# Oxidation of glucose, glutamate, and glutamine by isolated ovine enterocytes in vitro is decreased by the presence of other metabolic fuels[1,2]

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**ABSTRACT:** The objective of this study was to evaluate oxidative metabolism of glucose, glutamate, and glutamine by isolated ovine enterocytes in the presence of other metabolic fuels in vitro. A mixed mucosal primary cell culture containing enterocytes was isolated from crossbred wether sheep (n = 6) fed a mixed forage-concentrate diet and incubated for 90 min with 1 m*M* U-$^{14}$C-glucose, -glutamate, or -glutamine and additional substrates (water as negative control, acetate, propionate, butyrate, glucose, glutamate, or glutamine) at concentrations of 0.1, 1.0, and 10.0 m*M*. Oxidation of labeled substrates to $CO_2$ and net production of lactate and pyruvate in incubation media were measured. Oxidation of glucose and glutamine to $CO_2$ was decreased ($P < 0.05$) by 5 to 40% in the presence of additional substrates except acetate. Our observation that glutamine oxidation can be decreased by the presence of additional substrates is contrary to observations in the literature using enterocytes from nonruminants, indicating that ruminant enterocytes might rely on glutamine to a lesser extent as an energy source. Net glucose utilization was decreased ($P < 0.05$) 16% by propionate (10 m*M*) compared with control but was not affected by the other additional substrates. Glutamate oxidation to $CO_2$ was decreased 28% ($P < 0.05$) in the presence of propionate (10 m*M*) or by 17 and 33% in the presence of glutamine (1.0 and 10 m*M*, respectively), but not by that of the other additional substrates. Acetate did not affect the oxidation of glucose, glutamate, and glutamine. Propionate decreased ($P < 0.05$) the oxidation of glucose and glutamate only at the highest concentration (10 m*M*), indicating that the sparing effects of propionate on substrate oxidation are affected by its concentration in the incubation media. These observations indicate that ruminant enterocytes possess metabolic flexibility for oxidative metabolism of glucose, glutamine, and glutamate depending on the type and concentration of available additional substrates.

Key Words: Carbon Dioxide Production, Duodenum, Oxidative Metabolism, Sheep

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## Introduction

The small intestine is the primary site of digestion and absorption of dietary N, yet some dietary amino acids are extensively catabolized on the first pass by the mucosal cells of small intestine (Stoll et al., 1998; Wu, 1998). Although supplying additional metabolic fuels is expected to decrease oxidative metabolism and the use of individual amino acids by gut tissues (Seal et al., 1992; Seal and Parker, 1996; Noziere, et al., 2000), findings in the literature have not been consistent (Balcells et al., 1995; Taniguchi et al., 1995; Meijer et al., 1997). Nutrient uptake by the gut tissues affects hepatic metabolism and nutrient supply to peripheral tissues, yet the metabolic basis for oxidative metabolism in the gut tissues is not well understood.

Glucose and glutamine are major oxidative substrates for energy generation in gut tissues (Windmueller and Spaeth, 1980; Okine et al., 1995), and substrate preference for oxidative metabolism by enterocytes has been previously studied in vitro (Okine et al., 1995; Fleming et al., 1997; James et al., 1999). However, substrate-level metabolic interactions were evaluated only with a single concentration of glucose and glutamine for those studies, and, thus, little is known about metabolic interactions with other substrates and their concentration dependence on the preference for oxidative metabolism. Although the contribution of glutamate to mucosal oxidative metabolism was studied to

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[2] Mention of trade name, propriety product, or specific equipment does not constitute a guarantee or warranty by USDA and does not imply its approval to the exclusion of other products that may be suitable.

