Intestinal Atrophy Has a Greater Impact on Nitrogen Metabolism than Liver By-Pass in Piglets Fed Identical Diets via Gastric, Central Venous or Portal Venous Routes\textsuperscript{1,2}

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ABSTRACT Whole-body nitrogen metabolism is altered during parenteral feeding as a result of gut atrophy and/or lack of splanchnic first-pass metabolism. We developed in vivo models to describe the metabolic and physiologic effects of first-pass metabolism by the small intestine/liver, liver or non-splanchnic tissues. Fifteen 2- to 4-d-old piglets were fed identical diets continuously for 8 d via gastric (IG), portal (IP) or central venous (IV) catheters. Despite similar weight gain, IV and IP pigs had higher nitrogen output and hence lower nitrogen retention (80%) compared with IG pigs (87%) (P = 0.002). Body protein content was also higher in IG pigs (583 mg/g dry matter) compared with IV (550) and IP pigs (534) (P = 0.003). Despite similar intestinal lengths, total small intestinal and mucosal weights were ~40% lower in IV and IP pigs than in IG pigs. Free urea cycle amino acids were altered in plasma and mucosa, suggesting that limited arginine synthesis by an atrophied gut may have limited protein deposition. Although villous atrophy was observed in the duodenal and jejunal of IV and IP pigs, reduced crypt depth was observed only in IV pigs. Crypt depth was similar in all four gut sections from IG and IP pigs, suggesting that nutrient flux through the liver affects gut growth. Overall, metabolic responses to IV (non-splanchnic) and IP (liver) feeding were similar as a result of gut atrophy, whereas responses to IG (small intestine + liver) and IP (liver) feeding were different, suggesting that small intestinal atrophy affects nitrogen metabolism to a greater extent than liver by-pass. J. Nutr. 129: 1045–1052, 1999.

KEY WORDS: • nitrogen • small intestine • liver • route of feeding • neonatal piglets

Several studies have shown that total parenteral nutrition (TPN)\textsuperscript{4} feeding alters whole-body nitrogen metabolism compared with oral feeding (Duffy and Pencharz 1986, Jeewanandan et al. 1987, Lanza-Jacoby et al. 1982). In oral feeding, the small intestine and the liver metabolize nutrients extensively before delivery to the extraplanchnic tissues, whereas parenteral feeding involves infusion of total nutrients via a central vein, by-passing nutrient absorption and first-pass metabolism by splanchnic tissues. The quality and quantity of nutrients delivered to the extraplanchnic tissues differ substantially between the two routes of feeding, thus affecting whole-body nitrogen utilization and growth. The different nitrogen utilization as a consequence of parenteral feeding may be due to reduced gastrointestinal metabolism associated with gut atrophy and/or the lack of hepatic first-pass metabolism.

As a consequence of by-passing splanchnic organ metabolism, gut atrophy (Adeola et al. 1995, Goldstein et al. 1985, Johnson et al. 1975, Shulman 1988) or hypotrophy (Czemichow et al. 1992, Hughes and Dowling 1980) is a common observation during prolonged parenteral feeding. Although the effect of parenteral vs. oral nutrition on gut and organ growth has been studied (Adeola et al. 1995, Goldstein et al. 1985, Johnson et al. 1975, Shulman 1988), the relative importance of nutrient processing by the small intestine vs. the liver has not been elucidated.

The neonatal small intestine of piglets is very important in amino acid metabolism in that it is a major site of arginine synthesis (Stoll et al. 1998, Wu and Knabe 1995) and has a disproportionately high requirement for threonine (Bertolo et al. 1998, Stoll et al. 1998). Both of these factors are exacerbated in parenterally fed, compared with gastrically fed piglets (Bertolo et al. 1998, Brunton et al. 1998). Therefore, we hypothesized that small intestinal atrophy during parenteral feeding was the main factor leading to impaired nitrogen...
utilization. We chose to address this hypothesis by continuously feeding piglets identical diets via different routes in which first-pass metabolism of infused amino acids was different. We included intragastrically fed (IG) "control" piglets with intact splanchnic (small intestine and liver) nutrient absorption and first-pass metabolism as well as intravenously fed (IV) piglets with first-pass metabolism of nutrients by non-splanchnic tissues. To separate the metabolic contribution of the small intestine, we included intraportally fed (IP) piglets in which nutrient delivery to the liver and hepatic first-pass metabolism were maintained. By comparing the gut growth and nitrogen utilization among these groups, we could evaluate the importance of the small intestine compared with the liver for whole-body nitrogen metabolism when nutrients are presented enterally.

MATERIALS AND METHODS

Animals and surgical procedures. Fifteen intact male Yorkshire piglets were obtained from the University of Guelph's minimal disease herd at 2–4 d of age. Piglets were removed from the sow and transported to the laboratory where they immediately underwent surgery to implant catheters. All procedures used in this study were approved by the Animal Care Committee of the University of Guelph.

