Citroulline levels following proximal versus distal small bowel resection

Ivan M. Gutierrez a,b, Jeremy G. Fisher a,b, Offer Ben-Ishay a,b, Brian A. Jones a,b, Kuang Horng Kang a,b, Melissa A. Hull a,b, Nick Shillingford c, David Zurakowski b, Biren P. Modi a,b, Tom Jaksic a,b,*

a Center for Advanced Intestinal Rehabilitation (CAIR), Boston Children’s Hospital and Harvard Medical School, Boston, MA
b Department of Surgery, Boston Children’s Hospital and Harvard Medical School, Boston, MA
c Department of Pathology, Boston Children’s Hospital and Harvard Medical School, Boston, MA

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Purpose: Citroulline, a nonprotein amino acid synthesized by enterocytes, is a biomarker of bowel length and the capacity to wean from parenteral nutrition. However, the potentially variant effect of jejunal versus ileal excision on plasma citroulline concentration [CIT] has not been studied. This investigation compared serial serum [CIT] and mucosal adaptive potential after proximal versus distal small bowel resection.

Methods: Enterally fed Sprague-Dawley rats underwent sham operation or 50% small bowel resection, either proximal (PR) or distal (DR). [CIT] was measured at operation and weekly for 8 weeks. At necropsy, histologic features reflecting bowel adaptation were evaluated.

Results: By weeks 6–7, [CIT] in both resection groups significantly decreased from baseline (P < 0.05) and was significantly lower than the concentration in sham animals (P < 0.05). There was no difference in [CIT] between PR and DR at any point. Villus height and crypt density were higher in the PR than in the DR group (P < 0.02).

Conclusion: [CIT] effectively differentiates animals undergoing major bowel resection from those with preserved intestinal length. The region of intestinal resection was not a determinant of [CIT]. The remaining bowel in the PR group demonstrated greater adaptive potential histologically. [CIT] is a robust biomarker for intestinal length, irrespective of location of small intestine lost.

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Pediatric intestinal failure (IF) is an intrinsic bowel disease resulting in the inability to sustain growth, hydration, or electrolyte homeostasis. While mortality resulting from (IF) has improved [1], significant morbidity remains for patients dependent on parenteral nutrition (PN) or who require intestinal transplantation. Short bowel syndrome (SBS) represents a subset of intestinal failure caused by loss of intestine that results in insufficient length, and thus insufficient digestive and absorptive capacity. The most common etiologies of SBS are necrotizing enterocolitis, intestinal atresia, gastrochisis, and midgut volvulus [2]. This broad set of etiologies results in varying extents and locations of bowel loss. Classically, the functional capacity of the remaining small bowel has been difficult to predict.

Citroulline, a nonprotein amino acid, is principally synthesized from glutamine by enterocytes, the cells that make up the small intestinal mucosa. Citroulline is converted to arginine by the kidney, but renal excretion is quantitatively negligible [3–5]. Multiple experiments have demonstrated that the plasma concentration of citroulline, [CIT] is a reliable biomarker for bowel length in those who are enterally fed

[6–10]. Clinically, [CIT] above a threshold value (10–15 μmol/L) predicts enteral tolerance and the ability of children with SBS to wean from PN [11,12]. Low [CIT] is also an independent risk factor for catheter-associated blood stream infections in patients on PN [13].

As variability in [CIT] exists even in patients with similar bowel lengths, other factors may contribute to citroulline production. It is unclear whether citroulline is produced uniformly along the small bowel. One study of enzymatic activity showed that the rate controlling enzyme in citroulline production (pyrroline 5-carboxylate synthase) is more active in the proximal jejunum, suggesting that this is the predominant site of citroulline production [14]. However, a mucosal molecular study concluded that the highest level of citroulline transport occurs in the middle to lower ileum [15].

The anatomic location of the small intestinal segment lost, or more accurately, the location of the intestine that remains, may have a substantial impact on [CIT]. Therefore, quantifying the relative production of citroulline by intestinal segment is essential to interpreting test results in patients with SBS. The region of resection might significantly confound the interpretation of [CIT] in children with SBS.

However, the potentially variant effect of jejunal versus ileal resection on [CIT] has not been studied. In human subjects, it is difficult to control for the variety of other factors that affect bowel function, enteral tolerance, and potentially [CIT]. Rats have been used
extensively as a model in intestinal failure [16–20]. Citrulline metabolism appears to be similar in rats, and circulating levels decrease after enterectomy [21]. This investigation therefore sought to compare serial [CIT] in rats adapting to major proximal or distal bowel resection.

1. Methods

The Institutional Animal Care and Use Committee at Boston Children’s Hospital approved the study (Protocol #09-03-1303). Adolescent Sprague-Dawley rats were randomly assigned to one of three groups: proximal small intestine resection (PR), distal small intestine resection (DR), or sham operation. Postoperatively, weight and [CIT] were measured weekly for 8 weeks until the animals were euthanized.

