Milk Synthetic Response of the Bovine Mammary Gland to an Increase in the Local Concentration of Amino Acids and Acetate

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ABSTRACT

Rates of secretion of components into milk are a function of precursor concentrations and parameters that describe expression of the milk synthetic enzymes and their sensitivity to precursor concentrations. To establish the enzymatic sensitivities of milk fat yield and mammary acetate utilization to circulating acetate concentration, lactating cows were infused for 10 h with 0 or 40 g of acetate/h in an external iliac artery supplying one udder half. In addition, to investigate the possibility that energy supply influences the milk protein response to an elevated amino acid (AA) concentration, 2 different AA profiles were infused with and without acetate. Six cows, fed a total mixed ration of 21% crude protein ad libitum, were infused with AA at 0 g/h, 30 g/h in the profile of rumen microbes, or 30 g/h in the profile of milk proteins, in a 3 × 2 factorial arrangement with the 2 acetate treatments of 0 and 40 g/h, all in a 6 × 6 Latin square. Amino acid infusion caused a 60% increase, on average, in plasma concentration of AA entering the infused udder half. From the microbial AA profile, 49% of infused AA were taken up by the udder half, 42% of which occurred during the first pass. From the milk AA profile, 44% of infused AA were taken up by the udder half, 50% of which occurred during the first pass. There was an 8% increase in yield of milk protein with AA infusion, representing 7% capture, but no effect of the infused profile. Acetate infusion caused a decrease in the yields of milk protein and lactose when AA were infused, but not when AA were absent. Milk fat yields were not affected, although acetate concentrations in plasma entering the infused udder half increased by 123% and mammary uptakes increased by 128%. Mammary uptakes of long-chain fatty acids and β -hydroxybutyrate were not affected by acetate infusion, whereas glucose uptakes tended to increase. It was suggested that excess acetate may have been sequestered in adi-

pose tissue in the udder. Yields of both protein and fat in milk showed a low sensitivity to the concentration of their precursors in circulation. It was concluded that the K_m in Michaelis-Menten-type equations describing milk synthesis should be assigned a low value, and that the V_{max} is regulated to bring about changes in milk yield and composition.

Key words: amino acid, acetate, arterial infusion, milk composition

INTRODUCTION

Milk is synthesized in the mammary glands from precursors taken out of blood. The process of milk synthesis is biochemical and its kinetics can be described by Michaelis-Menten-type equations that consider enzyme activities and amounts in parameters such as K_m and V_{max} to quantify the relationship between concentrations of milk precursors and the rate of chemical reactions that use them, including synthesis and secretion of milk components (Baldwin, 1995). Nutritional effects on rates of milk component secretion such as from increased protein supply forage:concentrate ratio, and so on arise through an influence on the parameters or the substrate concentrations, or both, of the milk synthetic equations. To isolate the effect of the milk precursor concentration in nutritional responses, we have used a short-term infusion protocol in lactating cows, in which the arterial concentration of the precursor of interest is elevated in one udder half for 10 h by close arterial infusion (Cant et al., 2001, 2002). If the nutritional response involves a change in the level of expression of certain enzymes, then 10 h is too short a time for the parameters of milk synthesis to change significantly. Mammary utilization of milk precursors and composition of milk secreted during the 10-h interval can then be taken to represent primarily an effect of precursor concentration alone.

When arterial AA concentrations were increased 2- to 3-fold for 10 h in cows fed a diet of 16% CP and 1.7 Mcal of NE_L /kg, milk protein yield only increased 13% (Cant et al., 2001). Similarly, milk lactose yield was

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not affected by a 1.8-fold elevation of plasma glucose concentration for 10 h (Cant et al., 2002). These and similar observations of small responses in milk component yields to large changes in precursor concentrations *in vivo* led to the conclusion that, in general, milk component secretion is insensitive to precursor concentrations (Cant et al., 2003), which means that the K_m for utilization of the precursor must be substantially lower than the normal physiological concentration of the precursor. This conclusion lies in contrast to estimates of K_m that have been obtained from fitting the concentration dependencies of milk fat and lactose synthesis *in vitro*. In culture, mammary cells exhibit K_m values for biosynthetic use of acetate and glucose very similar to the physiological concentrations of the 2 precursors (Forsberg et al., 1984, 1985). The whole-cow simulation model of Baldwin (1995) assigns a K_m of 1.8 mM to the synthesis of milk fat from plasma acetate and a K_m of 3.0 mM to the synthesis of lactose from plasma glucose. These K_m values are equal to the reference concentrations of acetate and glucose, respectively, and would yield predictions of relatively large increases in fat and lactose yields in response to doubling of precursor concentrations.

The short-term effects of acetate concentration on milk fat synthesis *in vivo* have not been extensively studied. Over several days, 40 g of acetate/h infused into the rumen of cows stimulated yields of protein, fat, lactose, and total milk by approximately 12% (Rook and Balch, 1961). Compared with an isoenergetic infusion of intraruminal propionate, intraruminal acetate at 6 g/h for 10 d into goats increased the yield of milk fat by 36% with no effect on yield of protein or milk volume (Lough et al., 1983). However, in the short-term, Linzell (1967) reported that intravenous acetate infusion for 3 h into a fasted goat to restore the prefasting concentration of acetate did not affect yield of milk or fat. To establish the sensitivities of milk fat yield and mammary acetate utilization to circulating acetate concentration, we infused lactating cows with sodium acetate or saline in an external iliac artery feeding one udder half.

Synthesis of milk proteins in the mammary glands is an energy-demanding process, and the possibility exists that a low sensitivity to AA supply may be the result of an inadequate supply of metabolic energy. A second objective of our work was to reexamine short-term responses to elevated AA concentrations when acetate was also given to provide a supplemental source of energy. Third, given that the profile of AA available in circulation determines its quality for protein synthesis, we compared responses to 2 different profiles of infused AA, one based on the rumen microbial protein profile and one based on the milk protein profile.