Piglets were between 1.4 and 1.8 kg at arrival and were blocked by body weight among the three treatments. Using a modified method of Wykes et al. (1993) and Rombeau et al. (1984), custom-made Silastic catheters (Ed-Art, Don Mills, Canada) were installed with the use of aseptic technique. Catheters were tunneled under the skin from the point of exit on the left side of the chest to the points of entry into the blood vessels or stomach. Feeding catheters were installed in the stomach for IG piglets, in the jugular vein for IV piglets and in the umbilical vein for IP piglets; all pigs were fitted with a femoral vein catheter for blood sampling. In IG pigs, a Stamm gastrostomy was performed with a Silastic tube (Rombeau et al. 1984). The jugular catheter was inserted into the left jugular vein and advanced to the heart. The umbilical catheter was introduced transperitoneally into the umbilical vein and advanced to the portal-hepatic junction. The femoral catheter was introduced into the left femoral vein and advanced into the inferior vena cava just caudal to the heart. IV pigs also underwent a sham operation in which the abdomen and peritoneum were incised and sutured.

Piglets were housed individually in circular metabolic cages allowing visual and audio contact with other piglets. Toys and blankets were provided for environmental enrichment. The room was lighted from 0800 to 2000 h and was maintained at 28°C with supplemental heat provided by heat lamps. Piglets and cages were cleaned daily.

An elemental and complete diet (described in Wykes et al. 1993) was fed continuously via one of the feeding routes (IG, IV or IP) for 8 d after surgery. Diet was administered through a tether-swivel system (Alice King Chatham Medical Arts, Los Angeles, CA) using pressure-sensitive infusion pumps (IV, IP; lipid: Intralipid 20%, Pharmacia-Upjohn, Stockholm, Sweden) was infused simultaneously via the gastrostomy catheter, diluted 2.5-fold for 12 h, 1.5-fold for another 12 h and then full strength [480 mL/(kg · d)] for 7 d. The sterile TPN solutions were manufactured by the Parenteral Service Pharmacy at The Hospital For Sick Children, Toronto, Canada as previously described (Wykes et al. 1993). TPN was stored in the dark at 4°C until used; immediately before use, vitamins (MVI Pediatric, Rhone-Poulenc Rorer Canada, Montreal, Canada) and minerals (Micro-6 concentrate, Sabex, Boucherville, Canada) were added.

The IG diet was made as above, except that the solutions were not filtered sterilized. Furthermore, distilled water (1.5-fold dilution to lower osmolarity) and the appropriate amount of lipid were added to the final solutions. Infusion rates of the IG diets were increased so that energy and nitrogen intake rates were identical among groups (after adaptation). Piglets were weighed each morning and infusion rates were adjusted accordingly.

Tissue collection. Blood samples were collected into heparinized syringes on d 8 via the femoral catheter. Whole blood samples were centrifuged (3000 × g for 5 min) and plasma was collected and frozen at −20°C for further analyses. Urine was collected on ice in acidified containers for 24 h during the final 4 d of the protocol; volumes were measured and samples were stored at −20°C. Fecal samples for the 4-d period were also collected by placing a fine mesh wire screen in the funnels.

Piglets were killed by lethal injection of 750 mg of sodium pentobarbital on d 8. Liver and kidneys were removed,blotted dry, and weighed; samples were excised and frozen at −70°C for further analyses. The small intestine was removed from the mesenteric sheath, emptied, and the entire length and weight measured. The duodenum was removed, emptied, blotted dry, measured for length and weight and kept for mucosa measurements. A 1-cm segment was also kept in neutral buffered 10% formalin (Sigma Chemical, St Louis, MO) for histologic analyses. Excluding the first 10 cm of proximal jejunum, the next 60 cm of jejunum was excised for mucosa collection and another 1 cm was stored in buffered 10% formalin. Similarly, 60 cm of the ileal (minus the last 10 cm) and mid-jejunal segments was collected and kept on ice.

Mucosa analyses. Small intestinal segments were flushed with saline, blotted dry, and length and weight were accurately measured. The segment was uncoiled and its length was measured by suspension under its own weight. Segments were then slit lengthwise and the mucosa was scraped, weighed, frozen in liquid nitrogen and stored at −70°C for further analysis. For each of the segments, data were expressed as small intestinal weight per length of segment (mg/cm) and mucosal weight per length of segment (mg/cm). By using a calculation similar to that of Zhang et al. (1997), total mucosa weight was estimated by multiplying the mucosal weight per centimeter by the section length and summed. The section length for the proximal

