Influence of Stage of Lactation on Glucose and Glutamine Metabolism in Isolated Enterocytes From Dairy Cattle

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Pathways of glutamine and glucose metabolism in early-, mid-, and late-lactation dairy cows were evaluated by in vitro incubations of enterocytes for 2 hours with [U-^{14}-C]glutamine and [U-^{14}C]glucose. Enterocytes from early-lactation cows produced greater amounts of CO_2 from glutamine in concentrations that ranged from 2 to 8 mmol/L than enterocytes from either mid- or late-lactation cows. Enterocytes from early-lactation cows also produced greater amounts of CO_2 from 4 and 6 mmol/L glucose than enterocytes from either mid- or late-lactation cows. Glutamine was metabolized via glutaminolysis mainly to ammonia, alanine, aspartate, glutamate, and CO_2, and more of these products were produced in enterocytes from early-lactation cows than from pooled mid- and late-lactation (PML) cows. Glucose was metabolized mainly to lactate, as compared with pyruvate and CO_2. Lactate and CO_2 production were both greater in enterocytes from early-lactation cows than from PML cows. Glutamine as the sole substrate accounted for all the energy requirements of enterocytes from early-lactation cows but contributed only 31% in the presence of glucose. Similarly, glucose accounted for all the energy requirements of enterocytes from early-lactation cows and contributed 69% in the presence of glutamine. In enterocytes from all cows, the rate of adenosine triphosphate (ATP) production was greater in the presence of both glucose and glutamine compared with that in the presence of either substrate alone. The rate of production of ATP from glucose as the sole substrate was two to three times greater than that obtained from glutamine as the sole substrate in enterocytes from all groups of cows. Thus, we suggest that although glutamine is an important energy source for enterocytes, it is not quantitatively as important as glucose as an energy source for enterocytes from lactating cows.

IN A VARIETY of monogastric species, most of the energy required by cells that divide rapidly such as enterocytes is provided by oxidation of glucose and glutamine, with glutamine being the major energy source.[1-4] Thus, although glucose and glutamine may be metabolized by enterocytes at equimolar rates, glutamine oxidation to CO_2 accounts for 25% to 40% of total CO_2 production, whereas glucose oxidation accounts for only 6% to 10%.[1-3] In addition, glutamine oxidation by the small intestine in monogastric animals is not affected by increased availability of glucose in the intestinal lumen.[5] In lactating rats, there is a marked increase in phosphate-dependent glutaminase activity in the small intestine and an increased metabolism of glutamine, with glutamine sparing glucose.[6]

The importance of glucose and glutamine as energy sources for enterocytes from ruminant species has not been delineated. In vivo studies with ruminants indicate a consistent uptake of glucose[7,8] and glutamine[9] by the gut wall, which suggests that both may play important roles as metabolic nutrients for gut tissues. Thus, there is reason to believe that glutamine and/or glucose may play a role in functional and metabolic activities of the small intestine in dairy cattle. Indeed, glucose, volatile fatty acids, and glutamine have all been reported as metabolic nutrients for the ruminant small intestine.[10-12] In addition, glucose oxidation to CO_2 has been reported to be seven times greater than glutamine oxidation to CO_2, which in turn was decreased in the presence of butyrate and glucose in rumen papillae from Holstein steers.[13] However, the extent of metabolism of glucose and glutamine by enterocytes from the lactating cow has not been investigated in detail, although stage of lactation has been reported to influence transport and metabolism of glucose and amino acids by dairy cows.[11]

Metabolism in the wall of the small intestine is important because it influences the net presentation of nutrients to the liver for synthetic activities. Accordingly, the objective of the present studies was to examine the relative importance of glucose and glutamine as energy sources for enterocytes from cows at different stages of lactation.