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**Table 1.** Ingredient and chemical composition of experimental diet

| Ingredient | %, DM basis |
|---|---|
| Alfalfa hay | 55.0 |
| Ground corn | 40.0 |
| Soybean meal | 3.5 |
| Ammonium chloride | 0.5 |
| Premix of salt and trace mineral[a] | 0.5 |
| Dicalcium phosphate | 0.45 |
| Premix of vitamins A, D, and E[b] | 0.05 |
| Chemical composition | |
| DM, % | 88.6 |
| NDF, % DM | 32.8 |
| ADF, % DM | 22.6 |
| CP, % DM | 16.6 |
| Ether extracts, % DM | 4.4 |
| Ca, % DM | 1.11 |
| P, % DM | 0.35 |

[a]Minimum composition (%): NaCl, 92; Zn, 0.80; Fe, 0.55; Mn, 0.24; Cu, 0.067; I, 0.0067; Co, 0.0067; Se, 0.00016.
[b]Composition (IU/kg): vitamin A, 5,291,040; vitamin D, 1,322,760; vitamin E, 11,023.

some extent (Bergman, 1975), its interactions with other substrates have not been assessed in ruminants. As a result of the flow of ruminally produced VFA to the duodenum (Rupp et al., 1994), the use of VFA as additional metabolic fuels may also be important. Therefore, the objective of this study was to determine the extent to which oxidative metabolism of glucose, glutamate, and glutamine by isolated ruminant enterocytes is affected by the presence of acetate, propionate, butyrate, glucose, glutamate, or glutamine across a range of concentrations.

## Materials and Methods

### *Animal and Diets*

Duodenal mucosal enterocytes (**DME**) were collected from six growing crossbred wether lambs (Hampshire-Suffolk; 44.0 ± 1.9 kg) purchased from a commercial sheep farm in Maryland. Wethers were housed in individual pens at the USDA-ARS research facility (Beltsville, MD) and fed a pelleted diet containing 55% forage and 45% concentrate (DM basis; Table 1) ad libitum for at least 8 wk before slaughter. Average DMI, daily gain, and BW at slaughter were 2.1 ± 0.1 kg/d, 0.34 ± 0.04 kg/d, and 60.6 ± 1.6 kg, respectively. All animal procedures were preapproved by the Beltsville Agricultural Research Center Institutional Animal Care and Use Committee (Protocol No. 02-008).

### *Isolation of Enterocytes*

Animals were slaughtered using a stun gun followed by exsanguination. A segment of the duodenum (1 m) was taken between 1 to 2 m distal to the pylorus immediately after slaughter (within 5 min), and rinsed with isotonic buffer (Krebs-Ringer buffer with 25 m*M* HEPES; **KRB-HEPES**; oxygenated with $O_2$:$CO_2$ [95:5], pH 7.4, 37°C) to remove feed particles and mucus. Duodenal segments were split longitudinally and mucosa was scraped off from underlying musculature using a glass slide, minced, and placed in a 500-mL Erlenmeyer flask containing digestion solution (37°C; 250 mL; collagenase 300 units/mL; dispase 0.1 mg/mL; $CaCl_2$ 0.14 mg/mL). Following transport to the laboratory (approximately 10 min), the scraped mucosa in digestion solution was incubated in a forced-air orbital shaker (Model 3527 LabLine Instruments, Melrose Park, IL) at 37°C for 20 min. Liberated DME were separated from mucosal remnants by sequential filtration through a 1000-$\mu$m and a 300-$\mu$m polypropylene mesh (Spectra/Mesh, Spectrum Laboratory Products, Los Angeles, CA) without vacuum. Filtrate containing liberated DME was centrifuged at 60 × *g* for 6 min (Centra-MP4R, International Equipment Company, Neeham Heights, MA). Supernatant was discarded, and the pellet containing DME was washed with KRB-HEPES and centrifuged again at 60 × *g* for 6 min. The resulting pellet was suspended in KRB-HEPES, and cell yield and viability were determined by hemacytometer counting and 0.4% trypan blue dye exclusion techniques, respectively (Baldwin and McLeod, 2000). Cell yield and viability for this experiment were $2.5 \times 10^9 \pm 0.4 \times 10^9$ and 85.0 ± 2.0%, respectively (n = 6).

