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Application of a Mechanistic Model to Study Competitive Inhibition of Amino Acid Uptake by the Lactating Bovine Mammary Gland

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ABSTRACT

A mathematical model is used to describe uptake by a countertransport system and subsequent flow of three amino acids (AA), Phe, Val, and Met, from arterial blood to milk protein in the mammary gland of a lactating cow. The model suggests that total uptake of all AA is higher than net uptake and that a large proportion of the incoming AA is released from the cell directly back to blood. The model is used to predict which of the three AA is limiting the rate of milk protein synthesis and the response to increased arterial concentration of the first-limiting AA. Simulations are performed to predict possible outcomes of several experimental protocols to AA infusion, which might be used to test in vivo the responsiveness of the bovine mammary gland to an altered arterial concentration of AA. Of the three AA considered, arterial Met concentration appears to be first-limiting. The infusion profile that gives the greatest response in milk protein synthesis rate alters the arterial profile of AA such that it is identical to that of proteins originating in the mammary gland. Model construction can be simplified by acknowledging normal biological constraints.

(**Key words**: amino acid, transport, uptake, inhibition)

Abbreviation key: **MOP** = mammary origin proteins.

INTRODUCTION

The increased demand for protein from dairy products has stimulated interest in the process of protein synthesis in the lactating bovine mammary gland and in understanding which factors limit the rate of the overall process. Amino acids from arterial blood are the precursors of milk proteins that are synthesized in the mammary gland. Amino acid uptake by cells (4), including bovine mammary cells (3), is accomplished by a family of 13 different membrane-bound AA transporters. The AA specificity of these individual transporters varies widely; some transport only specific AA, and others transport a variety of AA. A complete understanding of AA uptake by the gland would entail consideration of all permutations involving greater than 20 amino acids and 13 different transporters. Therefore, simpler transport models are needed to advance understanding and to determine whether AA transport limits protein synthesis. The specificity of the AA transporters supports the need to treat each AA as a specific metabolite. However most previous modeling efforts have simplified the process by treating AA as a homogeneous group (2, 5, 10, 25).

Essential AA, which are used for milk protein synthesis, have been divided into two discrete categories (14); group I comprises AA that appear to be secreted stoichiometrically in milk with their net uptake by the mammary gland, and group II comprises AA that are secreted in milk in quantities lower than those taken up by the gland. It may be more than coincidence that four of the five AA designated to group I are transported primarily by the L system AA transporter (3, 4). The unique characteristic of this transporter is that it acts as a countertransporter and exports one AA residue out of the cell for each AA residue that it imports into the cell. The net rate of influx into a cell or rate of efflux out of a cell of total AA transported by this method is dependent on the relative concentration gradients of AA, which are transported by this system, on opposite sides of the

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membrane (3, 4). The rate of milk protein synthesis may be limited by the net rate of uptake of these essential AA, and, therefore, indirectly by the activity level of the L system transporter.

A mechanistic dynamic two-pool mathematical model of AA uptake by the lactating bovine mammary gland is described and used to estimate total uptake and net uptake of two group I AA, Phe and Met, and one group II AA, Val, which are transported primarily by the L system transporter. The model determines whether competitive inhibition, that is, the reduction in transport of one AA because of increased competition for limited transport capacity by another AA, may be a factor in limiting AA uptake and, therefore, in limiting the rate of milk protein synthesis. The model simulates the potential effects of increasing arterial concentrations of individual AA or specific groups of AA, as would be done with close arterial infusion of AA to the bovine mammary gland.

MATERIALS AND METHODS

Model Description

The model used is a modified version of a previously reported model (13) and represents the reference animal, a cow producing 30 $L \cdot d^{-1}$ of 3.34% total protein milk (Figure 1). The model considers AA utilization by the secreting alveolar cells producing all mammary origin proteins (**MOP**) found in bovine milk, which include caseins, α-LA, and β-LG. The cells have two intracellular pools containing either free AA or AA that is bound in milk protein. Oxidation and transamination of Met and Phe within the gland is assumed to be negligible (11), and oxidation of Val within the cell is set at 30% of net uptake (20). Transamination of Phe to Tyr was disregarded because of the low activity of Phe hydroxylase in mammary tissue of lactating ruminants when arterial Phe concentrations were within the normal physiological range (24).

The model is completely defined by Equations [A1] to [A45] given in Appendix 1; the mathematical notation employed is given in Tables A1, A2, and A3. The computer program used to solve the model was written in Advanced Continuous Simulation Language (1), and a fourth-order, fixed-step Runge-Kutta algorithm was used to integrate the differential equations numerically to predict pool sizes and the forward and reverse flux rates of AA from each pool to the next. The integration step length was made sufficiently small so as not to introduce numerical instability by bridging any discontinuities (8). The model

