Evaluation of Whole Blood and Plasma in the Interorgan Supply of Free Amino Acids for the Mammary Gland of Lactating Dairy Cows[1]

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ABSTRACT

We investigated the contribution of plasma and red blood cells to amino acid (AA) supply for milk protein synthesis during a combination of treatments that included abomasal infusion of casein and AA and utilization of a hyperinsulinemic-euglycemic clamp. Treatments resulted in substantial differences in circulating concentrations of AA, mammary uptake of AA, and rates of milk protein synthesis. Arterial concentrations of all AA in plasma were highly correlated with that of whole blood. Concentrations of AA in red blood cells were either higher (Asn+Asp, Gly, His, Leu, Met, Orn, Ser, Tau, Thr, and Tyr), lower (Ala, Arg, Cit, Cys, Ile, and Val), or similar (Gln+Glu, Phe, and Pro) to that of plasma. Arteriovenous difference measurements demonstrated that interorgan transfer of AA to the mammary gland was primarily by plasma. There was little involvement of red blood cells except for small quantities of Leu, Met, and Thr to the mammary gland; this contribution was greatest for Met and accounted for 14% of the total mammary uptake. Countercurrent transport of Gln + Glu, Asn + Asp, and Pro was also evident where these AA were extracted from plasma, but were released into red blood cells as blood passed through the mammary gland. This net influx of Gln+Glu, Asn+Asp, and Pro into red blood cells was equivalent to 26, 17, and 30% of their mammary uptake from plasma. Overall, the interorgan transport of free AA for the mammary gland was predominantly by plasma, and red blood cells were limited to minor contributions in mammary uptake for a few AA. Furthermore, arteriovenous differences of essential AA across the mammary gland were highly correlated between plasma and whole blood.

(**Key words:** amino acids, insulin, red blood cells, plasma)

Abbreviation key: AV = arteriovenous, **BCAA** = branched-chain AA, **CB** casein plus BCAA, **CB+I** = casein plus BCAA plus insulin treatment, **EAA** = essential AA, **Ht** = hematocrit, **NEAA** = nonessential AA, **RBC** = red blood cells, **Water** = water treatment, **Water + I** = water plus insulin treatment, **WB** = whole blood.

INTRODUCTION

Arteriovenous (**AV**) difference measurements allow the quantification of AA uptake by tissues or organs (20). Whether AA concentrations are measured in plasma or whole blood (**WB**) could affect the accuracy of this technique. Earlier research suggests that plasma and red blood cells (**RBC**) play important roles in the interorgan transport of AA in nonruminants and ruminants, although the importance of these two sources has been equivocal (8, 11, 12, 13, 17, 18). Lobley et al. (21) recently examined both mass and isotope transfers of AA in wether lambs; they observed a primary role for plasma in interorgan transfer of free AA, but found little evidence of any major or general involvement of the RBC.

Hanigan et al. (16) systematically compared the importance of plasma and RBC in the interorgan transfer of AA to the mammary gland of dairy cows. They concluded that RBC played a major role in the transfer of AA mass taken up by the mammary glands of lactating cows. They also observed that AV differences for individual AA from whole blood were poorly correlated with uptake measurements obtained with plasma and concluded that uptake data derived from plasma were inadequate (16). In contrast, other studies with lactating dairy cows and goats have suggested only a minimal role for RBC in mammary supply of AA, although these investigations have not involved extensive or systematic comparisons of plasma and WB (3, 4, 6, 27).

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Plasma has been utilized in the majority of research on mammary gland uptake of AA, so clarifying the importance of plasma and RBC in the interorgan transfer of AA is essential to evaluate published literature and for the design of future studies (7, 14, 20, 24, 26). Our objective was to conduct a systematic evaluation of the contribution of plasma and RBC to the transport and exchange of AA between the blood and the mammary gland of lactating dairy cows. To obtain a range in blood AA concentrations and mammary synthesis of milk protein, we employed a combination of treatments that included abomasal infusion of casein plus branched-chain AA (**BCAA**) and utilization of a hyperinsulinemic-euglycemic clamp. This provided us with a range in conditions over which plasma and RBC involvement in AA exchange might logically vary.

MATERIALS AND METHODS

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving dairy cows. Details of this study have been described previously (23, 24). Briefly, four multiparous, lactating, rumen-fistulated Holstein cows [220 ± 11 DIM ($\overline{X}$ ± SD)] were used in an experiment that consisted of two 12-d periods that were separated by 4 d. Abomasal infusions were applied throughout the entire 12-d period; the initial 4 d allowed for an acclimation interval, the second 4 d represented the baseline interval, and the third 4-d interval involved the hyperinsulinemic-euglycemic clamp. The abomasal infusions (6 L/d) and hyperinsulinemic-euglycemic clamp treatments were arranged as a two-way crossed factorial design where each cow was clamped twice at two levels of abomasal infusion. Thus, treatments were: 1) abomasal infusions of water (**Water**); (2) abomasal infusions of casein (500 g/d) plus BCAA (88 g/d) (**CB**); 3) water infusion plus insulin clamp (**Water+I**); and 4) casein plus BCAA infusion plus insulin clamp (**CB+I**). The casein plus BCAA infusions provided a daily nitrogen intake of 0.081 kg compared with an average of 0.69 kg from the diet.