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### Table 1

Amino acid (AA) profile of the diet fed to 2- to 4-d-old piglets

<table>
<thead>
<tr>
<th>L-Amino acid</th>
<th>mg/g AA</th>
<th>Semi-Indispensable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>31</td>
<td>Arginine</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>46</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Leucine</td>
<td>103</td>
<td>Proline</td>
</tr>
<tr>
<td>Lysine</td>
<td>82</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Methionine</td>
<td>19</td>
<td>Taurine</td>
</tr>
<tr>
<td>Phenylyalanine</td>
<td>80</td>
<td>Dispensable</td>
</tr>
<tr>
<td>Threonine</td>
<td>53</td>
<td>Alanine</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>21</td>
<td>Aspartate</td>
</tr>
<tr>
<td>Valine</td>
<td>53</td>
<td>Glutamate</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>488</strong></td>
<td><strong>Glycine</strong></td>
</tr>
<tr>
<td><strong>Serine</strong></td>
<td><strong>55</strong></td>
<td><strong>Serine</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>513</strong></td>
<td></td>
</tr>
</tbody>
</table>
jejunum and ileum were considered to be 25% each of the duodenum-
free total small intestinal length, and the medial jejunum was
considered to be 50% of the total. These proportions approximated the
total tissue weight distribution and were thus applied to the mucosal
weight data. Data were expressed as absolute values. Because mucosal
hydration was not different among groups (IG: 85.3%, IV: 84.6, IP:
83.9, pooled sd: 1.4), wet weight of mucosa was used in relevant
measurements.

Histological samples were prepared by the histology laboratory of
the Ontario Veterinary College at the University of Guelph. After
fixation in 10% buffered formalin, tissues were dehydrated in several
isopropanol concentrations, cleared in xylene and embedded in par-
affin wax. Transverse sections were cut using a standard microtome
knife (4- to 6-μm segments) and then stained with hematoxylin and eosin. Slides were examined under a light micro-
scope equipped with an ocular micrometer (Axioskop 20, Zeiss,
Oberkochen, Germany) at 100X magnification. Villi and crypts were
measured only when there was a complete longitudinal section of a
villus and its associated crypt. The heights of the best oriented villi
were measured from the tip to the crypt mouth and the depths of
associated crypts were measured from the crypt mouth to the base. At
least 10 villi and crypts were measured in each slide. To reduce
variability in measurements, all slides were examined by the same
technician.

Nitrogen balance. Nitrogen in urine and diet were analyzed by
combustion (Instrument FP-428, LECO Instruments, Mississauga,
Canada). Nitrogen balance (Nbal) was calculated as follows:

\[ N_{\text{bal}} [\text{mg/(kg \cdot d)}] = N_{\text{in}} [\text{mg/(kg \cdot d)}] - N_{\text{out}} [\text{mg/(kg \cdot d)}] \]

Nitrogen retention (Nret) was calculated as follows:

\[ N_{\text{ret}} (%) = \frac{N_{\text{bal}} [\text{mg/(kg \cdot d)}]}{N_{\text{in}} [\text{mg/(kg \cdot d)}]} \times 100 \]

Due to negligible fecal outputs, Nout was equal to urinary nitrogen
only.

Analyses of bodies. Entire piglet bodies (excluding tissue samples
removed) were frozen at −20°C, ground twice (model M802 2 HP
Hobart Mixer, Hobart Manufacturing, Don Mills, Canada), and
freeze-dried for 96 h (Virtis Coil 5 HP freeze dryer, Virtis, Gardiner,
NY). The dried sample was then regrind with a coffee grinder and
analyzed for fat, ash, analytical dry matter and total energy with
methods of the AOAC (1990). Total nitrogen was analyzed with the
use of combustion methods; a factor of 6.25 g protein/g nitrogen was
used to convert nitrogen to protein. Data for each pig were normal-
ized by reversed-phase HPLC. For plasma free amino acids, 200 μL
plasma was mixed with an internal standard (norleucine) and a
protein precipitant (0.5 mL trifluoroacetic acid/100 mL methanol),
mixed with a vortex mixer and centrifuged at 3000 x g for 5 min to
remove proteins. For mucosal (proximal jejunum) free amino acids,
norleucine was added to 100 μg of wet tissue, which was homoge-
nized in 1 mL trifluoroacetic acid/100 mL methanol and centrifuged
(5000 x g for 10 min). The pellet was homogenized and centrifuged
(5000 x g for 10 min), and the supernatants were pooled. For both
plasma and tissues, phenylisothiocyanate derivatives for reversed-
phase HPLC were prepared as described by Bidlingmeyer et al.

Statistical analyses. Data were analyzed by one-way ANOVA
followed by Tukey’s multiple comparisons between groups (Version
7.1, Minitab, State College, PA) and were considered significant at
P < 0.05. Pooled sd are presented from ANOVA analyses.