MATERIALS AND METHODS

Chemicals

D-[U-^{14}C]glucose (268 mCi/mmol) and L-[U-^{14}C]glutamine (200 mCi/mmol) were obtained from Moravek Biochemical (Brea, CA) and American Radiolabeled Chemicals (St Louis, MO), respectively. Radiochemical purity of L-[U-^{14}C]glutamine was determined to be greater than 99% by thin-layer chromatography. All other chemicals, enzymes, and reagents were purchased from Sigma Chemical (St Louis, MO).

Animals

Nine Holstein cows, three each in early lactation (< 100 days in lactation), mid-lactation (100 to 200 days in lactation), and late lactation (> 200 days in lactation), were used as tissue donors for isolation of enterocytes. Briefly, cows were killed at a local abattoir, and within 10 minutes a 10-cm segment of the small intestine was excised beginning 5 cm from the pylorus. Details of the removal and transport of duodenal tissue to the laboratory have been described elsewhere.[11]

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Preparation of Enterocytes

Enterocytes were isolated as described by Ameh and Thompson,[14] with slight modifications, within 25 minutes of removal of the duodenal tissue.[11] Briefly, 100 mL Krebs-Ringer HEPES buffer (pH 7.4) without $CaCl_2$ and saturated with O_2:CO_2 in a ratio of 19:1 (solution A) was flushed through the small intestine to remove mucus. The distal end of the duodenal segment was ligated with 3-0 Dexon (Ethicon, Somerville, NJ), and the segment was filled with pregassed solution A that contained 2.5 mg/mL bovine serum albumin (solution B). The proximal end was then ligated, and the duodenal segment was incubated in a beaker that contained 250 mL solution B for 15 minutes in a water bath at 37°C with constant gassing and agitation (60 to 80 oscillations/min). After the contents were discarded, the duodenal segment was again filled with pregassed solution B that contained 1.5 mg/mL hyaluronidase (solution C). The filled segment was placed in a container of crushed ice covered with polyethylene and gently patted with fingertips for 2 minutes to release enterocytes into the intestinal lumen. Enterocytes were washed three times in polypropylene tubes with solution B after centrifugation at 1,000 × *g* for 3 minutes each time. Washed enterocytes were resuspended in 15 mL solution B for subsequent metabolic studies. The majority of cells in the suspension were epithelial cells, as demonstrated by strong staining with antibodies to cytokeratin. In addition, the percentage of epithelial cells in the cell suspension as compared with other cells such as intraepithelial lymphocytes, as judged by differential counting of May-Grünwald-Giemsa–stained cytospin smears, was 89% ± 6% (n = 6 ± SD). Therefore, further purification steps were not performed. Aliquots of isolated enterocytes were used for determination of total cell count and cell viability. Viability as determined by 0.2% trypan blue exclusion was ≥ 86%.

