Amino Acid Availability Affects Amino Acid Flux and Protein Metabolism in the Porcine Mammary Gland[1]

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ABSTRACT A kinetic model was used to examine transmembrane flux kinetics of lysine, methionine and valine across the porcine mammary gland (MG) under dietary amino acid (AA) limiting, adequate and excess conditions. Lactating sows (3 per treatment) were offered three diets: lysine-deficient [LD, 4.9 lysine and 9.9 valine (g/kg diet)], adequate (Control, 9.7 and 10.2) and valine-excess (VE, 9.8 and 13.4). On d 18 of lactation, 2-^{15}N-lysine, 5-methyl-$^{2}H_3$-methionine and 1-^{13}C-valine were infused into a jugular vein for 20.5 h. Milk and arterial and mammary venous blood samples were collected at 2- and 1-h intervals, respectively. Compared with Control, milk yield and litter growth rate decreased ($P < 0.05$) in sows fed the LD diet. Model estimates of mammary protein synthesis (PS), breakdown (PB) and net PS decreased ($P < 0.05$) in sows fed the LD diet. Net uptake of lysine decreased ($P < 0.05$) in sows fed the LD diet as a result of decreases in inward and outward transport of lysine. Inward transport of methionine tended to be reduced ($P < 0.10$) in sows fed the LD diet, resulting in a decrease in net methionine uptake. In sows fed the VE diet, PB was reduced ($P < 0.05$) and PS unchanged compared with Control. Outward transport of valine and net lysine uptake were reduced ($P < 0.05$), but net valine uptake was unchanged in sows fed the VE diet compared with Control. In conclusion, the kinetic model provided estimates of PS that were similar to empirical measurements of milk protein output and mammary protein accretion. Transport of lysine and methionine by the porcine MG is closely linked to regulation of mammary PS. Lysine availability has little effect on the transmembrane flux of valine. J. Nutr. 132: 1224–1234, 2002.

KEY WORDS: • *amino acid uptake* • *protein turnover* • *lactation* • *compartmental kinetic model* • *mammary gland* • *pigs*

The porcine mammary gland (MG)[5] has a large demand for amino acids (AA). More than 95% of the indispensable AA requirement of sows is attributable to the mammary gland's requirements for milk synthesis (1). Imbalances created by excesses or deficiencies of dietary AA may limit milk protein synthesis and reduce lactation performance. To date, AA requirements of lactating sows have been derived largely from feeding trials, but these empirical approaches require large numbers of animals to detect small improvements in lactation performance. Lysine is often the first limiting AA for lactating sows, particularly when diets are based on corn and soybean meal as the main protein sources, but few studies have been able to identify further limiting AA other than lysine for lactation. Recently, valine has been suggested to be co-limiting in corn/soybean meal–based diets fed to sows nursing large litters (1,2). When synthetic lysine is provided to meet its requirement for sows nursing large litters, increasing dietary valine concentration such that the valine:lysine dietary ratio is >1.2:1 improves litter growth rate (2,3). Paradoxically, although the NRC (4) recommended a valine:lysine ratio of 1:1 in 1988, it decreased this ratio to 0.85:1 in 1998 (1).

Further progress in identification of limiting AA will require a mechanistic description of AA metabolism and a better understanding of the metabolic relationship between limiting AA and milk protein output. For example, if valine is co-limiting with lysine, why is valine always taken up by the mammary gland in excess of its output in milk (5)?

There are several potentially rate-limiting steps in milk protein synthesis, including AA supply to the MG, AA transport and intracellular metabolism. Many AA transport systems are bidirectional; therefore, net uptake (NU) of AA is a function of the balance between inward and outward transport. Uptake of certain indispensable AA by mammary epithelial cells is rate limiting for milk protein synthesis in vitro

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[5] Abbreviations used: AA, amino acids; A-V, arterio-venous; FY, phenylalanine and tyrosine; i.v., intravenous; LD, lysine-deficient; MG, mammary gland; MPE, molar percent excess; MPF, mammary plasma flow; PB, protein breakdown; PS, protein synthesis; VE, valine excess.

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(6,7). However, that may be not the case in vivo. For example, when histidine was severely limiting in the systemic supply, the extraction efficiency of histidine was increased in goat MG from 17 to 74%, avoiding a dramatic decrease in milk protein synthesis (8). To accomplish this, the rates of inward and outward transport of histidine across the MG were altered in favor of its net uptake. If these transport kinetics are unique to the limiting vs. nonlimiting AA, then it may be possible through removal of lysine or addition of valine to the diet to determine whether valine is a limiting AA for mammary protein synthesis in sows nursing large litters. Methionine utilization in growing pigs is increased under conditions of high intake of dietary branched-chain AA (9); hence, methionine utilization may also be increased in lactating sows by high dietary valine concentration. It is not known whether high intake of valine increases methionine transport and/or utilization by lactating mammary glands.

In the present study, we attempted to provide a mechanistic description of the transport properties of lysine, valine and methionine under conditions of dietary lysine limitation and valine excess, and to determine whether transport of these AA by the porcine MG limits or stimulates milk protein synthesis. To accomplish this, we designed the following three diets: a lysine-deficient diet such that lysine would be limiting, an adequate-lysine diet containing valine in a 1:1 ratio with lysine such that valine would be "limiting," and an adequate-lysine diet containing valine in a 1.3:1 ratio with lysine such that valine would be "nonlimiting" or "in excess." An arteriovenous tracer kinetic model was used to examine the specific transport kinetics of lysine, valine and methionine across the mammary gland. Our hypothesis was that the transport (inward and outward flux by the mammary gland) of lysine would be altered in favor of greater inward flux in pigs fed the lysine-deficient diet, and that valine would be altered in favor of greater outward flux in pigs fed the valine excess diet.

MATERIALS AND METHODS

This study was approved by the Michigan State University All-University Committee on Animal Use and Care.

Animals and diets. Nine Landrace × Yorkshire lactating sows (parity 2, mean ± SD body weight of 212.6 ± 12.0 kg on d 1 of lactation) were allocated to dietary treatments according to a randomized block design. Each block consisted of three sows; each sow in one block was provided free access to one of three diets from d 1 to 21 of lactation. The three diets contained the same concentrations of metabolizable energy, calcium, available phosphorus and indispensable AA other than lysine and valine. The dietary ratio of indispensable to dispensable AA-nitrogen (1:1) was constant (**Table 1**). The lysine-deficient diet (LD) contained 50% of the lysine content in the Control and valine-excess (VE) diets. The dietary ratio of valine to lysine was 1.05:1 and 1.37:1, for the Control and VE diets, respectively. Litters were cross-fostered within 48 h after birth to ensure 12 piglets per sow. Sows were housed in individual farrowing crates in a thermally controlled room (21°C), fed twice daily to appetite and provided free access to water. Food intake was recorded daily. Milk yield was estimated on d 21 by the weigh-suckle-weigh method (10). Piglets were weighed individually on d 1 and 21.

Cannulation. On d 9 ± 1 of lactation, a jugular vein, the right main anterior mammary vein and a carotid artery were cannulated as previously described (11). Halothane (1.5%) was administered as the anesthetic agent via tracheal tube during surgery. Antibiotic (Naxcel, Pharmacia and Upjohn, Kalamazoo, MI) and anti-inflammatory medicine (Banamine, Schering-Plough Animal Health Corp., Kenilworth, NJ) were administered intravenously (i.v.) for the first 3 d postsurgery. Food was offered gradually during the first 3 d postsurgery, and thereafter consumed ad libitum.

