


ORIGINAL ARTICLE

Nutrition and Growth

Intestinal microbial and metabolite profile in infants with small bowel stomas after bowel resection

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Funding information

Canadian Association of Pediatric Surgeons Research grant; Stollery Children's Hospital Foundation through the Women and Children's Health Research Institute

Abstract

Background: Infants with small bowel stomas (SBstoma) frequently struggle with absorption and rely on parenteral nutrition (PN). Intestinal absorption is difficult to predict based solely on intestinal anatomy. The purpose of this study was to characterize the microbiota and metabolic by-products within stoma effluent and correlate with clinical features and intestinal absorption.

Methods: Prospective cohort study collecting stoma samples from neonates with SBstoma ($N = 23$) or colostomy control ($N = 6$) at initial enteral feed (first sample) and before stoma closure (last sample). Gut bacteriome (16S rRNA sequencing), short-chain fatty acids (SCFAs) and bile acids (BAs) were characterized along with volume and energy content of a 48 h collection via bomb calorimetry (last sample). Hierarchical clustering and linear regression were used to compare the bacteriome and BAs/SCFAs, to bowel length, PN, and growth.

Results: Infants with $\leq 50\%$ small bowel lost more fluid on average than those with $> 50\%$ and controls (22, 18, 16 mL/kg/d, $p = 0.013$), but had similar energy losses (7, 10, 9 kcal/kg/d, $p = 0.147$). Infants growing poorly had enrichment of Proteobacteria compared to infants growing well (90% vs. 15%, $p = 0.004$). An increase in the ratio of secondary BAs within the small bowel over time, correlated with poor prognostic factors ($\leq 50\%$ small bowel, $> 50\%$ of calories from PN, and poor growth).

Conclusion: Infants with SBstoma and poor growth have a unique bacteriome community and those with poor enteral tolerance have metabolic differences compared to infants with improved absorption.

KEYWORDS

bile acids, microbiota, short-chain fatty acids, small intestine

1 | INTRODUCTION

A small intestinal enterostomy (SBstoma) is often required in newborns after intestinal resection. These infants frequently rely on parenteral nutrition (PN)

support due to excessive fluid and nutrient losses, even after stoma reversal. Although PN is necessary for growth and development, there are associated risks and the goal for any infant is to achieve full enteral nutrition. The length of the remaining small bowel is

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often the best predictor of how soon this will be achieved.¹ However, intestinal health is also important, and predicting the ability to wean from PN can be challenging. Potential factors affecting intestinal health include the intestinal pathology and degree of inflammation, comorbidities, and the intestinal microbiota.

The importance of the intestinal microbiota in overall gut health is recognized in conditions such as inflammatory bowel disease, necrotizing enterocolitis, and short bowel syndrome.^{2–4} In the setting of intestinal inflammation, alterations to the microbiota include decreased diversity and increased abundance of aerotolerant anaerobes.^{3,5,6} However, this has primarily been described in the colon whereas changes within the small bowel are less well-defined, but potentially important for absorption.⁷

In this study, we sought to characterize and correlate the small intestinal bacteriome and metabolic by-products in neonates with SBstoma with clinical features and markers of intestinal absorption. We hypothesize that infants with better enteral tolerance and growth will have a unique microbial environment and differences in metabolic biomarkers, which could affect long-term gut health.

2 | METHODS

2.1 | Patient selection and study design

This was a prospective cohort study enrolling infants under 6 months of age requiring an SBstoma, cared for in the neonatal intensive care units at BC Women's and Children's Hospital (Vancouver, BC, Canada) or Stollery Children's Hospital (Edmonton, AB, Canada) from January 2018 to December 2021. The study was approved by the BC Children's and Women's Research Ethics Board (REB H16-03374). Consent was obtained from all participating families. Only infants undergoing small intestinal resection were included. All stoma samples were collected with a syringe from the stoma appliance. Stoma bags are routinely emptied every 2–3 h therefore minimizing dwell time in the bag. Samples were immediately stored at -80° . Initial stoma samples were obtained postoperatively, after enteral nutrition initiation (15–20 mL/kg/d) (first sample) and then again just before stoma closure or when enteral feeding had plateaued (last sample). There was variation in time between first and last samples based on surgeon preference for timing of stoma closure or in the case of the two patients with total colonic Hirschsprung disease, when they reached stable enteral feeds. Documented clinical characteristics included age, diagnosis, intestinal anatomy, nutritional intake, anthropometrics, bloodwork, and any exposure to antibiotics. Variables used as markers of intestinal absorption included stoma volume (mL/kg/d) and

What is Known

- Infants with a small bowel stoma have variable intestinal absorption, and clinical trajectory can be difficult to predict.
- After intestinal resection, there are changes to the microbial community within the gut that may affect gut function.

