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Protein Metabolism in Lactating Goats Subjected to the Insulin Clamp

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ABSTRACT

A model of Leu and protein metabolism by the mammary gland and hind leg of lactating goats was constructed and evaluated from data collected by using [^{15}N, 1-^{13}C]Leu kinetics measured during amino acid (AA) infusion and a hyperinsulinemic-euglycemic clamp (IC). Goats were given continuous intravenous infusions of either saline or AA (65 g/d) for 7.5 d and from d 5 to 7.5 goats were subjected to IC. Arteriovenous kinetics were monitored on d 4 and 8 by continuous infusion (8 h) of [^{15}N, 1-^{13}C]Leu. Milk protein yield was increased by IC (+10%) and IC + AA (+21%), whereas AA infusion had no effect. The data were used to construct model equations that describe rates of protein synthesis and degradation, and from these equations, milk and muscle net protein synthesis were described. The model was unable to describe the observed responses in milk protein synthesis. Similar to observations in the literature, net protein gain by the hind leg increased with AA, IC, and IC + AA infusion, primarily through stimulation of protein synthesis by AA. For both tissues, IC depressed Leu oxidation, but only in the absence of AA infusion. Although the IC appears to regulate the ability of the mammary gland to coordinate blood flow and Leu catabolism in support of protein synthesis, our ability to construct a precise model describing mammary protein anabolism is still limited. In contrast, the response in protein anabolism of the hind-leg tissues of these midlactation goats was predicted well by the model, which indicate that the leg tissues were more sensitive to AA supply than the mammary gland.
(**Key words:** insulin, protein synthesis, amino acid metabolism, milk protein)

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Abbreviation key: BCAA = branched-chain amino acids, **IGF-1** = insulin-like growth factor-1, **MOP** = 4-methyl-2-oxopentanoate.

INTRODUCTION

In well-fed dairy ruminants, supplementation with protein or AA increases milk protein yield only marginally (Bequette et al., 2001; Griinari et al., 1997; Mackle et al., 1999), leading to speculation that the supplies of other nutrients are more limiting or that further endocrine stimulation is required to raise the metabolic set point for milk production (Cant et al., 1999). Support for the latter view comes from studies where plasma insulin and insulin-like growth factor-1 (**IGF-1**) have been elevated by use of the hyperinsulinemic-euglycemic clamp technique, where milk protein yield is increased by 15 to 28% in response to AA supplementation (Bequette et al., 2001; Griinari et al., 1997; Mackle et al., 1999).

The apparent shift in whole-body partition of AA in favor of milk protein synthesis led the group at Cornell (Griinari et al., 1997; McGuire et al., 1995) to propose that the insulin clamp establishes a new plane of metabolism through stimulation of the IGF system and coordination of tissue metabolism and physiology. Mammary blood flow and AA uptake are upregulated by the insulin clamp (Bequette et al., 2001; Mackle et al., 2000a). Recently, we demonstrated that the insulin clamp also upregulates blood flow and AA-uptake processes across the hind-leg tissues of lactating goats (Bequette et al., 2001), suggesting that the insulin clamp alters whole-body protein metabolism in a way that enhances the supply of AA to all productive tissues, not just to the mammary gland. What remains unclear is how the insulin clamp alters the metabolic use (protein synthesis and degradation, and AA catabolism) of AA by these and other tissues (Debras et al., 1989; Larbaud et al., 1996; Tauveron et al., 1994; Tesseraud et al., 1993), and the role of AA supply in this process.

To date there are few, if any, adequate biomathematical descriptions of AA use by ruminant tissues, espe-

cially muscle and the mammary gland, which are two potentially competing tissues for substrate supplies. Therefore, our objective was to determine whether or not we could use data collected by using an arteriovenous [^{15}N, 1-^{13}C]Leu kinetic technique to construct models describing Leu and protein metabolism by the mammary gland and a hind leg of lactating goats. The models were constructed from data collected in a previously published experiment with lactating goats subjected to an hyperinsulinemic-euglycemic clamp and infusion of AA (Bequette et al., 2001), and from additional isotope data reported herein.

MATERIALS AND METHODS

Goats, Diet, and Infusions

The Home Office Inspectorate (United Kingdom) and the Ethical Review Committee of the Rowett Research Institute approved all animal handling and experimental and surgical procedures. Four lactating goats were surgically prepared with flow probes (Transonics Systems Inc., Ithaca, NY) around the external iliac (8 mm) and the external pudic artery (6 mm) of the same hindquarter. The external pudic and perineal veins were ligated to eliminate contributions from these vessels to mammary blood flow and venous metabolite concentrations, and a carotid artery was elevated to a subcutaneous position (Bequette et al., 2001). Hind-leg blood flow (~60% of total hind-leg blood flow; B.J. Bequette, personal observation, 1999) was calculated from the difference between external iliac and pudic artery flows.

