Intestinal Inflammation Increases Gastrointestinal Threonine Uptake and Mucin Synthesis in Enterally Fed Minipigs

Didier Rémond, Caroline Buffière, Jean-Philippe Godin, Philippe Patureau Mirand, Christiane Obled, Isabelle Papet, Dominique Dardevet, Gary Williamson, Denis Breuillé, and Magali Faure

Abstract

The high requirement of the gut for threonine has often been ascribed to the synthesis of mucins, secreted threonine-rich glycoproteins protecting the intestinal epithelium from injury. This requirement could be even greater during intestinal inflammation, when mucin synthesis is enhanced. In this study, we used an animal model to investigate the effects of an acute ileitis on threonine splanchic fluxes. Eight adult multi-catheterized minipigs were fed with an enteral solution. Four of them were subjected to experimental ileitis involving direct administration of trinitrobenzene sulfonic acid (TNBS) into the ileum (TNBS-treated group) and the other 4 were not treated (control group). Threonine fluxes across the portal-drained viscera (PDV) were quantified with the use of simultaneous i.g.L-[15N]threonine and i.v.L-[U-13C]threonine infusions. Ileal mucosa was sampled for mucin fractional synthesis rate measurement, which was greater in the TNBS-treated group (114 ± 15%/d) than in the control group (61 ± 8%/d) (P = 0.02). The first-pass extraction of dietary threonine by the PDV and liver did not differ between groups and accounted for ~27 and 10% of the intragastric delivery, respectively. PDV uptake of arterial threonine increased from 25 ± 14 μmol·kg⁻¹·h⁻¹ in the control group to 171 ± 35 μmol·kg⁻¹·h⁻¹ in the TNBS-treated group (P < 0.001). In conclusion, ileitis increased intestinal mucin synthesis and PDV utilization of threonine from arterial but not luminal supply. This leads to the mobilization of endogenous proteins to meet the increased threonine demand associated with acute intestinal inflammation.

Introduction

The gastrointestinal tract is a highly secretory and proliferative tissue and, although it contributes only 3–6% of the body weight, it accounts for 20–35% of the whole-body protein turnover (1). The composition of secreted proteins largely affects its requirement for individual amino acids. For instance, the core proteins of intestinal mucins secreted in the lumen by the epithelium contain high amounts of threonine (2) and their production could explain the high rate of gastrointestinal threonine utilization (3,4). Whether mucins secreted in the upper part of the digestive tract can be digested, and their amino acids reabsorbed in the small intestine, remains conceivable. However, threonine recycling appears to be low (5) and threonine ileal loss particularly high, at least with respect to the whole-body threonine requirement (6). In inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease, the mucus layer and mucin production are often qualitatively and quantitatively impaired (7). Using models of chronic colitis in rats, we observed that although mucin synthesis was unchanged, the mucin composition was altered all along the gut, showing a decrease in threonine content (8). These data suggested that in inflammatory diseases, because of its involvement in the synthesis of acute phase proteins and proteins of the immune system (9), and possibly because of an increase in its ileal loss, threonine may become a limiting amino acid for mucin synthesis. Indeed, an increased supply of a mixture of amino acids, including threonine, serine, proline, and cysteine, increased mucin synthesis during colitis (10). Thus, an adequate threonine supply seems essential to restore gut barrier integrity and function during intestinal inflammation and therefore to improve recovery. It has been suggested that besides corticosteroid therapy, the use of enteral nutrition could be considered as a primary treatment in inflammatory bowel diseases (11). In this context, the definition of amino acid requirement, and particularly threonine requirement, by the gastrointestinal tract in the course of intestinal inflammation appeared crucial.

