Pediatric Surgery International (2019) 35:657–663
https://doi.org/10.1007/s00383-019-04475-4

ORIGINAL ARTICLE

Plasma citrulline is not a biomarker for intestinal adaptation in short bowel syndrome, studied in piglets: a model for human neonates

Marihan Lansing[1] · Justine M. Turner[1] · Pamela Wizzard[1] · Celeste M. Lavallee[2,7] · David W. Lim[3] · Mitsuru Muto[4] · Patrick N. Nation[5] · Paul B. Pencharz[6] · Ron O. Ball[7] · Paul W. Wales[1,8]

Accepted: 23 March 2019 / Published online: 1 April 2019

Abstract

Background There are no in vivo methods to measure adaptation in neonatal short bowel syndrome (SBS). We evaluated citrulline (Cit) levels in neonatal piglet surgical models of SBS.

Methods Piglets underwent 75% mid-intestinal resection with jejunoileal anastomosis (JI), 75% distal resection of ileum with jejunocolic anastomosis (JC) or sham surgery. Jugular and gastric catheters were inserted for parenteral and enteral nutrition. On D7, small intestine length and weight were measured, jejunum collected for histopathology and Cit level determined.

Results JI ($n=5$) compared to JC ($n=5$) had increased small intestinal length (JC − 17.5 cm; JI +22.0 cm; $p=0.02$) and mass (JC 43.1 mg/cm/kg; JI 51.3 mg/cm/kg; $p=0.02$), while Cit did not differ (JI 801.0 μM; JC 677.7 μM; $p=0.90$). Including non-resected shams ($n=4$), Cit correlated with length ($R^2=0.48$; $p=0.006$), but not for SBS alone ($R^2=0.11$; $p=0.4$), mass ($R^2=0.05$; $p=0.5$). A second experiment compared change in Cit levels from baseline to D7. Levels declined in sham ($n=8$) and JC ($n=10$) (sham − 110.1 μM; JC − 56.6 μM; $p=0.17$), regardless of intestinal lengthening (sham 29.9 cm; JC − 10.4 cm; $p=0.002$).

Conclusion Citrulline levels predict large differences in intestinal length and 'identify' SBS. However, citrulline cannot discriminate between adaptation in JI and JC, nor predict intestinal lengthening.

Keywords Neonates · Short bowel syndrome · Intestinal adaptation · Citrulline

✉ Justine M. Turner
Justine.turner@albertahealthservices.ca

[1] Department of Pediatrics, Edmonton Health Academy, University of Alberta, 11405-87 Ave, Edmonton, AB T6G 1C9, Canada

[2] Faculty of Medical Sciences, University of Toronto, Toronto, ON, Canada

[3] Department of Surgery, University of Alberta, Edmonton, AB, Canada

[4] Department of Pediatric Surgery, Kagoshima University, Sakuragaoka, Kagoshima, Japan

[5] Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada

[6] Departments of Pediatrics and Nutritional Sciences, University of Toronto, Toronto, ON, Canada

[7] Department of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, AB, Canada

[8] Division of General and Thoracic Surgery, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Abbreviations

SBS	Short bowel syndrome
EN	Enteral nutrition
PN	Parenteral nutrition
JI	Jejunoileal anastomosis anatomy
JC	Jejunocolic anastomosis anatomy

Introduction

In neonates, short bowel syndrome (SBS) occurs as a result of congenital or acquired conditions leading to a reduction in intestinal mass due to failure of development or from resection. As a result, children suffer intestinal failure as the available enterocyte mass is insufficient to absorb adequate fluids and nutrients to support survival and growth [1]. Children with SBS are; therefore, dependent on parenteral nutrition (PN). Massive resection of the intestine is a trigger for intestinal adaptation; a process that includes both structural and

functional changes that improve absorption to meet nutritional needs for growth and survival [2].

PN is a lifesaving nutrition replacement; however, the complications, such as cholestatic liver disease and sepsis, cause significant morbidity and potential mortality. Intestinal adaptation is required for patients to reach autonomy from PN. In the current era, new therapies are emerging that may promote intestinal adaption in children [3]. Therefore, an accurate method to measure adaptation would be clinically advantageous. However, in vivo measurement of structural adaptation, limited to gross assessment of small intestinal length or caliber, such as by fluoroscopy, is both inaccurate and hazardous for neonates due to radiation exposure [4]. Similarly, investigations to assess functional adaptation, such as 72 h fecal fat collections, are cumbersome and inconvenient [5].