$^{14}CO_2$ Production From Glutamine and Glucose Metabolism

Triplicate 1-mL enterocyte suspensions (3×10^6 viable cells) from each animal were incubated for 2 hours in 10-mL polypropylene tubes that contained 2.4 mL incubation medium (solution B) supplemented with insulin (0.5 IU/mL). The incubation medium also contained mean concentrations of various amino acids predetermined in duodenal digesta from cows in different stages of lactation.[11] Carbon dioxide production was measured at 2, 4, 6, and 8 mmol/L L-[U-^{14}C]glutamine (80 μCi/mmol) and at 2, 4, 6, and 8 mmol/L D-[U-^{14}C]glucose (80 μCi/mmol). In addition, L-[U-^{14}C]glutamine (4 mmol/L, 80 μCi/mmol) in the presence or absence of 6 mmol/L glucose or D-[U-^{14}C]glucose (6 mmol/L, 80 μCi/mmol) in the presence or absence of 4 mmol/L glutamine were included in incubation media to assess the interaction between glucose and glutamine metabolism by enterocytes. Incubations were performed at 37°C in a shaking water bath (60 to 80 oscillations/min) and were initiated by addition of the cell suspension and terminated by addition of 0.1 mL 70% perchloric acid. Samples into which 0.1 mL 70% perchloric acid was injected before addition of substrates were used as blanks (zero-time incubation). Incubation tubes (sealed except for inlets for gassing and outlets for collecting evolved CO_2) were continuously gassed with 95% O_2:5% CO_2. $^{14}CO_2$ was absorbed by 9 mL trapping agent (liquid scintillation cocktail that contained 5 g 2,5-diphenyloxazole, 0.2 g 1,4-bis-[2]-(5-phenyloxazolyl)benzene, 200 mL ethanolamine, 300 mL methyl cellusolve (2-methoxyethanol), and 500 mL toluene, for a total of 1,000 mL cocktail). At the end of the incubation period, shaking was continued for another 30 minutes to trap all the $^{14}CO_2$ evolved in the trapping agent after addition of 70% perchloric acid. $^{14}CO_2$ radioactivity was determined using a Beckman LS 5801 liquid scintillation counter (Beckman Instruments, Fullerton, CA) after the vials were washed twice with 3 mL trapping agent (for a total of 15 mL cocktail). Rates of net uptake of glucose and glutamine were measured in parallel incubations without ^{14}C-labeled substrates by measurement of removal of glucose and glutamine from the incubation media. Blank values were subtracted from sample values.

Measurement of Pyruvate, Alanine, Lactate, Ammonia, [^{14}C]glutamate, and [^{14}C]aspartate

Lactate and pyruvate concentrations in acid extracts of enterocytes plus medium were determined by enzymatic methods.[15,16] To minimize hydrolysis of glutamine to glutamate, acidified [^{14}C]glutamine-containing media used for analyses of ammonia, [^{14}C]glutamate, and [^{14}C]aspartate were immediately neutralized with 2 mol/L K_2CO_3 after CO_2 collection was completed. Samples were stored at −20°C and analyzed within 48 hours. This procedure resulted in ≤1% of the added glutamine being converted to glutamate, as measured by [^{14}C]glutamate production from [^{14}C]glutamine in the blanks. Ammonia content was measured by a spectrophotometric enzymatic method.[17] [^{14}C]glutamate and [^{14}C]aspartate were separated by Dowex AG 1 × 6 (200 to 400 mesh, acetate form) chromatography,[18] and the radioactivities were measured in a liquid scintillation counter. Amounts of [^{14}C]glutamate and [^{14}C]aspartate produced from [^{14}C]glutamine were calculated as radioactivity (disintegrations per minute) of either [^{14}C]glutamate or [^{14}C]aspartate × 1/specific activity of [^{14}C]glutamine. Levels of glutamate, aspartate, and alanine in incubation media were measured by high-performance liquid chromatography (HPLC)[19] using β-amino-*n*-butyric acid and ethanolamine as internal standards and amino-guanidopropionic acid as internal standard for glutamine. Apparent net production rates of various metabolites were calculated from differences in initial and final concentrations. Anion-exchange–column and HPLC methods gave similar estimates ($P > .05$) of production of glutamate and aspartate from glutamine. However, only results from HPLC analyses were used for statistical analyses.

Calculation of Adenosine Triphosphate Production

The rate of adenosine triphosphate (ATP) production from glutamine was calculated as outlined by Newsholme and Newsholme.[20] Briefly, 24 mol ATP are produced for every 5 mol CO_2 produced via complete oxidation of glutamine, which is equal to total CO_2 production from glutamine minus moles of aspartate produced because stoichiometrically 1 mol CO_2 is produced for 1 mol aspartate formed.[21] Oxidative deamination of glutamate to 2-oxoglutarate by glutamate dehydrogenase results in reduction of NAD(P) and thus a total theoretical maximum production of 12 ATP/mol aspartate produced from glutamine. However, in this study it was assumed that 9 mol ATP[22] were produced in the conversion of glutamine to aspartate, since our data indicated that glutamate was metabolized via aminotransferase reactions rather than through glutamate dehydrogenase. From glucose, 1 mol ATP was assumed to be produced per 1 mol lactate produced and 38 mol ATP for 6 mol CO_2 produced.[20]

