J. Dairy Sci. 84:241–255

Insulin Regulates Milk Production and Mammary Gland and Hind-Leg Amino Acid Fluxes and Blood Flow in Lactating Goats

B. J. Bequette,* C. E. Kyle,* L. A. Crompton,†
V. Buchan,* and M. D. Hanigan‡
*Rowett Research Institute, Greenburn Road,
Bucksburn, Aberdeen AB21 9SB, Scotland
†The University of Reading, Department of Agriculture,
Reading RG6 2AT, England
‡Dairy Research Group, Purina Mills Inc.
St. Louis, MO 63144

ABSTRACT

We investigated the roles of insulin and amino acid (AA) in regulating milk production and the uptake of AA and blood flow (BF) by the mammary gland and hind-leg of goats (n = 4). During two periods, either saline or AA (65 g/d) was infused i.v. for 7.5 d, and, beginning on d 5, goats were subjected to a hyperinsulinemic-euglycemic clamp. The insulin clamp elevated plasma insulin levels threefold and insulin-like growth factor-1 by 27%, and euglycemia was maintained by the infusion of glucose. Arterial, mammary, and tarsal vein blood samples were obtained on d 4 and 8 of each period, and blood flow was monitored continuously by probes. Insulin and insulin plus AA infusions increased the yields of milk by 13 to 18% and protein by 10 to 21%, but AA infusion alone had no effect. The insulin clamp reduced milk fat content by 21 to 31% and yield by 8 to 19%, and reduced the yields of milk fatty acids >C16. The insulin clamp increased mammary blood flow by 42%, but insulin and AA infusions both increased hind-leg BF by 29 to 52% and by 25%, respectively. Net uptakes of most plasma AA by the udder were reduced by insulin, whereas AA infusion had no effect. For the leg, the uptake of His and Thr were decreased by insulin, whereas the infusion of AA stimulated the uptake of total essential AA. Insulin increased the uptake of glucose by the udder but not by the leg. This study suggests that the udder and leg tissues respond differently to infusions of insulin and AA; the udder was more responsive to insulin, while the leg was more responsive to AA concentration (supply), at least in terms of AA uptake and net anabolism (protein gain or secretion).

(**Key words:** milk, insulin, amino acids, goats)

Abbreviation key: BCAA = branched-chain amino acids, **BF** = blood flow, **EAA** = essential amino acids.

INTRODUCTION

Lactating dairy cows convert dietary nitrogen and energy substrates into milk inefficiently (15 to 30%; 6). Not surprisingly, dietary supplementation with protein or energy leads to only marginal increases in milk production in otherwise well-fed cows but often large increases in production in cows fed deficient diets (see 6 for review). In cows fed protein deficient diets, Bequette et al. (6) calculated that only 35% of the supply of the additional AA beyond the liver was converted into milk protein. Thus, either substantial catabolism of AA occurs within the mammary gland or AA are being directed to the skin, muscle, kidneys, or adipose tissues where they are catabolized or stored as protein. However, few studies have examined AA metabolism during lactation, and most have involved examination of the mammary gland (5, 7, 8, 9, 10, 18, 21, 25, 28, 29, 32, 38).

Those studies seem to suggest that the supply of nutrients per se is not limiting for milk production. The endocrine system also appears to place a limitation on nutrient use through the coordination of tissue metabolism. This is splendidly demonstrated when the hyperinsulinemic-euglycemic clamp is applied to dairy cows and when the partition of AA into milk is enhanced considerably, while the incorporation of preformed fatty acids into milk is reduced (16, 17, 27, 28, 29, 30, 31). The technique involves the chronic infusion of insulin for 4 to 5 d at rates to elevate plasma insulin four- to fivefold, and infusion of exogenous glucose to maintain euglycemia. Dramatic improvements in efficiency and in some cases milk production have been observed when the insulin clamp is given alone. More-

Received February 1, 2000.
Accepted August 11, 2000.
Corresponding author: B. J. Bequette; e-mail address: bjb@rri.sari.ac.uk.

over, when casein is coinfused with the insulin clamp, the efficiency of converting the extra casein-AA supply into milk and milk protein yield are increased well above levels observed in response to growth hormone administration. Although insulin infusion was the variable altered in the above studies, changes in the IGF system were also observed (16, 27, 31). Regardless of the mode of action, the insulin clamp results suggest that the maximal capacity of the udder for milk protein synthesis can be increased, thereby altering the relationship between AA supply to the udder and the rate of milk protein synthesis. Such a shift would likely alter the partition of nutrients among the various postabsorptive tissues, but this has not yet been quantified in vivo.

The underlying mechanism(s) through which insulin acts to increase the partition of AA to the mammary gland and into milk protein is unclear. In studies involving infusions of insulin over hours or days, plasma concentrations of AA are reduced quite dramatically, with the branched-chain AA (**BCAA**) being most reduced (15, 16, 25, 27, 37, 38). Despite the lower plasma AA levels, mammary extraction and use of AA for milk protein synthesis is either maintained or increases (25, 38), suggesting that the udder has become more sensitive to nutrient supplies by increasing its ability to extract (transport) plasma AA or that blood flow (**BF**) had been increased. Furthermore, the apparent interaction between the insulin clamp and casein infusion, where a considerable proportion of the infused casein-AA are repartitioned into milk protein, also suggests that other nonmammary tissues have altered their sensitivity to these additional supplies of AA.

An aim of the present study was to compare the milk protein and fat yield and component responses of lactating goats subjected to a hyperinsulinemic-euglycemic clamp, with and without i.v. infusion of AA, to those obtained previously in dairy cows subjected to the insulin clamp. A further objective was to use this model to examine the role of insulin in regulating mammary and hind-leg metabolism including BF, AA transport and metabolism, and protein turnover, with a view to using these findings to improve our predictive model of nutrient utilization in the dairy cow (20). The present paper reports milk production, BF, and net uptake results for the udder and the hind-leg. Results of Leu metabolism and protein turnover are reported elsewhere (10).

MATERIALS AND METHODS

Goats, Diet, and Infusions

The Institute's Animal Welfare Committee and the veterinary inspectorate of the Home Office (United Kingdom) approved all surgical procedures and practices. Under general anesthesia, four (two primiparous and two multiparous) lactating goats were surgically fitted with transonic flow probes (Transonic Systems Inc., Ithaca, NY) around the external iliac (8 mm) and external pudic (6 mm) arteries of the same hind-quarter. The external pudic and perineal veins were also ligated on this side to eliminate their contributions to mammary BF, and the carotid artery was elevated to a subcutaneous position. Access to the external iliac and pudic arteries was by a lateral incision (~12 cm) in the inguinal region near the left udder. Probes were anchored to the surrounding tissues by attaching sutures to a surgical mesh silastic collar apparatus, which was glued to the probe cable. Cables were exited beneath the vulva and protected in a pouch glued to the rear of the goat. Blood flow to the hind-leg tissues was calculated from the difference between the external iliac and pudic artery flow probe measurements, and this represented ~60% of total leg flow based on comparisons to goats with probes placed around the common iliac artery (B. J. Bequette, personal observation). Goats were allowed to fully recover (~1 mo) before beginning experimentation.

At the initiation of the study, goats averaged 63.3 ± 6.9 kg of BW and were 142 ± 20 d postpartum. 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 (N × 6.25) and 11.3 MJ of metabolizable energy (calculated)/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). The concentrate comprised (g/kg, as-fed basis) barley, 395; whole corn, 150; wheat feed, 150; linseed flakes, 50; soybean meal (48% CP); Supersoy (67% CP; Norvite Feed Supplies, Insch, United Kingdom), 50; white fishmeal (67% CP), 50; molasses, 30; and a vitamin and mineral premix, 25 (Norvite Feed Supplies). Throughout the experiment and between infusion periods, the ration was delivered by automatic feeders (12 equal meals at 2-h intervals), and this level of feed intake was fixed for each goat. Feed intake was calculated to provide 95% of metabolizable energy and 143% of metabolizable protein requirements for maintenance and milk production (1). Milking was performed at 0830 and 1630 h.