Citrulline (Cit) is produced exclusively in intestinal enterocytes and blood levels have been proposed to reflect intestinal production in proportion to enterocyte mass [6, 7]. For this reason, researchers and clinicians have proposed Cit levels as a biomarker of intestinal adaptation and bowel length. The role of Cit as a marker of adaptation in neonates is even more controversial, because the neonatal gut has limited expression of the enzyme arginase (*ARG*), while there is greater expression of arginosuccinase synthase (*ASS*) until the age of 3–5 years [8]. This enzyme expression pattern suggests that the neonatal intestine is geared to release arginine (Arg) into the amino acid pool, rather than Cit. This would limit the utility of Cit as a marker of enterocyte mass in neonates.

The aim of this study was to determine the utility of Cit in predicting histological adaptation and intestinal lengthening in neonatal piglet models of SBS. We used our piglet models of SBS with and without total ileal resection, given that the two anatomical subtypes have markedly different potential for intestinal adaptation [9].

Materials and methods

The research was approved by the Faculty of Agriculture, life and Environmental Sciences Animal Policy and Welfare Committee, University of Alberta. Research was conducted in a biosecurity swine research facility, according to the guidelines of the Canadian Council of Animal Care. All piglets studied were male Landrace–Large White cross breed.

Experiment 1

Rationale and hypothesis Having consistently only observed adaptation in piglets with residual ileum, we hypothesized that if Cit is a useful marker of structural adaptation, it would discriminate between these two surgical models.

Allocations Piglets were randomly allocated to 75% resection of mid-intestine with jejunoileal anastomosis (JI, $n=5$) or 75% distal resection with jejunocolic anastomosis (JC, $n=5$) or to sham ($n=4$) with no resection.

Experiment 2

Rationale and Hypothesis Having consistently failed to observe intestinal growth in SBS piglets without ileum, we hypothesized that Cit would increase over time in sham piglets with developmentally normally growing intestines, but not in JC piglets without ileum.

Allocations Piglets were allocated to JC ($n=10$) or sham ($n=8$).

Surgical procedures and animal care All operations were performed under a general anesthetic using isoflurane (2–3%; Bensen Medical Industries Inc., Markham, Ontario, Canada). Following a longitudinal abdominal incision, the intestine was measured from ligament of Treitz to ileocecal valve along the anti-mesenteric border using a 60 cm 0-Silk suture. During surgery, all piglets had a 5-French jugular central venous catheter (Braintree Scientific Inc., Braintree, MA, USA) placed for parenteral nutrition and a 10-French gastrostomy tube placed for enteral nutrition (EN). Piglets were housed individually in metabolic cages in a 25–28 °C temperature and 12 h light/dark cycled room. To avoid sepsis and minimize pain, piglets were maintained on an antibiotic and analgesia regimen for 3 day post-operative. If presumed clinical sepsis occurred after day 3, additional broad-spectrum antibiotics were added to the regimen. All these methods have been previously published [10].

Parenteral and enteral nutrition (*EN*) formulas for both experiments were made in our laboratory and infused post-operatively, containing 16 g/kg/d amino acids, 10 g/kg/d lipid (Intralipid®), and 29 g/kg/d glucose. The calorie requirements of this piglet strain are 5 times that of a human; therefore, 10 g/kg/d of intravenous lipid would be the equivalent of 2 g/kg/d in a human neonate. In the EN, glucose was replaced with polycose to prevent osmotic diarrhea. PN was initiated at 50% and gradually increased over 24 h to 100%. EN was initiated and maintained at 20% on day 2 post-operative for all piglets. This amount was provided as trophic nutrition to support intestinal adaptation [11].

CIT levels: Experiment 1 plasma CIT was assessed at termination (D7) using Waters Empower 3 Chromatography Software (SPARC Biocentre, Hospital of Sick Children, Toronto). *Experiment 2 serum CIT* was measured at initiation of trial (D0) and at termination (D7) using liquid chromatography–mass spectrometry (LC/MS) (The Metabolomics Innovation Centre, University of Alberta, Edmonton). The change in analytical laboratory was for convenience only.

