Effect of Intravenous Amino Acid Infusion on Leucine Oxidation Across the Mammary Gland of the Lactating Goat

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ABSTRACT

Changes in the kinetics of leucine in the mammary gland were examined in four lactating goats (25, 38, 45, and 135 DIM) that were given an i.v. infusion of a mixture of 18 AA, not including leucine, to alter the availability of leucine to the gland relative to other AA. Arteriovenous monitoring of [1-^{13}C]leucine kinetics across one-half of the mammary gland was conducted on the last day (d 6 or 7) of the saline (control) and the AA infusion periods. Although blood flow to the mammary gland and the arterial concentration of most AA other than leucine were increased by the AA infusion, milk and protein yields did not change. For goats in early lactation (n = 3), arterial leucine concentrations fell considerably during AA infusion; however, the arteriovenous difference of leucine was maintained, resulting in uncommonly low leucine concentrations in venous plasma (8 μM). Whole body leucine flux (protein synthesis plus oxidation) was unaffected by AA infusion, but, because whole body leucine oxidation was reduced, whole body utilization of leucine for protein synthesis increased. The AA infusion reduced mammary oxidation of leucine to approximately one-third of control values. These results suggest that leucine oxidation can be reduced considerably without affecting milk protein output; thus, leucine oxidation may not be an irrevocable consequence of mammary metabolism. If catabolism of other AA either by the gland or in the whole body can be reduced, then the efficiency of milk yield can be improved.

(**Key words**: leucine metabolism, goat, mammary gland, protein metabolism)

Abbreviation key: **BCAA** = branched-chain AA, **ILR** = irreversible loss rate, **MOP** = 4-methyl-2-oxopentanoate, ***p*-AH** = *p*-aminohippuric acid, **PS** = protein synthesis.

INTRODUCTION

The branched-chain AA (valine, leucine, and isoleucine; **BCAA**) constitute approximately 22% of the milk protein residues, but, compared with many other AA, BCAA extraction by the mammary gland generally exceeds the quantity required for milk protein output (5, 8, 9, 16, 27). Studies in vitro and in vivo have shown that BCAA are catabolized by the mammary gland along pathways common to other tissues yielding transamination products, the appropriate oxo acids, and carbon skeletons for dispensable AA synthesis; a significant proportion appears as CO_2 (4, 19, 22, 23, 27).

The net uptake of leucine by the mammary gland almost always exceeds its secretion in milk protein, and this differential becomes even greater when milk protein output is increased by duodenal infusion of casein (8). The fate of this additional uptake and reasons for its occurrence are not known. In other ruminant species, leucine has been linked to the metabolic regulation of muscle protein, either directly via leucine (24) or its oxo acid (20) or indirectly through changes in peripheral tissue transamination or oxidation of leucine (11, 13). The oxidation of leucine by the mammary gland has been correlated with stage of lactation and dietary protein intake (4, 19), but it is unclear whether the additional uptake and catabolism of leucine by the mammary gland is essential for the synthesis of milk protein.

In the current study, the supply of other AA was manipulated to reduce leucine availability to the mammary gland relative to the availability of other AA. This manipulation tested leucine catabolism and its effect on protein metabolism in the mammary gland. If oxidation of leucine was crucial, then

Received June 26, 1995.
Accepted April 19, 1996.

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presumably oxidation would be maintained at substantial rates despite the limited availability of leucine; otherwise, milk protein output would decline. Coincidently, the changes in peripheral leucine concentration also allowed an assessment of the influence of arterial supply on AA extraction by the mammary gland.

MATERIALS AND METHODS

Goats and i.v. Treatment Infusions

Four nonpregnant, multiparous British Saanen goats were used. Mean (±SD) BW was 63 ± 8 kg. Each goat was housed individually in a metabolism crate for at least 2 wk prior to experimentation. Each goat had been surgically prepared during the preceding lactation with skin-covered loops established around a carotid artery and a superficial epigastric vein, including ligation of the vessels common to the mammary vein. Three goats were near peak lactation (25 to 45 d), and one goat was in midlactation (135 d). Earlier (58 d) in the current lactation and prior to bifurcation, this latter goat also had an ultrasonic flow probe (6 mm; Transonic Systems Inc., Ithaca, NY) placed around the external pudic artery on the same side as the mammary vein loop.

