CHAPTER 5.

SYSTEMATIC REVIEW RESULTS BY BIOMARKER CLASSIFICATIONS

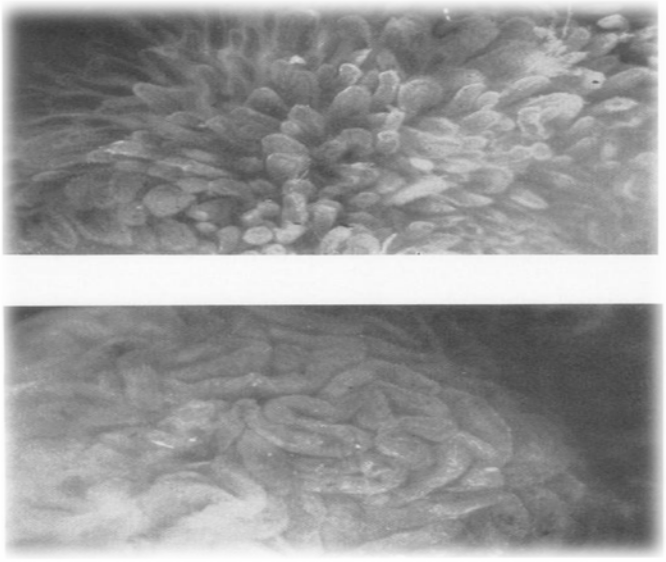
Denno, DM, VanBuskirk, KM, Nelson, ZC, Musser, CA, Tarr, PI. (2016). Environmental enteric dysfunction: Advancing current knowledge. St. Louis, MO: Washington University Libraries. http://dx.doi.org/10.7936/K7WQ0228

Chapter 5. Systematic Review Results by Biomarker Classifications

5.1 Markers of Absorption and Permeability: Overview

Tests of gut permeability and of absorption often overlap in concept, and are frequently performed simultaneously. For this reason, it is appropriate to provide background in tandem.

Proper functioning of the intestine depends on sufficient absorptive surface area and maintaining the barrier function and structural integrity of the lining of this organ. Absorptive surface is a function of individual villous surface area, as depicted in the photos below [3], and of gut length, which is probably not compromised in post-natally-acquired enteropathic syndromes. Absorption depends on the ability of various cellular mechanisms to assimilate nutrients from food that is ingested, using processes that rely on specialized pumps, pathways, and degradation. Integrity reflects sieve size, and presumably passive diffusion of large molecules across non-intact epithelia. To varying extents, these functionalities are hindered in EED, celiac disease, and small bowel Crohn's disease, among other disorders.



Microvilli of the small bowel, as seen with a magifying lens [3]. Normal finger-like projections are presented in the top panel. Enteropathy is characterized by flattened villi (bottom panel). Reproduced from *Gut*, Booth, C.C., Vol. 5, p. 46, 1964 with permission from BMJ Publishing Group Ltd.

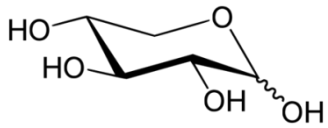
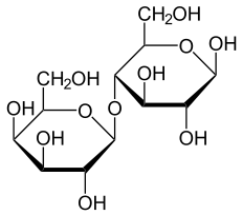
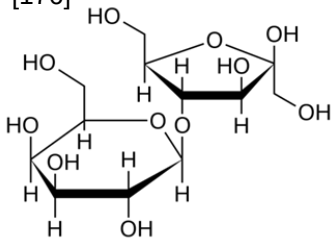
To perform its functions, the intestinal epithelium utilizes a layer of

highly specialized columnar epithelial cells connected by the apical junctional complex of tight junctions and adherens junctions. Theoretically, specific molecules can be chosen strategically to interrogate these various attributes. For example, breaches in integrity that enable passive diffusion into the host could be measured by ingesting a substance that is not found in the diet, and measuring its concentration in the blood or urine. Another detection strategy would be to use a molecule that is easily absorbed in health and disease, but where absorption is limited only by mucosal surface availability, and, similarly, measure this tracer in urine and blood. The optimal challenge substances would resist digestion in the gut, be nontoxic, and be easily measured. Ideally, one attribute (surface area) can and should be measured in parallel with the other (specific uptake). Lastly, it might be difficult to separate one function (i.e., permeability) from the other (absorption); therefore these two processes are discussed in tandem in this section.

5.1.1 Sugars as Tracers of Intestinal Function

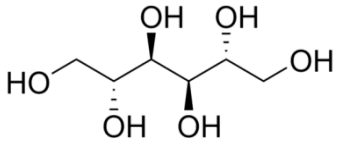
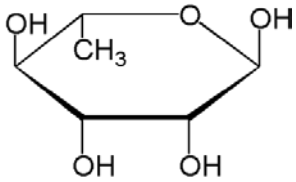
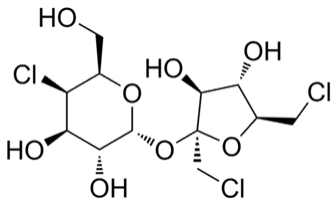
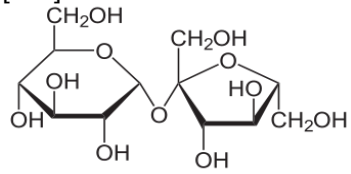
Historically, sugars have served well as tracers of intestinal function. These substances are nontoxic, easily detected in blood or urine, and, most importantly, neither made, nor degraded, by the host, so their presence in the body reflects gut uptake. Most of these sugars are assayed after ingestion of a load. Some of these sugars are “endomolecular,” i.e., consumed as part of a normal diet, but most are “xenomolecular,” i.e., foreign to natural diets, not metabolically necessary for the host, and absorbed without the benefit of specific transporters. Because they are foreign to the human diet, their use as a marker of intestinal function requires administration of a load to assay presence in body fluids. Moreover, depending on their size and the physiology of their assimilation, these sugars can be used to probe either amalgamated function, or specific processes or lesions (see Table 13).

Table 13. Sugar probes.

Sugar	Molecular weight	Molecular structure	Chemical (IUPAC) name	Formula	Endo- vs. Xeno-molecular[1]	Primary function assessed when found in blood and/or urine / Other comments
D-xylose	150.13	[174] 	D-xylose	$HOCH_2(CH(OH))_3CHO$	Xeno-molecular	Measure of small bowel (perhaps primarily jejunal) absorptive capacity. Sugar synthesized by wood.
Lactose	342.3	[175] 	β-D-galactopyranosyl-(1→4)-D-glucose	$C_{12}H_{22}O_{11}$	Endo-molecular	Measure of small bowel permeability, although might also reflect lactase deficiency.
Lactulose	342.30	[176] 	4-0-*β*-D-galactopyranosyl-D-fructofuranose	$C_{12}H_{22}0_{11}$	Xeno-molecular	Measure of small bowel permeability. Usually normalized to mannitol.

[1] We use the term xenomolecular for probes that would not be ingested in a normal dietary environment, while endomolecular probes are common and/or necessary dietary constituents. Therefore, endomolecular probes or their breakdown products are typical constituents in body analytes.

Table 13. Sugar probes.

Sugar	Molecular weight	Molecular structure	Chemical (IUPAC) name	Formula	Endo- vs. Xeno-molecular[1]	Primary function assessed when found in blood and/or urine / Other comments
Mannitol	182.17	[177] 	(2R,3R,4R,5R)-Hexan-1,2,3,4,5,6-hexol	$C_6H_{14}O_6$	Xeno-molecular	Measure of total small bowel absorptive capacity.
Rhamnose	164.16	[178] 	2R,3R,4R,5R,6S)-6-methyloxane-2,3,4,5-tetrol	$C_6H_{12}O_5 \cdot H_2O$	Xeno-molecular	Has been used in lieu of mannitol as a measure of total small bowel absorptive capacity.
Sucralose	397.64	[179] 	1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside	$C_{12}H_{19}Cl_3O_8$	Xeno-molecular	Measure of colonic permeability. Active ingredient in Splenda[R].
Sucrose	342.30	[180] 	Structural α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside	$C_{12}H_{22}O_{11}$	Endo-molecular	Measure of gastric permeability.

An early sugar that was employed as an indicator of intestinal function was D-xylose [181, 182], a pentose, which remains a standard for single sugar absorption testing worldwide. Humans do not synthesize D-xylose isomerase. Following an enteral challenge, the molecule is eliminated intact by renal clearance, after circulating in the blood. It is largely passively absorbed in the small bowel. The mechanism of clearance is glomerular filtration without demonstrated tubular reabsorption or renal tubular excretion. Hence, uptake of D-xylose can be assayed by seeking the peak in the serum or by collecting urine for assay. The assay for D-xylose currently relies on isotopic or photometric assays [183]. Its main limitation is that a single sugar cannot differentiate loss of absorptive function from loss of absorptive area (e.g., due to shortened bowel length). Other single sugars that indicate absorptive function include mannitol and rhamnose. Sugars that are not absorbed by an intact healthy gastrointestinal tract better reflect porosity including lactose and lactulose in the small intestine, sucrose in the stomach, and sucralose in the large intestine.

The second class of sugar absorption tests utilizes two sugars. One sugar reflects total small bowel absorptive capacity, and is most often mannitol or, occasionally, rhamnose. This "denominator" sugar requires no specific uptake mechanisms or host attributes except for available surface area. The “numerator” sugar in these dual tests is most often lactulose although lactose, sucrose, and sucralose have also been used. These large molecules enter the host by passive diffusion in the presence of alterations of the integrity of the small bowel (lactulose and lactose), stomach (sucrose), or colon (sucralose).

Dual sugar tests have additional advantages. A single molecule might be affected by factors that are not related to the permeability process, e.g., rates of gastric emptying and intestinal transit, bacterial degradation, or the sufficiency of urine collection. In contrast, a ratio of excretion of two molecules is not susceptible to these factors, because such processes act on both molecules similarly, and the ratio is the key retained metric. However, absolute values

might be low in these situations, and dynamic ranges for uptake are not well established. Finally, in adults, some data suggest that the use of hyperosmolar fluids (i.e., lactulose and mannitol) might affect absorption via solvent drag [184]. For purposes of comparison, the four grams of lactulose and one gram of mannitol dissolved in five milliliters of water, a commonly employed formulation [185] produces a solution that is 4,629 mOsm/kg as compared to normal plasma osmolality of 270-285 mOsm/kg.

Variations on the challenge and recover motif used in the classic sugar absorption studies warrant discussion. Some studies use indirect assessments of uptake. For example, non-absorbed D-xylose can be metabolized by microbes in the bacteria-rich colon, and possibly also in small bowel bacterial overgrowth, and measured as exhaled carbon dioxide.

We reviewed 36 studies that assessed small intestinal absorption or permeability using sugars as absorbed probes. Three studies measured lactose, an endomolecular sugar, either in the serum (n=1) or urine (n=2) among breastfeeding children. Of the 36 studies, 35 assessed malabsorption/permeability deficits by administering xenomolecular sugar challenges using lactulose, mannitol, rhamnose, or xylose (including one study each that also measured serum and urinary lactose). We reviewed five studies that reported xenomolecular probe results of single-only sugar tests, and 30 studies that reported results of dual sugar tests. The dual sugar tests included urinary L:M (n=25 studies), urinary or serum L:R (n=5), and urinary lactose:lactulose (n=1). The single sugar assay was used in six studies: urinary lactose (n=1) and D-xylose (two using serum measures, one using urine as a substrate, and two in which the body fluid from which the sugar was measured was not reported).

5.1.2 Endomolecular Nutrients as Tracers of Intestinal Function

Essential nutrients that are absorbed by healthy small intestine provide another opportunity to assess gut absorptive function. These types of marker assessments can provide

especially valuable information regarding the role of derangement of gut function among children with nutrient deficiencies, i.e., how much of the deficiency is because of lack of intake rather than malabsorption. Since these molecules are naturally found in body analytes, load administration requires tagging such as with the use of radiolabeled molecules, quantification of which can then be assessed in breath, blood, urine, and/or stool as surrogate markers of absorptive capacity. We reviewed six studies that utilized such methodologies including assessments of zinc (n=3), lipid (n=2) and iron (n=1) absorption.

We present in Evidence Tables 1 and 2 data from publications that utilized markers of absorption and permeability, respectively. Evidence Table 1 depicts data related to the systematic review from the articles that reported results of markers that were principally related to absorptive functions (such as D-xylose and fecal fat). Evidence Table 2 contains data on markers related to porosity or permeability. However, tests of gut permeability are often assessed simultaneously with markers of absorption. For example, the lactulose:mannitol ratio (L:M) and lactulose:rhamnose ratio (L:R) are often described as tests of permeability; however, by their nature they do include measures of absorption (via mannitol and rhamnose, respectively). For this reason, we present markers of permeability with or without concomitant assessment of absorption in Evidence Table 2. We identified 44 publications that assessed small intestinal absorption (13 studies) or permeability (primarily, sometimes also with some assessment of absorption based on dual sugar testing; 31 studies) in children in resource-poor settings.

5.2 Markers of Absorption

Data regarding markers of absorption are presented in Evidence Table 1.

Evidence Table 1. Markers of absorption.
Biomarkers in bold are primarily markers of malabsorption.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2002 Dini E et al. Sudan III and steatocrit in the detection of fecal fat in malnourished children Fecal fat by four different testing methods as a marker of malabsorption among children with varying nutritional status and well-nourished controls	Caracas, Venezuela 6 mo-9 yr olds with recruited from an outpatient nutrition center and well-nourished controls.	Case-control n=129; n=99 cases: • 30 with subclinical malnutrition • 34 with mild malnutrition • 30 with moderate malnutrition • 5 with severe malnutrition n=30 controls	<u>Stool Test</u>: Fecal fat, by method: • **Sudan III classic** • **Sudan III modified** • **Steatocrit classic** • **Steatocrit acid** Each subject underwent testing for all four methods.	Proportions testing positive for fecal fat ranged from 33%-41% overall, depending on test method used. The proportion testing positive varied by nutritional status across testing methods: • 80%-100% of severely malnourished subjects had a positive test • Similar proportions of subjects with subclinical, mild or moderate malnutrition tested positive, ranging from 30%-47% • 13%-27% of controls tested positive These differences appeared to be significant, but statistical comparison results were not entirely clear. Fecal fat did not vary based on quantity of fat intake. By all four methods, a high percentage of children with parasites tested positive (~60%) compared to children without parasites (25%). Associations were observed between infection with *Giardia lamblia* or *Blastocystis hominis* and fecal fat ($p<0.05$); this held true across diagnostic methods. The presence of diarrhea at time of testing was positively associated with fecal fat by all test methods ($p<0.02$ for all except steatocrit classic,	A majority of children studied tested negative for fecal fat. The highest percent testing positive was in those with severe malnutrition, followed by those with subclinical-moderate malnutrition. Controls had the lowest percent testing positive. Subjects with enteric parasites or those experiencing diarrhea at time of testing excreted fat significantly more often than uninfected children without diarrhea, although the magnitude of difference was not reported. There was some variation between the different testing methods, for example their relationship with a history of diarrhea in the year prior to testing.	Spanish language article. Control recruitment strategy was not well described. Proportions positive for fecal fat by history of diarrhea (current or previous) were not provided. Authors reported percent agreement between tests but did not report results of statistical testing of these estimates. Test results varied by subject characteristics; however, assessments adjusting for potential confounding were not reported.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				p=0.06). The relationship between fecal fat and history of diarrhea in the year prior to testing varied by test method: • Sudan III classic: p=0.134 • Sudan III modified: p<0.001 • Steatocrit classic: p=0.14 • Steatocrit acid: p=0.015 Agreement between all four methods was 72%. Agreement between at least three was 91.5%.		

2006
 	
Leite CA et al.
 	
Functional, microbiological and morphological intestinal findings among human immunodeficiency virus infected children
 	
Small intestinal and rectal biopsy to assess morphology and D-xylose as a marker of malabsorption among HIV-infected children
	Sao Paulo, Brazil
	
	5 mo-12 yr old (median 24 mo) HIV-infected subjects recruited from a hospital and clinic.
	
	All subjects had some degree of protein-energy malnutrition.
		Cohort
		
		n=11;
		
		n=5 patients with current or recent episode of diarrhea
		
		n=6 patients with no diarrhea in the 30 days preceding enrollment
			Blood Test:
			D-xylose
			(9 tested)
			
			Biopsy of small intestine by tethered capsule or endoscopy:
			Histopathology
			(10 tested)
			
			Rectal biopsy:
			Histopathology
			(6 tested)
				100% had low D-xylose absorption:
				• Mean: 15.6 mg/dL
				• SD: 5
				• Range: 8.9-24.4
				• Median: 14.2
				
				Small intestinal biopsy:
				• 100% had some degree of villous atrophy based on a I-IV grading system:
				  • Grade I: 3
				  • Grade I/II: 2
				  • Grade II: 1
				  • Grade II/III: 1
				  • Grade III/IV: 1
				  • 2 samples were too superficial to assess
				• Intraepithelial lymphocytes were increased in half of the biopsies.
				• Lymphocytic and polymorphonuclear (PMN) infiltration of the lamina propria were present in 10/10 and 7/10 biopsies, respectively.
					There was a high prevalence (100%) of abnormal D-xylose results among HIV-infected children, regardless of diarrhea status.
					
					All patients also had cellular infiltration of the lamina propria and varying degrees of villous atrophy.
					
					There was no correlation between D-xylose and degree of villous atrophy on biopsy.
						Portuguese language article.
						
						D-xylose <25 mg/dL was defined as indicative of malabsorption. This value is higher than what some references have noted as a cut-point [186]
						Investigators used a well-articulated system of grading villous atrophy.
						
						Results were not presented by diarrhea status, perhaps due to small sample size.

Evidence Table 1. Markers of absorption.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				Rectal biopsy: • 100% had normal architecture Lymphocytic and PMN infiltration were present in 6/6 and 4/6, respectively.		

2005
 	
López de Romaña D et al.
 	
Longitudinal measurements of zinc absorption in Peruvian children consuming wheat products fortified with iron only or iron and 1 of 2 amounts of zinc
 	
Zinc absorption among stunted, anemic children receiving zinc as well as iron and/or iron/zinc-fortified foods
	Lima, Peru
	
	3-4 yr olds residing in a poor community at the periphery of Lima with stunting and moderate anemia as a surrogate risk factor for zinc deficiency.
		RCT
		
		n=41;
		(31 completed both initial and follow-up absorption assay at 2 mo)
		
		Group 1:
		n=14 received wheat flour with iron fortification only
		(10 completed follow-up)
		
		Group 2:
		n=12 received wheat flour with iron and 3mg zinc/100g flour
		(9 completed follow-up)
		
		Group 3:
		n=15 wheat flour with iron and 9mg zinc/100g flour
		(12 completed follow-up)
			Urine Test:
			Zinc excretion to measure fractional absorption of zinc (FAZ) and total absorbed zinc (TAZ) following radiolabeled zinc administration
				Mean zinc parameters (SD) at initial assessment:
				• FAZ:
				  • Group 1: 0.34 (0.11)
				  • Group 2: 0.24 (0.05)
				  • Group 3: 0.13 (0.04)
				• TAZ (mg/d):
				  • Group 1: 0.71 (0.18)
				  • Group 2: 1.11 (0.21)
				  • Group 3: 1.34 (0.47)
				
				Neither mean FAZ nor TAZ changed significantly at subsequent assessments in any treatment group.
				
				In both the initial and subsequent assays, mean TAZ from zinc-fortified meals increased with increasing amounts of fortification ($p< 0.001$). However mean FAZ was inversely related to zinc intake from these meals ($p<0.001$).
				
				Non-fortified dinner FAZ and TAZ were significantly lower in the group receiving the most zinc-supplementation at the initial assay ($p=0.015$ and $p=0.012$, respectively) despite no difference in zinc intakes from the unfortified dinner by treatment group. This relationship between groups was not observed at the second assay; however, a significant decrease of 16% in mean FAZ
					Despite a reduction in FAZ with increasing fortification, TAZ increased as more zinc was consumed and with increasing concentrations of zinc fortification.
					
					Authors speculate that reduction in FAZ with increasing fortification could be due to factors such as saturation kinetics.
					
					Authors described a unexpected finding: subjects consuming more zinc from the zinc-fortified breakfast and lunch meals absorbed less zinc from the unfortified dinners during the initial absorption assay.
						Intestinal function could play a role in zinc (or other micronutrient) absorption; such factors were not explored in this study.
						
						The principal aim of this study was to determine appropriate extent of zinc fortification of a staple food in a specific community; we present only results relevant to this review.

Evidence Table 1. Markers of absorption.
Biomarkers in bold are primarily markers of malabsorption.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				and TAZ from the unfortified dinners was observed between initial and subsequent assays ($p<0.001$). Mean plasma zinc concentrations did not differ between treatment groups throughout the study period. The proportion with low fasting plasma zinc concentrations (<65µg/dL) was lower at the end of the study (3.3% vs. 20.5% initially, $p=0.046$).		
2002 Manary ML et al. Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet Zinc absorption in a sample of 10 asymptomatic children	Blantyre, Malawi 2–5 yr olds (mean age 43.6 mo, SD 7.7) from rural area attending immunization clinic. There was a high prevalence of stunting and low plasma zinc in this series.	Case-series n=10	Stool Test: **Endogenous fecal zinc (EFZ)** Urine Test: **Zinc excretion to measure fractional absorption (FAZ) and total absorption (TAZ) following radiolabeled zinc administration**	Mean (SD): • FAZ: 0.24 (0.04) • TAZ (mg/d): 1.30 (0.33) • EFZ (mg/d): 1.15 (0.33) Language in the discussion section strongly suggests, but does not explicitly state, that TAZ and EFZ were not correlated. Correlation analysis for these parameters was not reported.	EFZ was higher than would be expected for a zinc deficient cohort, and EFZ was not correlated with TAZ as would have been expected. While high-phytate diets leading to poor zinc absorption might explain these findings, the authors note that in a previous study (among a somewhat older age group) there were no differences in EFZ among children consuming high- or low-phytate diets [187]. They note that such perturbations in EFZ have also been reported in children with enteropathy due to cystic fibrosis [188] and suggest that a similar process could be going on in these Malawian children due to TE.	Authors note that the lack of comparable data from children of the age range in this study limits data interpretation. They also provide results per body weight due to presumed relationship; validity of such measures has not been established. Authors comment that the methods used for calculating absorption measures are sensitive and accurate, but quite difficult to conduct, especially among children.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2001 Mittal SK et al. Tropical sprue in north Indian children D-xylose and duodenal biopsy as markers of TS	New Delhi, India 0-15 yr old gastroenterology clinic patients with PD. Those with abnormal morphology on biopsy, abnormal D-xylose test, and clinical response to antibiotics were diagnosed as having TS. Those with abnormal morphology and response to gluten-free diet were diagnosed with CD. We include data on these subjects for comparative reasons.	Case-series n=94; (38 with repeat biopsies) <5 yr old: n=44	<u>Duodenal biopsy, method not specified:</u> Histopathology <u>Blood Tests</u>: • Hemoglobin • **D-xylose*** * Not specified whether from urine or serum, and units of measurement not provided.	36 (38.3%) were diagnosed with TS including 14/44 (31.8%) who were under 5 years of age. 18 (19.1%) were diagnosed with CD. Degree of villous atrophy among TS vs. CD patients: • Mild in 8/36 (22.2%) vs. 0 • Moderate in 23/36 (63.9%) vs. 4/18 (22.2%) • Severe in 5/36 (13.9%) vs. 14/18 (77.8%) Mean hemoglobin concentration (range) among TS patients was 8.3 g/dL (5.5-11) and did not differ from values of those with CD. Among the 22 TS patients, repeat biopsies showed: • 16 with normalization • 5 with improvement • 1 worsened despite marked clinical improvement The D-xylose test was abnormal in all TS patients by diagnostic definition.	More than half of the GI clinic patients with PD had some degree of villous atrophy. More than one-third and almost one-fifth of subjects were diagnosed with TS and CD, respectively. By study diagnostic definition, all TS patients improved with treatment. Among those who had repeat biopsies, almost three-quarters showed normalization of histology, while 23% had partial improvement and 1 patient had worsened pathology.	Biopsy results were not provided for patients without TS or CD. It was unclear if there were patients with abnormal D-xylose and histology who did not respond to antibiotic therapy and therefore were not diagnosed with TS. Cut-off points used to define abnormal D-xylose tests were not provided.
2002 Moya-Camarena SY et al. Effects of asymptomatic *Giardia intestinalis* infection on carbohydrate	Hermosillo, Sonora, Mexico 3-6 yr olds in a periurban setting attending preschool centers meeting inclusion criteria of no GI symptoms, no antibiotics in the	Case-control n=13; <5 yr old: n=5 n=7 asymptomatic cases infected	<u>Breath Tests*</u>: • Lactose HBT • **D-Xylose HBT**** <u>Urine Test</u>: **D-xylose****[1] * Reported as parts	Mean lactose HBT (SE): • Cases pre-treatment: 3.6 (0.75) ppm • Cases post-treatment: -0.85 (0.75) ppm ($p<0.05$ compared to pre-treatment) • Controls: 0.19 (0.81) ppm ($p<0.05$ compared to pre-treatment cases)	Lactose HBT concentrations were normal according to established cut-points among all subjects. However, lactose HBT was significantly higher among cases compared to controls and there	Statistical methods might not have been adequate to account for intra-subject correlation when comparing the same group of subjects (cases) before and after treatment.

[1] D-xylose results were expressed as % of dose administered.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
absorption in well-nourished Mexican children Lactose hydrogen breath test (HBT) as a marker of lactose absorption, and xylose breath test and urinary excretion as markers of xylose absorption in well-nourished children with asymptomatic giardiasis and non-infected controls	preceding 3 wk, and no SBBO by lactulose HBT and Indican test.	only with *G. intestinalis* n=6 controls without *Giardia* Cases were evaluated before and 3 wk after treatment with tinidazole. Post-treatment stools were verified for absence of parasites.	per million (ppm) post-substrate ingestion after subtraction of baseline, pre-substrate H_2 concentrations. A positive HBT is considered to be a rise of $\geq$20ppm in breath H_2 above baseline H_2 concentration. ** Investigators did not specify the xylose enantiomer used, however test functionality characteristics lead us to assume that it was the dextrorotary (D) enantiomer.	Mean xylose HBT (SE): • Cases pre-treatment: 2.2 (0.69) ppm for infected group • Cases post-treatment: -4.16 (0.69) ppm ($p<0.05$ compared to pre-treatment) • Controls: 1.13 (0.74) ppm (NS compared to pre-treatment cases) Mean urinary excretion of xylose (SE) among cases pre-treatment and post- treatment was 34% (3) and 46% (11), respectively (NS), well above cut-offs indicative of malabsorption.	was also a significant decrease in lactose HBT among cases after treatment. The clinical relevance of such mildly elevated HBT results in asymptomatically infected children is unclear. Results did not demonstrate xylose malabsorption by either urinary or breath measures among any group. While urinary results did not differ before and after treatment, case xylose HBT was significantly lower after treatment; again the clinical significance of such results is not apparent.	Investigators wished to exclude children with SBBO. As such, inclusion criteria restricted participants to those with adequate production of H_2 following ingestion of lactulose and with minimal urinary indoxyl sulfate excretion. The number of children excluded due to failure to meet these criteria was not reported.
2002 Murphy JL et al. Maldigestion and malabsorption of dietary lipid during severe childhood malnutrition Stool recovery of radiolabeled products as markers of lipid digestion and absorption, and bile salt deconjugation as a marker of SBBO among children with severe	Kingston, Jamaica 5-23 mo olds admitted to the Tropical Metabolism Research Unit of the University of the West Indies with severe malnutrition.	Case-series n=24 Subjects were divided into 3 groups of 8 children, each group receiving a different labeled triglyceride. Data were collected in three separate phases as described above in JL Murphy et al. 2001 [149].	Stool Tests: • **Total and fractionated ^{13}C following ingestion of one of three ^{13}C labeled triglycerides (TG): trilaurin, triolein, or trilinolein*** • ^{13}C stool assay following administration of labeled fatty acid ^{13}C glycocholate** * To assess fat excretion as a % of	Median total stool excretion of ^{13}C in phase 1 was 9% (range: 1%-29%) and did not vary between TG groups. Median ^{13}C excretion dropped 33%-99% in phase 2 and 86%-95% in phase 3 compared to phase 1 ($p<0.05$ each). Over the study period, there were significant associations between total lipid and the amount of ^{13}C labeled TGs in stool for some groups, but not for others. Median ^{13}C in TG and FA was similar across TG groups in all phases. 13C FA recovery was	High concentrations of ^{13}C (compared to healthy UK children) [190] were observed in half of the subjects at admission, reflecting impaired digestion or absorption. The differences in stool ^{13}C were wide but not as extreme as in a previous study by same investigators (also examined in this review) using a different TG (tripalmitin) substrate [149].	Authors state that the study was not powered to compare the different TGs, but they contend that medium chain trilaurin did not appear to be processed differently than the longer chain TGs triolein and trilinolein. Authors did not describe the method used to assign subjects to

Evidence Table 1. Markers of absorption.
Biomarkers in bold are primarily markers of malabsorption.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
malnutrition			dose administered. Also assessed proportion of ^{13}C in triglyceride (TG) and fatty acid (FA) fractions to distinguish excretion caused impaired digestion (presence of TG) vs. poor absorption (presence of FA). ** To assess bile salt deconjugation in the bowel caused by SBBO; conducted after the TG assessment and a 3 day washout period.	similar and reduced by ~2/3 compared to Phase 1. ^{13}C TG was not detectable in Phases 2 or 3. Statistical comparisons between phases were not reported. ^{13}C after radiolabeled glycocholate administration was detected in stool at quantities considered to be in excess of the 7% recovery of dose administered upper limit of normal in U.S. adults in [189]: • Phase 1: 13/24 (54%) • Phase 2: 5/24 (20.8%) Phase 3: 3/24 (12.5%)	^{13}C excretion did not significantly differ between TG groups and declined with improving clinical course. Similar to their previous study, significantly more ^{13}C in stool was recovered as FA than TG, reflecting impaired absorption over poor lipid digestion/ hydrolysis. Unlike in their previous study, there was evidence of SBBO as measured post-ingestion of ^{13}C glycocholate.	different TG groups. While it was noted that some subjects had positive stool cultures, details were not provided on the nature of the enteric infections.
2001 Murphy JL et al. Gastrointestinal handling and metabolic disposal of ^{13}C-labelled tripalmitin during rehabilitation from childhood malnutrition Fecal fat, stool recovery of radiolabeled products, and breath tests as markers of lipid digestion and absorption and bile salt deconjugation as a marker of SBBO among	Kingston, Jamaica 7-23 mo olds with malnutrition admitted to the University of the West Indies.	Case-series n=8 Data were collected in three separate phases (each lasting 9 days): 1. Within 48 hours of admission 2. During early rehabilitation 3. During late rehabilitation	Stool Tests: • **Fecal fat*** • **Total and fractionated ^{13}C assay after administration of ^{13}C tripalmitin (TP)**** • ^{13}C assay after administration of ^{13}C glycocholate (GCA)*** Breath Tests: • $^{13}CO_2$ after administration of ^{13}C glycocholate (GCA)*** or ^{13}C TP**** * In 72 hour stool collection (measured as total grams and as	Mean fecal fat (SD): • Phase 1: 2.4 g/day (3.6) or 5.9% (9.4) of dietary lipid intake • Phase 2: 1.7 (0.9) g/day, or 3.3% (2.4) of intake • Phase 3: 0.9 (0.6) g/day, or 1.4% (0.7) of intake • Differences between phases were not statistically significant. Total excretion of ^{13}C in stool also varied widely across patients (0%-44%) and did not differ between study phases. Correlation between fecal fat and ^{13}C ($r=0.48$; $p<0.05$) was observed. Lack of lipid digestion and absorption were assessed by	Mean fecal fat was not elevated compared to published norms [191, 192], during any study phase. There was wide variation in fecal fat at presentation, and wide variations in stool ^{13}C across subjects. Authors indicate that this is the first such assessment in malnourished children; previous studies on healthy children from the UK demonstrated average excretion of 6% [190]. The majority of excreted ^{13}C was in the form of FA rather than TG.	Statistical methods might be inappropriate for a small sample. All subjects were treated with antibiotics including metronidazole for presumptive SBBO; this might have affected GCA testing.

Evidence Table 1. Markers of absorption.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
children with severe malnutrition			% of dietary fat intake). ** To assess fat excretion as a % of dose administered. Also assessed proportion of ^{13}C in triglyceride (TG) and fatty acid (FA) fractions to distinguish excretion caused by impaired digestion (presence of TG) vs. poor absorption (presence of FA). *** To assess bile salt deconjugation in the bowel caused by SBBO; conducted after the TG assessment and a 3 day washout period. **** Expressed as a percentage of absorbed label (dose administered - label recovered in stool) to assess oxidation for acute energy needs.	measuring TG and FA fractions, respectively. Mean ^{13}C TG recovery (SD) (% of administered dose), number of patients excreting TG: • Phase 1: 0.7% (1.6), n=3 • Phase 2: 0.9% (2.8), n=1 • Phase 3: no recovery from any subjects, differences between phases were NS ^{13}C FA fraction in stool declined during rehabilitation. Mean ^{13}C FA recovery (SD): • Phase 1: 6.0% (7.3) • Phase 2: 4.8% (3.7) • Phase 3: 3.3% (3.8), differences between phases were NS Mean FA values were ~9x (NS), 5x ($p<0.001$), and 3x ($p<0.05$) higher than mean TG values in Phases 1, 2, and 3, respectively. Following administration of labeled TP, absorbed ^{13}C label by breath analysis was ~5% (range 0%-21.2%) and similar across study phases. Following the administration of labeled GCA, there was either no or minimal recovery of ^{13}C in stool and $^{13}CO_2$ on breath (as % of dose administered) in all phases.	Authors interpreted this to reflect failure of lipid absorption in the face of adequate digestion/ hydrolysis. Each form (FA and TG) was found in decreasing values as the study phases progressed, suggesting improved digestion and absorption, although results did not differ significantly. Fecal fat was correlated with concentrations of ^{13}C in stool. There was no evidence of SBBO or bile acid malabsorption. $^{13}CO_2$ excretion following administration of ^{13}C TP was minimal, suggesting a propensity for deposition in adipose tissues rather than oxidation for immediate energy needs. The authors report that this breath test has not been widely used, but that healthy UK children have breath excretion values from 15%-43% [190], compared to a mean of 5% and range 0%-21% in this cohort; the latter findings were more similar to results from kwashiorkor patients where ^{13}C-labeled oleic acid was	

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
					used as substrate [193].	

2000
 	
Nichols B et al.
 	
Contribution of villous atrophy to reduced intestinal maltase in infants with malnutrition
 	
Jejunal biopsy, maltase activity, and enzyme messenger RNAs among malnourished and well-nourished children. Assessed association between maltase and villous atrophy and other mucosal intestinal markers indicative of loss of enterocytes and enterocytic function.
	Sao Paulo, Brazil
	
	Cases were children (mean age 9.9 mo, SD 8.1) hospitalized with malnutrition refractory to dietary rehabilitation.
	
	Controls were children (mean age 3.6 mo, SD 1.0) with HAZ and WAZ scores >-2 and normal intestinal mucosa on biopsy, hospitalized for Kasai procedure for biliary atresia.
		Case-control
		
		n=33;
		
		n=24 cases
		
		n=9 controls
		
		Subjects were matched on height and weight; ages differed within matched sets.
			<u>Jejunal capsule biopsy:</u>
			• Histopathology*
			• Maltase activity
			• Intestinal messenger RNA (mRNA) abundances:
			  • Maltase-glucoamylase (MGA)
			  • Sucrase-isomaltase (SI)
			  • **Villin, a structural protein expressed only in enterocytes**
			  • **Sodium-activated luminal glucose-galactose transporter 1 (SGLT), a functional protein expressed only in enterocytes**
			  • β-actin
			
			* Mucosal atrophy was scored on a scale of 1 (absence of atrophy compared to an organ donor) to 4 (similar to children with active CD).
				Mean villous atrophy score (SD):
				• Cases: 2.6 (0.8)
				• Controls: 1.2 (0.5), p=0.006)
				
				WAZ score was correlated with villous atrophy (r=0.65, p- value not reported).
				
				13/25 [sic] cases and 0/5 controls had subnormal (defined as <94U/g protein) of maltase activity; mean maltase was 34% lower among cases (p=0.11). Maltase activity did not appear to decrease with WAZ score (further details not provided).
				
				However, in sub-analyses among those samples with an adequate β-actin, a housekeeping gene message, (n=10 cases, n=9 controls), cases' findings expressed as a mean percent of controls' (SD) included:
				• Villous length (reciprocal of atrophy score): 38.9 (41.6), p=0.004
				• Maltase activity: 37.1 (23.2), p=0.001
				• MGA mRNA: 45.1 (36.4), p=0.016
				• Villin mRNA: 52.5 (22.6), p=0.003
				• SGLT mRNA: 66.6 (23.1), p=0.057
				• β-actin: 88.2 (15.8), p=0.189
				
				Both villous length and maltase activity in a subset of cases were less than 40% of control values.
					The malnourished children had significantly greater villous atrophy than the younger controls.
					
					Among the subset tested for mRNA messages, maltase activity as well as the mRNA abundances for MGA, villin and SGLT were significantly correlated with case status and were correlated with villous atrophy.
					
					While maltase deficiency has been reported in malnutrition in other studies, authors assert that these are the first results that directly support the hypothesis that reductions in maltase activity are due to villous atrophy. This study also nicely correlates mRNA relative abundance with function.
						Tissue from patients requiring intestinal resection as part of their biliary atresia management provides an opportunity to assess presumably "normal" intestinal architecture. However, unless they mocked up *ex vivo* mucosal biopsies in these controls, resections will have lower proportions of villous to submucosa tissue compared to cases' samples derived from mucosal biopsies. While this probably doesn't affect histology, it might affect enterocyte functional assays and mRNA determination, as transmural tissue will bring in more diverse populations of cells; only some of them might have transcripts of interest. However, the bias is likely in a direction that would reduce effect size.

Evidence Table 1. Markers of absorption.
Biomarkers in bold are primarily markers of malabsorption.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
			Histology among controls was on surgically resected tissue.	MGA, villin, and SGLT mRNA abundances were correlated with villous atrophy score (r=0.73), (r=0.76), and (r=0.54), respectively (p-values not reported)[1]. MGA mRNA abundance was correlated with maltase activity (r=0.32).		It was unclear if control inclusion criteria included absence of atrophy or if all potential controls lacked atrophy. Statistical methods might not have adequately taken into account the small sample size and matching scheme. Subsets of subjects were investigated for various tests. For example, 10 cases had mRNA analyses based on β-actin adequacy. Another instance of selected testing was the subset of 22 and 15 cases that had WAZ score to histology and mRNA correlation analyses, respectively. Rationale for subset selection was not thoroughly described.
2001 Perin NM et al. Intestinal absorption	Florianopolis, Brazil 18 mo-14 yr old HIV-infected children with GI and non-GI	Cross-sectional n=104	Blood Test: **D-xylose**	Prevalence of an abnormal D-xylose result was 7.7%. Mean D-xylose (SD, range): 42.8mg/dL (14.4mg/dL, 16-73	D-xylose showed substantial variation across individuals.	Portuguese language article. D-xylose <25 mg/dL was defined as

[1] Villin and SGLT1 were assessed as a ratio with housekeeper gene β-actin.

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of D-xylose in children infected with the human immunodeficiency virus D-xylose as a marker of intestinal absorption among HIV-infected children with and without GI symptoms	symptoms of HIV infection recruited from a pediatric AIDS center.			mg/dL) D-xylose was not associated with age. Of the 8 children with abnormal results, 1 had diarrhea. Of 19 with diarrhea, 1 had an abnormal result. Of those with abnormal results, 50% had *Cryptosporidium* infection. Of the 33 subjects with *Cryptosporidium* infection, 4 had abnormal D-xylose results.		indicative of malabsorption.
2002 Poddar U et al. Celiac disease in India: Are they true cases of celiac disease? Duodenal biopsy, D-xylose, and fecal fat in children with symptoms of CD but normal mucosal biopsy results	Chandigarh, India 18 mo-14 yr olds with PD, FTT, or pallor from a hospital pediatric gastroenterology unit. Subjects with normal crypt:villous ratio on biopsy were of interest for this review.	Case-control n=47	<u>Endoscopic duodenal biopsy</u>: Histopathology <u>Stool Tests</u>: • **Fecal fat*** • **D-xylose**** * In 72-hour stool collection. ** Not specified whether from urine or serum, and units of measurement not provided.	38% had chronic inflammatory cell infiltrates in the lamina propria. 55% had abnormal D-xylose concentrations. 20% had abnormal fecal fat test. No results beyond proportion positive were reported for any of the above markers.	Among controls with normal mucosal architecture by biopsy, more than one-third had PD. D-xylose and fecal fat might not correlate well with duodenal biopsy results.	Relationships between fecal fat, D-xylose and biopsy results were not reported. While 38% of controls had PD, results for the markers studied were not stratified by PD for this group. Seven children with biopsies consistent with CD did not respond to gluten-free diet and were excluded from the study. Cut-off points used to define abnormal D-xylose tests were not provided.

Evidence Table 1. Markers of absorption.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2004 Sarker SA et al. *Helicobacter pylori* infection, iron absorption, and gastric acid secretion in Bangladeshi children Iron absorption among children with iron deficiency anemia with and without *H. pylori* infection	Dhaka, Bangladesh 2-5 yr old apparently healthy children from a periurban setting, screened for iron deficiency and *H. pylori*.	Case-control n=25: n=13 cases infected with *H. pylori* n=12 controls not infected with *H. pylori*	<u>Blood Test</u>: **Iron (absorption test)**	Mean[1] iron absorption from ferrous (Fe) sulfate and Fe fumarate: • Uninfected children: 15.6% and 5.4%, (p<0.001) • Infected children before treatment: 19.7% and 5.3%, (p<0.0001) • Infected children after treatment: 22.5% and 6.4%, (p<0.0001) • *H. pylori* treatment did not significantly affect absorption (Fe sulfate or fumarate), p=0.3	Iron absorption from Fe fumarate was significantly lower than from Fe sulfate. Results do not support the hypothesis that *H. pylori* infection influences absorption of water-soluble (Fe sulfate) or non-water-soluble (Fe fumarate) iron compounds.	Data on iron absorption among 2-5 yr olds are limited, making comparison of results from this study setting difficult.
2006 Sheng XY et al. Major variables of zinc homeostasis in Chinese toddlers Differences in zinc absorption in healthy toddlers with a high prevalence of zinc deficiency.	Xi-Chou (town) & Yun-Nan (province), China 19-25 mo olds recruited from a remote small town and 2 surrounding rural villages. 48% of children had plasma zinc concentrations below 2.5th percentile. Dietary zinc intake was low. There was a high prevalence of stunting among the subjects.	Cross-sectional n=43	<u>Stool Test</u>: **Endogenous fecal zinc (EFZ)** <u>Urine Test</u>: **Zinc excretion to measure fractional absorption of zinc (FAZ) and total absorbed zinc (TAZ) following radiolabeled zinc administration**	Mean (SD): • FAZ: 0.35 (0.12) • AZ (mg/d): 0.63 (0.24) • EFZ (mg/d): 0.67 (0.23) The quantity of absorbed zinc was lower than physiologic requirements. There was no statistically significant difference in any laboratory value between the town and village groups. Zinc absorption was ~80% of estimated physiologic requirement and equivalent to the amount of endogenous zinc excreted via the intestine; it was expected that absorbed zinc would exceed excreted zinc [144, 187, 194, 195]	Zinc absorption was lower than physiologic requirements and EFZ was higher than expected. The authors note that the results are difficult to explain and specifically state that they do not think (though without clear justification) that enteropathy is prevalent in the population and therefore could not be a contributing factor.	
Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.						

[1] Geometric mean.

Evidence Table 1. Markers of absorption.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting. **Abbreviations:** AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, Δ=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)						

Thirteen studies used markers of intestinal absorption among children in resource-limited settings. The range of markers included urinary, breath and serum markers of sugar absorption, fecal fat as a marker of lipid absorption, measures of endomolecular absorption, and presence of messenger RNAs in intestinal tissue as markers of intestinal surface area, and, by inference, absorptive capacity.

5.2.1 D-Xylose

While single molecule tests have disadvantages compared with dual molecule tests, as discussed above, the D-xylose test has been commonly used to assess malabsorption in developing settings. There has been further criticism of this test, including its potential hepatic metabolism of the sugar [196] and jejunal absorption [197].

D-Xylose was the most commonly reported measure of small bowel absorption in the literature that we reviewed and was employed in five studies. It was assessed either in serum (n=2) [136, 152] or urine (n=1) [147], although in two studies the substrate from which D-xylose was measured was not reported [146, 155]. Although one of these reports did not specify the xylose enantiomer [147], test functionality characteristics led us to assume it was the dextrorotary (D) enantiomer, as was used in the other studies. This study, in addition to assessment of urinary excretion of the sugar, also measured hydrogen excretion in breath before and after administration of the xylose. Three of the studies [136, 146, 147] provided demographic information stratified by age group and together included 58 children under five years of age. However, none of the studies presented D-xylose data by age group. Overall, the five studies included D-xylose results for 267 children up to the age of 14 years.

Two of the studies investigated the association of D-xylose with degree of villous atrophy on biopsy. One of these studies performed D-xylose tests in children with symptoms of celiac disease and normal architecture on duodenal biopsy, although approximately one-third had

chronic inflammatory cell infiltrates in the lamina propria [155]. This publication reported abnormal D-xylose results in 55% of these subjects (but did not provide information on the range of normal and abnormal concentrations, sampling site, or units of measure of D-xylose). Their results suggested that the D-xylose test might not correlate well with intestinal histopathology, especially crypt:villous architecture. The other study was among HIV-infected children with protein-energy malnutrition in Brazil [136]. All subjects' tissue had cellular infiltration of the lamina propria and varying degrees of villous atrophy on biopsy, and all subjects also had abnormally low D-xylose absorption (based on authors' reported cutoff value of <25 mg/dL). However, there was no correlation between the proportion of serum D-xylose absorption and the degree of villous atrophy on biopsy.

Another Brazilian study used the same definition of normal range for serum D-xylose among HIV-infected children with and without gastrointestinal symptoms. Eight percent of the children had D-xylose below the "normal" range in their blood following challenge [152]. The authors also examined the relationships between D-xylose absorption and history of diarrhea and *Cryptosporidium* infection. Of the eight children with abnormal D-xylose, one had diarrhea, and of 19 subjects with diarrhea, one had an abnormal result for D-xylose. Among subjects with abnormal results, 50% had *Cryptosporidium* infection, and of the 33 with *Cryptosporidium* infection (many of whom did not have diarrhea), four had an abnormal D-xylose test. They did not report statistical testing of association, but these clinical factors do not appear to be associated with D-xylose absorption in this cohort.

Moya-Camarena et al. performed urinary D-xylose and xylose breath hydrogen tests in well-nourished children with asymptomatic *Giardia intestinalis* infection and healthy controls recruited from preschool centers [147]. Results did not demonstrate any disturbance in urinary xylose absorption among infected cases or healthy controls. Urinary results did not differ before and after treatment for *Giardia*. Breath hydrogen levels decreased significantly with treatment,

indicating improvement. The clinical and physiologic implications of this difference, in the face of hydrogen breath test results well within the range of normal, are not apparent. The investigators did not report on the relationship between breath and urinary D-xylose results.

Finally, Mittal et al. used the D-xylose absorption test as part of their diagnostic criteria for tropical sprue among gastroenterology clinic patients presenting with persistent diarrhea. By definition, all of the children had abnormal xylose absorption results; however, information about the D-xylose dose, units of measure, cutoff point for normal, and even the identity of the sampled fluid (urine or serum) were not provided [146].