RESULTS

Body weight gain. The d 3 (adapted) and d 8 (necropsy) body
weights were not different among groups. Furthermore, the rates of body weight gain were not different among groups

Nitrogen balance. Regardless of the route of feeding, the
fecal output of all pigs was negligible because of the highly
absorbable nature of the diet. In rapidly growing animals,
receiving elemental diets, the most important source of nitro-
gen losses is via the urine due to the deamination and oxida-
tion of amino acids. Therefore, urinary excretion of nitrogen
was considered total output. The IV and IP groups had higher
nitrogen outputs (P < 0.001) and lower nitrogen retentions (P
= 0.002) compared with the IG group (Table 2). Measured
intakes (Table 2) as delivered by the infusion pumps were lower
than the amounts targeted (82–96% of targeted intake)
during the balance period, but actual intakes were not differ-
ent among treatment groups.

Analysis of bodies. Lower protein (P = 0.006) and higher
fat (P = 0.009) contents were observed in the bodies of IV and
IP pigs, compared with IG pigs (Table 3).

Organ and muscle weights. The liver (P < 0.001) and spleen
(P = 0.025) of IV and IP pigs were heavier than those from the
IG pigs (Fig. 1). Large intestines were heavier in IG
than in IP pigs, with IV pigs intermediate (P < 0.05); a trend
toward heavier kidneys in IV and IP pigs was also observed (P

TABLE 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Intragastric</th>
<th>Intravenous</th>
<th>Intraportal</th>
<th>Pooled sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>58.3</td>
<td>55.0</td>
<td>53.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Fat</td>
<td>30.6</td>
<td>35.1</td>
<td>36.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Ash</td>
<td>11.2</td>
<td>9.9</td>
<td>10.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Energy 27.2 27.5 27.4 0.92

1 Values are means, n = 5. Values in a row not sharing a letter differ
(P < 0.01, Tukey’s test).

TABLE 3

<table>
<thead>
<tr>
<th>Component</th>
<th>Intragastric</th>
<th>Intravenous</th>
<th>Intraportal</th>
<th>Pooled sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>58.3</td>
<td>55.0</td>
<td>53.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Fat</td>
<td>30.6</td>
<td>35.1</td>
<td>36.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Ash</td>
<td>11.2</td>
<td>9.9</td>
<td>10.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Energy 27.2 27.5 27.4 0.92

1 Values are means, n = 5. Values in a row not sharing a letter differ
(P < 0.01, Tukey’s test).
There were no differences among groups in weights of stomach or pancreas. The small intestines were not different in length among groups, yet the IV and IP groups had much lighter small intestines compared with the IG group relative to body weight (IG: 32.7 g/kg body weight, IV: 19.5, IP: 19.6, pooled SD: 3.9; \( P < 0.0001 \)) or to total small intestinal length (IG: 144 mg/cm, IV: 94, IP: 94, pooled SD: 15; \( P < 0.0001 \)).

**Figure 2** summarizes the gut parameters in the duodenum, proximal jejunum, medial jejunum and ileum. In all four sections, the total tissue weight per centimeter was lower in the IV and IP pigs compared with the IG pigs (\( P < 0.05 \)). In the duodenum and proximal jejunum, mucosa weight per centimeter was also lower for IV and IP vs. IG groups (\( P < 0.003 \)); a similar trend was observed in the medial jejunum (\( P = 0.11 \)). The mucosa as a percentage of total tissue weight was not different for any of the four sites (not shown). Total mucosa was 45 and 37% lower in the IV and IP pigs, respectively, compared with the IG pigs (\( P < 0.05 \)); similar differences were observed when total mucosa data were corrected for body weight (IG: 20.0 g/kg body weight, IV: 11.7, IP: 12.3, pooled SD: 4.3; \( P < 0.02 \)).

**Histology.** In the proximal jejunum, villous height was 25–30% lower in IV and IP pigs compared with IG pigs; a similar trend was observed in the medial jejunum (IG: 534 \( \mu \)m, IV: 410, IP: 420, pooled SD: 105; \( P = 0.17 \)). In the duodenum, villous height in IV pigs was lower than in IG pigs with IP pigs intermediate; there were no differences in the ileum. In the proximal jejunum, crypt depth was 18% lower in IV pigs compared with IG or IP pigs, which did not differ; a similar trend (IG: 144 \( \mu \)m, IV: 121, IP: 143, pooled SD: 16; \( P = 0.12 \)) was observed in the medial jejunum. Similarly, crypt depth in the duodenum of IG pigs (217 \( \mu \)m) was significantly higher than in IV pigs (185 \( \mu \)m) with IP pigs intermediate (211 \( \mu \)m), and crypt depth in the ileum of IP pigs (149 \( \mu \)m) was higher than that of IV pigs (125 \( \mu \)m) with IG pigs intermediate (143 \( \mu \)m).