Table 1. Ingredient and chemical composition of the TMR fed to all cows

Item	%
Ingredient composition (% as-fed)	
Alfalfa haylage	60.0
Corn	15.0
Wheat shorts	15.7
Barley	14.3
Soybean meal	5.2
Corn gluten meal	2.2
Soyplus	2.2
Molasses	1.5
Blood meal	0.7
Limestone	1.0
Tallow	0.4
Mineral mix	1.7
Chemical composition	
DM (% as-fed)	74.7
CP (% of DM)	21.2
Buffer solubility (% of CP)	37.9
NDF insolubility (% of CP)	9.7
ADF insolubility (% of CP)	5.8
NDF (% of DM)	29.1
ADF (% of DM)	20.5
Cellulose (% of DM)	17.2
Lignin (% of DM)	3.28
Fat (% of DM)	2.7
Ash (% of DM)	8.2
Calculated NE_L (Mcal/kg of DM)	1.67

MATERIALS AND METHODS

Animals, Treatments, and Sampling

All animal procedures were approved by the Animal Care Committee of the University of Guelph. Six non-pregnant, lactating Holstein cows (198 ± 23 DIM) were fitted with polyethylene catheters that terminated in left and right external iliac arteries, and polyurethane catheters in left and right subcutaneous abdominal veins draining the udder (Cant et al., 2001). The position of the arterial catheters was confirmed by unilateral appearance of Evans Blue dye in the venous catheter following rapid arterial injection.

After catheterization surgery, cows were fed a TMR (Table 1) of alfalfa haylage and a concentrate mixed in a 60:40 proportion, respectively, by weight. Hand-mixed TMR was fed *ad libitum* at each milking with refusals removed and weighed at 0730 h to calculate DMI. A grab sample of the TMR was acquired a number of times during the experiment for analysis. Each consignment of concentrate and each load of haylage were sampled for analysis.

Cows were milked in the same order at 0800 and 1800 h. A dual-bucket system was used with the milking claw divided into 2 sections so that milk from right and left udder halves was collected separately. Milk was weighed and a sample from each test bucket was collected for analysis.

Table 2. Composition of AA infusates (g/3 L)

Amino acid	Microbial AA profile	Milk AA profile
Glu	42.00	64.35
Asp	39.12	23.76
Ile	18.28	17.49
Leu	25.98	29.70
Met	8.35	7.92
Phe	32.07	30.36
Thr	18.60	15.84
Trp	5.12	4.95
Val	19.87	20.13
Ala	24.06	10.23
Cys	3.20	2.97
Gly	18.60	5.94
Pro	11.86	32.67
Ser	14.75	18.15
Arg·HCl	16.36	10.89
His·HCl	6.43	8.58
Lys·HCl	25.33	26.07

The experiment was designed as a 3×2 factorial within a 6×6 Latin square, with 3 AA treatments: 0 g/h, 30 g/h in the profile of rumen microbes (Clark et al., 1992), and 30 g/h in the profile of milk proteins (Hambraeus, 1982), and 2 acetate treatments: 0 and 40 g/h. Due to time constraints imposed by catheter patency, only 2 cows were subjected to the experiment at any one time. Thus, the 6×6 Latin square was accomplished in 3 consecutive, incomplete Latin squares. Infusion and sampling protocols were as previously used (Cant et al., 2001). Each period consisted of 10 h of continuous arterial infusion daily, followed by 14 h of rest.

Solutions were prepared the night before infusion in 3-L batches. They were: 9 g/L of saline, 110 g/L of AA in the microbial profile (Table 2), 110 g/L of AA in the milk profile (Table 2), 200 g/L of sodium acetate, 200 g/L of sodium acetate + 110 g/L of microbial AA, and 200 g/L of sodium acetate + 110 g/L of milk AA. The Gln, Asn, and Tyr of the 2 AA profiles were replaced with molar equivalents of Glu, Asp, and Phe, respectively. Individual AA, purchased from USBiochemical (Cleveland, OH) and Sigma Chemical Co. (Oakville, Ontario, Canada), were added to 2.5 L of Milli Q Ultrapure water [Millipore (Canada) Ltd., Mississauga, Ontario, Canada] containing 30 g of NaOH in the order listed in Table 2; each was dissolved before adding the next. All infusates were adjusted to pH 7.4 by adding either HCl or NaOH, subjected to a density determination by weighing 1-mL aliquots 5 times, filtered through a 0.22- μ m cellulose acetate filter, and stored in 4-L autoclaved, polypropylene bottles. The bottles were sealed and refrigerated overnight and were warmed before infusion.

Infusion into one arterial catheter began immediately after the 0800 h milking and continued until the 1800

h milking was complete. At 1500 h, 50 mL of 33% para-aminohippuric acid (PAH), as a marker to measure plasma flow rate, was injected through a 0.22- μ m syringe filter into the infusate bottle and mixed. At 1540, 1640, and 1740 h (approximately 8, 9, and 10 h into the infusion), blood samples were collected simultaneously from the arterial catheter contralateral to infusion and the ipsilateral venous catheter. A syringe pump was used to withdraw blood continuously over 10 min into sodium EDTA Vacutainers (Becton Dickinson, Franklin Lakes, NJ) on ice. Infusate weight was recorded at the precise beginning and end of the sampling interval. Blood was centrifuged for collection of plasma, which was frozen at -20°C until analyzed. Each infusate was sampled for chemical analysis.

Analytical Procedures

Milk samples were analyzed by infrared spectroscopy for protein, fat, and lactose content (AOAC, 1996). The 3 plasma samples from each blood vessel were analyzed individually for PAH and pooled for analysis of glucose, triacylglycerol, NEFA, and acetate concentrations by spectrophotometry, and for AA by reversed-phase HPLC, as previously described (Cant et al., 2001). Infusates were also analyzed for PAH and acetate accordingly.