Details of the abomasal infusates, the abomasal infusion procedure, and the hyperinsulinemic-euglycemic clamp technique have been described (23).

Arteriovenous Difference Measurements

Blood samples were collected simultaneously from an intercostal artery (arterial sample) and the caudal superficial epigastric vein (mammary venous sample) to measure AV differences of AA across the mammary gland. Details of sampling procedures and analytical techniques have been previously described (22, 24). Briefly, indwelling catheters (0.065 cm o.d. × 0.03 cm i.d. for intercostal artery catheter and 0.08 cm o.d. × 0.041 cm i.d. for mammary vein catheter; Micro-Renathane, Braintree Scientific Inc., Braintree, MA) were installed during the acclimation interval of the first period, and patency was maintained throughout the entire experiment. During each period, blood samples were collected on d 4 of the baseline interval and d 4 of the insulin clamp interval. On the two collection days, arterial and mammary venous blood samples were simultaneously collected at 1-h intervals over the 12 h between the a.m. and p.m. milkings. An aliquot (~5 ml) of the sampled blood was centrifuged to obtain plasma; hematocrit (**Ht**) value was determined and the remaining blood (~5 ml) was used as the WB sample. Equal volumes (0.65 ml) of either WB or plasma, and ice-cold sulfosalicylic acid (10%) containing the internal standard norleucine (1.5 μM) were mixed and vortexed extensively. Weights of all additions were recorded so samples could be corrected to the internal standard with specific gravity values. Samples were stored at −20°C until analysis.

Samples from the 12 hourly samples were composited at 2-h intervals to give a total of six pairs of arterial and venous samples within each day for each cow. These were then analyzed by HPLC as described by Mackle et al. (22) to give values for all AA except Asn, Gln, and Glu. In this initial analysis of WB samples, we observed that glutathione coeluted with the peaks for Asn, Gln, and Glu. To remove the glutathione peak and determine WB concentrations of Asn, Gln, and Glu, the six daily samples of arterial or venous WB were pooled within each day, and reanalyzed following the method of Joo et al. (19). Briefly, 1.2 ml of the pooled arterial and venous WB samples were freeze-dried, reconstituted in 0.52 ml of 0.4 N LiOH, and refrigerated for 1 h at 4°C to permit auto oxidation of glutathione. Next, 0.079 ml of 0.5 *M* Na_2SO_3 were added and the samples were chilled for 30 min; this step is performed to complete oxidation of glutathione to glutathione-S-sulfonate (19). Approximately 0.008 ml of concentrated HCl was added and pH was adjusted to 2.6 to 2.8 with 3 N LiOH. Samples were centrifuged at 8320 × g for 5 min at 4°C, filtered through a 2-μm filter, and frozen at −20°C until analysis.

Plasma concentrations of Trp were not quantified because of analytical difficulties in separating this AA from NH_4. In addition, concentrations of Lys and Trp in WB were not quantified due to their coelution in at least 50% of the samples.

Amino acids were grouped according to their essential importance for milk protein synthesis in the lactating cow (7). Essential AA (**EAA**) comprised Arg, Cys, His, Ile, Leu, Lys, Met, Phe, Thr, and Val. Nonessential

AA (**NEAA**) were Ala, Asn+Asp, Cit, Gln+Glu, Gly, Orn, Pro, Ser, and Tyr.

Calculation of Parameters and Statistical Analysis

Red blood cell concentrations of AA were calculated from the AA concentration of WB and plasma with correction for the Ht value as described by the equation of Hagenfeldt and Arvidsson (15):

$$[\text{RBC AA}] = \frac{[\text{WB AA}] - [\text{Plasma AA}] * (1 - \text{Ht})}{\text{Ht}}$$

This calculation assumes that the contribution of platelets and leukocytes to WB content of AA is negligible, and that the aqueous space in which RBC AA are dissolved is equal to the entire Ht value (9).

Uptake to output ratios for selected AA were derived using the calculated uptake of individual AA from plasma or WB, divided by the estimated output using AA concentrations for milk casein and whey protein as reported previously (24).

Data were analyzed by using the mixed model procedure of SAS (30) with cow and period as random effects, and insulin treatment and CB treatment as fixed effects in the model. The model used was described by:

$$Y_{ijkl} = \mu + \rho_i + \kappa_j + \iota_k + \beta_l + \iota\beta_{kl} + \varepsilon_{ijkl}$$

where μ = constant, ρ_i = period blocking effect, κ_j = cow blocking effect, ι_k = insulin treatment effect, β_l = CB treatment effect, $\iota\beta_{kl}$ = insulin by CB interaction, and ε_{ijkl} = error term.

Paired t-tests were performed to compare the concentrations of AA in plasma and RBC, and to determine whether the AV difference of AA from RBC was different from zero.

