

# Lysine Metabolism by the Mammary Gland of Lactating Goats at Two Stages of Lactation

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## ABSTRACT

An arteriovenous kinetics technique was used to monitor mammary gland lysine and protein metabolism in goats ( $n = 4$ ) at two stages of lactation ( $80 \pm 17$  vs.  $233 \pm 14$  DIM) in response to an i.v. infusion of lysine (Lys) plus methionine (Met). At each stage of lactation [ $2\text{-}^{15}\text{N}$ ] and [ $1\text{-}^{13}\text{C}$ ;  $6,6\text{-}^2\text{H}_2$ ] Lys kinetics were performed on the last day of 5-d i.v. infusion of saline followed by Lys (370 mg/h) plus Met (84 mg/h, LM). Milk and protein yields and dry matter intake were higher in early than in late lactation, but LM infusion did not affect these variables. Regardless of stage of lactation, the absolute and fractional oxidation rates of Lys by the mammary gland increased in response to LM infusion. When corrected for Lys oxidation, net uptake of Lys by the gland was less than milk protein Lys secretion. However, correction for the contribution of peptides (15.8%) to Lys uptake brought net Lys uptake close into balance with milk Lys secretion. The present data suggests that when Lys is in excess of requirements, the mammary gland appears to dispose of the extra supply via the oxidative mechanism.

**(Key words:** mammary gland, amino acid metabolism, goats, oxidation)

**Abbreviation key:**  $\alpha$ -AAA =  $\alpha$ -amino adipic acid, **p-AH** = para-aminohippurate, **EAA** = essential AA, **EPA** = external pudic artery, **GC-MS** = gas chromatography-mass spectrometry, **LM** = Lys + Met, **SAL** = saline.

## INTRODUCTION

Lysine and Met are often considered to be co-limiting AA for milk protein synthesis, particularly when maize-based diets are fed to lactating dairy cows (13, 19, 33, 35, 36). Lysine appears to be the first limiting AA when

corn-based rations are supplemented with protein sources of corn origin, and Met may be first limiting when all, or most, of the RUP intake is supplemented by legume or animal by-products, or both (34, 37). Most of the reported responses in milk and milk protein yields to infused Lys and Met have been in cows given diets prepared to be severely limiting in one, or both, AA (18, 19, 23, 28). Indeed, other workers (30, 32) failed to observe any productive responses to supplementing diets with these AA when the ration has been better formulated to meet requirements of high-yielding dairy cows. The overall inconsistency in productive response of dairy cows given extra essential AA (**EAA**) directly into the duodenum perhaps indicates that factors other than the supply of the EAA per se contribute to the responsiveness of the mammary gland. However, scarcity of information on the metabolic fate of EAA in the different tissues and the metabolic partitioning between the productive functions in various physiological situations makes the elucidation of these other factors problematical. Currently available systems for predicting requirements and responses in dairy cows (1, 14, 27) lack this information, which may be one reason why these systems tend to overpredict responses in milk output relative to observed data (13, 25).

In more recent studies in which arterial-venous differences have been measured across the mammary gland, data indicate that the gland often extracts Lys in greater amounts than it subsequently secretes in milk (20), suggesting that factors that control the metabolic fate of Lys in the mammary gland might influence responsiveness of the animal to additional supply of the AA. This study examined the metabolic fate of Lys and the production responses of lactating goats to an intravenous infusion of Lys plus Met in two stages of lactation. Some of the results have been reported elsewhere (24).

## MATERIALS AND METHODS

### Stable Isotopes

L-[ $2\text{-}^{15}\text{N}$ ] and [ $1\text{-}^{13}\text{C}$ ;  $6,6\text{-}^2\text{H}_2$ ] Lys-HCl, L-[ $5\text{-}^{13}\text{CH}_3$ ]Met, and L-[ $5,5,5\text{-}^2\text{H}$ ]Leu (all 99 atom%) were

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**Table 1.** Nutrient composition of the goats diet (% of DM).