Infusion protocol. On d 18 of lactation, sows were given a constant infusion of a mixture of L-[2-^{15}N] lysine · HCl (24.2 μmol/min), L-[S-methyl-$^{2}H_3$] methionine (5.2 μmol/min), and L-[1-^{13}C] valine (17.0 μmol/min) via the jugular vein. Previously, a nonprimed continuous isotope infusion in lactating sows showed that isotopic enrichments of plasma free leucine and casein-bound leucine reached a plateau at 12 h (12). Therefore, the infusion herein was extended to 20.5 h to allow repeated measurements over the plateau period. Amino acid tracers were purchased from MassTrace (Woburn, MA). The mixture of AA tracers was prepared in saline and filter sterilized through an in-line filter (surfactant-free cellulose acetate membrane, 0.20-μm pore size, Nalgene, Nalge Nunc, Rochester, NY). The infusion flow rate was controlled with a peristaltic pump (Minipuls 3, Gilson Medical Electronics, Middleton, WI). Matched sets of carotid arterial and mammary venous blood samples were obtained at 3-h intervals from 0 to 12 h of infusion and at 1-h intervals from 14 to 20 h of infusion. Blood samples were centrifuged at 1500 × *g* for 15 min at 4°C, plasma removed and stored at −20°C. A single MG (the 3rd right anterior) was milked-out completely by hand at 2-h intervals from 12.5 to 20.5 of infusion. Oxytocin (10 IU) was administered i.v. at each milking to allow milk removal. Milk samples were defatted by centrifugation at 1500 × *g* for 15 min at 4°C and stored at −20°C. A paired set of whole-milk samples were also obtained for determination of milk composition and AA concentration. Before isotope infusion, blood and milk samples were collected to obtain background samples for natural abundance of ^{13}C, ^{2}H or ^{15}N.

TABLE 1

Composition of experimental diets (as-fed basis)

Item	Lysine deficient	Adequate control	Valine excess
		g/kg diet	
Ingredient			
Corn	679.5	679.5	679.5
Soybean meal	125.0	125.0	125.0
Tallow	50.0	50.0	50.0
Solk floc[1]	25.0	25.0	25.0
Calcium phosphate	22.0	22.0	22.0
Calcium carbonate	10.0	10.0	10.0
Salt	2.5	2.5	2.5
Mineral and vitamin premix[2]	11.5	11.5	11.5
Amino acid mixture[3]	14.8	14.8	14.8
Lysine · HCl	0	8.0	8.0
Valine	4.9	4.9	9.3
Glutamic acid	24.1	36.8	42.4
Cornstarch	30.7	10.0	0
Nitrogen and indispensable amino acids			
Crude protein (N × 6.25)	138.1	151.1	158.8
Histidine	4.90	4.94	4.79
Arginine	7.08	7.15	6.83
Threonine	5.17	5.28	5.13
Valine	9.87	10.15	13.37
Methionine	3.15	3.26	3.39
Isoleucine	6.54	6.56	6.52
Leucine	12.90	12.91	12.51
Phenylalanine	8.00	7.87	7.73
Lysine	4.93	9.71	9.76
Tryptophan[4]	2.10	2.10	2.10

[1] The content of cellulose was 98.5%.

[2] Provided the following amounts of trace minerals and vitamins (mg/kg diet): copper, 5; iodine, 0.075; iron, 50; manganese, 5; selenium, 0.15; zinc, 50; retinyl acetate, 8.3; cholecalciferol, 0.0138; α-tocoperol, 44.1; menadione, 4.5; vitamin B-12, 0.033; riboflavin, 4.5; D-pantothenic acid, 17.6; niacin, 26.4; thiamin, 1.1; pyridoxine, 1.0; choline, 385.0; folic acid, 1.65; and D-biotin, 0.22.

[3] Provided the following amounts of indispensable amino acids (g/kg diet): histidine · HCl, 1.9; threonine, 3.2; DL-methionine, 1.4; isoleucine, 2.0; leucine, 1.6; phenylalanine, 2.5, tyrosine, 1.3, and tryptophan, 0.9.

[4] Calculated value (1).

Analytical techniques

Sample analyses. Feed samples were finely ground using a sample mill (Cyclotec 1093, Foss Tecator, Sweden). Total N in feed, defatted milk and casein was determined with a N analyzer (FP-2000, LECO, St. Joseph, MI) using EDTA (Sigma, St. Louis, MO) as a calibration standard. The concentration of total N in defatted milk was converted to true protein as follows: True protein in milk (%) = Total N in defatted milk (%) · (100 − lipid concentration in milk) · 6.38 · (1 − 0.15) × 10^{-2} where 6.38 corresponds to N% in milk protein and 0.15 corresponds to nonprotein N in sows milk (13–15). Milk lipid concentration was assayed using a midinfrared spectroscope (Multispec M, Berwind Instrument, York, UK).

Samples of feed and whole milk were hydrolyzed in 6 mol/L HCl at 110°C for 24 h before analysis by reverse-phase HPLC (Pico Tag, Waters, Milford, MA). Amino acids in the hydrolysates were derivatized with phenylisothiocyanate (Pierce, Rockford, IL), separated on a Pico Tag column (3.9 mm × 150 mm) and detected at 254 nm on a tenable absorbance detector (Waters 486). Norleucine (Sigma) was added as an internal standard before hydrolysis. Amino acid standard H (Pierce) was used as a calibration standard. The method was validated with a certified AA standard (NIST, Gaithersburg, MD).

Casein in defatted milk was precipitated at room temperature after adjusting the pH to 4.60 with 1.0 mol/L HCl, followed by centrifugation at 1500 × *g* for 15 min at 4°C. Precipitated casein pellets were washed twice with distilled water, solubilized at pH 7.0, freeze-dried and stored at −20°C. Casein was hydrolyzed in 6 mol/L HCl (containing 0.5 g/L dithiothreitol) at 110°C for 18 h. The hydrolysate was desalted on a cation-ion exchange resin column (AG, 50W-X8, H^+-form, Bio-Rad Laboratories, Hercules, CA) and amino acids eluted with 2 mol/L NH_4OH. Frozen plasma samples (200 μL) were thawed at 4°C, deproteinized with 100 μL of 10% sulfosalicylic acid and centrifugation at 1500 × *g* for 15 min at 4°C. The supernatant was desalted as above to isolate AA. Isolated AA from casein and plasma were freeze-dried and derivatized with methyl-*t*-butyldimethylsilyl trifluoroacetamide; isotopic enrichments were determined in the electron-impact mode by gas chromatography-mass spectrometry (Trio-1, VG Masslab, Manchester, UK) (16). Because the ^{15}N-lysine from the manufacturer was contaminated (~3%) with D-lysine, chiral separation was necessary (17). All isotopic enrichments were expressed as molar percent excess (MPE) with respect to the preinfusion natural abundance.

Plasma samples from 15 to 20 h of infusion from each sow were pooled for plasma AA assay. Glucosaminic acid was used as an internal standard. Plasma AA concentrations were determined on a Beckman 6300 Amino Acid Analyzer (Fullerton, CA). Plasma AA samples were precipitated as above and separated by a Beckman cation-ion exchange column charged in Li citrate buffer. Amino acids were measured spectrophotometrically after postcolumn derivatization with ninhydrin.

Plasma flow rate (L/h) was estimated by the Fick principle based on the assumption that the sum of milk phenylalanine and tyrosine (FY) outputs equals the sum of their mammary uptakes (18) as:

$$\begin{aligned}\text{Mammary uptake of plasma FY} &= \text{arteriovenous (A–V) difference of plasma FY}\\ &\times \text{mammary plasma flow} = \text{output of FY in milk}\\ &+ \text{amount of FY metabolized in the MG} \quad (1)\end{aligned}$$

There are four assumptions made in the balance between mammary uptake of plasma FY and its output in milk: *1*) The amount of FY metabolized in the MG is negligible. As demonstrated in the guinea pig mammary tissue, the activity of phenylalanine hydroxylation is negligible (19). Phenylalanine is not oxidized in the lactating goat MG (20). In addition, the amount of FY accretion in the lactating porcine MG is ~1 g/d (21), which is negligible compared with their output in milk (50 g/d) in the present study. *2*) Of total proteins in milk, ~5% is derived from nonmammary synthesized proteins (13–15). *3*) The contribution of vascular peptide-bound FY to mammary synthesized proteins was assumed to be negligible because 96% of casein-bound phenylalanine originates from plasma phenylalanine (20). *4*) Losses of FY from the MG via the lymph are negligible as is the output of free FY in milk (22). Therefore, Equation 1 was simplified as follows:

$$\begin{aligned}\text{Mammary uptake of plasma FY} &= \text{mammary A–V difference of plasma FY}\\ &\times \text{mammary plasma flow} = \text{Output of FY in milk} \cdot (1 - 0.05),\end{aligned}$$

Thus,

$$\begin{aligned}\text{Mammary plasma flow rate (L/h)} &= \text{Concentrations of FY in milk (mmol/L)}\\ &\times \text{milk yield (L/h)} \cdot (1 - 0.05)\\ &\times 10^3/\text{mammary A–V difference of plasma FY } (\mu\text{mol/L}) \quad (2)\end{aligned}$$

$$\begin{aligned}\text{Net output of AA} &= \text{Concentration of amino acid in milk (mmol/L)}\\ &\times (1 - 0.05) \cdot \text{milk yield (L/h)}\end{aligned}$$

Net output for each amino acid was calculated as described for FY as follows: Net output of AA = Concentration of amino acid in milk (mmol/L) · (1–0.05) · milk yield (L/h) where the contribution of nonmammary synthesized proteins was estimated to be 5.0% of the true protein concentration in milk (13–15).