Iliac plasma flow (IPF) at each of the 3 sampling timepoints was calculated from PAH analyses as

$$\text{IPF(L/h)} =$$

$$\frac{\text{volume infused over 10 min (L)} \times \text{infusate [PAH](mg/L)} \times 60 \text{ min/h}}{10 \text{ min} \times \{\text{venous [PAH](mg/L)} - \text{contralateral arterial [PAH](mg/L)}\}}$$

Mammary extraction of a plasma metabolite, calculated as the quotient of its arteriovenous difference and arterial concentration, is a consequence of both transmembrane transport and plasma flow. As an alternative to calculating extraction, then, the product of the first-order rate constant for capillary uptake (k) and the total volume of perfused capillaries ($\text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$) was calculated as $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}} = -\ln(1 - \text{extraction}) \times \text{IPF}$, according to Cant and McBride (1995).

Statistical Analyses

Variance in each observation (Y_{ijklm}) was analyzed by using the following model, with the GLM procedure of SAS Institute (2000):

$$Y_{ijklm} = \mu \dots + \text{sq}_i + \text{cow}(\text{sq})_{j(i)} + \text{per}_k + \text{aa}_l + \text{ac}_m \\ + \text{aa}_l \times \text{ac}_m + \varepsilon_{ijklm}$$

Table 3. Average yield and composition of milk from the infused udder half of 6 cows after 10 h of infusion into one external iliac artery of 0 g/h AA, 30 g/h in the profile of rumen microbes (microb AA), or 30 g/h in the profile of milk proteins (milk AA), with 0 (without) or 40 g/h (with) acetate

Item	Without acetate			With acetate			SEM	<i>P</i> -value ¹			
	No AA	Microb AA	Milk AA	No AA	Microb AA	Milk AA		AA	Ac	AA × Ac	Profile
Infusion interval, h	9.9	9.9	10.1	10.2	10.0	10.1	0.11	0.655	0.073	0.133	0.432
Yield, g/h											
Milk	540	575	564	523	520	528	18	0.812	0.003	0.795	0.665
Protein	18.5	20.6	20.7	18.9	19.6	19.7	0.6	0.063	0.026	0.695	0.421
Fat	23.1	26.0	23.2	26.6	23.5	25.0	1.3	0.700	0.708	0.209	0.802
Lactose	23.3	25.1	24.9	23.7	23.8	24.1	0.7	0.404	0.031	0.710	0.487
Percentage											
Protein	3.44	3.64	3.69	3.64	3.78	3.75	0.05	0.003	0.006	0.837	0.858
Fat	4.41	4.70	4.23	5.06	4.62	4.75	0.19	0.507	0.081	0.213	0.456
Protein:fat	0.80	0.78	0.88	0.72	0.83	0.81	0.04	0.133	0.471	0.269	0.366
Lactose	4.20	4.32	4.34	4.45	4.50	4.45	0.05	0.300	0.001	0.662	0.961

¹Probabilities from ANOVA of no effect of infusion of AA, infusion of acetate (Ac), and their interaction (AA × Ac), and probability from orthogonal contrast of no effect of profile of AA infusate (profile).

where $\mu \dots$ = mean, sq_i = *i*th square effect ($i = 1$ to 3), $cow_{j(i)}$ = *j*th cow effect within each *i*th square ($j = 1$ to 6), per_k = *k*th period effect ($k = 1$ to 6), aa_l = *l*th AA infusion effect ($l = 1$ to 3), ac_m = *m*th acetate infusion effect ($m = 1$ to 2), and ε_{ijklm} = error.

Effect of the AA profile was evaluated with an orthogonal contrast between *l*th AA treatments 2 and 3. Significances of effects were declared at $P \leq 0.05$ and tendencies were declared at $0.05 > P \leq 0.15$.

To test for carryover of treatment effects from one period to the next, the following ANOVA model was used:

$$Y_{ijkl} = \mu \dots + sq_i + cow(sq)_{j(i)} + per_k + prevtrt_l + \varepsilon_{ijkl}$$

where $\mu \dots$ = true grand mean, sq_i = *i*th square effect ($i = 1$ to 3), $cow_{j(i)}$ = *j*th cow effect within each *i*th square ($j = 1$ to 6), per_k = *k*th period effect ($k = 1$ to 6), $prevtrt_l$ = *l*th previous treatment effect ($l = 1$ to 6), and ε_{ijklm} = error.

No carryover effects were detected for any of the observed variables.

RESULTS

Feed intake averaged 29.8 kg/d (SEM = 1.4) over the experiment and was not affected by treatment. The infusion rate was maintained at 4.88 mL/min (SEM = 0.18) or 293 mL/h (SEM = 11).

Effects of AA Infusion

The only effect of AA infusion on milk components from the infused udder half was a tendency for an 8% increase in protein yield (Table 3). Because milk yield remained unchanged, protein percentage was greater

following AA infusion. The profile of the AA infusate made no difference in milk components.

Amino acid infusion caused arterial plasma glucose and insulin concentrations to increase (Table 4). There was a tendency for the $k \cdot Vol_{cap} \cdot N_{cap}$ product for glucose to increase, which, in combination with the elevated arterial concentration, brought about an increased mammary uptake of glucose by an average of 79 mmol/h. The microbial profile resulted in a greater plasma glucose concentration than the milk profile but had no effect on mammary glucose balance. Effects of AA infusion on acetate balance were similar to those for glucose balance (Table 4). The systemic arterial concentration was elevated, tended to be more elevated by the microbial profile than by the milk profile, the $k \cdot Vol_{cap} \cdot N_{cap}$ product increased, and so, mammary uptake of acetate increased by an average of 101 mmol/h. There was an interaction between main effects on BHBA uptake, in which AA infusion without acetate increased BHBA uptake and, when acetate was infused, decreased BHBA uptakes. Net utilization of long-chain fatty acids (LCFA) by the mammary glands was not affected, although arterial concentrations of triacylglycerol were decreased slightly by infusion of the microbial profile (Table 4).