Nutrient	Concentrate mix	Grass hay
CP	23.9	8.1
NDF	13.3	60.0
Ether extracts	2.7	3.0
OM	95.7	90.5
ME, MJ/kg	12.7	9.1
Met	0.60	0.20
Lys	1.70	0.85

purchased from Mass Trace (Somerville, MA). Specific mixtures of isotopes were dissolved in saline (9 g of NaCl/L, pH 7) and filter-sterilized (0.22- $\mu$ m filter units) into sterile glass bottles.

### Goats and Diets

Experiments were performed on four nonpregnant, multiparous British Saanen goats (BW = 65.1  $\pm$  6 kg) in late lactation (233  $\pm$  14 DIM) and then again early (80  $\pm$  17 DIM) in the following lactation. Goats had been surgically prepared with catheters (polyvinyl chloride, medical grade, i.d. 0.6 mm, o.d. 0.7 mm; Critchley Electrical Products, NSW, Australia) inserted into each external pudic artery. Transonic flow probes (6 mm; Transonic Systems Inc., Ithaca, NY) were placed around each external pudic artery (**EPA**); and a carotid artery was raised to a subcutaneous position (7, 8). At least 1 d prior to infusion of treatments or kinetic measurements, temporary catheters (polyvinyl chloride, medical grade, i.d. 0.8 mm, o.d. 1.2 mm, Critchley Electrical Products) were inserted into each jugular vein and into the elevated carotid artery and a subcutaneous mammary vein, respectively. Catheters were kept patent by flushing once daily with a sterile heparin-saline (200 U/ml) solution.

Goats were placed in metabolism crates and allowed at least 10 d of adaptation to the frequent feeding by automatic feeders (12 equal portions fed at 2-h intervals) and the daily routines of machine and hand milking (0830 and 1630 h). 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. Diets were formulated to meet metabolizable energy and protein requirements for maintenance and milk production (1, 2). The ration comprised molasses-treated chopped grass hay (10% on a fresh weight basis) and a pelleted concentrate (2:3 on DM basis, Table 1). The concentrate comprised (g/kg of DM): 395 of barley, 150 of whole corn, 150 of wheat feed, 150 of soybean meal, 50 of white fish meal, 50 of linseed flakes, and a vitamin and minerals premix (Norvite Feed Supplies, Inch, United Kingdom). Daily feed refusals were col-

lected and weighed, and feed intake was adjusted to allow 5% refusals.

### Experimental Procedure

The same experimental protocol was employed at both stages of lactation. Goats were given first given a 6-d i.v. infusion of saline (**SAL**, 15 g of saline/h) followed by (after 3 d of no infusion) a 6-d infusion of Lys (370 mg/h) plus Met (84 mg/h) (**LM**). Starting on d 5 of each infusion period they received a 30-h continuous i.v. infusion (50 g of solution/h) of [<sup>15</sup>N]Lys (25 mg/h), [<sup>13</sup>CH<sub>3</sub>]Met (10 mg/h) and [<sup>2</sup>H<sub>3</sub>]Leu (28 mg/h). During h 24 to 30 of this period they also received an i.v. infusion of [<sup>13</sup>C, <sup>2</sup>H<sub>2</sub>]Lys (60 mg/h) containing sodium-heparinate (6.6 kU/h). Over this same period *p*-aminohippurate (**p-AH**; 30 mg/g of solution) was infused into an EPA (only in late lactation). Starting at 23 h, each mammary gland was milked-out (no oxytocin given) by hand at 1-h intervals, the milk was weighed, and subsamples were taken for AA analyses and for casein isolation to determine AA enrichment. Starting at 25 h, blood was continuously (10 ml/h) withdrawn from an arterial source (carotid artery or the EPA) and from the mammary vein over five consecutive 1-h periods. Samples were collected into sealed syringes submerged in an ice bath. Flow probe voltages were logged (every 10 s), and data were integrated over the 1-h blood sampling periods. Flow probes were calibrated in vitro by the procedures outlined by the manufacturer. In the late-lactation experiment, blood flow was also measured by downstream dilution of *p*-AH in the mammary vein. All goats were kept standing during the blood sampling period.