Goats were offered a low protein ration [10.5% CP (N × 6.25)], consisting of fixed proportions (40:60, as-fed basis) of molasses-treated hay (10%, as-fed basis; 56 g of CP/kg) and pelleted concentrate (138 g of CP/kg). The daily ration was supplied in equal amounts at 2-h intervals from automatic feeders to establish approximate steady-state conditions of digestion and metabolism. Prior to experimentation, all goats were allowed ad libitum consumption of the low protein ration, which was calculated to satisfy 75% of the protein requirements and provided sufficient energy to meet requirements for maintenance, growth, and milk yield (1). At least 7 d prior to and throughout the experimental periods, intakes were fixed at the established ad libitum intake. Milking was performed at 0830 and 1630 h, weights were recorded, and subsamples were stored (10°C) for analyses of milk N.

A saline control (9 g of NaCl/L and 460 g of solution/d) or an AA mixture that was similar in composition to bovine milk casein but excluded leucine (63 g of AA in 460 g of solution/d; Table 1) was infused via a jugular vein catheter that had been inserted 48 h prior to infusion. The AA mixture also contained additional (+3.3 g/d) phenylalanine to replace tyrosine, which has a low solubility. All infusates were sterilized through 0.2-μm nitrocellulose filters into sterile plastic bags, which were then encased in aluminum foil.

TABLE 1. Mean daily rates of L-AA infusion.

AA	(g/d)
Met	1.9
Phe[1]	6.5
His	1.9
Trp	1.0
Thr	2.6
Val	4.2
Ile	3.1
Lys-HCl	6.3
Arg	2.4
Gly	1.2
Ala	1.9
Pro	5.6
Ser	3.4
Cys	0.4
Asn-H_2O	2.6
Gln	6.6
Glu	6.7
Asp	4.6
Total	62.9

[1]Includes additional (+3.3 g/d) Phe to replace Tyr, which has a low solubility.

At least 24 h prior to kinetics measurements, a catheter was inserted into the carotid artery and into the contralateral jugular vein for isotope infusion; two catheters were placed into the mammary vein loop (3). The mammary loop catheters were placed 20 cm between catheter tips, proximal and distal to the gland for infusion of *p*-aminohippuric acid (***p*-AH**) and blood sampling, respectively, and were retained in the same position for the two kinetics measurements. All catheters were maintained patent by regular flushing with saline containing heparin (200 IU/ml).

Measurement of Leucine Kinetics in the Mammary Gland

For periods of 6 or 7 d, three goats were infused first with saline and then with the AA mixture; a fourth goat received the reverse sequence. On the last day of each infusion period, kinetics of [1-^{13}C]leucine in the whole body and in the mammary gland were measured. On each occasion, [1-^{13}C]leucine (99 atom %; Tracer Technologies Inc., Somerville, MA), dissolved (35 mmol/kg) in sterile saline containing heparin (350,000 IU/kg), was infused (0.67 mmol/h) into a jugular vein for 8 h. Infusions began immediately following the morning milking. At 2 h, an infusion (600 mg/h) of *p*-AH (30 g/kg of sterile saline) into the mammary vein was initiated, except for the goat fitted with a flow probe. Blood flow for this goat

was monitored continuously throughout the 8-h isotope infusion. Starting at 3 h, at which time isotopic steady state in the plasma is usually achieved (3), blood samples were withdrawn continually (10 ml/h) at hourly intervals for the next 5 h from both the carotid artery and mammary vein catheters into sealed syringes submerged in an ice bath (14). Additionally, to inhibit clotting, an i.v. bolus dose of heparin (18,700 IU) had been given to each goat 15 min prior to collection of the first blood sample. Goats remained standing throughout sampling.

