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Transamination processes promote incomplete glutamine oxidation in small intestine epithelial cells

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The aim of the present study was to examine the influence of glucose on glutamine medabolism by intestinal epithelial cells. Cells were isolated from the proximal, mid, and distal small intestine of male, fed rats. The oxidation of [U-^{14}C]glutamine was greatest in the proximal small intestine, and the stimulatory effect of glucose on glutamine oxidation was most pronounced in this segment also. Amino-oxyacetate, an aminotransferase inhibitor, did not influence the oxidation of glucose when present alone, but increased glucose oxidation when present simultaneously with glutamine. Glutamine oxidation was suppressed by amino-oxyacetate in both the presence and absence of exogenous glucose. In particular, CO_2 production from glutamine was reduced by 48 and 79% in the absence and presence of glucose, respectively; succinate CO_2 ratios (CO_2 from [1,4-^{14}C]-succinate/[2,3-^{14}C]succinate, unlabeled substrate as specified) were reduced 59 and 71%, respectively, and the probability was doubled that glutamine carbon, which enters the TCA cycle, would complete a full turn of the cycle. These experiments confirmed earlier findings that glutamine carbon entering the TCA cycle is not entirely oxidized and showed that processes of transamination are essential for high rates of glutamine entry into the TCA cycle. Transamination appears also to be essential for efflux of intermediates from the TCA cycle and the synthesis of new compounds. Thus, transamination processes apparently facilitate the incomplete oxidation of glutamine in intestinal cells. These studies showed also that carbon that leaves the cycle re-enters predominantly via pyruvate dehydrogenase, rather than via pyruvate carboxylase, along the length of the small intestine. (J. Nutr. Biochem. 6: 27–37, 1995.)

Keywords: oxidation; enterocytes; glutamine; glucose

Introduction

Glutamine is the most abundant amino acid in the blood plasma, and it plays a vital role in whole body nitrogen and carbon metabolism. The splanchnic bed was found to be a large consumer of plasma glutamine in several studies with a variety of mammals,[1] and the small intestine has been shown to be the primary organ of glutamine metabolism.[2–5] Glutamine has been shown to have a trophic effect on the intestine when it has been compromised by chemical, physical, and surgical stresses.[6–10] The mechanism by which glutamine exerts its healing properties has not been clearly established although its role as the primary respiratory fuel has often been cited as one reason for its trophic effects. It was recently reported[11] that protein synthesis by isolated rat enterocytes was stimulated by the addition of glutamine or ketone bodies to an isolated rat enterocyte incubation medium, but not by the addition of glucose alone. Glutamine, glucose, and ketone-bodies have all been shown to be respiratory fuels, however.[2,4,5,12] Thus, these results suggest that energy alone is not the sole mechanism by which glutamine exerts its healing effects.

Several studies have shown that glutamine is not completely oxidized in the small intestine. Studies performed in vivo[2–5] showed that glutamine carbon was recovered as CO_2 and in metabolites such as organic acids and amino acids. Transamination processes have been suggested as one mechanism by which α-ketoglutarate is generated from glutamine-derived glutamate.[3,13] The identification of an

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$NAD(P)^+$-dependent malic enzyme in the mitochondria of small intestinal mucosa[14] has led to the suggestion that glutamine carbon probably leaves the tricarboxylic acid (TCA) cycle at malate where it is decarboxylated to pyruvate. Glutamine carbon may also leave the TCA cycle at oxaloacetate, however, and be converted to pyruvate via PEPCK and pyruvate kinase. Studies with isolated enterocytes[12,15] have shown also that glutamine is not completely oxidized and that glutamine carbon that leaves the cycle does not re-enter the TCA cycle to any significant extent.

Interactive metabolic effects between glucose and glutamine in the small intestine have been suggested in several studies. For example, oxygen consumption by enterocytes was greater when glucose and glutamine were provided simultaneously than when the substrates were provided individually.[13,16] Other studies have demonstrated that glutamine suppressed glucose oxidation, but glucose had no effect on glutamine oxidation.[17] Also, malate was shown to stimulate glutamine and glutamate oxidation in enterocyte mitochondria,[18] apparently via stimulation of aspartate aminotransferase.[19] Although these data suggest that the metabolism of glucose and glutamine are interrelated in cells of the small intestine, this interaction had not been extensively explored. We investigated the influence of aminotransferase activity on the interaction between the carbons of glutamine and glucose by employing the aminotransferase inhibitor amino-oxyacetate (AOA). This compound does not specifically inhibit alanine or aspartate transaminase but will inhibit other pyridoxal phosphate-dependent enzymes as well.[20] In intestinal epithelial cells, however, alanine and aspartate aminotransferases are the only pyridoxal phosphate-dependent enzymes known to be important for the metabolism of glutamine.[17,19]

There is substantial evidence that glutamine and glucose oxidation are not consistent along the length of the intestinal tract. For example, glutamine oxidation was found to be higher in cells isolated from the rat jejunum than from the colon[21]; glutamine utilization was higher in the proximal than in the distal segment of the small intestine[22]; and glutaminase-specific activity in the proximal small intestine was reported to be higher in one study[23] but not in another study.[24] Glucose oxidation was reported also to be higher in cells from the rat jejunum than from the colon[21]; and glycolytic enzyme activities in the intestinal mucosa were reported to decrease along a distal gradient.[23] Enzymes of the TCA cycle also decreased in a distal gradient along the small intestine.[23] Thus, it appears that epithelial cells of the proximal small intestine should metabolize glutamine and glucose at higher rates than epithelial cells of the distal small intestine, but these data were not available. Earlier studies from our laboratory showed that glutamine oxidation in cells of the rat jejunum was stimulated by glucose,[12] but in colonic cells glutamine oxidation was inhibited by glucose.[25] These results suggest that the metabolic interactions between glutamine and glucose may differ along the length of the small intestine. Differences in metabolism along the length of the small intestine may be responsible for the findings that supplementation of glutamine to enteral and parenteral solutions not containing glutamine does not result in a consistent pattern of healing by all segments of intestine.[6,8,26,27] Therefore, we examined the oxidation of glutamine by segment of small intestine as well as the interactive metabolism between glucose and glutamine.

In summary, the objectives of these studies were: (1) to determine whether glutamine oxidation by isolated intestinal cells decreased in a gradient fashion along the length of the small intestine; (2) to determine whether the glutamine molecule, once it enters the TCA cycle, is metabolized similarly by cells along the length of the small intestine; (3) to determine whether the stimulatory effect of glucose on glutamine was consistent along the length of the small intestine; and (4) to determine the role of transamination in the glutamine/glucose interactions.