Experimentation was initiated no less than 1 mo after surgery when goats averaged 63.3 (± 6.9) kg of BW and were at 142 (± 20) days of lactation. Goats were fed 95% of ad libitum intake a ration comprised (60:40, as-fed basis) of a pelleted dairy concentrate (223 g of CP and 11.3 MJ of metabolizable energy/kg of DM) and molasses-treated (20%, as-fed basis) hay (59 g of CP and 11.9 MJ of metabolizable energy/kg of DM). Throughout the experiment, the ration was delivered every 2 h in equal meals via an automatic feeder and feed intake was fixed during the period of the experiment. Animals were milked daily at 0830 and 1630 h.

Goats were assigned to intravenous infusions and the insulin clamp according to a balanced crossover design. The experiment consisted of two periods (7.5 d each, separated by 4 wk) of continuous jugular vein infusions of either saline (1 L/d, pH 7.4, equivalent to the sodium and chloride contents in the AA infusate) or a complete mixture of AA in the composition of cow's CN (1 L/d of a pH 7.4 solution containing 65 g of AA). The AA solution was filter (0.2-μm filter cartridges) sterilized and made fresh every 2 d. Beginning on d 5 of each period, a hyperinsulinemic-euglycemic clamp was maintained for 3.5 d. At least 1 d before each 7.5-d period, temporary catheters were inserted (10 cm) into the carotid artery, the contralateral jugular vein, the left mammary vein, and the tarsal vein of the left hind leg. Procedures for catheter insertion and maintenance were described in the companion paper (Bequette et al., 2001). Blood flow to the udder half and the hind leg was monitored continuously (24 h/d) throughout each 7.5-d period.

One week before the start of each infusion period, goats were placed in metabolic crates for acclimatization. Between infusion periods, animals were placed in floor pens and automatic feeders delivered the ration as before.

The insulin-clamp technique and blood-monitoring procedures for maintaining euglycemia were as described previously (Bequette et al., 2001). Milk yield was recorded at each milking and subsamples were taken for analysis of true protein (based on cow CN standards) as previously described (Bequette et al., 2001).

Tracer Protocol and Blood Collection

Measurements of [^{15}N, 1-^{13}C]Leu (99 atoms % ^{13}C and ^{15}N; Mass Trace, Somerville, MA) kinetics by an udder half and a hind leg were performed on d 4 and 8 (the last day) of each period. At 0830 h, goats were given an intrajugular dose of oxytocin (1 IU), and the mammary glands were milked out completely by machine and hand milking. Immediately, an 8-h continuous intrajugular infusion of [^{15}N, 1-^{13}C]Leu (440 μmol/h, containing heparin [6,500 IU/h]) was initiated. The addition of heparin prevented clotting during blood withdrawal. During the last 4 h of isotope infusion, blood from the carotid artery, mammary vein, and tarsal vein was continuously withdrawn over 1-h periods, by using a peristaltic pump, into sealed syringes submerged in an ice bath. Blood samples were immediately mixed by gentle hand rolling, and 2 × 1-ml samples were injected into evacuated 10-ml Vacutainers containing 0.5 ml of frozen lactic acid. Samples were reacted by vortex and kept at room temperature until analyzed for blood $H^{13}CO_3$ enrichment within 2 wk. Fresh blood samples were also analyzed for concentrations of plasma HCO_3^- (Radiometer ABL3 Blood Gas Analyzer; Radiometer Ltd., Crawley, UK) and packed cell volume. Plasma was harvested after centrifugation (2000 × g for 15 min at 4°C), and to known weights of plasma (0.7 g) were added equal known weights of a solution containing 100 μM L-norleucine and 20 μM 2-oxohexanoate. These samples and the extra plasma were stored at –20°C.

At the end of the 8-h infusion, the mammary glands were milked out after an intrajugular dose of oxytocin (2

IU), the milk from each gland weighed, and subsamples taken for determination of total AA content (Bequette et al., 1999a). Fifteen minutes later, the glands were milked out again by hand, and the CN isolated (Bequette et al., 1999a) for determination of [^{15}N, ^{13}C]Leu enrichment as a measure of mammary tissue intracellular pool activity. The repeated milking and 15-min delay served to provide a highly enriched sample not contaminated with unlabeled CN accumulated during the first 8-h of isotope infusion. Samples of blood, plasma, and CN were obtained on the day before isotope infusion to provide natural abundance background samples.

Analytical Methods

Plasma was analyzed for free AA concentrations as previously described (Bequette et al., 1999a). Plasma and CN samples were processed for gas chromatography-mass spectrometry of [^{15}N, 1-^{13}C] and [1-^{13}C]Leu, and plasma for [1-^{13}C] 4-methyl-2-oxopentanoate (**MOP**) as previously described (Bequette et al., 1999a). [1-^{13}C]MOP enrichment was determined from the quinoxalinol tertiary butyldimethylsilyl derivative under electron-impact ionization conditions on the mass spectrometer (Trio-1; VG Mass Lab, Manchester, UK). Plasma concentrations of MOP were also determined from gas chromatograph peak areas, corrected for the known addition of hexanoate. Enrichments of [^{15}N, 1-^{13}C] and [1-^{13}C]Leu were based on the tertiary butyldimethylsilyl derivative under electron impact conditions (Calder and Smith, 1988). Enrichments of singly ([1-^{13}C]) and doubly ([^{15}N, 1-^{13}C]) labeled Leu were determined from the peak area ratios of the ions at m/z (mass-to-charge ratio) 302, 303, and 304 for the unlabeled and singly and doubly labeled species, respectively. Calibration curves were generated from gravimetric mixtures of labeled and unlabeled Leu for both the singly and the doubly labeled Leu, and correction was made for the spillover from the [1-^{13}C]Leu (M + 1) into [^{15}N, 1-^{13}C]Leu (M + 2). All enrichments are expressed as mole percent excess. Total [^{13}C]Leu enrichment was the sum of singly and doubly labeled Leu.