In this study, we investigated threonine fluxes in the portal-drained viscera (PDV) using minipigs as an animal model. We

1 Supported by the Institut National de la Recherche Agronomique (France) and the Nestle Research Center (Switzerland).
3 Supplemental Appendix 1, Supplemental Figure 1, and Supplemental Tables 1–3 are available with the online posting of this paper at jn.nutrition.org.
4 To whom correspondence should be addressed. E-mail: dremont@clermont.inra.fr.
5 Abbreviations used: FSR, fractional synthesis rate; MPE, molar percent excess; MPO, myeloperoxidase; PDV, portal-drained viscera; TNBS, trinitrobenzene sulfonic acid.
hypothesized that an intestinal inflammation (ileitis), through an increase in mucin synthesis, would increase specifically threonine utilization by the digestive tract and possibly impair the reabsorption of endogenous protein secretions in the ileum. Using stable isotopically labeled threonine by both i.v. and intraduodenal routes, we investigated the effect of an acute ileitis on the partitioning of threonine uptake by the gastrointestinal tract, dietary vs. arterial supply, in minipigs undergoing enteral feeding with an elemental solution.

Materials and Methods

All procedures were in accordance with the guidelines formulated by the European Community for the use of experimental animals (L358–86/609/EEC) and the study was approved by the Regional Committee for Ethics in Animal Experimentation (CREEA d’Auvergue, Aubière, France).

Animals. The study involved 8 adult Pitman-Moore minipigs (CEGAV) (8–10 mo old; 26–30 kg body weight). They were housed in individual pens (1 × 1.5 m) separated by Plexiglass walls in a ventilated room with controlled temperature (20–23°C).

Surgical procedures. Before surgery, the minipigs were deprived of food for 24 h. One-half hour after premedication by i.m. injection of 7 mg/kg ketamine (Imalgène 1000; Rhône Mériex), 1.5 g/kg azaperone (Sresnil, Janssen Pharmaceutica), and atropine (Atropine 0.1%), Labaratoire Aguetant, anesthesia was induced via a snout mask with 10% isoflurane (Forene, Abbot) carried by oxygen for 10 min. After oral endotracheal intubation, the isoflurane concentration was reduced to 2% to maintain general anesthesia during the total surgery time. After midline laparotomy, minipigs were fitted with catheters (polyvinyl chloride; 1.1-mm i.d., 1.9-mm o.d.) in the portal vein, the aorta, and the caval vein. The portal vein catheter was placed via the splenic vein with the tip positioned in the liver hilus. This catheter was used for Ringer’s lactate (500–1000 mL) delivery during the remainder of the surgery. A transit time ultrasonic blood flow probe (Transonic Systems) was implanted around the portal vein (14-mm probe, A-series). Finally, a T-shaped cannula (silicone rubber; 12-mm i.d., 17-mm o.d.) was inserted into the ileum 15 cm upstream of the ileocecal valve and a catheter (silicone rubber; 1.57-mm i.d., 3.18-mm o.d.) was inserted into the stomach. Vascular catheters and probe cables were exteriorized through the skin on the right flank of the minipig, whereas ileal cannula and gastric catheter were exteriorized on the left flank. The first 4 d after surgery, minipigs were treated with an antibiotic (Clamoxyl, Pfizer Santé Animal) as a prophylactic measure and Fluixin (Finadynes, Shering-Plough) was administrated i.v. for postoperative analgesia. Feed allotments were progressively increased over 7 d up to the 500-g daily ration. A minimum of 2 wk was allowed for recovery from surgery before initiating the experiment.

Experimental ileitis. Ileitis was induced by intraluminal injection of trinitrobenzen sulfonic acid (TNBS) using a similar procedure used by Shibata et al. (12) in dogs. Minipigs were food deprived for 12 h. They were anesthetized by i.v. injection of 8 mg/kg ketamine, which was thereafter added if necessary to maintain anesthesia adequately during the full procedure of ileitis induction. The ileal cannula was removed, offering direct access to intestinal lumen. A fiberscope was introduced into the intestine toward the jejunum over ~70 cm. A tube fitted at its tip with 2 inflatable latex balloons at 20-cm intervals was slid into the intestine until its extremity passed beyond the fiberscope one. The first balloon was then inflated (20 cm2) and the fiberscope moved back to the 2nd balloon, which was inflated in turn before removing the fiberscope. The 20-cm segment closed between the 2 inflated balloons was rinsed out with 50% ethanol. A 30-mL solution of 120 mg/kg TNBS (Sigma-Aldrich chimie) dissolved in 50% ethanol was introduced into the isolated segment and kept in place for 30 min. The 2 balloons were then deflated, the tube removed, and the cannula was replaced. On the day of treatment, minipigs were not fed. One day before and for 3 d following TNBS challenge, arterial serum was collected for C-reactive protein determination as a marker of inflammation (acute phase response).