Blood samples were mixed by gentle hand-rolling, and duplicate 1-ml samples were injected into 10-ml Vacutainers containing 0.5 ml of frozen lactic acid, reacted rapidly by vortex, and kept at room temperature. Fresh blood samples were analyzed for plasma HCO<sub>3</sub><sup>-</sup> content and pH (Radiometer ABL3 Blood Gas Analyzer; Radiometer Ltd., Crawley, UK), and packed cell volume (%) was determined by hematocrit.

Samples of whole blood (0.5 g) were accurately weighed, and a known similar weight of a hemolyzing solution containing L-norleucine (100  $\mu$ M) was added as an internal standard for determination of AA concentrations. Whole blood was also prepared for gas chromatography-mass spectrometric (**GC-MS**) analyses by mixing 1 g with an equal weight of ice-cold distilled water. For *p*-AH determination, duplicate samples of blood (0.5 g) were accurately weighed and an equal weight of ice-cold water added. The remaining heparinized blood was centrifuged at 2000  $\times$  *g* for 15 min at 4°C. Plasma (0.5 g) was accurately weighed and a

known similar weight of the L-norleucine standard was added. Plasma (0.7 g) was also dispensed for GC-MS analyses. All samples were stored frozen at  $-20^{\circ}\text{C}$ . Immediately prior to isotope infusion, blood and milk samples were taken to assess the natural (background) abundance of the various isotopes.

Whole milk samples (0.5 g) were accurately weighed and a known similar weight of an L-norleucine standard (13.5 mM) was added for AA analysis. Samples were then hydrolyzed in 4 M HCl (3 ml) at  $110^{\circ}\text{C}$  for 18 h.

## Chemical Analyses

**Concentration and enrichment of AA.** Stored blood (plasma) was thawed at  $4^{\circ}\text{C}$  and deproteinized by centrifugation ( $13,000 \times g$  for 45 min) through filter units (10,000 nominal MW limit; Millipore Corp., Bedford, MA), and free AA concentrations in the protein-free supernatant were determined as previously described (22). Blood and plasma samples were prepared for GC-MS as previously described (4, 6, 10). The blood *p*-AH concentration was assayed by the Bratton-Marshall procedure as described by Smith et al. (38).

**Milk analyses.** Milk N concentration was determined on subsamples by macro-N (combustion) method. Milk crude protein was calculated as milk N  $\times$  6.38. Casein was isolated from milk by a modification of the method of McKenzie (26). Hydrolyzed whole milk and casein were prepared for AA analyses or GC-MS as previously described (6, 9).

Blood  $\text{H}^{13}\text{CO}_2$  enrichments were determined by using a VG isotope ratio-MS Sira-12 as described by (31). All enrichments were expressed as molar percentage excess against the appropriate natural abundance sample.

## Calculations

In this paper, only results for Lys metabolism will be reported.

**Plasma Kinetics.** The flux of Lys in plasma ( $F_p$ ) was calculated from isotope dilution of [ $^{13}\text{C}$ ,  $^2\text{H}$ ]Lys in plasma:

$$F_p \text{ (mmol/h)} = ((E_i/E_{a,Cl}) - 1) \times IR,$$

where  $E_i$  = the enrichment (molar percentage excess) of the infused isotope,  $E_{a,Cl}$  = the enrichment of [ $^{13}\text{C}$ ,  $^2\text{H}$ ]Lys in arterial plasma at plateau, and IR = the rate of isotope infusion.

**Mammary kinetics.** In the late-lactation experiment estimates of blood flow (kg/h) based on flow probe measurements were lower (by 28%) than those determined by the dye-dilution method. To account for this

underestimation in the early lactation goats (where PAH was not given), probe values were adjusted by using the following equation:

$$Y = 1.39X + 0.17, r^2 = 0.922 (P < 0.001, n = 35)$$

where X = probe value and Y = adjusted value. The intercept did not differ from zero ( $P > 0.95$ ).

Kinetic calculations were only performed for the udder half that was monitored. Net uptake (NU) of Lys was based on blood and plasma exchanges, calculated as:

$$\text{NU } (\mu\text{mol/h}) = (C_{a,l} - C_{v,l}) \times \text{BF}_{b \text{ or } p}$$

where C = the concentration ( $\mu\text{M}$ ) of free Lys (l) in arterial (a) and mammary venous (v) blood (b) or plasma (p) and  $\text{BF}_p = \text{BF}_b$  (l/h)  $\times$  (1 - (packed cell volume/100)) as appropriate.