Kinetic calculations

Whole-body and mammary gland transport kinetics of lysine, methionine and valine were calculated as previously described (8,23,24) with a few modifications. Whole-body flux (Fwb, see Glossary) of plasma AA was calculated from the average enrichment of the plasma free AA over the last 5 h of the isotope infusion (see **Figs. 1**, **2**, and **3**):

$$\text{Fwb} = (\text{Ei}/\text{Ea} - 1) \cdot \text{I} \quad (3)$$

where *Ea* and *Ei* are the isotopic enrichments of the AA in arterial plasma and in the infusate, respectively, where *I* is the rate of tracer infusion.

The apparent unidirectional flux of plasma AA across the MG (*Fmg*) was calculated as:

$$\text{Fmg (mmol/h)} = (\text{Ca} \cdot \text{Ea} - \text{Cv} \cdot \text{Ev}) \cdot \text{MPF}/\text{Ea} \quad (4)$$

where C is the concentration (mmol/L) and *E* is the enrichment (MPE) of the AA in arterial (*a*) and mammary venous (*v*) plasma, and MPF is mammary plasma flow (L/h).

Transmembrane flux of free amino acids in the MG. Transmembrane flux and intracellular kinetic calculations of free AA were based on a three-compartment model developed by Biolo et al. (23,24) and later adapted for the mammary gland by Bequette et al. (8) (**Fig. 4**). The assumptions and structure of the model were the same, except that the isotopic enrichment of milk casein-bound AA at plateau was used herein to represent the isotopic enrichment of the mammary intracellular pool.

The model here assumes that AA enter and leave the MG via the mammary artery (*Fa,o*) and the main mammary vein (*Fo,v*), respectively. *Fmg,a* and *Fv,mg* refer to the net movements of free AA from the mammary artery to the mammary intracellular free AA compartment and from this compartment to the mammary vein, i.e., inward and outward transmembrane flux, respectively. *Fv,a* refers to direct flow of AA from artery to vein that does not enter the intracellular free compartment. *Fmg,o* refers to the appearance rate of mammary intracellular free AA from endogenous sources [i.e., release from PB and de novo synthesis, if any]. *Fo,mg* refers to the disappearance rate of mammary intracellular free AA [i.e., the rate of utilization of the intracellular free AA for protein synthesis (PS), oxidation, and other metabolic fates, if any]. *Fa,o* and *Fo,v* were calculated as follows:

$$\text{Fa,o (mmol/h)} = \text{Ca} \cdot \text{MPF} \cdot 10^{-3} \quad (5)$$

$$\text{Fo,v (mmol/h)} = \text{Cv} \cdot \text{MPF} \cdot 10^{-3} \quad (6)$$

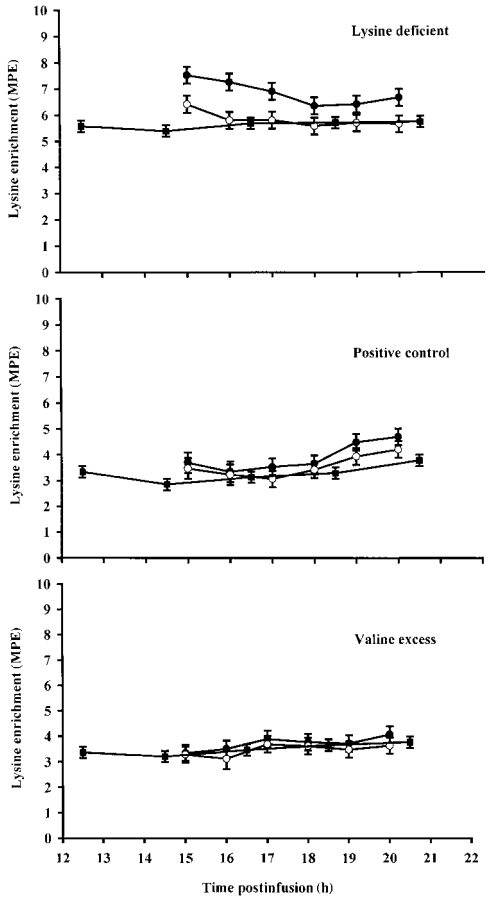

FIGURE 1 Enrichments of plasma free- and milk casein-bound lysine in sows fed the lysine-deficient diet (*upper panel*), the Control diet (*middle panel*) and the valine-excess diet (*lower panel*). Lines with closed circles, open circles and closed squares represent enrichments of plasma lysine in the carotid artery, plasma lysine in the mammary vein and casein-bound lysine in milk, respectively. MPE, molar percent excess. Values are means ± SEM, $n = 3$.

Net uptake (NU) of AA was calculated as:

$$\text{NU (mmol/h)} = \text{Fa,o} - \text{Fo,v, i.e.,}$$

$$\text{NU (mmol/h)} = (\text{Ca} - \text{Cv}) \cdot \text{MPF} \cdot 10^{-3} \quad (7)$$

Calculations of inward (*Fmg,a*) and outward (*Fv,mg*) transport rate by the MG were based on mass and isotopic transfers between the plasma and mammary intracellular pool. Intracellular pool AA enrichment is usually measured from tissues obtained by biopsy, for example, muscle (23,24). Biopsy of the MG leads to considerable bleeding because of its extensive vascular system. As an alternative, Bequette et al. (8) used the enrichment of casein-bound AA in milk, which was assumed to represent the enrichment of the immediate precursor pool within the MG at the site of milk PS. Thus, the enrichment of casein-bound AA in milk was also assumed to reflect the isotopic enrichment of the mammary intracellular free AA compartment at steady state. The average enrichment of casein-bound AA in milk over the last 4 h (16.5–20.5 h) of infusion was used. The net mass balance and tracer balance of AA across the MG were calculated as follows:

$$(\text{Ca} - \text{Cv}) \cdot \text{MPF} \cdot 10^{-3} = \text{Fmg,a} - \text{Fv,mg} \quad (8)$$

$$(\text{Ca} \cdot \text{Ea} - \text{Cv} \cdot \text{Ev}) \cdot \text{MPF} \cdot 10^{-3} = \text{Fmg,a} \cdot \text{Ea} - \text{Fv,mg} \cdot \text{Ec} \quad (9)$$

where *Ec* was the isotopic enrichment of casein-bound AA in milk at steady state. On the basis of the net mass and tracer balance across the MG, *Fmg,a* and *Fv,mg* could be solved from Equations 5, 6, 8 and 9:

$$\text{Fmg,a (mmol/h)} = \{[(\text{Ec} - \text{Ev})/(\text{Ea} - \text{Ec})] \times \text{Cv} + \text{Ca}\} \cdot \text{MPF} \cdot 10^{-3} \quad (10)$$

$$\text{Fv,mg (mmol/h)} = \{[(\text{Ec} - \text{Ev})/(\text{Ea} - \text{Ec})] \times \text{Cv} + \text{Cv}\} \cdot \text{MPF} \cdot 10^{-3} \quad (11)$$