Goats were assigned to intravenous AA infusions and insulin clamp treatments according to a balanced crossover design. The experiment consisted of two periods (separated by 4 wk) of continuous intravenous infusions of either saline (1 L/d, pH 7.4, equivalent to the Na and Cl load in the AA infusion) or a complete mixture of AA in the composition of cow's casein (1 L/d of a pH 7.4 solution containing 65 g of AA) for 7.5 d. The AA solution was filter (0.2-micron 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 period, temporary catheters (polyvinyl chloride, 0.8 mm i.d., 1.2 mm o.d., Critchely Electrical Products, Auburn, New South Wales, Australia) were inserted (10 cm) into the carotid artery, the contra-lateral jugular vein, and the left mammary vein, and a catheter was introduced into the tarsal vein of the left leg a known distance (~59 cm) until the tip was adjacent to the leg flow probe. This distance had been determined at the time of surgery for each goat. All catheters were kept patent by flushing daily with a heparin-saline solution (200 IU/ml of heparin). 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 kept in floor pens and fed by automatic feeders.

During each period, arterial blood samples (1 IU of heparin/ml of blood) were obtained each day at 0800 and 1700 h, plasma was harvested following centrifugation (2000 × *g* at 4°C for 15 min) and stored at –20°C. During the first 4 d of each period, these samples were also analyzed for plasma glucose with a handheld glucose meter (Glucotrend, Boehringer, Mannheim UK Ltd., East Sussex, England), to provide target glycemia levels for each goat during the insulin clamp. The solutions of insulin for infusion were prepared daily for each goat from frozen aliquots (1 mg of insulin/ml of sterile water containing 1% BSA, 50 μl of 6 N HCl was added to dissolve insulin) of porcine insulin (I-5523, lots 37H0665 and 106H0769; Sigma Chemical Co., St. Louis, MO). Aliquots (2.88 ml) were slowly thawed and made up to 240 ml with sterile saline containing 0.1% BSA. The insulin solution was infused (10 ml/h) i.v. through a sterile filter (0.45 micron) to deliver 120 μg of insulin per hour. During the insulin clamp, euglycemia was maintained by infusion of a glucose solution (40% glucose monohydrate solution, wt/vol; Baxter Viaflex, Glasgow, Scotland) at variable rates. Within 1 min, blood glucose was determined with the handheld glucose meter, and the glucose infusion rate was adjusted as appropriate. At the outset, blood sampling was frequent (5 to 15 min) until euglycemia and glucose infusion rates became more stable; subsequently, blood sampling was less frequent (1 h).

Milk yield was recorded at each milking, and subsamples were taken for analysis of fat, protein, and lactose with an infrared milk analyzer (Milko-Scan 133B mid infrared analyzer, N. Foss Electric, Hillerod, Denmark) and for total N by combustion analyses. Subsamples of milk from the p.m. following by the a.m. milking were composited (33:67) on d 3 and 7, and analyzed by GLC for milk fatty acids.

Mammary and hind-leg net fluxes of AA were performed on d 4 and 8 of each period. Beginning at 0830 h, goats were given in i.v. dose of oxytocin (1 IU), and the mammary glands were milked-out completely by machine and hand milking. Immediately, an 8-h continuous i.v. infusion of a solution containing heparin (6500 IU/h) was initiated. The addition of heparin was to prevent clotting during integrated blood withdrawal. Over the last 4 h of infusion, blood was continuously withdrawn over 1-h intervals from the artery, mammary vein, and tarsal vein catheters into sealed syringes submerged in an ice-bath (8). Plasma was harvested after centrifugation (2000 × *g* for 15 min at 4°C), and to a known weight of plasma (0.5 g) was added an equal known weight of a norleucine solution (100 nmol/g). These and extra plasma samples were stored at –20°C.

Analytical Methods

Plasma was analyzed for free AA concentrations as previously described (5). Daily plasma samples were analyzed for insulin and IGF-1. For insulin, an antiserum to porcine insulin (IM38, Amersham International, Aylesbury, England) and porcine insulin (I-3505, Sigma Chemical Co., Ltd., Poole, Dorset, England) as standard were used. Serial dilutions of caprine plasma demonstrated no deviation from parallelism with the standard curve. Plasma IGF-1 concentrations were determined as previously described (11). Recombinant IGF-I (IP9010, Peninsula Laboratories Europe, Ltd, Merseyside, England) was used as standard and rabbit anti-IGF-I antiserum was kindly supplied by B. T. Rudd (Birmingham and Midland Hospital for Women, Birmingham, England). No significant deviations from parallelism were observed when serial dilutions of caprine plasma were performed. Plasma glucose, urea, triacylglycerides, and FFA were determined on daily samples by enzymatic colorimetric kits (Labmedics Ltd., Romiley, Stockport, England) adapted to a selective chemistry analyzer.

Lipids were extracted from composited milk samples with chloroform and methanol, according to the Bligh and Dyer technique as modified by Kirk and Sawyer (24). Aliquots of the extracted fat were trans-esterified by heating at 105°C for 2 h in methanolic hydrogen chloride (14). The resulting fatty acid methyl esters were extracted into hexane:diethylether, concentrated under nitrogen, and separated by gas chromatography (Hewlett Packard 5890 gas chromatograph fitted with automatic sampler 7673A with HP 3396 chromatograph integrator and a flame ionization detector; Hew-

lett Packard, Palo Alto, CA) with a BPX-70 capillary column (60 m × 0.25 mm diameter) with temperature programming and flame ionization detection. Certified reference standards of pork and beef fat and maize and soya oil served as reference samples (Laboratory of the Government Chemist, Teddington, United Kingdom). Individual fatty acids were identified by reference to a mixture of 37 fatty acid methyl esters (Supelco, Poole, UK), and mixtures of other fatty acid methyl esters of known composition were analyzed within the same runs.

Calculations and Statistics

Net fluxes of plasma glucose and AA across an udder half or the hind-leg were calculated as [BF × (1-packed cell volume)] × (plasma arteriovenous difference). A rate parameter (K, ml/min) for removal of glucose and AA by an udder half and one hind-leg was calculated from the model of Hanigan et al. (20):

$$K_i = ([A_i] \times BF/[V_i]) - BF$$

where $[A_i]$ and $[V_i]$ represented arterial and venous (mammary or tarsal) plasma concentrations of the ith metabolite (nmol/ml) and BF represents mammary or hind-leg plasma flow (ml/min). The term K represents the ability of the tissue bed to clear plasma metabolite per unit time. In large part, K should describe the capacity of the transporter system, that is, the number and affinity of the transporters. However, a change in the effective perfusion of the tissue could expose more or fewer transporters to substrate, resulting in an apparent change in K, where more or fewer transporters are exposed to substrate and where the total number and affinity of those transporters may not have changed. Similarly, a change in the relationship between unidirectional uptake by and efflux from the tissue bed, e.g., where the relationship between unidirectional uptake and intracellular concentration has been altered, would result in a change in K, as it represents the net difference between unidirectional metabolite uptake and efflux. Consequently, although a change in K does indicate that the relationship between metabolite supply and net removal has been altered, it does not allow one to discriminate among potential changes in transport affinity or capacity, changes in effective perfusion, nor changes in the relationship between unidirectional metabolite uptake and efflux from the tissue bed.

Data were analyzed in Genstat 5 (Lawes Agricultural Trust, Rothamstead, UK) by ANOVA or by REML in the case of leg fluxes, for which one of eight experimental periods was missing due to catheter failure in one goat. The models included main effects of AA and insulin, and interactions of AA × insulin. Goat and period were declared random effects.

RESULTS

During the insulin clamp periods, plasma insulin was increased 3.3-fold, and euglycemia was maintained within 10% of baseline values (Table 1). The rate of glucose infusion required to maintain euglycemia reached a steady state at 24 to 36 h of the insulin clamp, and there was no effect of coinfusion of AA on glucose infusion rate [without AA = 13.39 vs. with AA = 13.94 g of glucose/h (SED 1.33)]. Infusion of AA tended ($P = 0.13$) to increase plasma insulin slightly, but there was no effect of AA infusion on plasma glucose, urea, FFA, triacylglycerides nor on IGF-1 concentrations (Table 1). Plasma IGF-1 was elevated 28% by the insulin clamp, reaching a maximum by 24 h of initiating the clamp (data not shown). Plasma urea and FFA were reduced by the insulin clamp.