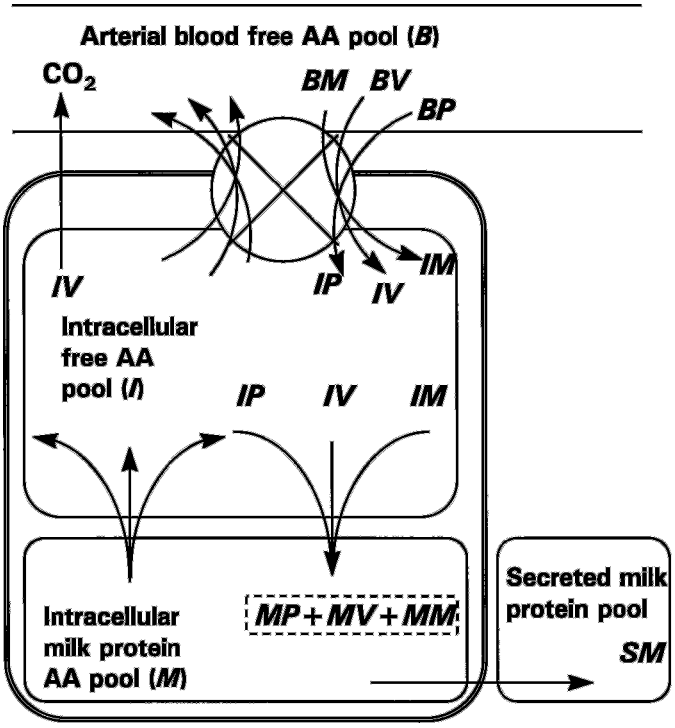

Figure 1. Schematic representation of the model. Square with double lines represents lactating mammary alveolar cell, squares with single lines represent total AA pools, and arrows represent fluxes of AA from one pool to another. Second letter of abbreviations indicate AA: M = Met, V = Val, and P = Phe.

utilizes simple kinetic equations to represent forward and reverse fluxes of AA from arterial blood through two intermediate pools into secreted milk protein. Energy-yielding substrates could also potentially limit the rate of MOP synthesis but were not considered in this model.

Biological Constraints and Assumptions

The description of a complex system by means of a model can be simplified substantially if known biological constraints are applied. For example, the rate of flux of individual AA relative to the rate of flux for other AA from the intracellular pool of free AA of the lactating cell into the intracellular pool of milk protein as protein translation occurs is stringently constrained because the AA profile of all MOP is fixed (17). The corollary is that intracellular milk protein, which subsequently undergoes hydrolysis within the cell, supplies a fixed AA profile. The assumption is made here that the L system transporter has equal affinity for all three AA; therefore, the influx and efflux of each AA are proportional to their individual proportion of total AA concentration on that particu-

lar side of the membrane. The total AA flux rate varies with the total concentration of all AA.

The uptake of total AA is calculated using Michaelis-Menten saturation kinetics (13) and the sum of arterial blood concentrations of the individual AA (Equation [A5]). Uptakes of each individual AA (Phe, Val, and Met, respectively) from arterial blood are then calculated as a proportion of the total flux (Equations [A6], [A7], and [A8]), based on the proportion of each individual AA in whole blood. The fluxes of each AA into blood from the intracellular pool of free AA are calculated similarly to total flux using the total intracellular concentration of free AA (Equation [A13]), which is then subdivided based on the proportion of each of the three individual AA (Equations [A14], [A15], and [A16]). Oxidation of Val is set at 30% of net Val uptake (Equation [A17]).

The output of each individual AA in MOP for the reference animal model is calculated in Table 1. Using the AA profile of each of these proteins, a midlactation cow producing 1.25 $L \cdot h^{-1}$ of milk with 3.34% protein would secrete 12.52 $mmol \cdot h^{-1}$ of Phe, 24.01 $mmol \cdot h^{-1}$ of Val, and 8.10 $mmol \cdot h^{-1}$ of Met.

Table 2 shows the method of calculation of maximum rate values for the utilization of each individual AA for MOP synthesis. The maximum rate of AA utilization is dependent on many factors, including the total number of lactating alveolar cells in a bovine udder, which has been estimated to be approximately 1.0×10^{13} (16). The number of mRNA for casein, which has previously been estimated to be approximately 1.0×10^5 $cell^{-1}$ (9), was increased for this model to 1.2×10^5 $cell^{-1}$ to account for whey protein mRNA. The product of number of cells and number of mRNA per cell gives a total of 1.2×10^{18} mRNA molecules being translated for MOP simultaneously in a complete bovine udder. The total number of mRNA is subdivided into MOP component protein mRNA by the relative proportions of each MOP found in milk (Table 1). The structure of the model assumes that the peptide elongation step is the limiting step in protein translation. Estimates of maximal initiation rate, which is suggested as being the limiting step in prokaryotic cells (7,19), were not found for ruminant tissues. Each molecule of mRNA can have many ribosomes associated with it that simultaneously perform translation; however, these ribosomes must be a minimum number of mRNA codons apart. The total number of active ribosomes is estimated by dividing the total number of codons per molecule of mRNA by 6, thus, assuming a minimum separation of 6 codons (22). The product of the number of mRNA units and the number of ribosomes per mRNA gives the total number of ribosomes that are operating simultaneously. Estimates of the rate of AA peptide bond formation by ribosomes range from 3 to 6 s^{-1} (21); therefore, a value of 4 s^{-1} was selected. Thus, the total rate of AA incorporation into MOP is the product of the number of active ribosomes and the rate of peptide bond formation. The incorporation rate of any single specific AA, however, would be equal to its individual proportion of the total AA profile, for example, 8 of 214 in the case of the simultaneous rate of Phe incorporation into α_{S1}-CN. Thus, the calculated maximum rate for the incorporation of Phe, Val, and Met into milk protein is 37.44, 74.43, and 28.20 $mmol \cdot h^{-1}$, respectively, based on the proportion of each individual MOP secreted. These values are used as maximum rate values for fluxes (Table A3).

The overall rate of MOP translation in the model is constrained by the intracellular concentration of the first-limiting AA. A potential rate of utilization of each individual AA, based on its intracellular free concentration at that point in time, is calculated at

TABLE 1. Secretion rate of Phe, Val, and Met in milk of a cow producing 1.25 $L \cdot h^{-1}$ of milk containing 3.34% protein.