At steady state, $Fa,o = Fmg,a + Fv,a$; and $Fo,v = Fv,a + Fv,mg$. Thus, Fv,a was calculated as follows:

$$\text{Fv,a} = \text{Fa,o} - \text{Fmg,a} \quad (12)$$

or

$$\text{Fv,a} = \text{Fo,v} - \text{Fv,mg} \quad (13)$$

Kinetics of intracellular free amino acids in the mammary gland. Total appearance rate (*Ra*) of mammary intracellular free AA was calculated by the intracellular tracer dilution approach. The only source of tracer appearing in the mammary intracellular free AA compartment was transported inward from plasma. Thus, any dilution in isotopic enrichment of tracer AA in casein is assumed to be from the intracellular free AA of endogenous sources (*Fmg,o*) (e.g., PB and de novo synthesis, if any). Therefore,

$$\text{Ra} \cdot \text{Ec} = \text{Fmg,a} \cdot \text{Ea, i.e.,}$$

$$\text{Ra (mmol/h)} = (\text{Fmg,a} \cdot \text{Ea})/\text{Ec} \quad (14)$$

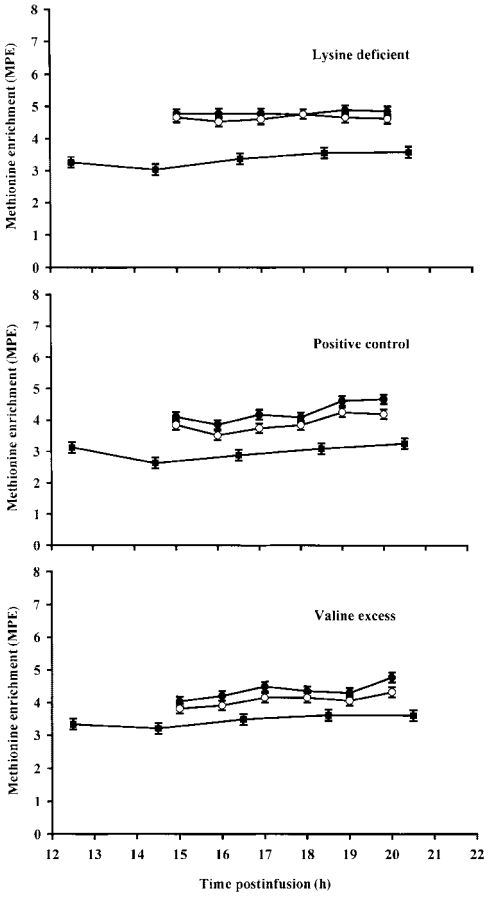

FIGURE 2 Enrichments of plasma free- and milk casein-bound methionine in sows fed the lysine-deficient diet (*upper panel*), the Control diet (*middle panel*), and the valine-excess diet (*lower panel*). Lines with closed circles, open circles and closed squares represent enrichments of plasma methionine in the carotid artery, plasma methionine in the mammary vein and casein-bound methionine in milk, respectively. MPE, molar percent excess. Values are means ± SEM, $n = 3$.

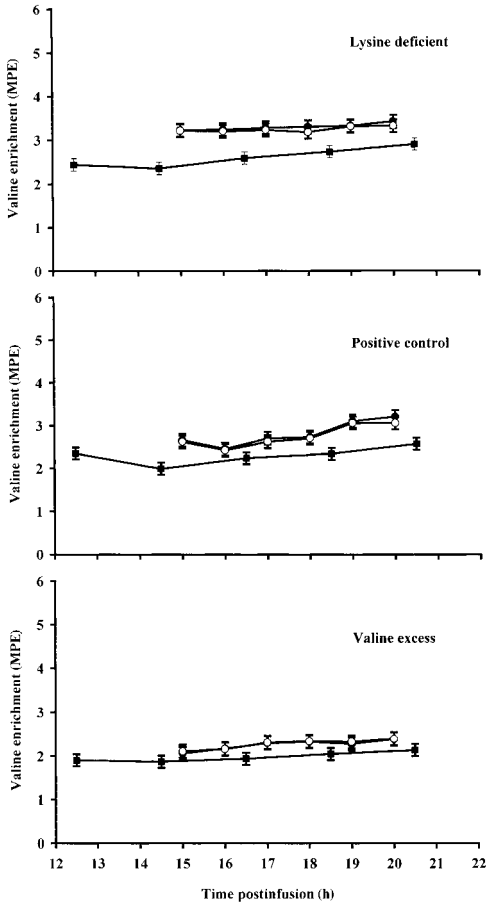

FIGURE 3 Enrichments of plasma free- and milk casein-bound valine in sows fed the lysine-deficient diet (*upper panel*), the Control diet (*middle panel*), and the valine-excess diet (*lower panel*). Lines with closed circles, open circles and closed squares represent enrichments of plasma valine in the carotid artery, plasma valine in the mammary vein and casein-bound valine in milk, respectively. MPE, molar percent excess. Values are means ± SEM, $n = 3$.

where *Ra* is the sum of inward transmembrane flux (*Fmg,a*) and the appearance rate of intracellular free AA from the endogenous sources (*Fmg,o*):

$$\mathrm{Ra} = \mathrm{Fmg,a} + \mathrm{Fmg,o}$$

Thus,

$$\mathrm{Fmg,o\ (mmol/h)} = \mathrm{Fmg,a} \cdot (\mathrm{Ea/Ec} - 1) \quad (15)$$

At steady state, the total fluxes into the mammary intracellular free AA compartment are equal to the total fluxes out of this compartment, i.e.,

$$\mathrm{Fmg,a} + \mathrm{Fmg,o} = \mathrm{Fv,mg} + \mathrm{Fo,mg}$$

Thus,

$$\mathrm{Fo,mg\ (mmol/h)} = (\mathrm{Fmg,a} - \mathrm{Fv,mg}) + \mathrm{Fmg,o} \quad (16)$$

i.e.,

$$\mathrm{Fo,mg\ (mmol/h)} = \mathrm{NU} + \mathrm{Fmg,o} \quad (17)$$

The disappearance rate (*Fo,mg*) of intracellular free AA could also be calculated directly as the tracer balance divided by the precursor enrichment (*Ec*):

$$\mathrm{Fo,mg\ (mmol/h)} = (\mathrm{Ca} \cdot \mathrm{Ea} - \mathrm{Cv} \cdot \mathrm{Ev}) \cdot \mathrm{MPF} \cdot 10^{-3}/\mathrm{Ec} \quad (18)$$

Protein synthesis and breakdown in the MG. The kinetic model can be used to derive rates of PS and PB within the MG provided that the indicator AA is not synthesized within tissue and that it is not used for any purpose other than for PS (i.e., oxidation). Protein equivalent fluxes of methionine were therefore used to derive rates of PS (constitutive plus milk protein) as:

$$\mathrm{PS\ (g/d)} = (\mathrm{Fo,mg} \cdot 0.149/0.0205) \cdot 24\ \mathrm{h}, \quad (19)$$

where 0.149 is the molecular weight of methionine (g/mol) and 0.0205 is the average of methionine in mammary tissue (21) and milk protein from this study (Tables 2 and 3).

Mammary PB was calculated similarly from the rate of intracellular appearance of methionine as:

$$\mathrm{PB\ (g/d)} = (\mathrm{Fmg,o} \cdot 0.149/0.0205) \cdot 24\ \mathrm{h} \quad (20)$$

Net production of protein in the MG. Net production of protein in the MG was derived from the three-compartmental kinetic model, i.e., the difference between PS and PB (Equations 19 and 20). The values for net production of protein derived from the model could be validated by estimates of accretion of mammary tissue proteins and output of mammary synthesized proteins in milk. Average accretion of mammary tissue proteins was reported to be14.81 g/d over a 21-d lactation period in lactating sows (21). The output of mammary synthesized proteins in milk was calculated as follows:

$$\begin{aligned}\text{Output of mammary synthesized proteins in milk (g/d)} &= \text{Milk yield (kg/d)} \cdot 1.15 \cdot \text{true protein concentration in milk (\%)} \\ &\quad \times (1 - 0.05) \cdot 10 \quad (21)\end{aligned}$$

where the estimate of milk yield was adjusted by 1.15 because the weigh-suckle-weigh method underestimates milk consumption compared with the D_2O dilution method (10,25). Again, the contribution of nonmammary synthesized proteins was estimated to be 5.0% of the true protein concentration in milk (13–15).