Kinetic Calculations

An arteriovenous difference technique combined with an intravenous infusion of [^{15}N, 1-^{13}C]Leu was used to measure Leu and protein metabolism by the mammary gland and a hind leg. Three-compartment kinetic models were constructed that allowed calculation of the fluxes of plasma unlabeled and labeled Leu, MOP, and CO_2 by the tissues (Figure 1). The model of Biolo et al. (1992) was used for the mammary gland and the model

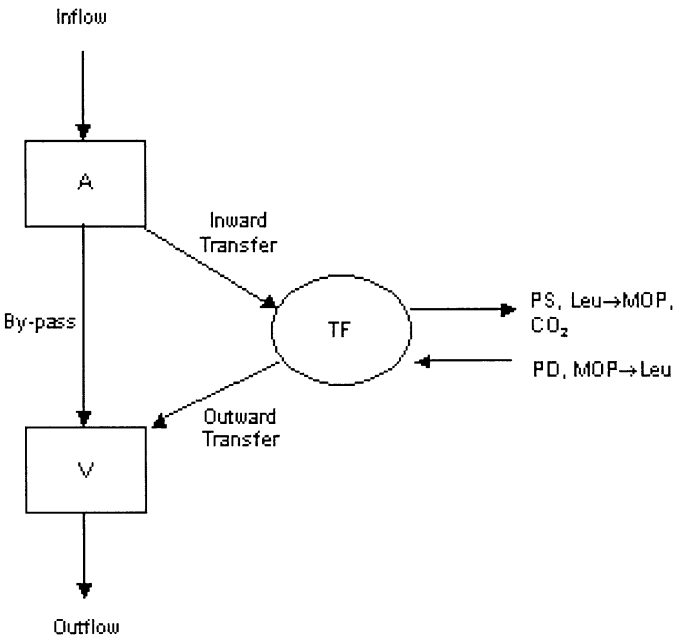

Figure 1. A three-compartment model of mammary gland and hind-leg metabolism of Leu. See Appendix for further description of fluxes. Abbreviations: A, arterial plasma pool; V, venous plasma pool; TF, tissue intracellular precursor free pool; PS, protein synthesis; PD, protein degradation; MOP → Leu, reamination of MOP to Leu; Leu → MOP, deamination of Leu to MOP; CO_2, oxidation of MOP.

of Cheng et al. (1985) was used for the hind leg. The two models differ only in the selection of intracellular precursor pool. For the mammary model, the enrichment of Leu in secreted milk CN obtained at the end of the 8-h isotope infusion was used to represent the intracellular free pool. We have found that the enrichment of AA in CN at 9 h of a continuous infusion of isotope approximates that of the mammary tissue's homogenate free pool (Bequette et al., 1999b). The hind-leg model uses the venous pool as a surrogate for the intracellular precursor. The mathematics for determining the individual fluxes for each model are given in the Appendix.

Dilution of the various labeled forms of Leu in the intracellular pool reflects the production of unlabeled Leu arising from protein degradation and transamination. Theoretically, if the total [^{13}C]MOP pool carries the same enrichment as the total [^{13}C]Leu pool, then the rate of isotope dilution of total [^{13}C]Leu from transamination would be zero and the rate of MOP reamination to Leu can be calculated by difference. Oxidation of Leu by the udder and the hind leg was computed from the release of the C-1 atom as CO_2. The venous [^{13}C]Leu enrichment was selected to represent activity at the site of decarboxylation in the mammary gland based on previous comparisons of the isotopic labeling

patterns of MOP, Leu, and CN-bound Leu (B. J. Bequette, unpublished data, 1994). For the hind leg, the venous enrichment of [^{13}C]MOP was used based on observations that muscle free and plasma MOP enrichments are similar (Watt et al., 1992). Transfers of Leu nitrogen and carbon from the plasma to the mammary gland were calculated from the model of Biolo et al. (1992) except that the enrichment of Leu in milk CN served as a measure of the intracellular pool. For the hind-leg tissues, flux calculations were based on gross (irreversible) removals from plasma. For the mammary model, inward transfer from plasma represents the only source of labeled Leu, thus intracellular (milk CN) dilution of [^{15}N, ^{13}C]Leu arises from protein degradation and de novo synthesis from MOP. Similar logic was applied to the hind-leg model except that the arterial to venous dilution of [^{15}N, ^{13}C]Leu was assumed to represent similar dilution occurring within the leg tissues.