### *Incubations and Metabolite Analyses*

Immediately after cell yield and viability were determined, a mixed mucosal primary cell culture containing enterocytes was incubated with either 1 m*M* D-[$^{14}$C(U)]glucose, 1 m*M* L-[$^{14}$C(U)]glutamate, or 1 m*M* L-[$^{14}$C(U)]glutamine (Moravek Biochemicals; Brea, CA). Each labeled substrate was added to a final concentration of 0.1 $\mu$Ci per flask (33.3 $\mu$Ci per millimole of substrate), and its quantitative contribution was approximately 0.01% of total substrates and was similar across treatments. Treatments were five types of additional substrates (**AS**) at three concentrations plus water control. The AS were acetate, propionate, butyrate, glucose, glutamate, and glutamine at 0.1, 1.0, and 10.0 m*M*, which were selected to reflect the wide range of possible substrate concentrations in the arterial blood (Quigley and Heitmann, 1991; Seal et al., 1992) or duodenal digesta (Okine et al., 1994; Rupp et al., 1994). Each treatment combination was applied to triplicate incubation flasks. Incubation media (KRB-HEPES with 0.12 *M* sodium bicarbonate) was fully oxygenated with $O_2$:$CO_2$ (95:5) and pH adjusted to 7.4 before addition to incubation flasks. Incubation was initiated by addition of 0.5 mL of the cell suspension ($1 \times 10^7$ viable cells) to 2.5 mL of incubation media freshly gassed (20 s under 95:5 $O_2$:$CO_2$) in 25-mL Erlenmeyer flasks. Flasks were sealed with a rubber serum cap fitted with a suspended center well containing filter paper, and placed into a heated (37°C) reciprocal-action shaking water bath (Precision Model 50 Jouan, Cedex, France). Incubations

**Table 2.** Effects of additional substrates at three concentrations on glucose metabolism by $1 \times 10^6$ of isolated duodenal mucosal cells in 90-min incubation (n = 6)

| | | Additional substrates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | Acetate | Propionate | Butyrate | Glutamate | Glutamine | SE | *P*-value |
| | Glucose oxidized to $CO_2$, nanomoles | | | | | | | |
| Control | 4.1 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 4.0 | 3.8 | 3.5 | 3.7 | 3.4 | 1.1 | 0.08 |
| 1.0 m*M* | — | 3.8 | 3.9 | 3.4* | 3.5* | 3.2* | 1.0 | 0.01 |
| 10.0 m*M* | — | 4.1 | 3.5* | 3.4* | 3.1* | 2.8* | 1.0 | 0.001 |
| | Lactate produced, nanomoles | | | | | | | |
| Control | 28.9 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 25.3* | 33.3* | 29.0 | 30.6 | 36.2* | 4.6 | 0.001 |
| 1.0 m*M* | — | 28.2 | 28.7 | 35.1* | 27.8 | 26.6 | 4.4 | 0.001 |
| 10.0 m*M* | — | 25.1* | 28.3 | 30.6 | 28.3 | 25.9 | 4.0 | 0.05 |
| | Pyruvate produced, nanomoles | | | | | | | |
| Control | 4.8 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 5.7 | 7.4 | 6.6 | 7.6 | 6.3 | 1.4 | 0.43 |
| 1.0 m*M* | — | 5.5 | 6.6 | 6.8 | 8.1 | 7.5 | 1.4 | 0.14 |
| 10.0 m*M* | — | 6.8 | ND[a] | ND | 9.0* | 9.5* | 1.4 | 0.001 |
| | Glucose utilized, nanomoles | | | | | | | |
| Control | 22.3 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 24.3 | 21.8 | 24.2 | 20.0 | 20.1 | 4.2 | 0.08 |
| 1.0 m*M* | — | 22.5 | 22.4 | 21.0 | 20.4 | 20.3 | 4.3 | 0.24 |
| 10.0 m*M* | — | 24.1 | 18.7* | 21.0 | 22.4 | 21.0 | 4.1 | 0.05 |
| | Glucose oxidized to $CO_2$, % of glucose utilized | | | | | | | |
| Control | 17.1 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 15.5 | 17.2 | 13.6 | 18.3 | 16.0 | 2.3 | 0.13 |
| 1.0 m*M* | — | 15.7 | 16.5 | 15.7 | 16.7 | 14.6 | 2.1 | 0.55 |
| 10.0 m*M* | — | 16.1 | 17.8 | 16.1 | 13.1* | 12.9* | 2.2 | 0.02 |

*Treatment means differ from control ($P < 0.05$).
[a]Not detected.

were terminated after 90 min by the addition of 0.2 mL of concentrated $HClO_4$ acid. Triplicate flasks were also prepared for assessment of endogenous metabolite concentrations (addition of cell suspension only) and 0-min metabolite production rates for each labeled substrate (acidified immediately after addition of cell suspension).