Experimental protocol. Throughout the experimental period, a liquid diet was enterally infused into the gastric catheter via a swivel system connected to a pump. The daily diet was prepared by mixing Nestlé f.a.a (40 mL/kg of body weight), which is an elemental nutrition support containing free amino acids, with Nutrifose FB (Roquette; 6 g/L of Nestlé f.a.a), which provided soluble dietary fiber. The mixture was made up to 1.2 L with water. The diet was infused at a rate of 200 mL/h for 6 h (between 0900 and 1500). The enteral feeding provided 2 g protein equivalent kg−1 d−1, 167.4 kJ kg−1 d−1, and 140 mg of threonine kg−1 d−1. The effect of intestinal inflammation was evaluated by measuring threonine fluxes 4 d before (d−4) and 3 d after (d 3) ileitis induction in 4 TNBS-treated minipigs (TNBS-treated group) and 4 control minipigs (control group) that did not undergo the procedure described above for ileitis induction but were submitted only to the period of starvation. On the days of sampling, baseline arterial and portal blood samples were drawn before starting the enteral nutrition. Once the infusion of the enteral solution was started, after an initial bolus equivalent to 1 h of infusion, L-[U-13C]threonine and L-[15N]threonine (Cambridge Isotope Laboratories) in sterile saline were infused (6 mL/h) at a rate of 4 μmol kg−1 h−1 in the caval vein and the stomach, respectively. Blood samples were then simultaneously taken from the artery and the portal vein at 60, 120, 210, 270, 300, 330, 360, and 390 min. Portal blood flow was continuously recorded during the sampling session.

At the end of the last sampling day, immediately after the last blood samples were taken, the minipigs were killed by i.v. injection of 125 mg/kg body weight of sodium pentobarbital (Dolethal, Vetoquinol). The abdomen was opened and the ileum was then rapidly excised, flushed with saline, and opened. Two 20-cm segments were isolated: one corresponding to the inflamed area, the other one being collected about 1 m upstream. For both segments, examination of the mucosa and scoring of macroscopically visible damage was performed according to the scale proposed by Rodriguez-Cabezas et al. (13). A small piece of the whole intestinal wall was fixed in Bouin’s (Pioneer Research Chemicals) for later histological examination and the mucosa of the remaining intestine was removed by scraping with a microscope slide, immediately frozen in liquid nitrogen, and stored at −80°C.

Sample processing. Blood samples were collected in prechilled syringes containing either EDTA-K (for whole blood analysis) or heparin (for blood gas analysis and plasma) as anticoagulant. Hemoglobin concentration and packed cell volume were immediately determined using an automatic blood-gas analyzer (ABL510, Radiometer). Plasma was separated by centrifugation at 1500 × g; 10 min at 4°C. Whole blood and plasma spiked with norvaline and norleucine, respectively, as internal standards were deproteinized with sulfosalicylic acid (190 mmol/L, final concentration) and stored at −80°C.

Histological examination and scoring of inflammation in ileum specimens. Fixed tissues, stored in 70% ethanol, were dehydrated, embedded in paraffin blocks, sectioned (5 μm thick), and successively stained with Harris hematoxylin, eosin-erythrosin, and safron for evaluation of epithelial damage. Slices were scored by a histopathologist unaware of the treatments on a scale from 0 to 20 according to criteria proposed by Millar et al. (14). Slices were equally stained with iron diamine/Alician blue for evaluation of the number of mucin-containing goblet cells.

Analytical methods. Biochemical analysis of ileum myeloperoxidase (MPO) activity was performed with a dianisidine-H2O2 assay (15). The C-reactive protein concentration in serum was determined by solid phase sandwich immunoassay (Tridelta Development).

