (COX) were infused into the mammary circulation of 4 lactating cows. Inhibitors were infused in a 4 × 4 Latin square design, where treatments were infusion for 1 h of saline, NOS inhibitor (N_ω-nitro-L-arginine methyl ester hydrochloride), COX inhibitor (indomethacin), or both NOS + COX inhibitors into one external iliac artery. *Para*-aminohippuric acid was also infused to allow for estimation of iliac plasma flow (IPF), of which approximately 80% flows to the mammary glands. Blood samples were collected before, during, and after inhibitor infusion from the contralateral external iliac artery and ipsilateral mammary vein. Inhibition of COX and NOS each produced a decrease in IPF, although the NOS effect was smaller and IPF continued to be depressed throughout the recovery period. The combination of COX and NOS inhibition produced a 50% depression in IPF and there was no carryover into the recovery period. Treatments that depressed IPF also increased arterial concentrations of acetate, β-hydroxybutyrate (BHBA), and glucose. Similarly, arteriovenous differences of acetate, BHBA, and glucose were all increased during IPF depression. To correct for a potential effect of arterial concentration, arteriovenous differences were normalized to arterial concentration, producing an extraction percentage. Inhibition of COX increased glucose extraction and tended to increase acetate and BHBA extraction. Dual inhibition only increased BHBA extraction and had no effect on mammary extraction of other metabolites. These extractions did not increase because clearances of glucose and TAG decreased as IPF decreased, and clearances of acetate and BHBA tended to decrease. Net uptake of TAG was depressed by dual NOS/COX inhibition, whereas uptakes of acetate, BHBA, and glucose were not affected by any of the treatments. To separate effects of flow from effects of arterial concentration, uptakes were regressed against IPF and arterial concentration simultaneously. According to the slopes of the regressions, a 10% decrease in IPF from the mean observed during saline infusion resulted in 3.8, 7.3, and 10.4% decreases in uptakes of acetate, glucose, and triacylglycerol, respectively. These findings indicate that mammary blood flow affects milk precursor uptake, and that clearance should not be assumed constant to predict mammary uptakes of milk precursors in situations where blood flow is changing.
Key words: mammary blood flow, dairy cow, mammary uptake

INTRODUCTION

Bovine mammary glands synthesize the major economic components of milk from acetate, BHBA, glucose, fatty acids, and amino acids taken out of blood. The ability to describe mechanistically the variation in mammary uptakes of these precursors is an important step to predict milk composition responses to changes in diet composition or cow management.

It is often assumed that the rate of blood flow to the mammary glands is an effector of net uptake of the milk precursors. Many studies have shown that mammary blood flow (**MBF**) varies according to hormonal or nutritional state of the dam (Linzell, 1974; Cant et al., 1993; Bequette et al., 2000; Rigout et al., 2002). There is controversy, however, as to how MBF can influence nutrient uptake, and whether the assumption is even correct. On the one hand, a strong, positive relationship exists between MBF and milk yield (Linzell, 1960; Kronfeld et al., 1968), but on the other hand, infusion of an nitric oxide (NO) donor into the mammary circulation to increase MBF for 6 h did not affect milk yields (Lacasse and Prosser, 2003). Nitric oxide is

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one of the main vasodilators released locally to match metabolic activity of the mammary glands with blood flow (Cieslar et al., 2014). The NO infusion experiment suggests that the MBF–milk yield connection is due to a pull, not a push, where the mammary glands exert local control over their own rate of blood flow to match it with milk synthetic activity, and MBF by itself has no effect on milk synthesis. This conclusion has profound implications for how variation in MBF should be accommodated in mathematical models to predict the milk synthetic response to arterial supply of nutrients.