Statistical analyses. Data were analyzed by the Mixed Procedure (SAS/STAT Version 6.12, SAS Institute, Cary, NC). The model for

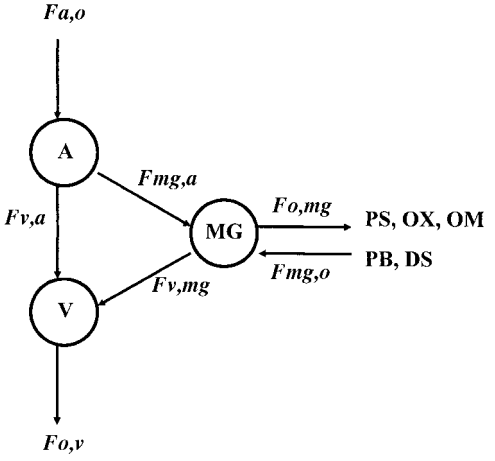

FIGURE 4 A three-compartmental model of amino acid kinetics across the porcine mammary gland during lactation. Free amino acid compartments in artery (A), main mammary vein (V), and mammary gland (MG) are connected by arrows indicating unidirectional fluxes of free amino acids between each compartment. Amino acids enter the MG via the mammary artery (*Fa,o*) and leave the MG via the main mammary vein (*Fo,v*). Other fluxes are designated as follows: *Fv,a*, direct flow of amino acids from artery to vein without entering the intracellular pool (by the arterial shunt); *Fmg,a* and *Fv,mg*, inward and outward transmembrane transport of amino acids from artery to the MG and from the MG to vein, respectively; *Fmg,o* the rate of intracellular amino acid appearance from endogenous sources [i.e., release from protein breakdown (PB) and de novo synthesis (DS), if any]; and *Fo,mg*, the rate of the intracellular amino acids disappearance [i.e., the rate of utilization of intracellular amino acids for protein synthesis (PS), oxidation (OX), and other metabolic fates (OM), if any].

TABLE 2

Productive performance in lactating sows fed lysine-deficient, control or valine-excess diets over a 21-d lactation period[1]

Item	Diet			SEM
	Lysine-deficient	Control	Valine-excess	
Feed intake, *kg/d*	5.34	5.71	5.83	0.56
Litter size on d 21, *n*	11.0	11.8	12.0	0.60
Litter growth rate, *kg/d*	1.69*	2.37	2.50	0.11
Milk yield, *kg/d*	6.26**	9.20	9.06	0.16
N in defatted milk, *g/100 g*	0.95*	1.01	0.99	0.01
Protein in milk, *g/100 g*	4.88*	5.11	5.03	0.04
Casein-N, *% of total N in milk*	49.30	53.28	52.69	2.30

[1] Values (least-square means) ($n = 3$) with superscripts (* $P < 0.05$, and ** $P < 0.01$) differ from Control.

total N concentration in defatted milk included block, dietary treatment and sampling time, and all two-way interactions with sampling time in a repeated statement. The model for other variables included block and dietary treatment. Based on the residual distribution, kinetic data were log-transformed before ANOVA. Least-square means were then converted to actual values. Differences between LD and Control treatments, and VE and Control treatments were considered significant at $P < 0.05$. Significant trends were considered at $P < 0.10$.

RESULTS

Milk production and litter growth. Sows fed the LD diet had lower milk yields ($P < 0.05$) and litter growth rates ($P < 0.01$) than sows fed the Control diet (**Table 2**). Milk protein concentration was lower ($P < 0.05$) in sows fed the LD diet. Production performance did not differ between sows fed the VE and Control diets. Mammary plasma flow rate, adjusted for sow body weight, was not affected by dietary AA availability (LD = 1.16 ± 0.14, Control = 1.28 ± 0.14, and VE = 1.40 ± 0.14 L/kg body · h).

Milk AA composition. Milk AA concentrations and output were lower in sows fed the LD diet ($P < 0.05$) and were not increased in sows fed the VE diet ($P > 0.05$) (**Table 3**). Amino acid composition was not affected by dietary amino acid availability when expressed as a percentage of the true protein in milk (data not shown).

Plasma AA and urea. Arterial plasma lysine concentrations were lower ($P < 0.01$) in sows fed the LD diet, whereas concentrations of some other indispensable AA and urea in sows fed the LD diet tended to increase ($P < 0.10$ to $P < 0.05$) (**Table 4**). Plasma valine was greater ($P < 0.01$) in sows fed the VE diet , whereas concentrations of other indispensable AA did not differ from sows fed the Control diet.

Lysine kinetics. Whole-body flux (*Fwb*) and gross removal (*Fmg*) of lysine by the mammary gland were lower ($P < 0.01$) in sows fed the LD diet compared with Control (**Table 5**). Compared with Control, inward (*Fmg,a*) and outward (*Fv, mg*) transport of lysine were also lower ($P < 0.05$) in sows fed the LD diet, resulting in a decrease ($P < 0.05$) in net uptake of lysine. The proportion of lysine net uptake to its arterial supply (*Fa,o*) was 120% greater in sows fed LD compared with Control (NU/*Fa,o*: 0.51 ± 0.05 vs. 0.23 ± 0.05, $P < 0.05$), indicating an increased extraction efficiency of plasma lysine by the MG of sows fed LD. The proportion of intracellular disappearance (*Fo,mg*) to total intracellular appearance (*Ra*) of lysine (i.e., intracellular lysine availability) increased in the LD-fed compared with Control-fed sows (*Fo,mg*/*Ra*: 0.67 ± 0.05 vs. 0.42 ± 0.05, $P < 0.05$), indicating an increased efficiency of intracellular lysine utilization for PS in sows fed the LD diet. In contrast, this proportion tended to decrease ($P < 0.10$) in sows fed VE compared with the Control (0.20 ± 0.05 vs. 0.42 ± 0.05). Compared with Control, partition of lysine to the MG tended to be reduced ($P < 0.10$) and net uptake of lysine was reduced ($P < 0.05$) in sows fed the VE diet . The fact that lysine net output to uptake ratio was apparently >1 ($P < 0.1$) may be attributed to additional lysine uptake in the form of peptides. Assuming that the ratio of inward (*Fmg,a*) to outward (*Fv,mg*) flux (I/O) reflects attempts by the MG to enhance the extraction of AA when they are limiting, decreases ($P < 0.05$) in the I/O ratio in sows fed the Control and VE diets suggest that lysine was adequate, i.e., no longer limiting at the level of the MG.

Methionine kinetics. Compared with Control, whole-body flux (*Fwb*) ($P < 0.10$), gross removal (*Fmg*) by the MG ($P < 0.05$) and partition of methionine to the MG ($P = 0.11$)

TABLE 3

Concentrations and output of indispensable amino acids in whole milk of lactating sows fed lysine-deficient control or valine-excess diets[1]

Amino acid	Diet			SEM
	Lysine-deficient	Control	Valine-excess	
		mmol/L		
Arginine	15.02	16.00	16.96	0.63
Histidine	8.25*	9.52	9.77	0.39
Isoleucine	16.13*	18.35	18.96	0.69
Leucine	33.72*	37.87	38.43	1.37
Lysine	25.02*	28.39	29.45	1.17
Methionine	6.23	6.95	7.71	0.36
Phenylalanine	12.12*	13.67	14.11	0.55
Threonine	17.52*	19.07	19.16	0.55
Valine	22.64†	25.40	26.09	1.00
		g/d		
Arginine	20.32*	30.20	28.59	1.21
Histidine	10.14**	15.98	15.55	0.42
Isoleucine	16.49**	25.92	25.51	0.73
Leucine	34.37**	53.63	51.76	1.36
Lysine	28.65**	44.78	44.09	1.36
Methionine	7.29**	11.32	11.69	0.24
Phenylalanine	15.57**	24.37	23.85	0.75
Threonine	15.67**	24.48	23.43	0.84
Valine	20.76**	32.09	31.30	0.82

[1] Values (least-square means) ($n = 3$) with superscripts differ (* $P < 0.05$ and ** $P < 0.01$) or tend to differ († $P < 0.10$) from Control.