There was no effect of treatment on DMI (Table 2). Daily milk yield and composition are reported in Table 3 and illustrated in Figure 1 as hourly rates over the a.m. and p.m. milking intervals. An unknown amount of milk had been removed from the glands for determination of [^{15}N, ^{13}C]Leu enrichment ~45 min after the arteriovenous measurement period. So that the data would not be biased, d 4 a.m. milk results were excluded from statistical analysis, but plotted in the figures. Except for a tendency ($P < 0.10$) for lower daily milk fat yield, the infusion of AA had no effect on milk production. Milk and protein yields increased gradually during the 3.5-d insulin clamp periods, reaching levels 13 to 18% and 7 to 21% higher than saline infusion. Milk crude (N × 6.38) protein and fat percentages were reduced by the insulin clamp, with the combined AA and insulin infusion resulting in the lowest fat percentage. The ratio of milk true protein to fat was increased by insulin. Recovery of the infused AA as true milk protein yield increased from 2% when AA alone was infused to 28% when the insulin clamp was combined with AA infusion. Lactose yield was increased by the insulin clamp.

In Figure 1, milk, protein, and fat yields are plotted as hourly rates over the p.m. (0830 to 1630 h) and a.m. (1630 to 0830 h) milking intervals compared with the arteriovenous kinetic measurements performed on d 4 and 8 during the p.m. interval. For milk and protein yields, p.m. and a.m. milking intervals did not differ ($P > 0.10$). Compared to saline, milk and protein yields were higher ($P < 0.005$) by 13 to 20% and 9 to 21% during the insulin and insulin plus AA infusion, and there was a tendency ($P = 0.07$) for milk protein yield

Table 1. Arterial plasma concentrations of hormones and metabolites.[1]

			Insulin clamp			Probability	
	Saline	AA	Saline	AA	SED	AA	Insulin
Insulin, μU/ml	88	105	291	337	29	NS	***
IGF-I, pmol/ml	14.8	14.5	20.0	19.5	1.6	NS[2]	***
Glucose, mmol/L	3.45	3.52	3.57	3.51	0.19	NS	NS
FFA, mmol/L	0.172	0.260	0.099	0.102	0.063	NS	*
TAG, mmol/L	0.086	0.116	0.114	0.087	0.038	NS	NS
Urea, mmol/L	8.38	8.87	6.72	6.74	0.91	NS	**

IGF-I, insulin-like growth factor-I; TAG, triacylglycerides.

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d. Means for glucose are based on samples during the arteriovenous measurement on d 4 and 8, and other values are the means for samples taken at 0800 and 1700 h on d 2 and 3 for Saline and AA infusions, and for d 6 and 7 for the insulin clamp treatments.

[2]$P > 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

to be higher during the a.m. milking interval when insulin plus AA were infused. Rates of fat yield were greater ($P < 0.05$) during p.m. than during a.m. intervals. Only during the a.m. interval was fat yield depressed by AA infusion (9%; $P = 0.08$) and the insulin clamp (14 to 25%; $P < 0.005$).

In general, the insulin clamp increased the proportion of milk fatty acids < $C_{17:0}$ and decreased those > $C_{17:0}$ (data not shown). Infusion of AA tended ($P = 0.07$) to increased the proportion of *trans*-$C_{18:2}$. Yields of milk fatty acids are given in Table 3. The insulin clamp decreased the yields of $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, *trans*-$C_{16:1}$, and >$C_{18:0}$ fatty acids. Infusion of AA tended to decrease the yields of $C_{14:0}$ and $C_{16:0}$.

Except for Thr, Arg, and Ile, infusion of AA increased arterial plasma concentrations of AA (Table 4). In contrast, infusion of insulin decreased concentrations of all AA shown in Table 4, except for Phe and Tyr. Total essential AA (**EAA**) were increased by AA infusion, whereas these were decreased 53% by the insulin. Total AA concentration (EAA + nonessential AA) was increased by 16% due to AA infusion, whereas insulin reduced levels by 44%. Of this drop in total AA concentration, the BCAA accounted for 44%. Thus, under

Table 2. Feed intake and milk production.[1]

			Insulin clamp			Probability	
	Saline	AA	Saline	AA	SED	AA	Insulin
DMI, kg/d	2.19	2.12	2.20	2.07	0.15	NS[2]	NS
Milk yield, g/d	2897	2932	3281	3412	187	NS	***
Milk composition, %							
CP[3]	3.41	3.42	3.21	3.25	0.10	NS	***
True protein	3.07	3.09	2.96	3.12	0.14	NS	NS
Fat	3.65	3.48	2.90	2.52	0.17	NS	***
Lactose	4.71	4.73	4.73	4.76	0.10	NS	NS
True protein:fat	0.85	0.90	1.04	1.23	0.09	NS	***
Component yield, g/d							
CP[3]	97	99	104	110	7	NS	**
True protein	87	90	96	105	7	NS	**
Fat	104	101	96	84	5	†	**
Lactose	136	139	154	162	9	NS	***

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d. Data are the means of samples taken on d 2 and 3, for Saline and AA infusion, and for d 6 and 7 for the insulin-clamp treatments.

[2]$P > 0.10$.

[3]Calculated as milk N × 6.38.

**$P < 0.01$.

***$P < 0.001$.

Table 3. Daily yields (g/d) of milk fatty acids.[1]

			Insulin clamp			Probability	
	Saline	AA	Saline	AA	SED	AA	Insulin
$C_{8:0}$	0.9	1.3	1.0	0.5	0.4	NS[2]	NS
$C_{10:0}$	9.2	9.8	8.7	6.5	1.1	NS	†
$C_{12:0}$	6.1	5.4	6.5	5.8	0.5	NS	NS
$C_{14:0}$	14.4	11.6	11.2	9.8	1.1	†	*
$C_{14:1}$	0.2	0.2	0.2	0.2	0.04	NS	NS
$C_{15:0}$	0.9	0.8	0.9	0.9	0.1	NS	NS
$C_{16:0}$	35.5	27.8	28.0	24.1	3.1	†	*
cis-$C_{16:1}$	1.8	1.9	1.3	1.4	0.6	NS	NS
trans-$C_{16:1}$	1.0	1.1	0.5	0.5	0.2	NS	**
$C_{17:0}$	1.0	1.0	0.8	0.9	0.2	NS	NS
$C_{18:0}$	13.5	12.7	5.7	4.8	1.9	NS	***
trans-$C_{18:1}$	2.5	2.5	1.1	1.1	0.3	NS	***
cis-$C_{18:1}$	26.8	28.1	13.7	14.7	4.9	NS	**
cis-$C_{18:2}$	3.4	3.1	1.9	1.9	0.4	NS	***
trans-$C_{18:2}$	0.43	0.43	0.23	0.25	0.04	NS	***
$C_{18:3}$	0.44	0.42	0.22	0.22	0.06	NS	***

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d. Data are from milk samples composited (33:67, p.m.:a.m.) from p.m. followed by a.m. milkings on d 3 for Saline and AA infusions, and d 7 for insulin clamp treatments.

[2]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

clamp conditions, the BCAA represented less [54 vs. 59% (2% SED); $P < 0.01$] of the total plasma EAA concentration. Coinfusion with AA attenuated the decreases in plasma His, Val, Lys, Met, Phe, Thr, Tyr, and Pro caused by the insulin clamp.

Daily (mean over 24 h) patterns of BF for half the mammary gland and the hind-leg are illustrated in Figure 2 (A and B). Mammary BF increased ($P < 0.05$) gradually and in close parallel to the increase in milk and protein yield, but infusion of AA alone had no effect. Leg BF tended ($P < 0.10$) to be higher by 31% during the insulin clamp periods. On the days of arteriovenous kinetic measurements, the insulin clamp increased mammary plasma flow by 42% ($P < 0.05$; Table 5) and the leg flow by 29 to 52% ($P < 0.05$; Table 6), and infusion of AA alone increased leg flow by 25% ($P < 0.05$).

Net fluxes of plasma glucose and AA across half the mammary gland are given in Table 5. Glucose uptake by the gland was increased by the insulin clamp. Infusion of AA tended ($P < 0.10$) to increase the uptake of Val and Asp but decrease the uptake of His ($P < 0.10$) and Arg ($P < 0.01$). Mammary uptake of His, Ile, Leu, Val, Lys, Thr, Gln, Gly, and Ser were reduced ($P < 0.05$) by the insulin clamp, whereas the uptake of Ala was increased.

Net fluxes of plasma glucose and AA across the hind-leg are given in Table 6. Hind-leg results were limited to seven of eight experimental periods due to the failure of a leg vein catheter for one goat during period 1 (saline and insulin clamp). Glucose uptake by the leg was not significantly affected by treatment. Infusion of AA increased the uptake of BCAA, total EAA, and Pro. Histidine and Thr uptakes were reduced by the insulin clamp, and, while uptakes of Gln and Gly were also reduced, the net balances of these two AA changed from positive to negative (i.e., production). In contrast, the uptake of Ala was increased by the insulin clamp.

