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Environmental Enteric Dysfunction is Associated with Altered Bile Acid Metabolism

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Abstract

Objectives—Environmental enteric dysfunction (EED), a clinically asymptomatic condition characterized by inflammation of the small bowel mucosa, villous atrophy, and increased gut permeability, is common among children in developing countries. Because of abnormal gut mucosa and altered gut microbiome, EED could potentially affect the metabolism and enterohepatic circulation of bile acids.

Methods—In 313 children, aged 12–59 months, EED was assessed by the dual sugar absorption test. Serum bile acids were measured using stable-isotope liquid chromatography-tandem mass spectrometry.

Results—In the overall study population, serum cholic acid and chenodeoxycholic acid were lower while glycocholic acid, taurodeoxycholic acid, glycodeoxycholic acid, glycolithocholic acid, and glyoursodeoxycholic acid were significantly higher at older ages. Independent of age, serum taurochenodeoxycholic acid, tauromuricholic acid, and glyoursodeoxycholic acid were significantly different between 244 children with and 69 children without EED. Total serum bile acids (median, interquartile range) were 4.51 (2.45, 7.51) and 5.10 (3.32, 9.01) $\mu\text{mol/L}$ in children with and without EED, respectively (age-adjusted, $P=0.0009$). The proportion of bile acids conjugated with taurine instead of glycine was higher in children with EED ($P<0.0001$).

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Conflicts of interest: The authors have no conflicts to declare.

Author contributions: R.D.S., I.T., L.F., and M.J.M. were responsible for the study design, statistical analyses, data interpretation, and writing the manuscript. I.T., K.M.M., and M.J.M. implemented the study in rural Malawi and collected samples and data. R.M., M.K., and M.I.O. contributed towards laboratory analyses of serum metabolites. M.G.F. and M.I.O. conducted the data analysis. All authors contributed to writing and approving the final manuscript.

Conclusions—EED is associated with altered bile acid metabolism in young children in rural Malawi. Further work is needed to determine the generalizability of these findings in other study populations.

Keywords

Africa; Children; Malabsorption; Malnutrition

Environmental enteric dysfunction (EED), a clinically asymptomatic condition characterized by inflammation of the small bowel mucosa, villous atrophy, malabsorption, and increased intestinal permeability, is common among young children in low-income countries (1). EED is considered a major factor contributing to childhood stunting (1–3). Histologic studies of EED show mucosal T-cell activation, crypt hyperplasia, shortening of villi, and elevated mucosal proinflammatory cytokines in the small intestine (4, 5). The gut microbiome in children with EED is presumed to be abnormal but has not yet been characterized (1).

Bile acids are essential as detergents to aid the intestinal absorption of dietary fats and fat-soluble vitamins and for signaling in a diverse set of signaling pathways that regulate nutrient metabolism (6). Because of abnormalities in enterocytes, low-grade inflammation, and increased intestinal permeability, EED could potentially affect the metabolism and enterohepatic circulation of bile acids. Bile acid malabsorption is known to impair the integrity and permeability of the intestinal mucosa (7–10) and could play a potential role in the pathogenesis of EED. Abnormalities in bile acid metabolism could also potentially affect the absorption of fat-soluble nutrients in EED. Recent advances in stable-isotope liquid chromatography-tandem mass spectrometry (LC-MS/MS) now allow accurate quantitation of serum bile acids. Using this platform, we addressed the hypothesis that children with EED have abnormalities in serum bile acid metabolism. We tested this hypothesis in young children in rural Africa.

Materials and Methods

Subjects and study design

The study design was cross-sectional. The study subjects consisted of a community-based sample of 313 children, aged 12–59 months, seen in six villages (Masika, Makhwira, Mitondo, Mbiza, Chamba, and Mayaka) in rural southern Malawi in 2011. Children were eligible for the study if they were between 12 and 59 months of age, had no evidence of congenital or chronic disease, caretaker-reported diarrhea, nor were under treatment for malnutrition. All children underwent anthropometry conducted by trained, experienced field workers. Weight was measured to the nearest 5 g using a digital scale (Seca 334, Chino, CA). Recumbent length (children < 24 months) or standing height was measured to the nearest 0.1 cm using a rigid height board (Seca 417). Height-for-age Z-scores (HAZ) and weight-for-height were calculated using World Health Organization growth standards (11). Stunting was defined as HAZ < −2 (11). Venous blood was drawn by study nurses and doctors. Serum samples were processed, aliquoted, and snap frozen in liquid nitrogen in cryovials within 4 h of blood drawing. Cryovials were transferred to storage at −80°C. Chichewa-speaking Malawian research nurses obtained written and oral informed consent