TABLE 4

Arterial concentrations of plasma amino acids and urea in lactating sows fed lysine-deficient control or valine-excess diets[1]

Amino acid	Diet			SEM
	Lysine-deficient	Control	Valine-excess	
		μmol/L		
Taurine	75.51*	19.89	20.51	9.04
Urea	5514.19†	2794.98	4329.01	816.71
Aspartic acid	34.94	47.51	48.59	6.84
Threonine	470.70†	225.00	224.63	67.48
Serine	111.05	88.88	72.85	7.63
Asparagine	57.58	61.39	67.11	13.45
Glutamic acid	368.08	411.96	398.73	77.45
Glutamine	512.73	559.21	450.32	37.68
Proline	266.28	256.11	250.98	32.75
Glycine	774.64	939.87	785.19	93.02
Alanine	466.62	685.86	666.15	55.19
Citrulline	103.86	86.79	86.62	17.11
Valine	529.96	664.85	1236.68**	60.88
Cystine	6.06†	2.20	4.23	0.91
Methionine	57.15	60.83	51.57	7.38
Isoleucine	109.80	87.38	98.70	11.02
Leucine	192.25	123.52	148.46	24.14
Tyrosine	155.88	88.73	68.31	36.94
Phenylalanine	101.56	61.12	64.27	14.77
Tryptophan	51.69	35.01	34.46	7.83
Ornithine	21.37	30.82	28.33	5.61
Lysine	32.67**	253.98	203.94	27.05
Histidine	108.98	113.21	101.08	9.07
Arginine	102.18	113.84	125.77	15.58

[1] Values (least-square means) ($n = 3$) with superscripts differ (* $P < 0.05$, and ** $P < 0.01$) or tend to differ († $P < 0.10$) from Control.

were reduced in sows fed the LD diet (**Table 6**). Inward transport (*Fmg,a*) ($P < 0.10$) and intracellular disappearance (*Fo,mg*) of methionine ($P < 0.05$), and consequently net uptake of methionine ($P < 0.05$), were reduced in sows fed the LD diet. The intracellular appearance (*Fmg,o*) of methionine decreased ($P < 0.05$) in sows fed the LD diet, indicating a decrease in PB in the MG compared with Control. Total appearance (*Ra*) ($P < 0.1$) and intracellular disappearance (*Fo,mg*) ($P < 0.05$) of methionine decreased, indicating a decrease in intracellular methionine availability for PS and other metabolism (if any) in the MG. More than 50% of the arterial supply (*Fa,o*) of methionine did not enter the intracellular methionine compartment, but flowed directly from the artery to the vein (*Fv,a*), resulting in a lower extraction efficiency of plasma methionine by the MG in sows fed the LD diet (NU/*Fa,o*: 0.19 ± 0.00 vs. 0.24 ± 0.00, $P < 0.01$). The greater proportion (>70%) of the total appearance rate (*Ra*) of the intracellular methionine was contributed by inward transport (*Fmg,a*) and a lesser proportion (<30%) by endogenous sources (i.e., PB) (*Fmg,o*).

Methionine kinetics in sows fed the VE diet were largely unaffected by valine availability, except for a decrease ($P < 0.05$) in methionine direct flow flux (by-pass) (*Fv,a*) to the MG and a decrease ($P < 0.05$) in the rate of appearance of methionine in the MG intracellular pool. The ratio of I/O for methionine was not affected by dietary lysine and valine availability. Compared with sows fed the Control diet, the contribution to the total intracellular methionine pool from inward transport (*Fmg,a*) was increased (*Fmg,a/Ra*, 0.82 ± 0.02 vs. 0.72 ± 0.02, $P < 0.05$) and that from mammary PB (*Fmg,o*) was decreased (*Fmg,o/Ra*: 0.18 ± 0.02 vs. 0.28 ± 0.02, $P < 0.05$).

Valine kinetics. Whole-body flux (*Fwb*), partition to and removal (*Fmg*) by the MG were greatest for valine, compared with lysine and methionine (**Table 7**). Similar to lysine and methionine, the rate of valine inward transport (*Fmg,a*) exceeded outward transport (*Fv,mg*) by >100% in sows fed the LD diet compared with Control. No differences in whole-body flux, mammary gross removal, and inward or outward transport of valine were found between sows fed the LD diet and those fed the Control diet. However, compared with sows fed the Control diet, the arterial valine supply (*Fa,o*) decreased ($P < 0.01$) and valine by-pass (*Fv,a*) from the artery to the vein tended to decrease ($P < 0.10$) in sows fed the LD diet. In sows fed the VE diet, both valine arterial supply (*Fa,o*) to the MG

TABLE 5

Plasma lysine kinetics in lactating sows fed lysine-deficient control or valine-excess diets[1]

Item	Diet			SEM
	Lysine-deficient	Control	Valine-excess	
		mmol/h		
Whole-body flux (*Fwb*)	14.89**	28.58	29.67	0.48
Mammary gross removal (*Fmg*) (apparent mammary unidirectional flux)	7.71**	15.07	9.58**	0.46
Partition to MG[2]	0.47†	0.54	0.33**	0.02
Net output/net uptake[3]	1.21	1.21	1.65[a]	0.16
Arterial supply (systemic entrance flux) (*Fa,o*)	11.22**	55.41	52.16	4.38
By-pass (direct flow flux) (*Fv,a*)	2.59	9.13	0.07	7.01
Inward transport (I) (*Fmg,a*)	9.65*	38.47	52.64	7.83
Outward transport (O) (*Fv,mg*)	4.18*	24.87	44.58	7.98
I/O	2.08*	1.56	1.10*	0.07
Net uptake (NU)	6.47*	10.91	7.10*	0.77
Intracellular disappearance rate (*Fo,mg*)	9.55**	17.43	10.35**	0.60
Intracellular appearance rate (*Fmg,o*)	2.57	5.98	3.29	1.07
Total appearance rate (*Ra*)	7.32*	48.17	58.23	8.41

[1] Values (least-square means) ($n = 3$) with superscripts differ (* $P < 0.05$, ** $P < 0.01$) or tend to differ († $P < 0.10$) from Control.
[2] Calculated as gross removal divided by whole-body flux.
[3] "a" indicates that value is different from 1 at $P = 0.058$.

TABLE 6

Plasma methionine kinetics in lactating sows fed lysine-deficient control or valine-excess diets[1]

Item	Diet			SEM
	Lysine-deficient	Control	Valine-excess	
		mmol/h		
Whole body flux (*Fwb*)	6.06†	6.93	6.86	0.23
Mammary gross removal (*Fmg*) (apparent mammary unidirectional flux)	2.42*	4.05	3.71	0.37
Partition to MG[2]	0.41	0.60	0.54	0.06
Net output/net uptake	1.04	0.99	1.00	0.16
Arterial supply (systemic entrance flux) (*Fa,o*)	11.43	13.77	13.37	0.84
By-pass (direct flow flux) (*Fv,a*)	7.83	7.55	6.95*	0.15
Inward transport (I) (*Fmg,a*)	3.42†	6.00	6.54	0.94
Outward transport (O) (*Fv,mg*)	1.43	2.73	3.35	0.68
I/O	2.39	2.21	1.95	0.09
Net uptake (NU)	1.96*	3.28	3.11	0.26
Intracellular disappearance rate (*Fo,mg*)	3.34*	5.58	4.51	0.41
Intracellular appearance rate (*Fmg,o*)	1.32*	2.29	1.39*	0.15
Total appearance rate (*Ra*)	4.74†	8.34	7.94	1.08