Net uptakes of milk precursors from blood by the mammary glands are routinely estimated as arteriovenous concentration differences across the mammary glands multiplied by MBF (Linzell, 1974). Net uptakes do not represent transport activity but represent intracellular sequestration of transported substrate via metabolic transformations. For example, glucose may be sequestered in lactose, glycerol, or CO_2 and H_2O. Transported substrate that is not sequestered intracellularly will return to the venous circulation and not contribute to the arteriovenous concentration difference. If MBF is not an effector of uptake, then a change in MBF should elicit a reciprocal change in arteriovenous difference. The arteriovenous difference may also be expressed as a proportion of arterial concentration, which is termed the extraction ratio. To our knowledge, there has been no experiment to test whether the arteriovenous difference or extraction ratio responds to a change in MBF in this way. There is a little indirect evidence of the response. When MBF was decreased in cows during fat feeding, extraction of arterial AA increased (Cant et al., 1993). Similarly, changes in MBF during duodenal Met or glucose infusions resulted in reciprocal changes in glucose and acetate extraction, respectively (Guinard and Rulquin, 1995; Rigout et al., 2002). However, when MBF was decreased by glucose infusion into the mammary arterial supply, extraction of acetate did not increase and acetate uptake declined (Cant et al., 2002). The possibility that a change in MBF to accommodate imbalanced supply of one milk precursor can influence the uptake of precursors of other components of milk is tantalizing but unproven. Theoretical models have been proposed in which MBF has an effect on nutrient uptake, either by changing the number of capillaries open to flow, and thereby exposing a different number of milk secretory cells to the arterial supply (Cant and McBride, 1995), or by changing the rate of replenishment of a well-mixed extracellular compartment with arterial metabolites (Hanigan et al., 1998). However, it remains unknown whether these theoretical models are correct in their assumptions concerning the role of MBF.

To decide whether and how to accommodate changing MBF in the prediction of nutrient uptakes from blood, we conducted an experiment in which MBF was modified by infusion of inhibitors of local vasodilatory mechanisms in the mammary glands. Two of the mechanisms by which the mammary glands control their own rate of blood flow to link it with metabolic activity are through local, endothelial production of NO and prostaglandin I_2 (**PGI_2**; Nielsen et al., 1995a; Cieslar et al., 2014). Briefly, adenosine released from the mammary epithelium when intracellular ATP is low activates endothelial NO synthase (**NOS**), and CO_2, H^+, and H_2O_2 released when metabolic activity is high activate endothelial NOS and cyclooxygenase (**COX**) to produce vasodilatory NO and PGI_2, respectively (Cieslar et al., 2014). Accordingly, we inhibited endothelial NOS with N_ω-nitro-L-arginine methyl ester hydrochloride (**L-NAME**), and endothelial COX with indomethacin, and measured arteriovenous differences of energy metabolites across the mammary glands. Possible outcomes ranged from a complete lack of effect of MBF on uptake, in which case extraction would increase, to a complete lack of effect on extraction, indicating depressed uptake.

MATERIALS AND METHODS

All animal procedures and holding facilities were approved by the Animal Care Committee at the University of Guelph (Guelph, ON, Canada). Four multiparous, lactating Holstein cows, at 580 ± 29.3 kg of BW and 54 ± 8.9 DIM (±SE), were fitted with polyurethane catheters in both left and right subcutaneous abdominal veins (milk veins) and polyethylene catheters in both iliac arteries, introduced through the saphenous arteries, as described by Maas et al. (1995).

After 2 d of recovery in box stalls, cows were housed in tie stalls with mattresses and wood shavings. They were milked and fed a corn-silage based TMR (50.5% DM, 17.7% CP, 32.6% NDF, 3.6% fat, 1.74 Mcal of NE_L/kg on DM basis) twice daily at 0800 and 1800 h. Refusals were weighed at 0730 h daily to determine feed intake. Milk was sampled at each milking and analyzed by infrared spectroscopy for protein, fat, and lactose (AOAC International, 1996).

Infusion Protocol

The trial was conducted over 2 periods, with 2 cows and 4 d in each period. Four infusion treatments were arranged in a 4 × 4 Latin square design, where treatments were infusion for 1 h of 90 mg/min NaCl, 25 mg/min L-NAME (Sigma Chemical Co., St. Louis, MO), 2