Methods of test performance and cutoff points were often not described adequately to enable comparing results across studies. For example, in two instances D-xylose abnormal cut points were specifically defined as <25 mg/dL [136, 152], This is higher than some references suggest as a cutoff point among infants and children [186]. Insufficient data were reported in these two studies for readers to assess how the proportion of those with D-xylose malabsorption might have shifted with differing cut points. In two other studies D-xylose results were only reported as normal or abnormal without defining a cutoff point [146, 155].

5.2.2 Endomolecular Challenge Absorption Tests

Six studies assessed absorption after administering labelled endomolecules (zinc (n=3), iron (n=1), and lipids (n=2)). These investigations rely on challenge with the molecule of interest, which is generally a stable isotope, and measure urinary excretion as an indicator of absorption, or fecal excretion, as an indicator of non-absorption. When examining stool, it is important to note that the fecal concentration could potentially represent uptake and subsequent excretion by the host. In one Peruvian study, there appeared to be saturation kinetics at high doses of zinc, and zinc dosing early in the day was related to diminished absorption of this divalent cation from unfortified foods later in the day [141]. Manary et al. identified an unexpectedly high fractional

excretion of the challenge zinc in children in Malawi [144]. In China, Sheng et al. demonstrated unexpectedly high fractional excretion of zinc in stool, and inferred diminished uptake of this nutrient, but also speculated that their population of children were not suffering from enteropathy [164]. Murphy et al. used ^{13}C labeled triglycerides (TG) as trilaurin, triolein, or trilinolein, and determined that in a group of Jamaican children, many of whom had bacterial overgrowth, the uptake of challenge lipids was impaired, but that the defect was not related to impaired intestinal lipolysis [148] Earlier, Murphy et al. demonstrated that during acute malnutrition, there is impaired lipid uptake from the gut, and that this defect improved during rehabilitation [149]. Unlike in the cohort in their later study, small bowel bacterial overgrowth was not an issue in this cohort. Sarker et al. studied labeled iron uptake in children with and without *Helicobacter pylori* infection, and found no evidence for diminished uptake among the infected children [163]. Similarly, treatment of the *H. pylori* infection did not improve uptake. They inferred that this gastric infection did not play a role in diminished uptake of iron by children in Bangladesh, but did note that there are limited data on the physiology of normal iron uptake in children in the age group studied (2-5 year olds).

5.2.3 Enterocyte-specific Proteins

Nichols et al. assessed intestinal tissue markers of digestion (see Evidence Table 3) as well as two markers of absorption: intestinal messenger RNA (mRNA) abundances of villin and sodium-activated luminal glucose-galactose transporter 1 transcripts [53]. These are structural proteins expressed only in enterocytes. mRNAs for these proteins are found in reduced quantities if intestinal surface area is diminished and hence reflect absorptive capacity. Nichols et al. compared the mRNA abundances in intestinal biopsies of children with refractory malnutrition to values found in controls that were neither stunted nor wasted, and found a reduction of more than 50% in mRNA abundances for both proteins among cases. It is important to note that the controls were children with biliary atresia who, as part of their

management for the condition, underwent intestinal resection. Without having performed an *ex vivo* mucosal biopsy in the control subjects, it is not possible to rule out a lower ratio of villous to submucosa tissue present in resected tissue which might affect mRNA determinations, as transmural tissue can be expected to contain a more diverse population of cells, only some of which might contain the transcripts of interest. However, the bias, if any, is likely in the direction that would reduce effect size.

5.2.4 Fecal Fat

Data on fecal fat was reported in three studies as a marker of malabsorption, including one study that measured the fat by four different methods, with a high degree of consistency in results across techniques [116]. That study, conducted among children with varying nutritional status in Venezuela, and another study conducted in India among children with symptoms consistent with and being evaluated for celiac disease [155], reported results as percent of subjects that tested positive while another study of severely malnourished Jamaican children [149] presented results as mean fecal fat excretion in grams per day and as percent of intake; hence, the results of this third study could not be compared to the other two. Positive results among the Venezuelan children varied from 13-27% (depending on laboratory method) among well-nourished children to 80-100% (again, depending on method) among the most severely malnourished children [116], while the Indian study detected fat in the stools of 20% of the children [155].

The Jamaican study found the fecal fat results to correlate with recovery of radiolabeled lipids in the stool after administration of ^{13}C tripalmitin. The Indian study found that 55% of subjects with symptoms of celiac disease but a negative serologic evaluation for that disorder had abnormal D-xylose absorption (although the authors did not report the units of measure,

cutoff point of normal-abnormal results, or whether or not they measured the sugar in the urine or serum) and 20% had abnormal fecal fat results; correlation testing was not reported.

5.2.5 Summary of Markers of Absorption

The assessed markers demonstrated normal and abnormal enteric function, depending on the marker and the study. The proportion of abnormal urinary D-xylose excretion results ranged from none among well-nourished children with asymptomatic *Giardia intestinalis* infection and healthy controls [147] to 100% among HIV-infected children in Brazil with protein-energy malnutrition [136]. Endomolecular markers also yielded varying results in different study populations. Apparent malabsorption was observed based on lipid uptake in a cohort of Jamaican children, some of whom had small bowel bacterial overgrowth [149]. In contrast, a study of iron uptake among children with and without *Helicobacter pylori* infection found no evidence of impaired absorption [163]. Malnourished children had reduced absorptive capacity relative to controls as inferred from reduced abundance of messenger RNA in biopsies for enterocyte-specific proteins [53]. Similarly malnourished children showed absorptive derangement by fecal fat test, with a notably higher proportion of positive tests than among well-nourished children [116].

Two of the fecal fat studies were the only ones that assessed more than one marker that is primarily a measure of absorptive capacity. The Jamaican study was the only one that reported testing for correlation between these tests of absorption [149]. Several studies compared the relationship of markers of absorption to biopsy. D-xylose [136, 146, 155] and fecal fat [155] corresponded poorly with histopathology. The papers suggested that there was only a short interval between the performance of these tests and the biopsies, but the exact length of time was not provided. Abundance of messenger RNA for two enterocyte-specific proteins, villin and sodium-activated luminal glucose-galactose transporter 1, did correlate with

villous atrophy in a study examining biopsy-derived markers [53]. None of the studies compared markers of absorption to other types of markers of enteric dysfunction (i.e., other than to biopsy or other measures of absorptive function).

Only one study reported data on an absorption marker in relation to the presence of diarrhea, finding no apparent association with D-xylose absorption, although this was not analyzed statistically [152]. None of the studies examined the association between absorption markers and growth outcomes. A Peruvian study was the only one to report an absorption marker as a clinical endpoint of an intervention trial, in this case a randomized, controlled trial of zinc supplementation [141]. It was also the only study to measure intra-individual longitudinal change outside of the context of intervention or convalescence. These investigators assessed zinc absorption longitudinally in controls without zinc supplementation and observed no change in absorption of this pentose sugar.

These absorption tests showed a range of logistical challenges for deployment in resource-limited settings. When methods were described, the D-xylose test was conducted by colorimetric or spectrophotometric means, whereas the tests of endomolecules involved more complex laboratory methods and the intestinal tissue markers, requiring biopsy, were most technically complicated and invasive. The D-xylose studies generally did not cite reference standards or compare their results to those from other studies. Two studies compared their fecal fat results to those in other published studies [116, 149]. One of these studies also conducted a lipid breath test and compared results to those previously published [149]. All of the zinc absorption studies discussed results in relation to expected values based on reference indices and other studies of zinc homeostasis [141, 144, 164]. In the iron absorption study, authors cited a lack of data available in the literature for subjects of similar age for comparison [163].

5.3 Markers of Permeability

Data regarding markers of permeability are presented in Evidence Table 2. As discussed above, many of the tests that assess permeability also entail a test component that assesses absorptive function.

Thirty-one studies assessed markers of intestinal permeability. All but one of these included assessments of dual sugar tests as either lactulose:mannitol (L:M) (n=25) or lactulose:rhamnose (L:R) (n=5); the other reported measures of lactose in the urine as lactose:creatinine. Two studies reporting L:M test results also reported other markers of permeability, either urinary lactose and lactose:lactulose or plasma endotoxin and IgG endotoxin-core antibody.

Evidence Table 2. Markers of permeability.

This category includes markers with dual assessment of absorption and permeability. Biomarkers in bold are primarily markers of permeability (with or without dual assessment of absorption).

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2004 Campbell DI et al. Intestinal inflammation measured by fecal neopterin in Gambian children with enteropathy: association with growth failure*, Giardia lamblia*, and intestinal permeability Fecal neopterin and L:M as markers of intestinal inflammation and permeability, respectively, and their correlation with growth status and *Giardia* recovery in the stool	Keneba, Gambia 2 mo olds from rural area followed until 15 mo of age.	Cohort n=72 Subjects were evaluated with twice-weekly questionnaire to determine diarrhea morbidity, clinic assessments of growth, and screening laboratory tests every 2 mo.	Stool Test: Neopterin Urine Tests: • **Lactulose**[1] • **Mannitol** • **L:M**	Mean neopterin concentration was negatively correlated with long-term height (r=-0.29, p<0.009) and weight (r=-0.36, p<0.007) gain, but not with giardiasis. Mean[2] L:M (CI): 0.31 (0.26, 0.34). Mean excretion of lactulose (CI): 0.20 (0.18, 0.23). Mean excretion of mannitol (CI): 3.0 (2.8, 3.2). Mean L:M was negatively correlated with long-term height gain (r value not provided, p<0.0001), but was not correlated with presence of *Giardia.* L:M and fecal neopterin were not correlated (p=0.11).	L:M and mean fecal neopterin concentration were not correlated. Mean L:M in the Gambian children was substantially higher than normal values in children in the UK. These high L:M ratios appear to be driven by mannitol excretion.	Study population might have some overlap with that of Campbell et al. also included in this review [110].
2003 Campbell DI et al. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation L:M as a marker of	Keneba, The Gambia All 2-11 mo olds were recruited from this rural village and followed up to 14 mo of age.	Cohort n=71	Urine Tests: • **Lactulose**[3] (53 tested) • **Mannitol** (52 tested) • **L:M** (52 tested) Blood tests: • Albumin • CBC • C-reactive protein (CRP)	At 8 wk of age: • Mean[4] L:M: 0.169 (CI: 0.145, 0.198; range: 0.058-0.657) • Mean lactulose recovery: 0.202 (SD=0.159; range: 0.009-0.640) • Mean mannitol recovery: 3.80 (SD=2.35; range: 0.52-8.58) L:M more than doubled between 12 wk-1 yr of age (r=0.44, p<0.001) and was driven by both increasing lactulose (r=0.18, p<0.001) and decreasing mannitol (r=-0.14, p<0.01) excretion with age.	Mean L:M ratios were elevated at 8 weeks of age, and more than doubled in the first year of life. Many markers of inflammation and endotoxin release were significantly correlated with L:M and lactulose recovery.	Presence of malaria parasites was assessed by blood smear at each study visit; the only parameter associated with malaria was CRP. Authors did not report investigating relationships between certain serum parameters

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Type of mean not specified.
[3] For lactulose and mannitol results, excretion measurement was not specified.
[4] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
intestinal permeability and its relationship with various inflammatory markers and endotoxin			• IgA • IgM • IgG • Plasma endotoxin • IgG endotoxin-core antibody	WAZ and HAZ scores were negatively correlated with L:M (r=-0.41, p<0.001), and primarily driven by lactulose excretion (r=-0.39, p<0.001). Laboratory values were consistent with chronic, low level immunostimulation: • 50% of platelet and 39% of leukocyte counts were elevated, especially mean lymphocyte counts which were almost twice expected values [198]. • While the mean[1] CRP was within the normal range, 25% of values were above the upper limit of normal (5 mg/L), and 17% were >10 mg/L [198]. • Mean IgG, IgA and IgM concentrations were near normal at 8 wk of age, but increased rapidly; all three were elevated above expected values in all other age groups [198, 199] • Mean[2] free plasma endotoxin concentration was twice the upper limit of normal [200] and IgG endotoxin-core antibody concentrations were also elevated [198] • However, mean albumin concentrations (and concentrations within SD) were generally within normal range [198]. L:M was correlated with IgG and IgA (r=0.41 and 0.41,	Poor growth was significantly correlated with L:M ratios, primarily due to lactulose excretion.	(blood counts, CRP concentrations) and L:M. Authors postulate that while general markers of inflammation cannot be specifically ascribed to a gut source, endotoxin and its related core antibody are potentially a direct measure of intestinal inflammation due to gut gram negatives as a primary source of endotoxin release among subjects without sources of extra-intestinal gram negative infection. Study population might have overlap with that of Campbell et al. 2004 also included in this review [15].

[1] Geometric mean.
[2] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				respectively, p<0.001), and IgM (r=0.28, p<0.02). IgG and IgA were also correlated with lactulose recovery (r=0.26 and 0.25, respectively, p<0.02). IgG endotoxin core antibody concentration was correlated with L:M and driven by lactulose recovery, (r=0.35, p<0.005 for both). Endotoxin concentrations were correlated with lactulose recovery (r=0.36, p<0.02) only.		

2003
 	
Campbell DI et al.
 	
Chronic T cell-mediated enteropathy in rural west African children: relationship with nutritional status and small bowel function
 	
L:M as a marker of intestinal permeability, small bowl biopsy with assessment of intestinal immune markers, and computerized morphometric analysis among rural Gambian children with differing degrees of malnutrition and compared to well-nourished UK children
	Fajara and Sibanar, The Gambia
	
	6 mo-3 yr old hospital- and clinic-based cases from rural communities.
	
	Case groups based on differences in nutritional status:
	1. WAZ score >-2, with GI complaints other than diarrhea
	2. Grade I protein energy malnutrition (PEM) (WAZ score -2 to -4) and unresponsive to nutritional supplements,
		Case-control
		
		n=40 cases:
		• Group 1: n=4
		• Group 2: n=11 (7 with diarrhea)
		• Group 3: n=25 (18 with diarrhea)
		
		n=34 with case tissue samples sufficient for cytokine immuno-reactivity tests:
		• Group 1: n=3
		• Group 2: n=8
		• Group 3: n=23
			<u>Endoscopic small bowel biopsy, site not specified</u>:
			• Histopathology
			• Morphometric assessment by computer analysis*
			• Intestinal tissue cytokines and immune markers:
			  • CD-3
			  • CD-4
			  • CD-8
			  • CD-19
			  • CD-25
			  • HLA-DR
			  • Perforin
			  • γδ T-cell receptor
			  • Syndecan-1
			  • TNF-α
			  • IFN-γ
			  • TGF-β
			  • IL-10
				Crypt-hyperplasia and villous atrophy were observed among all Gambian subjects, and the degree of histopathology did not differ among cases with differing nutritional status, nor was there a correlation with diarrhea.
				
				IEL[1] means were ~3-fold higher in Gambian than UK children.
				
				Median CD3, CD4, CD8, CD19, and CD25 cell counts were significantly higher (2-5x higher) among each case group compared to the UK controls.
				
				IEL, γδ, syndecan-1, HLA-DR, and perforin were detected among the Gambian children in varying degrees but were not reported for UK controls. Syndecan, CD3, and CD8 displayed a gradient proportional to malnutrition severity.
				
				All Gambian groups showed higher lamina propria cytokine-
					All Gambian subjects had evidence of enteropathy with crypt-hyperplasia and villous atrophy, and mean IELs >2 SD above UK norms, independent of nutritional status and diarrhea history.
					
					Elevation of cell-mediated intestinal markers and mucosal proinflammatory cytokines was present across the 3 Gambian groups, variably correlated with nutritional status.
					
					L:M ratios were elevated in all Gambian groups,
						Statistical methodology was not sufficiently detailed to determine what was compared (e.g. type of central tendency measure and variance calculations for L:M not stated).
						
						Duration of diarrhea not specified, but assumed to be persistent.
						
						Mucosal lymphocyte densities, cytokine immuno-reactivity, and L:M results were not stratified by history of diarrhea.

[1] These figures are presumed to represent IEL means; however, this was not explicitly stated.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
	with or without diarrhea 3. Grade II PEM (WAZ score <-4) with or without diarrhea Controls from UK* who were well nourished children with GI complaints other than diarrhea and with normal endoscopy results were also studied. * UK subjects are presented in this table due to comparisons of interest made in the review. However we do not include these subjects in the sample size for this review.		Urine Tests: • **Lactulose**[2] • **Mannitol** • **L:M** * Biopsy involved morphometric assessment by computer analysis of villous height, crypt depth, villous:crypt ratio, and intraepithelial lymphocyte (IEL) density (per 100 epithelial cells).	immunoreactive mononuclear cell density (~200-450/mm^2) than UK controls (30-80/mm^2). Among subjects with elevated cytokines, similar densities were seen for both pro-inflammatory (IFN-γ and TNF-α) and putative regulatory (IL-10 and TGF-β) cytokines. Epithelial expression of TGF-β was also enhanced compared to UK controls, but subjects with poorer nutritional status had lower densities of mucosal TGF-β+ cells, with median densities of 420 and 250 cells/mm^2 in the grade I and grade II PEM groups, respectively. L:M values[1]: • Group 1: 0.53 (0.4-1.3) • Group 2: 0.47 (0.02-2.20) • Group 3: 0.73 (0.14-2.2) • Not assessed among the UK controls Nutritional status was not associated with L:M, recoveries of lactulose or mannitol. L:M was correlated with mucosal B lymphocyte density (r=0.57, p<0.05), IEL (r=0.51, p<0.02), and perforin+ IEL (r=-0.64, p<0.03).	without apparent correlation to host nutritional status.	
2002 Campbell DI et al. Age-related association of small	Keneba, The Gambia and surrounding villages 2-60 yr olds	Cohort n=162; <5 yr old: n=26	Urine Tests: • **Lactulose**[2] • **Mannitol** • **L:M**	Mean[3] L:M (SE) in 2-5 yr old group: 0.353 (0.022). Mean lactulose and mannitol % recovery was ~0.45 and ~0.65, respectively.	Mean L:M in asymptomatic 2 to 5 yr olds was high and decreased significantly with increasing age, but	Subjects were free from diarrhea symptoms for at least one week prior to urinary assessments.

[1] Not clearly indicated if these figures represent mean (CI) or another measure of central tendency.
[2] Lactulose and mannitol results were expressed as % of dose administered.
[3] Type of mean not specified.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
intestinal mucosal enteropathy with nutritional status in rural Gambian children L:M and urinary lactulose and mannitol recovery as a marker of intestinal permeability and its association with nutritional status at varying ages. Also assessed correlation of change in L:M with nutritional status at 3.5 mo re-visit.	randomly selected from rural communities.	(23 were re-assessed)		L:M was highest in 2-5 yr age group and decreased with increasing age (up to age 20) ($p<0.001$), but never fell within referenced UK normal ranges [201]. Most of the improvement in L:M was driven by a reduction in lactulose excretion ($p<0.001$), which fell within expected UK ranges by age 10 yr. In contrast, although mannitol excretion slightly decreased with age, this trend did not reach statistical significance. In fact, excretion proportions were at all times ½ - ⅓ of expected UK values [201]. L:M was inversely correlated with HAZ score[1] ($r=-0.31$, $p<0.001$), but not with WAZ or body mass index (BMI) Z- scores. The correlation with HAZ score was mainly due to the higher lactulose excretion in subjects with poorer HAZ scores ($r=-0.22$, $p=0.001$) and held across all age groups. There was a small improvement in mean L:M (SE) between the two study time points from 0.198 (0.018) to 0.172 (0.010) ($p=0.026$ for change in L:M), driven by an improvement in mannitol recovery with no change in lactulose excretion. Indices of intestinal permeability within subjects showed a high	never fell within expected range of values. Among all age groups, L:M showed significant intra-subject correlation between tests conducted 3.5 months apart. Among all age groups, L:M was significantly inversely correlated with HAZ score, primarily driven by lactulose excretion.	The authors sought correlation between the mean L:M of the two visits and ΔBMIZ, ΔHAZ and ΔWAZ scores, but statistical calculations were not provided.

[1] Reported results were adjusted for age, sex, and visit.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

				degree of correlation between the two visits:
				• Lactulose: r=0.55, p<0.001
				• Mannitol: r=0.24, p<0.05
				• L:M: r=0.66, p<0.001
				
				Change in measures between visits (analysis not stratified by age):
				• Mean L:M (SD):
				  • Visit 1: -1.62 (0.66)
				  • Visit 2: -1.76 (0.55), (p=0.026)
				• Mean mannitol recovery (SD):
				  • Visit 1: 5.25 (2.69)
				  • Visit 2: 6.28 (3.03), (p=0.006)
				• Mean lactulose recovery (SD):
				  • Visit 1: 0.28 (0.20)
				  • Visit 2: 0.29 (0.18), NS (p-value not specified)		

2003 Chen P et al. Association of vitamin A and zinc status with altered intestinal permeability: analyses of cohort data from northeastern Brazil L:M as a marker of intestinal permeability pre- and post-vitamin A and zinc supplementation among children with history of PD or low WAZ score	Goncalves Dias favela in Fortaleza, Brazil 2-97 mo olds recruited from an urban shantytown.	Cohort n=75 with pre-supplement L:M and retinol concentrations measured: • 51 with pre-supplement circulating zinc concentrations measured • 20 with post-intervention* longitudinal follow-up of subset with history of PD or low WAZ score	Urine Tests: • **Lactulose**[1] • **Mannitol** • **L:M**	Baseline mean (SD): • L:M[2]: 0.29 (0.16) • Lactulose: 0.54 (0.29) • Mannitol: 2.07 (0.88) L:M was not correlated with age. L:M was inversely correlated with retinol (r=-0.55, p<0.0005), including after adjustment for zinc concentration and stratification on retinol concentrations. Retinol was correlated with mannitol (r=0.28, p=0.017) and lactulose (r=-0.22, p<0.063) excretion. Lactulose, mannitol and their combined ratio were not correlated with zinc concentrations.	Supplementation of vitamin A and zinc resulted in significant improvements in L:M among the cohort of children with a history of PD or low WAZ score who received post-supplementation assessment. Less than one-third of the subjects had post-intervention L:M assessments.	Longitudinal data were not reported stratifying on underlying condition (i.e. PD history vs. WAZ score). Follow-up data on L:M were not provided for the children with normal WAZ score or no history of PD. Unclear how long after supplementation the L:M testing was done.

[1] For lactulose and mannitol results, excretion measurement was not specified.
[2] Type of mean not specified.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
		* These subjects received a single oral dose of vitamin A and a 2-wk course of daily zinc supplements.		L:M improved after supplementation for the cohort of 20 children followed longitudinally with PD or low WAZ score: L:M mean (SD): • Pre-treatment: 0.28 (0.12) • Post-treatment: 0.19 (0.07) However, lactulose and mannitol excretion did not change significantly.		Post-supplementation L:M results in the text of the publication differed somewhat from what was reported in the publication table.
2007 Darboe MK et al. Effectiveness of an early supplementation scheme of high-dose vitamin A versus standard WHO protocol in Gambian mothers and infants: a randomised controlled trial L:M as a marker of intestinal epithelial integrity among infants receiving high-dose vitamin A or standard vitamin A protocol	Keneba, The Gambia Subjects recruited at birth from rural community. Age range during study was 0-12 mo.	RCT n=197 n=99 received high dose vitamin A protocol n=98 received standard dose vitamin A protocol	Urine Test: **L:M**	Mean[1] L:M and proportion with values >0.30 among those receiving standard doses of vitamin A, by age: • 2 mo: 0.195, 12% • 5 mo: 0.197, 13% • 7 mo: 0.212, 22% • 9 mo: 0.286, 30% • 12 mo: 0.322, 34% Mean L:M differed between the two groups only at 7 mo (0.276 in high-dose vitamin A group, p=0.014), although there was no difference in percentages with L:M >0.30.	L:M values rose by ~50% from age 2 mo to 1 yr and were not affected by dosing of vitamin A.	The L:M normal cutoff was defined higher than for most other L:M studies, as 0.30. This was derived from the mean plus 2 SD from a study of UK infants [202].
2001 Filteau SM et al. The effect of antenatal vitamin A and (beta)-carotene supplementation on gut integrity of infants	Durban, South Africa Pregnant, HIV-infected women between 28-32 wk gestation recruited from antenatal clinic. Infants were	RCT n=238 n=119 received vitamin A supplements (26 with HIV infection)	Urine Tests: • **Lactulose**[2] • **Mannitol** • **L:M** Subjects tested: • 1 wk: • Treatment:	Mean L:M[3] (CI) at 1 wk among infants without reports of illness was 0.12 (0.08, 0.17). L:M did not change with increasing age and did not significantly increase with reported morbidity. While a history of ever having been breastfed was an important	Mean L:M overall was within normal range. However, mean L:M for placebo-treated, HIV-infected infants by 14 weeks was significantly elevated to almost 0.5.	Specific sugar excretion was normalized to urinary creatinine to control for variation in renal function.

[1] Geometric mean.
[2] For lactulose and mannitol results, excretion measurement was not specified.
[3] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

of HIV-infected South African women
 	
L:M as a marker of intestinal permeability among infants of HIV-infected mothers enrolled in a vitamin A trial
	followed until 14 wk of age.	n=119 received placebo (29 with HIV infection)
		
		Treatment involved maternal vitamin A supplements during pregnancy and at delivery.
			n=104
			• Placebo: n=104
			• 6 wk:
			  • Treatment: n=100
			  • Placebo: n=105
			• 14 wk:
			  • Treatment: n=99
			  • Placebo: n=95
				contributor to L:M at 1 wk (ΔR^2=0.22, p=0.008), a significant effect was not seen at 6 and 14 weeks[1]. Current feeding status had a modest effect on L:M only at 14 wk (ΔR^2=0.06, p=0.04).
				
				Birth weight contributed significantly at 1 wk (ΔR^2=0.07, p=0.02), but current weight did not contribute significantly to L:M at any time point.
				
				HIV infection status by 14 wk was the major factor contributing to L:M at 6 wk (ΔR^2=0.22, p=0.008) and 14 wk (ΔR^2=0.21, p=0.01).
				
				Maternal HIV viral load during pregnancy was not consistently significantly correlated with infant L:M. Maternal lymphocyte counts and plasma retinol concentrations were not associated with infant L:M.
				
				While maternal vitamin A supplementation had no effect on L:M of uninfected infants, it appeared to prevent the increase in L:M of HIV-infected infants[2]:
				Mean L:M (CI):
				• Uninfected:
				  • Vitamin A group: 0.11 (0.08, 0.15)
				  • Placebo group: 0.09 (0.06, 0.12)
				• HIV-infected:
				  • Vitamin A group: 0.17 (0.13, 0.23)
				  • Placebo group: 0.50 (0.37, 0.68)
					While HIV infection did not affect mannitol excretion, it was associated with increased lactulose excretion.	

[1] Reported results were adjusted for confounding variables, unless otherwise noted.
[2] Reported results were adjusted for confounding variables included an interaction with HIV infection.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				Mannitol was not affected by vitamin A. HIV infection was not consistently significantly associated with mannitol across age groups. Lactulose also did not consistently differ between treatment groups or by HIV-status, although vitamin A prevention of increase in lactulose among HIV-infected infants neared significance at 14 wk (p=0.058)[1].		

2005
 	
Galpin L et al.
 	
Effect of *Lactobacillus* GG on intestinal integrity in Malawian children at risk of tropical enteropathy
 	
L:M and sucrose: lactulose ratio (SUC:L) as markers of intestinal and gastric permeability, respectively, in asymptomatic children presumed at risk of EED
	Mwenye, Malawi
	
	36-60 mo olds recruited from a rural community, excluding children with severe acute malnutrition or severe chronic illnesses.
	
	Subjects were considered at risk for EED due to residence in a location with high prevalence of EED.
	
	Presumed that if SBBO is etiology for EED, treatment with *Lactobacillus* will result in improved gut integrity.
		RCT
		
		n=164;
		
		n=81 received *Lactobacillus* GG (80 completed the study)
		
		n=83 received placebo (81 completed the study)
		
		Subjects received 30-days of *Lactobacillus* GG or placebo. Only the 161 subjects who completed the study had repeat testing.
			<u>Urine Tests</u>:
			• **Lactulose**[2]
			• **Mannitol**
			• Sucrose (SUC)
			• **L:M**
			• SUC:L
				At enrollment:
				• 73% had L:M >0.10
				• 40% had L:M >0.20
				• Mean[3] L:M (SD):
				  • Treatment: 0.18 (0.16)
				  • Placebo: 0.22 (0.20)
				• Mean lactulose (SD) in treatment group: 0.25 (0.17)
				• Mean mannitol (SD) in treatment group: 8.0 (4.5)
				• Mean SUC:L (SD):
				  • Treatment: 0.58 (0.64)
				  • Placebo: 0.60 (0.64)
				
				Mean excretion of sucrose (SD) increased from 0.057 (0.042) to 0.078 (0.058) in the treatment group (p=0.01), but similar results were observed in the placebo group. Otherwise there were no changes in lactulose, mannitol, L:M, or SUC:L after treatment or placebo.
					A high baseline prevalence of abnormal L:M was observed, with no change after intervention.
					
					High mannitol excretion (relative to UK norms) drove the abnormal L:M.
					
					There was little effect on SUC:L with intervention; sucrose excretion increased in both treatment and control groups.
						Difficult to interpret sucrose tests because there are limited data on laboratory values for these tests in young children.

2008	Dhamrai Upazila, Bangladesh	RCT	<u>Urine Test</u>:	Mean L:M[1] (SD) at baseline was 0.18 (0.24) in treatment groups, with	High L:M ratios overall with	High L:M ratios were defined as

[1] P-values are from reported results that were adjusted for confounding variables.
[2] Lactulose, mannitol, and sucrose results were expressed as % of dose administered.
[3] Arithmetic mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Goto R et al. Impact of anti-*Giardia* and anthelminthic treatment on infant growth and intestinal permeability in rural Bangladesh: a randomised double-blind controlled study L:M as a marker of intestinal permeability, IgG as a marker of chronic immune stimulation, and α-1-acid glycoprotein as an acute phase reactant among children undergoing anti-parasitic presumptive treatment vs. placebo. Also assessed markers' associations with growth parameters.	3-15 mo olds from a rural area were enrolled and followed in a 9-mo trial. There was a high prevalence of malnutrition in the study population.	n=222* n=75 received anti-*Giardia* and anthelminthic treatment n=59 received anti-*Giardia* treatment only n=88 received placebo * Those who fully participated and for whom data were analyzed are included in this review.	• **L:M** <u>Blood Tests</u>: • α-1-acid glycoprotein (AGP) • IgG • Albumin	no significant difference in placebo group or in testing post-intervention. Proportion with elevated L:M at any study time point varied between 58%-74%. >57% consistently elevated L:M ratios. Seasonal variation in L:M was observed ($p < 0.001$), with highest mean values in the monsoon season. L:M was associated with ΔWAZ and ΔWHZ scores at 24 weeks ($p=0.001$ and $p<0.001$, respectively, point estimates not provided.) Serum immune marker values were similar in all groups and did not change substantially with interventions. AGP concentrations were negatively associated with ΔWAZ score at 24 weeks ($p=0.004$, point estimate not provided), and were associated with ΔWHZ score at 12 weeks but not at 24 weeks.	substantial seasonal and within-infant variability. Interventions did not impact L:M or serum immune markers. There was improvement in weight with better L:M values, the degree to which this occurred was not reported.	greater than the upper CI for UK infants. Same study population as reported by this group in another study also included in this review [123].
2008 Goto R et al. Impact of intestinal permeability, inflammation status and parasitic infections on infant growth faltering in rural Bangladesh	Dhamrai Upazila, Bangladesh 3-15 mo olds from a rural area were enrolled and followed in a 9-mo trial. There was a high prevalence of	Longitudinal data extracted from an RCT [122]. n=298 Urine and blood samples were collected every 3 mo and	<u>Urine Test</u>: **L:M** <u>Blood Tests</u>: • α-1-acid glycoprotein (AGP) • IgG • Albumin • Hemoglobin	Mean[2] L:M: 0.15 L:M showed a decreasing trend with age ($p=0.003$), and was associated with female gender ($p=0.004$), HAZ score ($p=0.039$), and WAZ score ($p=0.019$), but not with giardiasis or any of the serum immune markers. IgG, AGP, and albumin were associated with giardiasis, but	Mean L:M was elevated. L:M was not associated with any of the tested serum markers of inflammation or with giardiasis. IgG rose with increasing age at the rate expected	Helminthiasis prevalence was very low; testing for association with markers was not performed. Giardiasis was defined as presence of a *Giardia*-specific IgM

[1] Geometric mean.
[2] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
L:M as a marker of intestinal permeability, IgG as a marker of chronic immune stimulation, and α-1-acid glycoprotein as an acute phase reactant. Also assessed laboratory values' associations with giardiasis and growth parameters.	malnutrition in the study population.	anthropometric measurements were collected monthly.		hemoglobin was not. Mean circulating albumin concentration was normal for age [203]. Compared to UK age-matched reference [199], rate of rise in IgG with increasing age was similar, but concentrations were consistently ~3g/L higher. IgG was not associated with growth parameters. Albumin was associated with HAZ score only (p=0.016). AGP was inversely associated with HAZ (p=0.011) and WAZ (p=0.005) scores.	(compared to UK norms) [199] but at higher concentrations across all ages.	response. Same study population as reported by this group in another study also included in this review [122]. Cut-off values representing elevated concentrations have not been determined for AGP. UK norms for 10 mo olds-adults are 0.88 g/L mean (0.21 SD) [204].
2002 Goto R et al. Poor intestinal permeability in mildly stunted Nepali children: Associations with weaning practices and *Giardia lamblia* infection L:M as a marker of intestinal permeability, and assessment of association with giardiasis, helminthiasis, nutritional practices, and growth status.	Kathmandu, Nepal 0-5 yr olds (mean age 3.8 yr) from two urban squatter settlements. 37% and 33% of subjects were stunted and underweight, respectively.	Cross-sectional n=210	Urine Tests: • **Lactulose**[1] • **Mannitol** • **Lactose**[2] (168 tested) • **L:M** (158 tested) • Lactose:lactulose ratio (157 tested)	L:M: • 92% had values >UK norms • Mean[3] L:M (SD, range): 0.26 (0.21, 0.04-1.71). • *Giardia*-infected versus uninfected means: 0.43 vs. 0.25, p=0.014 The duration of ingestion of solid foods (with or without concurrent breastfeeding) was not associated with L:M in multivariate analysis. L:M was correlated with longer duration of breastfeeding (r=0.27, p<0.019). Specifically, children who breastfed for >2 yr had higher L:M ratios than children who breastfed for shorter times (data not provided). L:M was not associated with:	L:M ratios were high overall. Wide individual variation was observed in L:M ratios. L:M was associated with giardiasis but not helminthiasis. Urinary lactose concentrations and lactose:lactulose ratios were significantly higher in breastfed subjects than in those that were not breastfed,	Low lactase activity was defined as lactose:lactulose ratio >0.4. Specific L:M data by WAZ and HAZ scores were not reported, although authors state that L:M was not associated with "growth status."

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Lactose results were expressed in mg/L.
[3] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				• History of diarrhea in the week preceding testing • Helminthiasis • Age • WAZ or HAZ scores Lactulose excretion ranged from 0.02–15.00. Mannitol excretion ranged from 0.5–15.00. 47% showed low lactase activity. Lactose values and lactose:lactulose ratios decreased with age (R^2=28%, p<0.0001), but were not associated with sex, ethnicity, and location nor were they associated with L:M. Mean[1] urinary lactose concentrations (mg/L) by feeding mode: • Breastfed: 172.5 • Non-breastfed: 44.5, p<0.0001 corrected for infant age Mean[2] lactose:lactulose ratio by feeding mode: • Breastfed: 2.76 • Non-breastfed: 0.31, p<0.0001 corrected for infant age Mean L:M by feeding mode: • Breastfed: 0.23 Non-breastfed: 0.28, non-significant, p-value not specified	despite similar intestinal permeability values. There were some unexpected findings: the duration of breastfeeding, and not the timing of introduction of solid foods, was correlated with L:M, and the correlation was direct, not inverse. Authors speculate that this could be due to higher mean age of their cohort compared to another study that demonstrated beneficial effect of duration of breastfeeding on reduced L:M in Guatemala [205].	
2000 Haase A et al. Dual sugar	Darwin, Australia Cases were >4 mo olds admitted to Royal Darwin	Case-control n=264; n=150 cases with	Blood Test: **L:R** Urine Test:	Among the subset with both blood and urine specimens: • Urine L:R: • Mean[3] (CI): • Cases: 12.4 (9.3, 16.5)	Children with diarrhea had significantly higher L:R ratios by both blood and urine	Authors used data from non-diarrheal controls from their clinical practice to derive cut-points for

[1] Geometric mean.
[2] Geometric mean.
[3] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
permeability testing in diarrheal disease Lactulose:rhamnose ratio (L:R) as a marker of intestinal permeability in children with or without diarrhea. Also directly compared blood and urine methods of L:R testing in a subset of subjects.	Hospital with diarrhea. Controls were patients admitted with non-GI illness. More than 75% of cases and controls were Aboriginal.	AD n=114 controls with no diarrhea	**L:R** Among cases: • 24 had both blood and urine L:R • 98 had blood L:R only • 28 had urine L:R only Among controls: • 25 had both blood and urine L:R • 36 had blood L:R only • 53 had urine L:R only A total of 49 subjects were tested with both blood and urine L:R methods to allow direct comparison of values.	• Controls: 6.7 (5.0, 8.8), p=0.004 • Distribution across ratios: • Low: n=31 • Intermediate: n=9 • High: n=9 • Blood L:R: • Mean[1] (CI): • Cases: 9.4 (6.7, 13.1) • Controls: 5.9 (4.4, 7.8), p=0.04 • Distribution across ratios: • Low: n=27 • Intermediate: n=11 • High: n=11 Among subjects with only urine tested: Mean[2] urine L:R (CI): • Cases: 15.7 (12.6, 19.6) • Controls: 6.7 (5.7, 8.0), p<0.0001 Among subjects with only blood tested: Mean[3] blood L:R (CI): • Cases: 12.8 (10.3, 16.0) • Controls: 3.7 (2.8, 4.9), p<0.0001 Even though blood L:R was consistently lower than urine L:R by a geometric mean (CI) of 1.09 (1.02, 1.16), there was strong correlation between L:R ratios in blood and urine as measured by: • Concordance correlation coefficient for agreement (CI) of 0.76 (0.64, 0.88) • Kappa statistic (CI) of 0.71 (0.51,	testing compared with controls without GI illness. There was substantial agreement between urine and blood L:R tests in the same subjects. Urine has been an established substrate for sugar excretion assessment as an indication of intestinal permeability. However, timed collection of urine is not a trivial task, especially among female children, and contamination with stool is problematic, especially in children with diarrhea. However, the much lower concentrations of probe sugars in blood compared to urine had posed a challenge to sensitive detection in blood. High performance liquid chromatography (HPLC) methods, as	L:R ratios used in this study: • Blood L:R: • Low= <7 • Intermediate= 7-12.5 • High= >12.5 • Urinary L:R: • Low= <10 • Intermediate= 10-18 • High= >18 Controls were significantly older than the cases, but authors suggest that age differences do not impact L:R test performance. Numbers of subjects do not always match up (e.g. numerator in test failure rate calculations does not match other such reported numbers). Analyses of those subjects who had both blood and urine testing were conducted on combined cases and controls. Analyses of those

[1] Geometric mean.
[2] Geometric mean.
[3] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				0.92) (when L:R ratios are divided into 3 ordered categories) • Sensitivity and specificity of blood tests of 81% (25/31) and 89% (16/18), respectively, when using the urine testing as the standard. The failure rate* for serum L:R testing (20/197, 10%) was lower than for urine testing (86/234, 37%) (p<0.0001). * Defined as emesis with rhamnose/lactulose oral challenge (same dose for urine and serum testing), urine leakage or contamination with stool, or plasma quantity from blood draw of insufficient quantity for analysis.	used in this study, now provide a more sensitive method of assessing blood specimens. The failure rate of L:R blood testing was significantly lower than that of urine testing.	with and without diarrhea would have been of interest. One would expect children with diarrhea to have higher rates of urine test failure due to contamination with stool and to have higher failure rates using either analytes due to higher rates of emesis. Spot blood testing might be more feasible than timed urine collections, but HPLC might not be feasible in resource-poor settings. This study appears to report on the same population as two other studies in this review which also assessed serum L:R as a marker of intestinal permeability [43, 58].
2003 Kukuruzovic R et al. Increased nitric oxide production in acute diarrhea is associated with abnormal gut	Darwin, Australia 1-6 yr old Aboriginal and non-Aboriginal hospital inpatients.	Case-control n=318; n=169 cases with AD (154 Aboriginal)	<u>Urine Test</u>: Nitric Oxide (NO)* <u>Blood Tests</u>: • **L:R** • Mean corpuscular volume (MCV)	NO among Aboriginal children with diarrhea was >3x higher than any other group and >5x higher than in non-Aboriginal controls. • NO was >3x and >2x higher among Aboriginal than non-Aboriginal children in the diarrhea (p<0.001) and no infections groups	NO_2 + NO_3:Cr ratio, as a measure of endogenous nitric oxide production, was used as a marker of gut permeability and inflammation, with	Positive stool RS was defined as <u>≥</u>0.5%. Abnormal L:R was defined as >7.6; no reference or derivation was

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
permeability, hypokalemia and malnutrition in tropical Australian aboriginal children Nitric oxide (NO) as a marker of intestinal permeability and inflammation, and lactulose:rhamnose ratio (L:R) as a marker of intestinal permeability and the relationship between NO and L:R, growth parameters, mean corpuscular volume (as a surrogate of iron deficiency), and stool reducing substances among children with and without diarrhea	Subjects were grouped as follows: 1. Children with AD 2. Children with no diarrhea but with non-GI infectious conditions 3. Children without GI or infectious conditions	n=149 controls: • 73 with non-GI infections (49 Aboriginal) • 76 with no infections (29 Aboriginal)	<u>Stool Test:</u> Reducing substances (RS)** (169 cases tested) * NO is an unstable free radical and is converted to nitrite and nitrate. Urine nitrate (NO_3)+ nitrite (NO_2) was expressed as a ratio with urine creatinine (NO_2 + NO_3:Cr) in order to account for differences in urine concentration. ** Measured only among children with profuse diarrhea.	(p<0.001), respectively, but there was no difference between them in the non-GI infections group. • NO was >3x and ~2x higher in the diarrhea compared to the no infections group among Aboriginals (p<0.001) and non-Aboriginals (p<0.03), respectively. • NO was virtually the same among the Aboriginal non-GI infections and no infections groups, as well as among the non-Aboriginal diarrhea and non-GI infections groups. 112/152 (74%) and 31/169 (18%) of children with AD had abnormal L:R ratios[1] and positive stool RS, respectively. NO and L:R were measured at "convalescence" on Day 5 among those with diarrhea: the mean improvement in NO was 21.7% compared with 54.6% for L:R (p=0.01). NO and L:R were correlated (n=193, r=0.37, p<0.001)[1]; the correlation was stronger for lactulose (effect ratio=1.47, p<0.001) than for rhamnose (effect ratio=0.80, p=0.02[2]). NO was not correlated with stool RS[3] or MCV, but was correlated with lower WAZ score (effect ratio=0.88, p=0.05).	an attempt to identify how much more it reflects as response to inflammation from GI vs. non-GI infections. Among non-Aboriginal controls, NO production was the same among those with diarrhea and non-GI infections (and higher compared to controls). NO was highest by far among Aboriginal children with diarrhea compared to any other group. Authors suggest that high basal concentrations of NO among Aboriginal children due to (clinically silent) enteropathy could explain the concentrations seen among Aboriginal controls in this study. NO appeared to decrease significantly more slowly than L:R among children	provided for this cut-point. Study population appears to be the same as in another Kukuruzovic, et al. study also included in this review which assessed serum lactulose:rhamnose as a marker of intestinal permeability [58].

[1] Reported results appear to have been adjusted for age and race.
[2] Reported results were adjusted for age and race.
[3] Reported results among children with diarrhea were adjusted for age and race.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
					recovering from diarrhea. NO was found to correlate with L:R. NO was more strongly correlated with lactulose than rhamnose.	
2002 Kukuruzovic RH et al. Milk formulas in acute gastroenteritis and malnutrition: a randomized trial Lactulose:rhamnose ratio (L:R) as a marker of intestinal permeability among children with diarrhea and/or malnutrition treated with milk formulas of varying composition and osmolality	Darwin, Australia Inpatient Aboriginal children <3 yr old with AD and/or WAZ score <-2. 60% of subjects had low WAZ score and 90% had diarrhea.	RCT n=177; n=60 received De-Lact formula n=65 received O-Lac formula n=52 received Alfaré formula Subjects were treated with one of three low-lactose formulas: • De-Lact, low-osmolality lactose-free formula • O-Lac, lactose-free formula • Alfaré, partially hydrolyzed formula	Blood Test: **L:R** L:R testing was repeated in 150 subjects at day 5: • De-Lact: n=48 • O-Lac: n=52 • Alfaré: n=50	Baseline mean[1] L:R (CI) in De-Lact group was 14.9 (10.4, 21.5), with no difference between groups. The mean improvement* in L:R (CI) was 13.0 (9.3, 16.6) with some significant differences between the various formulas: • De-Lact: 18.6 (10.6, 26.6) • O-Lac: 12.0 (7.5, 16.6), p=0.15 compared to De-Lact • Alfaré: 8.5 (2.1, 14.9), p=0.049 compared to De-Lact * Improvement in L:R was calculated as baseline L:R minus repeat L:R.	Authors noted that treatment with all of the low-lactose formulas studied resulted in improved L:R among this population at risk for enteropathy and growth failure. Improvement was most marked with the low osmolality formula, De-Lact.	Reported results did not appear to be harmonized with the method described for calculating improvement in L:R. Fully breastfed children were excluded. The study did not include a control arm (of standard care) to which change in L:R could be compared. Authors reiterate the advantages of serum over timed urine collection for assessment of L:R as discussed in another publication in this review [125].
2002 Kukuruzovic RH et al.	Darwin, Australia Cases were Aboriginal and	Case-control n=375 admissions for 306 children;	Blood Tests: • **Lactose** • **Lactulose**[2] • **Rhamnose**	27/75 (36%) of Aboriginal controls and 0 non-Aboriginal controls had abnormal L:R ratios.	Mean L:R ratios of Aboriginal children were approximately double those of non-	Positive stool RS was defined as ≥0.5%.