[1] Values (least-square means) with superscripts differ (* $P < 0.05$, ** $P < 0.01$) or tend to differ († $P < 0.10$) from Control.
[2] Calculated as gross removal/whole-body flux.

and by-pass (*Fv,a*) from artery to vein increased ($P < 0.01$) compared with the Control diet. Inward transport of valine (*Fmg,a*) and intracellular disappearance (*Fo,mg*) were unaffected; consequently, net uptake of plasma valine was not different from sows fed the Control diet. Outward transport (*Fv,mg*) was negligible ($P < 0.05$) in sows fed the VE diet compared with the Control diet. The appearance rate of the intracellular valine (*Fmg,o*) from endogenous sources (i.e., PB) decreased ($P < 0.05$) by 56.8% in sows fed the VE diet, indicating a decrease in mammary PB. In sows fed the VE diet, the proportion of inward transport (*Fmg,a*) to arterial supply of valine to the MG tended to decrease (*Fmg,a*/*Fa,o*: 0.26 ± 0.06 vs. 0.06 ± 0.06, $P < 0.10$); accordingly, the proportion of valine by-pass (from the artery to the vein) (Fv,a) to valine arterial supply (*Fa,o*) tended to increase (*Fv,a*/*Fa,o*: 0.74 ± 0.06 vs. 0.94 ± 0.06, $P < 0.10$). Thus, mammary extraction rate of plasma valine decreased numerically ($P = 0.09$) by 50% (NU/*Fa,o*: 0.14 ± 0.03 vs. 0.07 ± 0.03). The ratio of I/O did not differ in sows fed the LD and Control diets; for those fed the VE diet, however, this value could not be calculated because the outward transport of valine was negligible.

Protein synthesis and breakdown in the mammary gland. Estimation of protein turnover in the MG was based on the protein equivalent fluxes of methionine (**Table 8**). For all three treatments, the model estimates of net PS (including secretory plus constitutive proteins) by the mammary gland were similar to measured milk protein yield plus an estimate of mammary protein accretion from the study of Kim et al. (21). Protein synthesis and PB were reduced ($P < 0.05$) in sows fed the LD diet, resulting in a decrease in net protein balance. In sows fed the VE diet, PB was decreased ($P < 0.05$).

TABLE 7

Plasma valine kinetics in lactating sows fed lysine-deficient control or valine-excess diets[1]

Item	Diet			SEM
	Lysine-deficient	Control	Valine-excess	
		mmol/h		
Whole body flux (*Fwb*)	29.97	34.81	43.17*	1.85
Mammary gross removal (*Fmg*) (apparent mammary unidirectional flux)	20.11	23.08	24.02	4.65
Partition to MG[2]	0.64	0.69	0.55	0.13
Net output/net uptake[3]	0.46[b]	0.56[b]	0.45[b]	0.14
Arterial supply (systemic entrance flux) (*Fa,o*)	112.57**	150.91	333.99**	4.80
By-pass (direct flow flux) (*Fv,a*)	83.61†	111.15	313.68**	8.79
Inward transport (I) (*Fmg,a*)	28.26	36.21	23.47	8.44
Outward transport (O) (*Fv,mg*)	11.82	15.95	0.00*	4.21
I/O	2.34	2.23	—	0.09
Net uptake (NU)	15.30	20.19	23.99	4.35
Intracellular disappearance rate (*Fo,mg*)	24.02	27.25	27.12	4.99
Intracellular appearance rate (*Fmg,o*)	5.79	6.72	2.90*	0.67
Total appearance rate (*Ra*)	36.36	44.78	26.34	9.09

[1] Values (least-square means) with superscripts differ (* $P < 0.05$, ** $P < 0.01$) or tend to differ († $P < 0.10$) from Control.
[2] Calculated as gross removal divided by whole-body flux.
[3] Values different from 1 are indicated by "b" ($P < 0.01$).

TABLE 8

Mammary protein metabolism based upon protein equivalent fluxes of methionine in lactating sows fed lysine-deficient control or valine-excess diets[1]

Item	Diet			SEM
	Lysine-deficient	Control	Valine-excess	
Protein synthesis (PS), *g/d*	583.3*	975.1	787.1	71.6
Protein breakdown (PB), *g/d*	229.5*	399.6	243.1*	25.5
PS/BP	2.54	2.44	3.24**	0.01
Net protein balance, *g/d*	353.7*	575.5	544.0	46.2
Measured milk production,[2] *g/d*	355.0**	529.0	514.2	10.1

[1] Values (least-square means) with superscripts differ (* $P < 0.05$ and ** $P < 0.01$) from Control.
[2] Estimated from the sum of milk protein output (weigh-suckle-weigh) and an estimate (14.81 g/d) of mammary protein accretion (21).

DISCUSSION

Control of milk PS ultimately occurs within the epithelial cell at the level of translation and it is here that AA may become limiting. The intracellular availability of AA is determined by the activity of a range of transport processes, some of which handle multiple AA and transport AA into and out of the cell as well. Our questions were whether these transport processes limit or stimulate milk PS by the porcine mammary gland, and whether AA transport activity defines the limiting AA for milk PS. In an attempt to answer these questions, we employed an arteriovenous tracer kinetic model that had previously been applied to muscle and mammary gland (8,23,24). The kinetic model derives rates of transport of AA into and out of tissues, and rates of PS and PB. To assess the link between mammary transport of AA and milk PS, corn/soybean meal–based diets were formulated to create lysine limitation and valine excess. We found that mammary transmembrane flux of lysine and methionine under lysine-limiting and -adequate conditions was closely linked to milk PS. Although valine is implicated as the first limiting AA in diets fed to sows nursing large litters (2,3), this claim is equivocal (26). To further characterize mammary transport and to determine whether valine transport kinetics were the same as for lysine under conditions of limited and excess valine, we formulated the Control diet to contain valine in a 1:1 ratio with lysine, and thus possibly limiting in valine, and a third diet to contain valine in a 1.3:1 ratio with lysine, and thus possibly in valine excess. Valine was not limiting for mammary PS when fed in a ratio of 1:1 with lysine; within the physiologic range, lysine availability had little influence on mammary transport of valine.

In the present study, AA tracers were infused for 20.5 h, and isotopic enrichments reached their plateau in the plasma pool and in casein. The rates of transmembrane flux and protein turnover derived from this plateau period therefore should closely approximate true rates. Indeed, the model estimate of net protein balance between PS and PB, which was derived from the methionine kinetics, did not differ from the measured output of milk protein determined in this study by the weigh-suckle-weigh method plus an estimate of accretion of mammary protein (21). Therefore, the kinetic model employed is valid for the porcine mammary gland.

Our first question was whether milk protein output was associated with mammary transport of indispensable AA in vivo, and whether methionine and valine transport were linked to lysine availability. Compared with the lysine-adequate diet, milk protein output and litter growth rate were lower for sows fed the lysine-deficient diet. Correspondingly, net uptakes of plasma lysine and methionine by the mammary gland were also reduced for sows fed the lysine-deficient diet, and net uptake of plasma valine was numerically decreased. Mammary extraction of lysine did not approach 100% for sows fed the deficient diet, suggesting that there was a limit to mammary lysine transport. Indeed, inward transport of lysine was considerably lower in sows fed the deficient diet, as was outward transport. In lactating goats under histidine-limiting conditions, Bequette et al. (8) also observed large decreases in the bidirectional transport of histidine. The ratio of inward to outward flux increased under the deficient condition to favor inward transport. Herein, lysine arterial concentration (~30 μmol/L) in sows fed the lysine-deficient diet was 66% less than the K_m (~100 μmol/L) for lysine transport systems (y^+ and $B^{0,+}$) in human mammary gland (27). In chickens fed a lysine-limiting diet, capacities of transport systems y^+ and $b^{0,+}$ for lysine were down-regulated by 30% in jejunal brush border membrane vesicles (28). It is probable that these systems were also down-regulated in the present study, but due to the aggregated nature of the kinetic measurements, it is not known whether the response was a reflection of the number or activity of the transporters. What is clear, however, is that the processes for transporting lysine into as well as out of the cell were affected by arterial lysine availability. There was an apparent discrepancy in LD for the total appearance rate of intracellular lysine (*Ra*) and the sum of intracellular endogenous appearance rate (*Fmg,o*) and lysine inward transport (*Fmg,a*). However, because total appearance of intracellular lysine (*Ra*) was calculated according to Equation 14, where $Ra = Fmg,a \cdot Ea/Ec$, and the isotopic enrichment of casein-bound lysine (*Ec*) relative to arterial free lysine (*Ea*) was relatively high, as shown in Figure 1, the total appearance of intracellular lysine (*Ra*) may have been slightly underestimated. Lysine in the LD diet might be more efficiently channeled into casein synthesis due to its limiting status, and intracellular lysine less diluted by PB, thus resulting in a relatively higher isotopic enrichment of casein-bound lysine.