What is New

- Infants with impaired growth had an increased abundance of Proteobacteria and decreased Firmicutes within the small bowel effluent.
- There is a relative increase in secondary bile acids within the small bowel effluent over time in infants who remain primarily parenteral nutrition-dependent, who have <50% of the small intestine, and who are growing poorly.

energy losses (kcal/kg/d), PN dependence (>or ≤50% of caloric intake), small intestinal length (> or <50% expected length) and growth (poor growth: Z score for weight and height <−2 and a decline between first and last samples vs. adequate growth: Z score for weight and height >−2, with stable or improved Z scores between first and last samples).

2.2 | Absorption analysis

A 48-h stoma collection was obtained just before stoma closure (same time as the last sample) and from a group of control infants. Controls had normal small bowel anatomy but required a colostomy for anorectal malformation. Energy content within the 48-h stool collection was determined using bomb calorimetry standardized using benzoic acid tablets according to the manufacturer's recommendations (Parr 1341 Plain Jacket Bomb Calorimeter, see File S1).

2.3 | 16S rRNA sequencing of bacteriome

16S rRNA sequencing was performed as previously described.⁸ DNA was extracted using a modified version of Protocol Q, developed by the International Human Microbiome Standards Consortium. 16S rRNA gene amplicons sequencing data have been deposited in the National Center for Biotechnology Information Sequence Read Archive and are available for download under BioProject PRJNA970526 (see File S1).

2.4 | Bile acid (BA) and short-chain fatty acid (SCFA) analysis

BA and SCFA preparation was done using stoma samples and analyzed using liquid chromatography coupled to tandem mass spectrometry and gas chromatography, respectively. Additional information is provided in File S1.

2.5 | Statistical analysis

Patients' bacteriomes were analyzed using hierarchical clustering with the histograms plotted using gplots and factoextra packages of R software (R, version 4.1.2). BA and bilirubin analyses were done using a Pearson correlation coefficient (Graphpad Prism 9). All other analyses were done using Mann–Whitney *t* test using Graphpad Prism.

3 | RESULTS

Twenty-three infants with SBstoma, and six control infants with a colostomy were enrolled. One infant was excluded because intestinal length was not measured. Ten infants had ileostomies (>50% of their small bowel remaining) and 12 infants had jejunostomies (<50% of their small bowel remaining). The first SBstoma samples were collected at a median of 18 days postoperatively (interquartile range [IQR]: 14–25) and the last sample/48 h collection was obtained at a median of 70 days postoperatively (IQR: 41–89). At the time of the last sample 14 infants (64%) were receiving >50% calories from PN, 5 infants (23%) were receiving <50% calories from PN, and three infants (14%) had completely weaned from PN. Patient characteristics based on intestinal length are described in Table 1. Control infants had a median gestational age of 38 weeks and were a median of 6 days old at the time of 48-h collection. They were all exclusively fed with breastmilk and did not receive PN.

3.1 | Residual small bowel length does not always correlate with intestinal losses or microbial composition

Intestinal losses were assessed by both volume and energy lost over 48 h just before stoma closure when enteral nutrition had been maximized and was at steady state. Control patients had consistent volume (11–20 mL/kg/d) and energy (5–18 kcal/kg/d) losses. SBstoma patients had a wider range of losses that did not always correlate with bowel length. Three infants had high volume (40 mL/kg/d) but low energy losses (6–12 kcal/kg/d), two of whom had <30% of their small

intestine and all of whom had different underlying diagnoses (meconium peritonitis, necrotizing enterocolitis, and gastroschisis). There were also two infants with high energy losses (>40 kcal/kg/d) who had 80% and 95% of their small intestine respectively, one of whom had a segmental volvulus and the other necrotizing enterocolitis (Figure S1). In general, infants with >50% of the small bowel remaining had lower volume losses compared to infants with <50%. However, daily energy losses did not differ significantly (10 vs. 7 kcal/kg/d; $p = 0.295$). Infants with >50% of the small bowel also had higher Z score for weight (−0.09 vs. −1.96; $p = 0.002$) and were tolerating more enteral nutrition (67 vs. 33 kcal/kg/d; $p = 0.007$) at the final stoma collection, compared to infants with <50% (Table 1).