RESULTS

All cows completed the study in excellent health; catheters remained patent and all blood samples were obtained according to the study design. The hyperinsulinemic-euglycemic clamp elevated circulating insulin concentrations approximately fourfold above baseline concentrations and blood euglycemia was maintained (23). Abomasal infusion of casein plus BCAA affected arterial concentrations of several AA (24). However, this treatment had little effect on AV difference, the main variable of interest in the present study, and therefore, only least squares means for the insulin main effects are presented.

Arterial concentrations of AA in WB and plasma and calculated concentrations in RBC are presented in Table 1. In general, insulin clamp had similar effects on concentrations of AA in WB, plasma, and RBC, although there were some quantitative differences. Insulin clamp decreased ($P < 0.05$) concentrations of several EAA, including Cys, His, Ile, Leu, Met, Phe, and Val. Effects were most evident for the concentrations of BCAA, which were reduced by 50% in WB, 53% in plasma, and 50% in RBC. The insulin clamp had no effect on Thr concentrations in any of the blood fractions, and Arg concentrations in RBC were not affected but were very low initially (Table 1). For the NEAA, the insulin clamp decreased ($P < 0.01$) the concentrations of Cit, Orn, and Tyr, but increased ($P < 0.01$) the concentrations of Gly in WB, plasma, and RBC pools. Plasma concentrations of Tau were decreased ($P < 0.01$) while concentrations of Gln+Glu and Ser were increased ($P < 0.05$) by insulin clamp; concentrations of these AA in RBC were unaffected. Conversely, arterial concentrations of Ala and Pro in RBC were decreased ($P < 0.05$) by the insulin clamp, but plasma values for these AA were unaffected.

Comparison of the RBC:plasma ratio of AA concentrations indicates some AA (His, Leu, Met, Thr, Asn+Asp, Gly, Ser, Orn, Tau, and Tyr) were greater ($P < 0.05$) in RBC than in plasma, whereas concentrations of others (Arg, Cys, Ile, Val, Ala, and Cit) were less ($P < 0.05$) (Table 1). Only a few AA (Phe, Gln+Glu and Pro) were of similar concentration ($P < 0.1$) in plasma and RBC.

Figure 1 presents scatter plots of plasma arterial versus WB arterial concentrations for EAA and selected NEAA. In general, there were strong linear relationships between concentrations in plasma and WB with r^2 values ranging from 0.80 for Cys to 0.98 for Val. The intercepts of Ala, His, Thr, and Tyr are clearly greater than zero, consistent with their greater concentrations in WB than plasma. The r^2 values for the remaining NEAA measured in this study but not shown in Figure 1 were Asn+Asp (0.43), Cit (0.78), Gln+Glu (0.20), Gly (0.92), Pro (0.10), Ser (0.59), and Tau (0.16).

Table 2 presents AV differences of AA in plasma (corrected for Ht value) and WB pools for the Water and CB treatments compared to the two insulin clamp treatments (Water+I and CB+I). Insulin clamp treatment decreased ($P < 0.05$) the AV differences of His, Ile, Leu, Phe, Val, and Orn in both Ht-corrected plasma and WB (Table 2). Further, there were significant effects of insulin clamp treatment on AV difference of Thr, Asn+Asp, and Tyr for the WB pool; the apparent AV differences of these AA were lower ($P < 0.05$) during the insulin clamp (+Ins) treatment than during the control treatment (–Ins). Figure 2 presents scatter plots of AV differences for EAA and selected NEAA for Ht-corrected plasma and WB pools. Strong linear relationships were evident for most EAA with a range for r^2 of 0.54 to 0.93

Table 1. Least squares means for arterial concentrations (μM) of AA in whole blood, plasma, and red blood cells, and their ratio (RBC:PL ratio)[1].

	Whole Blood			Plasma			Red Blood Cells			RBC:PL ratio[2]	
AA	–Ins	+Ins[3]	SEM	–Ins	+Ins[3]	SEM	–Ins	+Ins[3]	SEM	Mean[4]	SEM
Essential											
Arg	59.1	49.5**	2.4	81.4	57.6***	4.7	6.1	0.8	6.1	0.05***	0.06
Cys	11.3	8.8***	1.3	18.7	16.1***	2.5	–6.5	–9.6†	3.3	–0.44***	0.08
His	79.9	65.5**	2.2	52.8	41.3**	2.2	146.0	126.6*	6.4	2.97***	0.14
Ile	137.1	69.2***	6.2	146.0	76.1***	4.9	116.5	51.9**	18.2	0.74**	0.07
Leu	203.0	100.8**	9.5	197.3	91.7***	8.6	218.4	123.5**	23.7	1.25*	0.08
Lys[5]	—	—	—	90.7	62.1***	4.1	—	—	—	—	—
Met	21.9	19.2**	1.8	19.7	17.5†	1.3	27.5	23.4*	3.5	1.39**	0.12
Phe	47.3	34.9***	1.6	47.9	37.9**	2.7	46.1	27.7**	4.9	0.88	0.09
Thr	113.7	103.6	6.5	102.5	94.9	7.5	141.8	126.1	9.1	1.39***	0.08
Val	322.5	161.4**	21.6	336.5	168.8**	17.9	290.5	143.0**	37.2	0.86**	0.05
Nonessential											
Ala	164.7	150.8	7.5	174.4	166.8	8.9	142.2	110.7*	10.6	0.75***	0.05
Asn+Asp	118.0	111.6	21.1	57.3	57.1	3.1	260.8	245.6	63.9	4.36***	0.46
Cit	75.2	54.1***	4.2	88.2	66.8**	3.1	44.3	19.7**	9.3	0.40***	0.06
Gln+Glu	236.6	250.0	5.9	230.7	261.2*	10.7	252.5	222.2	27.4	1.01	0.11
Gly	274.9	374.4**	14.2	201.3	306.3**	12.9	455.4	546.6**	28.4	2.04***	0.11
Ser	84.9	99.0***	1.8	69.7	84.5***	2.3	122.1	136.2	6.8	1.71***	0.10
Orn	84.4	54.4***	3.4	77.9	49.4***	4.7	100.8	66.9**	6.9	1.38**	0.11
Pro	110.3	91.5**	9.9	88.4	89.8	10.2	161.2	95.8**	51.5	1.66	0.35
Tau	140.3	126.8**	10.1	43.9	35.4**	2.6	372.4	358.1	29.1	9.43***	0.48
Tyr	60.1	47.6**	1.9	55.1	46.3**	2.7	73.0	50.8**	8.8	1.26†	0.12