Statistics

Data were analyzed in Genstat 5 (Lawes Educational Trust, Rothamsted, Herts, UK) by ANOVA for mammary data and by residual maximum likelihood for the hind-leg data where one of eight experimental periods was missing because of catheter failure. For both models, AA and insulin were declared fixed effects and goat and experimental period were declared random effects. The model was described by the following equation:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \beta\gamma_{jk} + \eta_{ij} + \varepsilon_{ijk}$$

where μ = constant, α_I = animal blocking effect, β_j = AA treatment effect, γ_k = insulin-clamp treatment effect, $\beta\gamma_{jk}$ = AA by insulin-clamp effect, η_{ij} = period blocking effect, and ε_{ijk} = error term. Individual treatment differences were detected by *t*-test by using the appropriate SED and degrees of freedom for within and between level comparisons.

RESULTS

Daily milk protein yields were increased ($P < 0.05$) by 10 and 21% by IC and IC + AA, respectively, whereas infusion of AA had no effect (Bequette et al., 2001). Hourly rates of milk protein secretion recorded during the p.m. milking intervals, corresponding to the time period of isotope kinetic measurements, were also increased ($P < 0.05$) by 9 and 21% by IC and IC + AA, respectively, and infusion of AA, again, had no effect (Figure 2).

Plasma insulin was significantly increased by 3.3-fold and plasma IGF-1 by 34% by the insulin clamp (Table 1). Euglycemia was maintained during IC and IC + AA. Plasma urea was significantly reduced (by 13 to 17%) by IC and IC + AA.

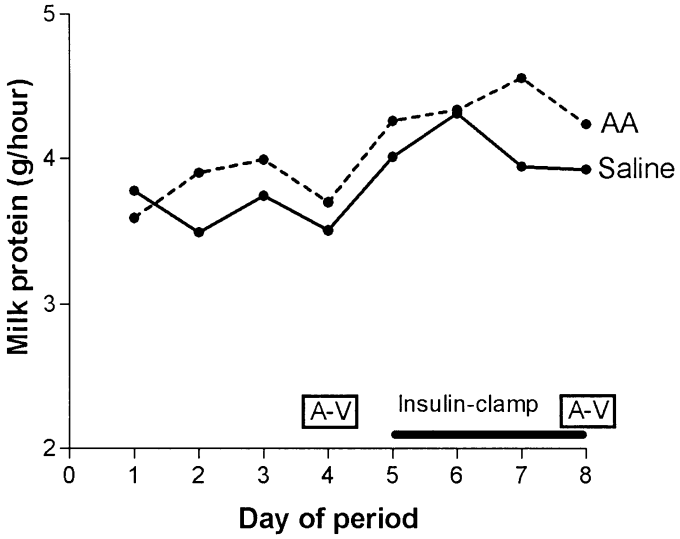

Figure 2. The hourly rates of milk protein yield during the p.m. milking intervals of each infusion period. Saline (solid line) or a complete mixture of AA (dashed line) were infused for 7.5 d, with goats subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d as indicated. Arteriovenous measurements were made on d 4 and 8 as indicated by A-V. The insulin clamp increased ($P < 0.05$ and greater) milk protein yield. Data was reported previously in the companion paper (Bequette et al., 2001).

Compared with saline infusion, plasma Leu was significantly increased by AA infusion, whereas concentrations were lower during IC and IC + AA. Plasma MOP was significantly reduced by IC and IC + AA. Compared with saline, mammary plasma flow was increased by IC ($P = 0.10$) and IC + AA ($P < 0.05$, Table 2). Mammary uptake of plasma Leu was significantly reduced by IC. Infusion of IC and IC + AA reduced ($P > 0.05$) the ratio of plasma net uptake of Leu to milk protein secretion. Absolute and fractional oxidation, and deamination and reamination of Leu were reduced by IC. The proportion of MOP reaminated back to Leu was decreased and that of MOP oxidized was increased by IC.

The [^{15}N, ^{13}C]Leu kinetic model estimates of mammary protein synthesis, degradation and net gain were not significantly different between treatments, however, there was a significant main effect of the insulin clamp to decrease net protein gain. Model estimates of net protein gain (secretion) are in contrast to the observed increase in milk protein yield due to IC and IC + AA (Bequette et al., 2001).

The hind-leg results were limited to seven of eight experimental periods because of the failure of a hind-leg vein catheter for one goat during period 1 (saline and IC infusions). Hind-leg plasma flow was significantly increased by AA, IC, and IC + AA (Table 3 and Figure

Table 1. Plasma metabolites and hormones.[1]

			Insulin clamp			Probability	
	Saline	AA	Saline	AA	SED	AA	Insulin
Leu, μM	133.4[a]	172.9[b]	63.3[c]	88.7[c]	12.3	†	***
MOP,[2] μM	7.6[a]	8.9[a]	5.4[b]	5.7[b]	0.9	NS[3]	**
Urea, mmol/L	8.38[a]	8.87[a]	6.72[b]	6.74[b]	0.91	NS	**
Glucose, mmol/L	3.45	3.52	3.57	3.51	0.19	NS	NS
Insulin, μU/ml	88[a]	105[a]	291[b]	337[b]	29	NS	***
IGF-1, pmol/ml	14.8[a]	14.5[a]	20.0[b]	19.5[b]	1.6	NS	***

[a,b,c]Values within a row having different superscript letters are different ($P < 0.05$).