from each child's caretaker before enrollment in the study. Community consent for the study also was obtained from the village chief and local health officials. The protocol for this study was approved by the College of Medicine Research Ethics Committee of the University of Malawi, the Human Research Protection Office of Washington University in St. Louis, and the Johns Hopkins School of Medicine Institutional Review Board. All authors had access to the study data and reviewed and approved the final manuscript.

Laboratory analyses

The dual sugar permeability test was used as the standard non-invasive measure of gut integrity (12), as described in detail elsewhere (3). Urinary lactulose and mannitol were measured using high performance liquid chromatography (3). A higher lactulose/mannitol (L:M) ratio indicates greater gut permeability. Children with an L:M ratio ≥ 0.15 were defined as having environmental enteric dysfunction (13). Serum bile acids were extracted and concentrations were assessed in a masked fashion using the Biocrates Bile Acids Kit (Biocrates Life Science AG, Innsbruck, Austria) following the manufacturers protocol for the API5500 liquid chromatography-tandem mass spectrometry (LC-MS/MS) System (SCIEX, Framingham, MA) running with Analyst 1.5.2 software and equipped with an electrospray ionization source, a Shimadzu CBM-20A command module, LC-20AB pump, and a Shimadzu SIL-20AC-HT autosampler and a CTO-10Ac column oven heater. Briefly, 10 μ L of the ISTD mix (10 stable isotope labeled internal standards) was pipetted onto a 96-well Biocrates plate and dried under nitrogen evaporator for 5 mins, followed by the addition of 10 μ L plasma-EDTA samples and calibration standards. The samples were dried under nitrogen at room temperature (RT) for 20 min. 100 μ L of methanol was added and incubated at RT on a shaker (600 rpm) for 20 min. The plate was centrifuged at $500 \times g$ for 2 min, resulting in 70 μ L of methanolic extract in the 96-deep well capture plate. The upper filter plate was removed and 60 μ L of water was added to each well of the capture plate. The capture plate was incubated at RT on a shaker (450 rpm) for 5 min. Twenty μ L of sample was injected into the ultrahigh pressure liquid chromatography column provided from the Biocrates Bile Acids Kit coupled with a Phenomenex ULTRA XB-C18 Security Guard Cartridge, 2.1mm ID. The mobile phase consisted of solvent A (ammonium acetate [10 mM] containing 0.015% formic acid) and solvent B (acetonitrile:methanol:water (6.5:3:0.5) containing ammonium acetate [10 mM] and 0.015% formic acid) with the following gradient: 0–0.5 min 35% B, 0.7 min 40% B, 3.0 min 45% B, 3.2 min 55% B, 5.5 min 65% B, 6.5 min 100% B, 8.5 min 100% B, 8.6 min 35% B, 11 min 35% B. Evaluation of the samples was carried out using the MetIDQ software. Concentrations were calculated using the Analyst/MetIDQ software and reported in μ mol/L.

Serum glycine and taurine were measured in a masked fashion using LC-MS/MS. Metabolites were extracted and concentrations measured using the AbsoluteIDQ p180 kit (Biocrates Life Sciences AG) following the manufacturers protocol for a 5500 QTrap (Sciex, Framingham, MA) mass spectrometer equipped with an electrospray ionization source, a CBM-20A command module, LC-20AB pump, and a SIL-20AC-HT autosampler, a CTO-10Ac column oven heater (all Shimadzu, Tokyo, Japan), and running Analyst 1.5.2 software (Biocrates), as described in detail elsewhere (14). The inter-assay and intra-assay coefficients of variation were $\sim 10\%$ for bile acids, glycine, and taurine.