After incubations were terminated, center wells were filled with 0.3 mL of benzethonium hydroxide to capture $CO_2$ during incubation at room temperature for 1 h. Center wells were placed in scintillation vials and filled with 4 mL of scintillant (BioSafe II, Research Products International, Mount Prospect, IL) before liquid scintillation counting (Tri-Carb 1500, Packard Instrument Company, Meriden, CN). Stock solutions containing labeled substrates were also counted for the determination of specific activity, and the extent of oxidation was calculated for each substrate. The incubation media were neutralized with 0.3 mL of 5.8 *M* $K_2CO_3$, and clarified supernatant ($2,300 \times g$ for 7 min) was used for analysis of lactate (Sigma Procedure No. 826-UV), pyruvate (Sigma Procedure No. 726-UV), and glucose (Sigma Procedure No. 510-A) concentrations. All assays were previously modified for use on a microtiterplate reader (CERES 900HDI, Bio-Tek Instruments, Winooski, VT), and were conducted on the day of incubation. Net glucose utilization was calculated from the reduction in glucose concentration in incubation media compared to 0-min control. Originally, the utilization of glutamate and glutamine were to be evaluated but are not reported because glutamate recovery in supernatant after centrifugation of incubation media was inadequate (i.e., measured glutamate concentrations for 0-min control was approximately two-thirds of expected value and variable).

Statistical analysis was conducted using the Fit model procedure of JMP (version 4.0, SAS Inc., Cary, NC). All data were analyzed for effect of AS type within each concentration of AS for each labeled substrate separately, and random effect of animal was also included in the model. Treatment effects were declared significant at $P < 0.05$, and pairwise comparisons between control and each treatment were conducted if overall treatment effect is significant.

## Results

Glucose oxidation was decreased in the presence of 1 m*M* butyrate, glutamate, and glutamine by 17, 15, and 22%, respectively. Glucose oxidation was also decreased in the presence of 10 m*M* propionate, butyrate, glutamate, and glutamine by 15, 17, 24, and 32%, respectively, compared with control (Table 2). Lactate

**Table 3.** Effects of additional substrates at three concentrations on glutamine metabolism by $1 \times 10^6$ of isolated duodenal mucosal cells in 90-min incubation (n = 6)

| | | Additional substrates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | Acetate | Propionate | Butyrate | Glutamate | Glutamine | SE | *P*-value |
| | Glutamine oxidized to $CO_2$, nanomoles | | | | | | | |
| Control | 6.0 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 5.8 | 5.7* | 5.5* | 5.2* | 5.3* | 1.2 | 0.001 |
| 1.0 m*M* | — | 5.5 | 5.5* | 5.6 | 4.7* | 4.9* | 1.2 | 0.0001 |
| 10.0 m*M* | — | 5.5 | 4.5* | 5.0* | 4.3* | 3.6* | 1.2 | 0.0001 |
| | Lactate produced, nanomoles | | | | | | | |
| Control | 4.1 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 4.5 | 4.7 | ND[a] | 8.4* | 6.1 | 1.2 | 0.01 |
| 1.0 m*M* | — | 3.0 | 7.4 | ND | 24.6* | 7.1 | 1.4 | 0.0001 |
| 10.0 m*M* | — | 4.7 | 6.3 | ND | 30.9* | 8.0 | 1.8 | 0.0001 |
| | Pyruvate produced, nanomoles | | | | | | | |
| Control | ND | — | — | — | — | — | — | — |
| 0.1 m*M* | — | ND | ND | ND | ND | ND | N/A[b] | N/A |
| 1.0 m*M* | — | ND | ND | ND | 6.9 | ND | N/A | N/A |
| 10.0 m*M* | — | ND | ND | ND | 15.4 | ND | N/A | N/A |