Blood samples were immediately mixed by gentle hand-rolling, and 2- × 1.5-ml samples were injected into evacuated 10-ml vacutainers containing 1 ml of frozen lactic acid and an antifoaming agent (9:1, vol/vol). Samples were stored frozen (21). 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. Two 0.5-g blood samples were then accurately weighed, and an equal weight of hemolyzing solution containing L-norleucine (100 μM) was added. The remaining heparinized blood was centrifuged at 2000 × g for 15 min at 4°C. Two 0.5-g aliquots of plasma were accurately weighed for p-AH determination. Another two 0.5-g samples were accurately weighed, and an equal weight of a solution containing 100 μM L-norleucine and 10 μM 2-oxohexanoate was added. All samples were stored at −20°C until analyzed. At least 24 h prior to isotope infusion, blood samples were taken to assess the natural abundance of ^{13}C in bicarbonate, leucine, and 4-methyl-2-oxopentanoate (**MOP**).

Analytical Procedures

Concentrations and ^{13}C enrichment determinations. Gravimetric procedures were used to quantify plasma p-AH concentrations assayed by the Bratton-Marshall procedure as described by Smith et al. (25). For determination of [1-^{13}C]leucine in blood and plasma and of [1-^{13}C]MOP in plasma, stored samples were thawed, and protein was removed immediately with 0.125 ml of sulfosalicylic acid (38%, wt/vol). The supernatants were then processed to yield oxo acid and AA fractions. Oxo acid was treated to yield the n-butyl heptafluorylbutyryl derivative, and the latter was treated to yield the n-butyldimethylsilyl derivative (7). Isotopic enrichments were quantified under electron-impact conditions on a gas chromatography mass spectrometer (Trio-1; VG Mass Lab, Manchester, England). Blood and plasma leucine and MOP (plasma only) concentrations were determined from gas chromatograph peak areas that were corrected for the known additions of norleucine and hexanoate. Concentrations of other AA were determined on pooled arterial blood samples by reverse-phase HPLC (Millipore, Waters Chromatography, Milford, MA) as described by Heinrickson and Meredith (12). Blood $H^{13}CO_3$ enrichments were determined on a VG isotope ratio-MS Sira-12 as described by Read et al. (21). All enrichments were expressed as atom percent excess against the appropriate natural abundance sample.

Milk analyses. Whole milk N concentrations were determined on subsamples by macro N (combustion) analyses. Milk protein output was calculated as milk N × 6.38.

Calculations

Irreversible loss rate (**ILR**; flux) for whole body leucine was calculated as previously described (4) based on either the enrichment of free leucine or MOP in arterial plasma. These values were converted to minimum estimates of whole body protein flux (grams per day) based on the mean leucine concentration in the body protein of growing sheep [66.9 g/kg; (15)].

Mammary gland blood flow (MBF) or plasma flow (MPF) was measured for one-half of the mammary gland based on downstream dilution of p-AH in the mammary vein loop (n = 3) or by an ultrasonic flow probe (n = 1). Plasma flow (dye-dilution method) was converted to mammary blood flow after correction for packed cell volume (PCV): MBF = MPF/(1 − PCV).

Calculations for mammary gland leucine kinetics were for only the one-half of the gland that was monitored and were similar to those employed previously for measurements across the mammary gland of the dairy cow (4). Net removal was the product of arterial (a) and venous (v) blood leucine or plasma MOP concentration difference and mammary blood or plasma flow, respectively. Leucine that was available for protein synthesis (**PS**; gain or secretion) was calculated as the net removal of leucine corrected for the net movements of the oxo acid (MOP) and oxidative losses:

$$\text{leucine available for PS (millimoles per hour)} = ((L_a - L_v) \times MBF) - [(M_v - M_a) + ((C_v \times E_{c,v} - C_a \times E_{c,a})/E_{m,v})] \times MPF$$

where L = the concentration (micromolars) of free leucine in blood, M and C = the respective concentrations (micromolars) of MOP and CO_2 in plasma, E = the enrichment (atom percent excess) of the metabo-

lite, and $E_{m,v}$ = the enrichment of MOP in mammary vein plasma, which was taken to be most representative of the isotopic activity at the site of decarboxylation in the tissues of the gland. Plasma flow was used to correct for oxidative losses (4, 11) and for the net movements of the oxo acid because MOP concentrations in goats and cows did not differ between blood and plasma (A. G. Calder, 1993, personal communication).