Assessment of structural adaptation On D7, under general anaesthesia, the same technique was used to re-measure bowel length. Following humane euthanasia, the intestine distal to the ligament of Treitz to the colon was resected, the contents discarded, and the small intestine weighed. To standardize the location of bowel samples, mucosal scrapings were collected from a 20 cm jejunal segment resected 20 cm distal to the ligament of Treitz. The mucosal scrapings were weighed and snap frozen with liquid nitrogen. Mucosal mass was calculated, adjusted for length and piglet total body weight (jejunum scraping weight/20 cm × 1000/total body weight; expressed in mg/cm/kg). Jejunal and ileal samples were collected and preserved in formaldehyde, for subsequent preparation and H&E staining for histological analysis by a certified veterinary pathologist, blinded to allocation. An average of ten villi height and crypt depth measurements were taken for each piglet and measured to the nearest 0.1 mm, using a micrometer eyepiece (Nikon Eclipse 80i).

Statistical analysis

Data are expressed according to normality as mean and total standard error or median with interquartile range (25th–75th percentiles) dependant on data distribution. Groups were compared by either student *t* test or Kruskal–Wallis tests, with Mann–Whitney tests for post hoc analysis. Linear regression was used to assess relationship between adaptive structural changes and plasma citrulline levels. All data were analysed using IBM SPSS 24 Statistics data editor. Results with $p < 0.05$ were considered significantly different.

Results

At baseline and follow-up, piglets were of similar age and weight (see Tables 1, 2). All piglets were healthy while on trial.

Structural adaptation in SBS piglets

As expected, in both experiments, JC animals demonstrated less potential for intestinal lengthening and adaptation (see Tables 1, 2). Particularly, of note is the lack of significant small bowel lengthening in JC piglets in both experiments, while this was observed in JI and sham piglets. While there was a trend for more villus lengthening in JI piglets, this did not reach a statistical significance.

Citrulline and adaptation

In Experiment 1 (Table 1), despite the differences in structural adaptation between the two SBS anatomical groups, day 7 plasma Cit levels were not different between JC, 677.7 µM (550.1–886.7) and JI, 801.0 µM (476.0–945.98) ($p = 0.92$). Plasma Cit level was significantly lower for both SBS groups compared to the Sham control, 1198.3 µM (886.9–1637.3) ($p = 0.014$). Plasma Cit level was correlated with bowel length at termination ($R^2 = 0.48$; $p = 0.006$) (Fig. 1) when all piglets were included in the analysis. However, when sham piglets were removed from the analysis and only SBS piglets considered, there was no correlation between plasma Cit and residual bowel length at termination ($p > 0.05$). Mucosal mass was not correlated with plasma citrulline levels ($p = 0.25$; $R^2 = 0.11$), nor was jejunal villus height ($p = 0.62$; $R^2 = 0.02$).

Citrulline and intestinal growth

In Experiment 2 (Table 2), the small intestine length in sham piglets grew as expected (increase length day 7 was noted in 7/8 piglets), while it did not grow in any of the JC piglets. However, in both groups, serum Cit levels declined markedly from baseline and were not statistically different at follow-up (sham − 110.1 µM and JC − 56.6 µM; $p = 0.12$). This despite the sham having on average 4 times longer small bowel length.

Discussion

Based on research indicating that Cit is exclusively produced in enterocytes, plasma levels of Cit have been proposed to reflect this intestinal production in proportion to enterocyte mass [12, 13]. Furthermore, it has been proposed that Cit level can reflect intestinal function [14]. This has led to the clinical uses of Cit level in short bowel syndrome to monitor adaptation [15]. In the current study, we used our novel neonatal piglet models of SBS with and without ileum and we did not identify an association between Cit levels and intestinal length, nor mucosal weight or villus height. Furthermore, we found that the direction of change of intestinal length and Cit level over time in the same animals was unrelated.