Statistical Analyses

Data were analyzed by paired *t* test, unpaired *t* test, or two-way ANOVA for glucose and glutamine, with Duncan's test for multiple mean comparisons using the general linear models procedure.[23] To facilitate statistical analyses and since the data between mid- and late-lactation cows (Table 1) did not significantly differ ($P > .05$), data from these two groups were pooled before subsequent statistical analyses (Tables 3, 4, and 5).

Table 1. Rate of $^{14}CO_2$ Production From [U-^{14}C]glutamine by Enterocytes From Cows at Different Stages of Lactation

[U-^{14}C]glutamine (mmol/L)	$^{14}CO_2$ Produced (nmol/2 h per 10^6 viable cells)			
	Early-Lactation	Mid-Lactation	Late-Lactation	SE‡
2	10.8*	7.0†	6.5†	1.2
4	14.1*	8.0†	8.0†	1.6
6	13.8*	9.8†	8.8†	0.9
8	14.5*	9.8†	9.3†	0.8

*†Means with different superscripts in the same row differ ($P < .05$).
‡Pooled SE for each stage-of-lactation mean (n = 3).

RESULTS

Glutamine and Glucose Oxidation

Enterocytes from early-lactation cows produced greater amounts of CO_2 from glutamine when incubated in the presence of 2 to 8 mmol/L glutamine than enterocytes from either mid- or late-lactation cows (Table 1). No significant difference in CO_2 production was observed at any glutamine concentration between mid- and late-lactation cows. The rate of CO_2 production from glutamine numerically increased with increasing glutamine concentration. Enterocytes from early-lactation cows incubated in the presence of 4 to 8 mmol/L glucose produced greater amounts of CO_2 than enterocytes from either mid- or late-lactation cows (Table 2). The amount of CO_2 produced from glucose was also significantly greater in enterocytes from mid-lactation cows than from late-lactation cows at glucose concentrations of 4 and 8 mmol/L (Table 2). The rate of CO_2 production from glucose increased ($P < .05$) with increasing glucose concentration in enterocytes from cows at each stage of lactation (Table 2).

Glutamine Metabolism

Data on glutamine metabolism in enterocytes incubated in the presence or absence of glucose are listed in Table 3. With glutamine as the sole substrate, there was a high recovery of glutamine carbons in products produced by enterocytes from early-lactation and pooled mid- and late-lactation (PML) cows, with the assumption that glutamine carbons can be mainly recovered in CO_2, glutamate, and aspartate and that a pathway must exist for incorporation of carbon atoms initially present in glutamine into pyruvate.[3] This high recovery allowed for evaluation of routes of glutamine metabolism in enterocytes of lactating cows. In the small intestine, the carbon skeleton of glutamine may be metabolized via two principal routes: by forming δ^1-pyrroline-5 carboxylate or by forming 2-oxoglutarate as a Krebs cycle intermediate. The former pathway leads to formation of citrulline, proline, and ornithine, the levels of which were not measured in the present study since almost all label could be accounted for by the 2-oxoglutarate pathway. In enterocytes from early-lactation and PML cows, 9% to 24% and 7% to 20% of metabolized glutamine carbons appeared in CO_2, respectively. In enterocytes from early-lactation and PML cows, 20% and 19% of glutamate produced from glutamine was converted to aspartate by metabolism of 2-oxoglutarate derived from glutamate to oxaloacetate and then to aspartate. With glutamine as the sole substrate, only minute amounts of lactate and pyruvate were formed from enterocytes from both early-lactation and PML cows. The amount of CO_2 produced from [U-^{14}C]glutamine in the presence of glucose

Table 2. Rate of $^{14}CO_2$ Production From [U-^{14}C]glucose by Enterocytes From Cows at Different Stages of Lactation

