The frequency of unilateral milking alters leucine metabolism and amino acid removal by the mammary gland of lactating goats

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ABSTRACT

The objective of this study was to test whether mammary AA metabolism and blood flow (BF) fluctuate in relation to changes in milk protein yield brought about by unilateral milking frequency. To investigate these relationships, the vascular systems of the mammary glands of 4 lactating goats were separated by ligation and flow probes were fitted around the external pudic artery of each gland. Goats were subjected to 3 consecutive periods (5 d each) during which 1) both glands were milked twice daily (2×; period 1); 2) glands were unilaterally milked, with gland A milked 8 times daily and gland B milked incompletely (30% of period 1 milk yield retained in the gland) once daily (8× vs. 1×; period 2); and 3) both glands milked twice daily (2×; period 3). On d 5 of periods 1 (2×) and 2 (8× vs. 1×), arteriovenous [$1\text{-}^{13}\text{C}$]Leu kinetics were monitored for each gland. Milk protein yield was greater for gland A compared with gland B. After 2× milking resumed in period 3, yields for both glands returned to period 1 levels. Similarly, BF increased (19%) for gland A (8×), whereas BF was decreased (21%) for gland B (1×). For both glands, BF returned to previous levels when 2× milking resumed in period 3. Mammary net uptakes and oxidation of Leu were greater for the gland milked 8× compared with that milked 1×. Net uptakes from blood of Ile, Leu, Val, Lys, Pro, and Arg were greater (39–79%) for the 8× compared with the 1× gland. In conclusion, the excess removal and oxidation by the mammary gland of some amino acids appears an obligatory requirement to support increases or decreases in milk protein yield when milking frequency is altered.

Key words: mammary, amino acid, stable isotope, blood flow

INTRODUCTION

The branched-chain AA (BCAA; Val, Leu, and Ile), Lys, and Arg are extracted by the mammary gland in excess of the requirement for milk protein output (Guinard and Rulquin, 1994; Bequette et al., 1996b, 1998). Studies in vitro and in vivo have shown that the BCAA are catabolized by the mammary gland along pathways common to other tissues yielding their corresponding oxoacids and nitrogen for dispensable AA synthesis, a proportion of which appears as CO_2 (Wohlt et al., 1977; Bequette et al., 1996a,b). Similarly, there is considerable catabolism of Lys by the mammary gland, with a portion appearing as CO_2 (Mabjeesh et al., 2000) and the amino group transferred to nonessential and essential AA (Lapierre et al., 2009). The excess net uptake of the BCAA by the mammary gland is further enhanced when milk protein output is increased by dietary protein supplementation or duodenal infusion of casein (Guinard and Rulquin, 1994; Bequette et al., 1996b). In several studies, oxidation of Leu and expression of the BCAA catabolic enzymes by the mammary gland were correlated with stage of lactation and dietary protein intake (Bequette et al., 1996b; DeSantiago et al., 1998; Tovar et al., 2001), suggesting a metabolic link between BCAA catabolism and milk production. Yet, in a study by Bequette et al. (1996a), mammary oxidation of Leu was reduced by manipulating the supply of Leu to the udder without affecting milk production. In that study, Leu oxidation was reduced considerably, but not eliminated, suggesting that a basal level of oxidation may be required to support milk biosynthesis. Similarly, even under limiting conditions, the transfer of Lys amino groups to other AA within the dairy cow mammary gland was maintained (Lapierre et al., 2009).

Nutrient-hormone signals coordinate metabolic and physiological function of the mammary gland to facilitate the partition and removal of nutrients by the gland for milk synthesis (Bauman and Currie, 1980). These peripheral signals are thought to establish the metabolic set point of the mammary gland and its capacity to extract nutrients for milk synthesis. Within the limits set by the peripheral signals, local regulatory mechanisms adjust rates of nutrient availability to match

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synthetic capacity. An example of this local regulation is the influence of unilateral and whole-gland milking frequency on milk yield (Wall and McFadden, 2008). In dairy cows where the milking frequency increased (Delamaire and Guinard-Flament, 2006), mammary net uptake of many nutrients increased as well. But, for those AA whose uptake is typically in excess (10–40%) of milk output, the proportion extracted in excess was maintained. In contrast, the excess uptake of glucose relative to lactose output was reduced. These observations suggest that the excess uptake of certain AA forms an obligate requirement to support milk synthesis, and that perhaps under increased milking pressure these AA serve as a substitute for the requirement for glucose. Little is currently known of the metabolic and physiological processes responsible for adjusting the rates of AA availability to support the variable rates of milk synthesis.