Protein synthesis was calculated as

$$(((L_a \times E_{l,a} - L_v \times E_{l,v}) \times MBF) + ((M_a \times E_{m,a} - M_v \times E_{m,v}) - (C_v \times E_{c,v} - C_a \times E_{c,a})) \times MPF)/E_y$$

where E_y = the substitutes for the true precursor. Two alternatives were considered, the enrichments of leucine and MOP in mammary vein plasma.

The fraction of leucine molecules that was extracted, including net isotope removed as MOP, and oxidized (fractional oxidation rate) was determined from the loss of the carboxyl carbon as CO_2:

$$((C_v \times E_{c,v} - C_a \times E_{c,a}) \times MPF)/(((L_a \times E_{l,a} - L_v \times E_{l,v}) \times MBF) + ((M_a \times E_{m,a} - M_v \times E_{m,v}) \times MPF)).$$

The absolute rate of leucine oxidation (millimoles per hour) by the gland was also determined from the loss of the carboxyl carbon as CO_2 with conversion to AA flow (loss) using mammary vein plasma MOP enrichment as the precursor:

$$100 \times (C_v \times E_{c,v} - C_a \times E_{c,a}) \times MPF/E_{m,v}.$$

Statistical Analysis

The statistical significance of the difference between treatments in kinetics parameters was assessed by paired t test and Student's t distribution; $P < 0.10$ (two-tailed test) was considered to be significantly different. Because the experimental period encompassed peak lactation (wk 3 to 8) for three of four goats, individual data files were fitted to a quadratic curve to model this period of the lactation cycle and to assess the effects of treatment on milk and protein yields. A treatment term was then added to the model to examine differences between the saline and AA infusion periods. No significant differences were found between treatments.

RESULTS

Milk Yield

No significant effect of the AA infusion on milk yield was found. For the control and AA infusion periods, total milk yields were 3.60 and 3.68 kg/d, and protein yields (grams of milk N × 6.38 per day) were 90.9 and 94.4 g/d, respectively.

Measurements of Whole Body Kinetics

The concentrations of leucine and MOP and other AA in blood and whole body leucine ILR are shown in Table 2. Concentrations of leucine in both arterial and mammary venous blood and plasma were lower during the AA infusion; however, because concentrations were slightly increased (blood, 96.4 vs. 102.8 μM; plasma, 93.5 vs. 99.1 μM) by AA infusion in the goat in late lactation, differences were not statistically significant for arterial blood and plasma. Despite the often low arterial concentrations, the differences between arterial and venous concentrations of leucine were maintained, and, as a result, the percentage leucine extraction was increased ($P < 0.05$) by AA infusion. Arterial plasma concentrations of MOP were reduced ($P < 0.05$) by AA infusion. For all goats, the blood concentrations of the essential AA, those most often considered to be limiting for milk PS (i.e., methionine, histidine, lysine, and phenylalanine), were increased (~1.2- to 3.3-fold) by AA infusion (Table 2).

Although concentrations of arterial leucine changed, whole body leucine ILR was unaffected by AA infusion (Table 2). Values calculated with plasma MOP were greater ($P < 0.001$; n = 8) than those estimates for plasma leucine as the precursor choice. The estimate based on MOP as the precursor reflected its intracellular pool origin, where leucine enrichment is diluted as a result of intracellular protein degradation. The protein equivalent of the ILR values ranged from 421 to 514 g/d, which was lower than previous estimates in goats at a similar stage of lactation [731 g/d; (3)]; however, the latter received higher intakes of protein.