Statistical analysis

Serum bile acid concentrations were skewed to higher values. Median and interquartile ranges (IQR) were used to describe serum bile acid concentrations. Serum glycine and taurine were normally distributed. Mean and standard deviation (SD) were used to describe serum glycine and taurine concentrations. Wilcoxon rank-sum tests were used to compare serum bile acid concentrations and bile acid ratios between groups. Student t-test was used to compare serum glycine and taurine between groups. Spearman correlations were used to examine the relationship of serum bile acids with continuous variables such as age and gut permeability as measured by the L:M ratio. Statistical analyses were conducted using R version 3.1.

Results

The characteristics of the 313 children in the study are shown (Table 1). There were nearly equal number of girls and boys. Most children were stunted. Spearman correlations between serum bile acids and age are shown (Table 2). Age was negatively correlated with cholic acid (CA) and chenodeoxycholic acid (CDCA) (both $P < 0.0001$) and positively correlated with taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA) and glycolithocholic acid (GLCA) (all $P < 0.0001$), glycocholic acid (GCA) ($P = 0.02$), and glyoursodeoxycholic acid (GUDCA) ($P = 0.03$). There were no significant differences in any serum bile acids between boys and girls (data not shown). The total serum bile acid composition is shown (Figure 1). The three most dominant serum bile acids were glycochenodeoxycholic acid (GCDCA), GCA, and GDCA. Deoxycholic acid (DCA), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), and CA were the next most abundant serum bile acids. GUDCA, tauromurocholic acid (TMCA), TDCA, CDCA, GLCA, and ursodeoxycholic acid (UDCA) comprised a relatively small portion of total serum bile acids.

Serum bile acid concentrations were compared between children with and without EED adjusting for age (Table 3). Serum TCDCA, TMCA, and GUDCA were significantly different between the two groups. Total serum bile acids were significantly lower in children with EED compared with children without EED ($P = 0.0009$). Mean (SD) serum glycine in children with and without EED was 339 (105) and 312 (69) $\mu\text{mol/L}$, respectively ($P = 0.06$). Mean (SD) serum taurine in children with and without EED was 186 (57) and 156 (50) $\mu\text{mol/L}$, respectively ($P < 0.0001$).

Serum bile acid ratios were examined in order to gain insight into bile acid conjugation (Table 4). The $(\text{TCA} + \text{TDCA})/(\text{GCA} + \text{GDCA})$, and $(\text{TCA} + \text{TDCA} + \text{TCDCA})/(\text{GCA} + \text{GDCA} + \text{GCDCA})$ ratios, which provide insight into the relative proportion of bile acids conjugated by taurine, were higher in children with EED compared to children without EED (both $P < 0.0001$).

Spearman correlations between the different serum bile acids, glycine, and taurine, are shown (Table 5). In general, the highest Spearman correlation coefficients were found between the same bile acid that was conjugated by either taurine or glycine (i.e., TCA and GCA, TCDCA and GCDCA, TDCA and GDCA), or primary bile acid conjugated by taurine

or glycine (i.e., TCA and TCDCA, GCA and GCDCA), or have similar conversion (i.e., 7 α -dehydroxylation, such as GCA and GDCA, TCA and TDCA). There were no significant correlations of glycine with any glycine-conjugated bile acid nor of taurine with any taurine-conjugated bile acid.

Discussion

This study suggests that young children with EED have alterations in bile acid metabolism. Total serum bile acids were ~12% lower in children with EED compared with children without EED. Lower serum bile acids in children with EED may be a reflection of injury to the ileum with impaired return via the portal circulation and spillover into the peripheral circulation. Higher serum bile acids most commonly reflect liver disease and impaired hepatic extraction. The lower serum bile acids in children with EED suggest that these children do not have liver disease, at least to the extent that could affect the hepatic extraction of bile acids.

The entire bile acid pool passes through the enterohepatic circulation 8–12 times per day, and under normal circumstances 3–5% of bile acids are lost in the stool (6, 17). Thus, each day up to a third of the bile acid pool is lost and must be synthesized. In healthy adults, the liver synthesizes 500 mg of cholesterol per day, of which 90% is utilized for bile acid synthesis and the remainder for synthesis of steroid hormones (18). If the modest reduction in serum bile acids is reflective of the total bile acid pool, higher hepatic bile acid synthesis may be required to compensate for this reduction.