Rate constants (K, 1/min per udder half or a hind-leg) for glucose and EAA uptake by half the udder and a hind-leg are compared in Table 7. There was a tendency ($P = 0.12$) for insulin to decrease the K for glucose uptake by the leg. Among the EAA, infusion of AA tended ($P < 0.10$) to reduce the K for His, Lys, and Phe uptake by the udder. Except for Met (no change) and Phe (reduced), the insulin clamp increased the K for EAA uptake by the udder. Insulin increased the K for leg uptake of Lys and the BCAA. Rate constants for nonessential AA uptake are compared in Table 8. There was no significant effect of treatment on the K for leg uptake of nonessential AA and K increased for only Ala during the insulin clamp. There was no effect of AA infusion on the K for mammary uptake of nonessential AA; however, the insulin clamp increased the K for Arg, Pro, and Ala uptake

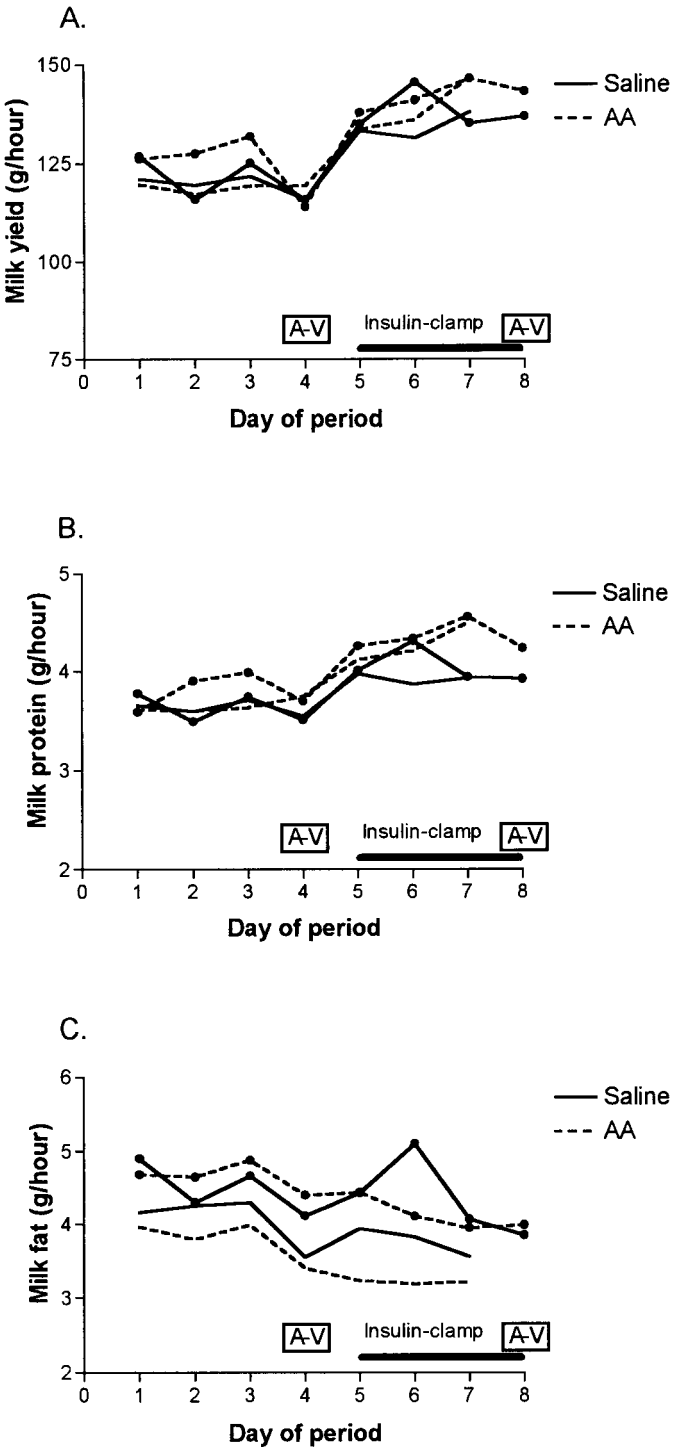

Figure 1. Hourly rates of milk (A), true protein (B) and fat (C) yields during the p.m. (symbol) and a.m. (no symbol) milking intervals during each infusion period. Saline (solid line) or a complete mixture of AA (dashed line) were infused for 7.5 d, with the hyperinsulinemic-euglycemic clamp given during the last 3.5 d as indicated. Arteriovenous measurements were made on days 4 and 8 as indicated by A-V. The insulin-clamp increased ($P < 0.05$ and greater) milk and protein yields during the p.m. and a.m. milking intervals, and decreased ($P < 0.005$) milk fat yield during the a.m. interval only.

by the udder while decreasing the K for Tyr, Gly, Gln, and Ser.

DISCUSSION

Attempts to model nutrient flows in the lactating cow require knowledge of the key points in this process where nutrient supply may limit productive capacity and where nutrient supply and (or) anabolic hormones may exert control (19, 20). Most attempts have only considered the kinetic responses of the gut, liver, and mammary gland to changes in the supply of AA or energy substrates, with little consideration of the control exerted by anabolic hormones (19, 20). The hyperinsulinemic-euglycemic clamp enhances milk production and efficiency in dairy cows (16, 17, 27, 31), presumably via mechanisms that favor the partition of nutrients to and uptake by the mammary gland while decreasing their partition to other tissues. Very little is known about the mechanism(s) that may be involved in this process. Herein, we used the insulin clamp, with or without infusion of AA, as a model to investigate the mechanisms controlling AA partition and metabolism in the lactating goat. Where possible, we have used the lactating goat instead of the cow to examine basic mechanisms regulating AA use by the mammary gland and for establishing kinetic parameters for modeling purposes. The hind limb was chosen because of its responsiveness to insulin status (37, 39) and because of the significant contribution of skeletal muscle to whole body protein metabolism in lactating animals.

Milk Production Responses

The insulin clamp has been applied to well-fed dairy cows (16, 17, 27, 31) and lactating ewes (2). The clamp herein was sufficiently long to attain large (+13 to 18%) increases in milk production. Whereas in the cow studies milk protein content was increased by the insulin clamp, we observed no effect of the clamp on milk (true and crude) protein content (Table 2). Nonetheless, the insulin clamp, with or without infusion of AA, increased true protein yield by 10 to 21%. Infusion of AA alone, however, failed to elicit a response in either milk or protein yield. In cows, the response to the clamp alone has been variable, with either no change (16, 17, 31) or an increase of 1.8 kg/d (+6.8%; 27) in milk yield. In the former studies, the lack of a response can probably be attributed to the large reduction in DMI caused by the insulin clamp. Similarly, in the lactating ewe study, where the insulin clamp failed to increase milk production, DMI was also depressed (2). In the current study, the clamp did not affect DMI. What appears to be consistent among these studies is

Table 4. Selected arterial plasma concentrations (μmol/l) of AA.[1]

			Insulin clamp			Probability[3]	
Item[2]	Saline	AA	Saline	AA	SED	AA	Insulin
His	41.1	40.0	14.2	42.2	6.0	*	*
Ile	107.5	123.4	47.3	65.7	11.7	NS[4]	***
Leu	133.4	172.9	63.3	88.7	12.3	†	***
Val	180.6	254.2	81.0	136.1	22.4	*	***
Lys	155.4	178.4	66.7	119.4	16.9	*	***
Met	21.1	25.1	13.9	23.9	1.8	**	*
Phe	30.5	50.0	33.2	54.9	3.9	**	NS
Thr	64.7	60.3	23.7	38.5	6.9	NS	***
Arg	114.3	117.4	59.8	79.3	9.9	NS	***
Tyr	57.2	56.7	59.4	73.3	4.2	†	*
Pro	137.0	180.0	82.0	162.1	11.2	**	**
BCAA	421.5	550.5	191.5	290.5	42.9	*	***
EAA	734.3	904.3	343.2	569.9	60.5	*	***
Total AA	1190	1380	664	1032	92	*	***

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d. Data are means (n = 4) of plasma samples taken during the 4-h arteriovenous measurement period.

[2]BCAA, branched chain AA (Leu + Ile + Val); EAA, sum of essential AA; Total AA, sum of essential plus nonessential AA.