The irreversible loss of Lys across the mammary gland ( $F_{mg}$ ) was based on plasma transfers as:

$$F_{mg} \text{ } (\mu\text{mol/h}) = [(C_{a,l} \times E_{a,Cl} - C_{v,l} \times E_{v,Cl})/E_{a,Cl}] \times \text{BF}_p$$

where  $E_{a,Cl}$  and  $E_{v,Cl}$  = the enrichment of [ $^{13}\text{C}$ ,  $^2\text{H}$ ]Lys in arterial and mammary venous plasma, respectively. Partition of plasma flux to the mammary gland ( $K_{mg}$ ) was calculated as:

$$K_{mg} \text{ (}/d) = F_{mg}/F_p$$

Fractional oxidation rate of Lys (FOR) was calculated as

$$\text{FOR} = (C_{v,c} \times E_{v,c} - C_{a,c} \times E_{a,c}) / (C_{a,l} \times E_{a,Cl} - C_{v,l} \times E_{v,Cl}).$$

The rate of oxidation of Lys (LO) was calculated as

$$\text{LO } (\mu\text{mol/h}) = ((C_{v,c} \times E_{v,c} - C_{a,c} \times E_{a,c}) \times \text{BF}_p) / E_{cas,Cl},$$

where the lowercase subscript c denotes the enrichment or concentration of  $\text{CO}_2$  in blood, and  $E_{cas,Cl}$  = the enrichment of the precursor at the site of oxidation. The enrichment of [ $^{13}\text{C}$ ,  $^2\text{H}$ ]Lys in casein was considered most appropriate. However, because the enrichment of [ $^{13}\text{C}$ ,  $^2\text{H}$ ]Lys in casein had not plateaued by the end of the 6-h infusion, but the long-term (30-h) infused [ $^{15}\text{N}$ ]Lys had, a predicted enrichment for [ $^{13}\text{C}$ ,  $^2\text{H}$ ]Lys in casein at plateau ( $E_{cas,Cl}$ ) was calculated from the relationship of the [ $^{15}\text{N}$ ]Lys in arterial plasma and casein at plateau as follows:

$$E_{cas,Cl} = E_{a,Cl} \times (E_{cas,Nl}/E_{a,Nl}),$$

**Table 2.** Feed intake and milk production of lactating goats.

Stage of lactation Infusion <sup>1</sup>	Early		Late		SE	Main effect	
	SAL	LM	SAL	LM		Lactation	Infusion
DMI, g/kg BW <sup>0.75</sup>	115.3	116.3	92.3	92.3	6.85	0.01	NS <sup>2</sup>
Milk production <sup>3</sup> kg/d	4.64	4.62	2.27	2.00	0.31	<0.001	NS
Protein yield, g/d	137.4	138.5	89.6	82.3	11.3	0.001	NS
Protein content, %	2.97	2.99	3.93	4.14	0.08	<0.001	NS
Experiment yield <sup>4</sup> , g/h	93.0	100.9	52.3	48.1	12.2	<0.001	NS

<sup>1</sup>SAL = i.v. saline infusion, LM = i.v. Lys plus Met infusion.

<sup>2</sup>NS = Not significantly different ( $P > 0.20$ ).

<sup>3</sup>Milk production on the days prior to kinetic measurements.

<sup>4</sup>Milk yield by an udder half during the 6-h blood sampling period.

where  $E_{cas,Nl}$  and  $E_{a,Nl}$  = the enrichments of [<sup>15</sup>N]Lys in casein and in arterial plasma at plateau, respectively.