Arterial $^{13}CO_2$ enrichments were much lower [1.7×10^{-3} vs. 6.01×10^{-3} atom % excess (SE difference, 0.508×10^{-3}; $P < 0.005$)] during the AA infusion. The rate of whole body CO_2 production was not measured, but, if it remained unaltered, then whole body leucine oxidation would be substantially reduced (possibly by as much as 72%) and, compared with measured leucine flux, whole body leucine would be more greatly utilized for PS with the AA infusion.

Mammary Gland Kinetics

Blood flow to one-half of the mammary gland tended ($P = 0.08$) to be higher with AA infusion (Table 3), and data (monitored daily) for the goat

with the fitted flow probe confirmed these changes (data not shown). Calculations of kinetics were restricted to the one-half of the gland that was monitored because of differences noted in milk yield between halves. Consequently, the commonly adopted procedure, which was to compare the net balance of arteriovenous AA with milk outputs, was not attempted. Also, for these goats, the contribution of the different mammary-derived milk proteins to the total N content of the milk was not known.

Although the net removal of leucine and MOP by the gland was not significantly affected by AA infusion, the utilization of leucine by the gland was altered. Fractional and absolute rates of leucine oxidation were reduced ($P < 0.05$) by AA infusion. This reduction increased ($P < 0.05$) the leucine that was available for PS (Table 3). Consequently, mammary gland PS, based on venous leucine ($P < 0.0001$) or venous MOP ($P < 0.01$), was increased because of the AA infusion.

DISCUSSION

Recently, Bequette et al. (4) observed that, although dietary protein supplementation increased the concentration and supply of leucine and other AA to the mammary gland, milk protein output of dairy cows was not enhanced. Instead, leucine oxidation by the gland was increased. The present study was undertaken to determine the effects of conditions in which the supply to the mammary gland of other AA was increased relative to leucine.

Infusion of the AA mixture did not significantly affect milk protein output (90.0 vs. 94.4 g/d), despite a significant increase in the arterial concentrations other essential AA (methionine, histidine, lysine, and phenylalanine). In contrast, for those goats in early lactation (n = 3), concentrations of leucine fell considerably as a consequence of AA infusion. Because the net availability of leucine in the whole body was not actually altered by AA infusion, the decline in concentration implied a greater removal of leucine by

TABLE 2. Leucine kinetics in the whole body and blood concentrations of selected AA in lactating goats (n = 4) receiving i.v. infusions (each of 6 or 7 d) of saline (control) and a mixture of 18 AA, excluding Leu.

Item	Control	AA	SED[1]	P
Leu, μM				
Arterial				
Blood	94.0	76.2	8.9	NS[2]
Plasma	93.2	75.1	9.4	NS
Venous				
Blood	54.4	35.9	6.2	0.06
Plasma	45.0	23.7	6.5	0.05
Net Leu extraction,[3] %				
Blood	43	54	3	0.05
Plasma	52	71	5	0.05
MOP,[4] μM	12.8	5.4	1.6	0.05
Selected AA,[5] μM				
Met	18.6	40.2	3.0	0.005
His	25.3	82.8	8.7	0.01
Lys	223.4	409.1	60.8	0.05
Phe	31.6	62.8	11.1	NS
Whole body ILR,[6] mmol/h				
ILR for Leu	9.14 (433)	8.88 (421)	0.54	NS
ILR for MOP	10.40 (493)	10.85 (514)	0.76	NS

[1]Standard error of the difference between treatment means.

[2]$P > 0.10$. Significance of difference was determined by paired t test.

[3]Net Leu extraction percentage was calculated from free Leu arterial (A) and mammary venous (MV) concentrations in blood and plasma: 100 × (A − MV)/A.

[4]Arterial plasma 4-methyl-2-oxopentanoate.

[5]Arterial blood concentrations.