[3]Interactions (AA × Insulin) were observed for His ($P < 0.05$), Tyr ($P < 0.10$), and Pro ($P < 0.05$).

[4]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

that the insulin clamp stimulates milk production only when the infusion of casein or AA alone fails to elicit a milk production response.

Recovery of the infused AA in milk protein was ~28% during the insulin clamp, but most of the production response had already been achieved when the insulin clamp had been given alone. Thus, in real terms, the insulin infusion improved the efficiency of converting feed N into milk N by 15%. In the cow studies (16, 27), feed N conversion efficiency was increased 16 to 50% by the insulin infusion. Although efficiency in those studies was increased (13 to 23%) by the combined insulin plus casein (and BCAA; 27) infusion, this improvement was less than the response to insulin infusion. Nonetheless, both studies reported 45% efficiency of recovery of the infused casein as milk protein during the clamp, which is a considerable improvement over many other studies in which recoveries of infused casein or AA have been only as high as 36% (see 6) when feeding purposely formulated protein deficient diets, and compared to the 2% recovery we observed with AA infusion.

We observed very similar changes in milk fat content, composition, and yield in response to the insulin clamp as previously observed in the cow studies (17, 27, 31), suggesting that insulin regulates adipose tissue and mammary fatty acid metabolism in the lactating goat in a manner similar to the cow. When insulin plus AA were infused, the observed depression in milk fat yield was associated primarily with reductions in the incorporation of $C_{10:0}$ (–29%), $C_{14:0}$ (–32%), $C_{16:0}$ (–32%), and $>C_{16:0}$ (–49%) fatty acids into milk. These modifications in milk fatty acid yield are similar to those observed when feeding high concentrate diets (23), with the one exception that trans-fatty acid yields are typically increased, not reduced, on high concentrate diets. Others (17, 27, 31) have interpreted these milk fatty acid responses to the insulin clamp as not being in support of the glucogenic-insulin theory of milk fat depression. On the basis of recent findings (4), the current theory of milk fat depression points to the inhibitory effect on milk fat synthesis of certain isomers of conjugated linoleic acid produced from rumen biohydrogenation, particularly when feeding high concentrate diets (22).

Hormone and Metabolite Concentrations

In previous studies in which dairy cows were subjected to the insulin clamp (16, 17, 27, 31), plasma insulin was elevated three- to fivefold and stimulated an increase in plasma IGF-1 but a reduction in IGF-binding protein-2. The IGF system has been implicated as a possible mediator of the galactopoietic response to the insulin clamp (30), but direct confirmation is still lacking. Herein, the clamp was maintained for

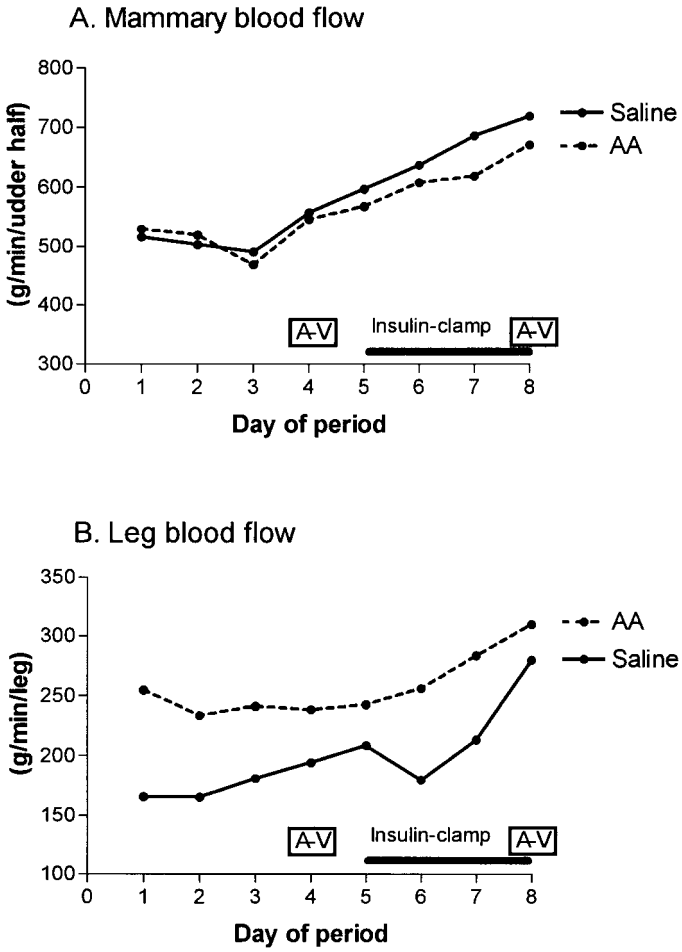

Figure 2. Temporal changes in BF to half the mammary gland (A) and a hind-leg (B). Saline (solid line) or a complete mixture of AA (dashed line) were infused for 7.5 d, with the hyperinsulinemic-euglycemic clamp given during the last 3.5 d as indicated. Arteriovenous measurements were made on days 4 and 8 as indicated by A-V. The insulin-clamp increased daily mammary ($P < 0.02$) and leg ($P < 0.10$) BF.

3.5 d in lactating goats with plasma insulin elevated threefold above baseline and plasma IGF-1 increased by 36%.

By 24 to 36 h of insulin infusion, the rate of exogenous glucose infusion required to maintain euglycemia had reached a steady state, as did plasma levels of IGF-1, urea, and FFA (temporal data not shown). The amount of glucose infused, reflecting insulin-stimulated glucose disposal plus that required to replace insulin-inhibited gluconeogenesis, was 2.1-fold the rate of milk lactose secretion. With one exception (3.5 g/kg; 31), there seems to be a remarkable consistency across studies and ruminant species in the amount of glucose infusion (g of glucose infused/kg of BW) required to maintain euglycemia under insulin clamp conditions (goats: present study, 5.1; 15, 4.8 to 5.8; cows: 16, 5.2; 27, 5.2), suggesting that tissue sensitivity to insulin in lactating ruminants is of a similar magnitude. As in previous studies in cows, coinfusion of AA with the clamp did not affect the rate of glucose infusion required to maintain euglycemia, implying that AA did not modulate insulin-stimulated glucose disposal. This is contrary to observations in humans, in which insulin-stimulated glucose disposal was reduced when AA were coinfused with the clamp (33).

Plasma total AA and EAA concentrations were dramatically reduced by 44 and 53%, respectively, when insulin was infused. This response has also been observed in the cow and goat studies. Casein was infused in one of the cow studies (16) to prevent the drop in plasma AA and to evaluate whether the lower plasma AA levels had limited the response to insulin. Although coinfusion of casein only partly compensated for the decrease in plasma concentrations of His, Thr, and BCAA, milk protein content and yield were substantially increased by the combined infusion, suggesting that AA supply was a limiting factor. In our study, coinfusion of AA with the clamp prevented the drop in plasma levels of His, Tyr, and Pro and lessened the decrease in the BCAA. Subsequently, the yield of milk protein was numerically the highest on the combined infusion (Table 2). Mackle et al. (26, 27) tested whether the BCAA might be limiting, but when the BCAA were infused alone or in combination with casein plus the insulin clamp, they did not observe an increase in milk protein yield above that observed when BCAA were not supplemented (16). Thus, either the basal diet fed to the cows in the Mackle et al. (26, 27) studies already supplied adequate quantities of BCAA to achieve maximal responses to the insulin clamp or other AA (e.g., His) were more limiting.

Mammary and Leg Metabolism

Transport and uptake of AA. Despite the reduced plasma AA concentrations during the insulin clamp, with and without infusion of AA, the rate of milk protein synthesis by the mammary gland increased. To compensate for the lower AA levels, mammary BF and tissue transport activity for several EAA was increased (Tables 7 and 8). Changes in hind-limb metabolism included an increase in leg BF and in transport activity for the BCAA and Lys. Thus, partition of AA towards the mammary and into milk protein did not appear to be facilitated by down-regulation of hind-leg AA transport activity.