In this study, the mammary glands of goats were unilaterally milked at different frequencies and efficiencies (30% of normal milk yield was left in the gland) to manipulate milk synthesis locally and to test whether Leu oxidation and the net uptake of other AA fluctuate in relation to milk yield. Thus, if Leu oxidation is essential for milk protein synthesis, then rates of oxidation would be expected to increase for the gland whose milk yield is increased by frequent milking.

MATERIALS AND METHODS

Goats and Milking Regimen

The Home Office Inspectorate (London, UK) and the Ethical Review Committee of the Rowett Research Institute (Aberdeen, UK) approved all animal handling, experimental, and surgical procedures. This experiment was conducted at the Rowett Research Institute in 1996. Four nonpregnant, multiparous British Saanen goats (BW = 66.3 ± 13.3 kg) in late lactation (approximately 5 mo) were used. In 2 separate surgeries earlier in lactation, the left carotid artery was raised to a subcutaneous position, deep and percutaneous vessels (arteries and veins) transecting the udder halves were ligated, and transonic flow probes (6 mm; Transonic Systems Inc., Ithaca, NY) were fitted around the external pudic artery of each gland (Bequette et al., 1999). After 1 mo of recovery, goats were placed in metabolism crates and allowed at least 7 d to acclimate to the frequent feeding by automatic feeders (12 $\times$ 2 h) and the daily routines of machine milking and hand stripping (0830 and 1630 h). They were fed a ration (Bequette et al., 2001) of chopped grass hay (containing 10% molasses on a fresh weight basis) and a pelleted concentrate (40:60 on a DM basis) in amounts that matched or exceeded recommended intakes (Agricultural and Food Research

Council, 1984) of energy and protein for maintenance plus milk production level at the start of the experiment.

In 3 consecutive periods (5 d each), the following milking regimens were imposed on each goat. In period 1, both glands were milked twice daily (2 $\times$; 0830 and 1630 h); in period 2, glands were unilaterally milked, with one gland milked 8 times daily (8 $\times$, gland A; 0900, 1100, 1300, 1500, 1700, 1900, 2100, and 2330 h) and the other gland milked incompletely (30% of period 1 milk yield was not removed from gland) once daily (1 $\times$, gland B; 0830 h); and in period 3, both glands were milked 2 $\times$ daily (0830 and 1630 h). During period 2, 2 goats had the left gland milked 8 $\times$ and the right gland milked incompletely 1 $\times$, and the other 2 goats had the right gland milked 8 $\times$ and the left gland milked incompletely 1 $\times$. Milk weights were recorded at each milking and subsamples were preserved with 0.1 mL of formaldehyde (40%, vol/vol) and stored at 4°C until analyzed. Milk taken between 1100 and 2330 h from the 8 $\times$ gland was composited and kept at 4°C, and a subsample was taken for milk analysis. Blood flow to each gland was monitored and logged every 12 s continuously for at least 16 h/d throughout the experiment (15 d).

At least 24 h before Leu and net uptake kinetic measurements, catheters were inserted (10 cm) into the carotid artery and into the contralateral jugular vein, and a catheter was placed into each subcutaneous abdominal vein. All catheters were maintained patent by regular flushing with heparin-saline solution (200 IU/mL).