[1] Geometric mean.
[2] Lactulose and rhamnose results were expressed as % of dose administered.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Small bowel intestinal permeability in Australian Aboriginal children Serum lactulose: rhamnose ratio (L:R), serum lactose, and stool reducing substances as markers of intestinal permeability among Aboriginal and non-Aboriginal children with and without diarrhea	non-Aboriginal children admitted to hospital with diarrhea. Controls were Aboriginal and non-Aboriginal children admitted without GI illnesses.	n=285 case admissions for AD (264 Aboriginal) n=90 control admissions with no diarrhea (74 Aboriginal)	• **L:R** • Hemoglobin • Mean corpuscular volume (MCV) <u>Stool Test</u>: Reducing substances (RS)* L:R testing was repeated on day 5 for a subset of Aboriginal subjects: • 174/264 admissions for acute diarrhea • 25/74 control admissions * Measured only among children with profuse diarrhea when "clinically indicated." Number tested not provided.	Mean[1] L:R at baseline: Cases: • Aboriginal: 16.4 • Non-Aboriginal: 7.9, p=0.002 compared to Aboriginal cases Controls: • Aboriginal: 4.6 • Non-Aboriginal: 2.5, p=0.02 compared to Aboriginal controls Mean improvement[2] in L:R (CI) at day 5 among those with repeat testing: • Aboriginal cases: 14.6 (11.2, 18.0) • Aboriginal controls: -0.63 (-4.0, 2.7) Mean lactulose recovery[3]: • Cases day 1: 0.085 (0.070–0.103) • Cases day 5: 0.039 (0.033–0.046) • Controls: 0.024 (0.019–0.029) All 3 values significantly differed from one another. Mean rhamnose recovery: • Cases day 1: 0.479 (0.424–0.542) • Cases day 5: 0.555 (0.498–0.616) • Controls: 0.585 (0.500–0.685) These values did not significantly differ from one another. Confidence intervals (CIs) in the authors' graphical representation of mean L:R at admission did not overlap, and the difference in means was particularly evident between Aboriginal and non-Aboriginal subjects.	Aboriginal children both among those with and without diarrhea, consistent with authors' suggestion that clinically silent enteropathy is prevalent among Aboriginal children. Mean L:R significantly improved over 5 days among Aboriginal cases. Children with severe diarrhea had higher mean L:R. Higher case L:R was driven more by high lactulose than by low rhamnose. Similarly, improvement in L:R among cases was primarily due to decreased lactulose. Stool RS and serum lactose were found in approximately one-quarter and one-third of Aboriginal cases, respectively. The latter was weakly associated with	Abnormal L:R was defined as >5.6, derived from 2 SD above the arithmetic mean for non-Aboriginal controls in this study. The rationale for the choice of 2 SD above the arithmetic, instead of the geometric, mean is not clear. Proportions of cases with abnormal concentrations were not reported. Analysis included data for 69 children with repeat admissions; this might violate independence assumptions for their statistical analysis methods. Repeat L:R testing was conducted on controls of both racial groups, but among cases it was only conducted on Aboriginal cases. This study appears to report on the

[1] Geometric mean.

[2] Improvement in L:R appears to have been calculated as baseline L:R minus repeat L:R, as described in another publication in this review; however, this was not expressly stated. Reference 134. Kukuruzovic RH, Brewster DR. Milk formulas in acute gastroenteritis and malnutrition: a randomized trial. J Paediatr Child Health, 2002. **38**(6):571-577.

[3] Figures reported parenthetically after the mean percent recoveries of lactulose and rhamnose were not specified as ranges or CIs.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				Factors associated with L:R among cases were[1]: • Acidosis (p=0.007) • Hypokalemia (p=0.035) • Diarrhea severity (p=0.001) Age and malnutrition were not associated with L:R. 38% and 27% of Aboriginal cases had positive serum lactose and stool RS, respectively. 12% of Aboriginal and non-Aboriginal controls combined had lactosemia. Presence of lactosemia was associated with L:R, adjusted relative risk (CI)=1.06 (1.03, 1.10)[2]. Stool RS, anemia, and MCV were not associated with L:R.	increased lactulose.	same population as in the Kukuruzovic, et al. 2003 reference also included in this review, which assessed nitric oxide excretion [43]. Authors reiterate the advantages of serum over timed urine collection for assessment of L:R, as discussed in another publication in this review [125].

2010
 	
Lima AA et al.
 	
Effects of vitamin A supplementation on intestinal barrier function, growth, total parasitic, and specific *Giardia* spp infections in Brazilian children: a prospective randomized, double-blind, placebo-controlled trial
 	
L:M as a marker of intestinal barrier function, and stool
	Fortaleza, Brazil
	
	2 mo-9 yr olds (mean 43 mo) from an impoverished urban community, eligible if HAZ score was <median for their community.
	
	Subjects were screened for intestinal parasites, and longitudinal anthropometrics were assessed.
		RCT
		
		n=79;
		
		n=40 received placebo (tocopherol)
		
		n=39 received vitamin A (retinyl palmitate)
		
		Subjects were treated every 4 mo.
			Urine Tests:
			• **Lactulose**[3]
			• **Mannitol**
			• **L:M**
			
			Stool Tests:
			• Lactoferrin
			• Cytokines:
			  • IFN-γ
			  • TNF-α
			  • IL-4
			  • IL-10
				Median L:M at baseline was 0.089. There was no significant change in L:M at 4 mo follow-up within either treatment group.
				
				No significant difference in L:M was observed between treatment groups.
				
				Both median lactulose and mannitol excretions decreased at 4 mo follow-up among the vitamin A compared to the placebo group:
				• Lactulose: 0.21 to 0.74, p=0.042
				• Mannitol: 3.06 to 8.25, p=0.008
				
				Overall proportion of lactoferrin was 23% initially. At 1 mo follow-up, there was no difference in prevalence between vitamin A (33%) and
					Frequency of stool lactoferrin varied between 23%-32%.
					
					While vitamin A supplementation was associated with reduced lactulose excretion, it was also associated with reduced mannitol excretion, with no overall effect on L:M.
					
					Vitamin A supplementation was not associated with presence of lactoferrin or
						Authors did not report testing for associations between urinary markers of intestinal permeability and concentrations of fecal cytokines, or between these markers and growth parameters or parasitosis.
						
						Cut-point values for lactoferrin positivity and abnormal L:M were not described.

[1] Reported results were adjusted for confounding variables, unless otherwise noted.
[2] Reported results were adjusted for severity of diarrhea, acidosis, hypokalemia, and age.
[3] For lactulose and mannitol results, excretion measurement was not specified.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
lactoferrin and specific intestinal immunological cytokines as markers of intestinal inflammation among nutritionally at-risk children who received either vitamin A or placebo				placebo (31%) groups. Cytokine concentrations did not significantly differ between placebo and vitamin A groups.	intestinal cytokine response.	Exclusively breastfed children were excluded from study participation due to assessment of stool lactoferrin.

2005
 	
Lima AA et al.
 	
Intestinal barrier function and weight gain in malnourished children taking glutamine-supplemented enteral formula
 	
L:M as a marker of intestinal permeability and various stool tests among children with malnutrition or PD who received either glycine or glutamine supplemented formula or placebo
	Fortaleza, Brazil
	
	2-60 mo olds hospitalized with WAZ score <-2, ~70% of whom had PD.
		RCT
		
		n=80;
		
		n=53 received supplemented formula
		• 27 with glycine
		• 26 with glutamine
		
		n=27 received nonsupplemented formula
			Urine Tests*:
			• **Lactulose**[1]
			• **Mannitol**
			• **L:M**
			
			Stool Tests**:
			• Lactoferrin
			• Leukocytes
			• Occult blood
			• Reducing substances (RS)
			
			* n=80 tested at enrollment, n=65 tested at day 10.
			
			** n=60 tested.
				Mean[2] L:M (SE):
				• Glutamine group:
				  • Baseline: 0.31 (0.10) (similar in all three groups)
				  • Day 10: 0.10 (0.02); significant decrease, (p=0.01)
				• No significant decrease in L:M in glycine and nonsupplemented formula groups at day 10
				
				Mean lactulose (SE):
				• Glutamine group:
				  • Baseline: 0.97 (0.46) (similar in all three groups)
				  • Day 10: NS decrease in all 3 groups
				
				Mean mannitol (SE):
				• Glutamine group:
				  • Baseline: 3.42 (0.64) (similar in all three groups)
				  • Day 10: NS decrease in all 3 groups
				
				Proportion of stool markers at baseline among all subjects:
				• Lactoferrin: 53.3%
				• Leukocytes: 11.7%
				• RS: 3.3%
				• Occult blood: 5.0%
					L:M significantly improved in the glutamine group only.
					
					>50% of subjects had intestinal inflammation by stool lactoferrin. Fecal leukocytes, RS, and occult blood were detected in fewer subjects than lactoferrin.
						The relationship between stool markers and L:M was not reported.
						
						Data were not stratified by history of PD.
						
						Fecal fat was assessed, but results were not reported.
						
						Cut-off values for lactoferrin positivity were not described.
						
						Exclusively breastfed children were excluded from study participation due to assessment of stool lactoferrin.

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Type of mean not specified.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2007 Lima NL et al. Wasting and intestinal barrier function in children taking alanyl-glutamine-supplemented enteral formula L:M as a marker of intestinal permeability in assessing alanyl-glutamine supplement to improve intestinal barrier function in malnourished children compared to glycine-containing placebo supplement	Parque Universitario, Fortaleza, Brazil 6 mo-8 yr olds (mean age 3.5 yr) from an urban setting with HAZ, WAZ, or WHZ scores <-1.	RCT n=107; n=51 received alanyl-glutamine treatment n=56 received glycine placebo	Urine Tests: • **Lactulose**[1] • **Mannitol** • **L:M**	L:M median (range) at baseline: • Treatment: 0.0385 (0.8922 [sic]) • Placebo: 0.0302 (5.5812 [sic]) Lactulose and mannitol excretion both significantly decreased in the treatment group only (p=0.05 for both sugars)[2]. L:M did not change significantly within or across groups days after treatment. Lactulose excretion was not associated with WHZ, WAZ or HAZ scores in either group[3]. Mannitol was not associated with growth parameters in the control group, but was associated with WHZ (r^2=-0.386, p=0.027) and WAZ (r^2=-0.385, p=0.027) scores in the supplemented group. Data for L:M and growth parameter association was not provided.	Even though lactulose excretion improved in the treatment group, mannitol excretion worsened with overall L:M not changing. Lactulose, mannitol and L:M did not change significantly in the placebo group.	Authors state that L:M median and range values were within the confidence interval for values of healthy children in the study community; no reference was cited. Although the authors defined persistent and chronic diarrhea in their methods, they did not report data stratified according to these conditions. Authors provide negative R^2 values when reporting Pearson's correlation analysis results; these likely actually represent r values.
2001 Northrop-Clewes CA et al. Anthelmintic treatment of rural Bangladeshi children: effect on host physiology, growth, and biochemical status	Jamalpur district, northern Bangladesh 2-5 yr olds from poor rural villages, sampled randomly from a larger cohort study.	RCT* n=109; n=54 received bimonthly empiric antihelminthic treatment n=55 received	Blood Tests • α 1-antichymotrypsin • Albumin • Total protein Urine Test: **L:M**	Mean L:M[4] at baseline: • Treatment: 0.22 • Placebo: 0.25 Seasonal variation in L:M was observed, with highest values following the monsoon season. Within-subject L:M analysis showed	L:M ratios were high overall and demonstrated seasonal variation. Intra-individual L:M values did not change significantly over time, nor were they associated with	Baseline study data were lost, so analysis began with samples taken at month 2. The relationship between the serum markers and intestinal

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Reported results were adjusted for age and season.
[3] Reported results were adjusted for age and season.
[4] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
L:M as a marker of intestinal permeability, and α 1-antichymotrypsin as a marker of inflammation and immune activation to treatment among children randomized to bimonthly antihelminthics or placebo.	Stools were assessed for helminthiasis and giardiasis. Growth was followed longitudinally.	placebo * Randomized at the village level.	Among 93 subjects with L:M at baseline: • 46 received treatment • 47 received placebo Among 66 subjects with repeated L:M testing: • 34 received treatment • 32 received placebo	no significant association with intestinal helminthiasis and no significant improvement in treatment or placebo groups over 1 yr. L:M was generally not associated with giardiasis (with the exception of one group at one study interval). L:M was inversely correlated with ΔHAZ and ΔWAZ scores at some of the follow-up intervals (r=-0.22, p<0.02 and r=-0.21, p<0.05, respectively, at 12 mo follow-up visit). Mean serum ACT, albumin and total protein were within normal ranges and were not associated with growth parameters. ACT and albumin concentrations did not significantly change with treatment, whereas total protein concentrations did (p<0.001).	helminthiasis or consistently associated with giardiasis. Inverse correlations were seen between L:M and growth parameters. Serum markers were within normal range. The only significant change in these markers was a decrease in total protein in the treatment group without concomitant change in albumin; this suggested a decrease in globulins (not directly measured), perhaps due to decreased inflammation.	permeability was not reported.
2009 Panter-Brick C et al. Pathways leading to early growth faltering: An investigation into the importance of mucosal damage and immunostimulation in different socio-economic groups in Nepal	Kathmandu, Nepal 3-18 mo olds in two cohorts: 1. All children in target age range from four squatter settlements Randomly selected, age-matched cohort from lower middle-class, periurban	Cohort n=86; n=48 in squatter cohort n=38 in lower middle-class cohort	Urine Test: **Lactose:Cr** Blood Test: Hemoglobin	Mean[1] Lactose:Cr (CI): • Squatter: 0.14 (0.12, 0.16) • Middle Class: 0.08 (0.07, 0.10) • Statistically significant difference between the 2 groups among the 6-12 mo olds (p=0.007) and 18-24 mo olds (p=0.002), but not among 12-18 mo olds. • For both SES groups, Lactose:Cr values decreased with increasing age (p<0.001). HAZ, WAZ, WHZ, and ΔWAZ scores were strongly associated with mean	Authors speculate that Lactose:Cr accounted for less of the deterioration in nutritional status among the squatter children because of several factors, including poorer nutritional intake, that impact the nutritional status of children with lower socio-economic	Specific sugar excretion was normalized to urinary creatinine to control for variation in renal function. Authors suggest that while Lactose:Cr might not be as accurate as L:M, it might be a more field-friendly assessment of

[1] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Lactose:creatinine ratio (Lactose:Cr) as a marker of intestinal permeability and hemoglobin, albumin, α-1-acid glycoprotein, and IgG as markers of immunostimulation. The latter were also assessed for their relationship to nutritional status.	households			Lactose:Cr ($p<0.001$ each) as was ΔHAZ score ($p=0.004$); ΔWHZ score was not. The strength and magnitude of association between ΔWAZ score and Lactose:Cr was most pronounced among the wealthier cohort and there was no association between ΔHAZ score and Lactose:Cr among the squatter children. Hemoglobin concentrations were inversely related to Lactose:Cr ($r^2=0.018$, $p<0.001$).	status.	mucosal damage compared to L:M, requiring only spot urine collection and no substrate dosing. However, L:M was not assessed in this study; direct comparison of the two tests was not possible. While hemoglobin concentration was inversely related to Lactose:Cr, testing for associations of other measured blood markers (IgG, AGP and albumin) with Lactose:Cr was not reported.
2000 Quadro L et al. Retinol and retinol-binding protein: gut integrity and circulating immunoglobulins L:M as a marker of small intestinal permeability, and its correlation with serum retinol among mildly malnourished children	Goncalves Dias favela in Fortaleza, Brazil 1-9 yr olds with mild malnutrition selected from a large cohort of children from an urban slum. They were recruited at birth and followed longitudinally. 19 (63%) had some degree of vitamin A deficiency—all of	Cross-sectional n =30	Urine Tests: • **Lactulose**[1] • **Mannitol** • **L:M**	80% of subjects had abnormal L:M, defined as >=0.030. Serum retinol was: • Inversely correlated with L:M (r=0.46, p=0.012) • Directly correlated with mannitol (r=0.66, p<0.01) Not correlated with lactulose (data not reported)	Children with low serum retinol had higher L:M, apparently mediated by mannitol excretion.	The L:M normal cutoff was defined lower than for most other L:M studies, as 0.030. The authors reference several studies regarding use of this cut point.

[1] Lactulose and mannitol results were expressed as % of dose administered.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
	these had mild deficiency, except for 2 with moderately low concentrations.					
2004 Rabbani GH et al. Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea L:M as a marker of intestinal permeability among infants with PD who are treated with green banana, pectin, or rice diet	Dhaka, Bangladesh 5-12 mo old males admitted to the hospital of the International Centre for Diarrhoeal Disease Research with PD but without other concurrent illnesses.	RCT n=57; n=19 received green banana and rice n=17 received pectin and rice n=21 received rice alone (placebo)	Urine Tests: • **Lactulose**[1] • **Mannitol** • **L:M**	Mean L:M (SD) by treatment group, pre- and post-treatment: • Banana: pre=0.50 (0.14), post=0.21 (0.12), p<0.01 • Pectin: pre=0.54 (0.17), post=0.23 (0.09), p<0.01 • Placebo: pre=0.41 (0.11), post=0.45 (0.13), p>0.6 Lactulose and mannitol excretion did not differ between groups at baseline. Lactulose excretion was not significantly reduced after intervention in the placebo group. Mean (SD): • Pre-treatment: 1.45 (0.12) • Post-treatment: 1.35 (0.15) Both treatment groups had 70-80% reduced lactulose excretion following treatment (p<0.01). Mannitol excretion increased in all groups compared to their pre-treatment values, but only significantly so in the banana and pectin groups (p<0.05). Mean mannitol % excretion (SD), pre- vs. post-treatment: • Banana: 1.82 (0.13) vs. 3.21 (0.16) • Pectin: 1.91 (0.12) vs. 3.2 (0.18) • Placebo: 2.10 (0.11) vs. 2.33	L:M values were high at baseline among the study population of inpatient young children with PD. Mean L:M significantly improved with the green banana or pectin intervention but were still above normal range following 7 days of treatment. The improvements in L:M were driven by both mannitol and lactulose, with the latter having an impact of greater magnitude. Authors cite studies postulating that the effectiveness of green banana in reducing diarrheal fluid loss is due to its high content of amylase-resistant starch, which undergoes bacterial fermentation into short-chain fatty	

[1] Lactulose and mannitol results were expressed as % of dose administered.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				(0.18) The banana and pectin groups stopped having diarrhea more often compared to controls (e.g. p<0.01 by day 4). Among the banana and pectin groups, stool reductions were associated with percent change in L:M before and after treatment (R^2=0.84 for pectin and R^2=0.86 for banana; p<0.05 for each).	acids in the colon, stimulating colonic salt and water absorption. Pectin is thought to work by a similar mechanism. Authors also suggest that short chain fatty acids might affect entero-hormones and growth factors, resulting in the observed changes in intestinal permeability [206].	
2009 Ritchie BK et al. 13C-sucrose breath test: novel use of a noninvasive biomarker of environmental gut health Sucrose breath test (SBT) as a marker of small bowel mucosal damage vis a vis sucrase activity among an Australian Aboriginal population. Also compared SBT with serum lactulose:rhamnose ratio (L:R)	Darwin and Adelaide, Australia 4 mo-5 yr old Aboriginal children admitted to hospital with diarrhea. Two control groups: 1. Aboriginal controls admitted to hospital with non-GI symptoms (50% had pneumonia) 2. Healthy, non-Aboriginal controls recruited from community	Case-control n=43; n=18 Aboriginal cases with AD n=25 controls: • 18 Aboriginal, without diarrhea • 7 non-Aboriginal, healthy	Blood Tests: • **L:R** (32 Aboriginal cases and controls tested) • C-reactive protein (CRP) • Mean Corpuscular Volume (MCV) • Hemoglobin Breath Test: ^{13}C sucrose breath test (SBT)	20/32 (63%) of Aboriginal children had abnormal L:R ratios. Mean[1] L:R (CI): • Diarrhea cases: 31.8 (24.9, 40.7) • Aboriginal controls without diarrhea: 11.4 (8.5, 15.5), significant difference (p<0.0001) SBT Mean (CI): • Diarrhea cases: 1.9% (0.9, 3.0), p<0.0001 compared to non-Aboriginal controls and p=0.004 compared to Aboriginal controls • Aboriginal controls: 4.1% (3.0, 5.2), p=0.032 compared to non-Aboriginal controls • Non-Aboriginal controls: 6.1% (4.8, 7.3) Significant differences were observed between all three groups. SBT results were not associated with wasting or with patient age or breastfeeding status.	SBT values were significantly lower and L:R values were significantly higher among Aboriginal children with diarrhea than among those without GI symptoms. SBT was also significantly lower among Aboriginal controls than among non-Aboriginal children without diarrhea. This is consistent with previous reports of high prevalence of clinically silent TE in this population. SBT was significantly inversely correlated with L:R.	Abnormal L:R ratios were defined as >16; no reference or derivation was provided for this cut-point. L:R test was not conducted among the non-Aboriginal controls. SBT/L:R correlation analysis was based on data for Aboriginal cases and controls combined; stratified analysis was not reported and could be of interest considering the large difference in L:R observed between these

[1] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				SBT and L:R were inversely correlated (r=0.67; CI: 0.42, 0.62; p<0.0001). L:R explained 45% of the variance in SBT; diarrhea explained 28% of variance. SBT was associated with increased MCV, relative risk (CI)=3.9 (2.8, 5.0). SBT was not associated with hemoglobin or CRP.		groups. Associations of MCV, CRP, and hemoglobin with SBT after adjusting for potentially confounding variables were not reported.

2001
 	
Rollins NC et al.
 	
Feeding mode, intestinal permeability, and neopterin excretion: A longitudinal study in infants of HIV-infected South African women
 	
L:M as a marker of gut mucosal integrity and urinary neopterin excretion as a marker of cell-mediated immunity in infants with and without HIV infection
	Durban, South Africa
	
	1, 6, and 14 wk old infants born to HIV-infected mothers.
		Cohort
		
		n=272
			Urine Tests:
			• **Lactulose**[1]
			• **Mannitol**
			• **L:M**
			• Neopterin
				Mean[2] L:M (CI):
				• HIV-infected subjects:
				  • 1 wk: 0.12 (0.06, 0.27)
				  • 6 wk: 0.24 (0.15, 0.38)
				  • 14 wk: 0.24 (0.14, 0.44)
				• Uninfected subjects:
				  • 1 wk: 0.13 (0.09, 0.19)
				  • 6 wk: 0.08 (0.06, 0.11)
				  • 14 wk: 0.09 (0.07, 0.13)
				
				HIV-infection by 14 wk of age was significantly associated with increased L:M.
				
				A non-significant, positive trend in neopterin excretion was observed among HIV-infected infants.
					L:M was generally normal (compared to UK values) for non-HIV-infected infants, but significantly increased among HIV-infected subjects, especially after 6 weeks.
					
					The increased L:M in HIV-infected infants was primarily driven by lactulose rather than mannitol.
					
					Higher neopterin excretion by HIV-infected infants was observed but this was not statistically significant.
						Assessment of association between L:M and neopterin was not reported.

2000
 	
Rollins NC et al.
 	
Vitamin A
	Durban, South Africa
	
	6-60 mo old inpatients or
		RCT
		
		n=139;
		
		n=66 received
			Urine Tests:
			• **Lactulose**[3]
			• **Mannitol**
			• **L:M**
			• Neopterin
				Mean[4] L:M:
				• Group 1:
				  • Day 0: ~1.8
				  • Day 3: ~2.4
				• Group 2:
					Mean L:M ratios were very high (~10x) (at baseline and at day 3 in both groups) compared to	Urine testing could only be conducted in the laboratory on certain days; hence only a subset of

[1] Lactulose and mannitol results were expressed in mg.
[2] Geometric mean.
[3] For lactulose, mannitol, and neopterin results, excretion measurement was not specified.
[4] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
supplementation of South African children with diarrhea: optimum timing for improving biochemical and clinical recovery and subsequent vitamin A status L:M as a marker of intestinal permeability and urinary neopterin, serum α-1 acid glycoprotein, and C-reactive protein as markers of inflammation among children with severe diarrhea	outpatients with severe diarrhea.	vitamin A on admission (group 1) n=73 received vitamin A after clinical improvement (group 2) Treatment involved vitamin A supplementation either on the day of admission or after acute diarrheal symptoms had resolved.	<u>Blood Tests</u>: • C-reactive protein (CRP) • α-1 acid glycoprotein (AGP) 49 subjects received urine testing: • Group 1: n=25 • Group 2: n=24 Blood and urine were tested on days 0 and 3.	• Day 0: ~1.2 • Day 3: ~0.7 There were no differences in mean L:M between groups or within groups between days 0 and 3, although there was a significant difference in paired analysis within individuals at the two time points (data not presented, and direction, magnitude and degree of significance not reported). Lactulose and mannitol excretion were assessed only in the paired analysis. Lactulose excretion decreased between days 0 and 3 (magnitude of effect and degree of significance not reported), while mannitol excretion showed no change. Mean[1] neopterin and AGP concentrations did not differ between groups or within groups on the different study days or in the paired analysis. When initial CRP (~2x higher in Group 2 compared to Group 1, $p<0.004$) was taken into account, mean CRP on day 3 did not differ between the 2 groups. However in the paired analysis, CRP concentrations were significantly different between days 0 and 3.	other studies in this review. Study authors suggested (via personal correspondence) that this could have been due to the severity of illness in the sample population (children hospitalized for diarrhea). Vitamin A administration did not result in significant improvement in L:M, neopterin, or AGP regardless of timing of vitamin A administration.	subjects underwent those tests. Group 2 patients had significantly higher CRP, non-significantly higher WBCs and AGP, and lower retinol and retinol-binding protein concentration compared to group 1 at baseline. Authors note that these parameters suggest that Group 2 patients might have been more ill at baseline. For the subset of 49 patients undergoing urine testing, the mean L:M and neopterin concentrations were lower among Group 2 than Group 1 subjects (NS). However, baseline differences in acute phase and vitamin A markers at baseline were not reported separately for these 49 subjects. Data for lactulose and mannitol excretion were not

[1] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
						reported separately. Rationale for additional analyses of these molecules expressed as ratios with creatinine was not explained. Authors suggest that their 3-day testing period (based on their previous work in a different setting [207] might have been too short to identify effect as demonstrated by McCullough et al. at 10 days after presentation [208].
2000 Thurnham DI et al. Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants L:M as a marker of intestinal integrity among children receiving vitamin A supplementation	Orissa State, India Subjects were recruited from 2 sources: 1. Hospital-based infants admitted for "diarrheal or respiratory disease," mean age 9 mo 2. Clinic-based infants with "minor ailments", age not specified	RCT n=174; n=94 hospital-based • 31 received vitamin A at day 1 • 32 received vitamin A at discharge (up to day 5) • 31 received placebo n=80 clinic-based* Clinic-based subjects were	<u>Urine Test</u>: **L:M** For hospital-based subjects, L:M was assessed at baseline, discharge from hospital, and 10 or 30 days after discharge. For clinic-based subjects, L:M was assessed at baseline, 4, and 8 wk.	Mean L:M was ~3-fold higher among hospitalized compared to clinic patients at baseline. Within the allocation groups, mean baseline L:M did not differ for either the hospitalized or clinic subjects. Among the hospital cohort, mean L:M declined significantly in the two vitamin A groups compared to the placebo group, and remained lower at day 30 among the treatment groups, but the difference was no longer significant compared to the placebo group. Among the clinic cohort, mean L:M reduction was accelerated in vitamin A-supplemented children. However, mean L:M did not significantly differ between treatment groups at any time point.	Mean L:M values, including post-intervention values, were 2-5 times higher than those observed in the UK [209]. L:M reduction was accelerated among vitamin A-supplemented children, but end-of-study mean values did not differ statistically between allocation groups in either the clinic or the hospital cohorts.	Precise numerical values were not reported, rather L:M results were portrayed in figures with units expressed in mg, making it difficult to compare these results to those of other studies. Information on study design, such as randomization scheme, was limited. The article also reported re-analyzed data from a 1991 report from

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
		randomized to receive vitamin A weekly for 8 wk or placebo. * Number of subjects in each treatment group was not specified.		The rate of decline in L:M was most steep among the vitamin A-treated hospitalized patients, in whom the mean L:M value decreased by 63% over 30 days, followed by placebo-treated hospitalized patients, with a decrease of 38% over 30 days. Mean L:M decreased by 57% in the vitamin A-treated clinic patients, while there was no change in L:M among the clinic placebo group.		The Gambia [209].

2009
 	
Trehan I et al.
 	
A randomized, double-blind, placebo-controlled trial of rifaximin, a nonabsorbable antibiotic, in the treatment of tropical enteropathy
 	
L:M, sucrose:lactulose, and sucralose:lactulose as markers of small intestinal, gastric, and colonic permeability, respectively, among those receiving rifaximin or placebo
	Limela, Malawi
	
	All 3-5 yr olds from the village were recruited.
		RCT
		
		n=144;
		
		n=72 received rifaximin for 7 days
		
		n=72 received placebo
		
		It was presumed that if SBBO is the etiology for enteropathy, treatment with rifaximin would result in improved intestinal integrity.
			Urine Tests
			• **Lactulose**[1]
			• **Mannitol**
			• Sucrose (SUC)
			• Sucralose (SCL)
			• **L:M**[2]
			• Sucrose:lactulose ratio (SUC:L)
			• Sucralose:lactulose ratio (SCL:L)
			
			Subjects were tested before and after treatment.
				At enrollment:
				• Mean mannitol (SD):
				  • Treatment: 9.57 (5.24)
				  • Placebo: 10.29 (6.62)
				• Mean lactulose (SD):
				  • Treatment: 0.30 (0.18)
				  • Placebo: 0.34 (0.25)
				• Mean SUC (SD):
				  • Treatment: 0.062 (0.04)
				  • Placebo: 0.074 (0.058)
				• Mean SCL (SD):
				  • Treatment: 0.51 (0.29)
				  • Placebo: 0.58 (0.53)
				• Mean L:M (SD):
				  • Treatment: 0.18 (0.12)
				  • Placebo: 0.17 (0.09)
				• Mean SUC:L (SD):
				  • Treatment: 0.50 (0.34)
				  • Placebo: 0.64 (0.90)
				• Mean SCL:L (SD):
				  • Treatment: 0.42 (0.32)
				  • Placebo: 0.39 (0.23)
				• For both groups combined:
				  • 76% had L:M >0.10
				  • 34% had L:M >0.20
				
				No significant post- intervention
					There was a high proportion with elevated L:M which did not change with rifaximin treatment.
					
					Baseline L:M measurements in this study resembled those of another Malawian population in similar environmental conditions [120].
					
					SCL excretion in this population was similar to that found in healthy American children (0.4%), while SCL:L was comparatively lower (0.8) and driven by lactulose [210]. SCL:L might be a better marker of colonic permeability [211-213]. Results
						Methodological differences in specimen collection and testing, in particular for SCL excretion, might account for some differences in values compared to other studies.
						
						This was the first use of SCL for site-specific absorption testing in a developing country setting.

[1] Lactulose, mannitol, SUC, and SCL results were expressed as % of dose administered.
[2] Type of mean for sugar ratios not specified.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				differences were observed in any fractional sugar excretion or dual sugar test, including among children with elevated pre-intervention L:M.	from this study potentially indicate that colonic permeability was normal. Few data exist on SUC excretion. Results in this trial are similar to those found in another Malawian population (0.06% SUC excretion) [120] and high compared to healthy older children from developed country settings (0.02-0.03%) [210, 212].	
2008 Vieira MM et al. Carotenoids, retinol, and intestinal barrier function in children from northeastern Brazil L:M as marker of intestinal barrier function, fecal lactoferrin and leukocytes as markers of intestinal inflammation, and CRP and AGP as acute phase reactants among children with varying vitamin A	Fortaleza, Brazil 2 mo-9 yr olds (mean age 41 mo) from an impoverished urban community, eligible if HAZ score <median for their community.	Cross-sectional n=102	Urine Tests: • **Lactulose**[1] • **Mannitol** • **L:M** (97 tested) Stool Tests: • Lactoferrin (93 tested) • Leukocytes Blood Tests: • C-reactive protein (CRP) • α-1-acid glycoprotein (AGP)	48.5% had abnormal L:M. L:M and excretion of each sugar separately did not vary with retinol concentration. L:M was associated with levels of common dietary carotenoids, primarily driven by lactulose. However, the association was not always statistically significant, and the direction of association varied depending on precursor. 40% of stool samples were positive for lactoferrin. 1% of stool samples were positive for fecal leukocytes. 30% of stool samples were positive	Almost half of subjects had increased L:M, and ~40% of subjects had increased lactoferrin. While serum retinol concentrations were not associated with L:M, serum carotenoids were; authors suggest that these retinol precursors might be more sensitive predictors of impaired intestinal function. However, the reported direction of	L:M threshold for abnormal values was defined as ≥0.0864 [214]. Cut-off values for lactoferrin positivity were not described. Relationships between acute phase proteins and measures of intestinal permeability or inflammation were not reported. Relationships between L:M and lactoferrin or fecal leukocytes as well as those between

[1] Lactulose and mannitol results were expressed as % of dose administered.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
status				for parasites but this had no impact on L:M results, lactoferrin, or acute phase reactants.	association varied, making interpretation of these results unclear.	retinol or carotenoids and lactoferrin or fecal leukocytes were not reported. Exclusively breastfed children were excluded from study participation due to assessment of stool lactoferrin.

2007
 	
Williams EA et al.
 	
A double-blind, placebo-controlled, glutamine-supplementation trial in growth-faltering Gambian infants
 	
L:M and plasma immunoglobulins and acute phase reactant proteins (albumin, C-reactive protein, and alpha-1-antichymotrypsin) in community-based Gambian infants enrolled in a glutamine trial
	West Kiang region, Gambia
	
	4-10 mo olds from a rural area followed during the 5-month rainy season and for 6 months afterward.
		Cohort
		
		n=72
		
		Glutamine or placebo of nonessential amino acids was orally administered twice daily during rainy season; L:M ratio was measured monthly, and plasma samples were collected 3 times.
			<u>Urine Tests</u>:
			• **Lactulose**[1]
			• **Mannitol**
			• **L:M**
			
			<u>Blood markers</u>
			• C-reactive protein (CRP)
			• Alpha-1 antichymotrypsin (ACT)
			• IgA
			• IgG
			• IgM
			• Albumin
				Mean[2] L:M (CI):
				• Baseline:
				  • Glutamine group: 0.33 (0.25, 0.43)
				  • Placebo group: 0.33 (0.26, 0.41)
				• Post-intervention:
				  • Glutamine group: 0.29 (0.23, 0.35)
				  • Placebo group: 0.26 (0.21, 0.32)
				
				Mean excretion of lactulose (CI):
				• Baseline:
				  • Glutamine group: 0.21 (0.16, 0.28)
				  • Placebo group: 0.20 (0.15, 0.26)
				• Post-intervention:
				  • Glutamine group: 0.17 (0.13, 0.21)
				  • Placebo group: 0.14 (0.11, 0.18)
				
				Mean excretion of mannitol (CI):
				• Baseline:
				  • Glutamine group: 2.65 (2.02,
					L:M values were elevated in this population, with no significant change after the intervention.
					None of the plasma markers differed significantly between treatment and placebo groups, either at baseline or at the end of supplementation.
					Growth outcomes did not differ significantly across treatment groups.
						The relationships between L:M and growth parameters, immuno-globulins, and acute phase proteins were not reported.

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Geometric mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				3.48) • Placebo group: 2.50 (1.87, 3.36) • Post-intervention: • Glutamine group: 2.48 (1.99, 3.11) • Placebo group: 2.14 (1.62, 2.82) L:M values did not differ significantly between treatment groups before or following intervention. However, a repeated measures ANOVA showed that during supplementation, L:M values were borderline elevated among the glutamine-supplemented group relative to the placebo group (p=0.05), counter to expectation. Neither ACT, CRP, albumin, nor immunoglobulins IgA, IgG, or IgM differed significantly between treatment and placebo groups, either at baseline or at the end of supplementation. Mean levels of IgA and IgG increased during the study (p <0.001), while IgM levels did not. Concentrations of each of these immunoglobulins did not differ between treatment and placebo groups. Plasma albumin, ACT, and CRP values showed no change over the course of the study. Proportions of children with elevated CRP ranged from 30-41% at different collection time points. The glutamine intervention had no effect on proportion of children with		

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				elevated CRP. Treatment and placebo groups experienced decreases in WAZ, HAZ, and MUAC coinciding with the rainy season; however, there was no significant difference observed between the groups for any of these parameters. Treatment and placebo groups did not differ in morbidity indices (i.e. percentage of time reported with a particular illness or illness overall).		
2000 Willumsen JF et al. Subclinical mastitis as a risk factor for mother-infant HIV transmission L:M as a marker of infant intestinal permeability and its relationship with subclinical maternal mastitis	Durban, South Africa HIV-infected breastfeeding mothers and their infants followed up to 14 wk of age. Women recruited from antenatal clinic via a vitamin A supplementation trial to reduce mother-to-child HIV transmission.	Cross-sectional analysis of baseline data prior to randomization for an RCT n=104 mothers n=108 infants (4 pairs of twins), (26 were HIV-infected by 3 mo of age)	<u>Urine Test:</u> **L:M**	There was no significant association between L:M and subclinical mastitis as measured by milk Na/K.	Subclinical mastitis was not associated with magnitude of L:M.	Actual L:M values were not reported but are found in a companion study, also included in this review [119].The study group in Willumsen, et al. represents a subsample of the study population reported in the companion study.
2000 Zhang Y et al. Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and *Cryptosporidium*	Lima, Peru 0-36 mo olds with watery diarrhea admitted to oral rehydration unit of hospital.	Case-control n=36; n=29 cases: • 15 with *C. parvum* alone • 7 with rotavirus alone • 7 with bacteria (alone or with	<u>Urine Test</u>: **L:M** Enrollment and convalescent (at day 20) L:M ratios were assessed.	Mean[1] L:M (SE) at day 1, day 20: • Rotavirus only: 0.67 (0.38), 0.19 (0.09) • Cryptosporidium only: 0.76 (0.43), 0.28 (0.14) • Bacterial infection: ranged from 0.2-0.87, 0.11-0.99 • Unknown etiology: 0.26 (0.12), 0.29 (0.18) Mean L:M ratios significantly	L:M ratios were significantly elevated in children with rotavirus or *Cryptosporidium* infection compared to those with diarrhea not caused by rotavirus, *Cryptosporidium*, or identifiable bacteria.	

[1] Arithmetic mean.

Evidence Table 2. Markers of permeability.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
L:M as a marker of intestinal permeability in children with diarrhea		rotavirus or *Cryptosporidium*) n=7 controls with unknown etiology		differed between the unknown etiology and both the rotavirus ($p<0.01$) and *Cryptosporidium* groups ($p<0.05$) at baseline, but not at day 20. Mean L:M ratios decreased between baseline and day 20 for both the rotavirus ($p<0.001$) and *Cryptosporidium* ($p<0.05$) groups. Among the group of subjects with enteric bacterial infections, the causative agents identified and mean L:M ratios (baseline, day 20) were: *Campylobacter jejuni* and rotavirus infection (0.86, 0.18), *C. jejuni* and *Cryptosporidium* infection (0.87, 0.53), *Salmonella* sp. (0.2, 0.11), *C. jejuni* (0.69, 0.99), and *Aeromonas* sp. (0.38, 0.11). The L:M ratios of this group of seven infants were not included in the statistical analyses.	Mean L:M did not change significantly among those with diarrhea of unknown etiology, but did significantly decrease among those infected with rotavirus or *Cryptosporidium*, reaching ratios similar to those with diarrhea of unknown etiology.	

Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting.
Further details on L:M studies can be found in Table 19.

Abbreviations: AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, ∆=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)

5.3.1 The Urinary Lactulose:Mannitol Ratio (L:M)

The urine L:M test is the permeability test that has been most extensively used to measure gut function in children in the literature identified for this review. This noninvasive test involves oral administration of a dose of both sugars (i.e., lactulose and mannitol), followed by a timed urine collection. As reviewed above, lactulose is a large sugar (a disaccharide) that is minimally absorbed from an intact small intestine. However, if permeability is altered, this disaccharide traverses intracellular spaces of the gut, is then cleared by glomerular filtration without renal tubular reabsorption, and becomes measurable in the urine. Mannitol is a sugar alcohol that is absorbed (via transcellular pathways) proportional to the small bowel absorptive capacity (i.e. surface area). Shortened microvilli diminish uptake and subsequent urinary excretion of mannitol, which, like lactulose, is filtered and not reabsorbed.

5.3.1.1 Technical Issues with the L:M Test

Prior to delving into the results of the L:M tests, it is important to note that all of the biomarkers examined in this review have particular and general performance considerations, perhaps none more so than the L:M test. These considerations for the L:M test are summarized in Table 14, which demonstrates the tremendous variability in methods, citation of methods, ranges of purported normal values of abnormal cutoff points, and the way that results were reported across the 25 studies that we reviewed that used L:M as a marker.

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2004 Campbell DI et al. Intestinal inflammation measured by fecal neopterin in Gambian children with enteropathy	2 mo olds from a rural area followed until 15 mo of age.	Not specified	1 hr after loading dose	A	5 hr	Enzymatic assay per Northrop CA et al. [215] and Lunn PG et al. [216]	Mean, type not specified, (CI)	
Normal value cut points[6]: <0.10 based on UK norm. Campbell DI et al. cited Travis & Menzies et al. [201] but it was unclear if the citation was related to normal values for lactulose and mannitol individually or also for L:M. Travis & Menzies did not report primary L:M data, but cited a mean (type not specified) of 0.016 (SEM 0.002), per Juby et al. [217], as normal.								
2003 Campbell DI et al. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function	2-11 mo olds from a rural village followed up to 14 mo of age.	Not specified	2 hr after loading dose	A	5 hr	Enzymatic assay per Northrop CA et al. [215] and Lunn & Northrop-Clewes [218]	Geometric mean (CI, range)	Gambian children [209]
Normal value cut points: Based on UK norms, value was not specified, per Freeman JV et al. [219], Weaver [220], Lunn PG et al. [202], and Lunn [52]. Freeman JV et al. [219] reported no data relevant to L:M. Weaver reported data for breastfed English infants and a derived median L:M from this data of ~0.2. Lunn PG et al. [202] reported a mean (SEM) L:M for UK 3-15 mo olds of 0.12 (0.02) based on "data from [their] laboratory", but did not portray the data or cite another reference. Text suggests this was a geometric mean, but this was not clearly stated. Lunn [52] described L:M patterns, but not specific values.								

[1] Some titles are abbreviated.
[2] A = 400 mg/kg of lactulose, 100 mg/kg of mannitol, B = not reported, C = 4 g lactulose, 1 g mannitol, D = 5 g lactulose, 1 g mannitol, E = 200 mg/kg of lactulose, 50 mg/kg of mannitol, F = 400 mg/kg of lactulose, 100 mg/kg of mannitol, G = 400 mg/kg of lactulose (maximum 4 g), 100 mg/kg of mannitol (maximum 1 g), H = 500 mg/kg of lactulose (maximum 5 g), 100 mg/kg of mannitol (maximum 1 g).
[3] Where authors cited references for these parameters, they are provided.
[4] Where authors cited references for these parameters, they are provided.
[5] Other than studies presented within the "Normal value cut points" row.
[6] Where authors cited references for normal value cut-points, they are provided along with the relevant information from those citations.

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2003 Campbell DI et al. Chronic T cell-mediated enteropathy in rural west African children: relationship with nutritional status and small bowel function	6 mo-3 yr old malnourished in- and out-patients from rural areas. Compared to well-nourished UK children with GI complaints other than diarrhea.	Not specified	Not reported	B	5 hr	Enzymatic assay per Sullivan PB et al. [221] and Lunn PG et al. [209]	Not clearly indicated	Asymptomatic Gambian children [209]
Normal value cut points: <0.10 based on UK norms, per Fagundes-Neto U et al. [17] and Beattie RM et al. [222]. We were unable to obtain the article by Fagundes-Neto U et al. to verify. No relevant L:M data were found in Beattie RM et al.								
2002 Campbell DI et al. Age-related association of small intestinal mucosal enteropathy with nutritional status in rural Gambian children	2-60 yr olds from rural communities.	Not specified	Not reported	C	5 hr	Enzymatic assay per Northrop CA et al. [215], and Lunn & Northrop-Clewes [218]	Mean, type not specified, (SE)	Adults in UK & tropics [57] and previous results in Gambian & UK subjects [202]
Normal value cut points: <0.10 based on UK norm per Freeman JV et al. [219] and Travis & Menzies [201]. Freeman JV et al. reported no relevant data. Travis & Menzies cited a mean (type not specified) L:M ratio of 0.016 (SEM 0.002), per Juby LD et al. [217], as normal.								
2003 Chen P et al. Association of vitamin A and zinc status with altered intestinal permeability	2-97 mo olds from an urban shantytown.	Not specified	Not reported	D	5 hr	HPLC per Barboza MS et al. [214]	Mean, type not specified (SD)	
Normal value cut points: Not reported.								

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2007 Darboe MK et al. Effectiveness of an early supplementation scheme of high-dose vitamin A versus standard WHO protocol in Gambian mothers and infants	0-12 mo olds from a rural community.	Inclusion: successfully obtained cord blood sample Exclusion: birth weight <2.2 kg, <37 wk gestation, congenital anomalies or severe peripartum illness	Not reported	E	5 hr	Enzymatic assay per Lunn PG et al. [209]	Geometric mean	
Normal value cut points: <0.30, authors derived this from Lunn PG et al. [202] UK infants' mean plus 2 SD. Lunn PG et al. stated a mean (type not specified) (SE) of 0.12 (0.02) based on "most recent data from this laboratory", data not shown or cited.								
2001 Filteau SM et al. The effect of antenatal vitamin A and (beta)-carotene supplementation on gut integrity of infants of HIV-infected South African women	Infants of HIV-infected women recruited from antenatal clinic and followed until 14 wk of age.	Not specified	Not reported	A	5 hr	Enzymatic assay per Lunn PG et al. [209] and Willumsen JF et al. [207]	Geometric mean (CI)	
Normal value cut points: Based on norms, values not specified, per Catassi C et al. [223] and Lunn PG et al. [209]. Catassi C et al. reported data from 72 Italian infants. Their L:M values were calculated in two ways: 1) absolute ratio of lactulose and mannitol in mg/dl with L:M mean (type not specified) (SD) ranging from 1.27 (0.73)-0.22 (0.21) and 2) ratio of lactulose and mannitol as percent dose ingested with L:M mean (type not specified) (SD) of 0.09 (0.08). Lunn PG et al. stated that UK infants' norm as a mean (type not specified) (SD) of 0.12 (0.09), but did not portray the related data or cite another reference for this UK norm.								

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2005 Galpin L et al. Effect of Lactobacillus GG on intestinal integrity in Malawian children at risk of tropical enteropathy	36-60 mo olds from a rural community.	Exclusion: evidence of severe acute malnutrition or severe chronic illness	Overnight	D	3 hr	Cation-exchange column and refractometer by modified method of Catassi C et al. [224] and Shulman RJ et al. [225]	Arithmetic mean (SD)	
Normal value cut points: <0.10 based on developed world norms between 0.03-0.12, per Goto R et al. [205], van Elburg RM et al. [226], Campbell DI et al. [112]. Goto R et al. reported data based on 158 Guatemalan infants aged 0-11 mo without diarrhea in preceding week; the L:M median ranged from 0.41-0.54. They also used 0.07 as a cut point for normal L:M based on another reference by Ford RP et al. [227] which did not report data relevant to L:M. van Elburg RM et al. reported L:M based on 30 Dutch 0-16 yr olds. L:M was calculated in two ways: 1) absolute ratio of lactulose and mannitol in mmol/mol creatinine with L:M mean (type not specified) (SD) 0.043 (0.045) and 2) ratio of lactulose and mannitol as percent dose ingested with L:M mean (type not specified) (SD) 0.034 (0.033). Campbell DI et al. reported mean (type not specified) (SE) L:M of 0.353 (0.022) based on 26 Gambian 2-5 yr olds without diarrhea. They also reference UK norms but do not report specific values (see above).								
2008 Goto R et al. Impact of anti-*Giardia* and anthelminthic treatment on infant growth and intestinal permeability in rural Bangladesh	3-15 mo olds from a rural community followed over 9 mos. Malnutrition was prevalent.	Not specified	Not reported	A	Usually 3 hr, but up to 5-6 hr if no urine output within 3 hr per Akram S et al. [228]	Enzymatic assay per Lunn PG et al. [216] and Northrop CA et al. [215].	Geometric mean (SD)	Gambian and Bangladeshi subjects per Lunn PG et al. [209] and Rousham EK et al. [229]
Normal value cut points: < upper CI for UK infants, value not specified, per Lunn PG et al. [209]. Lunn PG et al. stated that UK infants' norm as a mean (type not specified) (SD) of 0.12 (0.09), but did not portray the related data or cite another reference for this UK norm.								