**Nitrogen content of organs.** Total nitrogen per gram of liver or kidney did not differ among groups, but nitrogen per whole liver (\( P = 0.002 \)) or whole kidneys (\( P = 0.03 \)) relative to body weight was higher in IV and IP pigs compared with IG pigs. Total nitrogen per gram mucosa (from the proximal jejunum) was higher in IV and IP pigs, compared with IG pigs; however, total nitrogen per centimeter of proximal jejunum was higher in IG pigs than in IV or IP pigs (\( P < 0.004 \)). In addition, there was a trend for lower total nitrogen per total mucosa relative to body weight (\( P = 0.12 \)) in IV pigs than in IG pigs.
Effect of route of feeding identical diets on nitrogen content of organs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Intragastric</th>
<th>Intravenous</th>
<th>Intraportal</th>
<th>Pooled SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver, mg N/g</td>
<td>22.3</td>
<td>22.1</td>
<td>22.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Total2</td>
<td>783b</td>
<td>941a</td>
<td>1016a</td>
<td>81</td>
</tr>
<tr>
<td>Kidney, mg N/g</td>
<td>17.8</td>
<td>17.0</td>
<td>17.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Total2</td>
<td>143b</td>
<td>171a</td>
<td>177b</td>
<td>19</td>
</tr>
<tr>
<td>Mucosa, mg N/g</td>
<td>16.3b</td>
<td>18.9a</td>
<td>20.0a</td>
<td>1.7</td>
</tr>
<tr>
<td>mg N/cm</td>
<td>1.55a</td>
<td>0.90b</td>
<td>0.96b</td>
<td>0.26</td>
</tr>
<tr>
<td>Total2,3</td>
<td>328</td>
<td>214</td>
<td>247</td>
<td>83</td>
</tr>
</tbody>
</table>

1 Values are means, n = 5. Means in a row not sharing a letter differ (P < 0.01, Tukey’s test).
2 Total: mg nitrogen in whole wet tissue/kg body weight.
3 Total nitrogen in whole mucosa tended to be lower in intravenously fed pigs compared with intragastrically fed pigs (P = 0.12).

Amino acid analyses. Tables 5 and 6 display the free amino acid data for plasma (at necropsy) and small intestinal mucosa, respectively. Only urea cycle and related amino acids are displayed; complete amino acid data will be published in a separate paper (including all amino acids and their concentrations in mucosa, liver, kidney and plasma). After 8 d, the plasma ornithine concentration was lower in both IP and IV pigs than in IG pigs, and the plasma glutamine concentration was lowest in IP pigs, with IV pigs having intermediate concentrations (Table 5). In mucosa, citrulline and hydroxyproline concentrations were not different among groups (Table 6). The mucosal arginine concentration was higher in IG and IV pigs than in IP pigs, and the plasma glutamine concentration was lower in both IP and IV pigs than in IG pigs (Table 6).

DISCUSSION

To isolate the physiologic effects of route of feeding, we fed identical complete diets continuously via gastric, central vein or portal vein catheters. Intragastrically fed (IG) pigs represented the “control” group in which nutrient absorption and first-pass metabolism by the small intestine and liver occurred. Feeding via the portal vein (IP) represented a model in which nutrients are metabolized by the liver on first pass, but small intestinal absorption and first-pass metabolism are excluded. Nutrients infused into a central vein (IV) by-pass exclusive first-pass metabolism by the intestine or liver; these nutrients are therefore provided to non-splanchnic organs in concentrations that are not adjusted by splanchnic metabolism. We hypothesized that both IV and IP groups would experience gut atrophy, and hence lowered intestinal metabolic capacity. By using these different routes of feeding, we describe metabolic and physiologic effects due to different first-pass metabolism and/or lack of small intestinal metabolic capacity.

Body weight gain was not different due to route of feeding; however, we observed significantly lower protein deposition in both IV and IP piglets, compared with IG piglets, as indicated by the lower protein content of bodies (Table 3). The higher fat deposition in the parenteral groups (Table 3) and more protein in the IG piglets resulted in similar weight gain. Consistent with the body composition results, IV and IP pigs excreted more nitrogen than IG pigs and hence had lower nitrogen retentions (Table 2). In the only other study comparing the intragastric, intravenous or intraportal infusion of identical diets (King et al. 1983), intravenously and intraportally fed rats lost body weight and had a nonsignificant 25% reduction in nitrogen balance compared with intragastrically