We also investigated differences in intestinal bacteria based on intestinal length. Paired (first and last samples) 16S rRNA sequencing was available for SBstoma ($n = 19$) and control ($n = 4$) patients. Composition was described at the phylum level, with no significant difference in relative abundance between SBstoma patients with >50% versus <50% small bowel remaining (Figure 1A). Furthermore, alpha diversity was not significantly different between controls, or SBstoma patients with either >50% or <50% small bowel length. When comparing first and last samples for all SBstoma patients there was a significant decrease in the abundance of *Staphylococcus* ($p = 0.013$), along with a relative increase in Enterobacteriaceae (Figure 1B) over time. Finally, hierarchical clustering analysis of the gut microbiota did not demonstrate a relationship between SBstoma patients or controls at either time point (Figure 1C). We also compared infants based on other clinical parameters including gestational age (> or <32 weeks), underlying intestinal pathology (necrotizing enterocolitis vs. other), and PN dependency (> or <50% of calories) and did not find significant differences in the intestinal microbiota.

3.2 | Proteobacteria is enriched in infants with poor growth

A subset of infants ($n = 6$) had growth failure at the time of stoma closure. This was defined by a decline in Z scores for weight and length by one standard deviation between first and last stoma samples and by having Z scores at least two standard deviations below the mean. These infants had median Z scores for weight (−2.95; $p = 0.0004$) and length (−4.43; $p = 0.0003$), compared to infants with SBstoma growing adequately (weight −0.52 and length −0.52) with stable Z scores between first and last samples. When compared to those growing adequately, infants with poor growth had similar comorbidities, were of similar gestational age, and had no difference in the amount of enteral nutrition.

TABLE 1 Patient characteristics, nutrition, and growth based on small bowel length.

	Infants with >50% small bowel (n = 10)	Infants with <50% small bowel (n = 12)	Controls (n = 6)	p Value
Intestinal pathology (%)				NA
Necrotizing enterocolitis	5 (50)	5 (42)	0 (0)	
Intestinal perforation	3 (30)	0 (0)	0 (0)	
Segmental volvulus	1 (10)	1 (8)	0 (0)	
Small intestinal Hirschsprung's	0 (0)	2 (17)	0 (0)	
Meconium peritonitis	1 (10)	1 (8)	0 (0)	
Complex gastroschisis	0 (0)	2 (17)	0 (0)	
Small intestinal atresia	0 (0)	1 (8)	0 (0)	
Anorectal malformation	0 (0)	(0)	6 (100)	
Males, %	6 (60)	6 (50)	4 (67)	0.658
Median % small bowel remaining (IQR)	86 (81–95)	30 (16–48)	100 (NA)	4.89E–10
Median GA at birth (weeks, IQR)	25 (24–31)	30 (26–34)	38 (38–39)	0.314
Antibiotics exposure (%)				
Preoperative only	5 (50)	4 (33)	6 (100)	0.525
48 h short course	1 (10)	2 (17)	0 (0)	
Treatment for infection	4 (40)	6 (50)	0 (0)	
First stool sample (median, IQR)			48-h collection	
Age in days	26 (21–27)	45 (25–56)	8 (5–14)	0.063
Postoperative days	18 (14–22)	22 (15–28)	6 (3–12)	0.229
Z score weight	–0.09 (–0.90 to 1.14)	–1.96 (–2.35 to –1.53)	–0.11 (–0.3 to 0.1)	0.002
Z score length	–2.12 (–3.45 to –0.76)	–1.73 (–3.48 to –1.06)	0.25 (0.19–0.46)	0.917
Z score HC	–2.64 (–3.55 to –1.19)	–0.97 (–1.87 to –0.63)	–0.06 (–0.74 to 0.42)	0.512
PN kcal/kg/d	90 (80–96)	85 (73–93)	0 (0–0)	0.359
EN kcal/kg/d	22 (15–40)	28 (18–38)	98 (98–80)	0.426
Total kcal/kg/d	112 (107–117)	109 (100–122)	98 (98–80)	0.539
Last stool sample (median, IQR)				
Postoperative days	66 (56–79)	81 (60–86)		0.426
Z score weight	–0.69 (–1.69 to 0.23)	–1.73 (–2.95 to –1.7)		0.077
Z score length	–1.4 (–2.82 to 0.70)	–2.87 (–4.53 to –1.51)		0.362
Z score HC	–1.45 (–3.06 to 0.20)	–1.52 (–2.27 to –1.05)		0.764
PN kcal/kg/d	69 (0–96)	88 (68–93)		0.147
EN kcal/kg/d	67 (49–95)	33 (22–50)		0.007
Total kcal/kg/d	139 (137–156)	121 (115–130)		0.033

Note: p values < 0.05 are in bold. p Values refer to comparisons between infants with small bowel stoma.