*Treatment means differ from control ($P < 0.05$).
[a]Not detected.
[b]Not applicable.

production was decreased 13% by acetate at 0.1 m*M* and 10 m*M* compared to control but increased when glucose was incubated with propionate or glutamine at 0.1 m*M* or with butyrate at 1 m*M*, by 15, 25, and 21%, respectively. Pyruvate production was increased by approximately twofold when glucose was incubated with 10 m*M* of either glutamate or glutamine. When glucose was the sole substrate (control), lactate production accounted for the majority (69.9%) of glucose disappearance followed by $CO_2$ (17.1%) and pyruvate (10.5%) production, which is in agreement with previous observations (Okine et al., 1995). Glucose utilization averaged 21.7 nmol per million isolated enterocytes, or approximately 7% of glucose present in the incubation media (300 nmol per $1 \times 10^6$ cells). Thus, it is not likely that glucose utilization was limited by the availability of the substrate during incubations. Net glucose utilization was decreased by 10 m*M* propionate by 16% compared to control but was not affected by other AS. Glucose oxidation (percentage of net glucose utilization) was decreased in the presence of 10 m*M* glutamate and glutamine by 23 and 25%, respectively, indicating that catabolism of glutamate and glutamine, possibly associated with greater ammonia production, may affect glucose carbon metabolism.

Glutamine oxidation was decreased by 5 to 13% in the presence of 0.1 m*M* propionate, butyrate, glucose, or glutamine, by 8 to 22% in the presence of 1 m*M* propionate, glucose, or glutamine, and by 17 to 40% in the presence of 10 m*M* propionate, butyrate, glucose, or glutamine compared with control (Table 3). Presence of acetate at any concentration did not affect glutamine oxidation. Net lactate production for media containing labeled glutamine was not affected by AS with the exception of glucose, where approximately twofold to eightfold increases were observed. Similarly, pyruvate production was not detected except when glutamine was incubated with glucose at 1.0 and 10.0 m*M*.

Glutamate oxidation was decreased by glutamine at 1.0 and 10.0 m*M*, and propionate at 10.0 m*M* by 17, 33, and 28%, respectively (Table 4). Lactate production increased by fourfold to fivefold when glutamate was incubated with glucose at 1.0 m*M* and 10.0 m*M* compared to control, but was not detectable (<2.5 nmol·($1 \times 10^6$ cells)$^{-1}$·90 min$^{-1}$) when glutamate was incubated with propionate or butyrate, regardless of concentration. Pyruvate production was not detectable (<2.5 nmol·($1 \times 10^6$ cells)$^{-1}$ ·90 min$^{-1}$) except when glutamate was incubated with glucose at 1.0 and 10.0 m*M*.

## Discussion

Previous studies reported in the literature demonstrated that glucose and glutamine are important metabolic fuels for gut tissues (Windmueller and Spaeth, 1980; Okine et al., 1995), but it is not known whether gut tissues have a specific oxidative requirement for these substrates. Although several studies have evaluated substrate preference for in vitro oxidative metabolism by ruminant enterocytes (Okine et al., 1995) and nonruminant enterocytes (Wu et al., 1995; Fleming et al., 1997; James et al., 1999), comparisons were made with only a single concentration of glucose and glutamine. Thus, effects of AS concentration on substrate preference for oxidative metabolism are not yet established. In addition, although ruminant duodenal digesta contains relatively high concentrations of VFA (Rupp et al., 1994), a readily available metabolic fuel for the intestinal mucosa of ruminants, effects of VFA on substrate oxidative metabolism by enterocytes have