Plasma amino acid concentrations were measured by ion-exchange chromatography (Bio-Tek Instruments A.R.L). The threonine concentration in blood was measured by reversed-phase HPLC using precolumn derivatization with O-phthaldehyde reagent (16). For isotopic analysis, deproteinized blood samples (400 μL) were applied to a 3-mL bed

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volume column of AG50-X8 resin (100–200 mesh) in the H+ form. After a water wash, the amino acids were eluted with NH4OH and dried under vacuum. The isotopic measurements of L-[U-13C] threonine and L-[15N] threonine in plasma samples were carried out by GC-MS using N(O,S)-ethoxycarbonyl ethyl ester derivative. Briefly, the analyses were performed on a gas chromatograph (6890 series; Hewlett-Packard) using helium as carrier gas, coupled to a quadrupole mass spectrometer (MSD 5975; Agilent) operating in electron impact-ionization mode. Ions at masscharge ratios of 175, 176, and 177 for the M, M+1 (13C-threonine), and M+2 (15C-threonine) isotopomers, respectively, were monitored to determine the isotopic enrichment of 15N-threonine and 13C-threonine. To check and correct the accuracy of the 13C-isotopic measurement carried out in this condition, mixtures of a known amount of unlabeled threonine and a known amount of labeled [U-13C]threonine were used and analyzed by GC-MS. Moreover, because the isotopic enrichment is in the low range for the GC-MS device, a few samples were split in 2 and analyzed by GC-MS and GC-combustion-isotope ratio mass spectrometer and did not differ. The isotopic enrichments were calculated using the tracer/tracer approach and then subsequently transformed to a molar percent excess (MPE) value according to Rosenblatt et al. (17). This approach takes into account the isotopic contributions of labeled atoms and the natural abundance of the elements present in the derivatized molecules.

Mucins were purified from the ileal mucosa of minipigs and their amino acid composition was analyzed as previously described by Faure et al. (8). The 13C-isotopic enrichment of threonine in mucins was performed using N(O,S)-ethoxycarbonyl ethyl ester derivatives (18).

Calculations. Measurements were performed under steady-state conditions. Amino acid fluxes in fed minipigs were calculated using only the last 4 sampling times, 300–390 min after the onset of enteral feeding and tracer perfusions, when amino acid concentrations and [13C]threonine and [15N]threonine enrichment reached a plateau (Supplemental Fig. 1).

We estimated the arterial blood flow to the portal-drained vein viscera (BFa) as follows:

\[ BF_a = BF 	imes Hb_a / Hb_v \]

where BFa is measured blood flow (L·kg⁻¹·h⁻¹) in the portal vein and Hb a and Hb v are hemoglobin concentrations (g/L) in arterial and venous whole blood, respectively. We used the hemoglobin ratio to take in account water movement across the PDV.

Plasma amino acid net fluxes across the PDV (Fnet AA, μmol·kg⁻¹·h⁻¹) were calculated as

\[ F_{net} AA = ([AA]_a - [AA]_v) / ([AA]_a - [AA]_v) \]

where [AA] is the blood amino acid concentration (μmol/L), PF is plasma flow, and subscripts a and v refer to arterial and venous data, respectively. PF was calculated from blood flow with the relation PF = BF × (1 - PCV).

Whole-body threonine flux (Ra, μmol·kg⁻¹·h⁻¹) was derived from the equation

\[ Ra = \frac{[\text{Ei} \text{Thr}_{	ext{mucosa}}]}{[\text{Ei} \text{Thr}_{	ext{mucosa}}]} - 1 \]

where Inf is the rate of infusion of tracer (μmol·kg⁻¹·h⁻¹) and Ei Thrv and Ei Thrart are isotopic enrichments (MPE) in infused solution and arterial blood, respectively. Ra was calculated with i.v. tracer (Raiv) or i.g. tracer (Rag).

The difference between the whole-body fluxes calculated from the i.v. and the i.g. tracers was assumed to represent the first-pass splanchnic extraction of the i.g. tracer. Thus, the first-pass splanchnic extraction rate (Extraction, %) was calculated as

\[ \text{Extraction} = \frac{[\text{Rag} - \text{Rai}]}{\text{Rag}} \]

I.v. and i.g. infusion of labeled threonine allowed us to determine the unidirectional fluxes that underlie net fluxes across tissues. At the PDV level, it offers the possibility to distinguish the origin (luminal vs. arterial) of the threonine used by the mucosa. The unidirectional fluxes of threonine across the PDV were calculated from arterial and portal threonine isotopic enrichments and whole blood concentrations, and blood flow. A schematic model of the different fluxes is depicted (Fig. 1).
The C-reactive protein concentration in peripheral serum of TNBS-treated minipigs increased up to 200 mg/L within the first 24 h following TNBS challenge ($P < 0.01$). However, it returned to prechallenge values on d 3 (data not shown), indicating a transient acute phase protein response occurring in the liver following the local intestinal inflammation induction.