Measurement of Mammary Gland Leu Kinetics

On d 5 of periods 1 and 2, [$1\text{-}^{13}\text{C}$]Leu kinetics and AA net removal by each gland were monitored by an arteriovenous technique. On each occasion, [$1\text{-}^{13}\text{C}$]Leu (99 atom %; Tracer Technologies Inc., Somerville, MA), dissolved (35 mmol/kg) in sterile saline containing heparin (350,000 IU/kg), was infused (0.67 mmol/h) into a jugular vein for 8 h beginning at 0830 h. Starting at h 4 of infusion, at which time isotopic steady state in the plasma is usually achieved (Bequette et al., 1996a), blood samples were continuously withdrawn (10 mL/h) over 1-h periods for 4 h from the carotid artery and from the subcutaneous abdominal vein catheters of the 2 glands. Blood was withdrawn into sealed syringes submerged in an ice bath. Goats remained standing during the blood-sampling period.

Blood samples were immediately mixed by gentle hand-rolling, and duplicate samples (1.25 mL) were injected into evacuated 10-mL vacutainers containing 1 mL of frozen lactic acid, reacted by vortex to liber-

ate CO₂, and kept at room temperature until analyzed (within 1 wk) for blood H¹³CO₃ enrichment. Fresh blood samples were analyzed for concentrations of plasma HCO₃⁻ (Radiometer ABL3 Blood Gas Analyzer; Radiometer Ltd., Crawley, UK) and packed cell volume. A 0.5-g blood sample was then accurately weighed and an equal weight of hemolyzing solution containing 100 μM L-norleucine was added. The remaining heparinized blood was centrifuged at 2,000 × *g* for 15 min at 4°C. A 0.7-g plasma sample was accurately weighed and an equal weight of a solution containing 100 μM L-norleucine was added. Remaining plasma was stored along with the other samples at -20°C. At least 24 h before isotope infusion, blood samples were taken for assessment of the natural abundance of ¹³C in HCO₃⁻, Leu, and 4-methyl-2-oxopentanoate (MOP, the oxoacid of Leu).

Analytical Procedures

For determination of isotope enrichment of plasma [1-¹³C]Leu and [1-¹³C]MOP, stored samples were thawed and deproteinized after addition of 0.125 mL of sulfosalicylic acid [38% (wt/vol)]. The supernatants were processed to yield the oxoacid and AA fractions; the former was treated to yield the quinoxalinol-*n*-butyldimethylsilyl derivative, and the latter was treated to yield the *n*-butyldimethylsilyl derivative (Calder and Smith, 1988). Isotopic enrichments were quantified under electron-impact conditions on a gas chromatography-mass spectrometer (Trio-1; VG Mass Lab, Manchester, UK). Plasma Leu concentrations were determined from gas chromatograph peak areas corrected for the known additions of norleucine. Concentrations of other AA were determined on whole-blood samples by reverse-phase HPLC (Millipore; Waters Chromatography, Milford, MA) as described by Heinrikson and Meredith (1984). Blood H¹³CO₃ enrichments were determined on a VG isotope ratio-MS Sira-12 (VG Masslab, Altrincham, UK) as described by Read et al. (1984). All enrichments were expressed as atom percent excess against the appropriate natural abundance sample. Plasma glucose was determined by an enzymatic colorimetric kit (Labmedics Ltd., Stockport, UK) adapted to a selective chemistry analyzer. Plasma insulin was analyzed as described previously (Bruce et al., 1991). Whole-milk N concentrations were determined by Dumas combustion using an automated procedure (Foss Heraeus Macro N, York, UK) and milk protein output was calculated as milk N × 6.38.

Kinetic Calculations

Plasma irreversible loss rate of Leu based on arterial plasma Leu enrichment was calculated as described

previously (Bequette et al., 1996a). Blood flow (BF) was converted to plasma flow (PF) after correction for packed cell volume: $PF = BF \times (1 - \text{packed cell volume})$. Plasma Leu kinetics were calculated separately for each gland as described previously (Bequette et al., 1996a), except that account was not taken of the net movements of [¹³C]MOP because net release by the mammary gland of MOP was negligible. Net removal was the product of arterial (a) and venous (v) plasma Leu concentration difference and mammary PF for each gland. Net removal of other essential AA, including Leu, was based upon blood measurements and BF.