[1]Data taken from Bequette et al. (2001).

[2]MOP = 4-Methyl-2-oxopentanoate.

[3]Not significant; $P > 0.10$.

†$P < 0.10$.

**$P < 0.01$.

***$P < 0.001$.

3). Uptake of Leu (and other AA; Bequette et al., 2001) was significantly increased by AA infusion, whereas the IC and IC + AA treatments had no effect. Uptake of MOP was always negative, indicating net production by the hind leg, and it was not affected by treatment. Compared with saline, Leu oxidation was significantly reduced by IC whereas AA and IC + AA had no effect. Fractional oxidation of Leu was decreased by AA, IC, and IC + AA treatments. Deamination of Leu to MOP was reduced by IC, and reamination of MOP to Leu was lower for IC compared with the AA infusion.

Model estimates of hind-leg protein synthesis, degradation, and net protein gain are illustrated in Figure 3. Infusion of AA and IC + AA increased hind-leg protein synthesis, whereas no significant effects on protein degradation were observed. The hind leg was in protein balance (zero gain) during the saline control infusion (t-test $\neq 0$, $P > 0.10$), but AA, IC, and IC + AA increased net protein gain to produce a state of positive protein balance (t-test $\neq 0$, $P < 0.05$). A significant ($P < 0.05$) interaction between AA infusion and the insulin clamp for net protein gain was observed, indicating that the

Table 2. Mammary gland (half) Leu metabolism.[1]

			Insulin clamp			Probability	
	Saline	AA	Saline	AA	SED	AA	Insulin
Plasma flow, g/min	413[a,b]	390[a]	586[b,c]	589[c]	68	NS[2]	*
Leu and MOP[3] kinetics, μmol/h							
Leu uptake	1529[a]	1574[a,b]	1271[b]	1406[a,b]	181	NS	*
Leu uptake to milk output	1.14[a]	1.10[a]	0.85[b]	0.84[b]	0.09	NS	**
MOP uptake[4]	6.5	–8.4	–0.4	–17.5	12.0	†	NS
Leu oxidation	365[a]	371[a]	224[b]	350[a]	54	NS	†
FOR[3]	0.21[a]	0.24[a]	0.15[b]	0.24[a]	0.02	*	†
Leu → MOP	951[a]	1184[a]	502[b]	904[a]	162	†	**
MOP → Leu	593[a]	804[a]	277[b]	536[a]	132	NS	**
MOP reaminated, %	62.2[a,c]	68.7[a]	53.6[b]	58.0[b,c]	5.3	NS	**
MOP oxidized, %	38.7	35.1	46.7	40.7	6.4	NS	*
Protein metabolism, μmol of Leu/h							
Synthesis	3688	3946	3274	3406	400	NS	NS
Degradation	2517	2752	2228	2368	306	NS	NS
Net gain	1171	1194	1046	1038	165	NS	*

[a,b,c]Values within a row having different superscript letters are different ($P < 0.05$).

[1]Negative values represent net release or production of metabolite.

[2]Not significant; $P > 0.10$.

[3]MOP = 4-Methyl-2-oxopentanoate, FOR = fractional oxidation rate of Leu.

[4]MOP fluxes were significantly different from zero (t-test $\neq 0$; $P < 0.05$) for saline and insulin clamp + AA infusion.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

Table 3. Hind-leg Leu metabolism.[1]

			Insulin clamp			Probability[2]	
	Saline	AA	Saline	AA	SED	AA	Insulin
Plasma flow, g/min	190[a]	237[b]	246[b]	288[b]	21	*	*
Leu and MOP[3] kinetics, μmol/h							
Leu uptake	93[a]	277[b]	130[a,b]	239[a,b]	68	**	NS[4]
MOP uptake[5]	–8.7	–5.3	–5.6	–4.4	4.4	NS	NS
Leu oxidation	159[a]	115[a,b]	82[b]	111[a,b]	28	NS	**
FOR[3]	0.50[a]	0.27[b]	0.24[b]	0.25[b]	0.09	†	*
Leu → MOP	264[a]	275[a,b]	111[b]	202[a,b]	58	NS	***
MOP → Leu	104[a,b]	155[a]	31[b]	84[a,b]	45	NS	**
MOP reaminated, %	37.7	55.2	34.5	46.7	11.2	NS	NS
MOP oxidized, %	59.2	42.6	62.8	49.7	10.1	†	NS

[a,b]Values within a row having different superscript letters are different ($P < 0.05$).

[1]Negative values represent release or production of metabolite.

[2]Significant interactions (AA × insulin) were observed for Leu oxidation ($P < 0.01$) and FOR ($P < 0.05$).

[3]MOP = 4-Methyl-2-oxopentanoate, FOR = fractional oxidation rate of Leu.

[4]Not significant; $P > 0.10$.

[5]All MOP fluxes were significantly different from zero (t-test ≠ 0; $P < 0.05$).