**Plasma amino acid arterial concentrations and net fluxes across the PDV.** Except for glutamine, glutamate, and histidine, the arterial concentrations of the different amino acids were not affected by group, day of sampling, or their interaction (Supplemental Table 1). The arterial glutamate concentration in the control group was greater ($P = 0.049$) at d 3 (167 ± 7 μmol/L) than at d –4 (126 ± 9 μmol/L), whereas it was unchanged in the TNBS-treated group ($P = 0.202$). Conversely, for glutamine, the arterial concentration was lower ($P = 0.044$) at d 3 (235 ± 10 μmol/L) than at d –4 (357 ± 25 μmol/L) in the TNBS-treated group, but did not change in the control group ($P = 0.189$). The arterial threonine concentration was 728 ± 89 μmol/L. The portal blood flow tended to decrease between d –4 and d 3 in the control group ($P = 0.089$) but increased in the TNBS-treated group ($P = 0.024$) (Table 2).

**Threonine fluxes.** Whatever the tracer used in calculation, the whole-body flux of threonine was not affected by day of sampling or the treatments (Table 3). The first-pass splanchnic

<table>
<thead>
<tr>
<th>n</th>
<th>μmol·kg$^{-1}$·h$^{-1}$</th>
<th>% of i.g. infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow, ml·min$^{-1}$·kg$^{-1}$</td>
<td>30 ± 2</td>
<td>24 ± 2</td>
</tr>
<tr>
<td><em>Tyrosine</em></td>
<td>27 ± 1</td>
<td>84 ± 5</td>
</tr>
<tr>
<td><em>Cystine</em></td>
<td>33 ± 1</td>
<td>46 ± 4</td>
</tr>
<tr>
<td><em>Glycine</em></td>
<td>88 ± 2</td>
<td>146 ± 15</td>
</tr>
<tr>
<td><em>Aspartate</em></td>
<td>99 ± 2</td>
<td>16 ± 4</td>
</tr>
<tr>
<td><em>Alanine</em></td>
<td>111 ± 2</td>
<td>302 ± 30</td>
</tr>
<tr>
<td><em>Arginine</em></td>
<td>113 ± 3</td>
<td>77 ± 8</td>
</tr>
<tr>
<td><em>Proline</em></td>
<td>143 ± 3</td>
<td>95 ± 4</td>
</tr>
<tr>
<td><em>Serine</em></td>
<td>156 ± 3</td>
<td>80 ± 8</td>
</tr>
<tr>
<td><em>Glutamate</em></td>
<td>380 ± 8</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