1.1. Operative procedures

In all animals, a midline laparotomy was performed. The bowel was the eviscerated and small bowel was run from the ligament of Treitz (LOT) to the cecum. The small intestinal length was then measured along the antimesenteric border. The operations varied by study group. In the proximal resection group (PR), 50% of the small bowel was resected; the intestine was transected just beyond the LOT and again at the midpoint of the small bowel (measured halfway between the LOT and the ileocecal valve). Continuity was restored with end-to-end, single layer anastomoses. The distal resection animals (DR) underwent a similar procedure, with excision of small intestine from the midpoint to within 1 cm of the ileocecal valve. In the sham group, the bowel was transected at the midpoint and reanastomosed without resection.

1.2. Nutrition

All animals ate a standard solid chow diet (Prolab Isopro RMH 3000, LabDiet, St. Louis, MO) until 24 hours before operation, during which they were provided water only. They were allowed water for 12 hours postoperatively and then received a liquid diet ad libitum (Bio-Serv, Frenchtown, NJ) for the next 48 hours and then resumed solid food ad libitum. The liquid diet consisted of the following macronutrient composition: 18% protein, 12% fat, 70% carbohydrate. Solid food consisted of: 26% protein, 14% fat, and 70% carbohydrate. Neither diet contained significant citrulline.

1.3. Citrulline

Blood was collected by tail venipuncture in each animal at the time of operation and weekly thereafter. Serum citrulline was measured in μmol/L using a Waters Mass Track Amino Acid Analyzer (Waters, Milford, MA).

1.4. Histologic evaluation

At necropsy, a segment of small bowel adjacent to the anastomosis was preserved in formalin. Sections were embedded in paraffin, stained with hematoxylin and eosin, and reviewed by a gastrointestinal pathologist. Mucosal thickness, crypt depth, and villous height were measured in each sample with an AmScope 4-scale stage micrometer slide with divisions of 0.1 mm. Counts were done in well-oriented intestinal segments where full length crypt-villus units were identified. Care was taken to avoid areas adjacent to sites of anastomosis, where inflammation and fibrosis were intense. The parameters were evaluated in 10 distinct sites and the average was recorded. Crypt density was determined by counting the number of crypts in 50 μm stretches of mucosa. The rate of crypt fission, a potentially important mechanism of expansion of functional mass, was determined by counting the number of crypts showing fission among 100 enterocytes and expressed as a percentage. A bifurcating crypt with one or more bisecting fissures creating two or more flask-shaped bases, both communicating with a single crypt-villus junction was considered to be a crypt undergoing fission [22].

1.5. Statistical analysis

Animals surviving after 2 weeks were included for analysis. [CIT] data are presented as mean ± standard error of the mean. Weight and histologic variables are shown as mean ± standard deviation. All data conformed to a normal Gaussian-shaped distribution as evaluated using the Kolmogorov-Smirnov goodness-of-fit test. Between groups, [CIT], weight, and histologic data were compared using analysis of variance (ANOVA) with repeated measures where applicable to account for the same animals measured at different time points using the SPSS software package (version 21.0, SPSS Inc./IBM, Chicago, IL). Power analysis indicated that minimum sample sizes of 6–10 rats per group at each time point would provide 80% power to detect mean differences of 20 μmol/L between the three groups as well as changes in [CIT] from baseline in [CIT] of 20 μmol/L within PR and DR groups using ANOVA (version 7.0, nQuery Advisor, Statistical Solutions, Saugus, MA). All values of P < 0.05 (two-tailed) were considered significant.

2. Results

Each group began with similar numbers of animals (PR n = 20, DR n = 18, sham n = 21). Some animals were euthanized at 4 weeks and a subset was euthanized at 8 weeks. Notably, there were fewer animals in the DR group at week 8 than the other two experimental arms (PR n = 10, DR n = 6, sham n = 9). Bowel lengths were measured at operation before resections. All three groups had similar lengths (Table 1, P = 0.70). The baseline weights were the similar in the three groups (P = 0.76). No differences were seen between groups with regard to weights at any time point, including final weights (for week 8, P = 0.35). [CIT] at baseline were also similar (Table 1, P = 0.81). [CIT] values by group over time are shown in Fig. 1. In sham animals, [CIT] were similar throughout the experiment (P = 0.29). In both resection groups, [CIT] was similar to baseline in weeks 1–3 (P > 0.09). However, [CIT] was statistically lower than baseline after week 4 (P < 0.03) in animals that underwent resection. In short, a period of 4 weeks was required to observe any difference in [CIT] after resection.