**Table 4.** Effects of additional substrates at three concentrations on glutamate metabolism by $1 \times 10^6$ of isolated duodenal mucosal cells in 90-min incubation (n = 6)

| | | Additional substrates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Item | Control | Acetate | Propionate | Butyrate | Glucose | Glutamine | SE | *P*-value |
| | Glutamate oxidized to $CO_2$, nanomoles | | | | | | | |
| Control | 1.8 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 1.8 | 1.8 | 1.8 | 1.7 | 1.6 | 0.4 | 0.06 |
| 1.0 m*M* | — | 1.8 | 1.7 | 1.7 | 1.8 | 1.5* | 0.4 | 0.01 |
| 10.0 m*M* | — | 1.7 | 1.3* | 1.7 | 1.6 | 1.2* | 0.3 | 0.0001 |
| | Lactate produced, nanomoles | | | | | | | |
| Control | 6.3 | — | — | — | — | — | — | — |
| 0.1 m*M* | — | 6.3 | ND[a] | ND | 8.1 | 6.5 | 2.7 | 0.01 |
| 1.0 m*M* | — | 7.3 | ND | ND | 24.8* | 8.9 | 2.7 | 0.0001 |
| 10.0 m*M* | — | ND | ND | ND | 33.5* | 10.4 | 3.0 | 0.0001 |
| | Pyruvate produced, nanomoles | | | | | | | |
| Control | ND | — | — | — | — | — | — | — |
| 0.1 m*M* | — | ND | ND | ND | ND | ND | N/A[b] | N/A |
| 1.0 m*M* | — | ND | ND | ND | 6.1 | ND | N/A | N/A |
| 10.0 m*M* | — | ND | ND | ND | 10.2 | ND | N/A | N/A |

*Treatment means differ from control ($P < 0.05$).
[a]Not detected.
[b]Not applicable.

not been extensively studied. Reeds et al. (2000) suggested that glutamate contributes to intestinal oxidative metabolism to the same extent as glutamine, with nearly 100% of glutamate removed on first pass across the pig intestines. In ruminants, net flux data indicate that glutamate is an important contributor to intestinal metabolism (Tagari and Bergman, 1978); however, the contribution of glutamate to mucosal oxidative metabolism and its metabolic interactions with other substrates have not been determined. We hypothesized that ruminant gut tissues possess flexibility for oxidative metabolism by utilizing AS as oxidative fuels. The current experiment was designed to determine the extent to which oxidative metabolism of glucose, glutamate, and glutamine by isolated ovine enterocytes is affected by presence of AS across a range of concentrations.

### *Glucose Utilization*

Oxidation of U-$^{14}$C glucose to $CO_2$ was reduced by presence of all AS except acetate; however, net disappearance of glucose was not affected by AS except propionate (10 m*M*), which reduced glucose disappearance by 16% compared with control. Propionate carbon enters the Krebs cycle as succinyl CoA and can be metabolized to oxaloacetate and pyruvate, and thus might partially spare glucose carbon utilization for oxidation in the Krebs cycle or alanine synthesis. In contrast, the presence of acetate or butyrate did not affect glucose utilization possibly because acetate and butyrate enter the Krebs cycle as an acetyl CoA and the cells still require glucose carbon for synthesis of pyruvate and oxaloacetate. The addition of glutamate or glutamine did not affect net glucose utilization but decreased glucose oxidation (percentage of net glucose utilization) possibly because glucose carbon is used for other metabolic purposes. Glutamate and glutamine shifted the glucose catabolism toward pyruvate production in contrast to the report by Okine et al. (1995) with dairy cattle enterocytes, in which glutamine addition to glucose flasks resulted in a greater net release of alanine and glutamate with a decrease in pyruvate and lactate production. Fleming et al. (1991) suggested that the demand for pyruvate as an acceptor of amino N is likely increased because oxidative metabolism of glutamate and glutamine requires a transamination reaction. In the current experiment, alanine production was not measured; thus, the reason for increased pyruvate production resulting from glutamate or glutamine additions to incubation media containing glucose cannot be definitively established.