Protein component	Composition[1]		Protein mass[2]	Protein	Phe	Val	Met	Phe	Val	Met
	(% of total)	($g \cdot h^{-1}$)		($mol \cdot h^{-1}$)	(no.)[3]			($mmol \cdot h^{-1}$)		
α_{s1}-CN	31.3	13.07	23,615	5.53×10^{-4}	8	11	5	4.43	6.08	2.76
α_{s2}-CN	8.4	3.51	25,230	1.39×10^{-4}	6	14	4	0.83	1.96	0.56
β-CN + γ-CN	32.0	13.36	23,983	5.57×10^{-4}	9	19	6	5.01	10.58	3.34
κ-CN	10.5	4.38	19,025	2.30×10^{-4}	4	11	2	0.92	2.53	0.46
β-LA	9.6	4.01	18,283	2.19×10^{-4}	4	10	4	0.88	2.19	0.88
α-LG	3.8	1.59	14,176	1.12×10^{-4}	4	6	1	0.45	0.67	0.11
Nonmammary	4.4	1.84								
Total	100.0	41.75						12.52	24.01	8.10

[1]Composition of bovine milk protein (6).

[2]Molecular mass of each individual protein component (23).

[3]Number of molecules per molecule of each respective protein (15).

TABLE 2. Calculation of maximum rate of utilization of Phe, Val, and Met for milk protein in the bovine mammary gland.

Protein component	Total mRNA		mRNA	AA[1]	Rib[2]	Phe[3]	Val	Met	Phe	Val	Met
		(% of total)			(no.)					(mmol·h^{-1})	
α_{s1}-CN		31.3	3.92×10^{17}	214	35.67	8	12	6	12.51	18.78	9.38
α_{s2}-CN		8.4	1.06×10^{17}	222	37.00	9	15	6	3.79	6.31	2.52
β-CN + γ-CN		32.0	4.01×10^{17}	224	37.33	9	22	7	14.41	35.23	11.21
κ-CN		10.5	1.32×10^{17}	184	30.67	7	13	4	3.68	6.83	2.10
β-LG		9.6	1.20×10^{17}	177	29.50	4	12	5	1.92	5.77	2.40
α-LA		3.8	4.76×10^{16}	138	23.00	6	8	3	1.14	1.52	0.57
Total	1.20×10^{18}	100.0	1.20×10^{18}						37.44	74.43	28.20

[1]Number of AA per molecule of translated protein (14).

[2]Number of individual ribosomes translating each protein.

[3]The AA composition of all proteins is the same as in Table 1 with additional requirements for the signal peptide (14).

each iteration of the model (Equations [A18], [A19], and [A20]). A value is then calculated for the ratio of potential utilization compared with the maximum utilization rate (Equations [A21], [A22], and [A23]). These values are sorted using a Fortran procedure, and the lowest value is selected as being first-limiting (Equations [A24], [A25], and [A26]). The MOP synthesis proceeds at this rate, based on the sum of the AA flux rates (Equations [A27], [A28], and [A29]) and on the proportion of each AA in MOP. Conversely, the hydrolysis of intracellular milk protein and the associated flux of AA from the intracellular pool of milk protein into the intracellular pool of free AA are calculated as 30% of the total MOP synthetic rate (Equations [A36], [A39], and [A42]) (18).

Simulations Performed

Several experiments were simulated using the model. Initially the model was allowed to achieve steady state, thus, yielding predictions of normal flux rates, pool sizes, and concentrations in the mammary gland of the reference cow. Analyses were then performed to determine which arterial AA concentration, if any, was limiting the total MOP synthesis rate. Subsequently, simulations were run to determine the effects of competitive inhibition of AA uptake on all flux rates and pool sizes by increasing the arterial concentration of the AA that were not first-limiting (Table 5). Next, the arterial concentration of the first-limiting AA was increased in small increments to determine the level at which it was no longer first-limiting. Arterial concentrations were then increased in a coordinated pattern to determine the maximum rate of MOP synthesis.

A series of simulations were run using the model to show the likely effects of various experimental infusion protocols that might be used in vivo. Thus, the model was used to test for the effects of arterial infusion of AA as infusions of single AA or of various AA mixtures on the rate of MOP synthesis. The quantity of each AA that would be infused directly into the arteries supplying the two sides of the bovine mammary gland in an in vivo experiment was calculated both in millimoles per hour and grams per hour and is based on a blood flow ratio (liters) of mammary blood flow to milk produced of 750:1 (12).

The purpose of this simulation study was to develop a precise and robust method of identifying limiting AA or kinetic parameters that were limiting the rate of MOP synthesis. The choice of experimental protocol used in vivo, such as the AA profile of the infusion mixture, is important because several possible protocols are logical alternatives and merit consideration. The model allows us to test various experimental protocols to obtain an understanding of which ones would be most likely to yield useful results from an in vivo experiment.

The experimental infusion protocols tested here (Table 3) are compared with the reference cow and include the following: P1, normal uninfused reference cow; P2, infusion of three AA, on a molar basis, in a mixture of the same profile found in MOP, which would be similar to casein infusion and would be at an infusion rate such that arterial concentration of the first-limiting AA (Met) increased incrementally by 10%; P3, as P2, but Met concentration increased incrementally by 20%; P4, as P2, but Met concentration increased incrementally by 30%; P5, infusion of an AA mixture to attempt to bring the arterial concentration of all three AA up to the level of the AA that was initially the highest, Val at 3.71×10^{-4} mmol·ml^{-1}; P6, if the original arterial concentration of Val was the highest, and, concomitantly, first-

TABLE 3. Arterial concentrations under various infusion protocols and infusion rates of individual AA.