In SBS, retention of the ileum and ileocecal valve has been associated with the greatest potential for post-resection adaptation [16]. This was again confirmed in the first experiment, where the JI median percent change in length was + 15%, compared to − 13% in the JC piglets ($p = 0.016$). Similarly, mucosal mass was on average 12.1 mg/cm/kg higher in JI than JC piglets despite the same initial intestinal resection. While these structural changes by increasing surface area for absorption are potentially clinically relevant adaptive changes between the two surgical groups, plasma Cit levels failed to differentiate between the two groups. Although Cit levels at termination correlated with intestinal

Table 1 Piglet data outcomes first experiment

	Surgery type	Median	25th percentile	75th percentile	*p* value
Day 0 weight (kg)	JC	2.32	2.29	2.44	0.60
	JI	2.20	2.16	2.43	
	Sham	2.41	2.15	2.46	
Day 7 weight (kg)	JC	3.48	3.16	3.83	0.99
	JI	3.50	3.42	3.66	
	Sham	3.56	3.06	3.88	
Change in weight (kg)	JC	1.16	0.82	1.44	0.87
	JI	1.26	1.20	1.30	
	Sham	1.15	0.9050	1.43	
Length post-resection (cm)	JC	141.00	136.50	146.50	0.005
	JI	152.00	148.10	163.00	
	Sham	627.25	558.12	652.13	
Length at termination (cm)[a]	JC	125.00	118.25	128.60	0.003
	JI	173.00	157.00	189.25	
	Sham	646.50	576.63	670.62	
Change in length (cm)[a]	JC	− 17.50	− 26.50	− 8.90	0.08
	JI	22.00	3.40	31.25	
	Sham	25.00	− 17.75	49.00	
Jejunum scraping weight (g)[a]	JC	2.70	2.46	3.29	0.01
	JI	3.70	3.29	4.22	
	Sham	2.20	2.03	2.55	
Jejunum villus height (0.1 mm)	JC	7.12	6.21	7.57	0.10
	JI	7.82	6.93	10.96	
	Sham	6.31	5.39	7.02	
Mucosal mass (mg/cm/kg)[a]	JC	43.11	34.60	45.85	0.008
	JI	51.29	47.63	59.15	
	Sham	33.46	28.98	34.95	
Small bowel weight (g/kg)[a]	JC	7.27	6.52	7.92	0.005
	JI	14.23	11.54	14.28	
	Sham	28.85	28.00	32.96	
Day 7 citrulline (μM)	JC	677.67	550.09	886.70	0.056
	JI	801.04	476.05	945.88	
	Sham	1198.32	886.94	1637.33	
Day 7 arginine (μM)	JC	222.88	201.37	364.86	0.46
	JI	255.41	235.03	320.32	
	Sham	279.52	234.09	367.12	

JC Jejunocolic anastomosis, *JI* jejunoileal anastomosis

p value in table represents K. Wallis analysis comparing all three groups to each other

[a]Indicates significant difference between JI and JC allocation using Mann–Whitney post hoc; $p < 0.05$

length, the association was driven solely by the difference between the two resected groups and the sham piglets. Therefore, Cit could potentially identify an animal with a shortened gut (SBS), as opposed to normal length (sham), but could not discriminate structural adaptation occurring within the SBS groups. Furthermore, the utility of citrulline to monitor change in length over time was discounted in the second experiment, where citrulline levels declined and were not different despite normal expected growth in the sham and no growth in the resected piglets without ileum.

Similar to our study, using an experimental model of SBS in rodents, Gutierrez et al. examined 50% proximal resection, 50% distal resection, or sham surgical models over an 8-week period and determined the relationship between serum Cit levels and small intestinal length and histology [17]. They concluded that Cit level could differentiate between sham and resected rats, but was not at all related to histological adaptation. Therefore, emerging data from surgical animal models show that the discriminatory power of Cit level for structural adaptation is highly limited.