[U-^{14}C]glucose (mmol/L)	$^{14}CO_2$ Produced (nmol/2 h per 10^6 viable cells)			
	Early-Lactation	Mid-Lactation	Late-Lactation	SE§
2	13.3*	12.6*	13.0*	1.7
4	18.4*	15.6‡	13.6†	1.4
6	19.2*	17.1‡	14.3†	1.5
8	19.6*	17.8†	16.4†	1.2

*†‡Means with different superscripts in the same row differ ($P < .05$).
§Pooled SE for each stage-of-lactation mean (n = 3).

Table 3. Effect of Glucose on Glutamine Metabolism by Enterocytes From Cows at Different Stages of Lactation

	4 mmol/L [U-^{14}C]glutamine (nmol product/2 h per 10^6 viable cells)	
	No Glucose	+6 mmol/L Glucose
Glutamine uptake		
Early	11.3 ± 1.9†	10.2 ± 1.6†
PML	7.8 ± 1.7	7.3 ± 1.9
Products		
$^{14}CO_2$		
Early	14.1 ± 0.9†	10.1 ± 0.8*†
PML	8.2 ± 1.1	5.7 ± 0.6*
Glutamate		
Early	4.1 ± 0.7†	4.5 ± 0.5†
PML	2.9 ± 0.4	2.2 ± 0.6
Aspartate		
Early	2.3 ± 0.2†	1.9 ± 0.1*†
PML	1.5 ± 0.1	0.9 ± 0.004*
Ammonia		
Early	12.4 ± 2.3†	10.7 ± 1.0†
PML	8.0 ± 1.1	7.2 ± 0.9
Lactate		
Early	0.17 ± 0.02	17.1 ± 3.6*†
PML	0.15 ± 0.03	15.2 ± 1.2*
Alanine		
Early	5.0 ± 0.2†	7.7 ± 0.4*†
PML	3.4 ± 0.1	6.9 ± 0.1*
Pyruvate		
Early	0.14 ± 0.06	0.05 ± 0.003*
PML	0.15 ± 0.05	0.04 ± 0.005*
% of metabolized glutamine carbons in CO_2‡		
Early	23.6 ± 2.3	8.8 ± 1.9*
PML	20.1 ± 1.9	6.6 ± 2.1*

NOTE. Values are the mean ± SEM (n = 3 for early-lactation; n = 6 for PML).
*$P < .05$, significantly different from no-glucose group as analyzed by paired t test.
†$P < .05$, significantly different from PML group as analyzed by unpaired t test.
‡Calculated as nmol CO_2/[(nmol CO_2) + 5 (nmol Glu) + 4 (nmol Asp) + 3 (nmol Ala) + 3 (nmol Pyr) + 3 (nmol lactate)].

and glutamine as cosubstrates in enterocytes of both early-lactation and PML cows was lower than that formed in the presence of glutamine alone. Glutamate, aspartate, ammonia, and alanine were the major nitrogenous end products. Recovery of nitrogen in glutamine taken up in enterocytes from early-lactation and PML cows was 53% and 49% in ammonia, 21% for both in alanine, 18% for both in glutamate, and 10% and 9% in aspartate, respectively. Concomitant addition of glutamine and glucose to enterocytes from early-lactation cows produced an increase (54%) in the rate of net alanine formation and a decrease (18%) in aspartate formation. Glutamate and ammonia formation were not significantly affected by the presence of glucose. However, there was a 100-fold increase in the net amount of lactate produced from enterocytes at all stages of lactation in the presence of glucose.