[6]Whole body irreversible loss rate (ILR) with arterial plasma Leu or MOP atom percent excess as a reference ($P < 0.001$; n = 8). Values in parentheses represent the protein equivalent [grams of protein per day, assuming body protein comprises 66.9 g of Leu/kg; (15)] of ILR values.

TABLE 3. Leucine kinetics for one-half of the mammary gland of lactating goats receiving i.v. infusions (each of 6 or 7 d) of saline (control) and a mixture of 18 AA, excluding leucine.

Parameter	Control	AA	SED[1]	P
MBF,[2] kg/h	19.41	22.62	1.26	NS[3]
Kinetics of Leu and MOP,[4] μmol/h				
Net Leu removal	800	925	63	NS
Net MOP removal[5]	−4.5	16.5	11.5	NS
Leu for PS[6]	610	840	48	0.05
Oxidized Leu	185	100	22	0.05
PS[7]				
Venous Leu	755	1200	16	0.0001
Venous MOP	785	1105	61	0.01
Fractional Leu oxidation	0.19	0.07	0.01	0.005

[1]Standard error of the difference between treatment means.

[2]Mammary blood flow. Blood flow was measured by either a dye-dilution method (three goats) or ultrasonic flow probe (one goat).

[3]$P > 0.10$. Significance of differences determined by paired t test.

[4]4-Methyl-2-oxopentanoate.

[5]Negative values denote net release of metabolite.

[6]Based on blood removals and corrected for net MOP movements and oxidative losses [i.e., Leu available for protein synthesis (PS)].

[7]Calculations based on enrichments of either free Leu or MOP in venous plasma.

other tissues. Nonetheless, a response to the additional AA supply, particularly for goats fed the low protein diet (10.5% CP), would have been anticipated based on previous (8) milk responses when supplemental protein (casein) was infused into the duodenum. Unless leucine was limiting or close to limiting, the lack of a response to the additional supply would suggest that supply alone was not the major regulator. For example, a gastrointestinal event (e.g., hormonal or metabolic) that would not be stimulated by the jugular infusion route used in the current experiment might be required, or the uptake (extraction) and utilization of AA by the mammary gland for milk PS might have been limiting.

Leucine extraction by the mammary gland was probably not rate-limiting because, although arterial leucine concentrations were considerably reduced in three of four goats, sufficient quantities must have been extracted and available within the mammary gland to support the various requirements for milk yield; otherwise, milk protein output would have been reduced. Indeed, measurements of arteriovenous balance for the net uptake of leucine by the mammary gland have consistently shown that leucine is extracted in quantities much greater than those secreted in milk (5, 8, 9, 16), and leucine would probably not be in short supply for milk PS. However, it is still not known why many AA, such as leucine, are extracted by the gland in excess. Consequently, unless the excess AA uptake serves some anabolic or perhaps specialized (regulatory) role, this phenomenon would appear to be an inefficient process.

The mammary gland metabolizes leucine in a way that is similar to leucine metabolism by other tissues. Thus, in addition to incorporation into proteins [constitutive and milk proteins (3, 4, 19, 22, 27)], leucine can be catabolized to yield an amino group and carbon skeletons that can be used for the de novo synthesis of glutamine and alanine (10) and glutamic, aspartic, and organic acids (19, 22), respectively, plus oxidation to CO_2 (4, 19, 22), a process that could provide a small quantity of energy. For dairy cows given supplemental dietary protein, nearly all of the leucine extracted by the mammary gland in excess of milk output was oxidized (4), indicating that the gland was probably not accreting protein, which could have accounted for the additional uptake often observed with many AA. The possibility remained that the oxidation process occurred either as a passive response when leucine was extracted in excess of the net requirements (milk protein output) of the gland or that oxidation occurred to provide a source of amino groups for nonessential AA synthesis, and, thus, oxidation is only a consequence. In addition, that study (4) also did not indicate a specialized role involving leucine or its oxidation in the regulation of milk PS, which is a role implicated in muscle metabolism (11, 13, 20, 24). An alternative hypothesis emerged, suggesting an inverse relationship between milk protein output and leucine oxidation based on competition for substrate. Consequently, the obligatory nature of AA oxidation by the mammary gland is questionable.