**TABLE 4**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Intragastric</th>
<th>Intravenous</th>
<th>Intraportal</th>
<th>Pooled SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>165a</td>
<td>1014a</td>
<td>260b</td>
<td>448</td>
</tr>
<tr>
<td>Citrulline</td>
<td>759</td>
<td>325</td>
<td>912</td>
<td>395</td>
</tr>
<tr>
<td>Glutamate</td>
<td>6512a</td>
<td>3244b</td>
<td>2486b</td>
<td>1945</td>
</tr>
<tr>
<td>Glutamine</td>
<td>823a</td>
<td>661ab</td>
<td>170b</td>
<td>377</td>
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<tr>
<td>HydroxyPro</td>
<td>356</td>
<td>258</td>
<td>411</td>
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<td>Ornithine</td>
<td>201a</td>
<td>106b</td>
<td>58b</td>
<td>56</td>
</tr>
<tr>
<td>Proline</td>
<td>5988b</td>
<td>2748b</td>
<td>1491b</td>
<td>983</td>
</tr>
</tbody>
</table>

1 Values are means, n = 5. Means in a row not sharing a letter differ (P < 0.05, Tukey’s test).
2 Mucosal concentrations of citrulline tended to be lower in intravenously fed pigs compared with intraportally fed pigs (P < 0.10).

**TABLE 5**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Intragastric</th>
<th>Intravenous</th>
<th>Intraportal</th>
<th>Pooled SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>113</td>
<td>104</td>
<td>64</td>
<td>37</td>
</tr>
<tr>
<td>Citrulline</td>
<td>120</td>
<td>145</td>
<td>32</td>
<td>108</td>
</tr>
<tr>
<td>Glutamate</td>
<td>137</td>
<td>112</td>
<td>110</td>
<td>29</td>
</tr>
<tr>
<td>Glutamine</td>
<td>209a</td>
<td>161ab</td>
<td>116b</td>
<td>45</td>
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<tr>
<td>HydroxyPro</td>
<td>178</td>
<td>136</td>
<td>90</td>
<td>54</td>
</tr>
<tr>
<td>Ornithine</td>
<td>103a</td>
<td>45b</td>
<td>42b</td>
<td>15</td>
</tr>
<tr>
<td>Proline</td>
<td>638</td>
<td>661</td>
<td>621</td>
<td>204</td>
</tr>
</tbody>
</table>

1 Values are means, n = 5. Means in a row not sharing a letter differ (P < 0.05, Tukey’s test). Bold values are outside sow-fed piglet plasma range.
2 Data from Wykes et al. (1994).
3 Plasma concentrations of free arginine and hydroxyproline tended to be lower in intraportally fed pigs compared with intragastrically fed pigs (P < 0.12).
fed rats. In other studies, nitrogen balance was consistently similar between intravenous and intraportal diet infusion in adult humans (Lidstrom 1954), monkeys (Fairman et al. 1983), rabbits (Mulholland et al. 1952) and rats (Boraas et al. 1981). In the last-mentioned study with rats (Boraas et al. 1981), total body nitrogen was also identical after 10 d of intravenous or intraportal feeding. Furthermore, nitrogen balance during intravenous infusion of total nutrition has been shown to be lower than during oral feeding in rats fed identical diets (Lanza-Jacoby et al. 1982). Therefore, previous research in different adult species produced similar nitrogen balance results compared with this study in fast growing piglets, especially with respect to intravenous vs. intraportal feeding. These consistent results suggest that diet infusions that by-pass the gut result in similarly inefficient nitrogen utilization whether or not hepatic first-pass metabolism is maintained.

Lower nitrogen utilization by IV or IP infusion of diets could be explained by a variety of factors. Many anabolic hormones are stimulated when nutrients are presented luminally (Alverdy 1995); the absence of this stimulation in the parenteral groups may in part account for their lower nitrogen utilization. However, parenteral feeding results in a multitude of complex hormonal changes and their implications on overall protein synthesis have not been elucidated sufficiently. These hormonal changes have been suggested to be responsible for gut atrophy induced by parenteral nutrition (Alverdy 1995, Goldstein et al. 1985, Johnson et al. 1975); this atrophy reduces the metabolic capacity of the small intestine, resulting in lowered protein turnover during parenteral feeding in human neonates (Duffy and Pencharz 1986) and adults (Je-vanandam et al. 1987). We have demonstrated that, compared with orally fed pigs, intravenously fed pigs have ~30% lower phenylalanine (House et al. 1997) and lysine (House et al. 1998) requirements and ~60% lower threonine requirement (Bertolo et al. 1998). Stoll et al. (1998) demonstrated that the small intestine metabolizes approximately one third of dietary essential amino acids, consistent with our results for lysine and phenylalanine requirements. With respect to threonine requirements, due to high synthesis rates of specific proteins such as threonine-rich mucins, the gut may have a different amino acid requirement profile compared with whole-body protein (mostly muscle) requirements (Bertolo et al. 1998, Stoll et al. 1998). This profile difference for different tissues would result in an altered whole-body amino acid requirement profile during parenteral feeding.