Glucose Metabolism

Data on glucose metabolism in the presence or absence of glutamine are listed in Table 4. Glucose was metabolized mainly to lactate as compared with pyruvate and CO_2 in enterocytes from both early-lactation and PML cows. Fourteen percent to 19% and 16% to 18% of metabolized glucose carbons appeared in CO_2 in enterocytes from early-lactation and PML cows, respectively. Addition of glutamine to incubation media decreased ($P < .05$) the proportion of glucose metabolized to CO_2, lactate, and pyruvate and increased ($P < .05$) the formation of alanine and glutamate in enterocytes from both early-lactation and PML cows. No aspartate or only trace amounts were detected in enterocytes from both early-lactation and PML cows in the presence of glucose alone or in the presence of both glucose and glutamine.

Table 4. Effect of Glutamine on Glucose Metabolism by Enterocytes From Cows at Different Stages of Lactation

	6 mmol/L [U-^{14}C]glucose (nmol product/2 h per 10^6 viable cells)	
	No Glutamine	+4 mmol/L Glutamine
Glucose uptake		
Early	15.5 ± 1.8	14.7 ± 1.5
PML	13.6 ± 1.1	12.9 ± 1.7
Products		
$^{14}CO_2$		
Early	19.2 ± 1.3†	16.7 ± 0.9*†
PML	15.7 ± 1.3	13.5 ± 1.0*
Glutamate		
Early	0.3 ± 0.06	6.9 ± 0.3*†
PML	0.3 ± 0.05	4.5 ± 0.6*
Lactate		
Early	24.1 ± 1.8†	19.1 ± 1.8*†
PML	22.1 ± 1.7	14.3 ± 1.4*
Alanine		
Early	0.9 ± 0.08	2.9 ± 0.2*
PML	0.8 ± 0.04	2.4 ± 0.3*
Pyruvate		
Early	1.9 ± 0.3	0.07 ± 0.006*
PML	1.3 ± 0.4	0.08 ± 0.004*
% of metabolized glucose carbons in CO_2‡		
Early	18.9 ± 1.9	14.2 ± 1.7*
PML	18.0 ± 1.5	15.6 ± 1.4

NOTE. Values are the mean ± SEM (n = 3 for early-lactation; n = 6 for PML).

*$P < .05$, significantly different from no-glutamine group as analyzed by paired t test.

†$P < .05$, significantly different from PML group as analyzed by unpaired t test.

‡Calculated as nmol CO_2/[(nmol CO_2) + 3 (nmol lactate) + 3 (nmol pyruvate) + 3 (nmol Ala) + 5 (nmol Glu)].

Table 5. Theoretical Rates of ATP Production From Glutamine and Glucose in Enterocytes From Cows at Different Stages of Lactation

Substrate Present	ATP Generated (nmol/2 h per 10^{16} viable cells)	Contribution of Substrate to Total ATP Generation (%)	Percentage of Maximum ATP Generation (%)
Early-lactation cows			
6 mmol/L glucose	124.9 ± 9.8†	69	100
4 mmol/L Gln	56.5 ± 8.9†	31	
6 mmol/L glucose	145.7 ± 13.9*†	100	80
4 mmol/L Gln	77.3 ± 3.7*†	100	43
PML cows			
6 mmol/L glucose	99.8 ± 9.4	76	100
4 mmol/L Gln	31.1 ± 3.0	24	
6 mmol/L glucose	121.5 ± 10.3*	100	93
4 mmol/L Gln	45.7 ± 5.8*	100	35

NOTE. Data were calculated based on data in Tables 3 and 4. Values are the mean ± SEM, with n = 3 for early-lactation cows and n = 6 for PML cows. It was assumed that ATP generation was maximal in the presence of glucose and glutamine.

*$P < .05$, significantly different from corresponding means obtained for 6 mmol/L glucose plus 4 mmol/L glutamine as analyzed by paired t test.

†$P < .05$, significantly different from corresponding means for PML group as analyzed by unpaired t test.