† = $P < 0.1$; * = $P < 0.05$, ** = $P < 0.01$; *** = $P < 0.001$.

[1]Treatment least squares means represent without (–Ins) and with (+Ins) the insulin clamp, for the plasma and whole blood measurements; Measurements were performed on the last day of the 4-d periods.

[2]The ratio of plasma arterial AA concentrations:RBC arterial AA concentrations.

[3]Superscripts listed beside +Ins values within these columns show the F-test for the main effect of INS on arterial concentration of AA in WB, plasma or RBC.

[4]Superscripts listed beside mean ratios in this column show the probability that the arterial AA concentrations in RBC are different to that found in plasma.

[5]Lys concentrations were not determined in whole blood or RBC.

(excluding Cys). The r^2 values for the remaining NEAA not shown in Figure 2 were Asn+Asn (0.63), Cit (0.05), Gln+Glu (0.38), Gly (0.02), Pro (0.08), Ser (0.19), and Tau (0.10).

The mean AV differences for RBC are also presented in Table 2, and represent the calculated difference between Ht-corrected plasma and WB. Overall, AV difference for RBC was not different from zero for most AA. However, Leu, Met, and Thr were significantly greater ($P < 0.05$) than zero (Table 2), indicating mammary uptake of these AA from RBC. Conversely, the AV differences of Asn+Asp, Gln+Glu, and Pro from RBC were all significantly less ($P < 0.05$) than zero, suggesting that these AA were concentrated in RBC as they pass through the mammary gland.

DISCUSSION

Whether to analyze AA concentrations in plasma or WB pools deserves careful consideration during the study of AA kinetics. This is particularly pertinent to the development and use of computer models, which require accurate data to simulate nutrient use and productive output by the body. In the present study, the combination of dietary supplement of AA (abomasal infusions of casein plus BCAA) and endocrine manipulation (hyperinsulinemic-euglycemic clamp) resulted in substantial differences in AA concentration, mammary AA uptake, and milk protein yield in well-fed cows (23, 24). Relative to the water treatment, milk protein yield was increased by 28 g/d (+3%), 128 g/d (+15%), and 210 g/d (+25%) for the CB, Water+I, and CB+I treatments, respectively. Increased mammary blood flow and AA extraction efficiency provided the increased AA supply required to support treatment increases in milk protein yield. We hypothesized that the contribution of plasma and RBC to the uptake of AA by the mammary gland might vary according to the supply and demand of AA for milk protein synthesis and, thus, the data set provided an excellent opportunity to evaluate the role of plasma and RBC in the interorgan transfer of AA for mammary gland use.

The concentration of AA in RBC in our study was determined from the AA concentration of plasma and WB with correction for the Ht (15). We assume that contributions of platelets and leukocytes to AA content