Glucose is extensively metabolized in gut tissues, and understanding the metabolic basis for this apparent glucose requirement in gut tissues is essential to maximizing glucose conservation in gut tissues to improve overall energetic efficiency in ruminant animals. The current experiment showed that the utilization of glucose carbon can be spared by propionate in a concentration-dependent manner. Thus, propionate passage from the rumen could conceivably contribute to the conservation of glucose for peripheral utilization. Moreover, the oxidation of glucose carbon is decreased by propionate, butyrate, glutamate, and glutamine. Reduction in glucose oxidation in gut tissues might result in greater net availability of glucogenic precursor supply for the liver to synthesize glucose.

### *Glutamine Oxidation*

In similarity with our findings with glucose, the oxidation of U-$^{14}$C glutamine was decreased by all AS ex-

cept acetate, but the extent of reduction differed by type and concentration of AS. Our observations are contrary to studies using enterocytes isolated from rats (Fleming et al., 1997) and pigs (Wu et al., 1995). In rats, 5 m*M* glutamine reduced the production of $CO_2$ from 5 m*M* [U-$^{14}$C] glucose compared with basal glucose oxidation, whereas $CO_2$ production from [U-$^{14}$C] glutamine was not affected by glucose addition (Fleming et al., 1997). Similarly, Wu et al. (1995) incubated neonatal pig enterocytes in the presence of 2 m*M* [U-$^{14}$C] glutamine with or without 5 m*M* glucose, or 5 m*M* [U-$^{14}$C] glucose with or without 2 m*M* glutamine, and reported that glucose addition did not affect glutamine oxidation, whereas glutamine addition decreased glucose oxidation. In fact, several studies using isolated rat enterocytes have demonstrated that glucose increases glutamine entry into the Krebs cycle and oxidation rather than decreasing glutamine oxidation (Kight and Fleming, 1995; Beaulieu et al., 2002). The stimulatory effect of glucose on glutamine oxidation has been attributed to an increase in the flux through pyruvate and a stimulation of the transamination of glutamine amino N (Cremin and Fleming, 1997). These studies using isolated enterocytes from nonruminants consistently demonstrate that glucose oxidation can be spared by glutamine, but the presence of glucose does not decrease glutamine oxidation.

Similar to the current data from ovine enterocytes and in contrast to data from nonruminant enterocytes, Okine et al. (1995) reported that 6 m*M* glucose decreased 4 m*M* [U-$^{14}$C] glutamine oxidation and 4 m*M* glutamine decreased 6 m*M* [U-$^{14}$C] glucose oxidation by enterocytes isolated from dairy cows. One possible explanation for the apparent inconsistency across the literature regarding sparing effects of glucose on glutamine oxidation is found in the differences in substrate concentrations: both labeled substrate and AS. Rate of oxidation differs depending on the concentration of substrate, and each substrate has a different $V_{max}$ and $K_{ox}$ (Kight and Fleming, 1993; Baldwin and Mcleod, 2000). Thus, differences in the substrate concentration used can greatly influence the metabolism of the substrate and interpretation of the results.

An alternative explanation is that ruminant intestinal tissues have a specific requirement for glutamine oxidation that is lower than that of nonruminant intestinal tissues. This contention is supported by the differential glutamine utilization by gut tissues across species (Watford, 1999), and site along the gastrointestinal tract (Fleming et al., 1991). For example, glutamine oxidation by rat colonocytes was reduced by the addition of AS (Fleming et al., 1991), and a stimulatory effect of glucose on glutamine oxidation was observed in proximal but not distal small intestine (Kight and Fleming, 1995). Ruminant DME may have less reliance on glutamine as an oxidative fuel compared with nonruminant enterocytes; Gate et al. (1999) showed that the portal-drained viscera utilize the majority of glutamine primarily for macromolecule synthesis rather than the provision of energy.

### *Glutamate Oxidation*

In the current experiment, glutamate oxidation was only decreased by glutamine and propionate, suggesting that ruminant enterocytes have specific needs for glutamate oxidation that are not spared by the other AS. It is noteworthy that glutamine oxidation was reduced by all AS except acetate. Specific effects of AS on the oxidation of glutamine and glutamate were expected to be similar because glutamine catabolism leads to glutamate (Pinkus and Windmueller, 1977) and both glutamate and glutamine carbon enter the Krebs cycle as $\alpha$-ketoglutarate. The apparent differential effects of AS treatment on the oxidation of glutamate and glutamine may arise as a result of the lower glutamate oxidation relative to glutamine, making the detection of treatment effects more difficult. However, our observations cannot preclude the possibility that glutamate carbon is metabolized differently from glutamine carbon by enterocytes.