There is a paucity of data on serum bile acids, as measured by LC-MS/MS, in children. The distribution of serum bile acids in the present study was somewhat different than the distribution of serum bile acids described in a healthy reference population of Austrian children, aged 3–5 years (19). The main difference was that Malawian children had a lower proportion of TCDCA comprising total serum bile acids compared with the Austrian children. Such differences, which have not been described before, might be attributed to differences in the gut microbiota between these two groups of children. The gut microbiome can affect the composition of bile acids through dehydroxylation, epimerization, oxidation, esterification, desulfation, and other reactions in the production of secondary bile acids (20). Conversely, the bile acid pool can affect the composition of the gut microbiome (20). While the gut microbiome was not directly measured in the children in the present study, it is known that children with malnutrition in the same population in Malawi have lower levels of beneficial microbiota such as *Lactobacilli* and bifidobacteria (21). Exposure to Bacteroidales species and *Escherichia coli*, combined with a moderately malnourished diet can remodel the small intestine in mice to resemble features of EED found in humans (22).

There were modest differences in both serum taurine concentrations and proportion of bile acids conjugated with taurine instead of glycine in children with EED compared to children without EED. Serum concentrations of unconjugated bile acids were not different between children with and without EED, which suggests that small bowel bacterial overgrowth is not a significant problem, since elevated unconjugated bile acids occur in small bowel bacterial overgrowth (23). Although small bowel bacterial overgrowth has been hypothesized to play

an etiological role in EED, treatment of children with rifaximin had no effect on EED (24). We measured serum taurine and glycine to examine their relationship with conjugated bile acids. The present study shows no significant correlations between serum taurine and glycine and their respective conjugated bile acids. These findings, at least for taurine, are consistent with the concept that there is a partition between the systemic and enterohepatic taurine pools (25). In the present study, the ratio of glycine- to taurine-conjugated bile acids was ~12:1, which is higher than has been reported elsewhere (6, 19). The altered ratio may reflect increased bile acid turnover, however, it was more pronounced in controls compared to those with EED.

The strengths of this study are the community-based sample of children from a setting typical for EED, well characterized study groups for EED, and the use of LC-MS/MS for absolute quantitation of serum bile acids using a validated platform. The findings of this study cannot necessarily be generalized to other populations due to environmental, cultural, and dietary differences that could differ from the setting in Malawi. We are limited in comparison of our results with other pediatric populations, given the scarcity of studies that characterize serum bile acids in young children.

In conclusion, children with EED have altered bile acid metabolism. Further work is needed to confirm whether bile acid malabsorption is present in EED by measurement of fecal bile acid concentrations. The role of the gut microbiome in bile acid metabolism of children with EED remains uncharacterized and should be addressed in future studies.

Acknowledgments

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References

1. Keusch GT, Denno DM, Black RE, et al. Environmental enteric dysfunction: pathogenesis, diagnosis, and clinical consequences. *Clin Infect Dis*. 2014; 59(Suppl 4):S207–S212. [PubMed: 25305288]
2. Lunn PG, Northrop-Clewes CA, Downes RM. Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. *Lancet*. 1991; 338:907–910. [PubMed: 1681266]
3. Weisz AJ, Manary MJ, Stephenson K, et al. Abnormal gut integrity is associated with reduced linear growth in rural Malawian children. *J Pediatr Gastroenterol Nutr*. 2012; 55:747–750. [PubMed: 22732897]
4. Veitch AM, Kelly P, Zulu IS, et al. Tropical enteropathy: a T-cell-mediated crypt hyperplastic enteropathy. *Eur J Gastroenterol Hepatol*. 2001; 13:1175–1181. [PubMed: 11711773]
5. Campbell DI, Murch SH, Elia M, et al. Chronic T cell-mediated enteropathy in rural west African children: relationship with nutritional status and small bowel function. *Pediatr Res*. 2003; 54:306–311. [PubMed: 12788978]
6. Dawson PA, Karpen SJ. Intestinal transport and metabolism of bile acids. *J Lipid Res*. 2015; 56:1085–1099. [PubMed: 25210150]
7. Erickson RA, Epstein RJ Jr. Oral chenodeoxycholic acid increases small intestinal permeability to lactulose in humans. *Am J Gastroenterol*. 1988; 83:541–544. [PubMed: 3129932]