Infusion protocol	Arterial			Infusion rate					
	Phe	Val	Met	Phe		Val		Met	
	(mmol·ml)			(mmol·h^{-1})	(g·h^{-1})	(mmol·h^{-1})	(g·h^{-1})	(mmol·h^{-1})	(g·h^{-1})
P1	5.68×10^{-5}	3.71×10^{-4}	1.96×10^{-5}	0	0	0	0	0	0
P2	5.94×10^{-5}	3.76×10^{-4}	2.16×10^{-5}	2.4	0.40	4.7	0.55	1.9	0.03
P3	6.20×10^{-5}	3.81×10^{-4}	2.35×10^{-5}	4.9	0.80	9.4	1.10	3.7	0.06
P4	6.46×10^{-5}	3.86×10^{-4}	2.55×10^{-5}	7.3	1.21	14.1	1.65	5.5	0.10
P5	3.71×10^{-4}	3.71×10^{-4}	3.71×10^{-4}	294.6	48.67	0	0	329.4	5.76
P6	4.08×10^{-4}	4.08×10^{-4}	4.08×10^{-4}	329.3	54.40	34.7	4.07	364.1	6.37
P7	8.52×10^{-5}	5.57×10^{-4}	2.94×10^{-5}	26.6	4.40	174.4	20.40	9.2	0.16
P8	1.14×10^{-4}	7.42×10^{-4}	3.92×10^{-5}	53.6	8.86	347.8	40.80	18.4	0.22
P9	1.86×10^{-4}	3.71×10^{-4}	1.40×10^{-4}	121.1	20.01	0	0	112.9	1.97

limiting, protocol P5 would have no effect; therefore, another protocol in which the arterial concentration of all three would be increased to the level of the initial highest increased by 10% (i.e., all would be increased to 4.08×10^{-4} mmol·ml^{-1}); P7, increase the respective arterial concentration of all three AA by 50%; P8, increase of all arterial concentrations 100%; P9, infusion to achieve arterial concentrations of the same ratio as that required for MOP translation Phe:Val:Met of 1.33:2.64:1.00, respectively. Table 3 presents the target arterial concentration for each of the AA and the required infusion rate, in millimoles per hour or grams per hour to produce this concentration. Total infused quantities range from 9 to 728 mmol·h^{-1}, which is reasonable for infusion experiments with lactating dairy cows.

RESULTS AND DISCUSSION

This study demonstrates how mathematical modeling can aid the design of animal experiments and, in this case, provide guidance as to the quantities and profiles of AA to be used in AA infusion experiments. Using this simple mechanistic model, several key principles emerged regarding the biological limitations or the current understanding of the biological limitations of this complex process. The L system transporter has been shown to be independent of Na^+ (4). The rate of flux of all AA that pass through this transporter is controlled by the concentration of AA on both sides of the membrane. In the model the starting values for concentrations of free AA in the intracellular pool were set at the same values as those of whole blood. Dynamic models, such as this one with a small integration interval, reach equilibrium rapidly so that initial starting values are not of great consequence. When the three AA were considered as a homogeneous pool, the intracellular AA concentration that was predicted by the model for the reference cow model was 12-fold higher than that of whole blood (Table 4). The AA transporters usually exhibit concentrative uptake against a concentration gradient; however, the magnitude of the increased intracellular concentration that is predicted by the model was higher than expected. When considered individually, the intracellular concentrations of Phe, Val, and Met are 10-, 12-, and 5-fold higher than those of whole blood, respectively; the values of individual AA concentrations could not be predicted by the concentration of total AA only, which highlights two important points. Treatment of the total pool of AA as a single homogenous pool might not be an appropriate simplification and could lead to a misunderstanding of the biological processes. Also, because of the constraints imposed on AA transport and protein translation, the intracellular pool of free AA must act as a buffer between these two highly constrained systems, thus, allowing these relatively inflexible systems to continue to operate at acceptable efficiency.

Model results predict a profile of free AA in the intracellular pool that is different from that of whole blood or of synthesized protein (Figure 2) being required to support MOP synthesis. If the L system transporter has equal binding affinity for each of the

TABLE 4. Arterial concentrations of AA and concentrations of free AA in the intracellular pool and the ratio of intracellular to arterial concentration as predicted by the model.

AA	Concentration		Ratio
	Arterial	Intracellular	
	(mmol·ml^{-1})		
Phe	5.68×10^{-5}	5.60×10^{-4}	10
Val	3.71×10^{-4}	4.50×10^{-3}	12
Met	1.96×10^{-5}	9.77×10^{-5}	5
Total	4.47×10^{-4}	5.16×10^{-3}	12

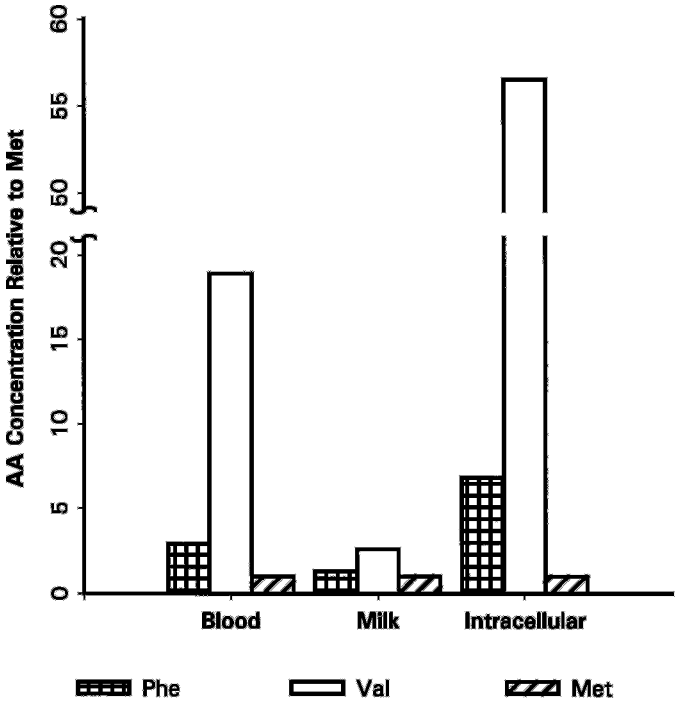

Figure 2. Profiles of AA in blood and milk and model predictions of the free AA in the intracellular pool relative to Met concentration.