Abbreviations: EN, enteral nutrition; GA, gestational age; HC, head circumference; IQR, interquartile range; NA, not available; PN, parenteral nutrition.

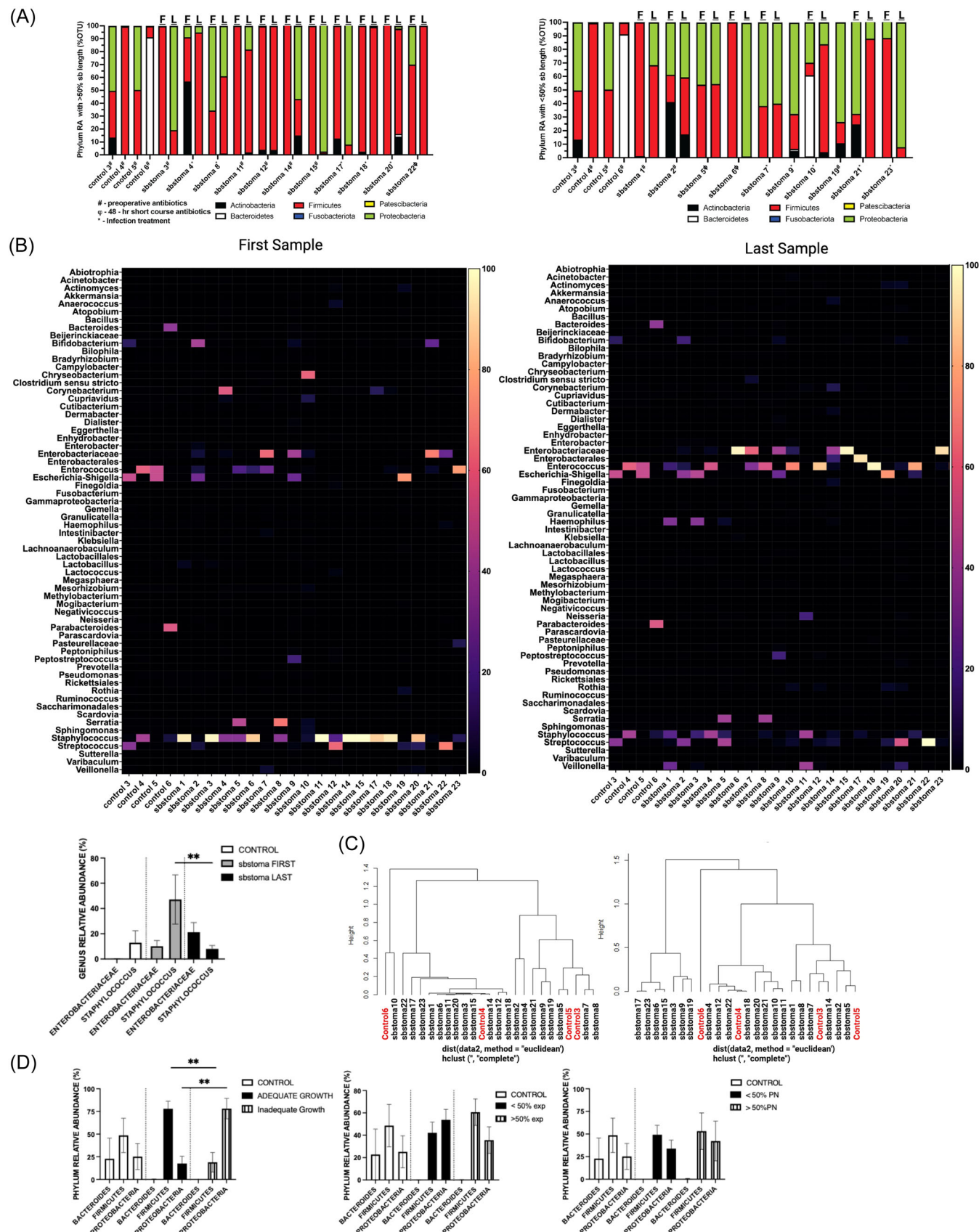


FIGURE 1 (See caption on next page).