8. Münch A, Ström M, Söderholm JD. Dihydroxy bile acids increase mucosal permeability and bacterial uptake in human colon biopsies. *Scand J Gastroenterol.* 2007; 42:1167–1174. [PubMed: 17852874]
9. Chen X, Oshima T, Tomita T, et al. Acidic bile salts modulate the squamous epithelial barrier function by modulating tight junction proteins. *Am J Physiol Gastrointest Liver Physiol.* 2011; 301:G203–G209. [PubMed: 21617116]
10. Stenman LK, Holma R, Eggert A, et al. A novel mechanism for gut barrier dysfunction by dietary fat: epithelial disruption by hydrophobic bile acids. *Am J Physiol Gastrointest Liver Physiol.* 2013; 304:G227–G234. [PubMed: 23203158]
11. de Onis M, Onyango A, Borghi E, et al. Worldwide implementation of the WHO Child Growth Standards. *Public Health Nutr.* 2012; 15:1603–1610. [PubMed: 22717390]
12. Denno DM, VanBuskirk K, Nelson ZC, et al. Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction: a systematic review. *Clin Infect Dis.* 2014; 59(suppl 4):S213–S219. [PubMed: 25305289]
13. Lord, RS., Bralley, JA., editors. *Laboratory Evaluations for Integrative and Functional Medicine.* Duluth, Georgia: Metamatrix Institute; 2008.
14. Schmerler D, Neugebauer S, Ludewig K, et al. Targeted metabolomics for discrimination of systemic inflammatory disorders in critically ill patients. *J Lipid Res.* 2012; 53:1369–1375. [PubMed: 22581935]
15. Hylemon PB, Zhou H, Pandak WM, et al. Bile acids as regulatory molecules. *J Lipid Res.* 2009; 50:1509–1520. [PubMed: 19346331]
16. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem.* 2003; 72:137–174. [PubMed: 12543708]
17. Jahnel J, Zöhrer E, Scharnagl H, et al. Reference ranges of serum bile acids in children and adolescents. *Clin Chem Lab Med.* 2015; 53:1807–1813. [PubMed: 25781695]
18. Fiorucci S, Distrutti E. Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. *Trends Mol Med.* 2015; 21:702–714. [PubMed: 26481828]
19. Smith MI, Yatsunenko T, Manary MJ, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science.* 2013; 339:548–554. [PubMed: 23363771]
20. Brown EM, Wlodarska M, Willing BP, et al. Diet and specific microbial exposure trigger features of environmental enteropathy in a novel murine model. *Nat Commun.* 2015; 6:7806. [PubMed: 26241678]
21. Bolt MJ, Stellaard F, Sitrin MD, et al. Serum unconjugated bile acids in patients with small bowel bacterial overgrowth. *Clin Chim Acta.* 1989; 181:87–101. [PubMed: 2721008]
22. Trehan I, Shulman RJ, Ou CN, et al. A randomized, double-blind, placebo-controlled trial of rifaximin, a nonabsorbable antibiotic, in the treatment of tropical enteropathy. *Am J Gastroenterol.* 2009; 104:2326–2333. [PubMed: 19491826]
23. Schneider SM, Joly F, Gehrhardt MF, et al. Taurine status and response to intravenous taurine supplementation in adults with short-bowel syndrome undergoing long-term parenteral nutrition: a pilot study. *Br J Nutr.* 2006; 96:365–370. [PubMed: 16923232]

What is Known

- Environmental enteric dysfunction affects millions of children in developing countries.
- The condition is characterized by inflammation of the small bowel mucosa, villous atrophy, malabsorption, and increased intestinal permeability.

What is New

- Environmental enteric dysfunction is associated with altered bile acid metabolism.
- Children with and without environmental enteric dysfunction differ in total serum bile acids, specific bile acid concentrations, and bile acid conjugation by taurine versus glycine.
- Altered bile acid metabolism is newly identified feature in the pathophysiology of environmental enteric dysfunction.