Protein synthesis (PS; gain or secretion) in the mammary gland was calculated as

$$PS (\mu\text{mol/h}) = ((C_{a,l} \times E_{a,Cl} - C_{v,l} \times E_{v,Cl}) - (C_{v,c} \times E_{v,c} - C_{a,c} \times E_{a,c}) \times BF_p) / E_{cas,Cl}$$

**Statistical Analyses**

Results were analyzed by a linear model that included 2 × 2 factorial arrangement. The model included main effects of goat, infusion (SAL vs. ML), stage of lactation (early vs. late), time of sampling, their interaction and residual error terms, the GLM procedure of SAS was used to run statistical analyses (39). When there was no effect of an interaction term or sampling time in all the variables tested, the model was reduced accordingly to include the proper main effects and the error residual term. Means are presented as least square means. Means were considered significantly different at  $P < 0.05$ .

**RESULTS**

**Animal Performance**

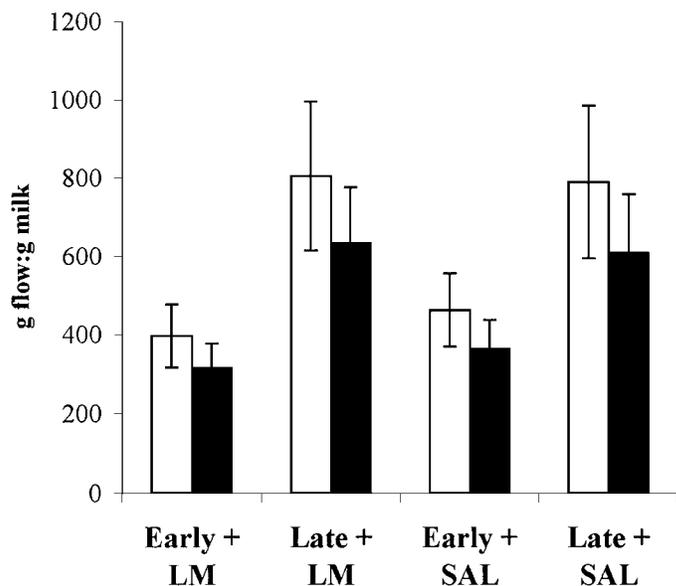
Dry matter intake (g/kg of metabolic BW), and milk volume and milk protein yields were higher in early compared to late lactation (Table 2). However, milk protein concentration was higher in late lactation. None of these variables responded to infusion of LM.

Mammary blood and plasma flows were not affected by infusion of LM and were similar at the two stages of lactation. The hourly milk secretion on the day of blood sampling was higher ( $P < 0.0001$ ) in early lactation than in late lactation and averaged 97 and 50 g/h, respectively (Table 2). The relationship of blood or

plasma flow to milk yield is presented in Figure 1. This relationship was lower ( $P < 0.0001$ ) in early than in late lactation but LM infusion had no effect on the ratio.

**Lys Kinetics and Metabolism**

**Plasma kinetics.** Plasma flux ( $F_p$ ) and arterial plasma concentration of Lys were higher in early than in later lactation (Table 3). During infusion of LM,  $F_p$  increased by 23 and 42% in early and late lactation (Table 3). The increase in  $F_p$  resulting from LM infusion was on average 3.37 mmol/h. This was greater than the



**Figure 1.** Mean ratios (±SE) of mammary blood (g/h; open bars) and plasma (g/h; bold bars) flows to milk yield (g/h) for half the udder in early and late lactating goats receiving continuous i.v. infusions of SAL and LM. Ratios were lower ( $P < 0.0001$ ) in early compared with late lactation, averaging 429 and 796 (SE = 130.3) kg blood/kg of milk and 338 and 619 (SE = 99.3) kg plasma/kg of milk, respectively.

additional Lys infused (2.53 mmol/h). Lysine arterial plasma concentration increased on infusion of LM by 60% in early lactation and 82% in late lactation. Partition of plasma Lys flux to the mammary gland ( $K_{mg}$ ) tended ( $P < 0.1$ ) to be higher in early compared to late lactation (0.15 vs. 0.14), and LM infusion decreased  $K_{mg}$ .

**Mammary Lys metabolism.** Net uptake of Lys from blood and plasma and secretion of Lys in milk were all higher in early than in late lactation. LM infusion did not affect milk Lys secretion and plasma net uptake; however, net uptake of Lys from blood was reduced by LM infusion.