Table 2 Piglet data outcomes second experiment

	Mean		Total SE	*p* value
	Sham $n=8$	JC $n=10$		
Age at surgery (days)	4.43	4.00	0.15	0.23
Weight at surgery (kg)	2.33	2.32	0.04	0.91
Weight at termination (kg)	3.89	3.78	0.07	0.44
Change in weight (kg)	1.56	1.46	0.04	0.24
Pre-resection small bowel length (cm)	569.81	591.67	10.18	0.27
Small bowel length at termination (cm)	599.75	137.45	56.21	< 0.001
Change in length (cm)	29.94	− 10.45	7.16	0.002
Jejunal scraping weight (g/20 cm)	2.27	2.91	0.12	0.002
Jejunum villus height (0.1 mm)	6.75	6.30	0.32	0.50
Jejunum crypt depth (0.1 mm)	1.54	1.73	0.07	0.21
Small bowel weight (g/kg)	24.69	7.27	2.12	< 0.001
Mucosal mass (mg/cm/kg)	29.19	38.82	1.80	0.004
Day 0 citrulline (μM)	168.95	109.82	18.26	0.14
Day 7 citrulline (μM)	58.89	53.21	4.39	0.41
Change in citrulline (μM)	− 110.06	− 56.61	15.59	0.12

Jejunocolic JC, data comparisons using student *t* tests

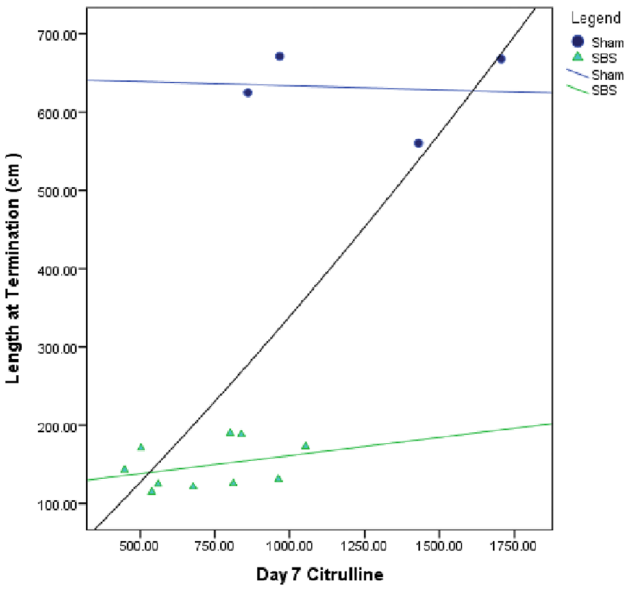

Fig. 1 Correlation between day 7 plasma citrulline (μM) and small intestinal length (cm) is shown: including Sham (blue circle) and SBS (green triangle), including both JI and JC. Slope of linear regression for all groups, including sham, is indicated by blue line and for SBS alone is indicated by green line

A number of clinical studies in the pediatric population also do not supportive of the utility of Cit level to assess adaptation or predict autonomy from PN. Stultz et al. [18], assessed Cit levels in pediatric patients, aged 1–44 months, both with and without intestinal resection, all receiving PN and all considered to have intestinal failure. The authors found that Cit level was related to changes in EN delivery and presumed that this indicated improved intestinal function, although they did not actually measure intestinal absorption [18]. In the current animal study, we did not measure intestinal function as absorption nor attempt to wean PN as our focus was the relationship with structural adaptation. Diamanti et al. prospectively assessed children with SBS between 6 months to 5.7 years and measured plasma Cit prospectively for 1 year [19]. They found a modest correlation ($R^2=0.22$) between baseline Cit level and small intestinal length measured at initial surgery. At follow-up, a correlation ($R^2=0.48$) with percentage enteral calories was found [19]. The main difference between these groups was the residual length, with a larger number of children with extreme short gut in the group that did not adapt. Therefore, similar to our findings, Cit may simply be a marker of large differences in small bowel length. Bailly-Botuha et al. also demonstrated that Cit level varied by large differences in bowel length, and yet, they did not find a relationship to enteral autonomy [20].

Few clinical studies have considered that differences in intestinal enzymatic ontogeny for Arg and Cit metabolism may account for the disparate findings in the literature for the utility of Cit as a biomarker of adaptation in pediatric SBS [19–22]. Kohler et al. studied enzymatic levels related to Arg metabolism in the intestinal biopsy tissue of children and adults undergoing intestinal surgery or endoscopy for disparate diseases such as intestinal atresia, meconium ileus, and cancer [8]. The age range included premature infants to adults 80 years. They confirmed that children older than 3–5 years do not express the enzyme ASS, pivotal for Arg synthesis from Cit, in the small intestine [8]. This means that it is only after this age that the intestine reliably exports

Cit for conversion to Arg in the kidney [23]. This change in physiology must be considered when interpreting the role of Cit to assess intestinal structure or function, as now discussed.