Although the insulin clamp increased milk protein yield over the period of the arteriovenous measurements (Figure 1B), net uptake of some AA by the udder was reduced. There are several possibilities to explain

Table 5. Plasma flow and net flux of plasma glucose and free AA across half the mammary gland.[1]

			Insulin clamp			Probability[3]	
Item[2]	Saline	AA	Saline	AA	SED	AA	Insulin
Plasma flow, g/min	413	390	586	589	68	NS	*
Glucose, mmol/h	21.1	19.2	25.1	25.4	3.1	NS[4]	*
Essential AA, μmol/h							
His	320	197	146	159	44	†	*
Ile	1103	1049	856	939	113	NS	*
Leu	1529	1574	1271	1406	181	NS	*
Val	1453	1567	1135	1360	152	†	†
Lys	1270	1124	855	996	163	NS	*
Met	280	275	263	266	33	NS	NS
Phe	382	395	381	340	42	NS	NS
Thr	742	594	476	476	65	NS	**
BCAA	4084	4190	3262	3705	398	NS	*
EAA	7078	6775	5384	5942	625	NS	*
Nonessential AA, μmol/h							
Arg	898	699	768	737	75	**	NS
Tyr	361	331	242	302	63	NS	NS
Pro	629	759	645	853	122	NS	NS
Asp	3	57	19	101	36	†	NS
Glu	693	688	889	602	325	NS	NS
Gln	922	703	249	–64	283	NS	*
Ala	849	551	1119	1148	148	NS	**
Gly	1277	385	–706	–46	795	NS	*
Ser	–244	–252	–826	–751	338	NS	*

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d. Positive values denote net uptake and negative values denote net release or production.

[2]BCAA, branched chain AA (Leu + Ile + Val); EAA, sum of essential AA.

[3]An interaction (AA × Insulin) was observed for Thr ($P < 0.10$).

[4]$P < 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

these observations. Firstly, the insulin clamp may have decreased the catabolism of AA within the udder, rather than stimulating further uptake of AA. This hypothesis is consistent with the observation that the uptake-to-output ratios for the BCAA, Lys, and Arg are reduced by infusion of insulin (10, see also 28). The udder catabolizes ~10% of Met, up to 5% of Phe, 20 to 34% of Lys, and 3 to 20% of Leu (see 6). Indeed, we observed in the current study that Leu oxidation by the udder was reduced by ~40% during the insulin clamp (10). This anticatabolic effect of insulin would serve to conserve more of the Leu taken up by the gland for milk protein synthesis, thus reducing the uptake-to-output ratio. The uptake of Ala by the udder was also increased by insulin, which may reflect an overall reduction in transaminase activity, thus requiring that more Ala be taken up from the blood supply to offset the lower synthesis of Ala de novo.

Secondly, our net uptake measurements were based on plasma free AA only and did not consider the potential contributions from circulating peptides or proteins. The uptake-to-output data (10) indicated that for most AA there was excess uptake of AA by the udder relative to milk protein yield, even during the insulin clamp. But, for Met and Phe, whose uptake was less than milk protein output and for which uptake did not change due to treatment, an additional source of these AA would be required. This source is likely to be circulating peptides or proteins. We have used indirect methods to demonstrate in vivo that peptides do contribute to the supply of some AA for casein synthesis (7). However, the peptide transport system, PepT1, has not been detected in the bovine udder (13), and until a functional mechanism for peptide uptake can be demonstrated, the issue of peptide use by the udder will continue to be debated.

Thirdly, our measurements were based upon plasma and did not consider the potential contribution of red blood cells (the packed cell volume) to the transport of AA. In our previous studies in goats, we have been unable to detect contributions of AA from red blood cells to the net uptake of AA by the udder, although red blood cells were found to participate in the carriage of some AA away from the gland (5, 7). With regards

Table 6. Plasma flow and net flux of plasma glucose and free AA across the hind-leg.[1]

			Insulin clamp			Probability	
Item[2]	Saline	AA	Saline	AA	SED	AA	Insulin
Plasma flow, g/min	190	237	246	288	21	*	*
Glucose, mmol/h	3.17	3.49	4.10	5.16	1.74	NS[3]	NS
Essential AA, μmol/h							
His	77	62	14	30	29	NS	**
Ile	94	206	107	176	43	**	NS
Leu	93	277	130	239	68	*	NS
Val	140	355	131	245	127	†	NS
Lys	173	230	105	200	87	NS	NS
Met	20	50	34	46	16	NS	NS
Phe	21	64	42	50	35	NS	NS
Thr	145	113	66	81	31	NS	**
BCAA	318	843	362	659	208	**	NS
EAA	744	1361	613	1067	335	*	NS
Nonessential AA, μmol/h							
Arg	92	157	81	69	66	NS	NS
Tyr	7	59	4	22	46	NS	NS
Pro	12	200	76	166	77	†	NS
Asp	–15	13	–7	18	16	NS	NS
Glu	281	128	238	119	178	NS	NS
Gln	10	119	–74	–221	170	NS	*
Ala	32	87	107	158	65	NS	**
Gly	394	295	–259	–219	416	NS	**
Ser	132	118	61	46	74	NS	NS

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d. Positive values denote net uptake and negative values denote net release or production.

[2]BCAA, branched chain AA (Leu + Ile + Val); EAA, sum of essential AA.

[3]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

to the role of insulin, or other hormones, in stimulating AA transport by red blood cells, there is no support. In fact, in cows subjected to the insulin clamp, the transfers of Thr, Asn + Asp, and Tyr from red blood cells (packed cell volume) to the mammary gland were reduced, rather than increased, compared with no infusion of insulin (29). Furthermore, Hanigan et al. (18) did not detect changes in the contributions of AA from red blood cells in cows administered growth hormone. Thus, the lower net uptake of AA observed during the insulin clamp herein does not appear to be due to the failure to monitor contributions from red blood cells.

The mammary gland and hind-leg tissues responded differently to AA infusion and the insulin clamp. Thus, while mammary uptake of AA and milk protein synthesis were not increased by AA infusion, the hind-leg responded to AA infusion by removing significantly greater amounts of the BCAA and total EAA (Table 6). The latter suggests that net protein gain by the hind-leg was stimulated by AA infusion, which is consistent with our estimates of net protein gain derived from the [^{15}N, ^{13}C]Leu kinetics (10). It would appear that these significant increases in removal of AA by the leg were due to increased BF and arterial concentrations, since no significant differences in the rate parameters for removal of AA were detected (Tables 7 and 8). Insulin infusion resulted in significant increases in hind-leg transport activity for the BCAA and Lys, which may explain the lack of a significant reduction in removal of these AA despite the large decline in arterial concentrations. Except for an increase in transport activity of BCAA and Lys in association with insulin infusions, one must conclude that the hind limb responds to AA supply in a passive manner. That is, the leg increases AA removal when supply is increased but decreases removal when supply is reduced. In consequence, BF appears to be a driving variable for the leg tissues.

Glucose uptake by the mammary gland was stimulated during insulin infusion. However, increased uptake apparently resulted from increased BF, as transport activity was not significantly affected. By contrast, previous work has not demonstrated significant changes in mammary glucose uptake during short-

Table 7. Rate constants (K, ml/min per udder half or a hind-leg) for plasma glucose and essential AA uptake by half the mammary gland and a hind-leg of lactating goats.[1]

			Insulin clamp			Probability[2]	
	Saline	AA	Saline	AA	SED	AA	Insulin
Glucose							
Mammary	143	120	88	171	46	NS[3]	NS
Leg	28	16	11	27	10	NS	NS
His							
Mammary	190	139	744	79	146	†	*
Leg	37	35	52	15	24	NS	NS
Ile							
Mammary	289	247	692	499	133	NS	*
Leg	13	32	49	64	13	NS	*
Leu							
Mammary	359	280	963	564	147	NS	**
Leg	9	32	54	62	13	NS	*
Val							
Mammary	205	168	507	274	100	NS	*
Leg	9	25	35	41	10	NS	*
Lys							
Mammary	223	159	584	237	101	†	*
Leg	19	23	45	40	9	NS	**
Met							
Mammary	558	472	805	308	115	NS	NS
Leg	19	55	69	44	31	NS	NS
Phe							
Mammary	515	234	294	129	96	†	*
Leg	13	28	23	17	17	NS	NS
Thr							
Mammary	361	385	983	356	170	NS	*
Leg	41	45	64	45	17	NS	NS

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d.

[2]Interactions (AA × Insulin) were observed for mammary K for His ($P < 0.05$), Val ($P < 0.10$), Lys ($P < 0.10$), Phe ($P < 0.10$), and Thr ($P < 0.05$).

[3]$P > 0.10$.