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Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2008 Goto R et al. Impact of intestinal permeability, inflammation status and parasitic infections on infant growth faltering in rural Bangladesh	3-15 mo olds from a rural community followed over 9 mos. Malnutrition was prevalent.	Not specified	1 hr before load	A	5 hr for first exam. Reduced to 3 hr subsequently if subject voided within first 90 minutes per Akram S et al. [228]	Enzymatic assay per Lunn PG et al. [216] and Northrop CA et al. [215]	Geometric mean, mean log L:M[7] (SD reported for logged value)	Gambian and Bangladeshi subjects per Lunn PG et al. [209] and Rousham EK et al. [229]
Normal value cut points: Geometric mean 0.12 for UK infants per Lunn PG et al. [209]. Lunn PG et al. stated that UK infants' norm as a mean (type not specified) (SD) of 0.12 (0.09), but did not portray the related data or cite another reference for this UK norm.								
2002 Goto R et al. Poor intestinal permeability in mildly stunted Nepali children: Associations with weaning practices and *Giardia lamblia* infection	0-5 yr olds from two urban squatter settlements. Malnutrition was prevalent.	Not specified	1 hr after load (with the exception of breastfeeding)	A	5 hr	Enzymatic assay per Lunn PG et al. [216]; Northrop CA et al. [215]; Blood et al. [230]; Lunn & Northrop-Clewes [218]	Geometric mean (range, geometric SE[8])	Bangladeshi subjects per Northrop-Clewes CA et al. [150] and Rousham EK et al. [229]. Gambian subjects per Lunn PG et al. [202] and Behrens RH et al. [231]. Guatemalan subjects[9] per Goto R et al. [205]
Normal value cut points: <0.12 based on UK geometric mean per Lunn PG et al. [202]. Lunn et al. stated that UK infants' norm as a mean (type not specified) (SD) of 0.12 (0.09), but did not portray the related data or cite another reference for this UK norm.								

[7] Authors present positive log L:M values, however, logged values would be expected to be negative for ratios between 0-1 as is the case for L:M ratios.
[8] Authors calculated the geometric standard error using the formula: antilog (mean + standard error of logged values) - geometric mean.
[9] For this study, medians were reported rather than means.

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2010 Lima AA et al. Effects of vitamin A supplementation on intestinal barrier function, growth, total parasitic, and specific *Giardia* spp infections in Brazilian children	2 mo-9 yr olds from an impoverished urban community.	Inclusion: HAZ score <community median Exclusion: exclusively breast-fed, participant in another study in past 2 yr, or febrile illness at enrollment	Not reported	F	Per Barboza MS et al. [214]	Per Lunn PG et al. [216] and Northrop CA et al. [215]	Median (range[10])	
Normal value cut points: Not reported.								
2005 Lima AA et al. Intestinal barrier function and weight gain in malnourished children taking glutamine-supplemented enteral formula	2-60 mo olds hospitalized with malnutrition, ~70% of whom had PD.	Inclusion: history of PD or WAZ score <-2 and consent to 10-day hospital stay Exclusion: exclusively breast-fed, intolerant to cow's milk, participant in another study in past 2 yr, illness at enrollment	3 hr before load	G	5 hr	HPLC per Barboza MS et al. [214]	Mean, type not specified, (SE)	
Normal value cut points: Not reported.								

[10] Authors used the term "range," but for some of the L:M medians, only one value was reported, apparently the maximum L:M value observed.

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2007 Lima NL et al. Wasting and intestinal barrier function in children taking alanyl-glutamine-supplemented enteral formula	6 mo-8 yr olds from an urban setting with HAZ, WAZ, or WHZ scores <-1.	Exclusion: exclusively breast-fed, participant in another study in the past 2 yr, sibling enrolled in this study, chronic or severe or febrile illness at enrollment	3 hr before load	D	5 hr	HPLC per Barboza MS et al. [214]	Median (range[i])	
Normal value cut points: Within the CI for values of healthy children in the community, values were not reported and no reference was provided.								
2001 Northrop-Clewes CA et al. Anthelmintic treatment of rural Bangladeshi children: effect on host physiology, growth, and biochemical status	2-5 yr olds from poor rural villages.	Not specified	2 hr before and after load	C	5 hr	Enzymatic assay per Northrop CA et al. [215], and Lunn & Northrop-Clewes [218]	Geometric mean, mean log L:M (SE reported for logged value)	Bangladeshi subjects per Northrop CA et al. [232]
Normal value cut points: <~0.15 based on UK norm per Lunn PG et al. [209]. Lunn PG et al. stated that UK infants' norm as a mean (type not specified) (SD) of 0.12 (0.09), but did not portray the related data or cite another reference for this UK norm.								
2000 Quadro L et al. Retinol and retinol-binding protein: gut integrity and circulating immunoglobulins	1-9 yr olds with mild malnutrition from an urban slum. Vitamin A deficiency was prevalent.	Inclusion: no sign of infection or other obvious illness	Overnight	D	5 hr	HPLC per Bao Y et al. [233]	None reported	
Normal value cut points: <0.030 based on norms per Lima AA et al. [234], Deitch [235], Ukabam & Cooper [236],Wyatt J et al. [237], Pearson AD et al. [238], and Ford RP et al. [227] Lima AA et al. reported mean L:M (type not specified) (SE) of 0.017 (0.002) based on data from 13 Brazilian adults without HIV infection. Deitch reported mean L:M from 8 healthy American adult controls. Mean L:M was 1.7; SE was only presented graphically. L:M was expressed as lactulose/mannitol x 100 and the ratio was calculated as units rather than % dose administered; type of mean was not otherwise specified. Baseline values of mannitol (from pre-test samples) were subtracted from test samples. Ukabam & Cooper reported separate lactulose and mannitol excretion means for 25 healthy adult controls, presumably from the UK based on address of corresponding author. They did not report data on the L:M ratio. Wyatt J et al. reported mean (type not specified) L:M (SE) of 0.018 (0.002) based on data from 30 healthy adults (presumably in Austria based on corresponding author's address.) They defined a normal cutoff of 0.030 based on mean L:M + 2 SD. Pearson AD et al. reported mean L:M (type not specified) (range) of 0.018 (0.005-0.028) based on data from 31 healthy 2-13 yr olds (presumably in UK based on corresponding author's address.) Ford RP et al. reported no data relevant to L:M.								

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2004 Rabbani GH et al. Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea	5-12 mo old males admitted to hospital with PD but without other concurrent illnesses.	Inclusion: male, PD Exclusion: concurrent illness	3 hr before load	D	5 hr	Enzymatic assay per Behrens et al. [239], and Yamanaka [240]	Mean, type not specified (SD)	

Normal value cut points: Based on norms, value not specified, per Lunn PG et al. [209], Barboza MS et al. [214], Behrens RH et al. [231], Roy SK et al. [241], Ford RP et al. [227]. Lunn PG et al. stated that UK infants' normal values are: mean (type not specified) (SD) 0.12 (0.09), but did not portray the related data or cite another reference for this UK value. Barboza MS et al. reported mean L:M (type not specified) (SD) of 0.0394 (0.0235) based on 15 Brazilian under-5s from a low SES area without diarrhea in the preceding 2 weeks. Behrens RH et al. reported mean L:M (type not specified) (+/-2SD) of 0.42 (0.2, 1.4) and 0.52 (0.2, 2.2) based on 255 tests on 60 healthy Gambian 0-18 mo olds with HFA >80% of NCHS median and 45 tests on 15 Gambian 0-18 mo olds with HFA between 60-80% of NCHS median. Roy SK et al. reported geometric mean L:M (CI) of 0.13 (0.1, 0.16) based on 53 asymptomatic Bangladeshi controls. Ages of controls were not described but diarrheal cases were aged 3-24 mo. Ford RP et al. reported no data relevant to L:M.

2001 Rollins NC et al. Feeding mode, intestinal permeability, and neopterin excretion: A longitudinal study in infants of HIV-infected South African women	1, 6, and 14 wk old infants born to HIV-infected mothers.	Not specified	Not reported	A	5 hr	Enzymatic assay per Willumsen JF et al. [207]	Geometric mean (CI)	

Normal value cut points: Based on norms, value not specified, per Catassi C et al. [223] and Behrens RH et al. [231]. Catassi C et al. reported mean L:M based on data from 72 Italian infants. Their L:M values were calculated in two ways: 1) absolute ratio of lactulose and mannitol in mg/dl with L:M mean (type not specified) (SD) ranging from 1.27 (0.73)-0.22 (0.21) and 2) ratio of lactulose and mannitol as percent dose ingested with L:M mean (type not specified) (SD) of 0.09 (0.08). Behrens RH et al. reported mean L:M (type not specified) (+/-2SD) of 0.42 (0.2, 1.4) and 0.52 (0.2, 2.2) based on 255 tests on 60 healthy Gambian 0-18 mo olds with HFA >80% of NCHS median and 45 tests on 15 Gambian 0-18 mo olds with HFA between 60-80% of NCHS median.

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2000 Rollins NC et al. Vitamin A supplementation of South African children with diarrhea: optimum timing for improving biochemical and clinical recovery and subsequent vitamin A status	6-60 mo old in- or out-patients with severe diarrhea. Intestinal permeability tests could be performed only for a subset of inpatients.	Exclusion: children with dysentery, metabolic acidosis, circulatory impairment, severe electrolyte disturbances, or inability to take oral fluids	Not reported	A	5 hr	Enzymatic assay per Willumsen JF et al. [207]	Geometric mean (CI)	
Normal value cut points: Not reported.								
2000 Thurnham DI et al. Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants	Inpatients with diarrheal or respiratory disease, mean age 9 mos. And out-patients with "minor ailments", age not specified.	Not specified	Not reported	B	5 hr	Not specified per Lunn PG et al. [209]	Mean, type not specified (SE)	
Normal value cut points: <0.12 based on UK norm per Lunn PG et al. [209]. Lunn PG et al. stated that UK infants' norm as a mean (type not specified) (SD) of 0.12 (0.09), but did not portray the related data or cite another reference for this UK norm.								
2009 Trehan I et al. A randomized, double-blind, placebo-controlled trial of rifaximin, a nonabsorbable antibiotic, in the treatment of tropical enteropathy	3-5 yr olds from a rural village.	Exclusion: chronic debilitating illness, congenital abnormalities, or severe acute malnutrition	Overnight	D	4 hr	HPLC per Shulman RJ et al. [212] and Scarpignato C et al. [242]	Mean, type not specified (SD)	
Normal value cut points: <0.10 based on norms of 0.03-0.12 per Campbell DI et al. [112], Goto R et al. [205], and Galpin L et al. [120]. Campbell DI et al. reported mean (type not specified) (SE) L:M of 0.353 (0.022) based on 26 Gambian 2-5 yr olds without diarrhea. They also reference UK norms but do not report specific values (see above). Goto R et al. reported L:M values based on 158 Guatemalan infants aged 0-11 mo without diarrhea in preceding week. L:M median ranged from 0.41-0.54. They also used 0.07 as a cut point for normal L:M based on another reference by Ford RP et al. [227] which did not report data relevant to L:M. Galpin L et al. reported arithmetic mean (SD) L:M of 0.18 (0.16) and 0.22 (0.20) based on 2 groups of 80 and 81 healthy Malawian 3-5 yr old children, respectively.								

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2008 Vieira MM et al. Carotenoids, retinol, and intestinal barrier function in children from northeastern Brazil	2 mo-9 yr olds from an impoverished urban community.	Inclusion: HAZ score <community median Exclusion: exclusively breast-fed, participants in another study in past 2 yr or febrile illness at enrollment	1 hr after load, fasted prior as well, but duration not specified.	H	5 hr	HPLC per Bao Y et al. [233], Lima, et al. [234], and Barboza MS et al. [214]	Mean (SD); however, only the proportion of normal values was reported rather than the mean	
Normal value cut points: <0.0864, calculated as mean + 2SD based on data from Barboza MS et al. [214]: mean L:M (type not specified) (SD) of 0.0394 (0.0235) based on 15 Brazilian under-5s from low SES area without diarrhea in preceding 2 weeks.								
2007 Williams EA et al. A double-blind, placebo-controlled, glutamine-supplementation trial in growth-faltering Gambian infants	4-10 mo olds from a rural area followed during the 5-month rainy season and for 6 months afterward	All age-eligible children in the study villages were included	None pre-dose (stating ethical considera-tions); 2 hr post-dose (exception of water allowed)	F	5 hr	Not specified. Williams et al. cited Travis and Menzies [201] generally regarding L:M assessment of intestinal permeability; it was unclear which aspects of Travis and Menzies' methods were being referenced.	Geometric mean, (CI)	
Normal value cut points: Not reported.								
2000 Willumsen JF et al. Subclinical mastitis as a risk factor for mother-infant HIV transmission	Infants of HIV-infected breastfeeding mothers, followed up to 14 wk of age.	Inclusion: breastfeeding	Not reported	B	Not specified	Enzymatic assay per Willumsen JF et al. [207]	None reported	
Normal value cut points: Not reported.								

Table 14. Comparisons of L:M test study methods and reporting frameworks.

Reference[1]	Population	Exclusion/ Inclusion Criteria	Fasting	Loading Doses of Lactulose and Mannitol[2]	Urine Collection Post Loading Dose Time Interval[3]	Laboratory Analysis Method[4]	Central Tendency Measure for L:M	Comparisons to Other Studies[5]
2000 Zhang Y et al. Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and *Cryptosporidium*	0-36 mo olds with watery diarrhea admitted to a hospital oral rehydration unit.	Inclusion: watery diarrhea and stool positive for rotavirus or *Cryptosporidium* Exclusion: dysentery, history of renal disease, or current antibiotic use	Not reported	A	5 hr	Enzymatic assay and spectrophotometer.	Arithmetic mean (SE). Also reported raw data allowing for further analysis by the reader.	

Normal value cut points: Not reported.

Abbreviations: AIDS=Acquired immune deficiency syndrome, CI=95% confidence interval, GI=Gastrointestinal, HAZ=Height-for-age Z (score), HFA=Height-for-age, HIV=Human immunodeficiency virus, HPLC=High performance liquid chromatography, hr=Hour(s), L=Lactulose, L:M=lactulose:mannitol, M=Mannitol, mo=Month(s), PD=Persistent diarrhea, SD=Standard deviation, SE=Standard error, TB=Tuberculosis, UK=United Kingdom, WAZ=Weight-for-age Z (score), WFA=Weight-for-age, WHZ=Weight-for-height Z (score), wk=Week(s), yr=Year(s)

An important initial methodological consideration is that administration of a loading dose is required; dosing among the studies we reviewed varied. Ideally, the dosing should be related to body mass, or at least to body weight, in view of the large range in body size and composition of the subjects to whom it is administered. Such dosing was reported to have been performed in only 13 of the 25 L:M studies we reviewed. It should be noted that either or both of these molecules can cause diarrhea via their hyperosmolar properties, and that sometimes the load causes vomiting. These side effects, although not serious, might be more prominent among children with certain characteristics thus skewing results. Indeed, the hyperosmolar effect is proposed to potentially interfere with absorption by causing solvent drag (retention of the measured solute in the gut), and has prompted consideration of a liquid meal with which to administer these exomolecular probes of intestinal absorption and permeability [184].

Because gastric emptying could delay kinetics of absorption, a standard and perhaps prolonged fasting interval should be employed. This presents a challenge when testing infants who cannot tolerate fasts of greater than 3-6 hours depending on age, especially if exclusively breastfed since breastmilk is easily and relatively quickly digested. Furthermore, even a three-hour fasting interval may not be tolerated among acutely malnourished children. However, fasting is most important for assessment of individual component measurements. Twelve of the 25 reviewed studies reported that the children were fasted prior to challenge, but the duration of fasting differed across studies.

There are additional theoretical concerns with hyperosmolar sugar absorption tests. First, there is evidence in humans that intestinal permeability can be altered by the administration of such solutes [243, 244]. Second, there is a small degree of endogenous mannitol production and excretion [243]. Third, the use of lactulose can accelerate orocecal transit time [245]. None of these factors were mentioned by any of these studies. These potential technical limitations have also not received much attention outside of the field of

environmental enteric dysfunction in the last two decades. Both the individual mannitol and lactulose component measures provide unique information as mentioned above, and thus should be reported separately; nearly three-quarters of the studies we reviewed did so. Ideally, the individual components should be presented as percent of dose administered, and also as an (ideally standardized) osmolarity of the ingested probe. Of the 18 studies reporting that components were measured separately, 11 presented results as such. Indeed, the report of Lima et al. exemplifies the merit of reporting such metadata, as a paradoxically inverse relationship between mannitol uptake and poor growth was identified [139].

Five-hour timed urine collections are considered standard. Timed urine collection among infants and young children presents a challenge in the best of circumstances and it can be even more challenging when subjects have loose stools, as is often the case among the very cohort of children for whom assessment of intestinal permeability is desired. Urine collection times varied across the studies in this review, and two did not report the information. Additionally, urinary bacteria, either representing clinical bacteriuria or contamination and replication prior to freezing, could alter sugar concentrations [246].

Laboratory detection method varied as described in Table 14. Limits of normal results have not been determined for populations in resource-limited settings. Comparisons are made to those derived from developed-country settings. Table 14 demonstrates the various norms, primarily from the United Kingdom (UK), that were cited and referenced within the studies that we reviewed. Furthermore, the results were reported in varying ways. For example, L:M values can change with age of the subject, and in the setting of low urinary flow, as in dehydration, the impermeability of the proximal tubule has not been adequately assessed in children. Stratification of results based on age was often non-existent or suboptimal for determining its contribution to high L:M values. Also, it was difficult to compare the same age groups between studies. Perhaps the most striking deficiency was the lack of standard reporting of central

tendency measures, which were variably provided as medians or means. The latter were calculated as arithmetic or geometric means; the geometric method is more appropriate for ratio measures such as L:M. Some studies did not report central tendency measures but only the proportion with abnormal results. Among the 25 L:M studies we reviewed, three reported no central tendency values, two reported medians, and 20 reported means. Of the studies reporting means, 10 reported the geometric mean, which is a mathematically preferred expression of a mean for ratios, while two reported arithmetic means, and eight reported means without specifying their type. Clearly, despite the relative abundance of recent L:M data, the wide variation in study and reporting methods hamper our ability to make inter-study comparisons.

5.3.1.2 Range of L:M Values Reported

The majority of studies that we reviewed found elevated L:M values in their subjects, consistent with reports from before the interval of publication of papers that we assessed for this systematic review (i.e., studies published prior to 2000). These elevated L:M values were largely compared to norms reported for Western (primarily from the UK) childhood values [201, 217, 220, 223, 226, 235, 237, 238] and less often related to presumed norms of children in developing-country settings [112, 120, 205, 214, 231, 234, 241].

Delineation of normal values is a challenge for several reasons. Normative curves are often established based on assessments among a small sample of children in a developed country. Two standard deviations are frequently used as cutoff point to define normal/abnormal levels. However, this may not always be an appropriate method for a variety of reasons. Genetic factors might or might not influence test results. Marker response to environmental exposures might, at least initially, reflect adaptive rather than pathologic responses.

As noted above, comparison of L:M values across the 25 studies that we reviewed was also challenged by differences in the way the values were reported (see Table 14). Among

studies reporting the ratio results as geometric means, the values (ratios) ranged from 0.08 to 2.4 and both extremes were published by the same author group in two different studies, although among different populations. The lowest (i.e. least abnormal) values were reported among HIV-negative South African infants under four months of age born to HIV-infected mothers [160, 161], while the highest values were found in children aged six to 60 months hospitalized with severe diarrhea, also in South Africa [161]. The fact that the highest values were an order of magnitude higher than in other studies reviewed led us to speculate that L:M values might have been expressed as a multiple for portrayal on the same graph with urinary neopterin and serum retinol-binding data.

Among children in rural Bangladesh, one study found a geometric mean L:M value of 0.15 [123]. In two different rural Bangladesh intervention trials, baseline L:M values were 0.18 in each of the anti-helminthic treatment groups and 0.16 in the placebo group in the same setting [122], but were 0.22 and 0.25 in a different rural setting also among anti-helminthic treatment and placebo groups, respectively [150].

In Nepal, the mean L:M value among mildly stunted children aged 0-60 months was 0.26, but did significantly differ between those infected with *Giardia* (0.43) vs. those without evidence of giardiasis (0.25) [124]. A series of studies conducted in The Gambia reported L:M geometric mean values of 0.169 [110], 0.353 [112], and 0.31 [15]. Another study demonstrated similar results, ranging from 0.26 to 0.37 in placebo-treated children, depending on month of the study [170]. These studies support the concept of widespread intestinal permeability issues among children in developing settings with considerable variation between studies, generally finding a greater degree of permeability in central African settings.

However, in contrast to the Asian and central African studies, some South African [119, 160] studies found L:M values that were similar to established norms (i.e., mean values from

healthy children in developed settings). These apparent contradictions could reflect the emergence of the economies in which these children reside, with concomitant improvement in gut health. Three [119, 160, 161] of the four L:M studies from South Africa presented geometric means (notably, each of these four studies were performed by the same investigator group). The fourth study [171] presented a subset of data published by Filteau et al. [119]. Filteau et al. [119] and Rollins et al. [160] assessed L:M among infants born to HIV-infected mothers and generally found normal values that did not increase with age. Filteau et al. also noted no change in L:M measures with increasing morbidity. However, in both studies the mean value of L:M for the infants who became infected with HIV was increased; the Rollins et al. observational study reported a mean of 0.24 and the Filteau et al. intervention trial reported a mean of almost 0.5 among those whose mothers had not received vitamin A supplementation.

Similarly, some South American [137, 139] studies found L:M values that were similar to mean values for healthy children. Six L:M studies from Brazil were conducted by one team of investigators [113, 137-139, 156, 169]. Three of the studies reported baseline L:M values but did not classify them as normal or abnormal [113, 137, 138]. Two studies assessing similar populations of mildly malnourished children from an impoverished urban community in Brazil [156, 169] reported that a high proportion of their subjects had abnormal L:M values. L:M median and range data collected a few years later in the same setting and with the same inclusion criteria were noted by the authors to be within the confidence interval for values of healthy children in the study community [139] (the reference for this confidence interval was not cited).

Several studies measured the variability of L:M values between and within individuals. Some of these identified wide ranges [122, 124], and Campbell et al. [112] found that L:M showed significant intra-subject correlation between tests conducted 3.5 months apart. In contrast, Northrop-Clewes et al. [150] observed that intra-individual L:M values in the placebo

group of an anti-helminthic trial did not change significantly over 12 months. Of note, L:M values might change with increasing age because of normal changes of physiologic maturation. However, the studies that we reviewed rarely reported data stratified by age, thereby prohibiting rigorous comparisons from one study to the next.

Very few studies investigated longitudinal change in a measure of central tendency of L:M values for their study sample. Campbell et al. found a small but significant improvement in mean L:M value between the two study time points (from 0.198 to 0.172, type of mean not mentioned), driven by an improvement in mannitol recovery with no change in lactulose excretion [112]. The interval between sampling was 3.5 months.

Three studies observed seasonal variation in L:M values. Two of these studies were conducted in rural Bangladesh; the timing of peak L:M values differed slightly between them. In one study [122], the highest values were observed during the monsoon season while in the other [150], the peak followed the monsoon season. A study in The Gambia reported monthly variation in geometric mean L:M values, ranging from 0.37 in July to 0.26 in October in placebo-treated children [170].

5.3.1.3 Associations between L:M and Growth Outcomes

The L:M test has been used extensively to assess small intestinal function in relation to nutritional status in children in resource-limited settings. Several studies have found inverse relationships between L:M and growth parameters, while others have not (Table 15). Goto et al. [123] and Campbell et al. [110] found that L:M was inversely associated with height-for-age Z-scores (HAZ) and weight-for-age Z-scores (WAZ). A longitudinal study by Goto et al. [122] found associations between change in L:M and changes in weight-for-age Z-scores (ΔWAZ) and weight-for-height Z-scores (ΔWHZ),showing improvement in growth parameters with decreased L:M values. A later study by Campbell et al. [15] found that long-term height gain was negatively

associated with mean L:M value. Northrop-Clewes et al. [150] observed that L:M was inversely correlated with change in height-for-age Z- (ΔHAZ) and ΔWAZ scores at 12 months of follow-up.

However, several studies did not find an association between L:M and growth parameters. Goto et al. [124] reported no association between L:M values and growth status, although they did not specify which of the assessed growth measures (HAZ and WAZ) were used to evaluate this. A 2003 study by Campbell et al. similarly found no association between L:M and grade of protein energy malnutrition [111]. The same group earlier demonstrated that L:M was inversely associated with HAZ after correcting for age, gender, and study visit, but not with WAZ or body mass index z-scores [112]. In these studies, the association with HAZ was mainly attributed to the greater lactulose excretion in subjects with poorer HAZ scores and was constant across all age groups. There was no significant association between L:M calculated as the mean of the two data collection points and change in nutritional status parameters.

Several factors complicate comparison of the relationship between L:M and growth parameters across studies. Anthropometric indices used in these assessments varied. Markers were sometimes associated with different anthropometric measures within the same study. In one instance [112], a relationship was identified between L:M and HAZ score but not WAZ score. When the relationship between L:M values and growth parameters is not reported, it is not clear if the anthropometric indicator was either not evaluated or whether a significant relationship was not identified (i.e., only positive results were presented). One study assessed association with single sugar excretion only, reporting no significant associations between HAZ, WAZ, or weight-for-height Z- (WHZ) scores and lactulose, but significant associations with both WAZ and WHZ scores and mannitol [139]. The article did not mention HAZ score when reporting the results of mannitol analyses, leaving unspecified whether the relationship was assessed and found to be nonsignificant, or not assessed at all.

Table 15. Associations between anthropometric indicators and biomarkers of EED.

Data presented are from one study unless otherwise indicated in parentheses.

Anthropometric Z- score	L:M	Lactulose	Mannitol	Lactose:creatinine	Stool lactoferrin	Stool neopterin	Urine nitric oxide	Intestinal maltase activity	Intestinal lactase activity	Histopathology
HAZ	Y (3) N (1)	N		Y					Y	By 2 of 3 digital morphometric measures[1]: Y
∆HAZ	Y (2)			Y	N	Y				
WAZ	Y (2) N (3)	N	Y	Y			N	N	Y	By 2 of 3 digital morphometric measures*: Y Endoscopy: N Histopathology: N (2) Y (1)
∆WAZ	Y (2)			Y		Y				
WHZ	Y	N	Y	Y						By 2 of 3 digital morphometric measures[1]: Y
∆WHZ				Y						
BMI	N									

Y=yes, a statistically significant relationship was found.
N=no, a statistically significant relationship was not found.

[1] The three digital morphometry measures included enterocyte height, enterocyte brush border, and enterocyte nucleus height. The two measures that were significantly associated with anthropometric indicators differed for each type of anthropometric indicator assessed.

5.3.1.4 Associations between L:M and Other Outcomes

Four studies assessed the potential association between L:M values and *Giardia* infection. Two found no association [15, 123]. A Bangladeshi antihelmintic trial [150] found that L:M and giardiasis were not associated overall; they only identified an association among the treatment group in their intervention trial at a single time point over the course of the 12-month study period. In the fourth study, the geometric mean L:M among mildly stunted Nepalese children from urban squatter settlements aged five years and under was 0.26, but statistically significantly differed between *Giardia*-infected and non-infected children (0.43 vs. 0.25, respectively) [124].

Several studies investigated the relationship between subject age and L:M values; three found no association [113, 119, 124]. Goto et al. demonstrated a decreasing L:M trend with age among a cohort of Bangladeshi 3-15 month olds [123]. Campbell et al. [112] compared Gambian 2-5 year olds with older children and found a significant association between age and L:M values as well as a small but statistically significant improvement in mean L:M over 3.5 months driven by improved mannitol excretion without change in lactulose excretion. They later [110] reported study results among another cohort of Gambian children that showed that L:M values showed a large and statistically significant increase between 12 weeks and one year of age, a change driven by both increasing lactulose and decreasing mannitol excretion. Darboe et al. [115] observed that L:M values rose by about 50% from age two months to one year, but did not analyze the trend statistically.

Various studies examined the L:M marker in the context of other conditions. No association was found between L:M and diarrhea history [124] or helminthiasis [150, 205]. Associations were found between L:M and infection with HIV [119, 160] and *Cryptosporidium* and rotavirus [172]. Two studies reported seasonal L:M variation in rural Bangladesh, with peaks during [122] or following [150] monsoon season.

5.3.1.5 Use of the L:M Test as an Endpoint for Intervention Trials

Multiple studies have used the L:M marker as an endpoint to assess intestinal permeability in randomized, controlled trials. The specific interventions varied widely, including micronutrient supplementation, probiotics, therapeutic diets, and antibiotic and anti-helminthic drug administration.

Four studies utilized L:M as an outcome in vitamin A supplementation intervention trials and found no association with L:M overall [115, 119, 137, 168] although Filteau et al. did identify a significant reduction in L:M values among HIV-infected children treated with vitamin A [119]. While Darboe et al. noted that a vitamin A-supplemented group experienced a reduction in lactulose uptake, they also observed an accompanying decrease in mannitol absorption; therefore the ratio of the two sugars did not change [115]. This finding demonstrates the value of reporting the absorption of the two sugars separately, in addition to reporting the ratio. In contrast, Chen et al. observed improved L:M values after treating a cohort with persistent diarrhea or low WAZ with a single dose of vitamin A and a two-week course of daily zinc [113].

Lima et al. conducted two studies using L:M as an outcome to assess the beneficial effects of formulas of different compositions used in therapeutic rehabilitation of children with persistent diarrhea and/or malnutrition. In the study assessing glycine-supplemented formula, L:M values improved in the treatment group [138]. However in a trial in which this group examined alanyl-glutamine-supplemented formula, even though lactulose excretion improved, mannitol excretion worsened and therefore L:M values did not change after the intervention [139]. In another trial of glutamine supplementation, conducted by Williams et al. in The Gambia, the intervention did not improve L:M values; in fact, analysis by repeated measures analysis of variance (ANOVA) indicated that L:M values were borderline elevated in the glutamine-supplemented group ($p=0.05$), contrary to what was expected [170].

Another dietary intervention trial examined the efficacy of green banana and pectin among children with persistent diarrhea [157]. While post-intervention L:M values were still elevated, both lactulose and mannitol excretion improved after treatment, with lactulose being the primary driver of the improvements seen in the ratio of these two sugars.

Four other trials examined the degree to which L:M values improved as a result of various interventions, with no effect observed: probiotics [120] and antibiotics (rifaximin) [13] to target small bowel bacterial overgrowth as a potential cause of impaired intestinal integrity in Malawi, and various anti-helminthic and anti-*Giardia* treatments in Bangladesh [122, 150]. Interestingly, a Brazilian vitamin A trial demonstrated reduced lactulose as well as mannitol excretion, but an unchanged overall ratio [137], thereby highlighting the value of reporting absorption of the sugars separately.

In many of these studies, power considerations were not addressed at all, or incompletely addressed, and were often subordinated to study realities. Several examples illustrate the issue. Goto et al. recruited 222 children, into two treatment groups and one placebo group [122]. They estimated their sample size based on baseline recent Bangladeshi infant data [247]. They calculated that to improve HAZ by 33%, a sample size of 68 was needed (using an α of 0.05, and 80% power). For WAZ, the sample size needed was 73. So, after allowing for attrition, they aimed to recruit approximately 100 infants in each group. The anti-*Giardia* treatment used in this study, secnidazole, is unpalatable, so there was concern that there might be more attrition in those receiving this treatment. Therefore the treatment groups were increased by about 10% relative to the control group (n = 142, 141 and 127, respectively). However, because of large loss of subjects to follow-up as well as other methodological issues, including inaccurate dosing by many subjects and switching to analysis on actual treatment received rather than on an "intent-to-treat" basis, the final group sizes were 75 (secnidazole & albendazole), 59 (secnidazole only), and 88 (control). Hence, the size of the second group fell

below sample size targets for adequate power, while the other groups were still of sufficient size for projected HAZ and WAZ outcomes.

Northrop-Clewes et al. [150] did not report sample size calculations, indicating that their target sample size of 120 was based on logistical reasons. They went on to state “the null effect of deworming in this study could perhaps have stemmed from an inadequate sample size or duration of follow-up. However, significant improvements were observed in studies with smaller samples (e.g., n = 23, n = 55, and n = 72; 42, 10, 11) and of much shorter duration (seven and nine weeks) [248-250]". This comparison draws attention to the potential role of publication bias in any review of the literature.

5.3.1.6 Associations between L:M and Other Markers

Five of the 25 publications examined L:M results in relation to other potential markers of intestinal dysfunction, including one study that found that it was not correlated with urinary lactose or lactose:lactulose [124]. Two studies assessed L:M and markers of systemic inflammation and found differing results [110, 123]. A fourth study found no correlation with stool neopterin [15]. The only study that we reviewed that examined L:M in relation to intestinal tissue reported a variety of intestinal tissue measurements by morphometry and reported positive correlation with mucosal B-lymphocyte density, intraepithelial lymphocytes (IEL), and IELs staining positive for perforin [111]. It is not clear if the other intestinal markers were not correlated or merely not assessed for correlation.

5.3.2 The Lactulose:Rhamnose Ratio (L:R)

Similar to the L:M, the lactulose:rhamnose ratio (L:R) has been used as an index of gut mucosal function. As in the L:M test, lactulose is used as the measure of barrier function, but rhamnose replaces mannitol as the marker of absorptive capacity. L:M is performed on urine samples and, as mentioned above, in timed collections, ranging from three to six hours. The L:R

test had been performed on similarly timed urine specimens as well. To our knowledge, there are no published data comparing rhamnose directly to mannitol in children with enteric dysfunction, but in adults with inflammatory bowel disease the two sugars are absorbed comparably [251].

5.3.2.1 The L:R Test as a Reflection of Issues in Serum or Urine Sugar Testing in Children

Recent advances in high-performance liquid chromatography (HPLC) now permit sensitive detection of lactulose, mannitol and rhamnose in the blood. While timed blood specimens have been evaluated in animals and human adults [252-256], the invasiveness of repeated phlebotomies presents practical and ethical challenges in pediatric research. However, collection of urine over a period of time in young children, while less invasive, is not a trivial task either. While application of urine “bag” collectors permits noninvasive capture of urine, loss of urine because of leakage around the bag is common, as is contamination by stool, especially among children with diarrhea. Indeed, it should be noted that the children in most need of intestinal function testing often have diarrhea, and the ingestion of the sugar loads themselves can lead to osmotic diarrhea.

Haase et al. compared the validity of L:R testing on one-time collections of blood to 5-hour urine collections [125] in children. They found that blood L:R values were consistently lower than urine L:R values by a geometric mean (95% confidence interval (CI)) of 1.09 (1.02, 1.16). There was substantial agreement between the two tests as measured by a kappa statistic of concordance (95% CIs) of 0.71 (0.51, 0.92). Assuming urine L:R as a gold standard, Haase et al. calculated sensitivity and specificity of blood L:R as 81% and 89% respectively.

As discussed above, test failure can occur for various reasons when tests rely on urine samples, particularly timed samples. However, test failure can also occur with blood specimens because of insufficient volumes collected to run the assay, and potentially from edema if blood

is obtained by finger stick [257]. In addition, the assay cannot be performed using either analyte if children vomit, or refuse to ingest, the sugars. Haase et al. defined test “failure” as emesis of the sugar load, urine leakage or contamination with stool, or blood collection insufficient for analysis (<0.25 mL of plasma). While they did not delineate the reasons for failure among the blood vs. the urine collections, they did observe a significantly higher failure rate for the urine (37%) compared to the blood assays (10%) ($p<0.0001$). Interestingly, a study published in 1999 (and therefore not included in the analysis of this systematic review) by the same authors also reported a 37% failure rate for urine L:R testing and in this earlier study they specified the proportions by cause of failure. They noted that the failure rate for a five-hour urine collection varied between 47% (32 of 68) in girls with acute diarrhea to 17% (14 of 82) in non-diarrheal controls. They also noted that causes of test failure consisted of vomiting or refusal to drink the probe sugar solution in 21 (9%) of the tests [258].

The remaining four studies that used L:R as a marker of gut permeability measured these ratios in blood specimens. We found that rates of technical failure of testing were not often reported in the sugar absorption/permeability studies that we reviewed. Of the four studies using serum L:R, two discussed failure to complete testing. One study specifically reported difficulty with venipuncture in two of 34 subjects [159]. In the other study, L:R was measured at baseline as well as following an intervention with specific milk formulas [134]. It was not entirely clear, but it did seem that baseline L:R testing was successfully completed on all subjects while there was a reported “failure” of post-intervention testing among 10 of 150 children. Reasons for test failure in the second determination were not reported. Among the five studies that used D-xylose (either urine or serum) [136, 146, 147, 152, 155] no mention was made of lack of completion of testing among the subjects enrolled. Only seven [124, 138, 139, 150, 161, 169, 172] of the 25 urinary L:M studies included in this review specifically mentioned the number of children who completed L:M testing compared to the number recruited into the study. It was not

always clear why incomplete L:M testing occurred, but it appears that in five of these studies there was a failure in testing because of improper compounding of the sugar solution, inability to collect urine during the testing period, urine leakage or stool contamination, or refusal to ingest the sugar solution. Similar to the study that expressly measured and reported failure rates in L:R testing [125], Goto et al. reported in 1999 (and therefore not included in this review) that the L:M test failure rates were 32% [205].

It should be noted that each of the five L:R studies in this review was conducted by the same researchers in Darwin, Northern Territories, Australia (one of their studies also included subjects from Adelaide, South Australia, Australia). It appears that some of the studies might represent data from overlapping cohorts of children. It should also be noted that while Australia clearly falls within the classification of a high income country, our inclusion definition of children in “developing-country setting” included marginalized or indigenous populations in a developed country who are plausibly exposed to the same environmental and/or infectious risks for EED as children in developed-country settings. In fact, research among Aboriginal populations in Australia has contributed a great deal to the EED field [58, 258].

5.3.2.2 Range of L:R Values Reported and Associations with Growth Outcomes

Many investigators report the L:R value multiplied by 100 for ease of reporting; this convention was followed within the articles examined for this review. The geometric mean was the measure of central tendency consistently used in these studies, facilitating comparison of results. However, while the L:M studies referenced various ranges of normal standards, none of the L:R studies cited such reference standards. Furthermore, the three studies that reported the proportion of children with abnormal L:R results used different cutoff points and did not cite references for these demarcations of normal and abnormal values that they employed. One study defined an abnormal L:R value as >16, with 20 of 32 children (63%) exhibiting values

above this threshold [159], another used 7.2 as their defined cutoff point with 112 of 152 subjects (74%) above that threshold [43], and a third explained the derivation of its cutoff point of >5.6 as two standard deviations above the arithmetic mean for non-Aboriginal children without diarrhea [58]. No citation was given for this standard value, implying that it might have been derived from the data collected in this study. However, the authors reported that none of the study's non-Aboriginal children without diarrhea had abnormal permeability values, suggesting that perhaps the cutoff point was derived from a different study. While their study included Aboriginal children with diarrhea, they did not report proportions of children with L:R values >5.6 for this group.

Three studies compared serum L:R values among primarily Aboriginal cases with diarrhea and controls without this disorder. Baseline geometric means for L:R (95% CIs) in the two studies ranged from 3.7 (2.8, 4.9) [134] to 5.9 (4.4, 7.8) [125] to 11.4 (8.5, 15.5) [159] among controls and 9.4 (6.7, 13.1) [125] to 12.8 (10.3, 16.0) [134] to 31.8 (24.9, 40.7) [159] among cases.

In a randomized trial of three different milk formulas in Aboriginal children with malnutrition and/or diarrhea, baseline geometric mean L:R values (95% CIs) were 14.9 (10.4, 21.5) in one treatment group; baseline values were similar in the other groups [134]. Although subjects were randomized to treatment groups, the study did not include a control arm of standard care to which change in L:R could be compared. Mean improvement in L:R (95% CIs) was 13.0 (9.3, 16.6) with some significant differences across treatment groups. This was the only study in this review using L:R as an outcome measure in an intervention trial.

A similar study reported that mean L:R values among Aboriginal children were approximately double those of non-Aboriginal children, whether examining across groups of subjects either with or without diarrhea, consistent with the authors' suggestion that clinically

silent enteric dysfunction is prevalent among Aboriginal children [58]. Geometric mean L:R among the children without diarrhea was 2.5 and 4.6 among non-Aboriginal and Aboriginals, respectively (p=0.02). Geometric mean L:R among those with diarrhea was 7.9 and 16.4 among non-Aboriginals and Aboriginals, respectively (p=0.002).

One study examined the relationship between L:R and growth and found no association with nutritional status or age. Associations were, however, found between high L:R and acidosis (p=0.007), hypokalemia (p=0.035) and diarrhea severity (p=0.001) among children with diarrhea [58].

5.3.2.3 Associations between L:R and Other Markers

Serum L:R was compared to other biomarkers for EED in three of the other four studies. Ritchie et al. found L:R to be significantly inversely correlated with the ^{13}C sucrose breath test (r=0.67; CI: 0.42, 0.62; p<0.0001) [159]. Kukuruzovic et al. studied urinary nitric oxide excretion in relation to L:R in Aboriginal and non-Aboriginal in-patients with acute diarrhea, non-gastrointestinal infections, or without infections or diarrhea [43]. They found that NO_2/NO_3:creatinine and L:R were correlated (r=0.37, p<0.001), after adjusting for age and race. The association was stronger for lactulose permeability, with an effect ratio (95% CIs) of 1.47 (1.29, 1.66), than it was for rhamnose malabsorption, with an effect ratio (95% CIs) of 0.80, (0.67, 0.97). NO_2/NO_3 concentrations decreased significantly less rapidly than L:R values among children recovering from diarrhea. Another study by Kukuruzovic et al. assessed the association between L:R and red cell indices, stool reducing substances, and lactosemia and identified an association only with the latter, but the degree of correlation was minimal [58].

5.3.2.4 Methodological Issues with the L:R Test

Overall, there were fewer issues with consistency in reporting of L:R results across studies, perhaps because all of the L:R studies in our review were performed by the same group

of investigators. Four of the five studies reported a measure of central tendency for L:R and it was consistently in the form of a geometric mean multiplied by 100. Dosing of the sugars was the same across the studies. The timing for serum sampling was similar, with most using a collection time of 90 minutes following administration of the dose; only one study differed slightly, with testing occurring in a range between 90 to 120 minutes. HPLC was the test method for the serum L:R throughout. However, in striking contrast to the L:M studies, reference standards for L:R values were not cited. The three studies that reported proportions with abnormal L:R values used markedly different cutoff values that were either not defined or given insufficient explanation.

The urinary L:R test has disadvantages typical of any urinary method for dual-sugar permeability testing; the serum method avoids these issues, and, indeed, Haase et al. found a lower test failure rate with this method.

5.3.3 Serum and Urinary Lactose

As mentioned above, we assigned markers to categories based on their best fit, recognizing that biomarkers could indicate derangement of more than one function. For example, lactose is not normally absorbed across the intestine, but rather requires breakdown to products that are absorbed, namely galactose and glucose. This digestion is catalyzed by lactase, an intestinal brush border enzyme. While the presence of lactose in the serum or urine would in most instances indicate a lack of lactase (as opposed to presence of lactose excessive to lactase saturation), it also, and primarily, reflects a permeability defect, because even if lactose is not broken down, its size should preclude traversing the mucosa unless a porosity defect also exists.

We identified three studies that measured lactose in the blood (n=1) or urine (n=2) in the context of putative intestinal injury. Kukuruzovic et al. tested markers of intestinal permeability,

including serum lactose, among hospitalized Aboriginal and non-Aboriginal children with and without diarrhea [58]. Circulating lactose was detected in 38% of Aboriginal cases and 12% of controls (Aboriginal and non-Aboriginal combined). Lactosemia was weakly associated with an abnormal L:R. Another study of children from two urban squatter settlements with high rates of malnutrition primarily focused on identifying lactase deficiency via urinary lactose:lactulose, finding that nearly half of subjects had low lactase activity [124]. Urinary lactose concentration, as well as lactose:lactulose ratio, decreased with increasing age, but neither was associated with the L:M. The authors noted that lactose concentrations and lactose:lactulose ratios were significantly higher in breastfed subjects than those not breastfed, despite similar L:M values. However, the paper did not specify the nature of the dietary lactose sources in the non-breastfed babies. That is to say, if the bioavailability of the lactose or the quantity ingested is low, mucosal function could appear to be inappropriately normal. The final study measured the lactose:creatinine (L:Cr) ratio in Nepali children living either in squatter settlements or lower middle-class periurban households [151]. Mean L:Cr was significantly higher among the squatter compared to the lower-middle class group. For both SES groups, L:Cr values decreased with increasing age ($p<0.001$). HAZ, WAZ, WHZ, and ΔWAZ scores were strongly associated with mean L:Cr ($p<0.001$ for each parameter) as was ΔHAZ score ($p=0.004$); ΔWHZ score was not. Interestingly, the strength and magnitude of association between ΔWAZ score and L:Cr was most pronounced among the wealthier cohort and there was no association between ΔHAZ score and L:Cr among the squatter children, perhaps because of poor diets accounting for more of an effect among the squatter children.

5.3.4 Summary of Markers of Permeability

Despite logistical challenges, the L:M and L:R tests and other tests of permeability have been utilized extensively to assess intestinal function in children in resource-limited settings.

L:M values were often, but not always, elevated above published developed country norms in community-based studies of asymptomatic children in resource-limited settings. The other tests of permeability generally were not compared to a reference standard, limiting comparison of results across studies and different settings.

The L:M test was more extensively studied than the other permeability markers and was used more widely as an endpoint for intervention trials, including evaluation of micronutrient supplementation, dietary interventions and probiotics, and anti-bacterial and anti-parasitic drug treatments. L:R was used as an outcome measure for one intervention study. With new HPLC laboratory methods that are sufficiently sensitive to detect lactulose, rhamnose and other sugars, dual sugar tests might become more widely used.

While we identified two studies that compared serum L:R to serum lactose [58] and L:M to urinary lactose and lactose:lactulose [124], we did not find comparisons of L:M and L:R test results; a head-to-head comparison of the two most extensively used permeability tests would be informative.

The relationship between L:M and age varied across studies; some observed an association [110, 112, 115, 123], while others did not [113, 119, 124]. The single study that investigated the relationship between L:R and age did not find an association [58]. Furthermore, while most studies found an inverse association between L:M and anthropometric status, results were mixed. One study investigated the relationship between L:R and growth, and did not observe an association [58].

As with the D-xylose and endomolecular tests of absorption, lack of consistency in what was used as a cutoff point for normal (or what was reported to have been used) in the dual sugar permeability tests made it very difficult to compare results from one study to the next. Across permeability tests, we found a wide spectrum of depth of reporting and insufficient

details were often provided. The L:M studies best portrayed issues with methodology, reporting, and poor comparability caused by wide differences in how the test was performed across studies, differences in subject preparation and feeding mode, dosage of test sugars, and timing of urine collection, all of which can influence test results. Further complicating comparability, the measures of central tendency for reporting L:M varied.

While L:R tests have been conducted by evaluating the different biological sampling media of urine or serum, the serum test was used much more widely in the last decade and serum tests have been conducted in a much more standard manner than the urinary L:M test. Urinary L:R has disadvantages typical of any urinary method for dual-sugar permeability testing; the serum method avoids these, and Haase et al. [125] found fewer test failures with this method.

We found only one example of the urinary lactose:creatinine test having been employed in a pediatric population in a resource-limited setting [151]. The lactose in the urine is waste from endogenous metabolism of ingested material, and the creatinine is also a waste product naturally present in the urine. Testing endogenous metabolites eliminates the need for a loading dose as well as a post-dose delay of multiple hours for sample collection, which are inherent parameters of dual-sugar tests. These aspects of the L:Cr test could provide advantages compared to dual-sugar tests in resource-limited settings. However, in comparing the tests, it will be important to consider other parameters such as sample processing, as well as the comparative accuracy and reproducibility of the different tests. Recent data on the use of the L:Cr ratio to assess intestinal permeability in pediatric populations in resource-limited settings is scarce. None of the studies from our review assessed both L:M and L:Cr ratios in the same individuals, so it is not possible to compare the tests directly from the available data.