Fig. 1.
Serum bile acid pool composition in 313 children, aged 12–59 months, from rural Malawi.

Table 1

Characteristics of the study population

Characteristic ¹	Total population	With EED	Without EED
	n = 313	n = 244	n = 69
Age, months	35.1 (11.7)	34.3 (11.9)	37.9 (10.5)
Female, %	50.2	50.8	47.8
Weight-for-height Z-score	0.3 (0.9)	0.2 (0.9)	0.3 (1.0)
Height-for-age Z-score	-2.4 (1.3)	-2.4 (1.3)	-2.4 (1.3)
Stunted, ² %	62	61	64
Caretaker is mother, %	96	95	97
Father is alive, %	96	97	90
Siblings, n	3.8 (1.7)	3.8 (1.7)	3.8 (1.7)
Individuals that sleep in same room as child, n	3.3 (1.5)	3.3 (1.4)	3.2 (1.7)
Home with a metal roof, %	20	18	28
Family owns bicycle, %	61	62	59
Animals sleep in house, %	37	35	43
Water from a clean source, %	71	67	83
Child uses pit latrine, %	79	80	75

¹ Mean (SD) or %² Height-for-age Z-score <-2.

Table 2

Spearman correlations for age and serum bile acids

Serum bile acid	Abbreviation	r^2	P
Cholic acid	CA	-0.32	<0.0001
Chenodeoxycholic acid	CDCA	-0.35	<0.0001
Glycocholic acid	GCA	0.13	0.02
Glycochenodeoxycholic acid	GCDCA	0.06	0.25
Taurocholic acid	TCA	-0.07	0.19
Taurodeoxycholic acid	TDCA	0.26	<0.0001
Taurochenodeoxycholic acid	TCDCA	-0.09	0.12
Deoxycholic acid	DCA	0.08	0.15
Glycodeoxycholic acid	GDCA	0.31	<0.0001
Glycolithocholic acid	GLCA	0.39	<0.0001
Tauromuricholic acid (alpha and beta sum concentration)	TMCA (a+b)	0.11	0.84
Glycoursodeoxycholic acid	GUDCA	0.12	0.03
Ursodeoxycholic acid	UDCA	-0.08	0.15

Serum bile acids in children with and without environmental enteric dysfunction (EED), adjusted for age

Table 3

Bile acid (μmol/L)	Abbreviation	With EED (n = 244)		Without EED (n = 69)		p ¹
		Median	IQR	Median	IQR	
Cholic acid	CA	0.11	0.03, 0.21	0.11	0.04, 0.25	0.07
Chenodeoxycholic acid	CDCA	0.033	0.001, 0.145	0.046	0.004, 0.158	0.15
Glycocholic acid	GCA	1.17	0.58, 2.30	1.50	0.85, 3.01	0.78
Glycochenodeoxycholic acid	GCDCA	1.95	0.94, 3.71	2.60	1.28, 4.00	0.50
Taurocholic acid	TCA	0.12	0.07, 0.24	0.13	0.05, 0.23	0.06
Taurodeoxycholic acid	TDCA	0.040	0.020, 0.100	0.050	0.020, 0.100	0.37
Taurochenodeoxycholic acid	TCDCa	0.13	0.06, 0.30	0.13	0.07, 0.27	0.004
Deoxycholic acid	DCA	0.15	0.52, 0.29	0.21	0.09, 0.38	0.76
Glycodeoxycholic acid	GDCA	0.60	0.23, 1.37	0.83	0.41, 1.80	0.50
Glycolithocholic acid	GLCA	0.025	0.005, 0.067	0.045	0.010, 0.100	0.64
Tauromuricholic acid, alpha and beta sum concentration	TMCA (a+b)	0.045	0.020, 0.090	0.041	0.020, 0.100	<0.0001
Glycoursodeoxycholic acid	GDCA	0.052	0.023, 0.12	0.085	0.036, 0.15	0.03
Ursodeoxycholic acid	UDCA	0.016	0.001, 0.031	0.021	0.004, 0.034	0.35
Total serum bile acids		4.51	2.45, 7.51	5.10	3.32, 9.01	0.0009

¹Wilcoxon rank-sum test. Interquartile range (IQR).