In this study, the amino acid profile of the diet (Table 1), which is based on milk protein, was adequate for the IG pigs as indicated by the 87% nitrogen retention; however, because the amino acid requirement profile for parenterally fed neonates is probably different, a diet based on milk protein may not be appropriate in parenteral feeding and may lead to catabolism of excess amino acids, as suggested by the lower nitrogen retentions (80%) in IV and IP pigs. Alternatively, the inadequate synthesis of semessential amino acids would also lead to catabolism of other amino acids that were consequently in excess. Arginine has been shown to be semissential in the piglet (Bål et al. 1986), and its synthesis occurs primarily in the small intestine (Stoll et al. 1998). Given the extensive gut atrophy in both IV and IP pigs in this study, arginine synthesis may have been inadequate. We are currently determining the arginine requirement of parenterally fed piglets. The primary precursors for arginine synthesis in the gut are glutamine (Bramley et al. 1998, Murphy et al. 1996) and glutamate (Reeds et al. 1997). Concentrations of both proline and glutamate in mucosa were low in both parenterally fed groups. However, mucosal arginine was lower only in mucosa of IP pigs. Glutamine may also be a precursor for arginine synthesis in the enterocyte (Wu et al. 1994), and its concentration was low only in mucosa of IP pigs, similar to the arginine concentration pattern. Although plasma arginine concentrations were not different among groups, ornithine levels were much lower in both IV and IP pigs in both plasma and mucosa (Tables 5 and 6); whether circulating ornithine serves as a precursor for arginine synthesis in the piglet is unknown. Because plasma urea was not different across groups (not shown) and symptoms of hyperammonemia were not observed (Brunton et al. 1998), arginine provided in the diet met the requirements for maintenance of the urea cycle, but may have been insufficient for maximal protein synthesis. Because both IV and IP pigs had similar nitrogen retentions, gut atrophy or by-pass, as opposed to hepatic by-pass, had more influence on inefficient utilization of dietary nitrogen.

Livers from IV and IP pigs were heavier than those of IG pigs (Fig. 1), consistent with results in rats (King et al. 1983). Enlarged livers after parenteral feeding may be indicative of hepatic steatosis, which is a common observation during long-term TPN and is probably dextrose related (Burgess 1992). Indeed, in the study by King et al. (1983), hepatomegaly after intravenous or intraportal nutrition in rats was due to increased liver lipid content. In this study, the protein concentration in the liver (as indicated by total nitrogen) did not differ across treatments with total hepatic protein significantly greater in IV and IP pigs (Table 4), consistent with the data of King et al. (1983). As shown in this study, TPN-induced hepatomegaly does develop in growing piglets and occurs whether nutrients are infused by the portal or central vein.

There were also gross differences in the weights of other organs (Fig. 1), which have been observed previously. Kidneys from the IV and IP pigs tended to be larger than those from the IG pigs. Parenteral infusion of total nutrition increases renal solute load and may cause enlargement of the kidneys; the present data are supported by Adeola et al. (1995) who also observed heavier kidneys and higher rates of protein synthesis in the kidneys of intravenously fed compared with orally fed piglets. The enlarged spleens of IV and IP pigs may have been caused by chronic, mild hemolysis due to infusion of hyperosmotic solutions. Large intestines of IG pigs were heavier than those of IP pigs (with IV pigs intermediate), also due to atrophy from lack of enteral nutrients. Unlike results from Adeola et al. (1995) and Goldstein et al. (1985), we did not observe smaller stomachs or pancreata after parenteral nutrition. With the exception of the liver, the mechanisms or implications of these gross changes in the various organs have not been studied.

Gross atrophy of the small intestine in IV and IP pigs was demonstrated in this study as indicated by low relative wet weights (Fig. 2). In both IV and IP pigs, the relative wet weight of the whole small intestine was ~60% of that in IG pigs; however, the total length of the small intestines was not different among the groups. These findings were similar to those of Goldstein et al. (1985) who found a 40% lower total small bowel weight (with no change in length) in 6-wk-old pigs fed identical diets intravenously for 3 wk compared with gastrically fed controls. In other studies, intravenously fed piglets (Adeola et al. 1995) and growing rats (Johnson et al. 1975) experienced a 40% lower total small intestinal weight compared with controls fed a nonpurified diet. Given the rapid growth rate of piglets and their small intestines in the first week of life (McCandless 1974), the lower intestinal weights after prolonged TPN feeding can be described as true atrophy, as opposed to the hypotrophy observed for adult animals fed prolonged parenteral nutrition (Hughes and Dowling 1980,
Czernichow et al. 1992). Indeed, Goldstein et al. (1985) observed that small bowel weights in piglets did not change after 3 wk of TPN. In this study, the lack of intestinal growth due to parenteral feeding was similar regardless of first-pass hepatic metabolism of nutrients in IP pigs.