Methods and materials

Animals

Male Sprague Dawley rats (Bantin & Kingman, Fremont, CA, and Simonsen Laboratories, Gilroy, CA) and male Fischer 344 rats (Simonsen Laboratories) weighing 265 to 325 g were allowed free access to a commercial diet (Rat Chow #5012, Ralston Purina, St. Louis, MO). The animals were anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL) at 5 mg/100 g rat and killed by thoracotomy. Animal handling procedures were approved by the Animal Care and Use Committee, University of California, Berkeley.

Chemicals

All chemicals and reagents were reagent grade. Radiochemicals obtained commercially were as follows: [1-^{14}C]acetate (ICN Radiochemicals, Irvine, CA), [2-^{14}C]acetate, D-[2-^{14}C]glucose, D-[6-^{14}C]glucose, D-[U-^{14}C]glucose, L-[U-^{14}C]glutamate, L-[1-^{14}C]glutamate, L-[U-^{14}C]glutamine, [U-^{14}C]α-methylglucose, [1,4-^{14}C]succinate, and [2,3-^{14}C]succinate (Dupont NEN, Boston, MA).

Preparation of isolated cells

The animals were killed the morning of experimentation in the fed state. The entire small intestine was removed: the proximal small intestine consisted of a 25 cm segment beginning 10 cm distal to the pylorus, the distal small intestine consisted of a 25 cm segment proximal to the ileo-cecal junction, and the mid-small intestine consisted of a 25 cm segment taken from the middle of the remaining small intestine. Cells were isolated from the mucosa as described previously[21] using chemical (EDTA) and gentle mechanical techniques. The procedure was modified to perform the EDTA incubation step for 10 min in the proximal and mid-small intestine, and for 20 min in the distal small intestine so that cells were completely removed but in a minimal time period. The resulting cell suspensions, containing primarily intact villi and rafts of cells, were kept on ice until incubations began at 37° C. This technique has been found to remove all epithelial cells. Dry weights of cells were determined by heating at 100° C for 2 to 3 h, and were calculated as the difference between the weight of a 1 mL cell suspension and the weight of a 1 mL Ca-containing KH buffer.

To characterize apparent viability of the cell suspensions, trypan blue staining and lactate dehydrogenase (E.C. 1.1.1.27) release (Sigma Diagnostics Kit DG 1340-UV, St. Louis, MO) were used to evaluate membrane integrity. Uptake of α-methylglucose[28] and CO_2 production were used to assess metabolic integrity. The results of the trypan blue staining showed that the cells were present predominantly as rafts of cells and intact villi, and that 95 to 99% of these cells excluded trypan blue. The free cells

were about 6 to 8% of the population, with ~65% viability. The α-methylglucose ratio (uptake in the absence of DNP/uptake in the presence of DNP, when uptake time is equivalent to time of incubation with substrate) for cells of the proximal, mid- and distal segments were 18 ± 2, 21 ± 2, and 8 ± 1, respectively. Lactate dehydrogenase (LDH) was determined in the supernatant and cell pellet at time zero and at the end of the incubation period. LDH release to the supernatant during incubations averaged 9 ± 1% in the proximal small intestine, 12 ± 2% in the mid-small intestine, and 21 ± 3% in the distal small intestine.

Carbon dioxide production

Aliquots of cell suspension (1 mL containing 2 to 4 mg of dry weight cells), ^{14}C-labeled substrate (~1.9 MBq/μmol), and unlabeled substrate (1 mL including tracer) were incubated 20 to 24 min at 37° C as previously described.[25] Reactions were stopped with perchloric acid (10%) and center wells containing NaOH (10 mmol/L) were used to trap carbon dioxide. The radioactivity was measured using a scintillation counter and, when necessary, total carbon dioxide production was calculated as previously described.[25]

Assessment of relative flux of carbon via pyruvate carboxylase

While the re-entry of glutamine-derived pyruvate into the TCA cycle is considered to be low, resulting in partial oxidation, the probability remains that it can re-enter the TCA cycle. We wanted to evaluate the predominant pathway of re-entry and to determine whether this was influenced by the presence of glucose or whether it differed by segment. The method of Kelleher and Bryan[29] was used to determine entry of carbon-containing compounds via pyruvate carboxylase (PCB) relative to entry via pyruvate dehydrogenase (PDH). The theoretical basis for this approach stemmed from the observations that the carbonyl and methyl carbons of acetate require different numbers of turns of the TCA cycle in order to be completely oxidized to CO_2.[30] Accordingly, if pyruvate is able to enter the TCA cycle via more than one pathway, and if this results in the label being incorporated into a different carbon moiety of citrate, then the number of turns necessary for complete oxidation would differ for the pyruvate molecules entering by these two pathways. The citrate labeling pattern should be the same for acetate and pyruvate molecules entering the TCA cycle via PDH, and this would result in similar "acetate" and "pyruvate" CO_2 ratios. By contrast, entry via PCB would result in different "acetate" and "pyruvate" CO_2 ratios. The degree of difference between these ratios was used[29] to derive the following formula for calculating carbon flux into the TCA cycle via PCB relative to carbon entry via PDH.

$$PCB' = 1 - PDH'$$

$$= \frac{2[(\text{Ac } CO_2 \text{ ratio}) - (\text{Pyr } CO_2 \text{ ratio})]}{[1 + (\text{Pyr } CO_2 \text{ ratio})][2\text{-}F][(\text{Ac}CO_2 \text{ ratio}) - 1]} \quad (1)$$

where

PCB′ = relative flux of ^{14}C-pyruvate into TCA cycle via PCB
PDH′ = relative flux of ^{14}C-pyruvate into TCA cycle via PDH
PCB′ + PDH′ = 1
acetate CO_2 ratio = $^{14}CO_2$ from [1-^{14}C]acetate/$^{14}CO_2$ from [2-^{14}C]acetate
pyruvate CO_2 ratio = $^{14}CO_2$ from [2-^{14}C]pyruvate/$^{14}CO_2$ from [3-^{14}C]pyruvate
F = ratio of randomized:nonrandomized oxaloacetate carbon due to flux through fumarase

The authors[29] stated that even if F is unknown, a nonzero value for the right side of the above equation indicates pyruvate carboxylation. The value of F is not known for intestinal cells, therefore values of 0.5 and 1.0 were used to estimate the probable extremes, and presented data were calculated with $F = 0.5$, as a conservative estimate.