AA that it transports, the system is tightly constrained. The different AA profile of the intracellular pool compared with that of blood is an indication that the profile of AA being exported to blood is different from the profile of AA being taken up from blood or being removed from the intracellular free pool for protein synthesis. When all AA are considered, along with AA oxidation and transamination, the ideal AA profile of arterial blood to support maximum protein synthesis rate may be quite different from that of MOP.

In such a constrained system, provision of an adequate supply of the first-limiting AA appears to be accomplished by a relatively high influx and efflux of all AA involved. The profile of AA in the influx or efflux changes little over time, and an increased total flux in both directions eventually leads to a difference in total net uptake that is adequate to support protein synthesis. At first, this system appears to be inefficient at removing AA from blood, but the L system transporter may conceivably be the best method that has evolved to handle AA with large side chains, such as the large branched or aromatic side-chain structures found in Phe, Tyr, Trp, Val, Ile, and Leu. This analysis also shows that, to understand the system completely, the experimenter must be familiar with flux quantities in absolute amounts, such as millimoles per day and as ratios of AA. In addition, an analysis that is totally dependent on one or the other alone can be quite misleading.

An increase in arterial concentrations of Phe and Val in 10% increments had minimal effects on the rate of MOP synthesis (Table 5). Competitive inhibition of uptake could limit MOP synthesis rate; however, a 30% increase in the arterial concentration of two AA that compete for transport capacity, but are not limiting, resulted in only a 3.6% decrease in MOP synthesis rate. One might expect that an increase in the arterial concentration of any AA transported by the L system transporter might increase the total transport rate and, therefore, might concomitantly increase the supply of the first-limiting AA; however, the model suggests that this scenario is incorrect.

Incremental increases in the arterial concentration of Met increased rate of MOP synthesis (Table 5), suggesting that, among the three AA discussed here, Met is most likely first-limiting. Increases in arterial concentrations of Met were associated with nonlinear (diminishing returns) increases in the rate of MOP synthesis up to the point at which Met was increased by 200%. An increase in arterial concentrations of Met above 200% slightly decreased the synthesis rate because of competitive inhibition and because Phe had become first-limiting. Maintenance of Met at the 200% increase level, as the arterial concentration of Phe increased, further increased the rate of MOP synthesis. This pattern continued until the arterial concentration of Phe had been increased by 30%; then, subsequent increases slightly reduced the overall synthesis rate. The model demonstrates clearly that small increases in the supply of the first-limiting AA

TABLE 5. Effect of increasing arterial concentrations of AA on synthesis rate of proteins of mammary origin.

Increase			Protein synthesis rate
Phe	Val	Met	
(%)			($g \cdot h^{-1}$)
0	0	0	41.75
10	10	0	41.29
20	20	0	40.84
30	30	0	40.39
0	0	50	54.51
0	0	100	62.83
0	0	150	68.24
0	0	200	68.58
0	0	250	68.52
10	0	200	70.70
20	0	200	71.84
30	0	200	71.82
30	0	250	73.84

TABLE 6. Flux rates between pools predicted by model under various infusion protocols.

Flux[1]	Infusion protocol								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
	(mmol·h^{-1})								
Total AA influx	334.1	339.6	344.9	350.3	616.1	649.5	448.7	541.4	460.3
Total AA efflux	276.7	278.1	279.9	281.7	500.9	532.7	376.6	459.4	348.0
Uptake efficiency, %	17.2	18.1	18.9	19.6	18.8	17.9	16.1	15.2	24.4
Uptake									
Phe F_{IPBP}	42.4	44.1	45.8	47.5	205.4	216.5	56.9	68.9	122.8
Val F_{IVBV}	277.1	279.4	281.7	284.0	205.4	216.5	372.2	448.7	245.0
Met F_{IMBM}	14.6	16.1	17.4	18.8	205.4	216.5	19.6	23.7	92.4
Output									
Phe F_{BPIP}	29.9	30.7	31.7	32.6	180.0	191.1	41.2	51.1	98.4
Val F_{BVIV}	241.5	241.4	241.5	241.6	133.4	144.3	327.6	398.0	175.6
Met F_{BMIM}	5.2	6.0	6.7	7.5	186.3	197.3	7.8	10.3	74.0

[1]F_{IPBP} = Phe flux from arterial blood free pool to intracellular free pool, F_{IVBV} = Val flux from arterial blood free pool to intracellular free pool, F_{IMBM} = Met flux from arterial blood free pool to intracellular free pool, F_{BPIP} = Phe flux from intracellular free pool to arterial blood free pool, F_{BVIV} = Val flux from intracellular free pool to arterial blood free pool, and F_{BMIM} = Met flux from intracellular free pool to arterial blood free pool.

could potentially allow dramatic increases in the overall rate of MOP synthesis and that eventually this limitation is overcome. Subsequently, the largest overall response comes from supplying the correct profile of all AA.