Infusion of AA caused an increased concentration in the general circulation of Phe, Val, Ile, Leu, Pro, and Ala, and tended to increase Arg and Ser (Table 5). The milk AA profile generated a greater Pro concentration, and tended to have greater Val, Ile, and Arg concentrations compared with the microbial AA profile. The concentrations of infused AA in the artery entering the infused glands were elevated an average of 60%. On the milk AA profile, close arterial plasma was greater in Leu, Pro, and Ser, lower in Asp, and tended to be

Table 4. Average plasma flow and balance of plasma metabolites across the infused udder half of 6 cows during the last 3 h of a 10-h infusion into one external iliac artery of 0 g/h AA, 30 g/h in the profile of rumen microbes (microb AA), or 30 g/h in the profile of milk proteins (milk AA), with 0 (without) or 40 g/h (with) acetate

Item	Without acetate			With acetate			SEM	<i>P</i> -value ¹			
	No AA	Microb AA	Milk AA	No AA	Microb AA	Milk AA		AA	Ac	AA × Ac	Profile
Iliac plasma flow, L/h	418	493	488	505	514	520	31	0.352	0.200	0.595	0.986
Insulin artery, pM	224	288	313	306	347	316	11	<0.001	<0.001	0.002	0.619
GH artery, µg/L	2.57	1.90	2.98	2.11	2.68	2.17	0.26	0.785	0.532	0.050	0.745
Glucose											
Artery, mM	3.65	3.87	3.81	3.90	3.95	3.93	0.04	0.016	0.002	0.621	0.034
Vein, mM	2.93	3.06	3.08	3.18	3.10	3.07	0.05	0.734	0.052	0.097	0.538
Extraction, %	19.8	21.1	19.4	18.7	21.7	22.0	1.3	0.520	0.804	0.277	0.540
k·Vol _{cap} ·N _{cap} , L/h	93	127	107	108	129	134	11	0.126	0.120	0.791	0.742
Uptake, mmol/h	300	412	355	369	436	449	32	0.086	0.061	0.780	0.588
Acetate											
Infusion, mmol/h	0	0	0	658	637	638	18	0.958	<0.001	0.663	0.812
Opposite artery, mM	1.33	1.49	1.44	1.83	2.02	1.79	0.08	0.042	<0.001	0.383	0.073
Artery, mM	1.30	1.50	1.44	3.17	3.29	3.04	0.08	0.252	<0.001	0.171	0.132
Vein, mM	0.32	0.35	0.34	1.02	0.88	0.83	0.05	0.199	<0.001	0.679	0.377
Extraction, %	77.7	78.2	76.6	67.7	73.0	73.1	1.6	0.142	0.013	0.838	0.549
k·Vol _{cap} ·N _{cap} , L/h	647	755	715	580	686	701	38	0.023	0.493	0.695	0.807
Uptake, mmol/h	421	579	541	1,078	1,220	1,142	52	0.043	<0.001	0.314	0.614
BHBA											
Artery, mM	0.89	0.90	0.85	0.93	0.94	0.79	0.12	0.534	0.932	0.969	0.423
Vein, mM	0.61	0.40	0.59	0.40	0.75	0.42	0.07	0.578	0.982	0.380	0.962
Extraction, %	31.7	60.0	50.3	59.6	36.5	49.2	5.9	0.306	0.544	0.090	0.687
k·Vol _{cap} ·N _{cap} , L/h	161	474	359	477	217	363	73	0.502	0.627	0.285	0.700
Uptake, mmol/h	112	331	306	265	219	190	17	0.084	0.177	0.022	0.048
Triacylglycerol											
Artery, µM	207	193	202	201	191	198	4	0.045	0.468	0.650	0.025
Vein, µM	157	147	151	154	146	155	5	0.314	0.829	0.746	0.213
Extraction, %	24.5	24.1	25.7	24.0	24.4	22.2	1.8	0.898	0.948	0.291	0.725
k·Vol _{cap} ·N _{cap} , L/h	120	159	146	136	148	130	16	0.461	0.466	0.273	0.774
Uptake, mmol/h	20.1	24.9	25.4	23.2	23.3	22.1	2.2	0.388	0.817	0.196	0.450
NEFA											
Artery, µM	135	146	136	139	156	137	10	0.278	0.412	0.992	0.385
Vein, µM	147	142	148	156	160	149	6	0.056	0.468	0.013	0.435
Long-chain fatty acids											
Artery, mM	755	726	743	743	729	731	17	0.419	0.940	0.746	0.193
Vein, mM	618	584	600	619	597	614	17	0.368	0.501	0.891	0.185
Extraction, %	18.6	19.7	19.6	17.3	18.6	16.4	1.7	0.811	0.383	0.832	0.710
k·Vol _{cap} ·N _{cap} , L/h	93	131	108	93	110	94	14	0.378	0.821	0.853	0.622
Uptake, mmol/h	58.6	80.0	70.1	60.0	69.5	60.4	8.1	0.324	0.681	0.821	0.680

¹Probabilities from ANOVA of no effect of infusion of amino acids (AA), infusion of acetate (Ac), and their interaction (AA × Ac), and probability from orthogonal contrast of no effect of profile of AA infusate (profile).

greater in Val and lower in Gly. There was an increased extraction of arterial Asp by the mammary glands during AA infusion, a decreased extraction of Ile, and a tendency toward a decreased extraction of Phe, Val, Leu, Ser, and Ala. Extraction of Ser was lower on the milk AA profile compared with the microbial AA profile and extractions of Phe and Val tended to be lower. Uptakes of Phe, Glu, and Asp were significantly increased more than 2-fold by AA infusion; uptakes of Leu, Arg, and Ser tended to be increased. On the milk AA profile, Asp uptake by the mammary glands was lower than on the microbial AA profile, and Phe and Ala uptakes tended to be lower.

Effects of Acetate Infusion

There was a significant effect of acetate on yield of milk protein and lactose, which was only manifest on the treatments with AA infusion (Table 3). Without AA, mean protein and lactose yields were approximately equal with or without acetate. With AA, protein and lactose yields were 5% lower with acetate than without. Thus, the effect of the acetate infusion was to prevent or attenuate the increase in lactose and protein yield brought about by the AA infusion. Milk fat yields were not affected by acetate infusion. Percentages of protein and lactose in milk were elevated and fat percentage tended to increase.