The fraction of Leu molecules extracted and oxidized (fractional oxidation rate) was determined from the loss of the carboxyl carbon as CO₂:

$$\frac{[(C_{c,v} \times E_{c,v} - C_{c,a} \times E_{c,a}) \times PF] / [(C_{l,a} \times E_{l,a} - C_{l,v} \times E_{l,v}) \times PF],$$

where C is the concentrations (μM) of free Leu (l) and CO₂ (c) in plasma and E is the enrichment (atom percent excess) of the metabolite.

The absolute rate of Leu oxidation (mmol/h) by each gland was determined from the loss of the carboxyl carbon as CO₂ with conversion to AA flow (loss) using mammary vein plasma Leu or MOP (m) enrichment as the precursor at the site of decarboxylation in the mammary tissues (Bequette et al., 1996a):

$$100 \times (C_{c,v} \times E_{c,v} - C_{c,a} \times E_{c,a}) \times PF / E_{m \text{ or } l,v}$$

Statistical Analysis

The experiment included 4 goats and 3 consecutive experimental periods of 5 d each. Treatments were assigned according to a randomized complete block split-unit (split-plot) design with repeated measures. Period 2 (d 6 to 10) was the treatment period, and periods 1 (d 1 to 5) and 3 (d 11 to 15) were the pre- and posttreatment periods, respectively. For Leu kinetics and AA net uptake, the mean of hourly values on d 5 and 10 for each goat (1, 2, 3, 4), treatment period (pretreatment, 2×; treatment, 8×, 1×), side (left, right), and gland (A, 2×, 8×, and B, 2×, 1×) combinations were used for statistical analysis. All statistical analyses were carried out using the MIXED procedure of SAS (SAS Institute, 2007). The statistical model included the fixed effects of gland, treatment, and treatment by gland interaction. The random portion of the model included the effects of goat, side, goat by gland, goat by treatment, and the residual variance. Residuals were examined for variance heterogeneity and separate residual variances were fit

Figure 1. Mammary blood flow and milk protein yields for the glands of goats that had 1) both glands milked twice daily (0830 and 1630 h) from d 1 to 5 (period 1); 2) glands unilaterally milked, with gland A milked 8 times daily (0900, 1100, 1300, 1500, 1700, 1900, 2100, and 2330 h) and gland B milked incompletely (30% of period 1 milk yield was not removed from gland) once daily (0830 h) from d 6 to 10 (period 2); and 3) both glands milked twice daily from d 11 to 15 (period 3). There was a gland by treatment interaction for blood flow ($P < 0.001$) and for milk protein yield ($P < 0.001$). For each gland, values with different superscript letters are different ($P < 0.05$).

as indicated. The treatment by gland interaction was the test for the effect of milking frequency.

Daily milk and protein yields and gland blood flows for each goat (1, 2, 3, 4), treatment day [pre-treatment d 3 to 5 (period 1); treatment d 8 to 10 (period 2); posttreatment d 13 to 15 (period 3)], side (left, right), and gland (A, B) combinations were used for statistical analysis. The statistical model included the fixed effects of gland, day, and day by gland interaction. The random portion of the model included the effects of goat, side, goat by gland, goat by day, and the residual variance. Residuals were examined for variance heterogeneity. Because the design included 15 daily observations of milk yields and mammary BF, compound symmetry and auto regressive structures were examined to identify an appropriate repeated measures structure. Two contrasts were included to interpret the milking frequency effect. The first was to determine whether the pre- and posttreatment effects were the same. If so, then a contrast was used that combined the pre- and posttreatment periods to examine the treatment by gland interaction to test for the effect of milking frequency. Values were considered significantly different if $P < 0.05$, and a trend was accepted if $P < 0.10$.