It should be noted that many elements of this chapter, especially pertaining to the L:M test, were published in 2014 [185].

5.4 Markers of Digestion

Twelve studies [43, 53, 58, 102, 109, 124, 132, 138, 147-149, 159] utilized a variety of markers that reflect intestinal digestive function. The particular data relevant to this review are listed for each of these studies in Evidence Table 3. Thirteen markers were assessed across the studies: eleven and two were markers of carbohydrate and lipid digestion, respectively. Testing for reducing substances in the stool was used in four studies as a marker of nonspecific poor digestion of sugars [43, 58, 132, 138]. Two other studies used biopsied intestinal tissue to measure specific disaccharidase activity or mRNA abundances [53, 109]. Three studies tested exhaled breath to assess maldigestion of lactose or sucrose [102, 147, 159].

One study employed urine lactose:lactulose ratio as a marker of lactase function [124]. Lastly, two studies, both by the same investigators, assessed intestinal lipid digestive capacity by measuring total triglyceride compared to fatty acids in the stool [148, 149].

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2002 Alves GM et al. Nutritional status and breath hydrogen test with lactose and lactulose in Terena Indian children Lactose hydrogen breath test (HBT) as a marker of lactase activity, and lactulose HBT as a marker of SBBO	Limão Verde and Córrego Seco, Mato Grosso du Sul, Brazil All children <10 yr old were recruited from these rural villages.	Cross-sectional n=264; <5 yr old: n=145 (However results were provided by <4 and ≥4 yr old age groups.)	<u>Breath Tests</u>: • **Lactose HBT** (251 tested) • Lactulose HBT (252 tested)	Lactose HBT: • Elevated: 27.1% among all subjects • Borderline: 43.0% among all subjects • 0% of subjects <4 yr had elevated or borderline results Lactulose HBT positive: • 11.5% of all subjects 8.6% of subjects <4 yr	The prevalence of lactase deficiency as measured by lactose HBT was >25%, but non-existent among those <4 yr of age. Prevalence of SBBO as assessed by lactulose HBT was ~10%.	Assessment of association between lactulose and lactose absorption was not reported.

2003
 	
Bustos M et al.
 	
Disaccharidase deficiency in Bolivian children with persistent diarrhea
 	
Jejunal biopsy and disaccharidase activities in children with PD and different forms of malnutrition
	Cochabamba, Bolivia
	
	3-34 mo old Amerindians hospitalized with PD and moderate or severe malnutrition in an urban setting.
		Cohort
		
		n=42 cases with PD and malnutrition:
		• 2 with kwashiorkor
		• 20 with marasmus
		• 20 with marasmic-kwashiorkor
		
		Children were assessed on admission and at three weeks, after diarrhea had resolved and anthropometrics were improving.
			<u>Jejunal tethered capsule biopsy:</u>
			• Histopathology
			• Disaccharidase activity:
			  • **Lactase**
			  • **Sucrose-Isomaltase**
			  • **Maltase**
			
			Histology was scored on a scale of 1 (normal) to 4 (severe morphological damage or flat mucosa).
				Most subjects had mild to moderate (score of 2-3) histological abnormalities, with one kwashiorkor patient having completely flat villi.
				
				Second biopsy showed a trend of improved mucosa, but difference was not significant based on histology score, intraepithelial lymphocyte density, or degree of infiltration of lamina propria.
				
				Percentages with enzymatic activity below normal at baseline, discharge:
				• Lactase: 64%, 59%
				• Sucrase-isomaltase: 97%, 90%
				• Maltase: 45%, 52%
				All changes were statistically significant.
				
				Lactase recovery was associated with admission HAZ (p=0.05) and WAZ (p=0.03) scores.
					Patients had diminished intestinal disaccharidase activity and substantial pathology on biopsy at admission and at three weeks, despite clinical improvements and tolerance of lactose-containing formula.	Spanish language article.
						
						Values for subnormal disaccharidase activity were not provided.
						
						The magnitude of lactase inverse association with growth parameters was not reported. Authors did not report whether they had tested for associations between maltase or sucrose-isomaltase and growth parameters.

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				Despite continued high disaccharidase deficiency prevalence at discharge, all children tolerated the lactose-containing formula challenge.		
2002 Goto R et al. Poor intestinal permeability in mildly stunted Nepali children: Associations with weaning practices and *Giardia lamblia* infection L:M as a marker of intestinal permeability, and assessment of association with giardiasis, helminthiasis, nutritional practices, and growth status.	Kathmandu, Nepal 0-5 yr olds (mean age 3.8 yr) from two urban squatter settlements. 37% and 33% of subjects were stunted and underweight, respectively.	Cross-sectional n=210	<u>Urine Tests</u>: • Lactulose[1] • Mannitol • Lactose[2] (168 tested) • L:M (158 tested) • **Lactose:lactulose ratio** (157 tested)	L:M: • 92% had values >UK norms • Mean[3] L:M (SD, range): 0.26 (0.21, 0.04-1.71). • *Giardia*-infected versus uninfected means: 0.43 vs. 0.25, p=0.014 The duration of ingestion of solid foods (with or without concurrent breastfeeding) was not associated with L:M in multivariate analysis. L:M was correlated with longer duration of breastfeeding (r=0.27, p<0.019). Specifically, children who breastfed for >2 yr had higher L:M ratios than children who breastfed for shorter times (data not provided). L:M was not associated with: • History of diarrhea in the week preceding testing • Helminthiasis • Age • WAZ or HAZ scores Lactulose excretion ranged from 0.02–15.00. Mannitol excretion ranged from 0.5–15.00.	L:M ratios were high overall. Wide individual variation was observed in L:M ratios. L:M was associated with giardiasis but not helminthiasis. Urinary lactose concentrations and lactose:lactulose ratios were significantly higher in breastfed subjects than in those that were not breastfed, despite similar intestinal permeability values. There were some unexpected findings: the duration of breastfeeding, and not the timing of introduction of solid foods, was correlated with L:M, and the correlation was direct, not inverse. Authors speculate that this could be due to higher mean age of their	Low lactase activity was defined as lactose:lactulose ratio >0.4. Specific L:M data by WAZ and HAZ scores were not reported, although authors state that L:M was not associated with "growth status."

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Lactose results were expressed in mg/L.
[3] Geometric mean.

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				47% showed low lactase activity. Lactose values and lactose:lactulose ratios decreased with age (R^2=28%, p<0.0001), but were not associated with sex, ethnicity, and location nor were they associated with L:M. Mean[1] urinary lactose concentrations (mg/L) by feeding mode: • Breastfed: 172.5 • Non-breastfed: 44.5, p<0.0001 corrected for infant age Mean[2] lactose:lactulose ratio by feeding mode: • Breastfed: 2.76 • Non-breastfed: 0.31, p<0.0001 corrected for infant age Mean L:M by feeding mode: • Breastfed: 0.23 Non-breastfed: 0.28, non-significant, p-value not specified	cohort compared to another study that demonstrated beneficial effect of duration of breastfeeding on reduced L:M in Guatemala [205].	

2002
 	
Kirkpatrick BD et al.
 	
Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children
	Port-au-Prince, Haiti
	
	<18 mo olds from a low SES setting recruited from the rehydration unit of GHESKIO HIV Center[3] with diarrhea and *Cryptosporidium* infection. Controls
		Case-control
		
		n=49;
		
		n=17 cases with *Cryptosporidium* and diarrhea (5 with PD)
		
		n=32 controls without
			Stool Tests:
			• **Reducing substances (RS)**
			• Lactoferrin
			• Cytokines:
			  • TNF-α receptor I
			  • IL-4
			  • IL-8
			  • IL-10
			  • IL-13
				Proportion RS-positive:
				• 33.3% cases
				• 64.7% diarrhea controls
				• 46.7% healthy controls, (p=0.2)
				
				Proportion lactoferrin-positive:
				• 83.3% cases
				• 60.0% diarrhea controls
				• 28.6% healthy controls, (p=0.01)
					Fecal lactoferrin was identified most often in children with diarrhea, especially in those with *Cryptosporidium.* While some fecal cytokines were detected in as many as 40% of healthy controls and 70% of controls with diarrhea, they were generally	Reported results were not stratified by persistent vs. acute diarrhea status.
						
						Cut-off values for lactoferrin positivity were not described.
						
						Stools from children

[1] Geometric mean.
[2] Geometric mean.
[3] The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections.

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Stool lactoferrin, reducing substances, leukocytes and cytokines as markers of intestinal inflammation of children with and without *Cryptosporidium infection*	recruited from an outpatient clinic without *Cryptosporidium* infection included those with and without diarrhea.	*Cryptosporidium*; • 17 with diarrhea (5 with PD) • 15 healthy	• IFN- γ <u>Blood Test</u>: WBC	IFN-γ was not recovered in any stools. All other fecal cytokines were significantly associated with *Cryptosporidium* cases compared to diarrhea and healthy controls. Additionally, TNF-α receptor I, IL-8, IL-13 were found in diarrhea and healthy controls, while IL-4 and IL-10 were not. Fecal lactoferrin was associated with the presence of TNF-α receptor I (point estimate not provided, p=0.03). Mean WBC counts were within normal range in all 3 groups.	associated with *Cryptosporidium* infection. The other stool tests did not discriminate by diarrhea or *Cryptosporidium* status.	who were breastfeeding were not tested for lactoferrin.
2003 Kukuruzovic R et al. Increased nitric oxide production in acute diarrhea is associated with abnormal gut permeability, hypokalemia and malnutrition in tropical Australian aboriginal children Nitric oxide (NO) as a marker of intestinal permeability and inflammation, and lactulose:rhamnose ratio (L:R) as a marker of intestinal	Darwin, Australia 1-6 yr old Aboriginal and non-Aboriginal hospital inpatients. Subjects were grouped as follows: 1. Children with AD 2. Children with no diarrhea but with non-GI infectious conditions 3. Children without GI or infectious conditions	Case-control n=318; n=169 cases with AD (154 Aboriginal) n=149 controls: • 73 with non-GI infections (49 Aboriginal) • 76 with no infections (29 Aboriginal)	<u>Urine Test</u>: Nitric Oxide (NO)* <u>Blood Tests</u>: • L:R • Mean corpuscular volume (MCV) <u>Stool Test:</u> **Reducing substances (RS)**** (169 cases tested) * NO is an unstable free radical and is converted to nitrite and nitrate. Urine nitrate (NO_3)+ nitrite (NO_2) was expressed	NO among Aboriginal children with diarrhea was >3x higher than any other group and >5x higher than in non-Aboriginal controls. • NO was >3x and >2x higher among Aboriginal than non-Aboriginal children in the diarrhea (p<0.001) and no infections groups (p<0.001), respectively, but there was no difference between them in the non-GI infections group. • NO was >3x and ~2x higher in the diarrhea compared to the no infections group among Aboriginals (p<0.001) and non-Aboriginals (p<0.03), respectively. • NO was virtually the same	NO_2 + NO_3:Cr ratio, as a measure of endogenous nitric oxide production, was used as a marker of gut permeability and inflammation, with an attempt to identify how much more it reflects as response to inflammation from GI vs. non-GI infections. Among non-Aboriginal controls, NO production was the same among those with diarrhea and non-GI infections (and higher compared to controls). NO was highest by far among Aboriginal children with diarrhea compared to	Positive stool RS was defined as <u>≥</u>0.5%. Abnormal L:R was defined as >7.6; no reference or derivation was provided for this cut-point. Study population appears to be the same as in another Kukuruzovic, et al. study also included in this review which assessed serum lactulose:rhamnose as a marker of intestinal permeability [58].

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
permeability and the relationship between NO and L:R, growth parameters, mean corpuscular volume (as a surrogate of iron deficiency), and stool reducing substances among children with and without diarrhea			as a ratio with urine creatinine (NO_2 + NO_3:Cr) in order to account for differences in urine concentration. ** Measured only among children with profuse diarrhea.	among the Aboriginal non-GI infections and no infections groups, as well as among the non-Aboriginal diarrhea and non-GI infections groups. 112/152 (74%) and 31/169 (18%) of children with AD had abnormal L:R ratios and positive stool RS, respectively. NO and L:R were measured at "convalescence" on Day 5 among those with diarrhea: the mean improvement in NO was 21.7% compared with 54.6% for L:R (p=0.01). NO and L:R were correlated (n=193, r=0.37, p<0.001)[1]; the correlation was stronger for lactulose (effect ratio=1.47, p<0.001) than for rhamnose (effect ratio=0.80, p=0.02[2]). NO was not correlated with stool RS[3] or MCV, but was correlated with lower WAZ score (effect ratio=0.88, p=0.05).	any other group. Authors suggest that high basal concentrations of NO among Aboriginal children due to (clinically silent) enteropathy could explain the concentrations seen among Aboriginal controls in this study. NO appeared to decrease significantly more slowly than L:R among children recovering from diarrhea. NO was found to correlate with L:R. NO was more strongly correlated with lactulose than rhamnose.	
2002 Kukuruzovic RH et al. Small bowel intestinal permeability in	Darwin, Australia Cases were Aboriginal and non-Aboriginal children admitted to hospital with diarrhea. Controls were	Case-control n=375 admissions for 306 children; n=285 case admissions for AD (264	<u>Blood Tests</u>: • Lactose • Lactulose[4] • Rhamnose • L:R • Hemoglobin • Mean corpuscular	27/75 (36%) of Aboriginal controls and 0 non-Aboriginal controls had abnormal L:R ratios. Mean[5] L:R at baseline: Cases: • Aboriginal: 16.4	Mean L:R ratios of Aboriginal children were approximately double those of non-Aboriginal children both among those with and without diarrhea, consistent with authors' suggestion that	Positive stool RS was defined as ≥0.5%. Abnormal L:R was defined as >5.6, derived from 2 SD above the

[1] Reported results appear to have been adjusted for age and race.
[2] Reported results were adjusted for age and race.
[3] Reported results among children with diarrhea were adjusted for age and race.
[4] Lactulose and rhamnose results were expressed as % of dose administered.
[5] Geometric mean.

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Australian Aboriginal children Serum lactulose: rhamnose ratio (L:R), serum lactose, and stool reducing substances as markers of intestinal permeability among Aboriginal and non-Aboriginal children with and without diarrhea	Aboriginal and non-Aboriginal children admitted without GI illnesses.	Aboriginal) n=90 control admissions with no diarrhea (74 Aboriginal)	volume (MCV) Stool Test: **Reducing substances** (RS)* L:R testing was repeated on day 5 for a subset of Aboriginal subjects: • 174/264 admissions for acute diarrhea • 25/74 control admissions * Measured only among children with profuse diarrhea when "clinically indicated". Number tested not provided.	• Non-Aboriginal: 7.9, p=0.002 compared to Aboriginal cases Controls: • Aboriginal: 4.6 • Non-Aboriginal: 2.5, p=0.02 compared to Aboriginal controls Mean improvement[1] in L:R (CI) at day 5 among those with repeat testing: • Aboriginal cases: 14.6 (11.2, 18.0) • Aboriginal controls: -0.63 (-4.0, 2.7) Mean lactulose recovery[2]: • Cases day 1: 0.085 (0.070–0.103) • Cases day 5: 0.039 (0.033–0.046) • Controls: 0.024 (0.019–0.029) All 3 values significantly differed from one another. Mean rhamnose recovery: • Cases day 1: 0.479 (0.424–0.542) • Cases day 5: 0.555 (0.498–0.616) • Controls: 0.585 (0.500–0.685) These values did not significantly differ from one another. Confidence intervals (CIs) in the authors' graphical representation of mean L:R at admission did not overlap, and the difference in	clinically silent enteropathy is prevalent among Aboriginal children. Mean L:R significantly improved over 5 days among Aboriginal cases. Children with severe diarrhea had higher mean L:R. Higher case L:R was driven more by high lactulose than by low rhamnose. Similarly, improvement in L:R among cases was primarily due to decreased lactulose. Stool RS and serum lactose were found in approximately one-quarter and one-third of Aboriginal cases, respectively. The latter was weakly associated with increased lactulose.	arithmetic mean for non-Aboriginal controls in this study. The rationale for the choice of 2 SD above the arithmetic, instead of the geometric, mean is not clear. Proportions of cases with abnormal concentrations were not reported. Analysis included data for 69 children with repeat admissions; this might violate independence assumptions for their statistical analysis methods. Repeat L:R testing was conducted on controls of both racial groups, but among cases it was only conducted on Aboriginal cases. This study appears to report on the same population as in the Kukuruzovic, et al. 2003 reference also

[1] Improvement in L:R appears to have been calculated as baseline L:R minus repeat L:R, as described in another publication in this review; however, this was not expressly stated. Reference 134. Kukuruzovic RH, Brewster DR. Milk formulas in acute gastroenteritis and malnutrition: a randomized trial. J Paediatr Child Health, 2002. **38**(6):571-577.
[2] Figures reported parenthetically after the mean percent recoveries of lactulose and rhamnose were not specified as ranges or CIs.

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				means was particularly evident between Aboriginal and non-Aboriginal subjects. Factors associated with L:R among cases were[1]: • Acidosis (p=0.007) • Hypokalemia (p=0.035) • Diarrhea severity (p=0.001) Age and malnutrition were not associated with L:R. 38% and 27% of Aboriginal cases had positive serum lactose and stool RS, respectively. 12% of Aboriginal and non-Aboriginal controls combined had lactosemia. Presence of lactosemia was associated with L:R, adjusted relative risk (CI)=1.06 (1.03, 1.10)[2]. Stool RS, anemia, and MCV were not associated with L:R.		included in this review, which assessed nitric oxide excretion [43]. Authors reiterate the advantages of serum over timed urine collection for assessment of L:R, as discussed in another publication in this review [125]

2005
 	
Lima AA et al.
 	
Intestinal barrier function and weight gain in malnourished children taking glutamine-supplemented enteral formula
 	
L:M as a marker of
	Fortaleza, Brazil
	
	2-60 mo olds hospitalized with WAZ score <-2, ~70% of whom had PD.
		RCT
		
		n=80;
		
		n=53 received supplemented formula
		• 27 with glycine
		• 26 with glutamine
		
		n=27 received
			Urine Tests*:
			• Lactulose[3]
			• Mannitol
			• L:M
			
			Stool Tests**:
			• Lactoferrin
			• Leukocytes
			• Occult blood
			• **Reducing substances (RS)**
				Mean[4] L:M (SE):
				• Glutamine group:
				  • Baseline: 0.31 (0.10) (similar in all three groups)
				  • Day 10: 0.10 (0.02); significant decrease, (p=0.01)
				• No significant decrease in L:M in glycine and nonsupplemented formula groups at day 10
					L:M significantly improved in the glutamine group only.
					
					>50% of subjects had intestinal inflammation by stool lactoferrin. Fecal leukocytes, RS, and occult blood were detected in fewer subjects than lactoferrin.
						The relationship between stool markers and L:M was not reported.
						
						Data were not stratified by history of PD.
						
						Fecal fat was assessed, but results were not reported.

[1] Reported results were adjusted for confounding variables, unless otherwise noted.
[2] Reported results were adjusted for severity of diarrhea, acidosis, hypokalemia, and age.
[3] Lactulose and mannitol results were expressed as % of dose administered.
[4] Type of mean not specified.

Evidence Table 3. Markers of digestion.
Biomarkers in bold are categorized as primarily markers of digestion.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

intestinal permeability and various stool tests among children with malnutrition or PD who received either glycine or glutamine supplemented formula or placebo		nonsupplemented formula	* n=80 tested at enrollment, n=65 tested at day 10.
			
			** n=60 tested.
				Mean lactulose (SE):
				• Glutamine group:
				  • Baseline: 0.97 (0.46) (similar in all three groups)
				  • Day 10: NS decrease in all 3 groups
				
				Mean mannitol (SE):
				• Glutamine group:
				  • Baseline: 3.42 (0.64) (similar in all three groups)
				  • Day 10: NS decrease in all 3 groups
				
				Proportion of stool markers at baseline among all subjects:
				• Lactoferrin: 53.3%
				• Leukocytes: 11.7%
				• RS: 3.3%
				Occult blood: 5.0%
						Cut-off values for lactoferrin positivity were not described.
						
						Exclusively breastfed children were excluded from study participation due to assessment of stool lactoferrin.

2002 Moya-Camarena SY et al. Effects of asymptomatic *Giardia intestinalis* infection on carbohydrate absorption in well-nourished Mexican children Lactose hydrogen breath test (HBT) as a marker of lactose absorption, and xylose breath test and urinary excretion as markers of xylose	Hermosillo, Sonora, Mexico 3-6 yr olds in a periurban setting attending preschool centers meeting inclusion criteria of no GI symptoms, no antibiotics in the preceding 3 wk, and no SBBO by lactulose HBT and Indican test.	Case-control n=13; <5 yr old: n=5 n=7 asymptomatic cases infected only with *G. intestinalis* n=6 controls without *Giardia* Cases were evaluated before and 3 wk after treatment with	Breath Tests*: • **Lactose HBT** • D-Xylose HBT** Urine Test: D-xylose**,[1] * Reported as parts per million (ppm) post-substrate ingestion after subtraction of baseline, pre-substrate H_2 concentrations. A positive HBT is considered to be a rise of ≥20ppm in breath H_2 above	Mean lactose HBT (SE): • Cases pre-treatment: 3.6 (0.75) ppm • Cases post-treatment: -0.85 (0.75) ppm (p<0.05 compared to pre-treatment) • Controls: 0.19 (0.81) ppm (p<0.05 compared to pre-treatment cases) Mean xylose HBT (SE): • Cases pre-treatment: 2.2 (0.69) ppm for infected group • Cases post-treatment: -4.16 (0.69) ppm (p<0.05 compared to pre-treatment) • Controls: 1.13 (0.74) ppm (NS compared to pre-treatment cases) Mean urinary excretion of xylose	Lactose HBT concentrations were normal according to established cut-points among all subjects. However, lactose HBT was significantly higher among cases compared to controls and there was also a significant decrease in lactose HBT among cases after treatment. The clinical relevance of such mildly elevated HBT results in asymptomatically infected children is unclear. Results did not	Statistical methods might not have been adequate to account for intra-subject correlation when comparing the same group of subjects (cases) before and after treatment. Investigators wished to exclude children with SBBO. As such, inclusion criteria restricted participants to those with adequate production of H_2 following ingestion

[1] D-xylose results were expressed as % of dose administered.

Evidence Table 3. Markers of digestion.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
absorption in well-nourished children with asymptomatic giardiasis and non-infected controls		tinidazole. Post-treatment stools were verified for absence of parasites.	baseline H_2 concentration. ** Investigators did not specify the xylose enantiomer used, however test functionality characteristics lead us to assume that it was the dextrorotary (D) enantiomer.	(SE) among cases pre-treatment and post- treatment was 34% (3) and 46% (11), respectively (NS), well above cut-offs indicative of malabsorption.	demonstrate xylose malabsorption by either urinary or breath measures among any group. While urinary results did not differ before and after treatment, case xylose HBT was significantly lower after treatment; again the clinical significance of such results is not apparent.	of lactulose and with minimal urinary indoxyl sulfate excretion. The number of children excluded due to failure to meet these criteria was not reported.
2002 Murphy JL et al. Maldigestion and malabsorption of dietary lipid during severe childhood malnutrition Stool recovery of radiolabeled products as markers of lipid digestion and absorption, and bile salt deconjugation as a marker of SBBO among children with severe malnutrition	Kingston, Jamaica 5-23 mo olds admitted to the Tropical Metabolism Research Unit of the University of the West Indies with severe malnutrition.	Case-series n=24 Subjects were divided into 3 groups of 8 children, each group receiving a different labeled triglyceride. Data were collected in three separate phases as described above in JL Murphy et al. 2001 [149].	<u>Stool Tests</u>: • **Total and fractionated ^{13}C following ingestion of one of three ^{13}C labeled triglycerides (TG): trilaurin, triolein, or trilinolein*** • ^{13}C stool assay following administration of labeled fatty acid ^{13}C glycocholate** * To assess fat excretion as a % of dose administered. Also assessed proportion of ^{13}C in triglyceride (TG) and fatty acid (FA) fractions to distinguish excretion caused impaired digestion (presence of TG) vs. poor absorption (presence	Median total stool excretion of ^{13}C in phase 1 was 9% (range: 1%-29%) and did not vary between TG groups. Median ^{13}C excretion dropped 33%-99% in phase 2 and 86%-95% in phase 3 compared to phase 1 ($p<0.05$ each). Over the study period, there were significant associations between total lipid and the amount of ^{13}C labeled TGs in stool for some groups, but not for others. Median ^{13}C in TG and FA was similar across TG groups in all phases. 13C FA recovery was similar and reduced by ~2/3 compared to Phase 1. ^{13}C TG was not detectable in Phases 2 or 3. Statistical comparisons between phases were not reported. ^{13}C after radiolabeled glycocholate administration was detected in stool at quantities considered to be in excess of	High concentrations of ^{13}C (compared to healthy UK children) [190] were observed in half of the subjects at admission, reflecting impaired digestion or absorption. The differences in stool ^{13}C were wide but not as extreme as in a previous study by same investigators (also examined in this review) using a different TG (tripalmitin) substrate [149]. ^{13}C excretion did not significantly differ between TG groups and declined with improving clinical course. Similar to their previous study, significantly more ^{13}C in stool was recovered as FA than TG, reflecting impaired	Authors state that the study was not powered to compare the different TGs, but they contend that medium chain trilaurin did not appear to be processed differently than the longer chain TGs triolein and trilinolein. Authors did not describe the method used to assign subjects to different TG groups. While it was noted that some subjects had positive stool cultures, details were not provided on the nature of the enteric infections.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
			of FA). ** To assess bile salt deconjugation in the bowel caused by SBBO; conducted after the TG assessment and a 3 day washout period.	the 7% recovery of dose administered upper limit of normal in U.S. adults in [189]: • Phase 1: 13/24 (54%) • Phase 2: 5/24 (20.8%) • Phase 3: 3/24 (12.5%)	absorption over poor lipid digestion/ hydrolysis. Unlike in their previous study, there was evidence of SBBO as measured post-ingestion of ^{13}C glycocholate.	
2001 Murphy JL et al. Gastrointestinal handling and metabolic disposal of ^{13}C-labelled tripalmitin during rehabilitation from childhood malnutrition Fecal fat, stool recovery of radiolabeled products, and breath tests as markers of lipid digestion and absorption and bile salt deconjugation as a marker of SBBO among children with severe malnutrition	Kingston, Jamaica 7-23 mo olds with malnutrition admitted to the University of the West Indies.	Case-series n=8 Data were collected in three separate phases (each lasting 9 days): 1. Within 48 hours of admission 2. During early rehabilitation 3. During late rehabilitation	<u>Stool Tests</u>: • Fecal fat* • **Total and fractionated ^{13}C assay after administration of ^{13}C tripalmitin (TP)**** • ^{13}C assay after administration of ^{13}C glycocholate (GCA)*** <u>Breath Tests</u>: • $^{13}CO_2$ after administration of ^{13}C glycocholate (GCA)*** • $^{13}CO_2$ after administration of ^{13}C TP**** * In 72 hour stool collection (measured as total grams and as % of dietary fat intake). ** To assess fat excretion as a % of dose administered. Also assessed	Mean fecal fat (SD): • Phase 1: 2.4 g/day (3.6) or 5.9% (9.4) of dietary lipid intake • Phase 2: 1.7 (0.9) g/day, or 3.3% (2.4) of intake • Phase 3: 0.9 (0.6) g/day, or 1.4% (0.7) of intake • Differences between phases were not statistically significant. Total excretion of ^{13}C in stool also varied widely across patients (0%-44%) and did not differ between study phases. Correlation between fecal fat and ^{13}C ($r=0.48$; $p<0.05$) was observed. Lack of lipid digestion and absorption were assessed by measuring TG and FA fractions, respectively. Mean ^{13}C TG recovery (SD) (% of administered dose), number of patients excreting TG: • Phase 1: 0.7% (1.6), n=3 • Phase 2: 0.9% (2.8), n=1 • Phase 3: no recovery from any subjects, differences between phases were NS	Mean fecal fat was not elevated compared to published norms [191, 192] during any study phase. There was wide variation in fecal fat at presentation, and wide variations in stool ^{13}C across subjects. Authors indicate that this is the first such assessment in malnourished children; previous studies on healthy children from the UK demonstrated average excretion of 6% [190]. The majority of excreted ^{13}C was in the form of FA rather than TG. Authors interpreted this to reflect failure of lipid absorption in the face of adequate digestion/ hydrolysis. Each form (FA and TG) was found in decreasing values as the study phases progressed, suggesting improved digestion and absorption, although	Statistical methods might be inappropriate for a small sample. All subjects were treated with antibiotics including metronidazole for presumptive SBBO; this might have affected GCA testing.

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			proportion of ^{13}C in triglyceride (TG) and fatty acid (FA) fractions to distinguish excretion caused by impaired digestion (presence of TG) vs. poor absorption (presence of FA). *** To assess bile salt deconjugation in the bowel caused by SBBO; conducted after the TG assessment and a 3 day washout period. **** Expressed as a percentage of absorbed label (dose administered - label recovered in stool) to assess oxidation for acute energy needs.	^{13}C FA fraction in stool declined during rehabilitation. Mean ^{13}C FA recovery (SD): • Phase 1: 6.0% (7.3) • Phase 2: 4.8% (3.7) • Phase 3: 3.3% (3.8), differences between phases were NS Mean FA values were ~9x (NS), 5x ($p<0.001$), and 3x ($p<0.05$) higher than mean TG values in Phases 1, 2, and 3, respectively. Following administration of labeled TP, absorbed ^{13}C label by breath analysis was ~5% (range 0%-21.2%) and similar across study phases. Following the administration of labeled GCA, there was either no or minimal recovery of ^{13}C in stool and $^{13}CO_2$ on breath (as % of dose administered) in all phases.	results did not differ significantly. Fecal fat was correlated with concentrations of ^{13}C in stool. There was no evidence of SBBO or bile acid malabsorption. $^{13}CO_2$ excretion following administration of ^{13}C TP was minimal, suggesting a propensity for deposition in adipose tissues rather than oxidation for immediate energy needs. The authors report that this breath test has not been widely used, but that healthy UK children have breath excretion values from 15%-43% [190], compared to a mean of 5% and range 0%-21% in this cohort; the latter findings were more similar to results from kwashiorkor patients where ^{13}C-labeled oleic acid was used as substrate [193].	
2000 Nichols B et al. Contribution of villous atrophy to reduced intestinal maltase in infants with malnutrition	Sao Paulo, Brazil Cases were children (mean age 9.9 mo, SD 8.1) hospitalized with malnutrition refractory to dietary rehabilitation. Controls were	Case-control n=33; n=24 cases n=9 controls Subjects were	<u>Jejunal capsule biopsy:</u> • Histopathology* • **Maltase activity** • Intestinal messenger RNA (mRNA) abundances: • **Maltase-glucoamylase**	Mean villous atrophy score (SD): • Cases: 2.6 (0.8) • Controls: 1.2 (0.5), p=0.006) WAZ score was correlated with villous atrophy (r=0.65, p- value not reported). 13/25 [sic] cases and 0/5 controls had subnormal (defined	The malnourished children had significantly greater villous atrophy than the younger controls. Among the subset tested for mRNA messages, maltase activity as well as the mRNA	Tissue from patients requiring intestinal resection as part of their biliary atresia management provides an opportunity to assess presumably “normal” intestinal

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Jejunal biopsy, maltase activity, and enzyme messenger RNAs among malnourished and well-nourished children. Assessed association between maltase and villous atrophy and other mucosal intestinal markers indicative of loss of enterocytes and enterocytic function.	children (mean age 3.6 mo, SD 1.0) with HAZ and WAZ scores >-2 and normal intestinal mucosa on biopsy, hospitalized for Kasai procedure for biliary atresia.	matched on height and weight; ages differed within matched sets.	**(MGA)** • **Sucrase-isomaltase (SI)** • Villin, a structural protein expressed only in enterocytes • Sodium-activated luminal glucose-galactose transporter 1 (SGLT), a functional protein expressed only in enterocytes • β-actin * Mucosal atrophy was scored on a scale of 1 (absence of atrophy compared to an organ donor) to 4 (similar to children with active CD). Histology among controls was on surgically resected tissue.	as <94 U/g protein) of maltase activity; mean maltase was 34% lower among cases (p=0.11). Maltase activity did not appear to decrease with WAZ score (further details not provided). However, in sub-analyses among those samples with an adequate β-actin, a housekeeping gene message, (n=10 cases, n=9 controls), cases' findings expressed as a mean percent of controls' (SD) included: • Villous length (reciprocal of atrophy score): 38.9 (41.6), p=0.004 • Maltase activity: 37.1 (23.2), p=0.001 • MGA mRNA: 45.1 (36.4), p=0.016 • Villin mRNA: 52.5 (22.6), p=0.003 • SGLT mRNA: 66.6 (23.1), p=0.057 • β-actin: 88.2 (15.8), p=0.189 Both villous length and maltase activity in a subset of cases were less than 40% of control values. MGA, villin, and SGLT mRNA abundances were correlated with villous atrophy score (r=0.73), (r=0.76), and (r=0.54), respectively (p-values not reported)[1]. MGA mRNA abundance was	abundances for MGA, villin and SGLT were significantly correlated with case status and were correlated with villous atrophy. While maltase deficiency has been reported in malnutrition in other studies, authors assert that these are the first results that directly support the hypothesis that reductions in maltase activity are due to villous atrophy. This study also nicely correlates mRNA relative abundance with function.	architecture. However, unless they mocked up *ex vivo* mucosal biopsies in these controls, resections will have lower proportions of villous to submucosa tissue compared to cases' samples derived from mucosal biopsies. While this probably doesn't affect histology, it might affect enterocyte functional assays and mRNA determination, as transmural tissue will bring in more diverse populations of cells; only some of them might have transcripts of interest. However, the bias is likely in a direction that would reduce effect size. It was unclear if control inclusion criteria included absence of atrophy or if all potential controls lacked atrophy. Statistical methods

[1] Villin and SGLT1 were assessed as a ratio with housekeeper gene β-actin.

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				correlated with maltase activity (r=0.32).		might not have adequately taken into account the small sample size and matching scheme. Subsets of subjects were investigated for various tests. For example, 10 cases had mRNA analyses based on β-actin adequacy. Another instance of selected testing was the subset of 22 and 15 cases that had WAZ score to histology and mRNA correlation analyses, respectively. Rationale for subset selection was not thoroughly described.
2009 Ritchie et al. 13C-sucrose breath test: novel use of a noninvasive biomarker of environmental gut health Sucrose breath test (SBT) as a marker of small bowel mucosal	Darwin and Adelaide, Australia 4 mo-5 yr old Aboriginal children admitted to hospital with diarrhea. Two control groups: 1. Aboriginal controls admitted to hospital with non-GI symptoms (50% had	Case-control n=43; n=18 Aboriginal cases with AD n=25 controls: • 18 Aboriginal, without diarrhea • 7 non-Aboriginal, healthy	<u>Blood Tests</u>: • L:R (32 Aboriginal cases and controls tested) • C-reactive protein (CRP) • Mean Corpuscular Volume (MCV) • Hemoglobin <u>Breath Test</u>: **^{13}C sucrose breath**	20/32 (63%) of Aboriginal children had abnormal L:R ratios. Mean[1] L:R (CI): • Diarrhea cases: 31.8 (24.9, 40.7) • Aboriginal controls without diarrhea: 11.4 (8.5, 15.5), significant difference (p<0.0001) SBT Mean (CI): • Diarrhea cases: 1.9% (0.9,	SBT values were significantly lower and L:R values were significantly higher among Aboriginal children with diarrhea than among those without GI symptoms. SBT was also significantly lower among Aboriginal controls than among non-Aboriginal children without diarrhea. This is	Abnormal L:R ratios were defined as >16; no reference or derivation was provided for this cut-point. L:R test was not conducted among the non-Aboriginal controls. SBT/ L:R correlation analysis

[1] Geometric mean.

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damage vis a vis sucrase activity among an Australian Aboriginal population. Also compared SBT with serum lactulose:rhamnose ratio (L:R)	pneumonia) 2. Healthy, non-Aboriginal controls recruited from community		**test (SBT)**	3.0), p<0.0001 compared to non-Aboriginal controls and p=0.004 compared to Aboriginal controls • Aboriginal controls: 4.1% (3.0, 5.2), p=0.032 compared to non-Aboriginal controls • Non-Aboriginal controls: 6.1% (4.8, 7.3) Significant differences were observed between all three groups. SBT results were not associated with wasting or with patient age or breastfeeding status. SBT and L:R were inversely correlated (r=0.67; CI: 0.42, 0.62; p<0.0001). L:R explained 45% of the variance in SBT; diarrhea explained 28% of variance. SBT was associated with increased MCV, relative risk (CI)=3.9 (2.8, 5.0). SBT was not associated with hemoglobin or CRP.	consistent with previous reports of high prevalence of clinically silent TE in this population. SBT was significantly inversely correlated with L:R.	was based on data for Aboriginal cases and controls combined; stratified analysis was not reported and could be of interest considering the large difference in L:R observed between these groups. Associations of MCV, CRP, and hemoglobin with SBT after adjusting for potentially confounding variables were not reported.

Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting.

Abbreviations: AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, Δ=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)

5.4.1 Sucrose and Lactose Breath Tests

Three studies used breath tests to detect the presence of lactose (n=2) [102, 147] or sucrose (n=1) [159] in the intestines after administration of a loading dose activity. These disaccharides should, in normal intestinal health, be split by the brush border enzymes lactase or sucrase to glucose and galactose or fructose, respectively. While these breath tests also reflect intestinal capacity to absorb these monosaccharides, we classified them as markers of digestive function first and foremost, because enzymatic cleavage of the ingested sugars is a cardinal intestinal process required for absorption to take place. Breath tests can measure various molecules in exhaled breath, most commonly hydrogen. Normally, very little hydrogen is detected in breath after an overnight fast; however, hydrogen is produced when undigested substances such as lactose are fermented by intestinal bacteria. The sucrose breath test (SBT) assessed in this review measured exhaled CO_2, a normal by-product of disaccharide metabolism which will be found in reduced amounts if there is reduced sucrase activity. Because genetically ordained age-associated lactose intolerance becomes common beyond infancy in many populations irrespective of intestinal mucosal health, the possibility exists that sucrase activity might serve as a better marker of overall intestinal function because such age-related decline in tolerances are not known to be associated with sucrose.

CO_2 exhalation in the SBT was significantly lower among Aboriginal children with diarrhea compared to those without gastrointestinal symptoms, as well as among Aboriginal controls compared to non-Aboriginal children without diarrhea. SBT results were not associated with age, breastfeeding status, or wasting [159]. SBT results were significantly inversely correlated with L:R values (r=0.67), but this was assessed among all Aboriginal subjects (cases and controls) combined. L:R values differed largely between these groups; it would be useful to investigate the relationship between SBT and L:R stratified on diarrhea status. SBT results were also associated with one other marker, mean corpuscular volume, but were not associated with blood C-reactive protein, an index of systemic inflammation, or with hemoglobin, concentrations of which can be reduced for a variety of reasons, including acute and chronic systemic inflammation. While this study suggests that SBT is a promising diagnostic test for EED, this was the only

publication that analyzed the SBT in children in a developing country setting or among marginalized populations during the time period under review.

Two studies utilized hydrogen breath tests (HBT) to assess lactase function [102, 147]. Each study also assessed either lactulose or D-xylose as substrates using the HBT. These two substrates for breath hydrogen analyses are primarily used to identify small bowel bacterial overgrowth (SBBO) and malabsorption, respectively, and as such they are discussed in those sections. Prevalence of abnormal lactose absorption differed between these two studies. Lactose absorption among indigenous Brazilian children with mild malnutrition was abnormal in more than one fourth of the subjects [102]. In well-nourished Mexican children, HBT values in cases with asymptomatic giardiasis were only mildly elevated but remained below concentrations that have been used as criteria for lactose malabsorption [147]. Cases had HBTs before and after anti-giardiasis treatment. The pre-treatment HBT excretion was significantly higher compared to controls without giardiasis and to post-treatment values. The clinical relevance of such mildly elevated HBT results in asymptomatically infected children remains unclear, however.

The discrepancy in results between the studies was unexpected, and the reason for it remains unclear. Both studies used the same cutoff for normal values, but the proportions of children with lactase deficiency were quite different. The differences might reflect numerous factors including differences in age, nutritional status, and age-related lactose intolerance. Another potential contributing factor is the exclusion of children with SBBO from the Mexican study but not from the Brazilian study, in which greater than 10% of the subjects had SBBO by lactulose HBT. An additional complication in assessing mucosal function, at least in children four years of age and older, is that there is a high prevalence of genetic lactase deficiency in many of the populations at risk for enteropathy, including in Latin America, Africa and Asia [259] and among Native Americans [260].

Neither of the HBT studies assessed the relationship of the test with growth outcomes in a statistically interpretable manner. While the Mexican study found normal D-xylose absorption by breath hydrogen testing in case (and control) subjects, neither this study nor the Brazilian study statistically analyzed the association between growth and the lactose HBT.

5.4.2 Stool Reducing Substances

Reducing substances are those that reduce copper salts in a hot solution to a chromogenic less oxidized state. These substances include glucose, lactose, fructose, galactose, and pentose. Like breath tests, reducing substances can indicate malabsorption, but also primarily identify maldigestion. This test has been used for decades to differentiate diarrhea secondary to infection from that caused by noninfectious intestinal dysfunction, particularly lactose intolerance. A condition of the test is that it must be performed on liquid stool.

Four studies [43, 58, 132, 138] assessed the prevalence of stool reducing substances to investigate digestive pathology. The proportion of positive tests for stool reducing substances among subjects varied widely across studies. Only 3.3% of Brazilian children hospitalized with malnutrition or persistent diarrhea had positive tests for stool reducing substances (definition of positive not provided) [138]. In contrast, tests in Haitian children with and without diarrhea were positive for stool reducing substances in one-third of those with diarrhea and cryptosporidiosis, in two-thirds of those with diarrhea but no evidence of *Cryptosporidium* infection, and in nearly half of controls without diarrhea [132], though the accuracy of this test on non-diarrheal stools is assumed to be inadequate because the non-absorbed sugars reportedly partition to the aqueous phase [261]. Hence, finding these sugars in solid stools, while unexpected, probably indicates of some degree of maldigestion. Again, cutoff points defining positivity were not provided, and there was no statistical difference between groups for this parameter (p=0.2).

Two studies investigated reducing substances among Aboriginal and non-Aboriginal Australian children with diarrhea; a positive result was defined as stool containing sugar concentrations $\geq$0.5 %. In the first of these, one-quarter of Aboriginal diarrheal cases tested positive for stool reducing substances [58]. In the subsequent study by the same authors almost one-fifth of children with profuse diarrhea tested positive [43].

Only one study of reducing substances examined its association with clinical outcomes, and this study found that the marker was associated with severity of diarrheal illness [58]. The same study found

that among subjects with diarrhea, a positive test for reducing substances was associated with high L:R by univariate analysis, but the association did not hold up in their multivariate model. Another study also examined the association between reducing substances and urinary nitric oxide among Aboriginal and non-Aboriginal children with acute diarrhea, but found none [43].

5.4.3 Intestinal Disaccharidases

Two studies assessed intestinal disaccharidase activity as a marker of digestive pathology on jejunal specimens obtained by capsule biopsy--Bolivian children with persistent diarrhea and malnutrition [109] and Brazilian children with refractory malnutrition [53]. Of note, the latter study utilized a unique control group, children without severe malnutrition undergoing intestinal resection as part of the management of their biliary atresia. Both studies assessed the proportion of children with maltase activities that were below normal, and the Bolivian study also measured sucrase-isomaltase and lactase activities. Both studies reported similar prevalences of abnormally low maltase activity in approximately half of the subjects with enteric dysfunction, but only one of these studies provided a defined cut point for abnormal (<94U/g protein) [53]. The deficiency in other disaccharidase activities was not as prevalent as that of maltase. In addition to maltase activity, the Brazilian study also measured abundance of messenger RNA for maltase-glucoamylase (MGA) and sucrase-isomaltase in small intestinal tissue, and found that they were correlated with the activity of maltase at the messenger RNA abundance.

The Brazilian study found no association between WAZ score and tissue maltase activity (no other details were provided), while the Bolivian study found that intestinal lactase concentrations were significantly and positively associated with WAZ and less strongly associated with HAZ at admission. Unfortunately, they did not detail the magnitude of these associations or report if they assessed maltase and sucrase-isomaltase association with growth parameters. Because intestinal disaccharidase activity is, by its nature, measured on intestinal tissue, it was relatively straightforward for these studies to assess marker association with intestinal histopathology. Maltase-glucoamylase (MGA) mRNA abundances were strongly correlated with both maltase activity and villous atrophy. The Bolivian study did not attempt to correlate disaccharidase activity with histopathology.

5.4.4 ^{13}C Assessment in Stool after Lipid Administration

Two studies by the same group of investigators in the same setting [148, 149] with a total sample size of 32 severely malnourished children assessed digestion by administering radiolabeled lipids. They then measured the amount of radiolabel excreted in the stool in the forms of triglyceride (TG) and fatty acid (FA), with recovery of fatty acids interpreted to indicate adequate lipid breakdown but poor absorption while recovery of triglycerides marked poor digestive function. Both studies found widely varying ^{13}C in stool, although the variation was less marked in their later study; however the sample size was only eight patients [148].

In both of these studies, the majority of excreted ^{13}C was in the form of FA rather than TG, reflecting a failure of lipid absorption in the face of adequate digestion. Each labeled lipid form (FA and TG) was found in decreasing concentrations as the study progressed, suggesting improved digestion and absorption, although concentrations did not differ significantly with time.

The two studies differed in their findings related to small bowel bacterial overgrowth; the first study [149] found no evidence for bacterial overgrowth while the second [148] did, as measured by the recovery of label in the stool after ingestion of ^{13}C glycocholate.

The first of these studies [149] additionally investigated fecal fat as a marker and found that it was associated with concentrations of ^{13}C in stool.

5.4.5 Urinary Lactose:Lactulose Ratio

We considered serum and urinary lactose to be primarily measures of gut permeability, as described above. As a large molecule, lactose should not enter the system unless there is a permeability defect. It should be recognized, however, that presence of lactose in the serum or urine could be complicated by lactase deficiency. However, lactase deficiency without a permeability defect would not be expected to result in the presence of lactose in the blood or urine. When urinary lactose is normalized against another marker for permeability, the lactose:lactulose ratio is primarily a marker of lactose digestion. One study assessed this marker and found that nearly half of subjects had low lactase activity,

defined as lactose:lactulose ratio >0.4 [124]. Urinary lactose and lactose:lactulose ratios significantly decreased with age and were significantly elevated among breast-fed compared to non-breastfed infants, adjusting for age, despite similar intestinal permeability values as measured by L:M. The markers were not associated with sex, ethnicity, or location. Lactose and lactose:lactulose ratios were not associated with L:M.

5.4.6 Summary of Markers of Digestion

A broad range of markers was used to assess digestive function in children in resource-limited settings. Intestinal disaccharidases were affected in subjects with persistent diarrhea and/or malnutrition. The sucrose and lactose HBTs showed varying degrees of digestive disturbance across studies with subjects of differing health conditions. Results for maltase activities were consistent across the two studies in which they were sought in intestinal tissues. Several different methods were used to assess intestinal enzyme capacity, and results between methods were consistent where comparable.

Few markers of digestion were investigated in relation to biopsy or tests of intestinal permeability. Breath tests in particular lacked comparison to other standard markers.

Varying associations were observed between markers of digestion and growth parameters. The sucrose breath test was not associated with growth [159]. The studies of intestinal disaccharidases found different results for different enzymes; one study found no association between maltase and wasting while the other found an association between lactase and growth parameters [53, 109]. The relationship between abnormal markers of digestion and growth outcomes was not reported for either hydrogen breath tests or stool reducing substances.