Table 5. Average balance of selected plasma amino acids across the infused udder half of six cows during the last 3 h of a 10-h infusion into one external iliac artery of 0 g/h AA, 30 g/h in the profile of rumen microbes (microb AA), or 30 g/h in the profile of milk proteins (milk AA), with 0 (without) or 40 g/h (with) acetate

Item	Without acetate			With acetate			SEM	P-value ¹			
	No AA	Microb AA	Milk AA	No AA	Microb AA	Milk AA		AA	Ac	AA × Ac	Profile
Lys											
Opposite artery, μM	54	68	79	63	59	60	7	0.339	0.451	0.922	0.359
Artery, μM	55	99	115	63	91	93	6	0.005	0.114	0.952	0.409
Extraction, %	38.4	30.8	36.2	9.2	34.5	21.1	10.2	0.655	0.183	0.345	0.821
Uptake, mmol/h	8.9	15	20	3.2	17	9.2	4.6	0.259	0.306	0.659	0.916
Phe											
Opposite artery, μM	33	64	71	37	65	61	4	<0.001	0.892	0.292	0.639
Artery, μM	33	100	106	37	99	93	5	<0.001	0.875	0.296	0.708
Extraction, %	39.8	38.4	23.8	34.4	32.9	25.0	5.1	0.063	0.428	0.876	0.100
Uptake, mmol/h	5.6	19	12	6.7	15	12	2.7	0.019	0.691	0.817	0.148
Tyr											
Opposite artery, μM	34	48	55	43	44	59	7	0.291	0.763	0.789	0.387
Artery, μM	34	48	55	43	44	59	7				
Extraction, %	30.4	27.0	29.2	29.5	29.6	27.6	3.7	0.308	0.237	0.375	0.404
Uptake, mmol/h	4.6	4.6	7.6	6.5	6.5	6.5	1.1	0.724	0.919	0.713	0.431
Thr											
Opposite artery, μM	142	177	207	130	156	144	17	0.169	0.066	0.587	0.750
Artery, μM	146	204	233	130	183	167	18	0.019	0.087	0.722	0.835
Extraction, %	18.3	7.4	19.0	2.9	15.5	4.5	8.4	0.790	0.459	0.469	0.504
Uptake, mmol/h	11	17	24	4.3	20	8.7	7.5	0.343	0.741	0.870	0.694
Val											
Opposite artery, μM	255	371	406	299	366	399	21	<0.001	0.500	0.356	0.085
Artery, μM	257	411	439	299	396	429	20	<0.001	0.630	0.491	0.062
Extraction, %	23.7	22.6	5.7	23.6	16.5	12.0	6.8	0.139	0.606	0.953	0.480
Uptake, mmol/h	25	46	21	38	35	25	14	0.705	0.533	0.990	0.567
Ile											
Opposite artery, μM	94	171	170	125	161	175	9	<0.001	0.067	0.602	0.146
Artery, μM	94	164	196	125	185	199	9	<0.001	0.109	0.699	0.117
Extraction, %	35.9	26.5	22.6	23.6	20.2	18.3	3.0	0.034	0.012	0.543	0.289
Uptake, mmol/h	14	22	22	15	19	18	3.0	0.251	0.289	0.889	0.802
Leu											
Opposite artery, μM	174	254	298	201	265	272	16	<0.001	0.601	0.451	0.176
Artery, μM	173	275	342	201	300	312	15	<0.001	0.581	0.414	0.041
Extraction, %	35.1	26.2	23.8	22.4	21.1	18.5	2.9	0.118	0.031	0.592	0.293
Uptake, mmol/h	25	36	39	23	35	32	4.9	0.052	0.740	0.946	0.950
Arg											
Opposite artery, μM	62	69	82	60	58	64	5	0.097	0.062	0.580	0.107
Artery, μM	65	87	94	60	75	75	5	0.004	0.083	0.644	0.457
Extraction, %	42.9	35.3	36.6	36.7	37.9	38.3	4.3	0.925	0.583	0.905	0.957
Uptake, mmol/h	11	15	17	12	16	16	2.2	0.064	0.472	0.966	0.679
Citrulline											
Opposite artery, μM	102	108	107	95	83	106	9	0.824	0.116	0.351	0.546
Artery, μM	102	108	107	95	83	106	9				
Extraction, %	16.1	11.0	8.0	14.8	17.4	14.8	5.3	0.462	0.023	0.449	0.858
Uptake, mmol/h	6.0	11	5.1	6.8	7.0	8.0	2.7	0.297	0.018	0.127	0.629
Pro											
Opposite artery, μM	71	94	168	61	76	103	13	0.001	0.013	0.383	0.002
Artery, μM	75	115	222	61	94	154	15	<0.001	0.025	0.539	<0.001
Extraction, %	14.3	9.1	12.4	10.7	30.9	9.3	9.7	0.540	0.906	0.867	0.275
Uptake, mmol/h	4.2	10	11	3.3	10	11	5.3	0.335	0.980	0.880	0.672
Ser											
Opposite artery, μM	63	72	86	51	57	61	6	0.079	0.004	0.864	0.151
Artery, μM	69	97	119	51	82	92	6	<0.001	0.014	0.666	0.018
Extraction, %	29.4	30.6	22.1	25.7	32.2	19.3	4.0	0.086	0.697	0.870	0.033
Uptake, mmol/h	8.5	15	13	7.1	14	9.8	2.3	0.138	0.515	0.951	0.431
Gly											
Opposite artery, μM	174	185	176	138	149	134	15	0.760	0.008	0.879	0.498
Artery, μM	192	237	191	138	192	148	15	0.052	0.011	0.797	0.082
Extraction, %	0.0	5.5	-2.0	-3.0	-48.4	-4.3	28.4	0.612	0.315	0.684	0.540
Uptake, mmol/h	0.9	8.5	-1.5	-1.1	-43	1.2	26	0.597	0.366	0.637	0.473