RESULTS

Statistical comparisons for daily milk and protein yields and daily mammary BF were based on the last 3 d of each period when physiological steady-state responses were attained. Protein yields (Figure 1) for gland A when milked 8x (period 2) were numerically higher than those when milked 2x (period 1), and yields returned to previous levels when 2x milking resumed (period 3); protein yields for gland B when milked 1x (period 2) were reduced by 23% ($P < 0.005$) compared with 2x milking (period 1), and yields returned to previous levels when 2x milking resumed (period 3). For gland A, daily BF was higher (16%; $P < 0.01$) during 8x milking (period 2) than during 2x milking (periods 1 and 3). For gland B, daily BF during 1x milking (period 2) was lower (12 to 14%) compared to 2x milking in period 1 ($P = 0.08$) and period 3 ($P < 0.05$). Daily BF for each gland returned to previous levels when 2x milking resumed in period 3.

Plasma irreversible loss rate of Leu (an estimate of whole-body protein synthesis) did not differ between periods 1 and 2 (11.39 mmol/h for period 1 and 11.32 mmol/h for period 2; SE of the difference of means = 0.72). Similarly, blood concentrations of most AA and glucose were not different between periods 1 and 2, except for Leu and Ile, which were lower ($P < 0.01$) by 10% (155.2 vs. 140.3 μM) and 12% (108.4 vs. 94.9 μM), respectively, during period 2. Concentrations of plasma

Table 1. Mammary blood and plasma flow and net uptakes of plasma glucose and blood AA by adjacent mammary glands unilaterally milked twice daily (2×) and at frequent (8×) or infrequent (1×) intervals¹

Item	Gland A		Gland B		SED	Gland by treatment probability
	2×	8×	2×	1×		
Blood flow (kg/h)	31.38	36.69*	29.86	26.56	1.33	0.008
Plasma flow (kg/h)	23.64	27.78*	22.54	20.17	1.03	0.008
Glucose (mmol/h)	33.0	33.4	26.1	21.3	2.4	NS
AA (μmol/h)						
His	194	268	178	150	75	NS
Ile	1,425	1,432	1,257	941*	132	0.05
Val	1,649	1,911	1,524	1,286	58	0.003
Leu	2,026	2,011	1,872	1,417*	111	0.03
Lys	1,417	1,492	1,326	1,060	49	0.005
Met	377	373	327	264	38	NS
Phe	557	563	503	392*	40	0.06
Tyr	392	451	324	328	25	NS
Thr	518	535	374	332	37	NS
Pro	702	793	583	530	30	0.02
Arg	856	1,102	806	793	58	0.02

¹Values are least squares means and the SE of the difference of means (SED) is the difference between treatments within gland. Both gland A and B were milked twice daily for a 5-d period (period 1), followed by a 5-d period (period 2) during which gland A was milked 8 times daily and gland B was milked incompletely (milk removed was equivalent to 70% of milk yield during period 1) once daily. Net uptake kinetics were monitored for each gland on the last day of each 5-d milking period.

*For within-gland comparisons, the value is significantly ($P < 0.05$) different from the value during period 1.

insulin were not different (52.0 μU/mL for period 1 and 51.8 μU/mL for period 2; SE of the difference of means = 14.4).

Mammary blood and plasma flows during the 8-h kinetic measurements followed the same trends as the daily BF values. Mammary net uptake of glucose did not differ between glands A (8×) and B (1×), but glucose uptake tended ($P = 0.09$) to be lower for gland B when milked 1× compared with 2×. There was a significant treatment by gland interaction for several AA (Table 1), indicating that net uptake of these AA from blood differed between 8× and 1× glands. Net uptake was different ($P < 0.05$) between glands A and B for blood Ile, Leu, Val, Lys, Pro, and Arg. Net uptakes of Ile, Leu, and Phe were lower ($P < 0.05$) for gland B when milked 1× compared with 2× milking.

Based on measurements on plasma, there was a tendency ($P < 0.10$) for net uptake of Leu to differ between 8× and 1× glands (Table 2), and net uptake of Leu was lower ($P < 0.05$) for gland B when milked 1× compared with 2×. Absolute and fractional oxidation of Leu by the mammary glands based on either Leu or MOP as precursor differed ($P < 0.05$) between glands A and B.