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

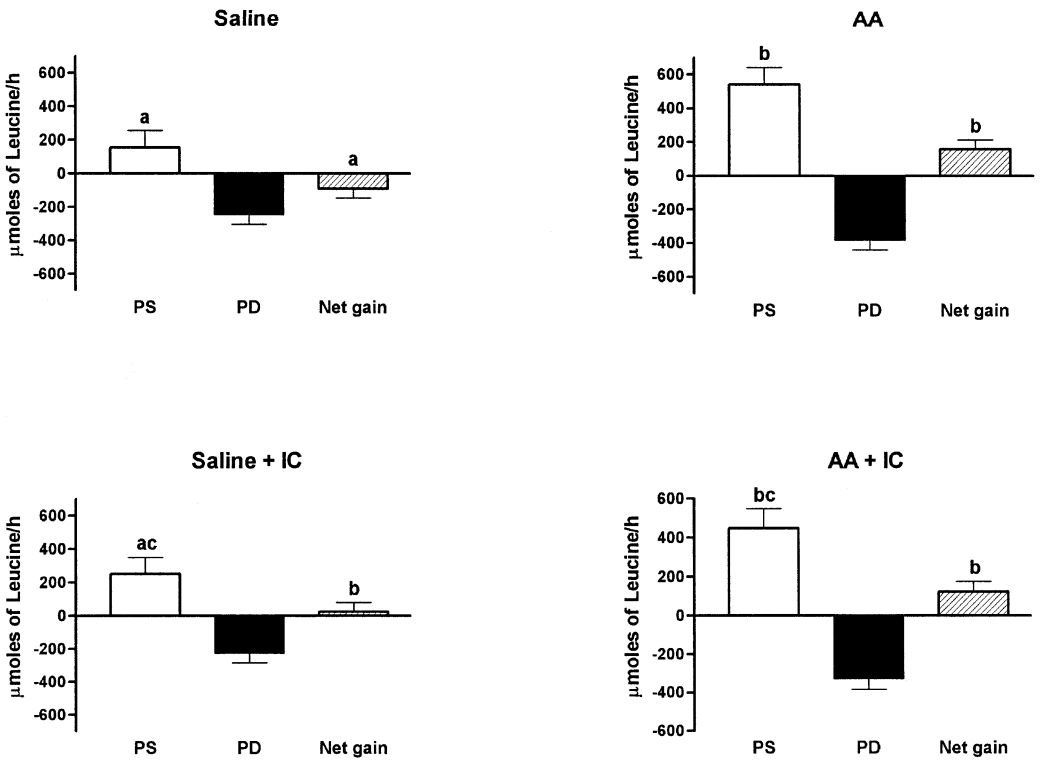

Figure 3. Effect of saline, amino acid (AA), the insulin clamp (Saline + IC), and AA + IC on protein synthesis (PS), degradation (PD), and net protein gain by the hind leg of lactating goats (n = 4). The SE of the difference between treatments was for PS 99, for PD 58, and for net gain 53 (5 df). Bars for PS, PD, and net gain having different letters are significantly different ($P < 0.05$) from other bars for PS, PD, and net gain.

insulin clamp had attenuated the protein anabolic response of the hind leg to AA infusion.

DISCUSSION

Griinari et al. (1997) proposed that regulation of nutrient partition by the insulin clamp involves coordinated responses to allow for increases in milk protein yield. Part of this coordinated response involves regulation of blood flow and AA uptake by the mammary gland and hind-leg tissues (Bequette et al., 2001; Mackle et al., 2000a). In the present study, we considered 1) whether the insulin clamp also coordinates protein and AA metabolism by the hind leg, in addition to that of the mammary gland, and 2) the role of AA supply in the protein anabolic responses of these two tissues. To define as precisely as possible the major limits to describing mammary and hind-leg protein synthesis, we developed an arteriovenous kinetic model based on [^{15}N, ^{13}C]Leu. Using the tracer data, we asked whether or not milk protein yield and muscle protein synthesis could be described. In particular, results generated from the mammary model were evaluated against measured milk protein yield (Bequette et al., 2001).

Mammary Metabolism

We reported previously that infusion of AA failed to stimulate milk protein yield of the goats in this study, whereas the insulin clamp increased protein yields by 10%, and when AA were coinfused with the clamp yields increased by 21% (Bequette et al., 2001; see also Figure 2). We anticipated that, in support of the increase in milk protein yield, both mammary uptake of AA and total gland net protein synthesis (milk protein secretion) would be increased by the insulin clamp. However, net uptake of plasma free Leu, Ile, Val, His, Lys, and Thr by the udder was reduced by the clamp (Bequette et al., 2001), and the [^{15}N, ^{13}C]Leu kinetic model predicted a decrease in mammary net protein gain or secretion.