When comparing [CIT] between groups, both the resection groups had significantly lower levels than the sham group by the end of the study. Starting at week 6, the DR group [CIT] was statistically lower than sham (P = 0.04) and remained lower through week

Table 1

<table>
<thead>
<tr>
<th></th>
<th>PR</th>
<th>DR</th>
<th>Sham</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Preoperative bowel length in cm (mean ± SD)</td>
<td>75 ± 11.0</td>
<td>74 ± 9.8</td>
<td>71 ± 7.9</td>
<td>0.70</td>
</tr>
<tr>
<td>Preoperative weight in grams (mean ± SD)</td>
<td>213 ± 102.0</td>
<td>202 ± 46.7</td>
<td>223 ± 85.3</td>
<td>0.76</td>
</tr>
<tr>
<td>Final weight in grams (mean ± SD)</td>
<td>501.4 ± 46.7</td>
<td>539 ± 43.8</td>
<td>530 ± 62.4</td>
<td>0.36</td>
</tr>
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was lower in the DR group than in the others (P < 0.02). There was a trend toward lower crypt fission rates in the DR group that did not achieve statistical significance (P = 0.09).

3. Discussion

Serum citrulline concentrations [CIT] appropriately discriminated animals undergoing bowel resection from those with intact small bowel. Citrulline concentrations significantly lower than sham animals were apparent 6 weeks after surgery in the distal resection (DR) group and 7 weeks afterwards in the proximal resection (PR) group (Fig. 1). The PR and DR group [CIT] were not statistically different at any time point. Despite resections, all animals grew appropriately with similar weekly weights between groups.

Multiple studies have documented that [CIT] is proportional to remaining bowel length in humans with SBS [7,8,12,23,24]. In this study, [CIT] was significantly lower in animals who underwent major bowel resection, hence [CIT] could effectively differentiate those animals from rats with intact length. This finding provides additional evidence that [CIT] is an accurate biomarker for bowel loss. Further, though such decreases in levels have been documented in prior studies of enterectomy in rats, proportionality has not been previously reported. In this study, the final [CIT] for animals that underwent 50% small intestinal resection was roughly half that of animals who retained full bowel length.

Interestingly, it took 4–5 weeks for the resected animals' [CIT] to drop statistically below their baseline values and 6–7 weeks to differentiate from levels in sham animals. All rats had initial decreases in [CIT]. This suggests that there may be a delay in the response of [CIT] after surgery. One hypothesis to explain this is that the period of relative bowel dysfunction that follows any abdominal operation...
causes an initial drop in [CIT]. Prior studies have demonstrated that [CIT] accurately reflects intestinal length in patients who are enterally fed, but may be inaccurate in a fasting state [11,25]. It is possible that postoperative pain and ileus contributed to decreased oral intake and thus less production of citrulline initially. This finding suggests that steady state citrulline concentrations are attained after an interval of postoperative bowel adaptation. The differences induced by the actual loss of enterocyte mass may hence be best evaluated after steady state values are attained. Weight gain in animals with reduced enterocyte mass may still be accomplished through increased enteral intake, which in the clinical context is termed hyperphagia. This behavior may account for the similar growth between animal groups in this study (Table 1).

At each weekly time point, [CIT] in PR and DR animals were similar. Mechanistically, this may mean that both remaining regions of bowel produce roughly equivalent amounts of citrulline. Alternatively, jejunum and ileum may have contrasting qualities the effects of which counteract each other (i.e. more inherent citrulline production in one segment and a greater ability to adapt in the other). If the jejunum produces more citrulline as some have suggested [14], then the remaining distal bowel in the PR group must have adapted to a greater extent.

Villus height and crypt depth have been used widely to quantitate small intestinal adaptation [26]. The crypt is the site of mucosal enterocyte production and thus the proliferation of crypts, quantified by crypt fission rate and crypt density, is a good histologic marker for adaptation [22]. Classically, ileum is thought to have greater adaptive potential than jejunum in mammals [27,28]. In the present study, the residual bowel in the PR group (distal intestine) had higher villi and crypt density (P < 0.02, Fig. 2). There was a trend toward increased crypt fission rate, but there was no statistical difference. However, prior studies suggest that maximal crypt fission occurs 6–14 days after insult and rates normalize by 6 weeks, thus an absence of a difference at 8 weeks should be expected. In amalgam, these histologic measures imply that the distal bowel remaining in PR rats underwent more substantial adaptive change than did the remnant proximal intestine in DR animals. Given these findings, it may be surmised that the relatively greater capacity of the jejunum to produce citrulline is ultimately matched by the ileum’s greater capacity for adaptation. The etiology of that adaptability is likely multifactorial, but may involve increased glucagon-like peptide-2 (GLP-2) activity [29,30]. Again, the acute response to surgery and poor postoperative enteral tolerance likely contribute to the initial drop in [CIT] in all animals.

Though this animal study has clear implications for human patients after small bowel resection, limitations exist. The model is not truly one of intestinal failure as all of the animals grew appropriately on full enteral nutrition. It is also possible that the effects of more substantial proximal or distal small bowel loss on [CIT] may be different than the effect of a 50% resection. Finally these animal data warrant corroboration through the examination of human surgical cohorts.

In this rat model, [CIT] proportionally decreased after 50% bowel loss, effectively differentiating animals undergoing resection from those with preserved intestinal length. More importantly, the specific anatomic segment of small bowel lost did not have an impact upon [CIT]. This supports the use of serum citrulline concentration as a robust biomarker of residual small intestinal length.

References