Bourdon et al. monitored Cit levels in very low birthweight preterm infants (< 32 weeks) [22]. The authors found a weak correlation between urinary Cit and gestational age plus postnatal age, suggesting that there is a relationship between Cit level and maturation [22]. However, Cit was not a reliable marker of intestinal function, based on lack of utility of the amino acid to predict enteral tolerance. Fitzgibbons et al. retrospectively studied pediatric SBS patients (median age 2.4 years; IQR 0.6–6.7 years) and concluded that Cit level correlated with intestinal length measured at baseline surgery and with later enteral tolerance [21]. They determined that a plasma Cit level ≥ 15 µmol/L was a positive prognostic factor for enteral autonomy [21]. However, it is important to note the limited number of young infants in this study ($n = 27$). The findings are likely driven by older children and may not apply to neonates and younger infants.

Studies examining the ability of Cit to predict adaptation in adults are generally more positive. In adults with SBS, Cit is expected to be excreted from the intestine to the kidney and it is, therefore, plausible that Cit does correlate more with gut length at this age. However, even in the adult age group, there is some concern over the discriminatory power of Cit to predict adaptation [24]. Fragkos and Forbes [15] recently published a meta-analysis exploring the role of Cit as a biomarker for adaptation. The pooled data were primarily from adult patients. The results demonstrated significant heterogeneity. The authors found that plasma Cit level did correlate with small bowel length (presumably at baseline). They claimed diagnostic utility for Cit to diagnose SBS versus healthy status and PN dependency versus autonomy; however, these differences were also readily discriminated clinically. The ability of Cit to predict small changes over time in intestinal function or structure that might be useful to guide treatment decisions or to assess new therapeutic interventions remains to be proven.

Conflicting findings in both pediatric and adult studies might be driven by methodological issues, including the relationship between the timing of Cit measurement and fasting. Fjermestad et al. studied adult patients with SBS with < 200 cm remnant small bowel length compared to healthy controls both fasting and postprandially [25]. In both SBS and healthy controls, the timing of Cit level postprandially impacted the measured level. Timing of Cit measurement is inconsistently reported in most studies. Another common limitation is related to sample size and statistical power to detect meaningful differences. Short bowel syndrome is a relatively rare condition and most publications represent single institution studies. Another consideration is the potential and often unknown impact of renal dysfunction on measured Cit levels. Up to 80% of adult intestinal failure, patients demonstrate evidence of renal dysfunction over time related to chronic PN exposure. Finally, enteral versus parenteral delivery of amino acids is also relevant for measured Cit levels. For example, first pass intestinal metabolism of glutamine improves Cit synthesis compared to parenteral glutamine [26].

Advantages of our experimental study design compared to the available clinical studies include: paired enteral feeding, the use of genetically and developmentally homogenous neonatal animals and our ability to measure length at follow-up (not simply at baseline as is most often reported in clinical studies). However, our study also had a limited sample size and 7 days.

In conclusion, we contend that Cit is not a useful biomarker of intestinal structural adaptation in neonates. Because Cit metabolism differs with age, it is possible that it would have greater validity as a biomarker of adaptation for older age groups. To clarify this issue further, studies examining the intestinal metabolism of arginine and citrulline in the neonatal intestine following gut resection are required. In addition, studies to examine correlation to functional adaptation should be performed. However, based on the current literature, it remains that controversial how discriminatory Cit may be as a biomarker for adaptation over time and pursuit of new biomarkers for adaptation is relevant and important in the current era of new trophic therapies.

Acknowledgements The authors wish to acknowledge the important contribution of Charlane Gorsak for technical assistance with the study and all the staff of the Swine Research and Technology Centre, University of Alberta.

Funding This work was Funded by the Canadian Institutes of Health Research (Grant Number: MOP-126179).