Approximately 20% of the Lys extracted by the gland was oxidized at both stages of lactation, and this proportion increased (~31%) in response to LM infusion. These kinetic data tended to be supported by the isotope balance data, which indicated that uptake of isotope was 17 to 58% greater than milk output and similarly, isotope balance tended to increase ( $P < 0.09$ ) in response to LM infusion. Thus, a greater amount of Lys was oxidized by the gland in early lactation, and at both stages of lactation LM infusion stimulated Lys oxidation.

Net uptake of free Lys from blood, corrected for oxidation losses, indicated that not enough Lys was extracted by the gland to account for milk Lys output. However, during SAL infusion, when the estimated contribution of vascular peptides to free Lys uptake was considered, enough Lys was available to account for Lys in milk protein. When LM was infused, however, insufficient free and peptide-bound Lys was apparently extracted for casein synthesis.

## DISCUSSION

Even though Lys and Met are reported to be co-limiting AA for milk protein synthesis, particularly when maize-based diets are fed to lactating dairy cows (13, 19, 33, 35, 36), the net removal of Lys by the mammary gland is often found to be in excess of its requirements for milk protein synthesis. This raises the possibility that, as a limiting AA, Lys may serve a role within the gland other than as a substrate for milk protein synthesis. However, little is known of the metabolism of Lys by the mammary gland, particularly in vivo. The objectives of the present study were 1) to develop a

**Table 3.** Whole body (plasma) and mammary metabolism of Lys by an udder half of lactating goats.

State of lactation Infusion <sup>1</sup>	Early		Late		SE	Main effect	
	SAL	LM	SAL	LM		Lactation	Infusion
	————— P —————						
Item <sup>2</sup>							
Whole body metabolism							
Plasma Lys, $\mu$ M	145	233	111	202	16.2	<0.001	<0.001
$F_p$ , mmol/h	12.70	15.62	7.91	11.21	0.44	<0.001	<0.001
$K_{mg}$	0.154	0.142	0.161	0.111	0.02	0.10	<0.001
Mammary blood flow, kg/h							
Whole blood	37.7	35.4	33.8	35.4	2.79	NS <sup>3</sup>	NS
Plasma	29.4	28.2	27.3	27.8	2.14	0.19	NS
Mammary metabolism, $\mu$ mol/h							
Net uptake (U)							
Blood	1781	1375	1272	943	225	<0.001	<0.001
Plasma	1317	1343	1167	1022	185	0.01	NS
Protein synthesis	1849	2020	1194	995	297	0.0001	NS
Milk Lys output (O)	1720	1706	1200	1020	245	0.0001	NS
Absolute oxidation	432	468	303	458	86	0.035	0.037
Fractional oxidation rate	0.20	0.28	0.20	0.34	0.06	NS	<0.001
Balance ratio (U:O)							
Isotope <sup>4</sup>	1.17	1.58	1.42	1.57	0.35	NS	0.09
Lysine							
Free AA <sup>5</sup>	0.85	0.57	0.91	0.53	0.18	NS	0.001
Free AA + peptides <sup>6</sup>	1.06	0.81	1.06	0.81	0.19	NS	0.002
Casein:plasma enrichment	0.86	0.81	0.85	0.85	0.04	NS	0.14

<sup>1</sup>SAL = Saline i.v. infusion, LM = Lys plus Met i.v. infusion.

<sup>2</sup> $F_p$  = Flux of Lys in plasma,  $K_{mg}$  = proportion of plasma flux partitioned to the udder.

<sup>3</sup>NS = Not significantly different ( $P < 0.20$ ).

<sup>4</sup>Calculated as: [<sup>15</sup>N]Lys output from plasma  $\div$  [<sup>15</sup>]Lys output in milk casein.

<sup>5</sup>Ratio of net uptake to milk Lys output, where Free AA = (blood free Lys uptake – oxidative losses) and

<sup>6</sup>Free AA + peptides = (blood free + peptide-bound Lys uptake – oxidative losses). Peptide uptake calculated as: milk Lys output  $\times$  (1 – ratio of [<sup>15</sup>N]Lys enrichment in casein to plasma). Milk output was corrected for 4% of milk protein AA derived from nonmammary synthesized proteins appearing in the milk (40).

tracer technique for monitoring the metabolism of Lys by the mammary gland in vivo and 2) to examine the metabolic fate(s) of Lys in response to a change in the supply of Lys (plus Met) to the gland. The experiments were conducted over two stages of lactation to examine whether the relationships between nutrient supply and milk output changed in response to lactational demand.