The two groups also had similar median residual small bowel length (55% vs. 75%, $p = 0.79$), stoma losses (16 vs. 22 mL/kg/d, $p = 0.73$ and 9 vs. 8 kcal/kg/d, $p = 0.999$) and antibiotic exposure. Median urine sodium concentration was higher for those with poor growth at the beginning of the study, but no different at the time of the last sample (Table 2). One patient with adequate growth was receiving ursodiol. Infants with poor growth had a higher abundance of Proteobacteria in their last SBstoma samples compared to infants growing adequately (90% vs. 15%, $p = 0.004$) and a lower abundance of Firmicutes (10% vs. 78%, $p = 0.001$). This bacterial signature was not seen when comparing infants based on bowel length or PN support (Figure 1D).

3.3 | Reduced intestinal length, poor enteral tolerance, and poor growth are associated with increased secondary BAs

BA and SCFA profiles within stoma effluent were analyzed. BA composition varied considerably among stoma samples. Secondary BAs were generally reduced or missing, with ursodeoxycholic acid and tauroursodeoxycholic acid being the most common, but detectable in only four patients. The relative abundance of secondary BAs in those four patients (SBstoma 6, 8, 21, and 23) was 55%, 90%, 84%, and 14%, respectively (Figure 2A and Table S1). Increased primary BAs negatively correlated with conjugated bilirubin levels ($r = -0.52$, $p = 0.02$), whereas secondary BAs positively correlated with serum conjugated bilirubin ($r = 6.082$, $p = 0.0001$) (Figure 2B). Infants with <50% of their small bowel had similar fold change in primary BAs (last/first stoma samples), but displayed a significantly greater increase in secondary BAs (last/first stoma samples; $p < 0.01$), compared to patients with >50% of their small bowel. This trend was also seen in infants with poor growth and in those requiring >50% of calories from PN (Figure 2C).

SCFAs also varied considerably among samples. Acetic acid was significantly higher in patients with >50% of the small bowel compared to <50% (Figure S2A). Although levels of propionate were 10-fold lower overall, there was a significant increase in propionate in infants with >50% of the small bowel and

in those receiving the majority of their calories from PN (Figure S2B). There were no differences in SCFA levels between infants with poor growth and those growing adequately, but there was increased butyrate in those with poor growth compared to controls (Figure S2C).

4 | DISCUSSION

Access to small bowel effluent in infants after small bowel resection is limited, and little is known about the small bowel microenvironment, including the microbiota and metabolic by-products. In this study, infants had variable microbial profiles despite similar characteristics such as bowel length, gestational age, or PN dependence. An exception was infants growing poorly who had increased abundance of Proteobacteria and a paucity of Firmicutes. We also found temporal changes to BAs and SCFAs within the intestinal lumen based on clinical characteristics.

It is often assumed that SBstoma losses, both volume and energy, reflect intestinal anatomy and that outputs increase as bowel length decreases, but this is not always the case. As expected, in controls, daily colostomy output was consistent, similar to what has been reported in healthy adults (2%–10% of caloric intake).⁹ Conversely, in infants after intestinal resection, energy losses are variable. One study found that children with <50% of the small bowel lost 20%–30% of their caloric intake.¹⁰ This was consistent with our findings where energy losses ranged from 20% to 40% of intake in those with <50% of small bowel length. However, exceptions included an infant with only 25% of their small bowel who had only 6% energy loss despite enteral caloric intake similar to the other infants with <50% of the small bowel. Conversely, another patient with 95% of the small bowel was losing 50% of caloric intake from the stoma. We performed 48-h stoma collection when intestinal function had stabilized and enteral nutrition had been maximized so that energy losses would reflect the best possible absorption for each infant. These findings suggest that intestinal length is not the only determinant of absorption. In general, caloric and volume losses are higher for infants with less small intestine, but we found that caloric losses cannot always be reliably predicted by

FIGURE 1 Microbiome analysis of infants with small bowel stoma. 16S microbiome analysis was done using samples collected from control infants and those with SBstoma. (A) Phylum composition based on expected bowel length (< or > 50%), showing first (F) and last (L) samples except controls, SBstoma 9 and SBstoma 19 with only one sample. (B) Heat map showing the change in bacterial genus from the first sample (left) and last sample (right) as well as specific analysis of Enterobacteriaceae and *Staphylococcus* relative abundance (SBstoma 9 and SBstoma 19 have only one sample). (C) Hierarchical analysis of phylum composition at first sample (left) and last sample (right). $N = 23$, * $p < 0.05$, ** $p < 0.01$. (D) Phylum relative abundance for Bacteroides, Firmicutes, and Proteobacteria at the first and last study samples compared between controls and adequate growth or poor growth based on body weight and length. $N = 22$, ** $p < 0.01$, expected bowel length (< or >50%) and percentage of PN at the end of the study (> or <50%). PN, parenteral nutrition; SBstoma, small bowel stoma.