Although the arterial concentration and supply of methionine to the mammary gland was not affected by dietary AA availability, the partitioning of plasma methionine flux to the mammary gland and net uptake and bidirectional transport rates of methionine were lower in sows fed the lysine-deficient diet. The phenomenon that methionine paralleled lysine in transport kinetics pattern suggests that regulation of the transport of these two AA may be linked, possibly through regulation of PS, as has been shown in other cell types (29). Interestingly, valine did not follow the same kinetics pattern as lysine and methionine when lysine was deficient. In fact, there were no differences in mammary net uptake, inward or

outward transport of valine under conditions of lysine deficiency or lysine adequacy, suggesting that lysine availability within the physiologic range does not affect valine kinetics.

Our second question was whether transport kinetics of valine, lysine and methionine were linked to valine status. The Control diet was formulated so that valine concentration relative to lysine was below those reported to be optimal for milk production (2,3), and the valine-excess diet was formulated so that valine would be nonlimiting and "appropriate" for sows nursing large litters (2,3). Increasing dietary valine concentration did not affect methionine kinetics and decreased net uptake of plasma lysine, which resulted from an increase in outward transport of lysine relative to its inward transport. Uptake of lysine by the mammary tissue is inhibited in vitro by high concentrations of neutral AA (e.g., leucine) (30–33), via stimulation of lysine outward flux (33). In addition, dietary supplementation of valine was shown to inhibit uptake of lysine by mouse jejunal brush border membrane (34). Recently, a Na^+-dependent AA transporter ($hATB^{0,+}$) cloned from human MG expressed the same kinetic properties of system $B^{0,+}$ (27). Note that arterial plasma concentrations of lysine and valine were ~250 and 650 μmol/L, respectively, in lactating sows fed the Control diet. In this connection, uptake of lysine by the MG might be inhibited by physiologic (or high) concentrations of plasma valine through stimulation of lysine outward transport. Because milk protein yield was not reduced as dietary valine increased, the lower rate of intracellular lysine disappearance likely reflected a reduction in lysine catabolism within the MG.

Valine inward transport was unaffected by dietary valine concentration. The transport system for valine in the lactating porcine MG has a K_m of 640 μmol/L in vitro (35), indicating that the AA transport system for valine may be saturated in vivo. Transport of the branched-chain AA into muscle is regulated in vivo mainly by their concentrations in the extracellular fluid and by intracellular metabolic removal, and is reduced in vitro by their high concentrations (36). Because the branched-chain AA may be toxic at high concentrations in the tissue, this down-regulation of transport for valine would ensure tissue protection from any risk of toxicity (37). Outward transport for valine was not increased in sows fed the valine-excess diet, and was negligible compared with sows fed the Control diet, possibly due to a very high concentration of valine in the extracellular space. Mammary venous plasma valine concentration, which is expected to be lower than that in the extracellular space, was as high as 1150 μmol/L in sows fed the valine-excess diet.

Our third question was whether PS and PB in the MG are regulated per se by AA availability. Based on the kinetics of methionine, model-derived PS was up to 2.0-fold greater than PB in the MG. Champredon et al. (38) reported similarly high rates of protein turnover in the goat mammary gland. Model estimates of net protein balance (including constitutive plus secretory proteins) were similar to the measured milk protein output plus mammary protein accretion. Both PS and PB were reduced in sows fed the lysine-deficient diet, and interestingly, this relationship remained constant in sows fed the Control diet. Milk protein output is significantly decreased in sows fed a lysine-deficient diet (39). The simultaneous and fixed nature of these two processes suggests that rates of milk protein output are directly associated with total PS and PB. Bequette et al. (40) observed a similar relationship in the bovine mammary gland. In sows fed the valine-excess diet, PB was significantly reduced, but due to a numerical decrease in synthesis as well, net protein balance or milk protein output was not affected. Valine may decrease mammary PB possibly through its intracellular metabolism (e.g., oxidation) and/or extracellular regulatory sites on the basolateral membranes of the mammary epithelial cells. Valine is taken up by the lactating porcine mammary gland in excess of milk protein output (5), suggesting that the excess valine is oxidized or utilized for the synthesis of other metabolites. To date, there have been no reports on valine metabolism in vivo by the porcine mammary gland. Richert et al. (41) did not find substantial CO_2 production in vitro from valine oxidation in the porcine mammary gland. In lactating cows and goats, however, branched-chain AA are oxidized extensively and oxidation accounts for the proportion of these taken up in excess (19,42–44). Branched-chain AA (e.g., leucine) inhibit muscle PB, presumably by their increased intracellular accumulation or their keto-acids (e.g., α-ketoisocaproate and isovalerate) (45–47). We did not measure the mammary intracellular concentrations of the branched-chain AA and their keto-acids, or plasma keto-acid concentrations. However, because the intracellular valine disappearance rate did not change with either the lysine-deficient or valine-excess diet, it is unlikely that valine oxidation by the MG increased in sows fed the valine-excess diet.

The inhibitory effect of high valine on mammary PB was more likely to occur on the plasma membranes because arterial plasma valine and urea-N concentration increased considerably without any increase in intracellular valine appearance in sows fed the valine-excess diet. High concentrations of extracellular leucine and α-ketoisocaproate inhibit PB in vitro, possibly through interactions with their regulatory sites on the plasma membrane in the liver and heart where the activity of branched-chain aminotransferase is minimal (48–50). Increasing dietary valine concentration does not increase milk protein output, but decreases mammary protein turnover mainly via a decrease in mammary PB. A lower rate of protein turnover can decrease energy costs. Whether improvement in litter growth rate in sows with a high valine intake (2,3) is related to this response requires further study. Valine is not limiting for mammary PS when fed in a ratio of 1:1 with lysine, and within the physiologic range, lysine availability has little influence on valine transport across the porcine mammary gland. The changes observed in transmembrane flux of lysine and methionine under lysine-limiting and -adequate conditions suggest that transport of these amino acids by the porcine mammary gland is closely linked to regulation of milk PS.

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APPENDIX

Glossary of terms used

Term	Unit	Definition
Fwb	mmol/h	Whole body irreversible loss flux or whole body flux of individual AA
Fmg	mmol/h	Mammary apparent unidirectional flux or gross removal of individual AA
Fa,o	mmol/h	Systemic entrance flux or arterial supply of individual AA
Fv,a	mmol/h	Direct flow flux of individual AA from mammary artery to mammary vein without entering intracellular fluid
Fo,v	mmol/h	Systemic exit flux or venous outflow of individual AA
NU	mmol/h	Mammary net uptake of individual AA
Fmg,a	mmol/h	Inward trans-membrane flux of free AA from mammary artery to mammary intracellular free AA compartment
Fv,mg	mmol/h	Outward trans-membrane flux of free AA from mammary intracellular free AA compartment to mammary vein
Fmg,o	mmol/h	Endogenous appearance rate of mammary intracellular free AA (e.g., from PB and de novo synthesis, if any)
Fo,mg	mmol/h	Disappearance rate of mammary intracellular free AA (e.g., for PS, oxidation, and other metabolism, if any)
Ra	mmol/h	Total appearance rate of mammary intracellular free AA, i.e., the sum of inward transmembrane transport (Fmg, a) and endogenous appearance flux (Fmg, o)