The sucrose breath test results were significantly associated with the L:R; the concentration of exhaled hydrogen was inversely associated with the L:R [159]. This test might be useful as a noninvasive marker of enteric dysfunction, although replication of these results is needed before a judgment as to their value can be made.

5.5 Markers of Intestinal Inflammation and Intestinal Immune Activation

Eighteen studies utilized a variety of markers that reflect intestinal inflammation or immune activation among children in resource-limited settings. The data relevant to this review are listed for each of these studies in Evidence Table 4. Eight types of markers were assessed across the studies, most of which were examined in stool. Lactoferrin was the most commonly assessed marker among these reports; nine studies utilized it to assess intestinal inflammation. Additionally, fecal cytokines, leukocytes, and neopterin were measured in five [101, 131, 132, 137, 140], four [104, 138, 158, 169], and one [15] studies, respectively. Two studies by the same group of investigators assessed fecal IgE [142, 143]. Intestinal tissue cytokines, immune and inflammatory cell markers, and duodenal aspirate immunoglobulins were investigated in one study apiece.

Evidence Table 4. Markers of intestinal inflammation and intestinal immune activation.
Biomarkers in bold are primarily markers of gut inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

2003
 	
Alcantara CS et al.
 	
Interleukin-8, tumor necrosis factor-alpha, and lactoferrin in immunocompetent hosts with experimental and Brazilian children with acquired cryptosporidiosis
 	
Fecal cytokines and lactoferrin as markers of intestinal inflammation among children with and without *Cryptosporidium parvum*.
	Fortaleza, Brazil
	
	3-43 mo olds recruited from a shantytown community who were screened for enteric pathogens.
	
	There was a high prevalence of malnutrition among the cases.
	
	(Adults experimentally exposed to *C. parvum* by ingestion were also studied; these data were not included in this review.)
		Case-control
		
		n=32;
		
		n=17 cases with *C. parvum:*
		• 4 with no diarrhea
		• 10 with AD
		• 3 with PD
		
		n=15 controls with no diarrhea or enteric pathogens and comparable HAZ and WAZ scores to cases
			<u>*Stool tests*</u>*:*
			• **Lactoferrin** (17 cases and 15 controls tested)
			• **IL-8** (13 cases and 15 controls tested)
			• **TNF-α** (10 cases and 0 controls tested)
				Lactoferrin positive:
				• 12/17 cases
				  • 1/4 cases without diarrhea
				  • 8/10 AD cases
				  • 3/3 PD cases
				• 3/15 controls (p=0.006 compared to cases)
				
				IL-8 detectable:
				• 3/13 all cases
				  • 0/2 cases without diarrhea
				  • 3/10 AD cases
				  • 0/1 PD cases
				• 6/16 controls (p=0.435 compared to cases)
				
				TNF-α detectable:
				• 0/10 cases
					The proportion of children who tested positive for fecal lactoferrin was greater in those with cryptosporidiosis, especially those symptomatic with diarrhea, than in uninfected controls, although 20% of the control group tested positive.
					
					Fecal IL-8 did not differ between those with and without *Cryptosporidium* infection. TNF-α was not elevated among children with *Cryptosporidium* infection.
						Direct comparisons between various stool tests were not reported.
						
						Lactoferrin results were graded based on agglutination reaction positivity with increasing dilution and were considered negative if there was no reaction at 1:25.
						
						Four subjects were breastfed and were tested for lactoferrin.

2009
 	
Amadi B et al.
 	
Reduced production of sulfated glycosaminoglycans occurs in Zambian children with kwashiorkor but not marasmus
 	
Duodenal biopsy including assessments of intestinal markers in children with PD and
	Lusaka, Zambia
	
	12.2-19.8 mo olds with PD and malnutrition admitted to the malnutrition ward of a teaching hospital.
		Case-control
		
		n=41*;
		
		n=41 cases with PD and malnutrition:
		• 18 with marasmus
		• 8 with marasmic kwashiorkor
		• 15 with kwashiorkor
		
		n=19 healthy control children from UK
			<u>Endoscopic duodenal biopsy:</u>
			• Histopathology
			• Densities in lamina propria and crypt epithelium:
			  • Cell proteins:
			  • Glycosaminoglycan (GAG)
			  • Enterocyte heparan sulfate proteoglycan (HSPG)
			  • Syndecan-1
			• Inflammatory cell markers:
			  • **CD3 IEL**
			  • **Ki67**
			  • **Human leukocyte**
				Biopsy findings among the Zambian compared to the UK children:
				• Villous height reduced
				• Crypt depth increased
				• ~50% reduction in crypt:villous ratio
				• Values for lamina propria cell densities were not reported for UK subjects
				
				No significant differences in crypt or villous measures or lamina propria cell densities were observed between nutritional groups or after nutritional rehabilitation.
					Mucosal architecture was markedly abnormal compared to UK controls but did not vary between marasmus and kwashiorkor presentations of malnutrition.
					
					Inflammatory cell densities were generally higher compared to UK children and showed different
						27 subjects were HIV positive; incidence was lower in the kwashiorkor group.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
different forms of malnutrition		* UK subjects are presented in this table due to comparisons of interest made in the review. However we do not include these subjects in the sample size for this review.	**antigen DR-1 (HLA-DR)**	Intestinal markers: • Inflammatory markers were seen in higher densities compared to the UK children. There were significant differences between the different nutritional groups in the specific types of inflammatory markers. • There was a significant reduction in GAGs and HSPG in the kwashiorkor group compared to UK children, but no significant differences between kwashiorkor and other presentations of malnutrition. • There was no difference in epithelial syndecan-1 protein expression between the malnutrition groups (data not available for UK controls).	patterns across the malnutrition presentations. Tissue concentrations of HSPG and GAG were reduced especially amongst children with kwashiorkor. Intestinal protein markers did not differ amongst the malnutrition groups.	

2000
 	
Azim T et al.
 	
Immune response of Bangladeshi children with acute diarrhea who subsequently have persistent diarrhea
 	
Immune activation tests, as well as transferrin and albumin as markers of nutritional status among children with and without PD
	Dhaka, Bangladesh
	
	7-12 mo olds with 6-8 days of watery diarrhea attending the International Centre for Diarrheal Disease Research.
	
	Cases were those who went on to develop PD, controls were those who did not.
	
	An additional group of subjects without diarrhea were recruited from a nutrition follow-up
		Case-control
		
		n=136;
		
		n=38 cases with PD
		
		n=98 controls:
		• 85 with AD
		• 13 with no diarrhea
			<u>Blood tests:</u>
			• IFN-γ
			• TNF-α
			• WBC (total and differential)
			• IgA
			• IgG
			• IgM
			• Transferrin
			• Albumin
			• Immune function tests:
			  • Neutrophil polarization response to chemotactic factor
			  • Neutrophil opsonization to yeast
			  • Mononuclear cell proliferation, spontaneous and in response to stimuli with
				WBC total and differential, immunoglobulin subtypes, cytokines, transferrin, and albumin did not differ between cases with diarrhea or controls, nor did stool leukocyte or erythrocyte counts.
				
				The percentages of neutrophils that polarized in response to stimulation were significantly higher in subjects with AD or PD compared to those without diarrhea; there was no difference between the two diarrhea groups.
				
				Opsonization did not vary between any groups.
					Some immune and inflammatory markers were associated with acute and/or persistent diarrhea.
					
					The only marker that was significantly associated with progression to PD was a negative DTH response to tuberculin antigen (odds ratio=3.8, CI: 1.4, 9.9). This was calculated from a logistic regression analysis that only
						The number of controls was relatively small and their nutritional status was not reported.

Evidence Table 4. Markers of intestinal inflammation and intestinal immune activation.
Biomarkers in bold are primarily markers of gut inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
	unit. Prevalence of malnutrition was high in all groups.		mitogens <u>Skin Test:</u> • Delayed-type hypersensitivity response (DTH) to tuberculin, tetanus, diphtheria, *Streptococcus, Proteus, Candida,* and *Trichophyton* <u>Stool tests:</u> • **Leukocytes** • Red blood cells	Monocyte spontaneous proliferation counts were less than half among children with no diarrhea compared to those with AD ($p<0.001$) or with PD ($p=0.011$); there was no difference between the two diarrhea groups. Monocyte proliferation in response to stimulation did not differ between the 3 groups. The proportion with DTH responses differed among the three groups only in response to tuberculin ($p=0.021$). More PD subjects had a negative tuberculin response than did subjects with AD ($p=0.024$).	included children with diarrhea.	
2007 Bushen OY et al. Heavy cryptosporidial infections in children in northeast Brazil: comparison of *Cryptosporidium hominis* and *Cryptosporidium parvum* Fecal lactoferrin as a marker of intestinal inflammation in children with *Cryptosporidium*	Goncalves Dias favela in Fortaleza, Brazil All newborns from an urban shantytown were recruited at birth and followed for up to 4 yr. This study included only children testing positive for *Cryptosporidium.*	Cohort n=42 (41 tested) Stools were collected at regular intervals as well as during episodes of diarrhea.	<u>Stool Test</u>: **Lactoferrin**	68.3% were lactoferrin-positive; there were no differences in positivity between subjects with *C. hominis* and *C. parvum* spp. 67.9% of lactoferrin-positive subjects had very high titers. Younger children were more often lactoferrin-positive ($p=0.03$). The difference was mediated by *C. parvum;* 87.5% of ≤1 year olds compared to 40.0% of older children with *C. parvum* were lactoferrin-positive ($p=0.04$). There was no difference among those infected with *C. hominis.* Lactoferrin was correlated with symptomatic infection among those with *C. parvum*: 78.6% of	Lactoferrin was correlated with younger age and symptomatic infection among those infected with *C. parvum.* Lactoferrin did not significantly predict growth outcomes. *Cryptosporidium* species-specific differences were observed in lactoferrin results. In contrast to *C. parvum*, there was no association between lactoferrin and symptomatic/ asymptomatic *C.*	Lactoferrin results were graded based on agglutination reaction positivity with increasing dilution and were considered negative if there was no reaction at 1:50 and highly positive at >1:400. Data were part of a larger study; similar data on lactoferrin in *Giardia*-infected children was published by A. Kohli, et al. (also included in this review), using a slightly different

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				symptomatic children compared to no asymptomatic children had a positive test (r=0.67, p=0.004[1]). Lactoferrin was not correlated with degree of oocyst shedding (p=0.28). Lactoferrin was correlated with ΔHAZ score among those with *C. parvum*, although this observation was not statistically significant (r=-0.39, p=0.13).	*hominis* infection (p=0.231). In addition, similar proportions of asymptomatic children with *C. hominis* had high fecal lactoferrin titers as had undetectable results.	grading scale for reporting lactoferrin results [133]. Rather than exclude breastfed children, Bushen et al. stratified results on breastfeeding status and found no difference in positive/ negative results, including when examined among younger and older children.
2004 Campbell DI et al. Intestinal inflammation measured by fecal neopterin in Gambian children with enteropathy: association with growth failure, *Giardia lamblia*, and intestinal permeability Fecal neopterin and L:M as markers of intestinal inflammation and permeability, respectively, and their correlation with growth status and	Keneba, Gambia 2 mo olds from rural area followed until 15 mo of age.	Cohort n=72 Subjects were evaluated with twice-weekly questionnaire to determine diarrhea morbidity, clinic assessments of growth, and screening laboratory tests every 2 mo.	Stool Test: **Neopterin** Urine Tests: • Lactulose[2] • Mannitol • L:M	Mean neopterin concentration was negatively correlated with long-term height (r=-0.29, p<0.009) and weight (r=-0.36, p<0.007) gain, but not with giardiasis. Mean[3] L:M (CI): 0.31 (0.26, 0.34). Mean excretion of lactulose (CI): 0.20 (0.18, 0.23). Mean excretion of mannitol (CI): 3.0 (2.8, 3.2). Mean L:M was negatively correlated with long-term height gain (r value not provided, p<0.0001), but was not correlated with presence of *Giardia.* L:M and fecal neopterin were	L:M and mean fecal neopterin concentration were not correlated. Mean L:M in the Gambian children was substantially higher than normal values in children in the UK. These high L:M ratios appear to be driven by mannitol excretion.	Study population might have some overlap with that of Campbell et al. also included in this review [110].

[1] Reported results were adjusted for confounding variables, unless otherwise noted.
[2] Lactulose and mannitol results were expressed as % of dose administered.
[3] Geometric mean.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Giardia recovery in the stool				not correlated (p=0.11).		

2003
 	
Campbell DI et al.
 	
Chronic T cell-mediated enteropathy in rural west African children: relationship with nutritional status and small bowel function
 	
L:M as a marker of intestinal permeability, small bowl biopsy with assessment of intestinal immune markers, and computerized morphometric analysis among rural Gambian children with differing degrees of malnutrition and compared to well-nourished UK children
	Fajara and Sibanar, The Gambia
	
	6 mo-3 yr old hospital- and clinic-based cases from rural communities.
	
	Case groups based on differences in nutritional status:
	• WAZ score >-2, with GI complaints other than diarrhea
	• Grade I protein energy malnutrition (PEM) (WAZ score -2 to -4) and unresponsive to nutritional supplements, with or without diarrhea
	• Grade II PEM (WAZ score <-4) with or without diarrhea
	
	Controls from UK* who were well nourished children with GI complaints other than diarrhea and with normal endoscopy results
		Case-control
		
		n=40 cases:
		• Group 1: n=4
		• Group 2: n=11 (7 with diarrhea)
		• Group 3: n=25 (18 with diarrhea)
		
		n=34 with case tissue samples sufficient for cytokine immunoreactivity tests:
		• Group 1: n=3
		• Group 2: n=8
		• Group 3: n=23
			<u>Endoscopic small bowel biopsy, site not specified</u>:
			• Histopathology
			• Morphometric assessment by computer analysis*
			• Intestinal tissue cytokines and immune markers:
			  • **CD-3**
			  • **CD-4**
			  • **CD-8**
			  • **CD-19**
			  • **CD-25**
			  • **HLA-DR**
			  • **Perforin**
			  • **γδ T-cell receptor**
			  • **Syndecan-1**
			  • **TNF-α**
			  • **IFN-γ**
			  • **TGF-β**
			  • **IL-10**
			
			<u>Urine Tests</u>:
			• Lactulose[1]
			• Mannitol
			• L:M
			
			* Biopsy involved morphometric assessment by computer analysis of villous height, crypt depth, villous:crypt ratio, and intraepithelial lymphocyte (IEL) density (per 100 epithelial cells).
				Crypt-hyperplasia and villous atrophy were observed among all Gambian subjects, and the degree of histopathology did not differ among cases with differing nutritional status, nor was there a correlation with diarrhea.
				
				IEL[2] means were ~3-fold higher in Gambian than UK children.
				
				Median CD3, CD4, CD8, CD19, and CD25 cell counts were significantly higher (2-5x higher) among each case group compared to the UK controls.
				
				IEL, γδ, syndecan-1, HLA-DR, and perforin were detected among the Gambian children in varying degrees but were not reported for UK controls. Syndecan, CD3, and CD8 displayed a gradient proportional to malnutrition severity.
				
				All Gambian groups showed higher lamina propria cytokine-immunoreactive mononuclear cell density (~200-450/mm^2) than UK controls (30-80/mm^2).
				
				Among subjects with elevated cytokines, similar densities
					All Gambian subjects had evidence of enteropathy with crypt-hyperplasia and villous atrophy, and mean IELs >2 SD above UK norms, independent of nutritional status and diarrhea history.
					
					Elevation of cell-mediated intestinal markers and mucosal proinflammatory cytokines was present across the 3 Gambian groups, variably correlated with nutritional status.
					
					L:M ratios were elevated in all Gambian groups, without apparent correlation to host nutritional status.
						Statistical methodology was not sufficiently detailed to determine what was compared (e.g. type of central tendency measure and variance calculations for L:M not stated).
						
						Duration of diarrhea not specified, but assumed to be persistent.
						
						Mucosal lymphocyte densities, cytokine immunoreactivity, and L:M results were not stratified by history of diarrhea.

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] These figures are presumed to represent IEL means; however, this was not explicitly stated.

Evidence Table 4. Markers of intestinal inflammation and intestinal immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
	were also studied. * UK subjects are presented in this table due to comparisons of interest made in the review. However we do not include these subjects in the sample size for this review.			were seen for both pro-inflammatory (IFN-γ and TNF-α) and putative regulatory (IL-10 and TGF-β) cytokines. Epithelial expression of TGF-β was also enhanced compared to UK controls, but subjects with poorer nutritional status had lower densities of mucosal TGF-β+ cells, with median densities of 420 and 250 cells/mm^2 in the grade I and grade II PEM groups, respectively. L:M values[1]: • Group 1: 0.53 (0.4-1.3) • Group 2: 0.47 (0.02-2.20) • Group 3: 0.73 (0.14-2.2) • Not assessed among the UK controls Nutritional status was not associated with L:M, recoveries of lactulose or mannitol. L:M was correlated with mucosal B lymphocyte density (r=0.57, p<0.05), IEL (r=0.51, p<0.02), and perforin+ IEL (r=-0.64, p<0.03).		
2001 Kapoor S et al. Giardiasis--clinical and diagnostic perspective Immunoglobulin concentrations in	New Delhi, India <12 yr olds admitted to hospital with PD and *Giardia*. Controls had no diarrhea and were hospitalized for	Case-control n=40; n=30 cases with PD and *Giardia* n=10 controls without diarrhea	<u>Duodenal secretion aspirates</u>: • **IgG** • **IgM** • **IgA** <u>Blood Tests</u>: • IgG • IgM	Higher mean concentrations of IgM were found in duodenal aspirates of cases compared to controls (p<0.05). Mean concentrations of duodenal IgA and IgG did not differ between cases and controls.	Differences in immunoglobulin concentrations were limited among children with PD infected with *Giardia* compared to children without such conditions.	The number of controls was small due to constraints in obtaining duodenal aspirate from children without GI symptoms.

[1] Not clearly indicated if these figures represent mean (CI) or another measure of central tendency.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
duodenal fluid and serum among children with PD and *Giardia* infection compared to those without diarrhea	non-GI conditions. Most cases were <7 yr old, with n=19 <3 yr old. Ages of controls were not specified.		• IgA			

2006
 	
Kirkpatrick BD et al.
 	
Childhood cryptosporidiosis is associated with a persistent systemic inflammatory response
 	
Fecal cytokines and lactoferrin as markers of intestinal mucosal inflammation among children with and without *Cryptosporidium* infection
	Port-au-Prince, Haiti
	
	<36 month olds recruited from GHESKIO HIV Center[1] with *Cryptosporidium* infection and healthy controls.
	
	Subjects were followed-up at 6 and 9 months after infection resolved.
	
	HIV status of subjects varied.
	
	There was a high prevalence of malnutrition in the study population.
		Cohort
		
		n=73;
		
		n=42 cases with diarrhea and *Cryptosporidium* infection (18 with PD)
		
		n=31 healthy controls without diarrhea and *Cryptosporidium*-negative
			<u>Stool Tests</u>:
			• **Lactoferrin**
			• Cytokines:
			  • **IFN-γ**
			  • **TNF-α**
			  • **TGF-β**
			  • **IL-4**
			  • **IL-8**
			  • **IL-10**
				Proportion lactoferrin-positive at enrollment:
				• Cases: 51.2%
				• Controls: 4.0%
				
				Cases had higher concentrations of TNF-α than controls at enrollment (p=0.04), but no difference was observed at follow-up.
				
				Controls had almost 2x higher fecal IFN-γ at enrollment (p=0.1); the magnitude of difference increased (to almost 3x) and was statistically significant at the 6- and 9-month follow-up (p=0.01 and p=0.03, respectively).
				
				No differences in fecal concentrations of TGF-β, IL-8, IL-4, or IL-10 were noted between groups.
					Lactoferrin was present among half of subjects with cryptosporidiosis and uncommon among those without such infection.
					
					Fecal TNF-α was higher among cases at enrollment but did not persist when infection resolved.
					
					Paradoxically, controls' comparatively higher fecal IFN-γ increased in both magnitude and degree of statistical significance at follow-up.
					
					Of the remaining fecal cytokines assessed, there were no differences between cases and controls.
						Cut-off values for lactoferrin positivity were not provided.
						
						Breastfed children (>85% of cases and controls) were included in testing. Proportions at follow-up were not reported.
						
						The association of fecal cytokines and lactoferrin with growth parameters, history of PD, and HIV status were not reported, nor was their association with each other.
						
						Various markers of systemic inflammation, including serum cytokines, were measured but their relationship with markers of intestinal inflammation was not reported.

[1] The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections.

Evidence Table 4. Markers of intestinal inflammation and intestinal immune activation.
Biomarkers in bold are primarily markers of gut inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

2002
 	
Kirkpatrick BD et al.
 	
Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children
 	
Stool lactoferrin, reducing substances, leukocytes and cytokines as markers of intestinal inflammation of children with and without *Cryptosporidium infection*
	Port-au-Prince, Haiti
	
	<18 mo olds from a low SES setting recruited from the rehydration unit of GHESKIO HIV Center[1] with diarrhea and *Cryptosporidium* infection. Controls recruited from an outpatient clinic without *Cryptosporidium* infection included those with and without diarrhea.
		Case-control
		
		n=49;
		
		n=17 cases with *Cryptosporidium* and diarrhea (5 with PD)
		
		n=32 controls without *Cryptosporidium*;
		• 17 with diarrhea (5 with PD)
		• 15 healthy
			Stool Tests:
			• Reducing substances (RS)
			• **Lactoferrin**
			• Cytokines:
			  • **TNF-α receptor I**
			  • **IL-4**
			  • **IL-8**
			  • **IL-10**
			  • **IL-13**
			  • **IFN- γ**
			
			Blood Test:
			• WBC
				Proportion RS-positive:
				• 33.3% cases
				• 64.7% diarrhea controls
				• 46.7% healthy controls, (p=0.2)
				
				Proportion lactoferrin-positive:
				• 83.3% cases
				• 60.0% diarrhea controls
				• 28.6% healthy controls, (p=0.01)
				
				IFN-γ was not recovered in any stools.
				
				All other fecal cytokines were significantly associated with *Cryptosporidium* cases compared to diarrhea and healthy controls.
				
				Additionally, TNF-α receptor I, IL-8, and IL-13 were found in diarrhea and healthy controls, while IL-4 and IL-10 were not.
				
				Fecal lactoferrin was associated with the presence of TNF-α receptor I (point estimate not provided, p=0.03).
				
				Mean WBC counts were within normal range in all 3 groups.
					Fecal lactoferrin was identified most often in children with diarrhea, especially in those with *Cryptosporidium.* While some fecal cytokines were detected in as many as 40% of healthy controls and 70% of controls with diarrhea, they were generally associated with *Cryptosporidium* infection. The other stool tests did not discriminate by diarrhea or *Cryptosporidium* status.	Reported results were not stratified by persistent vs. acute diarrhea status.
						
						Cut-off values for lactoferrin positivity were not described.
						
						Stools from children who were breastfeeding were not tested for lactoferrin.

2008 Kohli A et al. *Giardia duodenalis* assemblage, clinical presentation and	Goncalves Dias favela in Fortaleza, Brazil All newborns from an urban shantytown were	Cohort n=108 stool samples from 47 children Stools were	Stool Test: **Lactoferrin**	Proportion of positive lactoferrin results decreased with each new *Giardia* infection, p=0.015: 1.1st infection: 74.0% (15.2% of those testing positive had high titers)	Increased concentrations of lactoferrin were observed more frequently with first time *Giardia* infections.	Lactoferrin results were graded based on agglutination reaction positivity with increasing dilution; the following scale was

[1] The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections.

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Biomarkers in bold are primarily markers of gut inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
markers of intestinal inflammation in Brazilian children Fecal lactoferrin as a marker of intestinal inflammation in *Giardia*-infected children and its association with persistence of diarrhea	recruited at birth and followed for up to 4 yr. Those with *Giardia* recovered from stools were included in this study.	collected at regular intervals as well as during episodes of diarrhea.		2.2nd infection: 40.0% (5.3% of those testing positive had high titers) 3.3rd infection: 1 (20.0%) tested positive (at a high titers) Increasing titers of lactoferrin were associated with longer duration of diarrhea, p=0.017: • Negative: 2.2 days • Low: 9.7 days • High: 14.6 days Lactoferrin results did not differ between symptomatic and asymptomatic children at first infection, but those with symptoms had positive results with recurrent infections with greater frequency (75.0% vs. 0 in asymptomatic repeat infections, p=0.017.)	Concentrations were associated with longer duration of diarrhea, although these results were not presented separately for first and recurrent infections. Stool lactoferrin might be useful in predicting duration of diarrheal illness in *G. duodenalis*-infected children.	used: • High = positive at 1:400-1:3200 dilution • Low = positive at 1:25-1:200 • Negative = no reaction at 1:25 Stools from children who were breastfeeding were not tested. Data were part of a larger study; similar data on lactoferrin in *Cryptosporidium*-infected children were published by O.Y. Bushen, et al. (also included in this review); however, Bushen et al. used a slightly different grading scale for reporting lactoferrin results [108].
2010 Lima AA et al. Effects of vitamin A supplementation on intestinal barrier function, growth, total parasitic, and specific *Giardia* spp	Fortaleza, Brazil 2 mo-9 yr olds (mean 43 mo) from an impoverished urban community, eligible if HAZ score was <median for their community.	RCT n=79; n=40 received placebo (tocopherol) n=39 received vitamin A (retinyl	Urine Tests: • Lactulose[1] • Mannitol • L:M Stool Tests: • **Lactoferrin** • Cytokines: • **IFN-γ**	Median L:M at baseline was 0.089. There was no significant change in L:M at 4 mo follow-up within either treatment group. No significant difference in L:M was observed between treatment groups.	Frequency of stool lactoferrin varied between 23%-32%. While vitamin A supplementation was associated with reduced lactulose excretion, it was also	Authors did not report testing for associations between urinary markers of intestinal permeability and concentrations of fecal cytokines, or between these

[1] For lactulose and mannitol results, excretion measurement was not specified.

Evidence Table 4. Markers of intestinal inflammation and intestinal immune activation.
Biomarkers in bold are primarily markers of gut inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
infections in Brazilian children: a prospective randomized, double-blind, placebo-controlled trial L:M as a marker of intestinal barrier function, and stool lactoferrin and specific intestinal immunological cytokines as markers of intestinal inflammation among nutritionally at-risk children who received either vitamin A or placebo	Subjects were screened for intestinal parasites, and longitudinal anthropometrics were assessed.	palmitate) Subjects were treated every 4 mo.	• **TNF-α** • **IL-4** • **IL-10**	Both median lactulose and mannitol excretions decreased at 4 mo follow-up among the vitamin A compared to the placebo group: • Lactulose: 0.21 to 0.74, p=0.042 • Mannitol: 3.06 to 8.25, p=0.008 Overall proportion of lactoferrin was 23% initially. At 1 mo follow-up, there was no difference in prevalence between vitamin A (33%) and placebo (31%) groups. Cytokine concentrations did not significantly differ between placebo and vitamin A groups.	associated with reduced mannitol excretion, with no overall effect on L:M. Vitamin A supplementation was not associated with presence of lactoferrin or intestinal cytokine response.	markers and growth parameters or parasitosis. Cut-point values for lactoferrin positivity and abnormal L:M were not described. Exclusively breastfed children were excluded from study participation due to assessment of stool lactoferrin.

2005
 	
Lima AA et al.
 	
Intestinal barrier function and weight gain in malnourished children taking glutamine-supplemented enteral formula
 	
L:M as a marker of intestinal permeability and various stool tests among children with malnutrition or PD who received either glycine or glutamine
	Fortaleza, Brazil
	
	2-60 mo olds hospitalized with WAZ score <-2, ~70% of whom had PD.
		RCT
		
		n=80;
		
		n=53 received supplemented formula
		• 27 with glycine
		• 26 with glutamine
		
		n=27 received nonsupplemented formula
			<u>Urine Tests*</u>:
			• Lactulose[1]
			• Mannitol
			• L:M
			
			<u>Stool Tests**</u>:
			• **Lactoferrin**
			• **Leukocytes**
			• Occult blood
			• Reducing substances (RS)
			
			* n=80 tested at enrollment, n=65 tested at day 10.
			
			** n=60 tested.
				Mean[2] L:M (SE):
				• Glutamine group:
				  • Baseline: 0.31 (0.10) (similar in all three groups)
				  • Day 10: 0.10 (0.02); significant decrease, (p=0.01)
				• No significant decrease in L:M in glycine and nonsupplemented formula groups at day 10
				
				Mean lactulose (SE):
				• Glutamine group:
				  • Baseline: 0.97 (0.46) (similar in all three groups)
				  • Day 10: NS decrease in all 3 groups
					L:M significantly improved in the glutamine group only.
					
					>50% of subjects had intestinal inflammation by stool lactoferrin. Fecal leukocytes, RS, and occult blood were detected in fewer subjects than lactoferrin.
						The relationship between stool markers and L:M was not reported.
						
						Data were not stratified by history of PD.
						
						Fecal fat was assessed, but results were not reported.
						
						Cut-off values for lactoferrin positivity were not described.
						
						Exclusively

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Type of mean not specified.

Evidence Table 4. Markers of intestinal inflammation and intestinal immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

supplemented formula or placebo				Mean mannitol (SE):
				• Glutamine group:
				  • Baseline: 3.42 (0.64) (similar in all three groups)
				  • Day 10: NS decrease in all 3 groups
				
				Proportion of stool markers at baseline among all subjects:
				• Lactoferrin: 53.3%
				• Leukocytes: 11.7%
				• RS: 3.3%
				• Occult blood: 5.0%
						breastfed children were excluded from study participation due to assessment of stool lactoferrin.

2006 Long KZ et al. The effect of vitamin A supplementation on the intestinal immune response in Mexican children is modified by pathogen infections and diarrhea Fecal cytokines as markers of intestinal mucosal immune activation in children with and without GI pathogens receiving vitamin A or placebo	Mexico City, Mexico All 5-15 mo olds within a periurban community were eligible to be screened for participation.	RCT n=505 stool samples from 127 children; n=243 stool samples from 57 who received vitamin A supplementation n=262 stool samples from 70 who received placebo Participants were followed regularly; diarrhea history was tracked and stool samples were tested.	<u>Stool Tests</u>: Cytokines: • **IL-4** • **IL-6** • **IFN-γ**	Positive tests for fecal cytokines: • IL-4: ~55% • IFN-γ: ~50% • IL-6: ~40% There were no significant differences in proportions of these fecal cytokines between vitamin A-supplemented and placebo subjects. Vitamin A-supplemented children with diarrhea had lower median IFN-γ concentrations (odds ratio[1]=0.51; CI: 0.26, 0.99) and higher IL-4 concentrations (odds ratio=2.14; CI: 0.94, 4.87) compared to children with diarrhea in the placebo group in a nonrandomized analysis. IL-6 concentrations did not differ in this analysis. There were no differences between the two groups among those	Differences in fecal cytokine concentrations due to vitamin A supplementation were observed only in the subset of subjects with GI infection or diarrhea.	Pre-intervention fecal cytokine concentrations were not reported. Differences in fecal cytokines between all subjects with and without GI infections or history of diarrhea were not directly reported; all differences were described in terms of vitamin A interaction.

[1] The odds ratios represent odds that a cytokine (categorized into three levels: undetectable, <median, >median) will have a higher value among vitamin A-supplemented children.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				without diarrhea. Differences in median concentrations of fecal cytokines by types of enteric pathogens were also observed.		
2001 Mahmud MA et al. Sociodemographic, environmental and clinical risk factors for developing persistent diarrhea among infants in a rural community of Egypt Stool IgE as a marker of gastrointestinal allergy and its association with persistent vs. acute diarrhea	Bilbeis, Egypt Newborns recruited at birth from a rural village and followed for the first year of life. Surveillance of diarrhea symptoms identified 392 episodes of diarrhea, 41 (11%) of which were persistent.	Nested case-control within a cohort study n=392 episodes of diarrhea (including 41 episodes of PD) among 152 infants*) * Stool samples from each PD episode were counted as a case for analysis and compared to randomly selected AD episodes and to non-diarrheal samples.	Stool Test: **IgE**	Fecal IgE was detected more frequently in stools from episodes of PD compared to episodes of AD: odds ratio (CI)[1]=3.3 (1.0, 10.9). Fecal IgE was detected more frequently in stools from episodes of PD than in stools from children without diarrhea: odds ratio (CI)=4.84 (1.1, 21.7).	Fecal IgE was detected 3 times more frequently during episodes of PD than AD and 5 times more frequently in PD stools than in stools from those without diarrhea.	Sampling was based on episodes of diarrhea within a cohort of infants; individual infants could have contributed more than one diarrheal episode. Additionally, it appears that an individual could also have been included as a case of PD, a control with AD, or a non-diarrhea stool within the same analysis. Study population appears to be the same as in another Mahmud, et al. study also included in this review which reported the prevalence of fecal IgE by gender and age within the cohort [143].
2001 Mahmud MA et al.	Bilbeis, Egypt Newborns recruited at birth from a rural	Cohort n=152 followed for 29,036	Stool Test: **IgE**	Overall incidence of fecal IgE: 0.39/child-year By age group:	Substantial incidence of fecal IgE was observed in this setting in	Study population appears to be the same population as in another

[1] Reported results were adjusted for confounding variables, unless otherwise noted.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Increased fecal IgE among infants in a rural community of Egypt: an analysis of associated risk factors Fecal IgE as a marker of intestinal inflammation among children with diarrhea	village and followed for the first year of life.	infant days Stools were collected during episodes of diarrhea.		• < 3 mo: 0.28/child-yr • 3-6 mo: 0.42/child-yr • 6-9 mo: 0.16/child-yr • >9 mo: 0.12/child-yr Relative risks (CI): • 3-6 compared to >9 mo olds: 3.28 (1.03, 13.60) • Male gender: 1.82 (0.83, 4.18)	infants. IgE incidence peaked at 3-6 mo of age. Male gender was associated with fecal IgE.	Mahmud, et al. reference also included in this review which assessed the relationship between fecal IgE and PD [142].
2001 Rabbani GH et al. Increased nitrite and nitrate concentrations in sera and urine of patients with cholera or shigellosis To assess and compare nitric oxide as a marker of intestinal inflammation among children with cholera or shigellosis or healthy controls. Evaluated to assess nitric oxide production during infection of small bowel without inflammatory lesion (e.g., cholera) and during infection causing colon inflammation (e.g., shigellosis).	Dhaka, Bangladesh 2-6 yr olds with cholera or shigellosis admitted to the hospital. Controls were recruited from the healthy attendants of patients or from children of hospital staff. Mean age (SD) in yr: • Shigellosis cases: 3.8 (1.2) • Cholera cases: 4.2 (1.4) • Controls: 4.7 (1.8)	Case-control n=63; n=45 cases: • 24 with cholera • 21 with shigellosis n=18 healthy controls Samples were collected from cases on admission and upon discharge (after 7-10 days of treatment).	Urine Test: Nitric Oxide (NO)* Blood Tests: • Nitrite (NO_2) • Nitrate (NO_3) • WBC Stool Test: **Leukocytes** * Nitric oxide (NO) is an unstable free radical that is converted to nitrite and nitrate. Urine NO_2 + NO_3 were expressed as a ratio with urine creatinine in order to account for differences in urine concentration.	In children with shigellosis, median serum NO was ~8x higher at baseline than in controls and significantly differed from convalescent concentrations ($p<0.01$). Concentrations declined by 52% of baseline during the recovery period but did not return to values found in the controls (measure of statistical significance not reported). In children with cholera, median serum NO concentrations at baseline were ~4x higher than in control subjects. Recovery concentrations decreased 52% from baseline ($p<0.01$); convalescent values did not differ from the values in controls ($p<0.4$). Median urinary NO ratios were similar among those with *Shigella* and *V. cholerae* infection, both upon admission and discharge. Initial values were ~2x higher than upon discharge ($p<0.05$ and 0.01,	NO as measured by both serum and urinary NO_2 and NO_3 concentrations was significantly elevated at presentation during acute illness compared to 7-10 days after hospitalization in both cholera and shigellosis. Median serum NO concentrations in cholera patients were ~half of those with shigellosis both upon admission and upon discharge and concentrations were much higher in cases than in controls, Such striking differences were not observed for urinary NO results.	Some values reported in table format conflict with the text; columns of data appear to be transposed. Assessment for NO correlation with fecal leukocyte counts was not reported, nor was the correlation between urinary NO and total blood WBC.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				respectively). Control median NO was of an intermediate concentration between cases' admission and discharge median concentrations; the difference between control and case admission values was NS. Mean blood WBC counts (SD): • Shigellosis: 19.6 (3.3) • Cholera: 8.3 (2.8) • Controls: 7.1 (1.8) Mean fecal WBC/high power field (SD): • Shigellosis: 38 (17) • Cholera: 5 (2) • Controls: 3 (1) Serum NO correlated with blood WBC count in shigellosis cases at baseline (r^2=0.92, p<0.01), but only to a slight degree upon discharge (r^2=0.26, no p-value reported) and there was no correlation among the cholera cases. Serum NO correlated with stool volume at presentation (r^2=0.85, statistically significant per authors, p-value not reported).	Serum NO concentrations correlated with total blood WBC in shigellosis cases.	
2006 Samie A et al. *Cryptosporidium* species: preliminary descriptions of the prevalence and genotype distribution among school	Vhembe, South Africa 0.1-88 yr olds from semi-urban community included patients hospitalized with diarrhea or other GI complaints as well	Cross-sectional n=26 ≤5 yr old: • 22 hospital-based subjects • 4 school-based subjects	Stool Test: **Lactoferrin**	16/22 patients and 0/4 students were lactoferrin positive. While examination of lactoferrin association with history of diarrhea or with *Cryptosporidium* infection was not reported, only 3/22 and 16/22 hospitalized patients and 2/4 and 3/4 school children	Lactoferrin prevalence was high among children hospitalized with diarrhea or other GI symptoms, regardless of *Cryptosporidium* status.	Among the entire study cohort of all ages, lactoferrin results were similar among hospitalized patients regardless of *Cryptosporidium* status (influence of HIV infection was

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
children and hospital patients in the Venda region, Limpopo Province, South Africa Stool lactoferrin as a marker of intestinal inflammation among hospitalized patients and school children	as students attending nearby schools.			were positive for *Cryptosporidium* and had a history of diarrhea, respectively.	Lactoferrin was not found among school-recruited children, most of whom did have a history of diarrhea. Two of the four school children were *Cryptosporidium*-positive.	not reported). Among school children, lactoferrin was more frequently found to be positive among those infected with *Cryptosporidium;* statistical testing was not reported. Lactoferrin results were graded based on agglutination reaction positivity with increasing dilution and was considered negative if there was no reaction at 1:25. Some subjects were breastfed and were tested for lactoferrin.
2008 Vieira MM et al. Carotenoids, retinol, and intestinal barrier function in children from northeastern Brazil L:M as marker of intestinal barrier function, fecal lactoferrin and leukocytes as	Fortaleza, Brazil 2 mo-9 yr olds (mean age 41 mo) from an impoverished urban community, eligible if HAZ score <median for their community.	Cross-sectional n=102	Urine Tests: • Lactulose[1] • Mannitol • L:M (97 tested) Stool Tests: • **Lactoferrin** (93 tested) • **Leukocytes** lood Tests: • C-reactive protein (CRP)	48.5% had abnormal L:M. L:M and excretion of each sugar separately did not vary with retinol concentration. L:M was associated with levels of common dietary carotenoids, primarily driven by lactulose. However, the association was not always statistically significant, and the direction of association varied depending on precursor.	Almost half of subjects had increased L:M, and ~40% of subjects had increased lactoferrin. While serum retinol concentrations were not associated with L:M, serum carotenoids were; authors suggest that these retinol	L:M threshold for abnormal values was defined as ≥0.0864 [214]. Cut-off values for lactoferrin positivity were not described. Relationships between acute phase proteins and measures of intestinal permeability or

[1] Lactulose and mannitol results were expressed as % of dose administered.

Evidence Table 4. Markers of intestinal inflammation and intestinal immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
markers of intestinal inflammation, and CRP and AGP as acute phase reactants among children with varying vitamin A status			• α-1-acid glycoprotein (AGP)	40% of stool samples were positive for lactoferrin. 1% of stool samples were positive for fecal leukocytes. 30% of stool samples were positive for parasites but this had no impact on L:M results, lactoferrin, or acute phase reactants.	precursors might be more sensitive predictors of impaired intestinal function. However, the reported direction of association varied, making interpretation of these results unclear.	inflammation were not reported. Relationships between L:M and lactoferrin or fecal leukocytes as well as those between retinol or carotenoids and lactoferrin or fecal leukocytes were not reported. Exclusively breastfed children were excluded from study participation due to assessment of stool lactoferrin.

Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting.

Abbreviations: AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, Δ=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)

5.5.1 Fecal Lactoferrin

Fecal lactoferrin reflects infiltration of intestinal mucosa by polymorphonuclear neutrophils. The lactoferrin test might be more sensitive than assays for detection of leukocytes in stool because the former does not rely on the detection of whole cells, or on visualizing technology and trained observers. Its usefulness as a marker is enhanced by its relative stability in feces at room temperature.

We reviewed nine studies that utilized stool lactoferrin as a measure of intestinal inflammation. Populations in two of these studies appear to overlap [108, 133].

5.5.1.1 Prevalence of Fecal Lactoferrin

The proportion of lactoferrin-positive stools varied widely across publications whose study populations also varied in terms of nutritional status and health conditions such as diarrhea symptoms and infection with enteric parasites. The proportion of subjects whose stools tested positive for lactoferrin ranged from 13 to 74% [132, 133].

Two studies assessed stools of children whose HAZ scores were below the median and found that 23 to 40% were lactoferrin-positive [137, 169]. Another study found that 53% of subjects with malnutrition or persistent diarrhea had lactoferrin in their stools [138]. Samie et al. measured lactoferrin in 26 stools of children hospitalized with diarrhea or other gastrointestinal complaints and in stools of four primary school children, three of whom had diarrhea at the time of testing [162]. None of the schoolchildren and 73% of the inpatients had lactoferrin in their stools.

Several studies measured stool lactoferrin in children with parasitic infections. The proportion of Brazilian children infected with *Giardia* whose stools tested lactoferrin-positive decreased with each subsequent infection [133]. At first infection, 74% of subjects' stools were lactoferrin positive, 15% of which had high lactoferrin concentrations, defined as 1:400-1:3200

dilution in an agglutination reaction. At second infection, 40% were positive, of which 5% had high concentrations of this marker. At third infection, 20% tested positive, all of which had a high concentration of lactoferrin. Interestingly, lactoferrin results did not differ between symptomatic and asymptomatic children at first colonization by these parasites, but those with symptoms were more likely to produce stools that tested positive with recurrent infections. In a study among children from an impoverished Brazilian community with HAZ scores less than the median, nearly one-third of stools were positive for parasites, but parasitic infection was not associated with presence of stool lactoferrin [169].

Four studies measured stool lactoferrin in children with *Cryptosporidium* infection. One study tested stool lactoferrin in infants and children with *Cryptosporidium parvum* diarrhea, controls with diarrhea of different etiology, and healthy controls [132]. Lactoferrin was detected in 60% of stools from *C. parvum*-infected cases, 35% of diarrhea control stools, and 13% of healthy control stools. This difference across groups was statistically significant. Two other studies also found that the proportion of subjects with lactoferrin-positive stools was about 50 percentage points higher among cases compared to controls [101, 131]. Bushen et al. reported lactoferrin results only on *Cryptosporidium*-infected children; 68% were positive for lactoferrin and 68% of these subjects tested positive at a high titer, defined as positive at >1:400 dilution [108]. There were no differences in prevalence of lactoferrin-positivity between subjects with *C. hominis* and *C. parvum* spp.

5.5.1.2 Associations between Fecal Lactoferrin and Growth or Other Outcomes

Fecal lactoferrin did not significantly predict growth outcomes in the one study that assessed this relationship [108]. This was also the only study to assess the marker's relationship to breastfeeding and age. While there was no association with breastfeeding, younger subjects' stools were significantly more likely to be lactoferrin-positive. This difference, however, was

mediated by *C. parvum*; there was no age-associated difference among those infected with *C. hominis*.

Three studies found significant associations between fecal lactoferrin and cryptosporidiosis [101, 131, 132]. A fourth study reported a high proportion of positive stools from infected subjects but did not enroll uninfected children, so assessment of association with presence of infection was not possible [108]. That study did, however, test the relationship of fecal lactoferrin with oocyst shedding and found no association. A fifth study that had a small proportion of subjects infected with *Cryptosporidium* species did not report examining for association with fecal lactoferrin and infection, but based on the numerical data presented such an association was not mathematically possible [162].

Fecal lactoferrin was used as an outcome measure in one intervention trial testing the efficacy of vitamin A supplementation on intestinal barrier function, growth, and intestinal parasite infections [137]. No difference was observed in fecal lactoferrin results among treatment and placebo groups.

5.5.1.3 Association between Fecal Lactoferrin and Other Markers

Despite the number of studies that utilized fecal lactoferrin, only one study examined the association between fecal lactoferrin and another biomarker and found that fecal lactoferrin was significantly associated with the presence of tumor necrosis factor-α receptor I (TNF-αRI). Although the investigators also tested for other fecal cytokines, leukocytes and reducing substances, they did not report attempts to associate lactoferrin presence or concentration and these other markers in these subjects [132].

5.5.1.4 Methodological Issues with the Fecal Lactoferrin Test

The stool lactoferrin assay was generally performed with commercial kits. Methods described were similar across studies and results graded based on agglutination reaction positivity with increasing dilution. Six studies did not clearly report their cutoff for a positive test

[131, 132, 137, 138, 162, 169]. Two studies defined a positive threshold as a titer of >1:50 [101, 108], another as >1:25 [133]. Two studies defined high titers of positivity to be values >1:400 [108, 133].

Ingestion of breast milk might cause lactoferrin to be detected in the stool, in the absence of intestinal inflammation. Most of the lactoferrin studies in this review did not discuss this potential issue within the context of their research. One study that tested a wide age range of subjects did not mention the breastfeeding status of those being tested [162]. Five of the studies excluded breastfeeding subjects from lactoferrin testing [132, 133, 137, 138, 169]. Several studies, however, tested fecal lactoferrin in subjects known to be breast feeding [101, 108, 131], and one of these studies assessed the association between breastfeeding and stool lactoferrin but found none [108].

5.5.2 Fecal Cytokines

Five studies [101, 131, 132, 137, 140] measured concentrations of stool cytokines as indicators of intestinal inflammation; four of these studies investigated fecal cytokines in relation to gastrointestinal infection [101, 131, 132, 140]. The most commonly measured fecal cytokines were IFN-γ, TNF-α or TNF-αRI, and IL-4; each was assessed in four studies. IL-8 and IL-10 were each measured in three studies, while TGF-β, IL-6 and IL-13 were each measured in one.

5.5.2.1 Prevalence of Fecal Cytokines

Three of the five studies of fecal cytokines reported the proportion of subjects with positive tests. The proportion of samples with detectable cytokines in stool varied widely by cytokine and by study, ranging from 3% for IL-4 [132] to 55%, also for IL-4 [140]. In a study of children with and without gastrointestinal pathogens, about half of the stool samples were positive for IL-4, IFN-γ, and/or IL-6, respectively [140]. In contrast, in a study that measured a panel of cytokines in the stools of children with and without diarrhea, IFN-γ was not recovered in any stools and IL-4 was only detected in 3% of samples [132]. Only IL-13 and TNF-αRI were

detected in a substantial proportion of these subjects' stools; the former was detected in 32% and the latter in 52%. IL-8 was detected in 15% and IL-10 in 12% of stools. At 4-month follow-up in a subset of subjects, IL-8 was found in 6% and IFN-γ, IL-4, and IL-10 were not found in any stools. Among subjects in whom TNF-αRI was detected in stool during acute infection, this cytokine persisted at lower concentrations in stools of 50% of cases but was not detected in stools of controls. Some subjects previously negative for fecal TNF-αRI were positive at follow-up. In a study of fecal IL-8 and TNF-α in children with or without *C. parvum* infection, IL-8 was detectable in 31% of subjects without significant differences based on infection status [101]. TNF-α was only investigated in infected children and was detected in stools of 40%, all of whom had acute diarrhea; however, none had high fecal concentrations of the cytokine.