TABLE 2 Comparison of infants with poor growth versus adequate growth before stoma closure.

	Infants with poor growth (n = 6)	Infants with adequate growth (n = 16)	p Value
Intestinal pathology (%)			NA
Necrotizing enterocolitis	5 (83)	5 (31)	
Complex gastroschisis	1 (17)	2 (13)	
Intestinal perforation	0 (0)	3 (18)	
Meconium peritonitis	0 (0)	2 (13)	
Segmental volvulus	0 (0)	2 (13)	
Small intestinal Hirschsprung	0 (0)	2 (13)	
Comorbidities (%)			NA
None	1	8	
HIE	2	3	
IVH	2	3	
RDS	2	0	
IUGR	2	1	
Acute renal injury	0	2	
Urine sodium concentration (mmol/L)			
First sample	90 (58–113)	38 (25–43)	0.004
Last sample	43 (37–47)	25 (15–43)	0.425
Antibiotics exposure (%)			NA
Preoperative only	4 (67)	5 (31)	
48 h short course	0 (0)	3 (19)	
Treatment for infection	2 (33)	8 (50)	
ΔZ score for weight (first/last)	−1.02	−0.04	NA
ΔZ score for height (first/last)	−1.09	0.23	NA
Z score for weight (last)	−2.95 (−3.35 to −2.50)	−0.52 (−1.66 to 0.155)	0.0004
Z score for height (last)	−4.43 (−4.59 to −2.65)	−0.52 (−1.26 to 0.160)	0.0003
GA at birth (weeks)	25 (24–25)	30 (24–33)	0.141
% small bowel remaining	55 (30–95)	75 (24–86)	0.793
Total daily calories (kcal/kg/d)	131 (120–139)	129 (118–139)	0.963
% enteral calories	34 (17–59)	37 (27–54)	0.553
Stool output (mL/kg/d)	16 (11–41)	22 (14–31)	0.734
Energy loss in stool (kcal/kg/d)	9 (5–21)	8 (5–16)	0.999
SCFA concentration in the stool (μM/g)	247 (131–541)	264 (84–527)	0.913
Relative abundance proteobacteria (%)	90 (65–98)	15 (3–50)	0.004
Relative abundance firmicutes (%)	10 (4–16)	78 (44–95)	0.001

Note: All values are expressed as medians with interquartile range. p Values < 0.05 are in bold. Δ Z score (first/last) is the median change in Z score for weight and length between the first and last samples.

Abbreviations: GA, gestational age; HIE, hypoxic-ischemic encephalopathy; IUGR, intrauterine growth restriction; IVH, intraventricular hemorrhage; NA, not available; RDS, respiratory distress syndrome; SCFA, short-chain fatty acid.