Several research groups have expended considerable effort to produce an in vivo animal preparation by which arterial concentrations of the AA supplying the lactating mammary gland can be manipulated via arterial infusion. The model suggests that all AA flux rates within the gland would respond to altered arterial AA concentrations (Table 6) using the experimental infusion protocols discussed previously (Table 3). Pool sizes and pool size ratios of individual AA pools, in response to each of the infusion protocols, are shown in Tables 7 and 8, respectively.

The first three infusion protocols, P2 to P4, in which the infusate contains AA in the same proportions as found in MOP, allow an increased rate of MOP synthesis. An effect of the infusion of this AA profile is that the size of the intracellular pool of Met increases proportionally much more than that of the other AA (Table 7). Over the entire range of increase in arterial concentration of these three AA, the size of the intracellular pool of Val increases 1%, of Phe increases 9%, but of Met increases 50%. The increase in the total rate of AA uptake rate by the cells is greater than the increase in AA efflux into blood (Table 6); therefore, most of the increased Met uptake is going directly to MOP.

Infusion protocols P5 and P6 produce the greatest increase in the rate of MOP synthesis, which is not surprising considering that those protocols also require the highest infusion rates (Table 3) and result in the least efficient net uptake (Table 6), which is measured as the proportion of the AA uptake that goes directly to MOP synthesis. The higher infusion rate in protocol P6 than in P5 resulted in a small

TABLE 7. Amino acid pool sizes predicted by model under various infusion protocols.

Pool	Infusion protocol								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
	(mmol)								
Total intracellular AA	56.74	57.09	57.55	58.03	127.22	140.64	84.60	112.06	76.10
Intracellular pool									
Phe	6.13	6.31	6.51	6.72	45.83	50.44	9.26	12.46	21.51
Val	49.54	49.56	49.66	49.77	33.96	38.09	73.58	97.09	38.39
Met	1.07	1.22	1.38	1.55	47.43	52.10	1.75	2.50	16.19
Rate of MOP synthesis, g·h^{-1}	41.75	44.70	47.29	49.81	84.55	84.86	52.44	59.58	81.56

TABLE 8. Ratio of AA (Phe:Val:Met) in blood and intracellular pools of free AA under various infusion protocols.[1]

Protocol	Blood	Intracellular pool
P1	2.9:18.9:1.0	5.7:46.3:1.0
P2	2.9:17.4:1.0	5.2:40.6:1.0
P3	2.6:16.2:1.0	4.7:36.0:1.0
P4	2.5:15.1:1.0	4.3:32.1:1.0
P5	1.0:1.0:1.0	1.0:0.7:1.0
P6	1.0:1.0:1.0	1.0:0.7:1.0
P7	1.3:19.0:1.0	5.3:42.4:1.0
P8	2.9:189.0:1.0	5.0:38.8:1.0
P9	1.2:2.7:1.0	1.3:2.7:1.0

[1]The ratio of AA in mammary origin proteins is constant at 1.3:2.6:1.0.

increase in MOP synthesis rate. This increase is reasonable considering that the arterial level of all AA was increased to that of the AA that was initially the highest. In this case, that AA was not the first-limiting AA, and, therefore, protocol P5 overcomes the limitation. Importantly, the amount of AA that was infused to accomplish these protocols was of sufficiently small quantity to allow the procedure to be performed in vivo. Although not tested directly, these results suggest that high levels of infusion, particularly with a solution of inappropriate AA profile, with a goal of saturating one part of the system, may cause nonphysiological results and, thus, lead to false conclusions. Infusion rates must be considered carefully to produce arterial concentrations that are within a realistic physiological range so that useful results are obtained.

Protocols P7 and P8, which increased the arterial concentration of all three AA by 50 and 100% of the original value, allowed substantial increases in the rate of MOP synthesis. However, these results must be viewed with caution because the primary cause of the increased synthesis rate is the increased supply of the first-limiting AA. The increased supplies of Phe and Val have minimal effect on the rate of MOP synthesis. Increasing arterial Met concentration by 50 or 100% gave MOP synthesis rates of 54.25 and 61.72 $g \cdot h^{-1}$, respectively, which were slightly higher than the increases when all AA were increased simultaneously. Again, this result demonstrates how experimental results could be misinterpreted when acquired from infusion experiments using an in vivo preparation.

The final protocol tested was P9; arterial concentrations were altered such that the resulting arterial concentrations of AA were the same profile as that of MOP. Protocol P9 was the most efficient in terms of AA capture in MOP as a proportion of the total taken up by the lactating cells. Net uptake efficiency was also the highest of all the infusion protocols, yet still appeared low at approximately 24.4%. Infusion rates for this protocol were intermediate between the lowest and highest used in other protocols discussed, yet the predicted rate of MOP synthesis is very close to the maximum achieved with any of the infusion mixtures. These results suggest that the ideal AA pattern in arterial blood is similar to that of the profile in MOP and that careful calculation is needed to ensure infusions that attain this profile. The resultant profile for arterial AA in this protocol is quite different from the one achieved by infusion of a solution that contains the same profile as MOP.

CONCLUSIONS

This theoretical model provides useful insight into the limitations of MOP synthesis in the lactating bovine mammary gland. The model provides guidance in the selection of quantities and profiles of AA for infusion that merit experimental testing and suggests that some AA are of little value. Experiments that infuse high levels of AA or inappropriate AA profiles could lead to very small responses in MOP synthesis rate and to incorrect conclusions from experimental results. Experimentally imposed perturbations to the system must be within physiological limits in order to obtain useful results.