There are several possibilities that may explain these contradictory results, including a reduction in the oxidation of AA by the udder, uptake of AA from red blood cells, or other unaccounted sources of AA. In the present study and that of Mackle et al. (2000a), the net uptake-to-milk output ratio for Leu and other AA was reduced by the insulin clamp, which suggests that the udder used AA more efficiently. Indeed, we observed a decrease in mammary oxidation of Leu because of the insulin clamp, which supports the contention of Mackle et al. (2000a) that intramammary use of the branched-chain amino acids (**BCAA**) is decreased by the insulin clamp. However, Leu oxidation was elevated to control levels when AA were coinfused with the insulin clamp and where milk protein yield was increased further, thus creating a greater deficit in Leu supply for protein synthesis. Uptake of AA from red blood cells could supply some of this deficit, but for most AA this contribution appears to be small (0 to 5% for Leu; Bequette et al., 1999a; Mackle et al., 2000b) and the insulin clamp does not affect this contribution (Mackle et al., 2000b). Another possibility is that the insulin clamp stimulated the removal of circulating peptides and proteins by the udder (for review see Bequette et al., 1998), but to date there is little known of the factors regulating peptide use by the lactating gland in vivo nor whether insulin regulates peptide transport.

The discrepancy between measured milk protein yield and the kinetic model's estimation of mammary net protein gain or secretion may also be due to the above reasons, in particular, the potential contribution of additional Leu derived from red blood cells or from peptide uptake under insulin-clamp conditions. Another possibility is that the model estimate of Leu oxidation is in error. The model computes protein synthesis after correction for irreversible losses because of Leu oxidation. The estimate of oxidation is dependent on the precursor pool selected to represent the activity at the site of oxidation within the mammary gland, and here we considered the enrichments of venous Leu or its keto-acid (MOP), as others have done for the mammary gland and other tissues (Cheng et al., 1985; Harris et al., 1992; Oddy and Owens, 1996; Watt et al., 1992). However, for technical reasons, a direct comparison between the actual activity at the site of oxidation and the vascular pool choices has never been made for the mammary gland, and this must be further evaluated to confirm the validity of the model.

Hind-leg Metabolism

Despite the low concentrations of AA during the insulin clamp, hind-leg protein gain was stimulated by the insulin clamp. Hind-leg protein gain was stimulated as a result of a slight, but nonsignificant, increase in hind-leg protein synthesis with no clear effect on protein degradation. Larbaud et al. (1996) needed supraphysiological (44- to 70-fold above basal) levels of insulin to cause a reduction in muscle proteolytic activity in nonlactating goats subjected to the insulin clamp for 2.5 h, during which plasma IGF-1 was not affected. Studies in fed (Wester et al., 2000) and fasted (Douglas et al., 1991; Oddy et al., 1987) sheep do not provide evidence for increased protein synthesis in response to insulin (nonclamp conditions), even with net anabolism. Another possibility is that IGF-1 mediated the anabolic effects of the insulin clamp on the hind leg of the lactat-

ing goats. The data here are equivocal, however, with one study reporting inhibition of hind-leg protein degradation (estimated by the Phe model) of fasted and maintenance fed sheep (Oddy and Owens, 1996), whereas in another report IGF-1 stimulated hind-leg muscle protein synthesis (estimated by the Leu model) of fasted sheep (Douglas et al., 1991). In humans, coinfusion of AA with insulin or IGF-1 further stimulated net protein gain by the forearm (estimated by the Phe model), primarily via an enhancement of protein synthesis (Fryburg et al., 1995). We did not observe a further stimulation of protein gain by the hind leg of goats when AA was coinfused with the insulin clamp. Instead, the combined AA infusion and insulin clamp further stimulated milk protein yield, which suggests that the insulin clamp may have specifically enhanced the sensitivity of the mammary gland, rather than the leg tissues, to AA supply.

Infusion of AA did not stimulate milk protein yield. Instead, infusion of AA increased hind-leg protein synthesis (+248%) and degradation (+56%), which resulted in a substantial improvement in hind-leg net protein gain (+272%). Protein synthesis by the forearm of humans (Fryburg et al., 1995) and across the hind leg of sheep (estimated by the Phe model; Wester et al., 2000) is also increased by AA infusion. Our results in midlactation goats are in contrast to those reported for early (d 30) lactating goats, however, where infusion of AA elevated plasma AA concentrations two-fold, but where muscle protein synthesis (measured by the flooding-dose technique) was not stimulated (Tauveron et al., 1994). These apparently conflicting results may be attributable to the kinetic model used or because of differences in stage of lactation. If one assumes the latter, then it is possible that in early lactation (Tauveron et al., 1994) when extramammary tissues are less responsive to both AA supplies (Tesseraud et al., 1993) and to insulin (Debras et al., 1989), there is preferential diversion of AA toward milk protein synthesis. Subsequently, in later lactation (present study) when extramammary tissues become more responsive to anabolic signals (Tesseraud et al., 1993), then AA will be diverted toward these nonmammary tissues to replenish vital protein stores (mainly muscle).

Distribution and Regulation of Leu Metabolism

The mammary gland catabolizes a significant quantity of the BCAA (present study; Bequette et al., 1996, 1998) and, similar to observations in sheep (Harris et al., 1992; Oddy et al., 1987), we have found that the hind leg of lactating goats catabolizes a significant proportion (24 to 50%) of Leu extracted from plasma. When expressed on a per-tissue-weight basis [assuming 9.1% (Baracos et al., 1991) and 3.1% (B. J. Bequette, unpublished results, 1999) of BW as hind leg and mammary gland tissues, and 58.9% muscle in hind-leg tissues (Baracos et al., 1991)], the relative rates of Leu oxidation (2.8:1) and transamination (4.5:1) by the mammary gland are much greater than that of the hind-leg muscles, which would favor Leu catabolism by the mammary gland. Similar changes in the distribution of the BCAA catabolic enzymes have been reported to occur when rats enter into lactation (DeSantiago et al., 1998). Whether this shift in BCAA catabolism serves an important role in lactation has not been determined.