Preliminary experiments showed that glutamine suppressed pyruvate and glucose oxidation similarly, suggesting a common pool for exogenous pyruvate and glucose-derived pyruvate, and suggesting also that the "pyruvate" ratios can be assessed using the glucose isotopes under these conditions. As the [3-^{14}C]pyruvate isotope was not commercially available, the glucose isotopes [2-^{14}C]glucose and [6-^{14}C]glucose were used since they would be converted to [2-^{14}C]pyruvate and [3-^{14}C]pyruvate. In these experiments, PCB′ flux was determined for intestinal cells incubated in 20 mmol/L glutamine and 20 mmol/L glutamine + 20 mmol/L glucose. $^{14}CO_2$ production was quantified when cells were incubated separately in trace quantities of [1-^{14}C]acetate, [2-^{14}C]acetate, [2-^{14}C]glucose, and [6-^{14}C]glucose.

Estimation of flux of substrates into the TCA cycle and their fate

Acetate CO_2 ratios were calculated as described for Equation 1. Succinate CO_2 ratios were calculated as follows: $^{14}CO_2$ from [1,4-^{14}C]succinate/$^{14}CO_2$ from [2,3-^{14}C]succinate. Values for "A + T" were calculated from the acetate and succinate CO_2 ratios using formulas derived by others.[15,31] "A" is defined as the probability that TCA cycle carbon is incorporated into citrate via acetyl CoA, and "T" is defined as the probability that TCA cycle carbon is incorporated into citrate via oxaloacetate. Therefore, the sum of A + T is the probability that a compound will complete one turn of the TCA cycle by either remaining in the cycle or leaving as a four carbon fragment and re-entering via acetyl CoA. This provides an index of the probability that a molecule entering the TCA cycle will be completely oxidized (this must be distinguished from net oxidation which requires influx of acetyl CoA).

$$A + T = \frac{(2 \times {}^{14}CO_2 \text{ from } [2\text{-}^{14}\text{C}]\text{acetate}^*)}{({}^{14}CO_2 \text{ from } [1\text{-}^{14}\text{C}]\text{acetate}^{**} + {}^{14}CO_2 \text{ from } [2\text{-}^{14}\text{C}]\text{acetate})}$$

* = [2-^{14}C]acetate or [2,3-^{14}C]succinate
** = [1-^{14}C]acetate or [1,4-^{14}C]succinate (2)

Relative entry of substrate into the TCA cycle was calculated as described previously[12] using Equations 3 and 4.

TCA cycle entry of substrate, μmol carbon atoms/g · min

$$= \frac{(CO_2 \text{ production from } [U\text{-}^{14}\text{C}]\text{-labeled substrate})}{A + T} \quad (3)$$

Values were calculated for the treatments without addition of AOA, and these values were arbitrarily assigned a value of 1.0. Values for the treatments with AOA were then calculated as a percentage of the value of the treatments without AOA. These values are termed "relative TCA cycle entry of substrate."

Relative TCA cycle entry of substrate

$$= \frac{\text{TCA cycle entry of substrate with AOA}}{\text{TCA cycle entry of substrate without AOA}} \quad (4)$$

This approach assumes that the production of CO_2 from [U-^{14}C]-labeled substrates will equally reflect substrate oxidation in the TCA cycle regardless of the presence of AOA. This approach assumes also that the relationship between the value for A + T

and the probability of complete oxidation of the substrate will be unaffected by the presence of AOA.

Values for A + T were calculated from values determined experimentally for entry of glutamate into the TCA cycle (equivalent to CO_2 production from [1-^{14}C]glutamate in μmol/g · min) and its oxidation (equivalent to CO_2 production form [U-^{14}C]glutamate in μmol/g · min), where

$$A + T = \frac{CO_2 \text{ from [U-}^{14}\text{C]glutamate}}{CO_2 \text{ from [1-}^{14}\text{C]glutamate} \times \#\text{ C atoms/mole glutamate}} \quad (5)$$

Oxygen uptake by isolated cells

A YSI Model 53 oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH) was used to quantify oxygen uptake in aliquots of cell suspensions (containing 2 to 4 mg, dry weight) using standard methodology.[32] Cells were incubated 2 to 5 min without substrate (endogenous value), with substrate, or with an inhibitor as indicated. Calibration was performed using glucose oxidase and known quantities of glucose.

Influence of transamination on glutamine metabolism

Amino-oxyacetate (AOA) was used to inhibit transamination in intestinal cells in three sets of experiments. First, the influence of preincubation with AOA (0.5 mmol/L, 10 min, 37° C) on α-methylglucose ratio and CO_2 production from 5 mmol/L [U-^{14}C]glutamine was evaluated. To evaluate the effect of preincubation, cells with buffer ± AOA were incubated in a 37° C shaking water bath. Substrate with tracer was added after 10 min, and the incubation continued for 20 min. Second, the influence of concentration of AOA (range 0 to 5.0 mmol/L) on CO_2 production from 5 mmol/L [U-^{14}C]glutamine and CO_2 ratios from [1,4-^{14}C]-succinate and [2,3-^{14}C]succinate were also determined. Last, the influence of AOA was evaluated on CO_2 production from ^{14}C-labeled glucose and glutamine, succinate CO_2 ratios, and values for A + T. All but the first experiment were completed without the preincubation step.

To further examine the role of aminotransferase activity on the glucose and glutamine interactions, we evaluated the influence of alanine on the oxidation of [U-^{14}C]glutamine ± glucose. A high concentration of alanine, an endproduct of transamination, would be expected to mimic the effects of AOA.

Statistics

Data are presented as the mean ± SEM. Statistical analyses were performed using paired *t*-tests, and one- and two-factor repeated measures analysis of variance techniques.[33] Statistically significant results were followed-up using Tukey's Studentized Range Test at a procedure-wise error rate of 0.05. When a statistically significant interaction was found between segment and substrate, the follow-up procedure was performed on all four segment and substrate means.

Results

Oxidation of glutamine by segment of small intestine

The CO_2 production from 5 mmol/L [U-^{14}C]glutamine in the presence and absence of glucose was compared for three segments of small intestine. CO_2 production was significantly higher by cells from the proximal small intestine (15.6 ± 1.1 μmol CO_2/g · min) than by cells from the distal small intestine (7.2 ± 0.6 μmol CO_2/g · min) (*Figure 1*). In the presence of glucose and glutamine, CO_2 production from [U-^{14}C]glutamine was significantly stimulated in cells of the proximal but not the distal small intestine. In a subsequent experiment, glucose was shown to stimulate both glutamine and glutamate oxidation, although the extent of stimulation was somewhat less for glutamate than glutamine (12% and 21% stimulation for glutamate and glutamine, respectively) when substrates were present at 5 mmol/L.