Quan et al. (1998) suggested that the transport rate of glutamate to cells or mitochondria is slower than that of glutamine, and that glutamine and glutamate may be catabolized in different intracellular compartments due to the existence of multiple intracellular glutamate pools within enterocytes. Additionally, glutamate carbon entry to the Krebs cycle does not necessarily mean that complete oxidation will occur (Quan et al., 1998). For example, glutamate carbon might be utilized as a carbon skeleton for the synthesis of other nonessential amino acids (e.g., alanine or aspartate) after partial oxidation in the Krebs cycle. Nonetheless, a metabolic basis accounting for the differential effects of AS on glutamate and glutamine oxidation is not known, and further research is needed to determine whether enterocytes have specific needs for glutamate oxidation.

### *Effects of Volatile Fatty Acids*

In the current experiment, acetate did not affect the oxidation of glucose, glutamate, or glutamine, even though acetate oxidation to $CO_2$ by ovine DME in vitro has been observed previously (R. L. Baldwin, VI and K. R. McLeod, unpublished data), and the mesenteric-drained viscera (mostly small intestine) utilizes acetate from arterial circulation (Seal and Parker, 1994). Therefore, the lack of a sparing effect of acetate in the current experiment is not attributable to the limited capacity of DME to utilize acetate. Propionate reduced the oxidation of glucose and glutamate only when included at the highest concentration (10 m*M*). This may be attributable to a high $K_m$ or low cellular affinity for propionate. Seal and Parker (1996) evaluated the effect of intraruminal infusion of propionate at two dose levels on amino acid metabolism by gut tissues of steers. Con-

sistent with our observations, they reported that propionate infusion elicited a dose-dependent effect on the net absorption rate of glutamine and glutamate; propionate infusion at 1.0 mol/d increased the net absorption of glutamine and glutamate across mesenteric-drained viscera compared to propionate infusion at 0.5 mol/d.

The concentration of propionate in digesta flowing to the duodenum might be a potentially important factor affecting oxidative metabolism in the small intestine in vivo. High-concentrate diets often increase the net absorption of amino acids compared with high-forage diets (Seal et al., 1992), and this might be due in part to a greater propionate passage to the duodenum associated with feeding high-concentrate diets, thus reducing the oxidative metabolism of amino acids. Rupp et al. (1994) showed that total VFA concentration in duodenal digesta ranged from 7 to 14 m*M*, of which propionate represents 16 to 25%. Thus, physiological concentrations of propionate are expected to be within the range evaluated in the current experiment (1 and 10 m*M*). The presence of 10 m*M* propionate decreased the oxidation of glucose and glutamate, whereas 1 m*M* propionate did not. Nonetheless, these observations demonstrate that the oxidative metabolism and substrate preference of DME can be altered by other available substrates, and, thus, altering the site of nutrient delivery through diet formulation may be a viable method of preserving glucose and amino acid carbon past the intestinal barrier.

## Implications

Ruminant duodenal mucosal enterocytes possess the metabolic flexibility for oxidative metabolism of glucose, glutamine, and glutamate. All additional substrates except acetate decreased the oxidation of glucose and glutamine, whereas only glutamine and propionate decreased glutamate oxidation. Sparing effects of additional substrates on glutamine oxidation found in this experiment are distinct from experiments in which enterocytes from nonruminants were investigated and might suggest a different extent of reliance on glutamine as an energy source between ruminant and nonruminant enterocytes. The sparing effect of propionate on the oxidation of glucose, glutamate, and glutamine differed by its concentration, indicating that oxidative metabolism in duodenal mucosal enterocytes is potentially altered by diet formulation. More research is warranted to elucidate the metabolic basis for oxidation of glucose, glutamine, and glutamate in ruminant gut tissues.

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