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

term hyperinsulinemic-euglycemic clamps (21, 25, 38). Nonetheless, the lack of a change in mammary glucose transport activity is consistent with the inability to detect expression of the insulin-responsive glucose transporter in the mammary gland (40) and strengthens the current argument that insulin does not directly regulate glucose uptake by the udder. We also did not observe changes in hind-leg glucose removal or transport activity due to insulin infusion. This is consistent with previous observations in which the hind-limb tissues of lactating sheep were found to be resistant to insulin (39).

Tissue blood flow. During the insulin clamp, independent of whether or not AA were infused, mammary BF was increased by 42%. This is the first direct evidence that mammary BF is increased by the insulin clamp and confirms a recent report of a 42% increase (28) using an indirect method (the Fick principle) to estimate mammary BF. The clamp increased leg BF by 29 to 52%, and there was a tendency for infusion of AA to increase leg flow by 25%. Interestingly, although increases in mammary and hind-leg BF were both associated with insulin infusion, AA infusion only affected hind-limb flow. The mechanism(s) mediating these responses appears to be complicated, involving at least insulin, IGF-1, glucose, AA, or all of these.

We have previously found in lactating goats where insulin was infused i.v. for 4 h and where euglycemia was not maintained, that both mammary BF and the proportion of cardiac output directed to the udder were reduced (36). Similarly, in dairy cows, Metcalf et al. (32) observed a reduction in mammary BF when insulin was infused close arterial to the udder, but in this study euglycemia was also not maintained. By contrast, Hove (21) observed an increase in mammary BF in goats during a 10-h i.v. infusion of insulin and where

Table 8. Rate constants (*K*, ml/min per udder half or a hind-leg) for plasma nonessential AA uptake by half the mammary gland and a hind-leg of lactating goats.[1]

			Insulin clamp			Probability[2]	
	Saline	AA	Saline	AA	SED	AA	Insulin
Arg							
Mammary	203	160	455	276	70	NS[3]	**
Leg	17	25	35	20	20	NS	NS
Tyr							
Mammary	156	168	87	85	43	NS	*
Leg	5	21	3	6	17	NS	NS
Pro							
Mammary	100	102	192	115	21	NS	*
Leg	0	21	19	21	10	NS	NS
Asp							
Mammary	91	564	190	870	277	NS	NS
Leg	–29	92	14	122	64	NS	NS
Glu							
Mammary	986	615	890	684	391	NS	NS
Leg	187	51	180	90	90	NS	NS
Gln							
Mammary	57	45	17	1	17	NS	*
Leg	–1	4	–3	–9	10	NS	NS
Ala							
Mammary	130	102	228	190	54	NS	*
Leg	5	11	20	24	12	NS	*
Gly							
Mammary	28	11	–13	0	13	NS	*
Leg	8	5	–5	–4	10	NS	NS
Ser							
Mammary	–38	–37	–90	–74	31	NS	*
Leg	21	19	8	11	13	NS	NS

[1]Goats (n = 4) were infused i.v. with saline (Saline) or a complete mixture of AA for 7.5 d, and goats were subjected to a hyperinsulinemic-euglycemic clamp during the last 3.5 d.

[2]An interaction (AA × Insulin) was observed for mammary *K* for Pro ($P < 0.05$).

[3]$P > 0.10$.

*$P < 0.05$.

**$P < 0.01$.

an attempt was made to maintain euglycemia by infusing glucose at a fixed rate. In the current study, BF increased soon after initiating the insulin clamp, and we were able to detect when blood glucose concentrations had fallen below normal levels because mammary and hind-leg BF were low on the flow probe monitor. Blood flow returned to previously high levels soon after euglycemia was reestablished. Stimulation of glucose metabolism in the capillary endothelium of muscle by insulin is believed to result in activation of endothelium-derived nitric oxide production via metabolic coupling (3). Thus, when glucose concentrations are reduced under clamp conditions, the activity of this mechanism may be reduced and vice versa when glucose is at normal levels. Consequently, glucose is clearly involved in regulating BF to both the hind-leg and the udder.

The imbalance in plasma metabolite concentrations during the clamp may have also precipitated the changes in BF. We have previously observed that mammary BF increases when plasma His or Leu concentrations are reduced in goats, suggesting that the udder may be capable of sensing low concentrations of AA (8, 9). Thus, in an attempt to compensate for the AA deficiency caused by the insulin clamp, mammary BF increased. This hypothesis could be also tested by clamping AA concentrations during the insulin clamp. The hind-leg appears to respond positively in terms of BF to elevated AA concentrations (AA infusion) and to reduced AA concentrations (insulin infusion); however, thus negating the AA concentration hypothesis, at least with regards to the leg. The lack of a significant interaction between insulin and AA on leg BF also suggests that the two may act through a common mechanism(s).

Plasma concentrations of FFA, acetate, and BHBA are also reduced during the insulin clamp, which may have initiated the increase in mammary BF. According

to the theoretical model of mammary BF regulation developed by Cant and McBride (12), mammary BF will increase when energy supply is reduced. At the center of this model is the hypothesis that the mammary gland exerts control over local BF rate in order to maintain adenylate charge. Thus, when circulating energy metabolite concentrations are low, as occurs when insulin is infused, mammary tissue adenosine concentrations would be increased, which would cause dilation of the capillary microvasculature and an increase in BF.

The elevation in IGF-I caused by the clamp may have also caused the increase in mammary BF. Indeed, the IGF peptides have been shown (34, 35) to increase mammary BF and milk yield when infused close arterial to the udder, suggesting that free IGF-I or II may be direct or indirect effectors of milk synthesis via regulation of BF.

CONCLUSIONS

Insulin clearly affects AA metabolism in the ruminant. Although a number of changes appear to have occurred in mammary and leg tissues, the most significant appears to be the increase in BF. Insulin does not appear to be a direct regulator of milk protein synthesis as its concentrations were increased immediately upon initiation of the clamp, yet the maximum stimulation of milk protein yield did not occur until a day or two later (present results, 16, 27, 31). Mammary BF and milk protein yield initially increased in parallel with changes in IGF-1, but further increases in BF and yield occurred subsequently, without a further increase in IGF-1. Whether milk protein synthesis is regulated directly by BF or whether BF acts in support of protein synthesis is not possible to discern herein. Nonetheless, the IGF peptides have been shown (34, 35) to increase mammary BF and milk yield when infused close arterial to the udder. A further contributor to this regulation may also involve insulin and glucose since these clearly play a role in regulating IGF-1 and mammary BF. Infusion of AA stimulated anabolism by leg tissues, but not the mammary gland. Under insulin clamp conditions this response was reversed. The partition of AA to the udder under clamp conditions was not facilitated by down-regulation of AA transport activity by the leg tissues, nor by increased removal of AA by the udder, suggesting that metabolism of AA within the tissues may be a key point of regulation.

The coordination of nutrient partition in the lactating animal obviously involves interactions between nutrient supply and anabolic regulation by hormonal factors at the tissue level, which through intracellular signaling pathways determine the anabolic or catabolic fates of nutrients within target tissues such as the mammary gland and hind-limb tissues. Further progress towards maximal milk production in dairy cows and the ability to predict changes in milk production from alterations in dietary supply (19, 20) will depend on a clearer understanding of these metabolic pathways and identification of the nutrient and hormonal signals responsible for initiating the metabolic cascades.

ACKNOWLEDGMENTS

This work is part of a joint project at the Rowett Research Institute (Aberdeen, Scotland) and Reading University (Reading, England) cosponsored by a consortium of the Ministry of Agriculture, Fisheries and Food (London); the Biotechnology and Biological Sciences Research Council (Swindon); the Scottish Office Agriculture Environment and Fisheries Department (Scotland); Purina Mills, Inc. (St. Louis, MO); Hendrix Voeders B.V. (The Netherlands); and the Milk Development Council (Cirencester).