In the present study, the reduction in leucine oxidation from AA infusion did not increase milk protein output, but an increase in total gland PS did occur. If the changes in leucine oxidation can be extrapolated to include other AA, then the present results would not support the hypothesis of oxidative competition. Furthermore, oxidation is not likely to be associated with a requirement to provide amino groups for de novo AA synthesis; otherwise, milk protein output would have declined as leucine oxidation declined. The present results suggest that leucine oxidation might not be an obligatory event in the milk protein biosynthetic process, although there might be a basal level of oxidation required because leucine oxidation was not nullified (Table 3).

Although a basal level of oxidation might be necessary, a considerable proportion of that oxidation is probably a passive response to dispose of leucine (and possibly other AA) once supplied and extracted by the gland in excess of net requirements. Consequently, the changes in leucine oxidation during the present and previous lactation studies (4, 19) may reflect the subtle mechanisms that regulate the BCAA catabolic pathways, whereby alterations in either substrate concentration or supply, or hormonal influences, or both, may predominate. Hormonal influences may involve insulin, which inhibits leucine oxidation across the hind limb (composed predominately of muscle) of starved sheep (18), and infusion of AA induces insulinemia in lactating goats (26). Mammary gland and perhaps whole body leucine oxidation were reduced in the current study, but a consistent relationship with leucine concentration was not established. Additionally, because insulin status was not monitored in the present study, links with endocrine or substrate regulation, or both, cannot be elucidated. This relationship could be addressed, however, by manipulation of local mammary gland concentrations of insulin and AA (17).

One of the more noticeable features of the present study was the ability of the mammary gland to enhance leucine extraction, despite the often marked reductions in arterial concentration (Table 2), and to maintain the arteriovenous concentration difference of leucine. Therefore, arterial concentration might not be a major determinant of leucine uptake. Perhaps the venous concentration might provide a closer approximation of the limits to AA transport because venous concentration is probably a better indication of the lower end of the concentration gradient established across the tissues. In the present study, venous plasma leucine often fell to very low concentrations (8 μM), which suggested that the interactions of substrate and transporter that were necessary to maintain the intracellular-extracellular gradient for this AA were very efficient and perhaps tightly controlled. Leucine is transported via two systems and, under the conditions of the present study, uptake was probably mainly via the concentrative system, which is under hormonal regulation (2). Further investigation is required to determine whether such flexibility exists in transport activity for other AA, especially the limiting ones.

CONCLUSIONS

In this study, reductions in leucine catabolism by the mammary gland did not result in net changes in anabolism as assessed by milk protein output. This lack of association perhaps suggests that the AA oxidative mechanism is linked to other requirements not identified in the current study or that it is a passive response for the disposal of AA, which are supplied and extracted by the mammary gland surplus to requirements for milk PS. This relationship may not be consistent for all circumstances (e.g., situations in which leucine is even more limiting) or for all AA, because all AA are oxidized by the dairy cow but to varying extents (6). Identification of the factors regulating AA oxidation in the whole body or the mammary gland, therefore, has considerable importance for the improvement of milk yield efficiency.

ACKNOWLEDGMENTS

The work reported here formed part of a collaborative project funded by a consortium of the Ministry of Agriculture, Fisheries and Food, British Petroleum Nutrition Ltd., United Kingdom; the Milk Marketing Board of England and Wales; the Agricultural and Food Research Council; and the Scottish Office Agriculture and Fisheries Department. The authors gratefully thank Alan Walker and Jane Kelso for their care and diligence in conducting the goat experimentation. The expertise for surgery offered by D.A.H. Farningham and expertise for mass spectrometry analyses by S. Anderson and A. G. Calder are also gratefully acknowledged.

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