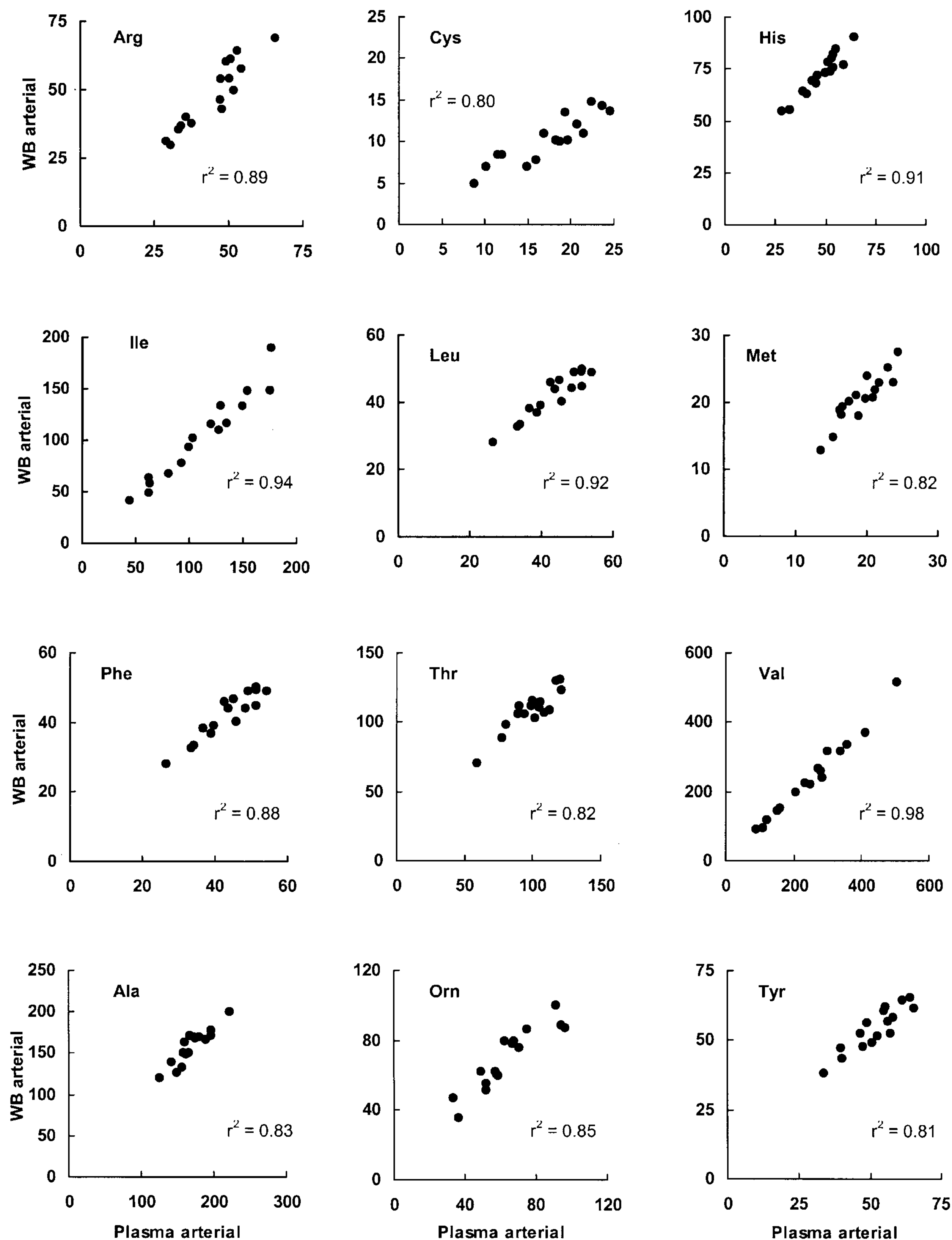

Figure 1. Relationship between plasma and whole blood arterial concentrations of essential AA and selected nonessential AA. Each point represents a single cow (n = 4) during each of four treatments as described in Materials and Methods section. WB = whole blood.

Table 2. Least squares means for arteriovenous difference (μM) of AA in whole blood, plasma, and red blood cells (RBC).[1]

	Whole Blood			Plasma[2]			RBC	
AA	–Ins	+Ins[3]	SEM	–Ins	+Ins[3]	SEM	Mean[4]	SEM
Essential								
Arg	26.5	23.2	2.6	25.2	22.5	2.6	1.0	0.7
Cys	1.6	1.2	0.3	1.3	1.2	0.1	0.2	0.2
His	10.0	7.1**	0.8	8.9	7.1*	0.7	0.5	0.5
Ile	38.1	26.5***	3.1	36.2	26.2**	1.7	1.1	1.3
Leu	57.5	37.7***	3.7	53.2	37.1***	2.2	2.5†	1.4
Lys[5]	—	—	—	35.7	28.6*	2.6	—	—
Met	9.2	7.7	0.7	7.7	6.8	0.5	1.2**	0.4
Phe	15.6	13.2*	1.6	15.3	13.2*	1.2	0.2	0.6
Thr	22.8	17.9***	1.8	20.1	17.8	1.9	1.4*	0.9[6]
Val	42.4	34.7**	2.5	43.1	33.1**	4.9	0.5	1.6
Nonessential								
Ala	13.3	17.3	1.8	13.0	19.8	3.1	–1.1	1.3
Asn+Asp	11.6	7.6*	1.7	13.9	11.6	1.1	–3.3***	0.8[6]
Cit	0.5	0.4	1.4	0.8	2.4	1.0	–1.2	1.2
Gln+Glu	37.8	37.6	6.0	44.1	46.5	4.4	–7.6*	3.4
Gly	0.1	–2.9	3.7	4.3	1.8	1.9	–4.5	2.8
Ser	18.6	14.1	2.4	19.3	14.7	1.2	–0.6	1.5
Orn	21.9	13.2***	1.3	21.1	12.5***	1.1	0.7	0.5
Pro	10.1	7.1	2.7	10.9	13.5	2.0	–3.6†	1.9
Tau	0.07	–0.84	1.6	0.2	0.0	0.6	–0.5	1.3
Tyr	13.6	9.6**	1.4	11.9	10.6	1.2	0.3	0.6[6]

† = $P < 0.1$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

[1]Treatment least squares means represent without (–Ins) and with (+Ins) the insulin clamp, for the plasma and whole blood measurements; only the mean AV difference is presented for RBC. Measurements were performed on the last day of the 4-d periods.