Table 4

Serum bile acid ratios in children with and without EED, adjusted by age

Bile acid ratio	With EED (n = 244)		Without EED (n = 69)		P ¹
	Median	IQR	Median	IQR	
(TCA + TDCA)/(GCA + GDCA)	0.10	0.07, 0.16	0.07	0.04, 0.11	<0.0001
(TCA + TDCA + TCDCa)/(GCA + GDCA + GCDCA)	0.09	0.06, 0.13	0.06	0.04, 0.10	<0.0001

CA	0.106																			
CDA	0.179	0.219 *																		
GCA	-0.169	0.226 *	-0.018																	
GCDA	-0.151	0.172	0.133	0.762 \$																
TCA	-0.018	0.305 \$	0.042	0.750 \$	0.638 \$															
TDCA	-0.119	0.118	-0.262 **	0.612 \$	0.317 \$	0.519 \$														
TCDA	-0.050	0.233 *	0.165	0.639 \$	0.862 \$	0.746 \$	0.314 \$													
DCA	-0.073	0.072	-0.017	0.051	-0.091	-0.061	0.196	-0.221 *												
GDCA	-0.238 *	0.078	-0.250 *	0.714 \$	0.656 \$	0.479 \$	0.561 \$	0.482 \$	0.333 \$											
GLCA	-0.260 **	-0.119	-0.321 \$	0.395 \$	0.415 \$	0.221 *	0.333 \$	0.195 *	0.399 \$	0.671 \$										
TMCA	-0.125	0.123	-0.265 **	0.609 \$	0.312 \$	0.519 \$	0.624 \$	0.340 \$	0.190	0.557 \$	0.333 \$									
GUDCA	-0.119	0.116	0.081	0.580 \$	0.679 \$	0.391 \$	0.331 \$	0.551 \$	0.072	0.540 \$	0.317 \$	0.325 \$								
UDCA	0.018	0.258 **	0.302 \$	-0.010	-0.009	0.065	0.029	-0.017	0.471 \$	0.018	0.012	0.023	0.234 *							
glycine	0.492 \$	0.168	0.133	-0.014	-0.064	0.032	-0.055	-0.062	-0.066	-0.123	-0.111	-0.055	-0.076	-0.015						
	taurine	CA	CDCA	GCA	GCDA	TCA	TDCA	TCDA	DCA	GDCA	GLCA	TMCA	GUDCA	UDCA						

CA	0.106																			
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TDCA	-0.119	0.118	-0.262 **	0.612 \$	0.317 \$	0.519 \$														
TCDC A	-0.050	0.233 *	0.165	0.639 \$	0.862 \$	0.746 \$	0.314 \$													
DCA	-0.073	0.072	-0.017	0.051	-0.091	-0.061	0.196	-0.221 *												
GDCA	-0.238 *	0.078	-0.250 *	0.714 \$	0.656 \$	0.479 \$	0.561 \$	0.482 \$	0.333 \$											
GLCA	-0.260 **	-0.119	-0.321 \$	0.395 \$	0.415 \$	0.221 *	0.333 \$	0.195 *	0.399 \$	0.671 \$										
TMCA	-0.125	0.123	-0.265 **	0.609 \$	0.312 \$	0.519 \$	0.624 \$	0.340 \$	0.190	0.557 \$	0.333 \$									
GUDCA	-0.119	0.116	0.081	0.580 \$	0.679 \$	0.391 \$	0.331 \$	0.551 \$	0.072	0.540 \$	0.317 \$	0.325 \$								
UDCA	0.018	0.258 **	0.302 \$	-0.010	-0.009	0.065	0.029	-0.017	0.471 \$	0.018	0.012	0.023	0.234 *							
glycine	0.492 \$	0.168	0.133	-0.014	-0.064	0.032	-0.055	-0.062	-0.066	-0.123	-0.111	-0.055	-0.076	-0.015						
	taurine	CA	CDCA	GCA	GCDCA	TCA	TDCA	TCDC A	DCA	GDCA	GLCA	TMCA	GUDCA	UDCA						