Results from simulated infusion protocols that were associated with increased MOP synthesis rate suggested that the profile of AA in the intracellular pool of free AA also changed. The proportion of the intracellular pool of free AA that was occupied by the first-limiting AA (Met in this case) increased substantially as the rate of MOP synthesis increased. The concentrations of individual AA in this pool do not increase in the same proportion as the increase in blood. Therefore, the model suggests that consideration of all AA as a single homogeneous pool may be an inappropriate simplification that could lead to erroneous conclusions.

The optimal efficiency of AA uptake that was predicted by the model indicated that only 24.4% of AA extracted from blood was directed toward MOP synthesis. However, this proportion could be quite efficient in biological terms, and, therefore, experimenters need to keep an open mind about what constitutes a realistic range, particularly in an area such as this, for which only minimal experimental data exist. As suggested earlier, this result is perhaps the most efficient system available for transporting bulky AA.

The model predicts that the profile of arterial AA supporting a maximum rate of MOP synthesis, is the

same AA profile as MOP. This result may be somewhat limited by the use of a simplified model and may be quite different when all AA are considered, particularly AA that appear to be used primarily for oxidation. The values predicted by the model would obviously be of much greater utility if comparison could be made with actual in vivo experimental data.

The overall process of MOP synthesis is complex, and reducing the process to its component parts facilitates understanding. This model suggests that the overall process may not be highly regulated but, rather, may be very highly restricted by several biological constraints. This distinction is subtle, yet very important, when attempting to find methods of manipulating the MOP synthesis system in the lactating cow. The model also demonstrates how systems that are inflexible, such as that of AA uptake and MOP translation, can be buffered by the intracellular pool of free AA.

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APPENDIX 1

Pool Dynamics: Input and Output Equations

Total AA concentration in arterial blood (T_{BA}):

$$\text{Concentration:} \quad T_{BA} = C_{BP} + C_{BV} + C_{BM}. \quad [A1]$$

Proportion of each AA in blood (P_{BP}, P_{BV}, and P_{BM}):

$$\text{Phe:} \quad P_{BP} = C_{BP}/T_{BA}. \quad [A2]$$

$$\text{Val:} \quad P_{BV} = C_{BV}/T_{BA}. \quad [A3]$$

$$\text{Met:} \quad P_{BM} = C_{BM}/T_{BA}. \quad [A4]$$

Total uptake of AA by cells from arterial blood (F_{IABA}):

$$F_{IABA} = V_{IABA}/(1 + (K_{IABA}/T_{BA})). \quad [A5]$$

Uptake of individual AA by cells from arterial blood (F_{IPBP}, F_{IVBV}, and F_{IMBM}):

Phe: $F_{IPBP} = F_{IABA}P_{BP}$. [A6]
Val: $F_{IVBV} = F_{IABA}P_{BV}$. [A7]
Met: $F_{IMBM} = F_{IABA}P_{BM}$. [A8]

Total concentration of free AA in the intracellular pool (T_{IA}):

$$T_{IA} = C_{IP} + C_{IV} + C_{IM}. \quad [A9]$$

Proportion of each free AA in the intracellular pool (P_{IP}, P_{IV}, and P_{IM}):

Phe: $P_{IP} = C_{IP}/T_{IA}$. [A10]
Val: $P_{IV} = C_{IV}/T_{IA}$. [A11]
Met: $P_{IM} = C_{IM}/T_{IA}$. [A12]

Total output of AA to arterial blood from cells (F_{BAIA}):

$$F_{BAIA} = V_{BAIA}/(1 + (K_{BAIA}/T_{IA})). \quad [A13]$$

Output of individual AA to arterial blood from cells (F_{BPIP}, B_{BVIV}, F_{BMIM}, and F_{OVIV}):

Phe: $F_{BPIP} = F_{BAIA}P_{IP}$. [A14]
Val: $F_{BVIV} = F_{BAIA}P_{IV}$. [A15]
Met: $F_{BMIM} = F_{BAIA}P_{IM}$. [A16]
Oxidation of Val: $F_{OVIV} = (F_{IVBV} - F_{BVIV}) \times K_{OVIV}$. [A17]

Theoretical maximum rate of incorporation of individual AA into milk protein ($MAXF_{MPIP}$, $MAXF_{MVIV}$, and $MAXF_{MMIM}$):

Phe: $MAXF_{MPIP} = V_{MPIP}/(1 + (K_{MPIP}/C_{IP}))$. [A18]
Val: $MAXF_{MVIV} = V_{MVIV}/(1 + (K_{MVIV}/C_{IV}))$. [A19]
Met: $MAXF_{MMIM} = V_{MMIM}/(1 + (K_{MMIM}/C_{IM}))$. [A20]

Ratio of rate of incorporation to maximum rate of incorporation into milk protein for each individual AA:

Phe: $R_{MP} = MAXF_{MPIP}/V_{MPIP}$. [A21]
Val: $R_{MV} = MAXF_{MVIV}/V_{MVIV}$. [A22]
Phe: $R_{MM} = MAXF_{MMIM}/V_{MMIM}$. [A23]

Procedure to determine which concentration of AA in the intracellular pool is limiting the maximum rate of milk protein synthesis ($MAXM_{SYN}$):

Set first value: $MAXM_{SYN} = R_{MP}$. [A24]
Test against next: if $R_{MV} < MAXM_{SYN}$, then $MAXM_{SYN} = R_{MV}$. [A25]
Test against next: if $R_{MM} < MAXM_{SYN}$, then $MAXM_{SYN} = R_{MM}$. [A26]