**Dispensable amino acids**

<table>
<thead>
<tr>
<th>n</th>
<th>μmol·kg$^{-1}$·h$^{-1}$</th>
<th>% of i.g. infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Histidine</em></td>
<td>42 ± 1</td>
<td>84 ± 8</td>
</tr>
<tr>
<td><em>Methionine</em></td>
<td>66 ± 2</td>
<td>36 ± 5</td>
</tr>
<tr>
<td><em>Phenylalanine</em></td>
<td>139 ± 3</td>
<td>82 ± 6</td>
</tr>
<tr>
<td><em>Lysine</em></td>
<td>157 ± 4</td>
<td>93 ± 10</td>
</tr>
<tr>
<td><em>Isoleucine</em></td>
<td>163 ± 4</td>
<td>81 ± 6</td>
</tr>
<tr>
<td><em>Valine</em></td>
<td>182 ± 4</td>
<td>78 ± 9</td>
</tr>
<tr>
<td><em>Threonine</em></td>
<td>193 ± 4</td>
<td>75 ± 5</td>
</tr>
<tr>
<td><em>Leucine</em></td>
<td>326 ± 7</td>
<td>79 ± 5</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 4 (d 3) or 8 (d –4).
2 Data were analyzed by a mixed-model ANOVA with subjects as random effect and day, group, day × group as factors. $P_1$  for a day effect; $P_2$  for a group effect; $P_{1×2}$  for an interaction between days and groups.
TABLE 3  Threonine whole body flux, and threonine fluxes across the PDV and the distal small intestine in enterally fed minipigs 4 d before and 3 d after induction of ileitis.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>TNBS</th>
<th>ANOVA</th>
<th>( P_{d} )</th>
<th>( P_{t} )</th>
<th>( P_{d \times t} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lg. infusion, ( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>194.5 ± 6.6</td>
<td>184.2 ± 10.8</td>
<td>199.1 ± 2.3</td>
<td>0.217</td>
<td>0.221</td>
<td>0.661</td>
</tr>
<tr>
<td>(^{15}\text{N}-\text{Threonine}</td>
<td>3.8 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.2</td>
<td>0.047</td>
<td>0.437</td>
<td>0.283</td>
</tr>
<tr>
<td>Whole-body flux, ( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R_{\text{IA}} )</td>
<td>302.0 ± 15.1</td>
<td>288.7 ± 29.1</td>
<td>302.5 ± 17.8</td>
<td>0.537</td>
<td>0.492</td>
<td>0.415</td>
</tr>
<tr>
<td>PDV flux, ( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>( F_{\text{net , Thr}} )</td>
<td>144.2 ± 9.3</td>
<td>149.8 ± 6.7</td>
<td>102.1 ± 12.5</td>
<td>0.043</td>
<td>0.422</td>
<td>0.003</td>
</tr>
<tr>
<td>Capt ( \text{Thr}_\text{IA} )</td>
<td>41.8 ± 9.4</td>
<td>24.9 ± 14.3</td>
<td>171.0 ± 3.6</td>
<td>0.005</td>
<td>0.040</td>
<td>0.001</td>
</tr>
<tr>
<td>Capt ( \text{Thr}_\text{SU} )</td>
<td>55.7 ± 7.7</td>
<td>52.3 ± 9.3</td>
<td>53.0 ± 11.4</td>
<td>0.115</td>
<td>0.957</td>
<td>0.385</td>
</tr>
<tr>
<td>Ap ( \text{Thr}_\text{IA} )</td>
<td>142.6 ± 7.7</td>
<td>136.0 ± 10.1</td>
<td>150.2 ± 9.4</td>
<td>0.907</td>
<td>0.275</td>
<td>0.303</td>
</tr>
<tr>
<td>Ap ( \text{Thr}_\text{SU} )</td>
<td>43.4 ± 9.3</td>
<td>38.6 ± 10.5</td>
<td>123.0 ± 18.6</td>
<td>0.012</td>
<td>0.057</td>
<td>0.008</td>
</tr>
<tr>
<td>First-pass extraction, % of i.g. infusion</td>
<td></td>
<td></td>
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<tr>
<td>Splanchnic</td>
<td>33.6 ± 4.8</td>
<td>38.7 ± 6.5</td>
<td>39.2 ± 7.9</td>
<td>0.156</td>
<td>0.852</td>
<td>0.490</td>
</tr>
<tr>
<td>Portal</td>
<td>28.0 ± 3.6</td>
<td>27.8 ± 4.3</td>
<td>25.9 ± 5.4</td>
<td>0.168</td>
<td>0.704</td>
<td>0.183</td>
</tr>
<tr>
<td>Hepatic</td>
<td>5.7 ± 1.7</td>
<td>11.9 ± 2.9</td>
<td>13.2 ± 4.3</td>
<td>0.083</td>
<td>0.780</td>
<td>0.704</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, \( n = 4 \) (d 3) or 8 (d −4).
2 \( R_{\text{IA}} \), threonine whole body flux calculated from intravenous tracer; \( F_{\text{net \, Thr}} \), threonine net flux; Capt \( \text{Thr}_\text{IA} \), arterial threonine uptake; Capt \( \text{Thr}_\text{SU} \), arterial threonine release; Ap \( \text{Thr}_\text{IA} \), non-luminal threonine release; Ap \( \text{Thr}_\text{SU} \), non-luminal threonine release.
3 Data were analyzed by a mixed-model ANOVA with subjects as random effect and day, group, day × group as factors. \( P_{d} \), \( F \) for a day effect; \( P_{t} \), \( F \) for a group effect; \( P_{d \times t} \), \( F \) for an interaction between days and groups.