5.5.2.2 Associations between Fecal Cytokines and Growth or Other Outcomes

None of the studies of fecal cytokines reported assessment of their relationship to growth outcomes in the children studied.

Three studies investigated the relationship between fecal cytokines and *Cryptosporidium* infection. While IFN-γ was not identified in any stools in a Haitian study, fecal TNF-αRI, IL-8, IL-13, IL-4 and IL-10 were significantly associated with *C. parvum* infection [132]. Interestingly, some of these cytokines (TNF-αRI, IL-8, IL-13) were found in children with non-*Cryptosporidium* diarrhea and in children without diarrhea while other cytokines (IL-4 & IL-10) were not. Fecal cytokines were not associated with presence of co-pathogens in the stool. A subsequent study by the same investigators measured concentrations of IFN-γ, TNF-α, TGF-β, IL-4, IL-8, and IL-10 at multiple time points over nine months in stools of children with and without *Cryptosporidium* infection [131]. In contrast to their previous study, there were no differences in fecal concentrations of TGF-β, IL-8, IL-4 or IL-10 between those children with and without *Cryptosporidium* infection. Also, in contrast to their previous study, IFN-γ was detected and concentrations in stools of controls were almost double those of cases at enrollment, but this

difference was not statistically significant. At six- and nine-month follow-up, however, concentrations in stools of controls increased to almost triple that of cases and the difference was significant. Fecal TNF-α was only assessed in the second study; those with cryptosporidiosis had significantly higher concentrations at enrollment, but this did not persist at follow-up, i.e., after infection had resolved. A Brazilian study only measured TNF-α among 10 *C. parvum*-infected children and found no samples with elevated concentrations. IL-8 was measured among those with and without *C. parvum* infection, but the proportions of those with detectable serum concentrations did not differ between the groups [101].

Two randomized, controlled trials of vitamin A supplementation used fecal cytokines as outcome measures [137, 140]. In one, IL-4, IL-6 and IFN-γ were measured as the primary endpoints of the intervention in children with and without gastrointestinal pathogens [140]. Overall no significant difference was observed in stool cytokine concentrations between vitamin A-supplemented and placebo subjects. However the relationship between fecal cytokine concentrations and vitamin A supplementation was modified by both gastrointestinal infection and diarrhea. Similarly in the second study, vitamin A supplementation was not associated with an intestinal cytokine response [137].

5.5.2.3 Associations between Fecal Cytokines and Other Markers

Fecal cytokines were compared to other markers in only one of these studies, finding that TNF-αRI was significantly associated with fecal lactoferrin [132].

5.5.3 Fecal Leukocytes

Four studies collected data on stool leukocytes. Two of these studies reported the proportion of tests positive for the presence of leukocytes. These values ranged from 1% among children with HAZ scores below the local median [169] to 11.7% among children hospitalized with malnutrition or persistent diarrhea [138]. Two studies compared fecal leukocytes across subject groups with different gastrointestinal conditions. In one study, where neither proportions

of positive nor fecal leukocyte counts were reported, the investigators found that fecal leukocytes did not differ between children with acute diarrhea, some of whom went on to develop persistent diarrhea, and controls without diarrhea [104]. The second of these two studies found that mean fecal leukocyte counts were nearly eight times higher in shigellosis cases than in cholera cases and nearly 13 times higher in shigellosis than in healthy controls [158].

5.5.4 Fecal Neopterin

One study measured fecal neopterin as an indicator of intestinal inflammation, following subjects from 2-15 months of age [15]. No association was observed between L:M and fecal neopterin. Mean neopterin concentrations were negatively associated with long-term height gain, but were not associated with *Giardia* recovery in stools.

5.5.5 Fecal IgE

Two studies assessed fecal IgE as a marker of intestinal inflammation; both appear to have been conducted on the same population. The first was a community-based cohort study that investigated incidence of fecal IgE by gender and age group [143]. The incidence of fecal IgE was substantial, at 0.39/child-years, and peaked at 3-6 months of age. The second study assessed the relationship between stool IgE and persistent and/or acute diarrhea [142]. Fecal IgE was detected greater than three times more frequently among stools from episodes of persistent diarrhea compared to stool from episodes of acute diarrhea (OR (95% CIs) =3.3 (1.0, 10.9)) and nearly five times more frequently in persistent diarrhea stools than in stools from those without diarrhea (OR (95% CIs) =4.8 (1.07, 21.7)).

5.5.6 Inflammatory Intestinal Cell Markers

One study assessed inflammatory cell markers in intestinal tissue [103]. Densities of cell proteins and inflammatory markers in the lamina propria and crypt epithelium in Zambian children with persistent diarrhea and different forms of malnutrition were compared across

groups and to those in healthy controls from the UK. Immunohistochemistry was performed on intestinal tissue obtained by endoscopic biopsy. The intestinal cell proteins studied were glycosaminoglycans (GAG), heparan sulfate proteoglycan (HSPG), and syndecan-1. The inflammatory cell markers measured were $CD3^+$ intraepithelial lymphocytes (IEL), human leukocyte antigen DR-1 (HLA-DR), and Ki67, a crypt epithelial nuclear proliferative marker.

Inflammatory markers were observed at higher concentrations in the Zambian subjects than in the UK controls. There were significant differences in specific types of inflammatory markers across malnutrition groups. There was a significant reduction in intestinal GAG and HSPG in the kwashiorkor group compared to UK children, but there were no significant differences in these values between the children with kwashiorkor and those with other types of malnutrition. There was no difference in epithelial syndecan-1 protein expression between the malnutrition groups; data for this marker were not reported for the UK controls.

These markers were not compared to other types of markers of intestinal function.

5.5.7 Intestinal Tissue Cytokines and Immune Markers

While scintigraphy and counts of intraepithelial white blood cells (WBCs) and leukocyte presence in the lamina propria provide assessments of intestinal inflammation, scintigraphy can be used more broadly to study intestinal pathology and as such is listed in section VII on nonspecific markers of intestinal injury. Because WBCs are sought in biopsies along with assessments of intestinal architecture, histopathology, including assessment of WBC infiltration, is listed in section VII as well.

Only one study assessed intestinal tissue cytokines and immune markers. Markers were compared between rural Gambian children with differing degrees of malnutrition and UK control subjects with normal endoscopic results [111]. They assessed cytokine immunohistochemistry of intestinal tissue using antibodies against syndecan-1, perforin, γδ T-cell receptor, CD-3, CD-4,

CD-8, CD-19, CD-25, HLA-DR, TNF-α, IFN-γ, TGF-β, and IL-10. Median $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD25^+$ cell counts were 2-5 times higher among each case group compared to the UK controls; these differences were statistically significant. IEL, γδ, syndecan-1, HLA-DR, and perforin were detected among the Gambian children in varying degrees but were not reported for UK controls. Syndecan, CD3, and CD8 displayed a gradient proportional to malnutrition severity. All Gambian groups had greater density of cytokine-immunoreactive mononuclear cells in the lamina propria than UK controls. Among subjects with elevated cytokines, similar densities were seen for both pro-inflammatory (IFN-γ and TNF-α) and putative regulatory (IL-10 and TGF-β) cytokines. Epithelial expression of TGF-β in Gambian subjects was also enhanced compared to UK controls, but subjects with poorer nutritional status had lower densities of mucosal TGF-β+ cells. A marker of intestinal permeability, L:M was significantly associated with mucosal B lymphocyte, IEL, and perforin-positive IEL densities.

5.5.8 Duodenal Aspirates for Immunoglobulins

One study measured immunoglobulin concentrations in duodenal fluid as a marker of intestinal inflammation [128]. Concentrations of duodenal immunoglobulin were compared in hospitalized children with both persistent diarrhea and *Giardia* infection and controls without diarrhea or gastrointestinal infection. Significantly higher mean concentrations of IgM were found in duodenal aspirates of cases compared to controls. Mean concentrations of duodenal IgA and IgG did not differ between cases and controls, however.

5.5.9 Summary of Markers of Intestinal Inflammation

A variety of markers were assessed with a preponderance of studies using fecal lactoferrin. Lactoferrin was measured among study populations with varying characteristics. Lactoferrin results varied considerably as did results of other measures of intestinal inflammation.

Of note, among studies that met our review inclusion criteria, there was a complete absence of research utilizing fecal calprotectin (a protein produced by neutrophils and monocytes). This marker has been found in stools of patients with inflammatory bowel diseases in developed country settings during the time period of this review [262-264], but we found no analyses of its prevalence and potential role as a diagnostic tool in children with EED.

A threshold for normal for tests of intestinal inflammation was often not defined by authors. Generally data on these markers were not collected longitudinally so it is not clear how much intra-individual variation exists. Only one study statistically compared markers of intestinal inflammation to each other, and found a relationship between TNF-αR1 and lactoferrin [132]. None of the intestinal inflammation studies compared these markers to biopsy results or to other tests of intestinal dysfunction such as permeability or absorption.

An important consideration in evaluating these tests is the overall feasibility of performing them, especially in resource-limited settings. For example, stool is a somewhat easier analyte to obtain than blood (especially non-capillary assessments) and even urine among young children. Duodenal aspirates are, of course, more invasive tests and examination of intestinal tissue can only be performed on biopsy specimens, limiting their feasible use. Nonetheless, stool is an imperfect analyte. Production of sample cannot be scheduled, and the mechanics of obtaining the specimen are sometimes challenging, necessitating using adhesive bags or collection from diapers. In addition, stool is probably more biohazardous than blood, and certainly more than urine, and often elicits handling concerns by laboratory staff and couriers.

5.6 Markers of Systemic Inflammation and Systemic Immune Activation

Markers of systemic immune function and inflammatory response might provide informative indirect evidence of precursors to, or consequences of, small intestinal injury.

Indeed, the hypothesis that local inflammation could be reflected in systemic markers is intriguing. There is precedent for associations between markers of organ-specific inflammation and systemic inflammation, with the best studied being coronary artery and inflammatory bowel diseases. The atherosclerotic lesion in coronary artery disease and the small and/or large bowel inflammation in Crohn's disease and ulcerative colitis are characterized by acute and chronic inflammatory changes, and, as such, could be analogous to the lesions found in EED. By extension, there is ample evidence of an association between systemic markers of inflammation and acute coronary artery events, including myocardial infarction, sudden cardiac death, and cerebrovascular accidents, and of intestinal disease activity in inflammatory bowel diseases. Concentrations of circulating C-reactive protein (CRP) have, indeed, emerged as strong predictors of such events in adults, and complement the use of lipid profiles, which are a more specific biomarker of coronary artery disease [265-267]. Systemic CRP or erythrocyte sedimentation rates are indices of inflammatory bowel disease activity [268, 269].

In the case of EED, there is justification for seeking evidence of systemic inflammation as a biomarker, based on the precedent of coronary artery disease and inflammatory bowel diseases. However, there are additional justifications: the pathophysiology of environmental enteric dysfunction might result in, or be associated with, a hyperpermeable gut. Such increased bowel permeability would expose the host to a variety of injurious substances, most particularly microbial inflammatory drivers, and the host response could either be a surrogate for inflammation, or reflect the process that leads to enteric dysfunction. For example, one or more bacterial molecules could be absorbed, leading to a pro-inflammatory host response, as manifested by elevated CRP, but such a response could be an appropriate reaction to the gut that enables the molecules to be absorbed.

To hone in on assessments of systemic inflammation and immune function that could be helpful as markers of enteric dysfunction, we reviewed those studies that examined the markers

among children with presentations such as persistent diarrhea, or investigations of the markers in relationship to markers of intestinal inflammation or dysfunction.

It should be noted that there can be considerable overlap between the primary assignment of many of these markers of systemic inflammation and nutrient testing (e.g., serum albumin, total protein, hemoglobin, and blood counts.) For example, while hypoalbuminemia, hypo- or hypergammaglobulinemia, and impaired erythropoiesis are indicators of nutritional status, they can also reflect systemic immune activation [270-273]; as such, they were assigned to this section. For purposes of this review, if a test could be related to inflammatory activation and measured the product of a nutrient (such as hemoglobin) and not the nutrient itself (such as iron), then we included the test in our review.

We identified a diverse set of markers from 23 studies that we assigned to the category of systemic inflammation but which could serve as biomarkers for intestinal inflammation. Relevant data for each of the studies in this category can be found in Evidence Table 5. The most commonly employed markers were CRP, albumin, hemoglobin, transferrin, and varying total and immunoglobulin class concentrations. Additional markers included circulating cytokines and/or chemokines, total oxidant status, circulating α-1-acid glycoprotein (AGP), serum mannose-binding lectin, and urinary neopterin. Three studies assessed nitric oxide [43, 158] and plasma endotoxin and IgG endotoxin-core antibody [110], which are markers that could reflect systemic inflammation driven by intestinal processes. One study also included data on multiple markers of cellular, as well as humoral, immunity [104].

Although we did not systematically seek studies that assessed systemic marker association with nutritional status, among the studies that used markers of systemic inflammation or immune activation that were included in this review, seven assessed systemic inflammatory markers in relation to anthropometric indices [43, 110, 122, 123, 130, 150, 151]. The relationships varied widely across markers and sometimes across different growth

parameters for the same marker. Some data associated circulating markers of systemic inflammation and stunting; the markers with the strongest associations were albumin and α-1-acid glycoprotein, each in an inverse relationship [123].

Six studies investigated association of systemic markers with persistent diarrhea [104, 106, 107, 114, 130, 166]. Those identified at significantly lower concentrations in children with persistent diarrhea relative to children without diarrhea included: serum total protein [106, 107], hemoglobin [106, 166], mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) [166], lymphocyte counts [166], and anti-oxidant concentrations [107]. Albumin concentrations were also lower in children with persistent diarrhea; this relationship was statistically significant in one study [106] but not in another [166]. Sample size differences could explain the effect discrepancy with six 6-24 month olds with persistent diarrhea in the latter [166] compared to 96 6-36 month olds in the former [106]. While albumin concentrations are known to increase with increasing age, the slightly older cohort in the study that found an association is unlikely to explain the discrepancy in results.

Markers found at higher levels in children with persistent diarrhea compared to those with no diarrhea were total oxidant status, thiobarbituric reactive substances, and DNA damage [107]. In addition, assays of neutrophil polarization and monocyte spontaneous proliferation showed increased responsiveness in children with acute or persistent diarrhea relative to those without diarrhea [104]. A negative delayed-type hypersensitivity response to tuberculin antigen was also associated with progression from acute to persistent diarrhea [104].

Only one study utilizing markers of systemic inflammation investigated both growth outcomes and persistent diarrhea; this was in relation to serum mannose-binding lectin [130], which was not significantly associated with either.

Five studies investigated the relationship of systemic inflammatory markers to gut permeability. Associations were found between IgG, IgA, IgM, and IgG endotoxin core antibody

and intestinal function as measured by the L:M ratio [110] and between hemoglobin concentration and the lactose:creatinine ratio [151]. Additionally a correlation was observed between urinary nitric oxide and the serum L:R ratio [43]. In contrast, anemia and mean corpuscular volume (MCV) were not associated with serum L:R [58], and associations were not found between the L:M ratio and other markers such as α-1-acid glycoprotein (AGP), total IgG, albumin, or hemoglobin concentrations [123]. Furthermore, only one of these studies included biopsy, but the authors did not report assessing the relationship between markers of systemic inflammation and histology [146].

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
Biomarkers in bold are primarily markers of systemic inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

2000
 	
Azim T et al.
 	
Immune response of Bangladeshi children with acute diarrhea who subsequently have persistent diarrhea
 	
Immune response tests, as well as transferrin and albumin as markers of nutritional status among children with and without PD
	Dhaka, Bangladesh
	
	7-12 mo olds with 6-8 days of watery diarrhea attending the International Centre for Diarrheal Disease Research.
	
	Cases were those who went on to develop PD, controls were those who did not.
	
	An additional group of subjects without diarrhea were recruited from a nutrition follow-up unit.
	
	Prevalence of malnutrition was high in all groups.
		Case-control
		
		n=136;
		
		n=38 cases with PD
		
		n=98 controls:
		• 85 with AD
		• 13 with no diarrhea
			<u>Blood tests:</u>
			• **IFN-γ**
			• **TNF-α**
			• **WBC (total and differential)**
			• **IgA**
			• **IgG**
			• **IgM**
			• **Transferrin**
			• **Albumin**
			• Immune function tests:
			  • **Neutrophil polarization response** to chemotactic factor
			  • **Neutrophil opsonization** to yeast
			  • **Mononuclear cell proliferation**, spontaneous and in response to stimuli with mitogens
			
			<u>Skin Test:</u>
			• **Delayed-type hypersensitivity response (DTH**) to tuberculin, tetanus, diphtheria, *Streptococcus, Proteus, Candida,* and *Trichophyton*
			
			<u>Stool tests:</u>
			• Leukocytes
			• Red blood cells
				WBC total and differential, immunoglobulin subtypes, cytokines, transferrin, and albumin did not differ between cases with diarrhea or controls, nor did stool leukocyte or erythrocyte counts.
				
				The percentages of neutrophils that polarized in response to stimulation were significantly higher in subjects with AD or PD compared to those without diarrhea; there was no difference between the two diarrhea groups.
				
				Opsonization did not vary between any groups.
				
				Monocyte spontaneous proliferation counts were less than half among children with no diarrhea compared to those with AD ($p<0.001$) or with PD ($p=0.011$); there was no difference between the two diarrhea groups.
				
				Monocyte proliferation in response to stimulation did not differ between the 3 groups.
				
				The proportion with DTH responses differed among the three groups only in response to tuberculin ($p=0.021$). More PD subjects had a negative tuberculin response than did subjects with AD ($p=0.024$).
					Some immune and inflammatory markers were associated with acute and/or persistent diarrhea.
					
					The only marker that was significantly associated with progression to PD was a negative DTH response to tuberculin antigen (odds ratio=3.8, CI: 1.4, 9.9). This was calculated from a logistic regression analysis that only included children with diarrhea.
						The number of controls was relatively small and their nutritional status was not reported.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2003 Bitarakwate E et al. Serum zinc status of children with persistent diarrhoea admitted to the diarrhoea management unit of Mulago Hospital, Uganda Serum protein, albumin, and hemoglobin among children with and without PD	Kampala and Mpigi, Uganda 6-36 mo olds with PD, recruited from hospital, and healthy controls recruited mainly from the local population.	Case-control n=192; n=96 cases with PD n=96 healthy controls	<u>Blood Tests</u>: • **Albumin** • **Total protein** • **Hemoglobin**	PD cases: 7.47.9% low serum protein 8.69.7% low serum albumin 9.Low mean hemoglobin (10.5 g/dL) For controls, means of all three laboratory values were within normal range; percent of subjects with abnormal values are not reported. All three test results were significantly lower in children with PD than in controls (p< 0.01 for each comparison).	Decreased albumin, serum total protein and hemoglobin concentrations were associated with PD.	
2010 Bukhari AS et al. DNA damage and plasma homocysteine concentrations are associated with serum metabolites and mineral constituents' profiles in children with persistent diarrhea Serum proteins, metabolites, and levels of DNA damage among children with and without PD	Faisalabad, Pakistan 3-6 yr olds admitted to hospital with PD and healthy controls.	Case-control n=72; n=36 cases with PD n=36 healthy controls	<u>Blood Tests</u>: Serum proteins and metabolites: • **Albumin** • **Globulin** • Homocysteine • **Total protein** • Total cholesterol, HDL, LDL, triglycerides • AST, ALT • T3, T4 • **Total oxidant status (TOS), Total anti-oxidant status (TAS), and thiobarbituric reactive substances (TBARS)** • **DNA damage to lymphocytes**	Mean values significantly higher among PD cases than in healthy controls: 10.LDL 11.Homocysteine 12.TOS 13.TBARs 14.DNA damage Mean values significantly lower among PD cases than in healthy controls: 15.Total protein 16.T4 17.TAS	Multiple serum markers were associated with PD, especially DNA damage to lymphocytes (p=0.0001). The authors speculate that zinc deficiency, more commonly found in the children with PD, might be responsible for increased homocysteine concentrations and play an important role in mediating DNA damage.	Control recruitment strategy was not well described. TOS, TBARS and TAS were incompletely defined. Some values differed by gender in both the case and control groups: 18.Triglycerides 19.Total cholesterol 20.HDL 21.T3 Multiple markers studied; analyses did not appear to address potential confounding.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2003 Campbell DI et al. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation L:M as a marker of intestinal permeability and its relationship with various inflammatory markers and endotoxin	Keneba, The Gambia All 2-11 mo olds were recruited from this rural village and followed up to 14 mo of age.	Cohort n=71	<u>Urine Tests:</u> • Lactulose[1] (53 tested) • Mannitol (52 tested) • L:M (52 tested) <u>Blood tests</u>: • **Albumin** • **CBC** • **C-reactive protein (CRP)** • **IgA** • **IgM** • **IgG** • **Plasma endotoxin** • **IgG endotoxin-core antibody**	At 8 wk of age: • Mean[2] L:M: 0.169 (CI: 0.145, 0.198; range: 0.058-0.657) • Mean lactulose recovery: 0.202 (SD=0.159; range: 0.009-0.640) • Mean mannitol recovery: 3.80 (SD=2.35; range: 0.52-8.58) L:M more than doubled between 12 wk-1 yr of age (r=0.44, p<0.001) and was driven by both increasing lactulose (r=0.18, p<0.001) and decreasing mannitol (r=-0.14, p<0.01) excretion with age. WAZ and HAZ scores were negatively correlated with L:M (r=-0.41, p<0.001), and primarily driven by lactulose excretion (r=-0.39, p<0.001). Laboratory values were consistent with chronic, low level immunostimulation: • 50% of platelet and 39% of leukocyte counts were elevated, especially mean lymphocyte counts which were almost twice expected values [198]. • While the mean[3] CRP was within the normal range, 25% of values were above the upper limit of normal (5 mg/L), and 17% were >10 mg/L [198]. • Mean IgG, IgA and IgM concentrations were near normal at 8 wk of age, but increased rapidly; all three were elevated above expected values in all other	Mean L:M ratios were elevated at 8 weeks of age, and more than doubled in the first year of life. Many markers of inflammation and endotoxin release were significantly correlated with L:M and lactulose recovery. Poor growth was significantly correlated with L:M ratios, primarily due to lactulose excretion. Authors postulate that while general markers of inflammation cannot be specifically ascribed to a gut source, endotoxin and its related core antibody are potentially a direct measure of intestinal inflammation due to gut gram negatives as a primary source of endotoxin release among subjects without sources of	Presence of malaria parasites was assessed by blood smear at each study visit; the only parameter associated with malaria was CRP. Authors did not report investigating relationships between certain serum parameters (blood counts, CRP concentrations) and L:M. Study population might have overlap with that of Campbell et al. 2004 also included in this review [15].

[1] For lactulose and mannitol results, excretion measurement was not specified.
[2] Geometric mean.
[3] Geometric mean.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				age groups [198, 199]. • Mean[1] free plasma endotoxin concentration was twice the upper limit of normal [200] and IgG endotoxin-core antibody concentrations were also elevated [198]. • However, mean albumin concentrations (and concentrations within SD) were generally within normal range [198]. L:M was correlated with IgG and IgA (r=0.41 and 0.41, respectively, p<0.001), and IgM (r=0.28, p<0.02). IgG and IgA were also correlated with lactulose recovery (r=0.26 and 0.25, respectively, p<0.02). IgG endotoxin core antibody concentration was correlated with L:M and driven by lactulose recovery, (r=0.35, p<0.005 for both). Endotoxin concentrations were correlated with lactulose recovery (r=0.36, p<0.02) only.	extra-intestinal gram negative infection.	
2002 Clark TD et al. Risk factors and cumulative incidence of anaemia among human immunodeficiency virus-infected children in Uganda	Kampala, Uganda 9 mo old HIV-infected children followed at Mulago hospital until 36 mo of age. More than 40% were stunted and/or underweight at	Cohort n=225	Blood Test: **Hemoglobin**	While chronic diarrhea was associated with moderate anemia in a univariate analysis (odds ratio=2.5, CI: 1.0, 6.3), it was either not associated with moderate anemia (hemoglobin <9 g/dL) in a multivariate model or not included in the model	While there was a high prevalence of anemia (<11 g/dL) and moderate anemia (<9 g/dL) (92% and 35% at 9 months, respectively) among this cohort of HIV-infected children, chronic diarrhea	The association between chronic diarrhea and other assessed hematologic markers (any degree of anemia, mean corpuscular volume, and mean corpuscular hemoglobin

[1] Geometric mean.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Association of chronic diarrhea with moderate anemia in HIV-infected children	enrollment.				appears to have not been associated with anemia in the multivariate analysis. However, the text did not specifically state whether chronic diarrhea was tested in the multivariate model.	concentration) was not reported.
2008 Goto R et al. Impact of anti-*Giardia* and anthelminthic treatment on infant growth and intestinal permeability in rural Bangladesh: a randomised double-blind controlled study L:M as a marker of intestinal permeability, IgG as a marker of chronic immune stimulation, and α-1-acid glycoprotein as an acute phase reactant among children undergoing anti-parasitic presumptive treatment vs. placebo. Also assessed markers' associations with	Dhamrai Upazila, Bangladesh 3-15 mo olds from a rural area were enrolled and followed in a 9-mo trial. There was a high prevalence of malnutrition in the study population.	RCT n=222* n=75 received anti-*Giardia* and anthelminthic treatment n=59 received anti-*Giardia* treatment only n=88 received placebo * Those who fully participated and for whom data were analyzed are included in this review.	<u>Urine Test</u>: L:M <u>Blood Tests</u>: • **α-1-acid glycoprotein (AGP)** • **IgG** • **Albumin**	Mean L:M[1] (SD) at baseline was 0.18 (0.24) in treatment groups, with no significant difference in placebo group or in testing post-intervention. Proportion with elevated L:M at any study time point varied between 58%-74%. >57% consistently elevated L:M ratios. Seasonal variation in L:M was observed (p <0.001), with highest mean values in the monsoon season. L:M was associated with ΔWAZ and ΔWHZ scores at 24 weeks (p=0.001 and p<0.001, respectively, point estimates not provided.) Serum immune marker values were similar in all groups and did not change substantially with interventions. AGP concentrations were negatively associated with ΔWAZ score at 24 weeks (p=0.004, point estimate not provided), and were associated with ΔWHZ score at 12 weeks but not at 24 weeks.	High L:M ratios overall with substantial seasonal and within-infant variability. Interventions did not impact L:M or serum immune markers. There was improvement in weight with better L:M values, the degree to which this occurred was not reported.	High L:M ratios were defined as greater than the upper CI for UK infants. Same study population as reported by this group in another study also included in this review [123].

[1] Geometric mean.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
growth parameters.						
2008 Goto R et al. Impact of intestinal permeability, inflammation status and parasitic infections on infant growth faltering in rural Bangladesh L:M as a marker of intestinal permeability, IgG as a marker of chronic immune stimulation, and α-1-acid glycoprotein as an acute phase reactant. Also assessed laboratory values' associations with giardiasis and growth parameters.	Dhamrai Upazila, Bangladesh 3-15 mo olds from a rural area were enrolled and followed in a 9-mo trial. There was a high prevalence of malnutrition in the study population.	Longitudinal data extracted from an RCT [123] n=298 Urine and blood samples were collected every 3 mo and anthropometric measurements were collected monthly.	Urine Test: L:M Blood Tests: • **α-1-acid glycoprotein (AGP)** • **IgG** • **Albumin** • **Hemoglobin**	Mean[1] L:M: 0.15 L:M showed a decreasing trend with age (p=0.003), and was associated with female gender (p=0.004), HAZ score (p=0.039), and WAZ score (p=0.019), but not with giardiasis or any of the serum immune markers. IgG, AGP, and albumin were associated with giardiasis, but hemoglobin was not. Mean circulating albumin concentration was normal for age [203]. Compared to UK age-matched reference [199], rate of rise in IgG with increasing age was similar, but concentrations were consistently ~3g/L higher. IgG was not associated with growth parameters. Albumin was associated with HAZ score only (p=0.016). AGP was inversely associated with HAZ (p=0.011) and WAZ (p=0.005) scores	Mean L:M was elevated. L:M was not associated with any of the tested serum markers of inflammation or with giardiasis. IgG rose with increasing age at the rate expected (compared to UK norms) [199] but at higher concentrations across all ages.	Helminthiasis prevalence was very low; testing for association with markers was not performed. Giardiasis was defined as presence of a *Giardia*-specific IgM response. Same study population as reported by this group in another study also included in this review [122]. Cut-off values representing elevated concentrations have not been determined for AGP. UK norms for 10 mo olds-adults are 0.88 g/L mean (0.21 SD) [204].
2007 Jain S et al. Fecal occult blood screening in children with severe malnutrition Fecal occult blood	Delhi, India Children (ages unspecified) admitted with severe malnutrition and age-matched healthy controls recruited from an immunization clinic.	Case-control n=80; n=50 cases with severe malnutrition n=30 healthy controls	Stool Test: Occult blood Blood Test: **Hemoglobin**	Fecal occult blood test was positive in 30/50 (60%) cases and 0/30 controls. Among cases positive for fecal occult blood, 20 (66.7%) were found to have hemoglobin <8 g/dL. Enteric infections: • Parasitic infections were detected	A high proportion of severely malnourished children had a positive fecal occult blood test, compared with no positives among healthy controls.	Among cases, half had a presenting complaint of diarrhea (duration not specified), but the authors did not report results stratified by diarrhea duration.

[1] Geometric mean.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
among severely malnourished children compared to healthy controls				in 14/50 (28%) of cases, 12 (85.7%) of whom tested positive for fecal occult blood. • Bacterial infections were detected in 18/50 (36%) of cases, 13 (72.2%) of whom tested positive for fecal occult blood. • Of the remaining 18 for whom an enteric pathogen was not identified, 5 (27.8%) tested positive for fecal blood. Among the 30 cases with fecal occult blood, 16 were breastfed, 11 were fed cow's milk, and 3 were fed formula.	Malnourished children with identifiable pathogens more often tested positive for fecal occult blood, although approximately 25% of those without an identifiable pathogen also tested positive. Presence of fecal blood did not appear to vary by feeding mode (e.g. breast milk, cow's milk, or formula), although data presented were limited.	Authors did not provide differences in proportions of occult blood among those with and without specific enteric pathogens. Statistical analysis was not provided; data were reported as proportions only. Matching scheme was not defined.
2001 Kapoor S et al. Giardiasis--clinical and diagnostic perspective Immunoglobulin concentrations in duodenal fluid and serum among children with PD and *Giardia* infection compared to those without diarrhea	New Delhi, India <12 yr olds admitted to hospital with PD and *Giardia*. Controls had no diarrhea and were hospitalized for non-GI conditions. Most cases were <7 yr old, with n=19 <3 yr old. Ages of controls were not specified.	Case-control n=40; n=30 cases with PD and *Giardia* n=10 controls without diarrhea	<u>Duodenal secretion aspirates</u>: • IgG • IgM • IgA <u>Blood Tests</u>: • **IgG** • **IgM** • **IgA**	Higher mean concentrations of IgM were found in duodenal aspirates of cases compared to controls ($p<0.05$). Mean concentrations of duodenal IgA and IgG did not differ between cases and controls.	Differences in immunoglobulin concentrations were limited among children with PD infected with *Giardia* compared to children without such conditions.	The number of controls was small due to constraints in obtaining duodenal aspirate from children without GI symptoms.
2006 Kirkpatrick BD et al. Serum mannose-binding lectin	Port-au-Prince, Haiti <36 mo old inner-city residents recruited from the rehydration unit at the State	Case-control n=99; n=49 cases with *Cryptosporidium*	<u>Blood Test</u>: **Mannose-binding lectin (MBL)**	Serum MBL concentrations were lower in cases than in healthy controls ($p=0.002$) and diarrhea controls ($p=0.045$). Percentage MBL-deficient:	While cryptosporidiosis was associated with MBL deficiency, MBL concentrations were not significantly	MBL deficiency was defined as concentrations <u>≤</u>70 ng/mL.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
deficiency is associated with cryptosporidiosis in young Haitian children Mannose-binding lectin as a marker of innate immune activation among children with and without *Cryptosporidium* infection	University Hospital or from GHESKIO HIV Center[1]. All subjects were HIV-negative.	infection (22 with PD) n=9 diarrhea controls negative for *Cryptosporidium* n=41 healthy controls without diarrhea and *Cryptosporidium*-negative		• 36.7% cases • 9.8% healthy controls • 0 diarrhea controls Cryptosporidiosis was associated with MBL deficiency (odds ratio=22.4; CI: 3.1, 160.8[2]). Among cases, the proportion of those with PD was nearly double among those with MBL deficiency compared to those without MBL deficiency, but these results were not significant (p=0.13). MBL deficiency was not associated with duration of diarrhea (p=0.37) among those with cryptosporidiosis nor with anthropometric status among either cases or controls.	associated with mean duration of diarrhea or history of PD.	

2002
 	
Kirkpatrick BD et al.
 	
Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children
 	
Stool lactoferrin, reducing substances, leukocytes and cytokines as markers of intestinal inflammation of children with and without
	Port-au-Prince, Haiti
	
	<18 mo olds from a low SES setting recruited from the rehydration unit of GHESKIO HIV Center[3] with diarrhea and *Cryptosporidium* infection. Controls recruited from an outpatient clinic without *Cryptosporidium* infection included those with and without diarrhea.
		Case-control
		
		n=49;
		
		n=17 cases with *Cryptosporidium* and diarrhea (5 with PD)
		
		n=32 controls without *Cryptosporidium*;
		• 17 with diarrhea (5 with PD)
		• 15 healthy
			<u>Stool Tests</u>:
			• Reducing substances (RS)
			• Lactoferrin
			• Cytokines:
			  • TNF-α receptor I
			  • IL-4
			  • IL-8
			  • IL-10
			  • IL-13
			  • IFN-γ
			
			<u>Blood Test</u>:
			• **WBC**
				Proportion RS-positive:
				• 33.3% cases
				• 64.7% diarrhea controls
				• 46.7% healthy controls, (p=0.2)
				
				Proportion lactoferrin-positive:
				• 83.3% cases
				• 60.0% diarrhea controls
				• 28.6% healthy controls, (p=0.01)
				IFN-γ was not recovered in any stools.
				
				All other fecal cytokines were significantly associated with *Cryptosporidium* cases compared to diarrhea and healthy controls.
				
				Additionally, TNF-α receptor I, IL-8, IL-13 were found in diarrhea and healthy controls, while IL-4 and IL-10
					Fecal lactoferrin was identified most often in children with diarrhea, especially in those with *Cryptosporidium.* While some fecal cytokines were detected in as many as 40% of healthy controls and 70% of controls with diarrhea, they were generally associated with *Cryptosporidium* infection. The other stool tests did not discriminate by diarrhea or	Reported results were not stratified by persistent vs. acute diarrhea status.
						
						Cut-off values for lactoferrin positivity were not described.
						
						Stools from children who were breastfeeding were not tested for lactoferrin.

[1] The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections.
[2] Reported results were adjusted for confounding variables, unless otherwise noted.
[3] The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Cryptosporidium infection				were not. Fecal lactoferrin was associated with the presence of TNF-α receptor I (point estimate not provided, p=0.03). Mean WBC counts were within normal range in all 3 groups.	*Cryptosporidium* status.	
2003 Kukuruzovic R et al. Increased nitric oxide production in acute diarrhea is associated with abnormal gut permeability, hypokalemia and malnutrition in tropical Australian aboriginal children Nitric oxide (NO) as a marker of intestinal permeability and inflammation, and lactulose:rhamnose ratio (L:R) as a marker of intestinal permeability and the relationship between NO and L:R, growth parameters, mean corpuscular volume (as a surrogate of iron deficiency), and stool reducing substances among children with and without diarrhea	Darwin, Australia 1-6 yr old Aboriginal and non-Aboriginal hospital inpatients. Subjects were grouped as follows: • Children with AD • Children with no diarrhea but with non-GI infectious conditions • Children without GI or infectious conditions	Case-control n=318; n=169 cases with AD (154 Aboriginal) n=149 controls: • 73 with non-GI infections (49 Aboriginal) 76 with no infections (29 Aboriginal)	Urine Test: **Nitric Oxide (NO)*** Blood Tests: • L:R • **Mean corpuscular volume (MCV)** Stool Test: Reducing substances (RS)** (169 cases tested) * NO is an unstable free radical and is converted to nitrite and nitrate. Urine nitrate (NO_3)+ nitrite (NO_2) was expressed as a ratio with urine creatinine (NO_2 + NO_3:Cr) in order to account for differences in urine concentration. ** Measured only among children with profuse diarrhea.	NO among Aboriginal children with diarrhea was >3x higher than any other group and >5x higher than in non-Aboriginal controls. • NO was >3x and >2x higher among Aboriginal than non-Aboriginal children in the diarrhea (p<0.001) and no infections groups (p<0.001), respectively, but there was no difference between them in the non-GI infections group. • NO was >3x and ~2x higher in the diarrhea compared to the no infections group among Aboriginals (p<0.001) and non-Aboriginals (p<0.03), respectively. • NO was virtually the same among the Aboriginal non-GI infections and no infections groups, as well as among the non-Aboriginal diarrhea and non-GI infections groups. 112/152 (74%) and 31/169 (18%) of children with AD had abnormal L:R ratios and positive stool RS, respectively. NO and L:R were measured at "convalescence" on Day 5 among those with diarrhea: the mean improvement in NO was 21.7%	NO_2 + NO_3:Cr ratio, as a measure of endogenous nitric oxide production, was used as a marker of gut permeability and inflammation, with an attempt to identify how much more it reflects as response to inflammation from GI vs. non-GI infections. Among non-Aboriginal controls, NO production was the same among those with diarrhea and non-GI infections (and higher compared to controls). NO was highest by far among Aboriginal children with diarrhea compared to any other group. Authors suggest that high basal concentrations of NO among Aboriginal children	Positive stool RS was defined as ≥0.5%. Abnormal L:R was defined as >7.6; no reference or derivation was provided for this cut-point. Study population appears to be the same as in another Kukuruzovic, et al. study also included in this review which assessed serum lactulose:rhamnose as a marker of intestinal permeability [58].

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				compared with 54.6% for L:R (p=0.01). NO and L:R were correlated (n=193, r=0.37, p<0.001)[1]; the correlation was stronger for lactulose (effect ratio=1.47, p<0.001) than for rhamnose (effect ratio=0.80, p=0.02[2]). NO was not correlated with stool RS[3] or MCV, but was correlated with lower WAZ score (effect ratio=0.88, p=0.05).	due to (clinically silent) enteropathy could explain the concentrations seen among Aboriginal controls in this study. NO appeared to decrease significantly more slowly than L:R among children recovering from diarrhea. NO was found to correlate with L:R. NO was more strongly correlated with lactulose than rhamnose.	
2002 Kukuruzovic RH et al. Small bowel intestinal permeability in Australian Aboriginal children Serum lactulose: rhamnose ratio (L:R), serum lactose, and stool reducing substances as markers of intestinal	Darwin, Australia Cases were Aboriginal and non-Aboriginal children admitted to hospital with diarrhea. Controls were Aboriginal and non-Aboriginal children admitted without GI illnesses.	Case-control n=375 admissions for 306 children; n=285 case admissions for AD (264 Aboriginal) n=90 control admissions with no diarrhea (74 Aboriginal)	<u>Blood Tests</u>: • Lactose • Lactulose[4] • Rhamnose • L:R • **Hemoglobin** • **Mean corpuscular volume (MCV)** <u>Stool Test</u>: Reducing substances (RS)* L:R testing was	27/75 (36%) of Aboriginal controls and 0 non-Aboriginal controls had abnormal L:R ratios. Mean[5] L:R at baseline: Cases: • Aboriginal: 16.4 • Non-Aboriginal: 7.9, p=0.002 compared to Aboriginal cases Controls: 1.Aboriginal: 4.6 2.Non-Aboriginal: 2.5, p=0.02 compared to Aboriginal controls Mean improvement[1] in L:R (CI) at day 5 among those with repeat testing:	Mean L:R ratios of Aboriginal children were approximately double those of non-Aboriginal children both among those with and without diarrhea, consistent with authors' suggestion that clinically silent enteropathy is prevalent among Aboriginal children. Mean L:R significantly	Positive stool RS was defined as ≥0.5%. Abnormal L:R was defined as >5.6, derived from 2 SD above the arithmetic mean for non-Aboriginal controls in this study. The rationale for the choice of 2 SD above the arithmetic, instead of the geometric, mean is not clear.

[1] Reported results appear to have been adjusted for age and race.
[2] Reported results were adjusted for age and race.
[3] Reported results among children with diarrhea were adjusted for age and race.
[4] Lactulose and rhamnose results were expressed as % of dose administered.
[5] Geometric mean.

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permeability among Aboriginal and non-Aboriginal children with and without diarrhea			repeated on day 5 for a subset of Aboriginal subjects: • 174/264 admissions for acute diarrhea • 25/74 control admissions * Measured only among children with profuse diarrhea when “clinically indicated." Number tested not provided.	• Aboriginal cases: 14.6 (11.2, 18.0) • Aboriginal controls: -0.63 (-4.0, 2.7) Mean lactulose recovery[2]: • Cases day 1: 0.085 (0.070–0.103) • Cases day 5: 0.039 (0.033–0.046) • Controls: 0.024 (0.019–0.029) All 3 values significantly differed from one another. Mean rhamnose recovery: • Cases day 1: 0.479 (0.424–0.542) • Cases day 5: 0.555 (0.498–0.616) • Controls: 0.585 (0.500–0.685) These values did not significantly differ from one another. Confidence intervals (CIs) in the authors' graphical representation of mean L:R at admission did not overlap, and the difference in means was particularly evident between Aboriginal and non-Aboriginal subjects. Factors associated with L:R among cases were[3]: • Acidosis (p=0.007) • Hypokalemia (p=0.035) • Diarrhea severity (p=0.001) Age and malnutrition were not associated with L:R.	improved over 5 days among Aboriginal cases. Children with severe diarrhea had higher mean L:R. Higher case L:R was driven more by high lactulose than by low rhamnose. Similarly, improvement in L:R among cases was primarily due to decreased lactulose. Stool RS and serum lactose were found in approximately one-quarter and one-third of Aboriginal cases, respectively. The latter was weakly associated with increased lactulose.	Proportions of cases with abnormal concentrations were not reported. Analysis included data for 69 children with repeat admissions; this might violate independence assumptions for their statistical analysis methods. Repeat L:R testing was conducted on controls of both racial groups, but among cases it was only conducted on Aboriginal cases. This study appears to report on the same population as in the Kukuruzovic, et al. 2003 reference also included in this review, which assessed nitric oxide excretion [43]. Authors reiterate the advantages of serum over timed

[1] Improvement in L:R appears to have been calculated as baseline L:R minus repeat L:R, as described in another publication in this review; however, this was not expressly stated. Reference 134. Kukuruzovic RH, Brewster DR. Milk formulas in acute gastroenteritis and malnutrition: a randomized trial. J Paediatr Child Health. 2002. **38**(6):571-577.
[2] Figures reported parenthetically after the mean percent recoveries of lactulose and rhamnose were not specified as ranges or CIs.
[3] Reported results were adjusted for confounding variables, unless otherwise noted.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				38% and 27% of Aboriginal cases had positive serum lactose and stool RS, respectively. 12% of Aboriginal and non-Aboriginal controls combined had lactosemia. Presence of lactosemia was associated with L:R, adjusted relative risk (CI)=1.06 (1.03, 1.10)[1]. Stool RS, anemia, and MCV were not associated with L:R.		urine collection for assessment of L:R, as discussed in another publication in this review [125].
2001 Mittal SK et al. Tropical sprue in north Indian children D-xylose and duodenal biopsy as markers of TS	New Delhi, India 0-15 yr old gastroenterology clinic patients with PD. Those with abnormal morphology on biopsy, abnormal D-xylose test, and clinical response to antibiotics were diagnosed as having TS. Those with abnormal morphology and response to gluten-free diet were diagnosed with CD. We include data on these subjects for comparative reasons.	Case-series n=94; (38 with repeat biopsies) <5 yr old: n=44	<u>Duodenal biopsy, method not specified:</u> Histopathology <u>Blood Tests</u>: • **Hemoglobin** • D-xylose***** * Not specified whether from urine or serum, and units of measurement not provided.	36 (38.3%) were diagnosed with TS including 14/44 (31.8%) who were under 5 years of age. 18 (19.1%) were diagnosed with CD. Degree of villous atrophy among TS vs. CD patients: • Mild in 8/36 (22.2%) vs. 0 • Moderate in 23/36 (63.9%) vs. 4/18 (22.2%) • Severe in 5/36 (13.9%) vs. 14/18 (77.8%) Mean hemoglobin concentration (range) among TS patients was 8.3 g/dL (5.5-11) and did not differ from values of those with CD. Among the 22 TS patients, repeat biopsies showed: • 16 with normalization • 5 with improvement • 1 worsened despite marked clinical improvement The D-xylose test was abnormal in all TS patients by diagnostic definition.	More than half of the GI clinic patients with PD had some degree of villous atrophy. More than one-third and almost one-fifth of subjects were diagnosed with TS and CD, respectively. By study diagnostic definition, all TS patients improved with treatment. Among those who had repeat biopsies, almost three-quarters showed normalization of histology, while 23% had partial improvement and 1 patient had worsened pathology.	Biopsy results were not provided for patients without TS or CD. It was unclear if there were patients with abnormal D-xylose and histology who did not respond to antibiotic therapy and therefore were not diagnosed with TS. Cut-off points used to define abnormal D-xylose tests were not provided.