[2]Plasma AV differences of AA are corrected for hematocrit value and are presented on μM of whole blood basis.

[3]Superscripts listed beside +Ins values within these columns show the F-test for the main effect of Ins on AV difference.

[4]Superscripts listed beside mean ratios in this column show the probability that the AV difference of RBC differs from zero.

[5]Lys arteriovenous differences were not determined in whole blood.

[6]There were significant ($P < 0.05$) insulin effects on AV difference of AA from RBC for Thr, Asn+Asp and Tyr; their apparent AV difference from RBC was lower during +INS treatment.

in WB are negligible and that the distribution space for the AA is equal to the Ht value. Darmaun et al. (9) pointed out that the first assumption may lead to overestimation of certain AA in RBC, because leukocytes or platelets may contain concentrated amounts of specific AA such as Tau. In the present study, apparent concentrations of Tau were about ninefold greater in RBC than in plasma (Table 1). The second assumption may lead to an underestimation of AA concentrations in the intracellular water of RBC because the cell membrane of RBC accounts for 25 to 30% of the Ht value (9). Nonetheless, this second error would be systematic across all AA, and overall the approach provides a reasonable estimate of intracellular AA concentrations in RBC.

We found concentrations of His, Leu, Met, Gly, Ser, Orn, and Tyr were all moderately greater in RBC than plasma, whereas concentrations of Tau and Asn+Asp were substantially greater (Table 1). Concentrations of Ala, Arg, Cit, Ile, and Val were less in RBC than plasma. These data are in general agreement with results from other studies in mature and growing sheep (17, 21) and growing calves (8). In our study (Table 1) and others (6, 8, 17), Arg concentration in RBC was approximately zero. Lobley et al. (21) chose not to present Arg concentrations in WB and suggested the low concentrations were due to metabolism by arginase that is released from RBC during hemolysis. Cys concentrations in RBC were less than zero (Table 1), similar to results with calves (8). However, these findings may be artifacts related to analytical problems or oxidation of Cys in WB. Cys is a component of the gamma-glutamyl cycle (25) needed for the synthesis of glutathione, an important peptide found in RBC, thus it seems likely that some free Cys would be present in the RBC.

Changes in AA concentrations in plasma and RBC caused by the insulin clamp, were qualitatively and quantitatively similar, except for Arg, Ala, Gln+Gln,