The stimulatory effect of glucose on glutamine was evaluated when substrates were present at physiologically relevant and thus unequal concentrations (*Table 1*). Plasma concentrations were simulated with 0.5 mmol/L glutamine and 5 mmol/L glucose.[4] Postprandial luminal concentrations were simulated with 5.0 mmol/L glutamine[34] and 70 mmol/L glucose. This concentration of glucose provided a safety margin over the concentration of 50 mmol/L that has been reported.[35,36] The stimulatory effect of glucose on glutamine oxidation was lower when glutamine was present at 0.5 mmol/L than at 5.0 mmol/L. At each glutamine concentration (0.5 and 5.0 mmol/L), the stimulatory effect was greater when glucose was present at 70 mmol/L than at 5.0 mmol/L.

Total CO_2 production from both glutamine and glucose was calculated as the CO_2 production from [U-^{14}C]glutamine + glucose and [U-^{14}C]glucose + glutamine. In the presence of both substrates, total CO_2 production was 2.4 fold higher in the proximal small intestine than in the distal small intestine (*Table 2*). The date for [U-^{14}C]glutamine in glutamine alone are presented for comparison (*Table 2*) and to illustrate similar differences between segments. The production of CO_2 from glucose and glutamine primarily represent mitochondrial respiration since we found that less than 5% of the glucose is metabolized by intestinal epithe-

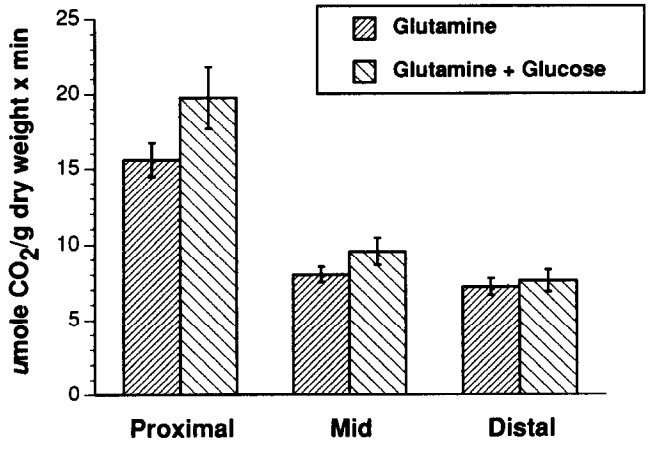

Figure 1 Carbon dioxide production from 5 mmol/L [U-^{14}C]glutamine ± 5 mmol/L glucose using cells isolated from the proximal, mid- and distal small intestine of fed rats. Values are means ± SEM, n = 6 for proximal and distal segments, n = 2 for mid.

lial cells via the pentose phosphate pathway (unpublished data).

The acetate CO_2 ratios were used to calculate values for A + T according to Equation 2. When analyzed statistically, interactions between substrate and segment were not significant for A + T, so data could be pooled to increase statistical power (*Table 2*). The data that follow in parentheses in the text represent the pooled means, and therefore, differ from those in *Table 2*. Values for A + T were significantly lower with glutamine and glucose than with glutamine alone (0.336 and 0.393, respectively, $S_p = 0.033$), and values were lower in the proximal than in the distal segment (0.288 and 0.443, respectively, $S_p = 0.026$).

Table 1 Influence of glucose on CO_2 production from [U-^{14}C]glutamine when substrates are present at physiologically relevant concentrations

Substrate concentrations (mmol/L)		CO_2 production from [U-^{14}C]glutamine	Difference
Glutamine	Glucose	(μmol/g · min)	%*
0.5†	0.0	9.46 ± 0.33**	
0.5	5.0‡	9.69 ± 0.87	+2.4%
0.5	70.0§	10.41 ± 0.21	+10.0%
5.0‖	0.0	15.21 ± 1.75	
5.0	5.0	17.99 ± 0.25	+18.2%
5.0	70.0	19.04 ± 2.22	+25.2%

*Difference, % = (CO_2 value for glutamine + glucose)/(CO_2 value for glutamine alone) × 100
†0.5 mmol/L glutamine is used as an estimate of plasma glutamine concentration.
‡5.0 mmol/L glucose is used as an estimate of plasma glucose concentration.
§70.0 mmol/L glucose is used as an estimate of postprandial luminal glucose concentration.
‖5.0 mmol/L glutamine is used as an estimate of postprandial luminal glutamine concentration.
**Mean ± SEM, $n = 2$.

Relative flux of carbon into the TCA cycle by pyruvate carboxylase

To calculate PCB′ flux, acetate and pyruvate CO_2 ratios were determined using acetate and glucose isotopes, respectively. Both acetate and glucose CO_2 ratios were significantly lower with glutamine as substrate than with glutamine and glucose together, and the degree of difference was similar in the proximal and distal segments of the small intestine as indicated by the lack of a significant interaction between the two main factors (*Table 2*). Consequently, PCB′ flux was not significantly different for the four treatments and mean values ranged from 6 to 11%. Thus, relative flux of pyruvate carbon into the TCA cycle was predominantly by PDH in both segments of the small intestine when the substrate was either glutamine alone or glutamine and glucose.

Comparison of the effects of glucose, pyruvate, and lactate on glutamine metabolism

The presence of glucose significantly stimulated glutamine oxidation (by ~25%) when these substrates were present in equimolar concentrations of 5 mmol/L or 20 mmol/L (*Table 3*). In subsequent experiments, equimolar concentrations of lactate also significantly stimulated glutamine oxidation. The magnitude of change in glutamine oxidation was similar for glucose, lactate, and pyruvate (*Table 3*).

Influence of transamination on glutamine metabolism

The influence of preincubating cells in AOA was evaluated to determine whether this would increase the effects of this

Table 2 Metabolic differences between epithelial cells of the proximal versus distal small intestine

Substrate:	Glutamine		Glucose + Glutamine		*p*-values for F statistic*		
Segment:	Proximal	Distal	Proximal	Distal	Segment	Substrate	Interaction
CO_2 production (μmol/g · min)†							
[U-^{14}C]glucose	—	—	5.2 ± 0.4	3.0 ± 0.2			
[U-^{14}C]glutamine	16.0 ± 1.0	7.5 ± 0.5	20.0 ± 1.5	7.6 ± 0.5			
Total	16.0	7.5	25.1	10.6			
Acetate CO_2 ratio‡	5.73 ± 0.51	3.12 ± 0.17	6.30 ± 0.11	4.14 ± 0.49	0.001	0.031	NS
Value for A + T§	0.30 ± 0.01	0.49 ± 0.02	0.28 ± 0.003	0.40 ± 0.03	0.001	0.034	NS
Glucose CO_2 ratio‖	3.79 ± 0.21	2.67 ± 0.19	4.30 ± 0.30	3.77 ± 0.51	NS	0.012	NS
PCB′ flux (as % of PDH + PCB)**	11 ± 1.8%	8 ± 2.8%	10.0 ± 1.8%	6.0 ± 3.7%	NS	NS	NS