At both stages of lactation, the i.v. infusion of Lys plus Met failed to increase milk protein yield, despite a 60 to 82% increase in the arterial concentration and supply of these AA to the mammary gland. Thus, the argument would be that Lys and Met were probably not limiting for milk production under the conditions of the present study wherein goats were fed a ration consisting primarily of maize, barley, soybeans, and fishmeal as protein sources. The major response of the gland during the LM infusion was to oxidize more Lys, both on an absolute and fractional basis. However, infusion failed to stimulate any increase in Lys uptake by the gland, so during LM infusion less Lys was available within the mammary gland for protein synthesis compared with SAL infusion.

In earlier studies, Bequette et al. (9) had suggested that the oxidative process within the udder might compete with milk protein biosynthesis for AA supplies. Subsequent work from this group has tended to dismiss this hypothesis; when Leu oxidation by the mammary gland was reduced, milk protein synthesis was not altered (8). A simple interpretation of the present results seems to be that since Lys supply was in excess of milk protein synthetic requirements (i.e., no response in milk production to their addition), the oxidative process served only as a mechanism to dispose of the extra supply of Lys.

Oddy et al. (29) reported that in well-fed lactating goats the fractional oxidation of Leu by the mammary gland was lower in early compared to late lactation (0.08 vs. 0.34,  $P < 0.05$ ). In contrast, during the control periods (SAL infusion) in the present study the fractional oxidation rate of Lys was similar in early and late lactation, despite large differences in DMI and milk production of the goats. These data could be interpreted as suggesting that an obligatory, perhaps minimal proportion of Lys uptake is required to be oxidized. If such a requirement exists, it will be necessary to reevaluate the models currently used to predict the efficiency and postabsorptive requirements of Lys for milk production (21). An obligatory requirement to oxidize Lys will need to be tested in the same way as Bequette et al. (8) examined Leu oxidation but, if substantiated, this should help to simplify the current models of AA metabolism being developed for the mammary gland (16, 21).

Because the LM infusion did not alter milk production, it was not surprising that the infusion did not

change total gland protein synthesis at either stage of lactation. From early to late lactation, however, gland protein synthesis fell by 840  $\mu\text{mol Lys/h}$ , while milk protein-Lys output decreased by only 603  $\mu\text{mol Lys/h}$ . This differential (237  $\mu\text{mol Lys/h}$ ) suggests that additional biosynthetic processes are associated with milk protein synthesis. Champredon et al. (12) and Baracos et al. (5) have reported that total gland protein synthesis is 42 to 72% higher than net milk protein output. Present data indicate 39%  $((237 \div 603) \times 100)$  extra nonmilk protein synthesis between the stages of lactation. However, when we compared total gland protein synthesis to milk protein output within either stage of lactation, we observed that total synthesis was similar to milk protein output. The latter suggests that little, if any, nonmilk protein synthesis occurs within the gland, which would seem to be highly unlikely given the need to maintain mammary tissue protein mass and other constitutive processes.

The latter anomaly may relate to some limitations of the arterio-venous kinetic technique and model. The validity of the arterio-venous technique for measuring protein and AA metabolism depends on several assumptions. One is that only the vascular free AA contributes to tissue anabolic and catabolic pathways, but this assumption has recently been challenged by several reports that have compared the isotopic enrichment of milk protein AA and plasma free AA (4, 7). Bequette et al. (6) estimated that in late-lactation goats, fed the same diet as reported herein, ~12% of Lys and ~17% of Met for casein synthesis derived from vascular non-free AA sources. In the present study, when net uptake of blood free Lys was corrected for oxidative losses and then compared to milk Lys output, the supply of Lys for milk protein synthesis was short by 16 to 45%.