The total weight per centimeter of each section of the small intestine was also lower in both parenteral groups. With the exception of the ileum, the mucosal weight per centimeter at each section was also lower in IV and IP pigs compared with IG pigs. Similar reductions have previously been observed in IV-fed piglets (Shulman 1988), growing rats (Johnson et al. 1975) and adult rats (Czernichow et al. 1992). Consistent with these data, both parenteral groups had lower protein per centimeter of small intestine than IG pigs (Table 4). In this study, the estimated total mucosa from these data suggested that IV- and IP-fed pigs had ~60% of the mucosa in IG pigs, a reduction similar to that found for total small intestinal relative weight. The significant reduction in total and mucosal weights for the whole small intestine and for the sections suggests that both the muscularis and mucosa components were similarly atrophied or stunted in their growth. The implication of this finding is that any attempt at preventing or reversing gut atrophy in the growing gut should target not only mucosal growth and function, but also address the stunted growth of the muscularis.

Small intestinal atrophy also occurred at the histologic levels of villous height and crypt depth. Compared with IG pigs, IV pigs had shorter villi and reduced crypt depths in all sections (including a trend for lower crypt depths in the medial jejunum, \( P = 0.12 \)) (Fig. 3). Histologic gut atrophy has been observed in previous studies involving parenterally fed growing animals. Goldstein et al. (1985) found lower mucosal depth, villous height and cell number per microvillus in the proximal jejunum of 6-wk-old TPN-fed piglets, compared with controls fed identical diets by gastrostomy; however, they did not observe any differences in crypt depth. In the study by Shulman (1988), TPN-fed piglets experienced reduced villous surface area and mucosal height in the jejunum and ileum compared with controls fed a nonpurified diet. In several other studies in adult rats (Bark et al. 1994, Czernichow et al. 1992), dogs (Feldman et al. 1976) and humans (Guedon et al. 1986), histologic gut hypotrophy has been observed after prolonged parenteral feeding. By employing identical diets, we have shown that IV feeding leads to histologic abnormalities of the growing small intestine that result from route of feeding alone.

Feeding intraportally led to similar atrophy of the villi in the jejunal sections, but not in the duodenum or ileum, compared with IV pigs (Fig. 3). However, unlike in IV pigs, IP pigs did not experience atrophy of the crypts in any of the gut sections because IG and IP pigs had similar crypt depths. To our knowledge, no previous studies have investigated the effects of IP infusion of nutrients on small intestinal atrophy. The only difference between the two parenterally fed groups is hepatic first-pass metabolism. The infusion of nutrients directly to the liver may promote crypt growth by stimulating the secretion of gut hormones or growth factors, such as gastrin or insulin-like growth factor. This study is the first to describe the effects of portal nutrition on gut histology; the less atrophic consequences of nutrient infusion via the portal vein, compared with the central vein, warrant further investigation into the amino acid profile of TPN solutions and their influence on gut metabolism.

In this study, small intestinal atrophy occurred in both parenterally fed groups regardless of whether the liver first metabolized the infused nutrients. This gross atrophy was due to route of feeding, not diet, and resulted in a much lower total metabolic capacity of the small intestine. However, we also demonstrated that this gut atrophy is histologically, and perhaps functionally different between IV- and IP-fed pigs. Hepatic metabolism appeared to improve or maintain crypt cell proliferation. If atrophy is due primarily to lack of enteral stimulation, as proposed by others (Alverdy 1995, Goldstein et al. 1985, Johnson et al. 1975), then portal infusion of total nutrition would not confer any benefits to small intestinal integrity and function. These data suggest that the liver must play an important role for some aspects of normal gut growth. Overall, gross atrophy of the gut and villi dramatically reduces the total metabolic capacity of the small intestine, which in turn appears to alter interorgan amino acid metabolism. This lack of gut metabolic capacity contributed to the changes in nitrogen metabolism observed in this study, and further research should have as a goal better nitrogen retention through adjustment of the amino acid profile of the diet. The goal should be to achieve a nitrogen retention of ≥75%.

This study supports our hypothesis that during parenteral feeding, reduced small intestinal metabolism is more responsible for lower nitrogen utilization than by-passing hepatic first-pass metabolism. The IV and IP groups were similar in that gut atrophy occurred because of a lack of luminal nutrients over time. However, they differed in that the IP route of feeding maintained hepatic first-pass metabolism, unlike the IV route. If hepatic first-pass metabolism were most important for nitrogen metabolism, then the IP and IG groups would have responded similarly because they both maintain hepatic first-pass metabolism. However, with respect to nitrogen metabolism, the IG and IP groups were different, whereas the IV- and IP pigs responded similarly. Thus, the small intestine and its metabolic capacity appear to be more important in regulating nitrogen or amino acid metabolism than the liver. Therefore, in our overall goal of developing an ideal amino acid profile appropriate for parenterally fed individuals, we should consider amino acid metabolism by the small intestine and provide a nutrient profile that accommodates such a physiologic state.

**LITERATURE CITED**