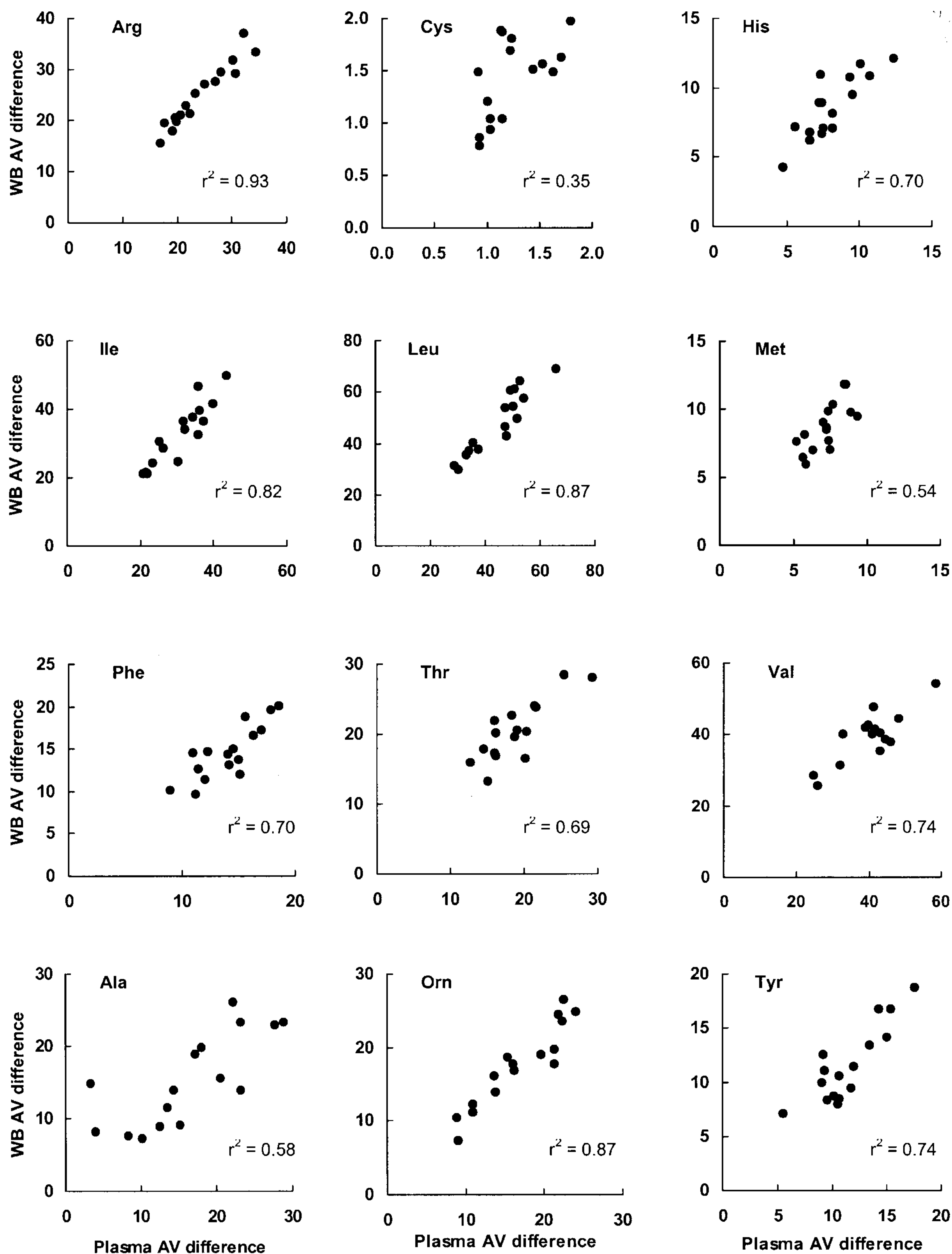

Figure 2. Relationship between plasma and whole blood arteriovenous differences of essential AA and selected nonessential AA. Each point represents a single cow (n = 4) during each of four treatments as described in Materials and Methods section. WB = whole blood, AV = arteriovenous.

and Pro. For example, during the insulin clamp concentrations of BCAA were substantially reduced in both plasma (–53%) and RBC (–50%). Concentrations of Gly and Ser were increased in both plasma (+52% for Gly; +21% for Ser) and RBC (+20% for Gly; +12% for Ser). These qualitative and quantitative similarities suggest an equilibrium exists between the plasma and RBC pools. Others have observed nutritional effects on concentration of AA in RBC in calves (8), rats (2), and humans (31). Although we generally found changes in AA concentrations in RBC reflected those occurring in plasma (μM basis; Table 1), Azar et al. (2) reported differential effects when rats were fasted or fed a protein-free diet.

Relative to our objective, we found that plasma represented the major pool from which free AA were taken up by the mammary gland (Table 2). The RBC made a minimal contribution and of the EAA measured, only Leu, Met, and Thr had AV differences for RBC that were significantly different from zero (Table 2). Relative to their total uptake by the mammary gland, the RBC AV differences of Leu, Met, and Thr accounted for 5.3, 14.0, and 7.0%, respectively. In a previous study with lactating dairy cows, Hanigan et al. (16) systematically compared plasma and WB and found extensive involvement of RBC in the mammary uptake of many AA. However, a less extensive study conducted by the same research group showed little involvement of RBC (6). For example, mammary uptakes of Ile, Lys, and Met using plasma alone would be underestimated by 79, 26, and 20% with data of Hanigan et al. (16), whereas with data of Cant et al. (6) one can calculate these AA would be overestimated by 220, 31, and 140%, respectively. In agreement with our results, a recent study by Pacheco-Rios et al. (29) utilized lactating cows fed only pasture and found plasma was the major pool of free AA used by the mammary gland. In addition, several recent studies have reported AV differences for plasma and WB across the mammary glands of lactating goats and cows. Although these investigations did not represent systematic comparisons, based on the similarity of AV differences between plasma and WB, the authors concluded that plasma was the primary means of interorgan transport of AA with RBC playing little or no significant role (3, 4, 27).

We were particularly interested in the interorgan transport of AA during the insulin clamp because circulating concentrations of EAA are reduced by 30 to 50% during this treatment (14, 24). Our results indicated that plasma was the predominant source of AA taken up by the mammary gland during the insulin clamp, just as it was for the noninsulin treatments (Table 2). The insulin clamp had no effect on AV difference for most AA from RBC, except for Asn+Asp, Thr, and Tyr. The latter two AA had greater AV differences during the baseline period, although the AV difference of Tyr from RBC was not different from zero. The negative AV difference of Asn+Asp was greatest during the insulin clamp, indicating this pair of AA was concentrated in the RBC. Data on effects of nutritional signals such as insulin on RBC involvement in AA transport are scarce. Aoki et al. (1) found that in humans insulin was capable of markedly increasing forearm muscle uptake of Glu from RBC. Conversely, insulin reduced the uptake of AA by RBC in trout (5).