Compliance with ethical standards

Conflict of interest Author Marihan Lansing declares that she has no conflict of interest. JM Turner has research grant funding from GLyPharma Therapeutic, Inc., and from Empire Biotechnologies Inc. Author Pamela Wizzard declares that she has no conflict of interest. Author Celeste M. Lavallee declares that she has no conflict of interest. Author David W. Lim declares that he has no conflict of interest. Author Mitsuru Muto declares that he has no conflict of interest. Author Patrick N. Nation declares that he has no conflict of interest. Author Paul B. Pencharz declares that he has no conflict of interest. Author Ron O. Ball declares that he has no conflict of interest. Author Paul W. Wales has research grant funding from GLyPharma Therapeutic, Inc., and from Empire Biotechnologies Inc.

Human and animal rights All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. O'Keefe SJ, Buchman AL, Fishbein TM, Jeejeebhoy KN, Jeppesen PB, Shaffer J (2006) Short bowel syndrome and intestinal failure: consensus definitions and overview. Clin Gastroenterol Hepatol 4(1):6–10. https://doi.org/10.1016/j.cgh.2005.10.002
2. Sukhotnik I, Siplovich L, Shiloni E, Mor-Vaknin N, Harmon CM, Coran AG (2002) Intestinal adaptation in short-bowel syndrome in infants and children: a collective review. Pediatr Surg Int 18(4):258–263. https://doi.org/10.1007/s003830100695
3. Chandra R, Kesavan A (2018) Current treatment paradigms in pediatric short bowel syndrome. Clin J Gastroenterol 11(2):103–112. https://doi.org/10.1007/s12328-017-0811-7
4. Algin O, Evrimler S, Arslan H (2013) Advances in radiologic evaluation of small bowel diseases. J Comput Assist Tomogr 37(6):862–871. https://doi.org/10.1097/RCT.0b013e318299658c
5. Raman M, Fenton T, Crotty P, Ghosh S, Rioux K, Hundal R (2015) A novel method to identify fat malabsorption: the Serum retinyl palmitate test. Clin Chim Acta 438:103–106. https://doi.org/10.1016/j.cca.2014.08.001
6. Crenn P, Coudray-Lucas C, Cynober L, Messing B (1998) Postabsorptive plasma citrulline concentration: a marker of intestinal failure in humans. Transpl Proc 30(6):2528
7. Crenn P, Messing B, Cynober L (2008) Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. Clin Nutr 27(3):328–339. https://doi.org/10.1016/j.clnu.2008.02.005
8. Kohler ES, Sankaranarayanan S, van Ginneken CJ, van Dijk P, Vermeulen JL, Ruijter JM, Lamers WH, Bruder E (2008) The human neonatal small intestine has the potential for arginine synthesis; developmental changes in the expression of arginine-synthesizing and-catabolizing enzymes. BMC Dev Biol 8:107. https://doi.org/10.1186/1471-213x-8-107
9. Lim DW, Levesque CL, Vine DF, Muto M, Koepke JR, Nation PN, Wizzard PR, Li J, Bigam DL, Brubaker PL, Turner JM, Wales PW (2017) Synergy of glucagon-like peptide-2 and epidermal growth factor coadministration on intestinal adaptation in neonatal piglets with short bowel syndrome. Am J Physiol Gastrointest Liver Physiol 312(4):G390–G404. https://doi.org/10.1152/ajpgi.00281.2016
10. Josephson J, Turner JM, Field CJ, Wizzard PR, Nation PN, Sergi C, Ball RO, Pencharz PB, Wales PW (2015) Parenteral soy oil and fish oil emulsions: impact of dose restriction on Bile flow and brain size of parenteral nutrition-fed neonatal piglets. JPEN J Parenter Enteral Nutr 39(6):677–687. https://doi.org/10.1177/0148607114556494
11. Cisler JJ, Buchman AL (2005) Intestinal adaptation in short bowel syndrome. J Investig Med 53(8):402–413
12. Crenn P, Vahedi K, Lavergne-Slove A, Cynober L, Matuchansky C, Messing B (2003) Plasma citrulline: a marker of enterocyte mass in villous atrophy-associated small bowel disease. Gastroenterology 124(5):1210–1219