Theoretical Rates of ATP Production From Glutamine and Glucose

Rates of ATP production based on metabolites produced from glutamine and glucose metabolism are listed in Table 5. Glutamine as the sole substrate accounted for all the energy requirements of enterocytes from early-lactation cows and contributed as little as 31% in the additional presence of glucose. Similarly, in enterocytes from early-lactation cows, glucose as the sole substrate accounted for all the energy and contributed 69% in the additional presence of glutamine. Similar rates of ATP production were calculated for enterocytes from PML cows. However, rates of ATP production were greater for enterocytes from early-lactation cows as compared with PML cows. In enterocytes from all cows, the rate of ATP production was greater in the presence of both glucose and glutamine than when either glucose or glutamine were present alone. In addition, the rate of ATP production from glucose as a sole substrate was two to three times greater than that obtained with glutamine as the sole substrate in enterocytes from all cows.

DISCUSSION

General Discussion

In the present study, we systematically investigated glucose and glutamine metabolism in enterocytes from dairy cows at different stages of lactation. Previous research demonstrated that exposure of either serosal or mucosal surfaces of enterocytes to substrates may alter metabolic fates of glucose and glutamine.[24] Isolated enterocytes, as used in the present study, cannot be used to distinguish between possible different metabolic fates of substrates that enter cells across functionally distinct brush-border or basolateral membranes. In addition, other cell types in vascular, perfused–small intestine experimental models may have significant metabolic contributions to the experimental model. However, isolated cells provide an opportunity for experimental control of substrate concentrations. The extent of nutrient metabolism by in vitro preparations of small intestine from various species has been shown to be complementary to observations obtained in vivo and therefore reflects metabolic activities in the whole mucosa.[25] Mean concentrations of various amino acids predetermined from duodenal digesta of cows in different stages of lactation were provided in the incubation media. This decision was made to ensure that enterocytes from cows in different stages of lactation were bathed with the same concentrations of amino acids to eliminate confounding effects of different amino acid concentrations on glucose and glutamine metabolism.

Glutamine and Glucose Oxidation

The significance of glutamine in the metabolism of enterocytes might be attributed to its dual metabolic roles: as a source of energy via partial oxidation and as a source of carbon and nitrogen precursors for biosynthetic processes. When glutamine was the sole substrate for enterocytes, oxidation of glutamine increased by 34%, 40%, and 43% in early-, mid-, and late-lactation cows, respectively, when glutamine concentration increased from 2 to 8 mmol/L (Table 1). This indicates a glutamine concentration-dependence of enterocytes from all cows. Glucose concentration-dependence of enterocytes from all cows was also demonstrated (Table 2).

Metabolic Fates of Glutamine and Glucose and Their Interactions

When glutamine was the sole substrate for enterocytes from early-lactation and PML cows, ammonia production accounted for 53% and 49% of the nitrogen from glutamine metabolized to glutamate, respectively. Since the ammonia produced was roughly equivalent to glutamine uptake, further metabolism of glutamate derived from glutamine must largely be via transaminase reactions rather than through glutamate dehydrogenase. All glutamine metabolized by enterocytes first had to be converted to glutamate by the action of phosphate-dependent glutaminase, which has been shown to be present in intestinal mucosa and in the intestinal wall of ruminants at relatively high levels of activity.[26,27] Based on the end products that result from metabolism of glutamine (Table 3), it seems that glutamine in the ruminant small intestine is used in a manner similar to that proposed for the small intestine of various monogastric species. For example, when glutamine was the sole substrate, approximately 30% of glutamate metabolized was converted into aspartate in enterocytes of both early-lactation and PML cows (Table 3). In the current study, alanine was quantified by HPLC and ^{14}C incorporation into the alanine peak from glutamine and glucose was not measured. It is therefore difficult to account for the carbon skeleton of alanine produced when glutamine was the sole substrate for enterocytes. However, it is well established that glutamate metabolism in the small intestine yields alanine.[25,28,29] In addition, Hanson and Parsons[3] have suggested possible pathways for incorporation of glutamine carbon into pyruvate. Indeed, shifts in the yield of alanine by inclusion of glucose suggest that carbons of alanine may be provided either by degradation of glutamate to pyruvate or by glycolytic pyruvate. However, the suggestion of incorporation of glutamine carbon into pyruvate is equivocal, since only 3% of ^{14}C from ^{14}C-glutamine was found in alanine in rat jejunum in vivo and most of the carbon in alanine may be derived from lactate and glucose.[29]