Continued

Table 5 Continued. Average balance of selected plasma amino acids across the infused udder half of six cows during the last 3 h of a 10-h infusion into one external iliac artery of 0 g/h AA, 30 g/h in the profile of rumen microbes (microb AA), or 30 g/h in the profile of milk proteins (milk AA), with 0 (without) or 40 g/h (with) acetate

Item	Without acetate			With acetate			SEM	<i>P</i> -value ¹			
	No AA	Microb AA	Milk AA	No AA	Microb AA	Milk AA		AA	Ac	AA × Ac	Profile
Ala											
Opposite artery, μM	227	257	262	199	243	243	13	0.025	0.102	0.899	0.580
Artery, μM	237	319	284	199	290	264	14	0.001	0.207	0.931	0.536
Extraction, %	19.9	16.0	6.2	12.0	12.4	3.6	4.0	0.085	0.295	0.814	0.085
Uptake, mmol/h	21	27	8.1	12	17	6.9	6.1	0.257	0.387	0.829	0.110
Glu											
Opposite artery, μM	87	111	129	88	89	124	20	0.294	0.563	0.648	0.482
Artery, μM	76	127	212	88	140	198	22	0.005	0.665	0.914	0.151
Extraction, %	37.1	41.0	51.8	40.9	29.9	60.5	14.4	0.824	0.976	0.818	0.816
Uptake, mmol/h	16	27	51	15	34	64	9	0.016	0.900	0.580	0.278
Asp											
Opposite artery, μM	14	15	14	8	14	9	2	0.154	0.186	0.833	0.596
Artery, μM	15	68	48	8	65	41	3	<0.001	0.384	0.397	<0.001
Extraction, %	12.3	79.8	70.7	5.8	78.4	67.1	11.6	<0.001	0.905	0.848	0.400
Uptake, mmol/h	2.5	26	16	0.36	26	14	1.4	<0.001	0.765	0.414	<0.001

¹Probabilities from ANOVA of no effect of infusion of amino acids (AA), infusion of acetate (Ac), and their interaction (AA × Ac), and probability from orthogonal contrast of no effect of profile of AA infusate (profile).

Plasma acetate concentrations were elevated 33% in the general arterial circulation and 123% in the close arterial circulation of the infused udder half (Table 4). Extraction of arterial acetate by the infused udder half was decreased by the infusion but the $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$ product was not actually affected, so the 123% increase in mammary acetate uptake was due entirely to the increase in arterial acetate concentration. Arterial glucose and insulin concentrations were elevated by acetate infusion (Table 4), the $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$ tended to be increased and so mammary uptake of glucose increased 18%. There was no change in BHBA, triacylglycerol, or LCFA supply or utilization by the mammary glands (Table 4).

During acetate infusion, there were decreased concentrations in the arterial circulation of Pro, Ser, and Gly and tendencies for decreases in Thr, Arg, citrulline (Cln), and Ala (Table 5). Isoleucine concentration tended to increase with acetate. Close arterial concentrations were similarly affected. Proline, Ser, and Gly decreased, Lys, Thr, Arg, and Cln tended to decrease, and Ile tended to increase. Extraction of the close arterial Ile and Leu decreased and extraction of Cln increased. The only AA uptake affected by acetate infusion was a 23% increase in Cln uptake.

DISCUSSION

Sensitivity to AA Concentrations

Amino acid concentrations were elevated by an average of 60% in the artery supplying one udder half, which is considerably less than the 280% elevation produced

by close arterial infusion of 30 g of AA/h previously reported (Cant et al., 2001). In the previous experiment, cows were fed a diet of 16% CP compared with 21% this time, and so basal concentrations of most AA in arterial plasma were initially greater. Regardless, the small but detectable increase in milk protein yield, despite a 60% increase in circulating AA concentrations, confirms the low sensitivity of product formation to precursor concentration. In other words, formulation of the Michaelis-Menten equation(s) describing milk protein production should consider a K_m for AA lower than the normal physiological concentration of AA. The strongest treatment effect was a 2.1 g/h increase in milk protein yield in response to 30 g/h of supplemental AA, accounting for approximately 7% of the infusate. In longer infusion experiments, in which a greater proportion of the added AA is recovered in milk protein (Hanigan et al., 1998), the parameters of the Michaelis-Menten equation, or what we have called the setpoint (Cant et al., 1999), must have changed.

Aside from the capture in milk protein, 2 other marginal efficiency measures can be calculated from the mammary arteriovenous difference data. One measure is the extraction of arterially infused AA on the first pass through the mammary glands, which was calculated as $(\text{ex}_i A_i - \text{ex}_o A_o) / (A_i - A_o)$, where ex_i and ex_o are the arteriovenous extraction percentages on the infused and opposite sides, respectively, and A_i and A_o are the respective arterial concentrations. Because ex_o was not measured, it was estimated from the slope of the change in extraction percentages over the change in arterial AA concentrations between control and AA infusions

Table 6. Marginal efficiencies of utilization of infused AA by the infused udder half of six cows during the last 3 h of a 10-h infusion into one external iliac artery of 30 g/h AA in the profile of rumen microbes (microbial AA) or milk proteins (milk AA)

AA	Microbial AA			Milk AA		
	Infusion rate, mmol/h	First-pass extraction, % of infused	Increase in net uptake, % of infused	Infusion rate, mmol/h	First-pass extraction % of infused	Increase in net uptake, % of infused
Lys	14.7	48	68	16.3	36	52
Phe	16.5	34	66	16.8	11	35
Thr	13.3	14	82	12.2	15	71
Val	14.4	8	62	15.7	-29	-54
Ile	11.8	7	51	12.2	2	45
Leu	16.8	10	68	20.7	6	55
Arg	8.0	26	50	5.7	30	87
Pro	8.8	37	71	26.0	9	28
Ser	11.9	40	56	15.8	10	23
Gly	21.0	-89	-81	7.2	-60	-1
Ala	22.9	-9	24	10.5	-45	-86
Glu	24.3	29	62	40.0	74	105
Asp	25.0	98	98	16.3	90	83
Weighted average		21	49		22	44

as $ex_c + (A_o - A_c)(ex_i - ex_c)/(A_i - A_c)$, where ex_c and A_c are the extraction percentages and arterial concentrations during control infusions. First-pass extractions of infused AA ranged from -89 to 98% (Table 6) and for many AA tended to be lower than extraction of the basal arterial concentration. This decline in percentage extraction of AA as arterial concentrations increase has been noted previously (Hanigan et al., 1992; Tesseraud et al., 1992) and is likely a consequence of the bidirectional transport of AA across the mammary glands. Amino acids taken into mammary cells have a greater chance of returning to the circulation when the proportion incorporated into protein is decreasing (Bequette et al., 2001), as was occurring during AA infusion in this study. Net first-pass extraction of the entire infusate, on a molar basis, averaged 21% for the microbial AA profile and 22% for the milk AA profile.