If adjustments are made to account for the contribution of vascular peptide-bound Lys to casein synthesis (by the precursor-product labeling technique; 3), the supply of Lys comes nearly into balance with its requirements for casein synthesis (Table 3). Furthermore, the calculated contribution of peptides to milk protein-Lys synthesis was not significantly affected by altering the supply of free Lys. This is in contrast to our previous studies with [ $^{13}\text{C}$ ]Phe (6) in which increasing the supply of free Phe to the gland reduced the contribution of peptide-bound Phe from 11 to 3%. While currently there is little direct confirmation of the existence of a mammary peptide transport system(s), evidence does seem to be mounting to suggest that peptide-bound AA can be used by the mammary gland and that the transport systems carrying these peptides seem to be selective for peptides having specific AA sequences and which appear to differ in their regulation by free AA supply.

Another assumption inherent in the arteriovenous mammary model is that the activity of the precursor pool at the sites of protein and AA metabolisms can be determined. The inability to directly monitor the intracellular pool activity by biopsy has necessitated that these data be derived from measurements of the appropriate metabolites disappearing from or returning to the vascular blood pools (red blood cells and plasma) via the tissues. As an alternative, we monitored the enrichment of Lys in secreted milk casein at plateau. This choice gave higher estimates of protein synthesis (data not shown) compared to the vascular alternatives (arterial and venous). One possible limitation to the use of the enrichment in casein is that it will reflect the activity of the secretory cells only, and these cells may differ in metabolic activity compared to the nonsecretory cells of the gland.

The estimation of tissue AA oxidation also requires an appropriate measurement of the activity within the tissue at the sites of AA oxidation or interconversion. In the case of Lys, the enrichment of  $\alpha$ -amino adipic acid ( $\alpha$ -AAA), the secondary product of Lys catabolism that undergoes intracellular oxidation, has been monitored by others to assess Lys oxidation in the whole body of humans (15). Herein, we used the adjusted enrichment of [ $^{13}\text{C}$ ,  $^2\text{H}$ ]Lys (based on [ $^{15}\text{N}$ ]Lys in casein), which should reflect the intracellular enrichment of the  $\alpha$ -AAA, again, at least within the secretory cells of the gland. One unavoidable limitation of the arteriovenous kinetic technique is that the secondary metabolite,  $\alpha$ -AAA, which is produced primarily within the liver from the [ $^{13}\text{C}$ ]Lys, may be extracted and oxidized by the gland, thus leading to an overestimation of Lys oxidation. This contribution was apparent in the present study in the LM infusion since the Lys net balance data, where free plus peptide-bound Lys uptake and Lys oxidation were considered, did not match milk Lys outputs.

In this study we took the opportunity to examine changes in mammary blood flow in the same animals in early (80 DIM) and in late (233 DIM) lactation when milk production differed nearly twofold. Conventional wisdom would suggest that metabolic activity is directly linked to the regulation of tissue blood flow to supply  $\text{O}_2$  to fuel metabolism (17). One would expect, therefore, that as milk production decreased (as lactation proceeded), metabolic activity and  $\text{O}_2$  consumption would also decrease and blood flow rate would decrease accordingly. This latter is the conceptual basis of a recent model developed by Cant and McBride (11) to predict changes in mammary blood flow rate and nutrient uptake from changes in mammary metabolic activity and milk production (i.e.,  $\text{O}_2$  consumption and adenylate charge). In the current experiments, however, the ratio

of blood flow to milk yield was much lower in early compared to later lactation (400:1 vs. 800:1), suggesting that the metabolic activity to blood flow relationship does not apply, at least between stages of lactation.

## CONCLUSIONS

An arteriovenous tracer technique, developed to monitor Lys metabolism by the mammary gland of lactating goats, indicated that both blood free (85%) and peptide-bound (15%) Lys contribute to mammary gland protein metabolism. This contribution of peptides to Lys uptake by the gland does not seem to be altered by the supply of free Lys. A considerable proportion (20 to 35%) of Lys uptake was oxidized and this mechanism is possibly a disposal route for excess AA taken up by the gland. The findings in this study would seem to indicate that previous assumptions of a simple unidirectional (non-metabolized) transfer of blood free AA into milk proteins needs to be reconsidered to take account of 1) substantial uptake of peptides and 2) variable oxidation of intracellular free Lys.

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