Insulin and the BCAA have been linked to regulation of BCAA catabolism by muscle of rats (Aftring et al., 1986; Hutson et al., 1980) and sheep (Harris et al., 1992; Oddy et al., 1987). Herein, Leu oxidation by the udder and the hind leg of lactating goats was reduced by the insulin clamp. However, it is unclear whether insulin, IGF-1, or the low plasma AA concentrations resulting from the insulin clamp caused the reduction in BCAA catabolism. Based on the finding that mammary and hind-leg Leu oxidation increased to control levels when AA were coinfused with the insulin clamp, our data suggest that AA or Leu supply (concentration) may be important regulators of BCAA catabolism by these tissues.

CONCLUSIONS

In the present study, we examined the mechanisms through which the insulin clamp coordinates protein and AA metabolism by the mammary gland and the hind-leg tissues. These processes were upregulated under insulin-clamp conditions such that the ability of the mammary gland, but not the hind leg, to respond to AA supply was enhanced. When the endocrine system was not enhanced by the insulin clamp, additional supplies of AA were diverted toward net protein accretion by hind-leg tissues rather than toward milk protein synthesis. However, the present study used data from only one experiment and therefore cannot be used to describe across a wider range of metabolic activities. It is clear that as a field we still lack a sufficiently detailed description of the biological mechanisms and pathway kinetics involved in muscle and milk protein synthesis to describe them precisely in biomathematical models. Such biomathematical approaches must continue to be built, expanded, evaluated, and improved so that we can continue to design more useful experiments to meet the goal of describing such processes.

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APPENDIX

The mathematical models for calculating the kinetics for Leu metabolism by the mammary gland and a hind leg are given below, where C and E represent the concentration (μmol/L) and enrichment (atom % excess) of metabolite, respectively, subscripts A, V, and M represent arterial, venous, and milk CN, respectively, Leu and DLeu stand for total [^{13}C]Leu and [^{15}N, ^{13}C]Leu enrichments, respectively, and where F is plasma flow (kg/h).

Mammary model

Leu inflow (Leu_{in}) = $C_A Leu \times F$
Leu outflow (Leu_{out}) = $C_V Leu \times F$
Leu balance = $Leu_{in} - Leu_{out}$
MOP inflow (MOP_{in}) = $C_A MOP \times F$
MOP outflow (MOP_{out}) = $C_V MOP \times F$
MOP balance = $MOP_{in} - MOP_{out}$
Leu carbon flux (C-flux) = $([(E_M Leu - E_V Leu)/(E_A Leu - E_V Leu)] \times C_V Leu + C_A Leu) \times F$
Leu nitrogen flux (N-flux) = $([(E_M DLeu - E_V DLeu)/(E_A DLeu - E_V DLeu)] \times C_V Leu + C_A Leu) \times F$
Reamination (T_R) = N-flux – C-flux
Oxidation (Ox) = $[(C_V CO_2 \times E_V CO_2 - C_A CO_2 \times E_A CO_2)/E_V Leu)] \times F$
Net transamination (T_N) = Ox + MOP balance
Deamination (T_D) = $T_N + T_R$
Protein degradation (PD) = $([(\text{N-flux} \times E_A DLeu)/E_M DLeu] - \text{N-flux}) - T_R$
Protein synthesis (PS) = Leu balance + MOP balance + PD – Ox
Net protein gain = PS – PD

Hind-leg model

Leu inflow (Leu_{in}) = $C_A Leu \times F$
Leu outflow (Leu_{out}) = $C_V Leu \times F$
Leu balance = $Leu_{in} - Leu_{out}$
MOP inflow (MOP_{in}) = $C_A MOP \times F$
MOP outflow (MOP_{out}) = $C_V MOP \times F$
MOP balance = $MOP_{in} - MOP_{out}$
Leu carbon flux (C-flux) = $Leu_{in} \times (E_A Leu \times C_A Leu - E_V Leu \times C_V Leu)/(E_A Leu \times C_A Leu)$
Leu nitrogen flux (N-flux) = $Leu_{in} \times (E_A DLeu \times C_A Leu - E_V DLeu \times C_V Leu)/(E_A DLeu \times C_A Leu)$
Reamination (T_R) = (N-flux – C-flux)/[1 – ($E_V Leu - E_V$-MOP)/$E_V Leu$]
Oxidation (Ox) = $[(C_V CO_2 \times E_V CO_2 - C_A CO_2 \times E_A CO_2)/E_V MOP] \times F$
Net transamination (T_N) = Ox + MOP balance
Deamination (T_D) = T N + T_R
Protein degradation (PD) = N-flux – T_R
Protein synthesis (PS) = Leu balance + PD – T_N
Net protein gain = PS – PD