Calculate the actual flux of each individual free AA from the intracellular pool to intracellular milk protein (F_{MPIP}, F_{MVIV}, and F_{MMIM}):

Phe: $F_{MPIP} = V_{MPIP} \times MAXM_{SYN}$. [A27]
Val: $F_{MVIV} = V_{MVIV} \times MAXM_{SYN}$. [A28]
Met: $F_{MMIM} = V_{MMIM} \times MAXM_{SYN}$. [A29]

Free Phe in the intracellular pool (IP):

Concentration: $C_{IP} = Q_{IP}/Wa$. [A30]
Inputs: F_{IPBP} Equation [A6]; F_{IPMP} Equation [A36].
Outputs: F_{BPIP} Equation [A14]; F_{MPIP} Equation [A27].
Differential equation: $dQ_{IP}/dt = F_{IPBP} + F_{IPMP} - F_{BPIP} - F_{MPIP}$. [A31]

Free Val in the intracellular pool (IV):

Concentration: $C_{IV} = Q_{IV}/Wa$. [A32]
Inputs: F_{IVBV} Equation [A7]; F_{IVMV} Equation [A39].
Outputs: F_{BVIV} Equation [A15]; F_{MVIV} Equation [A28]; F_{OVIV} Equation [A17].
Differential equation: $dQ_{IV}/dt = F_{IVBV} + F_{IVMV} - F_{BVIV} - F_{MVIV} - F_{OVIV}$. [A33]

Free Met in the intracellular pool (IM):

Concentration: $C_{IM} = Q_{IM}/Wa$. [A34]
Inputs: F_{IMBM} Equation [A8]; F_{IMMM} Equation [A42].

Outputs: F_{BMIM} Equation [A16];
F_{MMIM} Equation [A29].
Differential equation: $dQ_{IM}/dt = F_{IMBM} + F_{IMMM} - F_{BMIM} - F_{MMIM}$. [A35]

Phe in intracellular milk protein (MP):

Inputs: F_{MPIP} Equation [A27].
Outputs $F_{IPMP} = F_{MPIP} \times k_{PDEG}$; [A36]
$F_{SPMP} = F_{MPIP} - F_{IPMP}$. [A37]
Differential equation: $dQ_{MP}/dt = F_{MPIP} - F_{IPMP} - F_{SPMP}$. [A38]

Val in intracellular milk protein (MV):

Inputs: F_{MVIV} Equation [A28].
Outputs: $F_{IVMV} = F_{MVIV} \times k_{PDEG}$; [A39]
$F_{SVMV} = F_{MVIV} - F_{IVMV}$. [A40]
Differential equation: $dQ_{MV}/dt = F_{MVIV} - F_{IVMV} - F_{SVMV}$. [A41]

Met in intracellular milk protein (MM):

Inputs: F_{MMIM} Equation [A29].
Outputs: $F_{IMMM} = F_{MMIM} \times k_{PDEG}$; [A42]
$F_{SMMM} = F_{MMIM} - F_{IMMM}$. [A43]
Differential equation: $dQ_{MM}/dt = F_{MMIM} - F_{IMMM} - F_{SMMM}$. [A44]

Secreted milk protein (SP):

Inputs: F_{SPMP}.
Differential equation: $dQ_{SP}/dt = F_{SPMP}$. [A45]

TABLE A1. Symbols for entities represented in the model.

Symbol	Definition
BP	Free Phe in blood
BV	Free Val in blood
BM	Free Met in blood
BA	Total free AA in blood (BP + BV + BP)
IP	Free Phe in the intracellular pool
IV	Free Val in the intracellular pool
IM	Free Met in the intracellular pool
IA	Total free AA (IP + IV + IP)
MP	Phe bound to milk protein in the intracellular pool
MV	Val bound to milk protein in the intracellular pool
MM	Met bound to milk protein in the intracellular pool
OV	Oxidation of Val within cell
SP	Secreted Phe bound to milk protein
SV	Secreted Val bound to milk protein
SM	Secreted Met bound to milk protein
MAXF	Theoretical maximum flux from free AA in the intracellular pool to AA bound to milk protein for each individual AA
MAXM	Theoretical maximum rate of milk protein synthesis acknowledging first-limiting AA
PDEG	Degradation of intracellular milk protein

TABLE A2. Notation.

Notation[1]	Definition	Units
C_i	Concentration of i	millimoles per milliliter
F_{jk}	Flux rate of j ← k transaction	millimoles per hour
k_{jk}	Mass action constant for j ← k transaction	per hour
K_{jk}	Michaelis-Menten constant for j ← k transaction	millimoles per milliliter
Q_i	Quantity of i	millimoles
V_{jk}	Maximum rate for j ← k transaction	millimoles per hour
Wa	Mammary cell water volume	milliliters

[1]The subscripts i, j, and k take values from Table A1.

TABLE A3. Kinetic parameters.

Parameter[1]	Value
C_{BP}	5.68×10^{-5}
C_{BV}	3.71×10^{-4}
C_{BM}	1.96×10^{-5}
V_{IABA}	1.4235×10^{3}
K_{IABA}	1.459×10^{-3}
V_{BAIA}	1.4235×10^{3}
K_{BAIA}	2.148×10^{-2}
V_{MPIP}	37.44
K_{MPIP}	1.07×10^{-4}
V_{MVIV}	74.43
K_{MVIV}	1.07×10^{-4}
V_{MMIM}	28.20
K_{MMIM}	1.07×10^{-4}
K_{OVIV}	0.30
K_{PDEG}	0.30
Wa	1.095×10^{4}

[1]See Table A2 for units.