Amino acids infused into the external iliac artery that bypass the udder half become available for the entire body to use, and a second marginal efficiency measure depends on the division of infused AA among all tissues of the body. This efficiency is the proportion of infused AA taken up on a net basis by the infused udder half, and it ranged from -86 to 105% for the individual AA (Table 6). From the microbial AA profile infusate, 49% of the AA were taken up by the udder half, 42% of which occurred during the first pass. From the milk AA profile infusate, 44% of infused AA were taken up by the udder half, 50% of which occurred during the first pass. These proportions are overestimates to the extent that external iliac blood is diverted through the femoral artery to the hind leg, which we have estimated to be, at most, 20%. Thus, it appears that approximately 60% of the infused AA was used elsewhere in the body and, given

only 7% capture in milk protein, the majority of AA taken up by the mammary glands were not secreted as milk protein. We conclude that, although milk protein production exhibits a low K_m for AA, mammary AA utilization in other pathways (i.e., catabolism) exhibits a higher K_m .

In addition to changes in mammary AA utilization, AA infusion caused insulin concentrations to increase in arterial plasma and stimulated the mammary uptake of the energy metabolites glucose and acetate. Insulin stimulates milk protein secretion when administered continuously over several days (Grinari et al., 1997), so the possibility exists that the small increase in milk protein yield observed over 10 h was due to this hormonal effect, and not to the AA per se. However, short-term infusions of insulin have not resulted in any detectable changes in mammary AA utilization (Laarveld et al., 1981; Tesseraud et al., 1992). Nor does insulin appear to influence glucose or acetate utilization by mammary tissue in the short term (Laarveld et al., 1985). Insulin concentration was most likely increased in response to elevated glucose concentrations, as well as the increase in AA concentration, because both are potent secretagogues of the hormone. The glucose and acetate concentrations likely increased as products of disposal of the 60% of the infused AA not used by mammary tissue. The approximately 20% increase in $k \cdot Vol_{cap} \cdot N_{cap}$ products for glucose and acetate clearance by the mammary glands (Table 4) remains unexplained.

Few differences between the microbial and milk AA profiles were observed. The milk AA profile infused was substantially greater in Glu and Pro content and lower in Asp, Ala, Gly, and Arg (Tables 2 and 6). The difference between infusates in Pro content resulted in a

greater Pro concentration in the general circulation of cows infused with the milk AA profile but none of the other differences were manifest (Table 5). In the close arterial plasma, the Pro, Asp, and Gly differences between infusates were retained. These AA are not generally considered to be important to milk synthesis, although the excessive uptake of Arg by the mammary glands for synthesis of the Pro and Glu of milk protein has prompted some investigation of Pro responses. The main effect of postruminal Pro supplementation (80 g/d) of cows appears to be a disproportionately large increase in milk fat yield (Bruckental et al., 1991), which has been shown to be a symptom of an AA imbalance rather than a corrected deficiency (Weekes et al., 2006). Arteriovenous differences of Arg were reduced during Pro infusion (Bruckental et al., 1991) and a similar sparing of the conversion of Arg to Pro in the mammary glands and elsewhere in the body may have occurred in this experiment such that Arg concentrations rose in both the general and the close arterial plasma with the milk AA profile compared with the microbial AA profile (Table 5), despite being infused at a slower rate. The branched-chain AA also tended to be greater in both infused and noninfused arteries with the milk AA profile compared with the microbial AA profile. This effect might be indicative of differences in hepatic AA utilization that consume little Val, Ile, and Leu. None of the differences in AA concentrations between infusate profiles had an effect on yield or composition of milk.

Sensitivity to Acetate Concentration

First-pass extraction by the infused udder half of the 645 mmol/h acetate infused into one external iliac artery was calculated from the difference between extraction percentages on the treatments with and without acetate, as for the AA above, to be 65%. The proportion of infused acetate that was taken up by the infused udder half, on a net basis, was 98%. Again, both these values are overestimates because of a fraction of blood in the external iliac artery that does not flow to the mammary glands. Nevertheless, it is apparent that a very high proportion of the acetate infusion was used by the mammary glands. In addition, because there was no change with acetate infusion in the $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$ product representing clearance of plasma acetate by the mammary glands, the uptake of acetate can be said to be a direct consequence of the arterial acetate concentration. More specifically, the hypothesis holds that acetate uptake follows first-order, mass-action kinetics. This hypothesis was also upheld by close arterial glucose infusions during which IPF decreased by 16% but the first-order rate constant for mammary acetate up-

take did not change, so acetate uptake decreased (Cant et al., 2002).

When glucose infusion caused acetate uptake by the mammary glands to decrease, there was no concomitant decrease in milk fat yield (Cant et al., 2002). Likewise, the increase in acetate uptake induced by acetate infusion in this experiment caused no significant change in milk fat yield. Linzell (1967) also reported no change in milk or fat yield of a fasted goat infused for 3 h with acetate. In a preliminary experiment, acetate infusion at graded levels up to 36 g/h into the external iliac artery of 4 cows for 10 h did not affect milk fat yields or percentages (Maas et al., 1995). The evidence is strong that fat yield is not sensitive to acetate concentrations or uptake by the mammary glands. In this experiment we add evidence to indicate that net BHBA and LCFA disappearance across the mammary glands is also not affected by a high acetate concentration. Thus, the additional acetate does not appear to induce a change in the use of other fat precursors.