DISCUSSION

In the present study, even though the 2 udder halves were exposed to the same concentration of nutrients and hormonal signals, local limitations on milk synthe-

sis were overridden in the gland milked 8× but reduced in the gland milked incompletely (1×). Milk protein yields were higher for the 8× compared with the 1× gland. As planned, milk protein yields were reduced by 23% for the 1× gland. Others have shown that increased milking frequency (3× to 8×) of cows and goats over hours, weeks, or months increases milk yield, although the magnitude of the response can be variable (Maltz et al., 1984; Wilde et al., 1987; Bar-Peled et al., 1995). Endocrine and autocrine factors were considered possible regulators of the frequent milking response. Bar-Peled et al. (1995) compared the hormone (growth hormone, IGF-1, prolactin, insulin, and oxytocin) profiles of cows milked 3× versus 6× daily and observed an increase in oxytocin and a decrease in insulin in the more frequently milked cows at wk 10 of treatment. At 1 and 6 wk of treatment, there were no differences in hormone profiles. In the present study, frequent and infrequent milking regimens were imposed on the goats at the same time by employing the unilateral milking model (Wall and McFadden, 2007), which allowed both glands to be exposed to the same peripheral signals. Despite the glands being milked at different intervals, no differences were observed in insulin concentrations, and, except for slightly lower blood concentrations of Leu and Ile during period 2, no changes in other AA and glucose were observed. Thus, local mechanisms were involved. It was proposed (Wilde et al., 1995) that a local autocrine mechanism involving an inhibitory protein may regulate milk yield when milking frequency

Table 2. Plasma leucine flux and metabolism by adjacent mammary glands unilaterally milked twice daily (2×) and at frequent (8×) or infrequent (1×) intervals¹

Item ²	Gland A		Gland B		SED	Gland by treatment probability
	2×	8×	2×	1×		
Net uptake (μmol/h)	1,867	1,837	1,769	1,359	197	0.10
Leu oxidation (μmol/h)	485	533	381	301	20	0.008
MOP oxidation (μmol/h)	446	507	382	301	34	0.03
Fractional oxidation	0.172	0.206	0.172	0.174	0.010	0.02

¹Values are least squares means and the SE of the difference of means (SED) is the difference between treatments within gland. Both gland A and B were milked twice daily for a 5-d period (period 1), followed by a 5-d period (period 2) during which gland A was milked 8 times daily and gland B was milked incompletely (milk removed was equivalent to 70% of milk yield during period 1) once daily. Leucine kinetics were monitored for each gland on the last day of each 5-d milking period.

²Leucine oxidation was calculated with either Leu or 4-methyl-2-oxopentanoate (MOP) used as precursor at the site of oxidation within the mammary tissues.

is altered. However, there have been no follow-up studies to confirm the role of this inhibitor. More recently, investigations identified local changes in global gene expression that underpin the milk responses to milking frequency, in particular those associated with cell cycle regulation, immune function, neovascularization, and nutrient transport and metabolism (Connor et al., 2008).

Within a day of commencing 8× milking, daily BF to the 8× gland increased in parallel to milk protein yield, whereas BF to the 1× gland was lower compared with that of 2× milking. Effects on milk yield and BF of each gland were transient because both returned to previous levels upon resuming 2× milking. Changes in milk yield have not always been associated with changes in mammary BF, and this has fueled the debate over whether BF drives or acts in support of milk synthesis. For example, short-term (8 h) frequent (hourly) milking of the mammary glands of goats increased milk yield by 15%, but this effect was not paralleled by changes in mammary BF (Maltz et al., 1984). By contrast, short-term infusion of IGF increased milk yield and mammary BF (Prosser et al., 1994), and, in longer term studies (5–7 d), infusions of insulin and administration of growth hormone increased milk yield and mammary BF to similar extents (Fullerton et al., 1989; Bequette et al., 2001). In the present study, glands A and B were both exposed to the same concentration of blood factors, but their rates of BF and milk yield were regulated differentially. The parallels between milk yield and BF over the long term confirms that mammary BF is intimately linked to local regulation of milk synthesis and that this increase in BF is required for long-term maintenance of milk synthesis (Connor et al., 2008). One can only speculate that local stimulation or inhibition of BF serves as part of a regulatory mechanism for adjusting rates of nutrient supply to support anabolic needs of the gland.