extraction of dietary threonine was ~37% and was not significantly affected by the TNBS challenge. Similarly, the first-pass extractions of threonine by the PDV and by the liver were not affected and accounted for 27 and 10% of the dietary threonine, respectively. The TNBS challenge induced a 5-fold increase in PDV uptake of arterial threonine, which increased from 2 to 12% of the arterial influx. In the TNBS-treated group, the contribution of the PDV to the whole-body flux of threonine increased from 13 to 58% between d −4 and d 3 (\( P = 0.001 \)), whereas it was not affected in the control group (\( P = 0.354 \)). The PDV release of nondiarythreonine on d 3 was 2-fold greater in the TNBS-treated group than in the control group.

**Ileal mucin synthesis.** Finally, the number of mucin-containing goblet cells was greater (t test, \( P < 0.05 \)) in the treated area of the TNBS-treated group (220 ± 14 goblet cells/mm intestine) than in the control group (168 ± 28 goblet cells/mm intestine). The FSR of ileal mucins in the TNBS-treated group (114 ± 15%/d) was nearly twice that of the control group (61 ± 8%/d; \( P = 0.021 \)). The amino acid composition of collected mucins was not affected by ileitis (\( P > 0.10 \)). Threonine accounted for 10.4 ± 2.3% of the total amino acid content of mucins (Supplemental Table 3).

**Discussion**

Threonine, a nutritionally indispensable amino acid, seems to be of critical importance for gut function given its high occurrence in the mucins secreted along the length of the digestive tract (1). In the present study, we demonstrated that increased mucin synthesis in the case of intestinal inflammation substantially and specifically affects the gastrointestinal tract uptake of threonine.

Determination of the quantitative utilization of threonine by the gastrointestinal tract was not possible in humans, so we used the minipig as an animal model for adult humans. In this model, as previously observed for calves (19) and pigs (20), threonine accounted for 10% of the total amino acids in intestinal mucins. This contribution was much lower than that reported in humans (2). Nevertheless it is still high compared with other body proteins such as muscle proteins (5–6% of threonine).

The experimental approach combined the arterio-venous difference technique with the infusion of labeled threonine. Simultaneous infusion of different tracers in the systemic circulation and stomach allowed differentiation of the unidirectional fluxes (uptake, release) that constitute the net flux at the level of the PDV. To determine the effect of a local inflammation of the intestine, we developed an experimental model of ileitis. This model, involving luminal administration of TNBS dissolved in ethanol, was initially set up to induce recto-colic inflammation in rats (21) and was thereafter adapted to produce ileitis in dogs and pigs (12,22). The approach used in the present study was similar to the one reported in dogs (12), but the tube was inserted via a permanent external fistula in the distal ileum instead of using the rectal route. Macroscopic and histological examination demonstrated the effectiveness of the procedure in ileitis induction.

The PDV first-pass extraction of dietary threonine in the present study with adult minipigs was low (30%) compared with the one in growing pigs on solid food (50%) and in enterally fed piglets (70%) (4,5). Besides the type of feeding, the physiological development stage could be a major determinant of this first-pass extraction. Indeed, in humans, splanchnic first-pass extraction of leucine, another indispensable amino acid, decreases from 50% in neonates to 25% in adults (23,24). In preterm infants receiving enteral nutrition, as in enterally fed piglets, a first-pass splanchnic extraction of threonine of 70% was reported (25). Unfortunately, no measurement of first-pass splanchnic extraction of threonine is available in adult humans, but our data suggest that it could be much lower than previously thought. The high threonine requirement of neonates could be...
related to enhanced mucin synthesis, because the neonate's acquired immune system is not fully functional in the intestine and the neonate is dependent on the innate defenses of mucus (26).