*p-values of the F-statistic were calculated using log transformed data.
†CO_2 production from substrates at 5 and 20 mmol/L was not significantly different. Therefore, data in this table include equivalent numbers of replicates from both concentrations. Values for glutamine + glucose include data from [U-^{14}C]glutamine plus data for [U-^{14}C]glucose in solutions of equimolar concentrations of these two substrates. When appropriate, values include mean ± SEM, otherwise only mean values are provided; $n = 8$.
‡Calculated as the ratio of $^{14}CO_2$ from [1-^{14}C]acetate/[2-^{14}C]acetate, when incubated separately but under identical conditions (i.e., same specific activity and weight of cells, and unlabeled substrates at 20 mmol/L). Values are means of $n = 4$.
§Calculated as described by Equation 2 in the Methods and materials section using acetate CO_2 ratios presented in this table.
‖Used synonymously with the pyruvate CO_2 ratio for calculating PCB′ flux, when the glucose CO_2 ratio represents the ratio of $^{14}CO_2$ from [2-^{14}C]glucose/[6-^{14}C]glucose, when incubated separately but under identical conditions. Values are means of $n = 4$.
**Calculated as described by Equation 1 in the Methods and materials section, with $F = 0.5$.

Table 3 Influence of glucose, pyruvate and lactate on glutamine oxidation by epithelial cells of the proximal small intestine

	CO_2 production from [U-^{14}C]glutamine (μmol/g · min)		
	Experiment 1	Experiment 2	Experiment 3
Glutamine	15.6[a] ± 1.1	15.5 ± 0.76	16.7[a] ± 1.8
Glutamine + Glucose	19.7[b] ± 2.1	20.2 ± 1.2	20.0[b] ± 2.1
Glutamine + Pyruvate		20.6 ± 0.65	
Glutamine + Lactate			21.3[b] ± 1.9

Values are means ± SEM for 6, 2, and 4 animals, respectively, for the three experiments. Means within experiments 1 and 3 with different superscripts are significantly different, with $P = 0.013$ and $P = 0.006$, respectively. All substrates were present at 5 mmol/L for Experiment 1 and at 20 mmol/L for Experiments 2 and 3.

Table 4 Influence of preincubating cells* in the absence and presence of amino-oxyacetate (AOA; 0.5 mmol/L) on metabolic functions of epithelial cells of the proximal small intestine

			P-value of *F*-statistic		
	−AOA	+AOA	Preincubation	AOA	Interaction
α-methylglucose uptake ratio†			0.037	0.072	NS
No preincubation	17.8 ± 0.09	23.6 ± 0.94			
With preincubation	4.9 ± 0.38	7.1 ± 0.50			
CO_2 production‡			0.068	NS	NS
No preincubation	11.3 ± 2.84	6.2 ± 0.66			
With preincubation	9.3 ± 3.00	4.9 ± 0.90			

*To evaluate the influence of "preincubation," cells were incubated 10 min at 37° C in buffer with or without AOA, then substrates were added as required for the two procedures and incubated for an additional 20 min. Values are means ± SEM of two experiments.
†α-methylglucose uptake is calculated as the ratio of dpm from [U-^{14}C]α-methylglucose in cell pellets without DNP/with DNP.[27]
‡Cells were incubated with [U-^{14}C]glutamine in 5 mmol/L glutamine and CO_2 is expressed as μmol/g · min.

inhibitor on metabolism of intestinal cells. Preincubating isolated cells for 10 min significantly decreased the α-methylglucose ratio (by ~65%), but this difference did not appear to be due to the presence of AOA since the same degree of suppression was noted in treatments with and without AOA, as evidenced by the lack of a statistically significant interaction between these two main factors (*Table 4*). Preincubation reduced CO_2 production from [U-^{14}C]glutamine by ~20%, with and without AOA, but this degree of change was not statistically significant ($p = 0.068$). AOA reduced CO_2 production from glutamine by ~55%, although this did not achieve statistical significance due to the small number of experiments. This decrease was noted both with and without preincubation and was not likely due to loss of cellular integrity, however, since the α-methylglucose uptake ratio was not lowered by AOA. Also, LDH release was unaltered by the presence of this concentration of AOA (single experiment, data not shown), and the addition of AOA did not influence oxygen uptake from endogenously available substrates even though AOA reduced oxygen uptake in the presence of exogenous substrates (*Table 5*). The values were similar regardless of whether AOA was added after or before the addition of exogenous substrate, however. Overall, these data suggested that preincubation had negative effects on membrane integrity, cell viability, and metabolic function of isolated epithelial cells. Thus, further experiments with AOA were performed without the preincubation step.

The optimal concentration of AOA to add to the incubation medium for cells isolated from the proximal small intestine was assessed by evaluating CO_2 production from 5 mmol/L [U-^{14}C]glutamine with 0 to 5 mmol/L AOA and the CO_2 ratios from [1,4-^{14}C]succinate and [2,3-^{14}C]succinate in 20 mmol/L glutamine + 20 mmol/L glucose with 0 to 1 mmol/L AOA. The CO_2 production from glutamine was reduced in the presence of AOA in a concentration-dependent manner, ranging from 24% reduction with 25 to 100 μmol/L AOA to 50% with 0.5 to 5 mmol/L AOA (data not shown). Succinate CO_2 ratios decreased as the concentration of AOA increased with a maximal and consistent value for AOA concentrations of 1 to 5 mmol/L (data not shown). All further experiments were performed using AOA at 1 mmol/L since this concentration had maximal effect on the oxidative fate of the glutamine carbon entering the TCA cycle. Also, this concentration of AOA did not appear to be toxic to the cells since oxygen uptake was maintained above the value for endogenous fuels (*Table 5*).