With the assumption that glutamine carbons are mainly recovered in CO_2, glutamate, aspartate, and alanine, metabolized glutamine carbons that appeared in CO_2 were estimated to be 9% to 24% and 7% to 20% in enterocytes from early-lactation and PML cows, respectively. In addition, with the assumptions that there was insufficient endogenous substrate to provide the carbon skeleton of alanine production in the absence of glucose and that a pathway must exist for incorporation of glutamine carbons into pyruvate,[29] it could be calculated that 45% of glutamate produced in enterocytes from early-lactation cows would need to be metabolized to pyruvate probably via oxaloacetate by phosphoenolpyruvate carboxykinase and pyruvate kinase and/or via malate by malic enzyme, with pyruvate being converted in part to alanine and lactate. These enzymes have been identified in intestinal mucosal.[30] Differences in metabolic fates of glutamine in enterocytes from lactating and nonlactating monogastric species have been reported to be due to differences in morphology and number of enterocytes.[31] Although these changes together with an enlarged small intestine contribute to an increased demand for glutamine, changes in the number of cells may not be as important in the present study because glutamine metabolism is expressed per number of viable cells.

With the assumption that glucose carbons contributed only to the production of lactate, alanine, pyruvate, and glutamate, it was calculated that 14% to 19% and 16% to 18% of metabolized glucose carbon appeared in CO_2 in enterocytes of early-lactation and PML cows, respectively. The presence of glutamine decreased the percentage of metabolized glucose carbons that appeared in CO_2 by 24% in enterocytes from early-lactation cows and by 13% in enterocytes from PML cows. However, the presence of glucose decreased the percentage of metabolized glutamine carbons that appeared in CO_2 by 63% and 67% in enterocytes from early-lactation and PML cows, respectively

(Table 3). These results agree with previous studies that demonstrated greater glucose utilization as compared with glutamine in sheep small intestine and in rumen papillae from steers.[12,13,32]

The high levels of lactate produced in the presence of glutamine plus glucose as cosubstrates (Table 3) and in the presence of glucose alone (Table 4) are intriguing because enterocyte preparations were continuously gassed with the 95% O_2:5% CO_2 mixture to prevent hypoxic conditions. It has been suggested that most of the intraluminally absorbed glucose is absorbed intact into the portal vein and is not metabolized to lactate.[33] However, it has also been reported that the small intestine metabolizes glucose to lactate.[24]

Glutamine and Glucose as Potential Energy Substrates

The following lines of evidence from the present study (Table 5) suggest that relative to glucose, glutamine may be less important for the provision of ATP in enterocytes from early-lactation cows: (1) The rate of ATP production from glutamine was two to three times lower than that from glucose when either was the sole substrate, and it was also lower as compared with the use of glucose plus glutamine as cosubstrates; (2) When both glucose and glutamine were present as compared with when either one was the sole substrate, the rate of ATP production from glutamine was decreased to a greater extent as compared with that from glucose for early-lactation cows. Potential ATP production from both glucose and glutamine showed a trend in PML cows similar to that observed for early-lactation cows.

In conclusion, in view of the present data we suggest that in enterocytes from lactating cows, glutamine may not be quantitatively as important as glucose in the provision of ATP, as calculated on the basis of measured metabolites. Glutamine may therefore play a role in providing both carbon and nitrogen for biosynthetic processes in the lactating cow. Indeed, glutamine has been hypothesized as a potential limiting amino acid for milk production,[34] and some nonessential amino acids such as glutamine and essential amino acids are probably conserved from utilization by the small intestine and therefore remain available for tissue and milk protein synthesis.

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