PDV, liver, and consequently splanchic first-pass extraction of dietary threonine were not affected by ileitis. The lack of response of the liver in terms of threonine utilization agrees with a focused inflammation in the digestive tract. Liver first-pass extraction of threonine accounted for only 20% of the total splanchic extraction. This value is in agreement with that in growing pigs (4), confirming the prominent role of the PDV in the splanchic first-pass extraction of dietary threonine. Because the enteral solution used in the present study contained only free amino acids, the largest part of the infused threonine was probably rapidly absorbed in the proximal part of the small intestine. Under these conditions, ileitis did not affect PDV first-pass extraction of dietary threonine, suggesting that neither absorption processes nor dietary threonine utilization by this gut segment was affected.

Ileitis produced a 5-fold increase in PDV uptake of arterial threonine. Whereas the majority of the utilized threonine derived from the intestinal lumen in noninflamed minipigs, as previously observed in piglets and growing pigs fed normal-protein diet (3,4), ~80% of the threonine taken up by the PDV was derived from arterial supply during the course of ileitis. Such a large increase cannot be related to only the small inflamed area. Ileitis through systemic or nervous signals could have induced an upregulation of intestinal protein synthesis, and mucin synthesis in particular, upstream and downstream from the inflamed area.

Different pathways could be involved in the metabolic fate of the threonine taken up by the PDV, depending on the tissue. Indeed, the intestine does not seem to oxidize threonine, whereas the pancreas, which is drained by the portal vein, can degrade threonine through the L-threonine 3-dehydrogenase pathway (3,27). In agreement, it was shown that PDV oxidize some of the threonine that is taken up from the arterial delivery, but this visceral threonine oxidation accounts for <15% of the whole-body threonine oxidation (4). The major metabolic fate of threonine in the PDV is therefore protein synthesis and, more particularly, mucin synthesis. Threonine utilization for mucin synthesis could have been relatively low in our specific conditions, because our minipigs received a low-fiber, nonprotein enteral solution and it is has been reported that intestinal mucin synthesis and secretion are stimulated by nutritional factors, such as fiber or dietary peptides (28,29). Indeed, the mucin FSR in the ileum was low (61%/d for the control group on d ~4) compared with the values reported for conventionally fed pigs (20) or rats (30) (~140%/d). Nevertheless, a large part of the increase in threonine utilization by the PDV in the course of ileitis was probably related to the increased mucin synthesis. This was supported by the specific increase in PDV retention of threonine, serine, proline, glutamate, and leucine, which are major constituent amino acids of mucins.

The present study showed that in noninflamed minipigs, ~20% of the threonine that is released in the portal blood is not of dietary origin. This endogenous release of threonine has 2 possible origins: reabsorption of threonine of secreted proteins or proteolysis in gastrointestinal tissues. Because degradation by the digestive enzymes of part of the mucins secreted in the upper part of the gut is conceivable, a recycling of threonine is likely. However, such recycling was not evident in the portal blood of enterally fed piglets (5), suggesting that either mucins are particularly resistant to digestive proteases or threonine released from mucin degradation is immediately reincorporated into intestinal proteins. Thus, although in the present work it was not possible to distinguish its origin, the increased endogenous release of threonine over the time course of ileitis suggested an increased proteolysis in gastrointestinal tissues.

Thus, the main change in threonine trafficking within the splanchic area induced by ileitis was the increase in PDV uptake of arterial threonine, which increased from 10 to 60% of the whole-body flux of threonine. Because the latter was not affected by ileitis, the increase in gastrointestinal utilization of arterial threonine would be accompanied by a proportional decrease in threonine utilization by peripheral tissues.

In conclusion, our data indicate that during acute ileitis, the gastrointestinal tract requirement for threonine is largely increased and that in the course of enteral nutrition containing free amino acids, the arterial supply is the preferential source of threonine used to meet the additional need. Because mucosal and mucin protein synthesis in the proximal small intestine increases with luminal threonine concentration (31), increased enteral threonine supply would probably increase intestinal first-pass extraction of threonine, limiting the availability of additional dietary threonine for gut segments that do not benefit from luminal threonine supply (stomach, ileum, large intestine). Thus, during acute intestinal inflammation, the optimal solution could be a combined approach using enteral nutrition (with adequate threonine supply) to maintain intestinal mass and integrity (26) and parenteral nutrition to supply additional threonine to meet the increased PDV requirement. Finally, the increased threonine requirement in our study during conditions of local gut inflammation, only a 20-cm segment in the ileum, suggests this increase would be even more dramatic in clinical conditions.

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