In preliminary experiments, the influence of AOA on glutamine oxidation was found to vary by segment of small intestine. In particular, glutamine oxidation was reduced by AOA to a greater extent by cells of the proximal small intestine (50.5%) than by cells of the distal small intestine (14%), and this was observed when glutamine was the sole exogenous substrate. When both glutamine and glucose were present, the suppression of glutamine oxidation by AOA was again greater in the proximal small intestine

Table 5 Influence of AOA on oxygen uptake by epithelial cells of the proximal small intestine

Substrate*	AOA added before substrate			AOA added after substrate		
	Endogenous†	AOA‡	Substrate*	Endogenous	Substrate	AOA
			(Oxygen uptake, μmol/g · min§)			
Glutamine	20.1 ± 1.4	19.7 ± 1.3	25.6 ± 1.6	17.5 ± 1.7	27.8 ± 1.8	22.6 ± 1.7
Glutamine + Glucose				16.6 ± 1.0	32.4 ± 2.4	27.9 ± 2.1

*Substrates were added to concentrations of 5 mmol/L.
†Endogenous respiration refers to oxygen uptake measured prior to the addition of substrates or AOA.
‡AOA was added to a concentration of 1 mmol/L.
§Mean ± SEM, $n = 2$.

Table 6 Influence of AOA* on substrate oxidation by epithelial cells isolated from the proximal small intestine

Substrate:	Glucose		Glutamine		Glucose + Glutamine	
	−AOA	+AOA[1]	−AOA	+AOA	−AOA	+AOA
CO_2 production†						
[U-^{14}C]glucose	11.5 ± 1.4	11.6 ± 1.4^{NS}	—	—	5.0 ± 0.5	11.3 ± 1.5[a]
[U-^{14}C]glutamine	—	—	15.2 ± 0.9	7.5 ± 0.8*	19.4 ± 0.5	4.3 ± 0.2[a]
Total	11.5	11.6	16.4	8.5	24.3	15.4
CO_2 ratio‡	1.32 ± 0.09	1.10 ± 0.03^{NS}	3.98 ± 0.01	1.62 ± 0.21ND	4.91 ± 0.32	1.42 ± 0.02[a]
A + T value§	0.87 ± 0.03	0.95 ± 0.01^{NS}	0.40 ± 0.001	0.77 ± 0.06ND	0.34 ± 0.01	0.83 ± 0.01[a]
Relative TCA cycle entry‖						
Glucose	1.00	0.92 ± 0.06^{NS}	—	—	1.00	0.93 ± 0.09^{NS}
Glutamine	—	—	1.00	0.28 ± 0.02ND	1.00	0.09 ± 0.006[a]

*AOA was present at 1 mmol/L. Values are means ± SEM for 2 to 7 experiments. [a]A statisically significant effect of AOA, when analyzed using a paired *t*-test. NS indicates that the effect of AOA was not statistically significant. ND indicates that statistics could not be performed as only 2 experiments were completed.
†Cells were incubated in 20 mmol/L glucose, glutamine, or glucose + glutamine as indicated, with trace quantities of the ^{14}C-labeled isotopes. CO_2 production was calculated from $^{14}CO_2$ and is presented as μmol/g · min.
‡The ratio of $^{14}CO_2$ production from [1,4-^{14}C]succinate/[2,3-^{14}C]succinate was determined from cells incubated under identical conditions in the 20 mmol/L concentrations of the substrates indicated. This succinate CO_2 ratio is synonymous with the acetate CO_2 ratio.
§Values for A + T were calculated from the succinate CO_2 ratios reported above, using Equation 2 in the Methods and materials section.
‖Relative entry of substrates into the TCA cycle was calculated using Equations 3 and 4 in the Methods and materials section.

(79.5%) than in the distal small intestine (49.7%). All remaining experiments were performed using cells of the proximal small intestine since the effects of AOA were more pronounced in this segment.

The influence of AOA on CO_2 production differed for ^{14}C-labeled glucose and glutamine and was dependent on which unlabeled substrates were present (*Table 6*). In the presence of glucose alone, CO_2 production from [U-^{14}C]glucose was unaffected by AOA. When both glucose and glutamine were present, however, CO_2 production from [U-^{14}C]glucose was significantly increased (~2 fold) by the presence of AOA. CO_2 production from glucose in the presence of AOA was similar for treatments with (11.3 μmol/g · min) and without (11.6 μmol/g · min) glutamine, and these values were comparable to CO_2 production from glucose when neither glutamine nor AOA were present (11.5 μmol/g · min). AOA significantly decreased CO_2 production from [U-^{14}C]glutamine for all treatments containing glutamine: by 51% when glutamine was the sole substrate and by 78% when both glutamine and glucose were present.

The CO_2 ratios were generally reduced by the presence of AOA (*Table 6*). There were variations in the degree of change, however, with no significant increase in the values of A + T for glucose alone, but marked increases for glutamine alone, or for glutamine + glucose. The largest effect was observed with both glutamine and glucose, and the value for A + T of only 0.34 was increased to 0.83 when AOA was present. This latter value was similar to the value observed for glucose alone when AOA was absent.

The influence of AOA on the rate of entry of substrates into the TCA cycle was estimated using two approaches. First, the CO_2 data and the values of A + T were used to calculate relative entry using Equation 4. These values should be interpreted as relative flux rather than as absolute flux since the values for A + T do not provide an absolute value for the probability of complete oxidation. These calculations indicate that AOA did not significantly influence the entry of glucose carbon into the TCA cycle (*Table 6*). By contrast, AOA profoundly reduced the entry of glutamine carbon into the TCA cycle and this decrease was statistically significant when both glutamine and glucose were present. For the second approach, the influence of AOA on CO_2 production from [1-^{14}C]glutamate was assessed. This approach assumes that glutamine-derived glutamate mixes into a common pool with exogenous glutamate so that they will have a common metabolic fate. This assumption was supported by finding that AOA reduced to similar degrees CO_2 production from [U-^{14}C]glutamate and

Table 7 Influence of AOA on glutamate entry into the TCA cycle

Isotope: Substrate, 5 mmol/L:	[1-^{14}C]glutamate Glutamate	[U-^{14}C]glutamate Glutamate	[U-^{14}C]glutamine Glutamine
		CO_2 production (μmol/g · min)	
−AOA	2.18 ± 0.3*	5.59 ± 1.1	16.3 ± 1.8
+AOA†	0.82 ± 0.3	2.86 ± 1.1	7.44 ± 1.9
% decrease	−62.2	−48.8	−54.3

*Mean ± SEM, n = 3.
†AOA added to a concentration of 1 mmol/L.

CO_2 production from [U-^{14}C]glutamine (49% and 54%, respectively) (*Table 7*). In this same experiment, AOA reduced CO_2 production from [1-^{14}C]glutamate by 62%, indicating that AOA reduced glutamate entry into the TCA cycle. If glutamine-derived glutamate and exogenous glutamate are metabolized similarly, AOA would also reduce entry into the TCA cycle of glutamine-derived glutamate. Since [1-^{14}C]glutamine was not commercially available, this was not measured directly, however.