REFERENCES

1 Agricultural and Food Research Council. 1993. Energy and Protein Requirements of Ruminants. An Advisory Manual Prepared by the AFRC Technical Committee of Responses to Nutrients. CAB Int., Wallingford, United Kingdom.
2 Back, P. J., D.D.S. Mackenzie, S. R. Davis, and P. M. Harris. 1998. The effects of insulin-nutrient supply interactions on ewe lactation. Proc. N.Z. Soc. Anim. Prod. 58:205–208.
3 Baron, A. D., and M. G. Clark. 1997. Role of blood flow in the regulation of muscle glucose uptake. Annu. Rev. Nutr. 17:487–499.
4 Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebo and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. 278:R179–R184.
5 Bequette, B. J., F.R.C. Backwell, A. G. Calder, J. A. Metcalf, D. E. Beever, J. C. MacRae, and G. E. Lobley. 1997. Application of a U-carbon-13-labelled amino acid tracer in lactating dairy goats for simultaneous measurements of the flux of amino acids in plasma and the partition of amino acids to the mammary gland. J. Dairy Sci. 80:2842–2853.
6 Bequette, B. J., F.R.C. Backwell, and L. A. Crompton. 1998. Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. J. Dairy Sci. 81:2540–2559.
7 Bequette, B. J., F.R.C. Backwell, C. E. Kyle, L. A. Crompton, J. France, and J. C. MacRae. 1999. Vascular sources of phenylalanine, tyrosine, lysine, and methionine for casein synthesis in lactating goats. J. Dairy Sci. 82:362–377.
8 Bequette, B. J., F.R.C. Backwell, J. C. MacRae, G. E. Lobley, L. A. Crompton, J. A. Metcalf, and J. D. Sutton. 1996. Effect of intravenous amino acid infusion on leucine oxidation across the mammary gland of the lactating goat. J. Dairy Sci. 79:2217–2224.
9 Bequette, B. J., M. D. Hanigan, A. G. Calder, C. K. Reynolds, G. E. Lobley, and J. C. MacRae. 2000. Amino acid exchange by the mammary gland of lactating goats when histidine limits milk production. J. Dairy Sci. 83:765–775.

10 Bequette, B. J., L. A. Crompton, and M. D. Hanigan. 1999. Mammary and hind-leg protein metabolism in lactating goats given intravenous infusions of insulin and amino acids. J. Dairy Sci. (Suppl. 1):37.
11 Bruce, L. A., T. Atkinson, J.S.M. Hutchinson, R. A. Shakespear, and J. C. MacRae. 1991. The measurement of insulin-like growth factor 1 in sheep plasma. J. Endocrinol. 128:R1–R4.
12 Cant, J. P., and B. W. McBride. 1995. Mathematical analysis of the relationship between blood flow and uptake of nutrients in the mammary glands of a lactating cow. J. Dairy Res. 62:405–422.
13 Chen, H., E. O. Wong, and K. E. Webb, Jr. 1999. Tissue distribution of a peptide transporter mRNA in sheep, dairy cows, pigs, and chickens. J. Anim. Sci. 77:1277–1283.
14 Christie, W. W., ed. 1982. Lipid Analysis. 2nd ed. Pergamon Press, Oxford, UK.
15 Debras, E., J. Grizard, E. Aina, S. Tesseraud, C. Champredon, and M. Arnal. 1989. Insulin sensitivity and responsiveness during lactation and dry periods in goats. Am. J. Physiol. 256:E295–E302.
16 Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, D. M. Barbano, and W. A. House. 1997. The role of insulin in the regulation of milk protein synthesis in dairy cows. J. Dairy Sci. 80:2361–2371.
17 Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, and D. L. Palmquist. 1997. The role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076–1084.
18 Hanigan, M. D., C. C. Calvert, E. J. DePeters, B. L. Reis, and R. L. Baldwin. 1992. Kinetics of amino acid extraction by lactating mammary glands in control and sometribove-treated Holstein cows. J. Dairy Sci. 75:161–173.
19 Hanigan, M. D., J. P. Cant, D. C. Weakley, and J. L. Beckett. 1998. An evaluation of postabsorptive protein and amino acid metabolism in the lactating cow. J. Dairy Sci. 81:3385–3401.
20 Hanigan, M. D., J. France, D. Wray-Cahen, D. E. Beever, G. E. Lobley, L. Reutzel, and N. E. Smith. 1998. Alternative models for analyses of liver and mammary transorgan metabolite extraction data. Br. J. Nutr. 79:63–78.
21 Hove, K. 1978. Effects of hyperinsulinemia on lactose secretion and glucose uptake by the goat mammary gland. Acta Physiol. Scand. 104:422–430.
22 Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of trans-C-18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2104–2114.
23 Kennelly, J. J., B. Robinson, and G. R. Khorasani. 1999. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in early-lactation holstein cows. J. Dairy Sci. 82:2486–2498.
24 Kirk, R. S., and R. Sawyer, eds. 1991. Pearsons Composition and Analysis of Foods. 9th ed. Longman Scientific and Technical, Harlow, UK.
25 Laarveld, B., D. A. Christensen, and R. P. Brockman. 1981. The effect of insulin on net metabolism of glucose and amino acids by the bovine mammary gland. Endocrinology 108:2217–2221.
26 Mackle, T. R., D. A. Dwyer, and D. E. Bauman. 1999. Effects of branched-chain amino acids and sodium caseinate on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82:161–171.
27 Mackle, T. R., D. A. Dwyer, K. L. Ingvartsen, P. Y. Chouinard, J. M. Lynch, D. M. Barbano, and D. E. Bauman. 1999. Effects of insulin and amino acids on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82:1512–1524.
28 Mackle, T. R., D. A. Dwyer, K. L. Ingvartsen, P. Y. Chouinard, D. A. Ross, and D. E. Bauman. 2000. Effects of insulin and postruminal supply of protein on use of amino acids by the mammary gland for milk protein synthesis. J. Dairy Sci. 83:93–105.
29 Mackle, T. R., D. A. Dwyer, K. L. Ingvartsen, P. Y. Chouinard, D. A. Ross, and D. E. Bauman. 2000. Evaluation of whole blood and plasma in the interorgan supply of free amino acids for the mammary gland of lactating dairy cows. J. Dairy Sci.83:1300–1309.
30 McGuire, M. A., D. A. Dwyer, R. J. Harrell, and D. E. Bauman. 1995. Insulin regulates circulating insulin-like growth factors and some of their binding proteins in lactating cows. Am. J. Physiol. 269:E723–E730.
31 McGuire, M., J. M. Griinari, D. A. Dwyer, and D. E. Bauman. 1995. The role of insulin in the regulation of mammary synthesis of fat and protein. J. Dairy Sci. 78:816–824.
32 Metcalf, J. A., J. D. Sutton, J. E. Cockburn, D. J. Napper, and D. E. Beever. 1991. The influence of insulin and amino acid supply on amino acid uptake by the lactating bovine mammary gland. J. Dairy Sci. 74:3412–3420.
33 Pisters, P.W.T., N. P. Restifo, E. Cersosimo, and M. F. Brennan. 1991. The effects of euglycemia hyperinsulinemia and amino acid infusion on regional and whole body glucose disposal in man. Metabolism 40:59–65.
34 Prosser, C. G., S. R. Davis, V. C. Farr, L. G. Moore, and P. D. Gluckman. 1994. Effects of close-arterial (external pudic) infusion of insulin-like growth factor-II on milk yield and mammary blood flow in lactating goats. J. Endocrinol. 142:93–99.
35 Prosser, C. G., I. R. Fleet, A. N. Corps, E. R. Froesch, and R. B. Heap. 1990. Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor-I into the mammary gland of the goat. J. Endocrinol. 126:437–443.
36 Randles, W. G., C. E. Kyle, M. D. Hanigan, L. A. Crompton, and B. J. Bequette. 1998. The effects of nutritional status and insulin on the partition of blood flow to the mammary gland and milk composition in lactating goats. Page 2 *in* Proc. Br. Soc. Anim. Sci. Animal Science.
37 Tauveron, I., D. Larbaud, C. Champredon, E. Debras, S. Tesseraud, G. Bayle, Y. Bonnet, Ph. Thieblot, and J. Grizard. 1994. Effect of hyperinsulinemia and hyperaminoacidemia on muscle and liver protein synthesis in lactating goats. Am. J. Physiol. 267:E877–E885.
38 Tesseraud, S., J. Grizard, B. Makarski, E. Debras, G. Bayle, and C. Champredon. 1992. Effect of insulin in conjunction with glucose, amino acids and potassium on net metabolism of glucose and amino acids in the goat mammary gland. J. Dairy Res. 59:135–149.
39 Vernon, R. G., A. Faulkner, W. W. Hay, Jr., D. T. Calvert, and D. J. Flint. 1990. Insulin resistance of hind-limb tissues in vivo in lactating sheep. Biochem. J. 270:783–786.
40 Zhao, F. Q., W. T. Dixon, and J. J. Kennelly. 1996. Localization and gene-expression of glucose transporters in bovine mammary-gland. Comp. Biochem. Physiol. (B: Biochem. Mol. Biol.) 115:127–134.