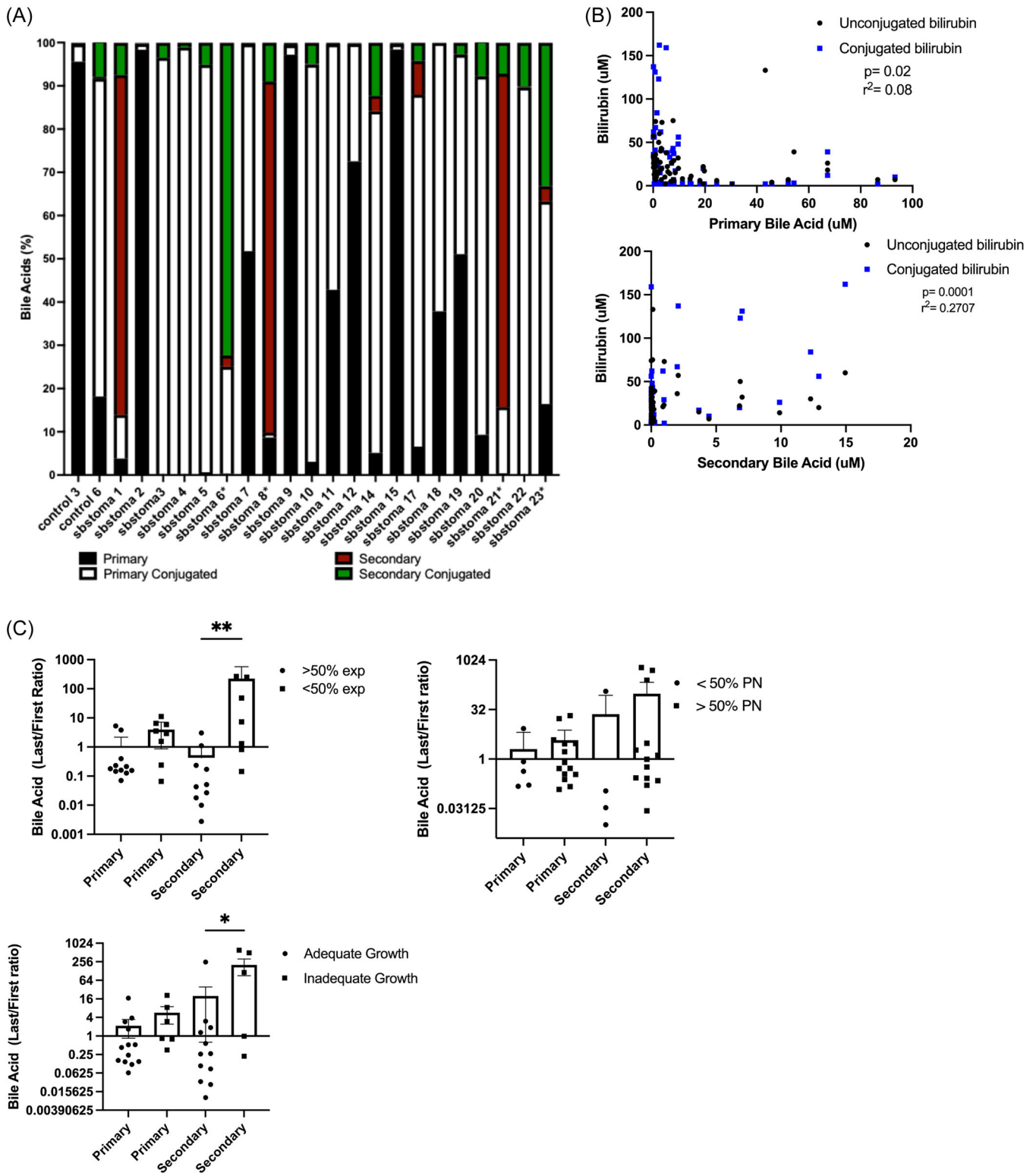


FIGURE 2 Bile acid analysis of infants with small bowel stoma. Stoma stool samples were analyzed using LC-MS/MS for (A) composition of primary and secondary bile acids. Asterisk (*) denotes those patients with secondary bile acid composition $>10\%$. (B) Pearson correlation coefficient was used to compare primary or secondary bile acids to conjugated bilirubin levels. (C) Bile acid ratio between the first and last samples collected was analyzed depending on expected bowel length ($>$ or $<50\%$), percentage of PN at the end of the study ($>$ or $<50\%$), or adequate growth or poor growth based on body weight and length. Ratios are based on relative abundance of bile acids. $N = 22$, $*p < 0.05$, $**p < 0.01$. LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; PN, parenteral nutrition.

intestinal length or stoma volume alone. Importantly, assumptions about intestinal absorption based solely on stoma output volume may be inaccurate.

We also examined potential correlations between clinical variables, gut bacterial abundance, and bacterial by-products (BAs, SCFAs). BA analysis of SBstoma effluent demonstrated predominantly primary with few secondary BAs, which was expected as infants are known to have few fecal secondary BAs.¹¹ Infants with poor prognostic indicators (less small bowel, increased PN dependence, higher conjugated bilirubin and those growing poorly) all had a relative increase in secondary BAs within the small bowel over time. There are only a few known anaerobic bacterial families capable of converting primary BAs to secondary BAs in healthy humans and these include Lachnospiraceae and Ruminococcaceae, which produce deoxycholic and lithocholic acid, the most abundant secondary BAs in humans.¹² Recent data suggest that these BAs play a role in reducing intestinal inflammation, protecting against insulin resistance, and improving motility by binding to TGR5 receptors located throughout the gut.¹³ In a study of adults with ulcerative colitis and ileal-pouch reconstruction, decreased secondary BAs were associated with pouchitis and inflammation of the small intestine.¹² However, both lithocholic acid and deoxycholic acid were essentially undetectable in our population. One possibility to explain the shift in BA composition in infants with poor prognostic indicators is an overall increase in bacterial load. Infants reliant on PN after bowel resection are at risk for small intestinal bacterial overgrowth (SIBO),^{14,15} which could potentially result in increased conversion of primary to secondary BAs.