The interorgan transport of AA has been investigated in several species. In general, the RBC appear to actively participate in the transfer of some AA between blood and some tissues (1, 8, 10, 11, 12, 13, 16, 17, 33). However, RBC involvement in AA transport and exchange in ruminants, particularly in cattle, is not well characterized, and results have been inconsistent for specific AA (6, 8, 16, 17, 18, 21, 29). Heitmann and Bergman (17) compared AA transport in both plasma and WB across the portal-drained viscera, liver, kidneys, and hindquarters in nonpregnant, nonlactating ewes fed at maintenance. They concluded that RBC transport of AA, per unit volume, was always concomitant to, and usually at the same rate as, plasma transport across all organs examined (17). However, Lobley et al. (21) investigated AA transfers across the splanchnic bed of growing lambs using AV difference and net isotope movement techniques and found no major or general involvement of RBC in the transport of free AA from the liver. Similary, Houlier et al. (18) found absorbed AA were transported to liver mainly by plasma in preruminant calves and Danilson et al. (8) found that RBC were important in the interorgan transport to the hindlimb muscle bed for only a few AA in growing calves. Variations in physiological state, nutritional status, and analytical methodology may explain some of the differences between studies.

Treatments used in our study resulted in a range in circulating concentrations of AA, and we found that AV differences of EAA between plasma and WB were generally highly correlated ($r^2 = 0.54$ to 0.93; Figure 2). Arteriovenous differences for NEAA were also correlated although relationships were weaker. Heitmann and Bergman (17) also reported that AA uptake across the portal-drained viscera, liver, kidneys, and hindquarters in sheep were highly correlated between plasma and WB. However, Hanigan et al. (16) found plasma and WB AV differences across the mammary gland were poorly correlated (r^2 ranged from only 0.0001 to 0.16 for all AA) and concluded that uptake data for plasma did not adequately represent whole blood. Causes of the differences between our work (Figure 2) and that of Hanigan et al. (16) are not apparent.

This difference is of importance in evaluating published research because the majority of studies on AA uptake by the ruminant mammary gland have based measurements on plasma. Our study involves both endocrine manipulation and alterations in AA supply, and results clearly demonstrate that correlations between plasma and WB AA for AV differences were generally strong (Figure 2), despite substantial treatment affects on circulating AA concentrations and milk protein yield.

Another interesting feature of our AV difference data is the apparent concentration of Asn+Asp, Gln+Glu, and Pro in RBC as blood passed through the mammary gland (Table 2). Because blood passes only briefly (3 to 9 s) through the microcirculation of an organ and because equilibration of AA between plasma and RBC is slow, it is proposed that AA are transferred directly between the organ and RBC, rather than having plasma as an intermediate pool (9, 11, 32). Countercurrent transport or opposite flow of some AA between plasma and RBC pools and an organ, has been previously observed in calves (8), dairy cows (16), fish (28), dogs (11), and humans (13). However, evidence for counterexchange of AA was not observed in sheep (17, 21). In the current study, the increase in AA concentration in RBC was equivalent to 26, 17, and 30% of the Asn+Asp, Gln+Glu, and Pro extracted by the mammary gland from plasma (Table 2). It is not possible to ascertain whether both or only one of the AA from the Asn+Asp and Gln+Glu pairs was involved in this countercurrent transport. These AA were paired for comparison of plasma and WB to avoid possible errors caused by the instability of Gln and Asn.

Results from our study also have other implications. First, an important analytical consideration is whether to use WB or plasma in the calculation of blood flow by using the Fick principle (20). Phe and Tyr are often used as indicators in this calculation because they are assumed to be stoichometrically transferred from blood to milk; plasma AV differences of Phe and Tyr would overestimate mammary blood flow if RBC were also contributing to their AV differences. Our data show that because AV differences of Phe and Tyr from RBC were not different from zero (Table 2), mammary blood flow estimates made with WB would not differ from those calculated with plasma (24). Second, uptake of Met is often regarded as a limiting AA because of high extraction rates from plasma and close uptake to output ratios (7, 26). We found that 14% of the total mammary Met uptake was derived from RBC. This contribution of RBC to Met uptake by the mammary gland would change the uptake to output ratio reported previously (24) from slightly below (0.95) to slightly above unity (1.08). Thus, the mammary gland appears to obtain a portion of this important AA from the RBC pool, and this will need to be considered when estimating limiting AA.

CONCLUSIONS

To examine the contribution of plasma and RBC to mammary AA supply, we measured AV differences during AA supplementation (abomasal infusion of casein plus BCAA) and endocrine manipulation (hyperinsulinemic-euglycemic clamp) in well-fed cows. Results demonstrated that plasma was the primary pool for interorgan transport of AA to the mammary gland. The RBC made minimal contributions for most EAA, except for small quantities of Leu, Met, and Thr; RBC accounted for 14% of the total mammary uptake of Met and 5 to 7% for Leu and Thr. In contrast, Gln+Glu, Asn+Asp, and Pro were extracted from plasma, but also were concentrated in RBC as blood passed through the mammary gland. This net influx of Gln+Glu, Asn+Asp, and Pro in RBC was equivalent to 26, 17, and 30% of their mammary uptake from plasma, respectively. Insulin clamp treatment had minimal effects on AV differences of most AA from RBC, although AV differences of Thr and Tyr were decreased and the negative AV difference of Asn+Asp increased. Overall, the interorgan supply of AA for the mammary gland was primarily by plasma, and the AV differences for mammary gland uptake of EAA from plasma and whole blood were highly correlated. The contribution of RBC to the supply and exchange of AA by the mammary gland was limited to only a few AA but may need to be considered to obtain a complete AA balance for the mammary gland.

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