The implication of no effect of acetate concentration, for mechanistic modeling of milk synthesis using Michaelis-Menten-type equations, is that the K_m value should be set much lower than the physiological concentration of plasma acetate. This conclusion conflicts with the K_m of 1.2 mM estimated from conversions of [^{14}C]acetate to fatty acids by mammary tissue incubated *in vitro* (Forsberg et al., 1984). However, Baldwin (1995) reported that the simulation model MOLLY, with a K_m for acetate conversion to milk triacylglycerol of 1.8 mM, predicted an approximately 40% increase in milk fat yield when blood acetate concentration was increased over the range we investigated, from 1.3 to 3.3 mM. A K_m one order of magnitude smaller (i.e., 0.18 mM) would yield an 8% increase in predicted milk fat yield. The Baldwin (1995) model was designed to describe longer-term responses to dietary perturbations, not within a day, as in this experiment. Longer-term infusions of acetate have indeed resulted in increased milk fat yields (Rook and Balch, 1961; Lough et al., 1983). Our suggestion is that the V_{max} for milk fat synthesis from acetate must increase to bring about this response. The V_{max} represents enzymatic capacity, which would be expected to increase over the course of a few days, and not within the 10-h timeframe of our experiment. The combination of an increase in V_{max} for each increase in acetate concentration over the long term could generate an aggregate response curve that appears very similar to a saturating Michaelis-Menten-type curve (Cant et al., 1999). Thus, the K_m that is used for long-term simulations of mammary responses to circulating concentrations of milk precursors may express a different level of aggregation than the short-term K_m and the two should not be confused.

If acetate uptake increased 123% but milk fat yield and BHBA and LCFA uptakes were not significantly affected, where did the additional acetate go? There is a time delay between uptake of acetate from the blood plasma and output in milk of the fat synthesized from it. Thus, some of the infused acetate may have been incorporated into milk fat that was secreted after our 10-h infusion period. Fat secreted during the 14-h milking interval after acetate infusions ceased was 13.6 g higher than after the other infusions ceased, which, if 40% by weight of the fat is synthesized from acetate, could account for only 151 mmol of acetate uptake. Even in conjunction with 105 mmol of acetate estimated to have been used in additional milk fat synthesis during the infusion period, most of the 6,392 mmol of uptake remains unaccounted for. Aside from fat, the other main product of acetate utilization in mammary tissue is CO₂ (Scott et al., 1976; Forsberg et al., 1984) in oxidative phosphorylation of adenosine nucleosides. But that would generate a lot of ATP whose use must be explained.

We have hypothesized in the past that the mammary glands exert control over the local rate of blood flow to match ATP production with its utilization (Cant and McBride, 1995). According to this hypothesis, it was predicted that IPF would decrease by 32% during close arterial infusion of 90 g/h glucose, and it was actually observed to decrease by 16% (Cant et al., 2002). Simulations with the mammary model described by Cant et al. (2003), from concentrations of metabolites observed in this experiment, yield a predicted decrease in IPF to 322 L/h to maintain ATP balance on the acetate infusion treatment, whereas it actually remained at 505 L/h (Table 4). The data may be sufficient to disprove the hypothesis, although without a change in plasma flow there remains a problem of energy balance across the udder half to be resolved.

In addition to faster uptake of acetate that was not incorporated into a milk component, the high acetate concentration also stimulated uptake of glucose by 18% but lactose yields in milk were not affected. Presumably, then, the additional glucose was oxidized to yield ATP. Forsberg et al. (1985) observed a dramatic increase in rates of glucose oxidation by slices of mammary tissue incubated *in vitro* with increasing concentrations of acetate. Between the concentrations of 1.3 and 3.3 mM observed in our experiment, production of ¹⁴CO₂ from [1-¹⁴C]glucose increased approximately 25% and, from [2-¹⁴C]glucose, approximately 10% (Forsberg et al., 1985). The particular stimulation of glucose utilization in the pentose phosphate cycle implicates faster NADPH removal in fat synthesis from acetate yet, although we observed a similar increase in mammary glucose oxidation at the elevated acetate concentration,

no change in milk fat synthesis was apparent. The energy balance problem and the stimulation of glucose oxidation can both be resolved if a good portion of the acetate that disappeared between artery and vein was taken up into adipose cells of the udder half and converted to triacylglycerol. An udder half may contain 1 to 3 kg of adipose tissue that responds in size to the nutritional status of the cow (Gibb et al., 1992) and, if it behaves similarly to subcutaneous depots, is more lipogenic in late lactation than early (McNamara and Hillers, 1986). In addition, when acetate is the primary substrate of lipogenesis in ruminant adipose tissue and glucose is used only for synthesis of the glycerol backbone of stored lipids (Vernon, 1980), the glucose we infused previously (Cant et al., 2002), in contrast to acetate here, would not have been taken up into mammary adipose and an IPF response was induced.

CONCLUSIONS

Yield of fat in milk was not affected by a 123% increase in arterial acetate concentration, despite a 128% increase in acetate uptake into mammary tissue. Yield of protein in milk was elevated 8% by a 60% increase in arterial AA concentrations and a 64% increase in mammary AA uptake. Acetate supply did not affect the response to AA infusion. The profile of circulating AA was not found to have a significant mass-action effect on yield of milk protein. These almost negligible responses highlight the low sensitivity of milk component output to a change in the concentration of its precursor arriving at the mammary glands. It would suggest that one should look to effects on mammary enzyme expression to explain longer-term, diet-mediated effects on milk component output. The implication for mechanistic modeling of milk synthesis using Michaelis-Menten-type equations is that the precursor concentration has little effect, via a low *K_m* value, and the *V_{max}* is regulated to bring about changes in component yields. The absence of an effect of acetate infusion on IPF led to the suggestion that mammary adipose growth may have accounted for a large proportion of the infused acetate utilization.

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