13. Crenn P, Coudray-Lucas C, Thuillier F, Cynober L, Messing B (2000) Postabsorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. Gastroenterology 119(6):1496–1505. https://doi.org/10.1053/gast.2000.20227
14. Curis E, Crenn P, Cynober L (2007) Citrulline and the gut. Curr Opin Clin Nutr Metab Care 10(5):620–626. https://doi.org/10.1097/MCO.0b013e32829fb38d
15. Fragkos KC, Forbes A (2018) Citrulline as a marker of intestinal function and absorption in clinical settings: a systematic review and meta-analysis. United Eur Gastroenterol J 6(2):181–191. https://doi.org/10.1177/2050640617737632
16. Belza C, Fitzgerald K, de Silva N, Avitzur Y, Steinberg K, Courtney-Martin G, Wales PW (2017) Predicting intestinal adaptation in pediatric intestinal failure: a retrospective cohort study. Ann Surg. https://doi.org/10.1097/sla.0000000000002602
17. Gutierrez IM, Fisher JG, Ben-Ishay O, Jones BA, Kang KH, Hull MA, Shillingford N, Zurakowski D, Modi BP, Jaksic T (2014) Citrulline levels following proximal versus distal small bowel resection. J Pediatr Surg 49(5):741–744. https://doi.org/10.1016/j.jpedsurg.2014.02.056
18. Stultz JS, Tillman EM, Helms RA (2011) Plasma citrulline concentration as a biomarker for bowel loss and adaptation in hospitalized pediatric patients requiring parenteral nutrition. Nutr Clin Pract 26(6):681–687. https://doi.org/10.1177/0884533611425682
19. Diamanti A, Panetta F, Gandullia P, Morini F, Noto C, Torre G, Lezo A, Goffredo B, Daniele A, Gambarara M (2011) Plasma citrulline as marker of bowel adaptation in children with short bowel syndrome. Langenbeck's Arch Surg 7:1041
20. Bailly-Botuha C, Colomb V, Thioulouse E, Berthe MC, Garcette K, Dubern B, Goulet O, Couderc R, Girardet JP (2009) Plasma citrulline concentration reflects enterocyte mass in children with short bowel syndrome. Pediatr Res 65(5):559–563. https://doi.org/10.1203/PDR.0b013e31819986da
21. Fitzgibbons S, Ching YA, Valim C, Zhou J, Iglesias J, Duggan C, Jaksic T (2009) Relationship between serum citrulline levels and progression to parenteral nutrition independence in children with short bowel syndrome. J Pediatr Surg 44(5):928–932. https://doi.org/10.1016/j.jpedsurg.2009.01.034
22. Bourdon A, Rougé C, Legrand A, Robert CD, Piloquet H, Vodovar M, Voyer M, Rozé J-C, Darmaun D (2012) Urinary citrulline in very low birth weight preterm infants receiving intravenous nutrition. Br J Nutr 108(7):1150–1154. https://doi.org/10.1017/s0007114511006660
23. Rabier D, Kamoun P (1995) Metabolism of citrulline in man. Amino Acids 9(4):299–316. https://doi.org/10.1007/bf00807268
24. Luo M, Fernández-Estívariz C, Manatunga AK, Bazargan N, Gu LH, Jones DP, Klapproth JM, Sitaraman SV, Leader LM, Galloway JR, Ziegler TR (2007) Are plasma citrulline and glutamine biomarkers of intestinal absorptive function in patients with short bowel syndrome? JPEN J Parenter Enteral Nutr 31(1):1–7
25. Fjermestad H, Hvistendahl M, Jeppesen PB (2017) Fasting and postprandial plasma citrulline and the correlation to intestinal function evaluated by 72-hour metabolic balance studies in short bowel jejunostomy patients with intestinal failure. JPEN J Parenter Enteral Nutr. https://doi.org/10.1177/0148607116687497
26. Melis GC, Boelens PG, van der Sijp JRM, Popovici T, De Bandt J-P, Cynober L, van Leeuwen PAM (2007) The feeding route (enteral or parenteral) affects the plasma response of the dipetide Ala-Gln and the amino acids glutamine, citrulline and arginine, with the administration of Ala-Gln in preoperative patients. Br J Nutr 94(1):19–26. https://doi.org/10.1079/BJN20051463