Except for a small decrease in blood Leu (10%) and Ile (12%), concentrations of AA and insulin were not affected during the unilateral milking period. The ability to manipulate local rates of milk protein synthesis by milking frequency, without the confounding influences of changes in peripheral blood concentrations of nutrients and hormones, provided an opportunity to examine local regulation of metabolic activity (Wall and McFadden, 2008). Metabolic activity per cell increased for glands milked 3× compared with 2× daily (Wilde et al., 1987), and a large number of metabolism-related genes are upregulated (Connor et al., 2008). Whether net removal of certain AA by the gland and metabolism of Leu are up- or downregulated specifically to accommodate the increase or decrease in milk protein synthesis and cell activity is not known. Net uptake from blood of Ile, Leu, Val, Lys, Pro, and Arg was greater for the gland milked 8× compared with the gland milked 1×. These general patterns were of similar magnitude as the changes in milk protein yield.

It is of interest that milking frequency altered the net uptake by the mammary gland of Ile, Leu, Val, Lys, and Arg because these AA are typically extracted by the gland in excess of requirements for milk protein output. If this excess uptake does not serve an obligatory function in support of milk synthesis, then one would expect the gland to alter the extent of catabolism of these AA in addition to altering net uptake. Almost all these AA were catabolized by the mammary gland but to varying extents depending upon stage of lactation, level of milk production, protein-AA intake, and hormonal status (Bequette et al., 1998). The BCAA were removed by the mammary gland in excess of milk protein output (Guinard and Rulquin, 1994; Bequette et al., 1996b), and for this reason catabolism of the BCAA, particularly Leu, was most studied. There appears to be a positive relationship between milk protein yield and the extent of Leu catabolism (Bequette et

al., 1996b), suggesting a metabolic link between milk yield and Leu catabolism. One exception has been our previous observation in goats where mammary Leu oxidation was reduced to low levels when Leu supply was made limiting (19 vs. 7% of mammary Leu uptake) without an effect on milk production (Bequette et al., 1996a). Because Leu oxidation still occurred, albeit at low levels, it is questionable whether a basal level of oxidation might be required. The frequent milking model provided a test to this theory; thus, if Leu oxidation was obligatory to support milk protein synthesis, then oxidation would be maintained or increased for the gland whose milk protein output was increased by frequent milking. Indeed, the fractional rate of Leu oxidation was maintained for the gland milked 8× and for the gland milked 1×, suggesting that Leu oxidation fluctuates in relation to the demands of milk synthesis. That Leu oxidation was maintained suggests that the oxidative process does not fluctuate to spare Leu and possibly other AA for milk protein synthesis, and, more importantly, that Leu oxidation is not a passive or ancillary mechanism of the mammary gland. Indeed, the mammary gland is a major site of BCAA catabolism in the lactating animal; gene expression, protein, and activities of the BCAA catabolic enzymes increased dramatically during lactation (DeSantiago et al., 1998; Tovar et al., 2001), further supporting the view that BCAA catabolism is a required process. Our conclusions are further supported by recent observations that Lys catabolism by the mammary gland of dairy cows was reduced, but not eliminated, when Lys was made limiting and milk protein yield decreased (Lapierre et al., 2009).

CONCLUSIONS

The present study employed the unilateral milking model to identify physiological and metabolic processes inherently regulated by the mammary gland to support its anabolic needs. Manipulation of milk protein output by altering milking frequency was associated with long-term, local (gland-specific) regulation of mammary BF. Shifts in milk protein yield by glands milked frequently or infrequently were supported by increases or decreases in the mammary net removal of several AA, notably those AA whose extraction by the gland is in excess of milk protein output. Oxidation of Leu by the mammary glands fluctuated in direct relationship to changes in milk protein yield brought about by altered by milking frequency, suggesting that Leu oxidation is a required process for milk biosynthesis but that changes in oxidation do not appear to facilitate changes in AA availability within the mammary gland.

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