The other metabolites analyzed, SCFAs, are by-products of carbohydrate fermentation by intestinal bacteria. SCFAs are typically concentrated in the colon and distal ileum, where they serve as a source of calories.¹⁶ Additionally, SCFAs enhance intestinal adaptation in animal models of neonatal short bowel syndrome, where butyrate drives intestinal epithelial changes in the small bowel.^{17,18} Infants produce mostly acetate from carbohydrate fermentation and as expected, we found higher levels of acetate in stoma samples from both the control group and infants with >50% of their small intestine.¹⁹ Although levels were lower in general, we did observe an increase in propionate concentration in those with higher PN needs and those with >50% of the small bowel at the end of the study period. Propionate's role has been less well studied than acetate and butyrate, but there is evidence that it induces differentiation of T regulatory cells, and supplementation has been associated with decreased inflammation in systemic diseases such as multiple sclerosis and atherosclerosis.^{20,21} Propionate can be synthesized from succinate, lactate, and/or deoxy sugars through either the succinate, acrylate, or

propanediol pathways, respectively.^{22,23} Infants who have lost small intestine and remain PN dependent, often have increased lactate levels within the bowel,²⁴ which could potentially be metabolized to propionate. The clinical significance of increased propionate remains unclear.

In healthy infants, the intestinal microbiota evolves over the first few years of life from predominantly aerobic to strictly anaerobes.²⁵ Factors that alter this process include the mode of delivery, prematurity, and the initial diet to name a few.²⁶ Several studies have identified a decrease in the overall diversity,²⁷ an increase in Proteobacteria,^{2,24} and in some cases Lactobacillaceae, in bowel resection patients.^{2,28} In this study, distinct phylum level changes were not observed based on intestinal length, gestational age, intestinal pathology, or PN needs. However, infants with poor growth, with a decline in their Z scores for weight and length during the study, had significantly more Proteobacteria and fewer Firmicutes despite similar clinical characteristics (Table 2). In all infants with poor growth, the predominant bacteria (90% abundance) included Enterobacteriaceae, Enterobacterales, or *Escherichia*. Whether this microbial environment contributes to impaired growth, or whether the underlying intestinal conditions support this profile is uncertain.

The impact of the intestinal microbiota on human metabolism has been well studied, yet remains controversial. Recent studies identified a link between Firmicutes and energy harvest, demonstrating an increased ratio of Firmicutes to Bacteroidetes in obesity,^{29,30} while other studies have found no correlation.^{31,32} A study looking at the microbiota in children with short bowel syndrome found that those with <35 cm of small bowel had a higher abundance of Proteobacteria (58%) and poor linear growth.³³ Similar to our cohort, most of the Proteobacteria were Enterobacteriaceae. Additionally, the small intestinal microbiota in children with malnutrition and malabsorption (tropical sprue) is enriched with Proteobacteria,^{34,35} and preterm infants with poor growth have a shift from *Staphylococcus* to Proteobacteria, specifically Enterobacteriaceae, and are in a metabolic state similar to fasting, with a reliance primarily on fatty-acid oxidation.³⁶ It may be that the microbiota in infants who are growing poorly preferentially metabolize lipids, and additional calories in the form of carbohydrates, may go unused.³⁷ Next steps include analyzing intestinal samples from infants with poor growth using shotgun sequencing to provide an in-depth understanding of bacterial changes.

This study is limited by its small size and heterogeneous population. However, all infants were managed with common clinical protocols, in a similar environment and were all receiving breast milk during the study. We were able to demonstrate that infants with poor prognostic indicators, including significant reliance

on PN, poor growth, and less small bowel, had a unique microbial signature and metabolic profile that could potentially be used to target new therapies and predict outcomes in the future.

ACKNOWLEDGMENTS

This study was funded in part by the Canadian Association of Pediatric Surgeons Research grant and the Stollery Children's Hospital Foundation through the Women and Children's Health Research Institute.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Piper HG, Bording-Jorgensen M, Veniamin S, et al. Intestinal microbial and metabolite profile in infants with small bowel stomas after bowel resection. *J Pediatr Gastroenterol Nutr*. 2024;1-11. doi:10.1002/jpn3.12327