Alanine reduced glutamine oxidation when present in equimolar concentrations with glutamine (*Table 8*). Glutamine oxidation was reduced by 35% when only glutamine and alanine were present. In the same experiment, alanine reduced glutamine oxidation by 26% when glucose was present also in the incubation medium.

Table 8 Influence of alanine on glutamine oxidation by epithelial cells of the proximal small intestine

Substrate (20 mmol/L)	CO_2 production, [U-^{14}C]glutamine (μmol/g · min)*	Decrease with alanine (%)
Glutamine	16.7 ± 1.8	
Glutamine + Alanine	10.9 ± 1.5	−34.7
Glutamine + Glucose	20.0 ± 2.1	
Glutamine + Glucose + Alanine	14.8 ± 1.3	−26.0

*Mean ± SEM, n = 4.

Discussion

Glutamine has been reported to be a major respiratory fuel for cells of the mammalian small intestine, and these as well as other studies have shown that glutamine carbon is partially oxidized within the TCA cycle. Our objective was to extend these findings to explore the oxidative pattern of glutamine by segment of small intestine, to further evaluate the glutamine and glucose metabolic interaction that has been reported by us and others, and to evaluate the role of transamination processes in determining the oxidative pattern of glutamine in the presence and absence of glucose. Data from these studies support a model describing the processes that facilitate the metabolic interactions between glucose and glutamine in intestinal epithelial cells. According to this model (*Figure 2*), the processes of transamination facilitate entry of glutamine-derived glutamate into the TCA cycle (designated as 1 in *Figure 2*), allowing a substantial flux of glutamine carbon into the TCA cycle. Also, the processes of transamination facilitate efflux of TCA cycle intermediates from the cycle (designated as 2 and 3 in *Figure 2*) which results in incomplete oxidation of substrates since there is little re-entry into the cycle. Thus, the processes of transamination appear to be responsible for high rates of entry of glutamine carbon into the TCA cycle and for incomplete oxidation.

Transamination facilitates the metabolic interaction between glutamine and glucose

The importance of transamination for entry into the TCA cycle of glutamine-derived glutamate is supported by several findings. First, in the presence of AOA, CO_2 production from [U-^{14}C]glutamine was reduced, and the oxidation of glutamine carbon was more complete as indicated by the lower CO_2 ratio (*Table 6*). Thus, it is evident that the entry of glutamine carbon into the TCA cycle was significantly reduced in the presence of the aminotransferase inhibitor, AOA. The calculations based on these data substantiate this reasoning (*Table 6*). AOA reduced glutamine carbon entry into the TCA cycle by 72% when glutamine was the sole exogenous fuel, and by 91% when both glutamine and glucose were present. An alternative approach was taken to determine the influence of AOA on glutamine entry into the TCA cycle. This second approach involved measuring CO_2 production from [1-^{14}C]glutamate since this carbon is released by the α-ketoglutarate dehydrogenase complex immediately following entry into the TCA cycle. Glutamate was used in place of glutamine in this second approach since the [1-^{14}C]glutamine isotope was not commercially available. The data showed that AOA reduced CO_2 production from [1-^{14}C]glutamate, suggesting that AOA reduced entry of glutamate into the TCA cycle (*Table 7*). Since the addition of AOA reduced the rate at which glutamine carbon enters the TCA cycle using both approaches, these results emphasize the importance of transamination processes for facilitating the entry of glutamine carbon into the TCA cycle.

Data presented here strongly suggest that glucose and glutamine interact metabolically to influence the entry of glutamine into the TCA cycle (designated as 1 of *Figure 2*). It is likely that the amino group of glutamate is transferred to glucose-derived pyruvate in this step, resulting in the formation of alanine (glucose carbons and glutamate nitrogen) and α-ketoglutarate (glutamine carbons). The possibility cannot be ruled out, however, that the glutamate amino

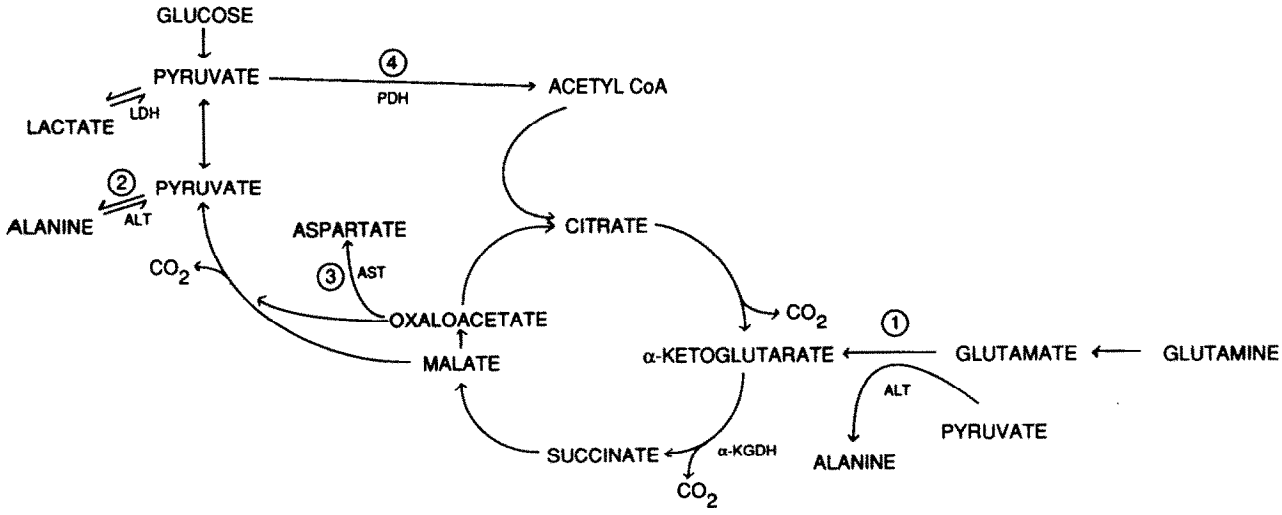

Figure 2 Diagram illustrating the metabolic interactions between glutamine and glucose in rat small intestine epithelial cells. Abbreviations are as follows: ALT = alanine aminotransferase; AST = aspartate aminotransferase; α-KGDH = α-ketoglutarate dehydrogenase; LDH = lactate dehydrogenase; PDH = pyruvate dehydrogenase. Pathways designated with numerals are discussed in the text.

group is transferred to glucose-derived oxaloacetate, with the consequent formation of aspartate; earlier work in enterocyte mitochondria suggested that this is of minor importance when compared with alanine formation, however.[19] Glucose stimulated glutamine oxidation in these experiments (*Figure 1*) and in our recent studies.[12] The present experiments demonstrate that stimulation occurs at physiologically relevant substrate concentrations (*Table 1*) although the effect was only significant at higher concentrations. When AOA was present in the incubation media, this stimulation was abolished (*Table 6*). Thus, it appears that glucose stimulates glutamine oxidation by providing three and/or four carbon intermediates to facilitate the disposal of the amino nitrogen of glutamine/glutamate via transamination reactions. This interaction would not occur in the presence of an aminotransferase inhibitor and, indeed, the stimulatory effect of glucose on glutamine oxidation was not observed in the presence of AOA. Glutamine oxidation was stimulated also by pyruvate and lactate (*Table 3*). This is consistent with the model that is proposed (*Figure 2*) since the pyruvate used in the transamination process (designated as 1) should not need to be derived specifically from glucose. The suppressing effect of alanine on glutamine oxidation (*Table 8*) also supports the model (*Figure 2*) since alanine should suppress the aminotransferase-catalyzed conversion of pyruvate to alanine (designated as 1). It appears that glucose interacts metabolically with glutamine at a step that is beyond the deamidation reaction since glucose stimulated glutamate oxidation (text of results). While the stimulation was greater from glutamine than glutamate, this difference may be due to a slower entry into the cell of the charged amino acid.[3]

The data presented here suggest that transamination processes are important in directing glutamine-derived TCA cycle intermediates out of the cycle with little probability of re-entry, which would be needed for net oxidation. Specifically, values for A + T were higher for glutamine in the presence than in the absence of AOA (*Table 6*) showing that AOA increases the probability that glutamine carbon is completely oxidized. Similar conclusions can be made from the studies with glutamate. In particular, values for CO_2 production from [1-^{14}C]glutamate and [U-^{14}C]glutamate (*Table 7*) were substituted into Equation 5 to calculate the value for A + T. The calculated values for A + T were 0.51 and 0.70 for glutamate in the absence and presence of AOA, respectively. These calculated A + T values are similar to the experimentally determined values for glutamine (0.40 and 0.75, respectively; *Table 6*). For both substrates, AOA increased the probability that carbon atoms entering the TCA cycle will either stay within the cycle for one complete turn ("T") or leave the cycle but re-enter to complete one full turn ("A"). Since glucose and glutamine carbon likely form common pools of TCA cycle intermediates, AOA could influence the fate of the glucose carbon. This effect was mediated through glutamine metabolism, however, since the value for A + T was not influenced by AOA when glucose was the sole exogenous substrate. These data suggest that transamination processes are necessary for the glucose, glutamine, and glutamate carbon to be directed out of the TCA cycle and into newly synthesized compounds derived from TCA cycle intermediates.

Concentrations of 0.5 to 5.0 mmol/L of AOA were essential to reach a constant rate of inhibition of glutamine oxidation. Cellular integrity was not impaired in this range, as the α-methylglucose ratio was increased by 38% (*Table 4*), which may represent an increased demand by the cells for glucose, since the metabolism of glutamine for energy production was suppressed. In our hands, AOA at concentrations of 10 mmol/L or higher resulted in incomplete buffering of the media and reduced the pH, with probable loss of cell viability and metabolic function. Since there were no apparent advantages to using AOA at concentrations greater than 1 mmol/L, and since other workers reported that this concentration abolished the formation of alanine by enterocytes,[37] 1 mmol/L AOA was used in all subsequent experiments.

The relative importance of aminotransferase activity to convert glutamate to α-ketoglutarate as compared with the

importance of glutamate dehydrogenase is still debated. Several groups have suggested that glutamate dehydrogenase is not a significant enzyme for α-ketoglutarate formation,[3,13,17,38] although high concentrations of AOA may have confounded the data of some of these studies by unintentionally reducing cell viability and metabolic function. Others have provided evidence of flux through glutamate dehydrogenase.[37] Our results show that glutamate carbon is able to enter the TCA cycle at a reduced rate in the presence of an aminotransferase inhibitor. This could result from incomplete aminotransferase inhibition and/or from flux through glutamate dehydrogenase. Our data suggest also that exogenous glutamate and glutamine-derived glutamate are channelled similarly at this junction (*Table 7*). The labeled metabolites will need to be analyzed before the relative flux through these two pathways can be quantitatively determined.

Previous data[12,15] suggested that little of the glutamine carbon re-enters the TCA cycle. The present results show that the glutamine carbon that does re-enter does so primarily via pyruvate dehydrogenase (PDH) rather than via pyruvate carboxylase (PCB) (*Table 2*). The relative importance of PDH for re-entry of glutamine carbon is not affected by the metabolic interaction between glucose and glutamine and is important for cells from both the proximal and distal segments of the small intestine.

Glutamine metabolism and the effects of glucose vary by segment of small intestine

These studies demonstrated a gradient effect along the length of the small intestine for glutamine metabolism. In particular, carbon dioxide production from glutamine was significantly greater in the proximal than distal small intestine (*Figure 1, Table 2*) which is consistent with our previous observation that glutamine oxidation was greater in cells isolated from the jejunum than colon.[21] This may be accounted for by the decreasing gradient along the intestinal tract for glutaminase.[23,24] The interactive effects of glucose on glutamine oxidation varied also along the length of the small intestine as glucose significantly increased carbon dioxide production from glutamine only in the proximal small intestine (*Table 2, Figure 1*). Flux of glutamine carbon into the TCA cycle and its oxidation were much higher in the proximal than in the distal small intestine (*Table 2*) which may be necessary for metabolizing dietary glutamine and the endogenous glutamine which must be primarily metabolized in cells of the proximal small intestine since a greater proportion of arterial blood passes through arteries in the proximal than in the distal small intestine.[40] Cells of the proximal segments may need a higher metabolic and synthetic capacity to support their higher rates of cellular proliferation,[39] and their higher need for synthesis of membrane cholesterol and phospholipids to aid in lipid absorption and metabolism. Thus, the metabolic interactions between glucose and glutamine would be of most benefit to the animal in the proximal small intestine and would be of relatively less importance in distal segments.

In conclusion, the oxidation of glutamine is greater in cells of the proximal than of the distal small intestine, and the stimulatory effect of glucose on glutamine oxidation is limited to the proximal small intestine. Transamination appears to be of major importance in this metabolic interaction. These processes appear to facilitate entry of large quantities of glutamine carbon into the TCA cycle by using glucose-derived pyruvate as the amino group acceptor, and this is associated with efflux of glutamine carbon from the TCA cycle with little re-entry and incomplete oxidation of glutamine carbon. Thus, the interactive effects of glucose and glutamine metabolism may be mediated by aminotransferase activity.

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