[1] Reported results were adjusted for severity of diarrhea, acidosis, hypokalemia, and age.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2001 Northrop-Clewes CA et al. Anthelmintic treatment of rural Bangladeshi children: effect on host physiology, growth, and biochemical status L:M as a marker of intestinal permeability, and α 1-antichymotrypsin as a marker of inflammation and immune activation to treatment among children randomized to bimonthly antihelminthics or placebo.	Jamalpur district, northern Bangladesh 2-5 yr olds from poor rural villages, sampled randomly from a larger cohort study. Stools were assessed for helminthiasis and giardiasis. Growth was followed longitudinally.	RCT* n=109; n=54 received bimonthly empiric antihelminthic treatment n=55 received placebo * Randomized at the village level.	Blood Tests: • **α 1-antichymotrypsin (ACT)** • **Albumin** • **Total protein** Urine Test: L:M Among 93 subjects with L:M at baseline: • 46 received treatment • 47 received placebo Among 66 subjects with repeated L:M testing: • 34 received treatment • 32 received placebo	Mean L:M[1] at baseline: • Treatment: 0.22 • Placebo: 0.25 Seasonal variation in L:M was observed, with highest values following the monsoon season. Within-subject L:M analysis showed no significant association with intestinal helminthiasis and no significant improvement in treatment or placebo groups over 1 yr. L:M was generally not associated with giardiasis (with the exception of one group at one study interval). L:M was inversely correlated with ΔHAZ and ΔWAZ scores at some of the follow-up intervals (r=-0.22, p<0.02 and r=-0.21, p<0.05, respectively, at 12 mo follow-up visit). Mean serum ACT, albumin and total protein were within normal ranges and were not associated with growth parameters. ACT and albumin concentrations did not significantly change with treatment, whereas total protein concentrations did (p<0.001).	L:M ratios were high overall and demonstrated seasonal variation. Intra-individual L:M values did not change significantly over time, nor were they associated with helminthiasis or consistently associated with giardiasis. Inverse correlations were seen between L:M and growth parameters. Serum markers were within normal range. The only significant change in these markers was a decrease in total protein in the treatment group without concomitant change in albumin; this suggested a decrease in globulins (not directly measured), perhaps due to decreased inflammation.	Baseline study data were lost, so analysis began with samples taken at month 2. The relationship between the serum markers and intestinal permeability was not reported.
2009 Panter-Brick C et al.	Kathmandu, Nepal 3-18 mo olds in two	Cohort n=86;	Urine Test: Lactose:Cr	Mean[2] Lactose:Cr (CI): • Squatter: 0.14 (0.12, 0.16) • Middle Class: 0.08 (0.07, 0.10)	Authors speculate that Lactose:Cr accounted for less of	Specific sugar excretion was normalized to

[1] Geometric mean.
[2] Geometric mean.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Pathways leading to early growth faltering: An investigation into the importance of mucosal damage and immunostimulation in different socio-economic groups in Nepal Lactose:creatinine ratio (Lactose:Cr) as a marker of intestinal permeability and hemoglobin, albumin, α-1-acid glycoprotein, and IgG as markers of immunostimulation. The latter were also assessed for their relationship to nutritional status.	cohorts: • All children in target age range from four squatter settlements • Randomly selected, age-matched cohort from lower middle-class, periurban households	n=48 in squatter cohort n=38 in lower middle-class cohort	Blood Test: **Hemoglobin**	• Statistically significant difference between the 2 groups among the 6-12 mo olds (p=0.007) and 18-24 mo olds (p=0.002), but not among 12-18 mo olds. • For both SES groups, Lactose:Cr values decreased with increasing age (p<0.001). HAZ, WAZ, WHZ, and ΔWAZ scores were strongly associated with mean Lactose:Cr (p<0.001 each) as was ΔHAZ score (p=0.004); ΔWHZ score was not. The strength and magnitude of association between ΔWAZ score and Lactose:Cr was most pronounced among the wealthier cohort and there was no association between ΔHAZ score and Lactose:Cr among the squatter children. Hemoglobin concentrations were inversely related to Lactose:Cr (r^2=0.018, p<0.001).	the deterioration in nutritional status among the squatter children because of several factors, including poorer nutritional intake, that impact the nutritional status of children with lower socio-economic status.	urinary creatinine to control for variation in renal function. Authors suggest that while Lactose:Cr might not be as accurate as L:M, it might be a more field-friendly assessment of mucosal damage compared to L:M, requiring only spot urine collection and no substrate dosing. However, L:M was not assessed in this study; direct comparison of the two tests was not possible. While hemoglobin concentration was inversely related to Lactose:Cr, testing for associations of other measured blood markers (IgG, AGP and albumin) with Lactose:Cr was not reported.
2001 Rabbani GH et al. Increased nitrite and nitrate concentrations in sera and urine of patients with cholera	Dhaka, Bangladesh 2-6 yr olds with cholera or shigellosis admitted to the hospital. Controls were	Case-control n=63; n=45 cases: • 24 with cholera • 21 with shigellosis	Urine Test: **Nitric Oxide (NO)*** Blood Tests: • **Nitrite (NO_2)** • **Nitrate (NO_3)** • **WBC**	In children with shigellosis, median serum NO was ~8x higher at baseline than in controls and significantly differed from convalescent concentrations (p<0.01). Concentrations declined by 52% of baseline during the recovery period but did not return to values found in the controls (measure of	NO as measured by both serum and urinary NO_2 and NO_3 concentrations was significantly elevated at presentation during acute illness compared to 7-10 days after	Some values reported in table format conflict with the text; columns of data appear to be transposed. Assessment for NO correlation with

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or shigellosis To assess and compare nitric oxide as a marker of intestinal inflammation among children with cholera or shigellosis or healthy controls. Evaluated to assess nitric oxide production during infection of small bowel without inflammatory lesion (e.g., cholera) and during infection causing colon inflammation (e.g., shigellosis).	recruited from the healthy attendants of patients or from children of hospital staff. Mean age (SD) in yr: • Shigellosis cases: 3.8 (1.2) • Cholera cases: 4.2 (1.4) • Controls: 4.7 (1.8)	n=18 healthy controls Samples were collected from cases on admission and upon discharge (after 7-10 days of treatment).	<u>Stool Test</u>: Leukocytes * Nitric oxide (NO) is an unstable free radical that is converted to nitrite and nitrate. Urine NO_2 + NO_3 were expressed as a ratio with urine creatinine in order to account for differences in urine concentration.	statistical significance not reported). In children with cholera, median serum NO concentrations at baseline were ~4x higher than in control subjects. Recovery concentrations decreased 52% from baseline ($p<0.01$); convalescent values did not differ from the values in controls ($p<0.4$). Median urinary NO ratios were similar among those with *Shigella* and *V. cholerae* infection, both upon admission and discharge. Initial values were ~2x higher than upon discharge ($p<0.05$ and 0.01, respectively). Control median NO was of an intermediate concentration between cases' admission and discharge median concentrations; the difference between control and case admission values was NS. Mean blood WBC counts (SD): • Shigellosis: 19.6 (3.3) • Cholera: 8.3 (2.8) • Controls: 7.1 (1.8) Mean fecal WBC/high power field (SD): • Shigellosis: 38 (17) • Cholera: 5 (2) • Controls: 3 (1) Serum NO correlated with blood WBC count in shigellosis cases at baseline ($r^2=0.92$, $p<0.01$), but only to a slight degree upon discharge ($r^2=0.26$, no p-value reported) and there was no correlation among the cholera cases. Serum NO correlated with stool volume at presentation	hospitalization in both cholera and shigellosis. Median serum NO concentrations in cholera patients were ~half of those with shigellosis both upon admission and upon discharge and concentrations were much higher in cases than in controls, Such striking differences were not observed for urinary NO results. Serum NO concentrations correlated with total blood WBC in shigellosis cases.	fecal leukocyte counts was not reported, nor was the correlation between urinary NO and total blood WBC.

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				(r^2=0.85, statistically significant per authors, p-value not reported).		
2009 Ritchie BK et al. 13C-sucrose breath test: novel use of a noninvasive biomarker of environmental gut health Sucrose breath test (SBT) as a marker of small bowel mucosal damage vis a vis sucrase activity among an Australian Aboriginal population. Also compared SBT with serum lactulose:rhamnose ratio (L:R)	Darwin and Adelaide, Australia 4 mo-5 yr old Aboriginal children admitted to hospital with diarrhea. Two control groups: • Aboriginal controls admitted to hospital with non-GI symptoms (50% had pneumonia) Healthy, non-Aboriginal controls recruited from community	Case-control n=43; n=18 Aboriginal cases with AD n=25 controls: • 18 Aboriginal, without diarrhea • 7 non-Aboriginal, healthy	Blood Tests: • L:R (32 Aboriginal cases and controls tested) • **C-reactive protein (CRP)** • **Mean Corpuscular Volume (MCV)** • **Hemoglobin** Breath Test: ^{13}C sucrose breath test (SBT)	20/32 (63%) of Aboriginal children had abnormal L:R ratios. Mean[1] L:R (CI): • Diarrhea cases: 31.8 (24.9, 40.7) • Aboriginal controls without diarrhea: 11.4 (8.5, 15.5), • significant difference (p<0.0001) SBT Mean (CI): • Diarrhea cases: 1.9% (0.9, 3.0), p<0.0001 compared to non-Aboriginal controls and p=0.004 compared to Aboriginal controls • Aboriginal controls: 4.1% (3.0, 5.2), p=0.032 compared to non-Aboriginal controls • Non-Aboriginal controls: 6.1% (4.8, 7.3) • Significant differences were observed between all three groups. SBT results were not associated with wasting or with patient age or breastfeeding status. SBT and L:R were inversely correlated (r=0.67; CI: 0.42, 0.62; p<0.0001). L:R explained 45% of the variance in SBT; diarrhea explained 28% of variance. SBT was associated with increased MCV, relative risk (CI)=3.9 (2.8, 5.0).	SBT values were significantly lower and L:R values were significantly higher among Aboriginal children with diarrhea than among those without GI symptoms. SBT was also significantly lower among Aboriginal controls than among non-Aboriginal children without diarrhea. This is consistent with previous reports of high prevalence of clinically silent TE in this population. SBT was significantly inversely correlated with L:R.	Abnormal L:R ratios were defined as >16; no reference or derivation was provided for this cut-point. L:R test was not conducted among the non-Aboriginal controls. SBT/L:R correlation analysis was based on data for Aboriginal cases and controls combined; stratified analysis was not reported and could be of interest considering the large difference in L:R observed between these groups. Associations of MCV, CRP, and hemoglobin with SBT after adjusting for potentially confounding variables were not reported.

[1] Geometric mean.

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				SBT was not associated with hemoglobin or CRP.		

2001
 	
Rollins NC et al.
 	
Feeding mode, intestinal permeability, and neopterin excretion: A longitudinal study in infants of HIV-infected South African women
 	
L:M as a marker of gut mucosal integrity and urinary neopterin excretion as a marker of cell-mediated immunity in infants with and without HIV infection
	Durban, South Africa
	
	1, 6, and 14 wk old infants born to HIV-infected mothers.
		Cohort
		
		n=272
			Urine Tests:
			• Lactulose[1]
			• Mannitol
			• L:M
			• **Neopterin**
				Mean[2] L:M (CI):
				• HIV-infected subjects:
				  • 1 wk: 0.12 (0.06, 0.27)
				  • 6 wk: 0.24 (0.15, 0.38)
				  • 14 wk: 0.24 (0.14, 0.44)
				• Uninfected subjects:
				  • 1 wk: 0.13 (0.09, 0.19)
				  • 6 wk: 0.08 (0.06, 0.11)
				  • 14 wk: 0.09 (0.07, 0.13)
				
				HIV-infection by 14 wk of age was significantly associated with increased L:M.
				
				A non-significant, positive trend in neopterin excretion was observed among HIV-infected infants.
					L:M was generally normal (compared to UK values) for non-HIV-infected infants, but significantly increased among HIV-infected subjects, especially after 6 weeks.
					
					The increased L:M in HIV-infected infants was primarily driven by lactulose rather than mannitol.
					
					Higher neopterin excretion by HIV-infected infants was observed but this was not statistically significant.
						Assessment of association between L:M and neopterin was not reported.

2000
 	
Rollins NC et al.
 	
Vitamin A supplementation of South African children with diarrhea: optimum timing for improving biochemical and clinical recovery and subsequent vitamin A status
	Durban, South Africa
	
	6-60 mo old inpatients or outpatients with severe diarrhea.
		RCT
		
		n=139;
		
		n=66 received vitamin A on admission (group 1)
		
		n=73 received vitamin A after clinical improvement (group 2)
			Urine Tests:
			• Lactulose[3]
			• Mannitol
			• L:M
			• **Neopterin**
			
			Blood Tests:
			• **C-reactive protein (CRP)**
			• **α-1 acid glycoprotein (AGP)**
				Mean[4] L:M:
				• Group 1:
				  • Day 0: ~1.8
				  • Day 3: ~2.4
				• Group 2:
				  • Day 0: ~1.2
				  • Day 3: ~0.7
				
				There were no differences in mean L:M between groups or within groups between days 0 and 3, although there was a significant difference in paired analysis within individuals at the two time points (data not
					Mean L:M ratios were very high (~10x) (at baseline and at day 3 in both groups) compared to other studies in this review. Study authors suggested (via personal correspondence) that this could have been due to the severity of illness in the sample	Urine testing could only be conducted in the laboratory on certain days; hence only a subset of subjects underwent those tests.
						
						Group 2 patients had significantly higher CRP, non-significantly higher WBCs and AGP, and lower retinol

[1] Lactulose and mannitol results were expressed in mg.
[2] Geometric mean.
[3] For lactulose, mannitol, and neopterin results, excretion measurement was not specified.
[4] Geometric mean.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
Biomarkers in bold are primarily markers of systemic inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
L:M as a marker of intestinal permeability and urinary neopterin, serum α-1 acid glycoprotein, and C-reactive protein as markers of inflammation among children with severe diarrhea		Treatment involved vitamin A supplementation either on the day of admission or after acute diarrheal symptoms had resolved.	49 subjects received urine testing: • Group 1: n=25 • Group 2: n=24 Blood and urine were tested on days 0 and 3.	presented, and direction, magnitude and degree of significance not reported). Lactulose and mannitol excretion were assessed only in the paired analysis. Lactulose excretion decreased between days 0 and 3 (magnitude of effect and degree of significance not reported), while mannitol excretion showed no change. Mean[1] neopterin and AGP concentrations did not differ between groups or within groups on the different study days or in the paired analysis. When initial CRP (~2x higher in Group 2 compared to Group 1, p<0.004) was taken into account, mean CRP on day 3 did not differ between the 2 groups. However in the paired analysis, CRP concentrations were significantly different between days 0 and 3.	population (children hospitalized for diarrhea). Vitamin A administration did not result in significant improvement in L:M, neopterin, or AGP regardless of timing of vitamin A administration.	and retinol-binding protein concentration compared to group 1 at baseline. Authors note that these parameters suggest that Group 2 patients might have been more ill at baseline. For the subset of 49 patients undergoing urine testing, the mean[2] L:M and neopterin concentrations were lower among Group 2 than Group 1 subjects (NS). However, baseline differences in acute phase and vitamin A markers at baseline were not reported separately for these 49 subjects. Data for lactulose and mannitol excretion were not reported separately. Rationale for additional analyses of these molecules expressed as ratios with creatinine was not explained.

[1] Geometric mean.
[2] Geometric mean.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
						Authors suggest that their 3-day testing period (based on their previous work in a different setting [207] might have been too short to identify effect as demonstrated by McCullough et al. at 10 days after presentation [208].

2003
 	
Soliman SM et al.
 	
Role of micronutrient mixture in acute and persistent diarrhea in infants and its impact on nutritional status
 	
Blood cell and albumin markers among infants with acute, persistent or no diarrhea, and effects on these markers after 10 days of supplementation with micronutrient mixture (containing vitamin A, zinc and other micronutrients) among subjects with and without diarrhea
	Cairo, Egypt
	
	6-24 mo olds with diarrhea were recruited from Al-Sahel teaching hospital malnutrition clinic.
	
	5/6 PD cases and 10/14 AD cases had some degree of malnutrition.
	
	Infants with PD had significantly lower vitamin A and zinc stores compared to controls. Those with AD had significantly lower vitamin A stores.
		Case-control
		
		n=30;
		
		n=20 cases:
		• 6 with PD
		• 14 with AD
		
		n=10 healthy controls*
		
		* Controls were age- and sex-matched to cases.
			Blood Tests:
			• **Complete blood count and differential**
			• Red cell measures:
			  • **Mean corpuscular volume (MCV)**
			  • **Mean corpuscular hemoglobin (MCH)**
			  • **Mean corpuscular hemoglobin concentration (MCHC)**
			  • **Transferrin saturation**
			• **Albumin**
				Mean baseline hemoglobin was significantly lower in infants with AD and PD ($p<0.05$ for each group) than in controls
				
				Mean MCV and MCH were lower in those with AD ($p<0.025$ and $p<0.01$, respectively) and PD ($p<0.01$ for both markers) compared to controls.
				
				Mean lymphocyte counts among PD cases were low compared to those of controls ($p<0.01$).
				
				Other markers did not vary significantly at baseline. However, among infants with PD, mean albumin was abnormally low, although it was not significantly different compared to controls or those with AD.
				Mean albumin (g/dL) (SE):
				• PD: 2.9 (0.27)
				• AD: 3.29 (0.25)
				• Controls: 3.37 (0.21)
				
				Following micronutrient supplementation, mean hemoglobin, MCV, lymphocyte counts and albumin increased in both diarrhea
					Albumin and many hematologic markers were low at baseline among infants with diarrhea, especially in those with PD, compared to those without diarrhea; some of these differences were statistically significant. Parameters generally normalized after micronutrient supplementation.	Information on control recruitment and anthropometrics were not specified.
						
						Sample size was small when stratified by case/control groups, especially for PD cases (n=6). Controls were reported to have been matched to cases, yet there were half the number of controls than cases and statistical testing (student's t-test) was not commensurate with matched case-control methodology.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				groups to concentrations on par with control baseline concentrations (albeit increases were NS except within the PD group). MCH improved to concentrations on par with the control group only among the infants with AD.		
2008 Vieira MM et al. Carotenoids, retinol, and intestinal barrier function in children from northeastern Brazil L:M as marker of intestinal barrier function, fecal lactoferrin and leukocytes as markers of intestinal inflammation, and CRP and AGP as acute phase reactants among children with varying vitamin A status	Fortaleza, Brazil 2 mo-9 yr olds (mean age 41 mo) from an impoverished urban community, eligible if HAZ score <median for their community.	Cross-sectional n=102	Urine Tests: • Lactulose[1] • Mannitol • L:M (97 tested) Stool Tests: • Lactoferrin (93 tested) • Leukocytes Blood Tests: • **C-reactive protein (CRP)** • **α-1-acid glycoprotein (AGP)**	48.5% had abnormal L:M. L:M and excretion of each sugar separately did not vary with retinol concentration. L:M was associated with levels of common dietary carotenoids, primarily driven by lactulose. However, the association was not always statistically significant, and the direction of association varied depending on precursor. 40% of stool samples were positive for lactoferrin. 1% of stool samples were positive for fecal leukocytes. 30% of stool samples were positive for parasites but this had no impact on L:M results, lactoferrin, or acute phase reactants.	Almost half of subjects had increased L:M, and ~40% of subjects had increased lactoferrin. While serum retinol concentrations were not associated with L:M, serum carotenoids were; authors suggest that these retinol precursors might be more sensitive predictors of impaired intestinal function. However, the reported direction of association varied, making interpretation of these results unclear.	L:M threshold for abnormal values was defined as >0.0864 [214]. Cut-off values for lactoferrin positivity were not described. Relationships between acute phase proteins and measures of intestinal permeability or inflammation were not reported. Relationships between L:M and lactoferrin or fecal leukocytes as well as those between retinol or carotenoids and lactoferrin or fecal leukocytes were not reported. Exclusively breastfed children were excluded from study participation due to assessment of stool lactoferrin.

[1] Lactulose and mannitol results were expressed as % of dose administered.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
Biomarkers in bold are primarily markers of systemic inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

2007
 	
Williams EA et al.
 	
A double-blind, placebo-controlled, glutamine-supplementation trial in growth-faltering Gambian infants
 	
L:M and plasma immunoglobulins and acute phase reactant proteins (albumin, C-reactive protein, and alpha-1-antichymotrypsin) in community-based Gambian infants enrolled in a glutamine trial
	West Kiang region, Gambia
	
	4-10 mo olds from a rural area followed during the 5-month rainy season and for 6 months afterward.
		Cohort
		
		n=72
		
		Glutamine or placebo of nonessential amino acids was orally administered twice daily during rainy season; L:M ratio was measured monthly, and plasma samples were collected 3 times.
			<u>Urine Tests</u>:
			• Lactulose[1]
			• Mannitol
			• L:M
			
			<u>Blood markers</u>
			• **C-reactive protein (CRP)**
			• **Alpha-1 anti-chymotrypsin (ACT)**
			• **IgA**
			• **IgG**
			• **IgM**
			• **Albumin**
				Mean[2] L:M (CI):
				• Baseline:
				  • Glutamine group: 0.33 (0.25, 0.43)
				  • Placebo group: 0.33 (0.26, 0.41)
				• Post-intervention:
				  • Glutamine group: 0.29 (0.23, 0.35)
				  • Placebo group: 0.26 (0.21, 0.32)
				
				Mean excretion of lactulose (CI):
				• Baseline:
				  • Glutamine group: 0.21 (0.16, 0.28)
				  • Placebo group: 0.20 (0.15, 0.26)
				• Post-intervention:
				  • Glutamine group: 0.17 (0.13, 0.21)
				  • Placebo group: 0.14 (0.11, 0.18)
				
				Mean excretion of mannitol (CI):
				• Baseline:
				  • Glutamine group: 2.65 (2.02, 3.48)
				  • Placebo group: 2.50 (1.87, 3.36)
				• Post-intervention:
				  • Glutamine group: 2.48 (1.99, 3.11)
				  • Placebo group: 2.14 (1.62, 2.82)
				
				L:M values did not differ significantly between treatment groups before or following intervention. However, a
					L:M values were elevated in this population, with no significant change after the intervention.
					
					None of the plasma markers differed significantly between treatment and placebo groups, either at baseline or at the end of supplementation.
					
					Growth outcomes did not differ significantly across treatment groups.
						The relationships between L:M and growth parameters, immuno-globulins, and acute phase proteins were not reported.

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Geometric mean.

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
Biomarkers in bold are primarily markers of systemic inflammation and/or immune activation.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				repeated measures ANOVA showed that during supplementation, L:M values were borderline elevated among the glutamine-supplemented group relative to the placebo group ($p=0.05$), counter to expectation. Neither ACT, CRP, albumin, nor immunoglobulins IgA, IgG, or IgM differed significantly between treatment and placebo groups, either at baseline or at the end of supplementation. Mean levels of IgA and IgG increased during the study ($p<0.001$), while IgM levels did not. Concentrations of each of these immunoglobulins did not differ between treatment and placebo groups. Plasma albumin, ACT, and CRP values showed no change over the course of the study. Proportions of children with elevated CRP ranged from 30-41% at different collection time points. The glutamine intervention had no effect on proportion of children with elevated CRP. Treatment and placebo groups experienced decreases in WAZ, HAZ, and MUAC coinciding with the rainy season; however, there was no significant difference observed between the groups for any of these parameters. Treatment and placebo groups did not differ in morbidity indices (i.e.		

Evidence Table 5. Markers of systemic inflammation and systemic immune activation.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				percentage of time reported with a particular illness or illness overall).		

Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting.

Abbreviations: AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, Δ=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)

5.7 Markers of Microbial Drivers

Markers of microbial environments, such small bowel bacterial overgrowth (SBBO), were reviewed, while markers for specific enteric organisms were beyond the scope of this review. The data from each of the studies relevant to this review are listed in Evidence Table 6.

Evidence Table 6. Markers of microbial drivers.
Biomarkers in bold are primarily markers of microbial drivers.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2002 Alves GM et al. Nutritional status and breath hydrogen test with lactose and lactulose in Terena Indian children Lactose hydrogen breath test (HBT) as a marker of lactase activity, and lactulose HBT as a marker of SBBO	Limão Verde and Córrego Seco, Mato Grosso du Sul, Brazil All children <10 yr old were recruited from these rural villages.	Cross-sectional n=264; <5 yr old: n=145 (However results were provided by <4 and ≥4 yr old age groups.)	Breath Tests: • Lactose HBT (251 tested) • **Lactulose HBT** (252 tested)	Lactose HBT: • Elevated: 27.1% among all subjects • Borderline: 43.0% among all subjects • 0% of subjects <4 yr had elevated or borderline results Lactulose HBT positive: • 11.5% of all subjects • 8.6% of subjects <4 yr	The prevalence of lactase deficiency as measured by lactose HBT was >25%, but non-existent among those <4 yr of age. Prevalence of SBBO as assessed by lactulose HBT was ~10%.	Assessment of association between lactulose and lactose absorption was not reported.
2000 Fagundes-Neto U et al. Studies of the small bowel surface by scanning electron microscopy in infants with persistent diarrhea Scanning electron microscope (SEM) and light microscope (LM) analyses of small intestinal biopsy among infants with PD with and without SBBO	Sao Paulo, Brazil 2-10 mo olds with PD and protein calorie malnutrition consecutively admitted to Sao Paulo Hospital.	Case-series n=16	Jejunal secretions aspirate: **Bacterial concentrations** Jejunal tethered capsule biopsy: Histopathology by LM and SEM Rectal tethered capsule biopsy: Histopathology	68.7% had bacterial overgrowth (concentration >10^4 colonies/mL): 3 had enteropathogenic *E. coli* while the rest had colonic microflora. All small intestine specimens had morphological abnormalities on LM: • 43.7% moderate villous atrophy • 56.3% subtotal villous atrophy SEM revealed abnormalities of varying intensity: • Among the 11 with SBBO, villous atrophy ranged from Grade II (n=4), Grade III (n=2), to Grade IV (n=3). • For the 5 subjects without SBBO, villous atrophy ranged from Grade I (n=1) to Grade 2 (n=4). • A mucous-fibrinoid pseudo-membrane over enterocytes was noted in 7 of the 11 with SBBO and none of the others. • Other abnormalities noted on SEM included: • Mucus and debris covered large	Histological abnormalities were noted in all subjects by LM and SEM. Degree of villous atrophy noted on SEM seemed to be correlated with SBBO (no statistical tests were reported). Authors speculate that the mucous-fibrinoid pseudo-membrane partially covering enterocytes is consistent with a malabsorptive process, with the findings of fat droplets on enterocytes surfaces, and with the state of malnutrition of the subjects.	Inconsistent reporting of proportions of histopathologic findings among all subjects and by SBBO status; assessment of potential relationship with SBBO between different histologic findings was not possible.

Evidence Table 6. Markers of microbial drivers.
Biomarkers in bold are primarily markers of microbial drivers.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				areas of the villous surface • Derangement of the enterocytes (in some cases cell borders were not clearly defined) • Reduced height and number (or absence in some places) of microvilli • Lymphocytes and fat droplets were observed over the surface of enterocytes (18%)[1] 10 subjects had colitis on rectal biopsy; this was not associated with SBBO or degree of small intestinal pathology on SEM.		
2002 Murphy JL et al. Maldigestion and malabsorption of dietary lipid during severe childhood malnutrition Stool recovery of radiolabeled products as markers of lipid digestion and absorption, and bile salt deconjugation as a marker of SBBO among children with severe malnutrition	Kingston, Jamaica 5-23 mo olds admitted to the Tropical Metabolism Research Unit of the University of the West Indies with severe malnutrition.	Case-series n=24 Subjects were divided into 3 groups of 8 children, each group receiving a different labeled triglyceride. Data were collected in three separate phases as described above in JL Murphy et al. 2001 [149].	<u>Stool Tests</u>: • Total and fractionated ^{13}C following ingestion of one of three ^{13}C labeled triglycerides (TG): trilaurin, triolein, or trilinolein* • **^{13}C stool assay following administration of labeled fatty acid ^{13}C glycocholate**** * To assess fat excretion as a % of dose administered. Also assessed proportion of ^{13}C in triglyceride (TG) and fatty acid (FA) fractions to distinguish excretion	Median total stool excretion of ^{13}C in phase 1 was 9% (range: 1%-29%) and did not vary between TG groups. Median ^{13}C excretion dropped 33%-99% in phase 2 and 86%-95% in phase 3 compared to phase 1 ($p<0.05$ each). Over the study period, there were significant associations between total lipid and the amount of ^{13}C labeled TGs in stool for some groups, but not for others. Median ^{13}C in TG and FA was similar across TG groups in all phases. 13C FA recovery was similar and reduced by ~2/3 compared to Phase 1. ^{13}C TG was not detectable in Phases 2 or 3. Statistical comparisons between phases were not reported. ^{13}C after radiolabeled glycocholate	High concentrations of ^{13}C (compared to healthy UK children) [190] were observed in half of the subjects at admission, reflecting impaired digestion or absorption. The differences in stool ^{13}C were wide but not as extreme as in a previous study by same investigators (also examined in this review) using a different TG (tripalmitin) substrate [149]. ^{13}C excretion did not significantly differ between TG groups	Authors state that the study was not powered to compare the different TGs, but they contend that medium chain trilaurin did not appear to be processed differently than the longer chain TGs triolein and trilinolein. Authors did not describe the method used to assign subjects to different TG groups. While it was noted that some subjects had positive stool cultures, details

1 These SEM results were not presented separately for those with and without SBBO.

Evidence Table 6. Markers of microbial drivers.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
			caused impaired digestion (presence of TG) vs. poor absorption (presence of FA). ** To assess bile salt deconjugation in the bowel caused by SBBO; conducted after the TG assessment and a 3 day washout period.	administration was detected in stool at quantities considered to be in excess of the 7% recovery of dose administered upper limit of normal in U.S. adults in [189]: • Phase 1: 13/24 (54%) • Phase 2: 5/24 (20.8%) • Phase 3: 3/24 (12.5%)	and declined with improving clinical course. Similar to their previous study, significantly more ^{13}C in stool was recovered as FA than TG, reflecting impaired absorption over poor lipid digestion/ hydrolysis. Unlike in their previous study, there was evidence of SBBO as measured post-ingestion of ^{13}C glycocholate.	were not provided on the nature of the enteric infections.
2001 Murphy JL et al. Gastrointestinal handling and metabolic disposal of ^{13}C-labelled tripalmitin during rehabilitation from childhood malnutrition Fecal fat, stool recovery of radiolabeled products, and breath tests as markers of lipid digestion and absorption and bile salt deconjugation as a marker of SBBO among children with severe	Kingston, Jamaica 7-23 mo olds with malnutrition admitted to the University of the West Indies.	Case-series n=8 Data were collected in three separate phases (each lasting 9 days): • Within 48 hours of admission • During early rehabilitation • During late rehabilitation	<u>Stool Tests</u>: • Fecal fat* • Total and fractionated ^{13}C assay after administration of ^{13}C tripalmitin (TP)** • **^{13}C assay after administration of ^{13}C glycocholate (GCA)***** <u>Breath Tests</u>: • **$^{13}CO_2$ after administration of ^{13}C glycocholate (GCA)***** • $^{13}CO_2$ after administration of ^{13}C TP****** * In 72 hour stool	Mean fecal fat (SD): • Phase 1: 2.4 g/day (3.6) or 5.9% (9.4) of dietary lipid intake • Phase 2: 1.7 (0.9) g/day, or 3.3% (2.4) of intake • Phase 3: 0.9 (0.6) g/day, or 1.4% (0.7) of intake • Differences between phases were not statistically significant. Total excretion of ^{13}C in stool also varied widely across patients (0%-44%) and did not differ between study phases. Correlation between fecal fat and ^{13}C ($r=0.48$; $p<0.05$) was observed. Lack of lipid digestion and absorption were assessed by measuring TG and FA fractions, respectively. Mean ^{13}C TG recovery (SD) (% of administered dose), number of patients excreting TG:	Mean fecal fat was not elevated compared to published norms [191, 192] during any study phase. There was wide variation in fecal fat at presentation, and wide variations in stool ^{13}C across subjects. Authors indicate that this is the first such assessment in malnourished children; previous studies on healthy children from the UK demonstrated average excretion of 6% [190].	Statistical methods might be inappropriate for a small sample. All subjects were treated with antibiotics including metronidazole for presumptive SBBO; this might have affected GCA testing.

Evidence Table 6. Markers of microbial drivers.
Biomarkers in bold are primarily markers of microbial drivers.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
malnutrition			collection (measured as total grams and as % of dietary fat intake). ** To assess fat excretion as a % of dose administered. Also assessed proportion of ^{13}C in triglyceride (TG) and fatty acid (FA) fractions to distinguish excretion caused by impaired digestion (presence of TG) vs. poor absorption (presence of FA). *** To assess bile salt deconjugation in the bowel caused by SBBO; conducted after the TG assessment and a 3 day washout period. **** Expressed as a percentage of absorbed label (dose administered - label recovered in stool) to assess oxidation for acute energy needs.	• Phase 1: 0.7% (1.6), n=3 • Phase 2: 0.9% (2.8), n=1 • Phase 3: no recovery from any subjects, differences between phases were NS ^{13}C FA fraction in stool declined during rehabilitation. Mean ^{13}C FA recovery (SD): • Phase 1: 6.0% (7.3) • Phase 2: 4.8% (3.7) • Phase 3: 3.3% (3.8), differences between phases were NS Mean FA values were ~9x (NS), 5x ($p<0.001$), and 3x ($p<0.05$) higher than mean TG values in Phases 1, 2, and 3, respectively. Following administration of labeled TP, absorbed ^{13}C label by breath analysis was ~5% (range 0%-21.2%) and similar across study phases. Following the administration of labeled GCA, there was either no or minimal recovery of ^{13}C in stool and $^{13}CO_2$ on breath (as % of dose administered) in all phases.	The majority of excreted ^{13}C was in the form of FA rather than TG. Authors interpreted this to reflect failure of lipid absorption in the face of adequate digestion/hydrolysis. Each form (FA and TG) was found in decreasing values as the study phases progressed, suggesting improved digestion and absorption, although results did not differ significantly. Fecal fat was correlated with concentrations of ^{13}C in stool. There was no evidence of SBBO or bile acid malabsorption. $^{13}CO_2$ excretion following administration of ^{13}C TP was minimal, suggesting a propensity for deposition in adipose tissues rather than oxidation for immediate energy needs. The authors report that this breath test has not been widely	

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
					used, but that healthy UK children have breath excretion values from 15%-43% [190], compared to a mean of 5% and range 0%-21% in this cohort; the latter findings were more similar to results from kwashiorkor patients where ^{13}C-labeled oleic acid was used as substrate [193].	

Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting.

Abbreviations: AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, Δ=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)

5.7.1 Lactulose Hydrogen Breath Test (HBT)

Alves et al. utilized the lactose hydrogen breath test (HBT) to measure of lactose absorption and the lactulose HBT as a measure of SBBO in a community-based study of indigenous children in Brazil [102]. Prevalence of SBBO in the study subjects was 12% among subjects below four years of age, and overall 9% had SBBO. The authors did not report on the relationship between lactulose and lactose absorption.

In a study of intestinal function among well-nourished children with asymptomatic giardiasis and healthy controls in Mexico, Moya-Camarena et al. utilized the lactulose HBT and the indican test to exclude subjects with SBBO. No further data were reported on results of the lactulose HBT; therefore we did not include this study in this section of our review [147].

5.7.2 $^{13}CO_2$ in Breath or Stool after Administration of ^{13}C Glycocholate as Marker for SBBO

Murphy et al. [149] assessed gastrointestinal function by stool tests with [149] or without [148] breath tests in children hospitalized for rehabilitation of malnutrition. The $^{13}CO_2$ breath test was performed after administering ^{13}C glycocholate (GCA) to assess bile salt deconjugation in the bowel caused by SBBO. The test was conducted after a similar assessment with triglyceride and a subsequent 3-day washout period. Results were expressed as percent of administered dose. In their initial study, following the administration of labeled GCA, there was no or minimal recovery of ^{13}C in stool and $^{13}CO^2$ on breath in all study phases. The authors interpreted these results to indicate that the GCA was not malabsorbed and that the subjects did not show evidence of bile salt deconjugation. In the subsequent larger study, however, ^{13}C from labeled GCA was recovered in the stool in more than one-third of the children, indicating the presence of SBBO [148].

5.7.3 Intestinal Aspirates for Bacterial Concentrations

Fagundes-Neto et al. analyzed jejunal aspirates and small intestinal biopsies of hospitalized infants with persistent diarrhea and malnutrition by scanning electron microscopy (SEM) and light microscopy [118]. Based on bacterial concentration in jejunal aspirates, more than two-thirds of subjects had bacterial overgrowth (concentration $>10^4$ colonies/mL), and three were infected with enteropathogenic *E. coli* while the rest had colonic microflora in their small bowel. Histological abnormalities were noted in all subjects by light microscopy and SEM. The degree of villous atrophy noted on SEM seemed to correlate with SBBO, but statistical testing was not provided.

Fagundes-Neto et al. also reported a mucous-fibrinoid pseudo-membrane that partially covered enterocytes [118]. They speculated that it could indicate a malabsorptive process, based on the findings of fat droplets on enterocyte surfaces and the malnourished condition of the subjects.

Reporting of proportions of histopathologic findings among all subjects and by SBBO status, as well as statistical assessment of potential correlation with SBBO between different histologic findings, would have benefitted the analysis.

5.8 Markers of Nonspecific Intestinal Injury

The matter of small bowel biopsies and EED is complex. From initial investigations nearly five decades ago, the histopathologic appearance of the small bowel defined the entity. Sentinel papers from Southeast Asia and elsewhere formed the basis of our understanding of the entity on which we have focused in this review [274, 275]. Also, there is an extensive tradition of pathological assessments of the bowel and other organs in a variety of syndromes and diseases, and histological assessment is often considered the gold standard to which biomarkers are compared. Histology certainly can inform the nature of lesions. In the digestive system, microscopic evaluation of tissue

can often help differentiate infectious from noninfectious inflammations, assess the degree of allergic reaction if present, suggest the principal effector cells, and identify malignant potential (rare in these subjects). Specific diagnoses can also be made by histologic evaluation of the small bowel; well-known examples are celiac disease and Crohn's disease.

In view of the potential value of relating biopsies to biomarkers, we sought to find any relation between this putatively definitive test (small bowel biopsy) and any laboratory test or abnormality. The data relevant to this review are listed for each of these 18 biopsy studies in Evidence Table 7. We also include other markers of non-specific intestinal injury in Evidence Table 7, including three studies utilizing fecal occult blood or red blood cells and one employing Tc-99m dextran scintigraphy.

Evidence Table 7. Markers of non-specific intestinal injury.
Biomarkers in bold are primarily markers of non-specific intestinal injury.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

2009
 	
Amadi B et al.
 	
Reduced production of sulfated glycosaminoglycans occurs in Zambian children with kwashiorkor but not marasmus
 	
Duodenal biopsy including assessments of intestinal markers in children with PD and different forms of malnutrition
	Lusaka, Zambia
	
	12.2-19.8 mo olds with PD and malnutrition admitted to the malnutrition ward of a teaching hospital.
		Case-control
		
		n=41*;
		
		n=41 cases with PD and malnutrition:
		• 18 with marasmus
		• 8 with marasmic kwashiorkor
		• 15 with kwashiorkor
		
		n=19 healthy control children from UK
		
		* UK subjects are presented in this table due to comparisons of interest made in the review. However we do not include these subjects in the sample size for this review.
			<u>Endoscopic duodenal biopsy:</u>
			• **Histopathology**
			• Densities in lamina propria and crypt epithelium:
			  • Cell proteins:
			  • **Glycosaminoglycan (GAG)**
			  • **Enterocyte heparan sulfate proteoglycan (HSPG)**
			  • **Syndecan-1**
			• Inflammatory cell markers:
			  • CD3 IEL
			  • Ki67
			  • Human leukocyte antigen DR-1 (HLA-DR)
				Biopsy findings among the Zambian compared to the UK children:
				• Villous height reduced
				• Crypt depth increased
				• ~50% reduction in crypt:villous ratio
				• Values for lamina propria cell densities were not reported for UK subjects
				
				No significant differences in crypt or villous measures or lamina propria cell densities were observed between nutritional groups or after nutritional rehabilitation.
				
				Intestinal markers:
				• Inflammatory markers were seen in higher densities compared to the UK children. There were significant differences between the different nutritional groups in the specific types of inflammatory markers.
				• There was a significant reduction in GAGs and HSPG in the kwashiorkor group compared to UK children, but no significant differences between kwashiorkor and other presentations of malnutrition.
				• There was no difference in epithelial syndecan-1 protein expression between the malnutrition groups (data not available for UK controls).
					Mucosal architecture was markedly abnormal compared to UK controls but did not vary between marasmus and kwashiorkor presentations of malnutrition.
					
					Inflammatory cell densities were generally higher compared to UK children and showed different patterns across the malnutrition presentations.
					
					Tissue concentrations of HSPG and GAG were reduced especially amongst children with kwashiorkor. Intestinal protein markers did not differ amongst the malnutrition groups.
						27 subjects were HIV positive; incidence was lower in the kwashiorkor group.

2000 Azim T et al.	Dhaka, Bangladesh 7-12 mo olds with 6-8 days of watery	Case-control n=136;	<u>Blood tests:</u> • IFN-γ • TNF-α • WBC (total and	WBC total and differential, immunoglobulin subtypes, cytokines, transferrin, and albumin did not differ between	Some immune and inflammatory markers were associated with	The number of controls was relatively small and their nutritional

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

Immune response of Bangladeshi children with acute diarrhea who subsequently have persistent diarrhea
 	
Immune activation tests, as well as transferrin and albumin as markers of nutritional status among children with and without PD
	diarrhea attending the International Centre for Diarrheal Disease Research.
	
	Cases were those who went on to develop PD, controls were those who did not.
	
	An additional group of subjects without diarrhea were recruited from a nutrition follow-up unit.
	
	Prevalence of malnutrition was high in all groups.
		n=38 cases with PD
		
		n=98 controls:
		• 85 with AD
		• 13 with no diarrhea
			differential)
			• IgA
			• IgG
			• IgM
			• Transferrin
			• Albumin
			• Immune function tests:
			  • Neutrophil polarization response to chemotactic factor
			  • Neutrophil opsonization to yeast
			  • Mononuclear cell proliferation, spontaneous and in response to stimuli with mitogens
			
			<u>Skin Test:</u>
			• Delayed-type hypersensitivity response (DTH) to tuberculin, tetanus, diphtheria, *Streptococcus, Proteus, Candida,* and *Trichophyton*
			
			<u>Stool tests:</u>
			• Leukocytes
			• **Red blood cells**
				cases with diarrhea or controls, nor did stool leukocyte or erythrocyte counts.
				
				The percentages of neutrophils that polarized in response to stimulation were significantly higher in subjects with AD or PD compared to those without diarrhea; there was no difference between the two diarrhea groups.
				
				Opsonization did not vary between any groups.
				
				Monocyte spontaneous proliferation counts were less than half among children with no diarrhea compared to those with AD ($p<0.001$) or with PD ($p=0.011$); there was no difference between the two diarrhea groups.
				
				Monocyte proliferation in response to stimulation did not differ between the 3 groups.
				
				The proportion with DTH responses differed among the three groups only in response to tuberculin ($p=0.021$). More PD subjects had a negative tuberculin response than did subjects with AD ($p=0.024$).
					acute and/or persistent diarrhea.
					
					The only marker that was significantly associated with progression to PD was a negative DTH response to tuberculin antigen (odds ratio=3.8, CI: 1.4, 9.9). This was calculated from a logistic regression analysis that only included children with diarrhea.
						status was not reported.

2005 Bhatnagar S et al. Celiac disease with mild to moderate histological changes	Delhi, India 1-18 yr olds with a presentation consistent with CD (combination of chronic diarrhea,	Case-series n=107	<u>Endoscopic duodenal biopsy:</u> **Histopathology**	70 had normal histology (defined as crypt:villous ratio 1:2-3, absence of lymphoid lamina propria infiltration, and minimal intraepithelial lymphocytes (IEL). 37 had mild changes (defined as	More than one quarter of children with chronic diarrhea had normal small intestinal mucosa; at follow-up their	

Evidence Table 7. Markers of non-specific intestinal injury.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
is a common cause of chronic diarrhea in Indian children Duodenal biopsy among children with chronic diarrhea	abdominal distension, and growth failure), recruited from a pediatric gastroenterology clinic. Subjects negative for CD-specific antibodies were of interest for this review.			mild blunting of villi with crypt:villous ratio of 1:1*). A specific etiology was identified in only n=5: • 2 with giardiasis • 1 with lymphangiectasia • 2 with chronic pancreatitis Only children with CD had moderate or severe histologic changes. * Along with increased IEL and lymphocytic lamina propria infiltration.	growth had improved and their diarrhea had resolved. No definitive diagnosis was reached for 86% of subjects with abnormal histology (albeit most had mild findings).	

2003
 	
Bustos M et al.
 	
Disaccharidase deficiency in Bolivian children with persistent diarrhea
 	
Jejunal biopsy and disaccharidase activities in children with PD and different forms of malnutrition
	Cochabamba, Bolivia
	
	3-34 mo old Amerindians hospitalized with PD and moderate or severe malnutrition in an urban setting.
		Cohort
		
		n=42 cases with PD and malnutrition:
		• 2 with kwashiorkor
		• 20 with marasmus
		• 20 with marasmic-kwashiorkor
		
		Children were assessed on admission and at three weeks, after diarrhea had resolved and anthropometrics were improving.
			<u>Jejunal tethered capsule biopsy:</u>
			• **Histopathology**
			• Disaccharidase activity:
			  • Lactase
			  • Sucrose-Isomaltase
			  • Maltase
			
			Histology was scored on a scale of 1 (normal) to 4 (severe morphological damage or flat mucosa).
				Most subjects had mild to moderate (score of 2-3) histological abnormalities, with one kwashiorkor patient having completely flat villi.
				
				Second biopsy showed a trend of improved mucosa, but difference was not significant based on histology score, intraepithelial lymphocyte density, or degree of infiltration of lamina propria.
				
				Percentages with enzymatic activity below normal at baseline, discharge:
				• Lactase: 64%, 59%
				• Sucrase-isomaltase: 97%, 90%
				• Maltase: 45%, 52%
				All changes were statistically significant.
				
				Lactase recovery was associated with admission HAZ (p=0.05) and WAZ (p=0.03) scores.
					Patients had diminished intestinal disaccharidase activity and substantial pathology on biopsy at admission and at three weeks, despite clinical improvements and tolerance of lactose-containing formula.	Spanish language article.
						
						Values for subnormal disaccharidase activity were not provided.
						
						The magnitude of lactase inverse association with growth parameters was not reported. Authors did not report whether they had tested for associations between maltase or sucrose-isomaltase and growth parameters.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				Despite continued high disaccharidase deficiency prevalence at discharge, all children tolerated the lactose-containing formula challenge.		

2003
 	
Campbell DI et al.
 	
Chronic T cell-mediated enteropathy in rural west African children: relationship with nutritional status and small bowel function
 	
L:M as a marker of intestinal permeability, small bowl biopsy with assessment of intestinal immune markers, and computerized morphometric analysis among rural Gambian children with differing degrees of malnutrition and compared to well-nourished UK children
	Fajara and Sibanar, The Gambia
	
	6 mo-3 yr old hospital- and clinic-based cases from rural communities.
	
	Case groups based on differences in nutritional status:
	1. WAZ score >-2, with GI complaints other than diarrhea
	2. Grade I protein energy malnutrition (PEM) (WAZ score -2 to -4) and unresponsive to nutritional supplementation, with or without diarrhea
	3. Grade II PEM (WAZ score <-4) with or without diarrhea
	
	Controls from UK* who were well nourished children with GI complaints other than diarrhea and with normal endoscopy results
		Case-control
		
		n=40 cases:
		• Group 1: n=4
		• Group 2: n=11 (7 with diarrhea)
		• Group 3: n=25 (18 with diarrhea)
		
		n=34 with case tissue samples sufficient for cytokine immunoreactivity tests:
		• Group 1: n=3
		• Group 2: n=8
		• Group 3: n=23
			<u>Endoscopic small bowel biopsy, site not specified</u>:
			• **Histopathology**
			• **Morphometric assessment by computer analysis***
			• Intestinal tissue cytokines and immune markers:
			  • CD-3
			  • CD-4
			  • CD-8
			  • CD-19
			  • CD-25
			  • HLA-DR
			  • Perforin
			  • γδ T-cell receptor
			  • Syndecan-1
			  • TNF-α
			  • IFN-γ
			  • TGF-β
			  • IL-10
			
			<u>Urine Tests</u>:
			• Lactulose[1]
			• Mannitol
			• L:M
			
			* Biopsy involved morphometric assessment by computer analysis of
				Crypt-hyperplasia and villous atrophy were observed among all Gambian subjects, and the degree of histopathology did not differ among cases with differing nutritional status, nor was there a correlation with diarrhea.
				
				IEL[2] means were ~3-fold higher in Gambian than UK children.
				
				Median CD3, CD4, CD8, CD19, and CD25 cell counts were significantly higher (2-5x higher) among each case group compared to the UK controls.
				
				IEL, γδ, syndecan-1, HLA-DR, and perforin were detected among the Gambian children in varying degrees but were not reported for UK controls. Syndecan, CD3, and CD8 displayed a gradient proportional to malnutrition severity.
				
				All Gambian groups showed higher lamina propria cytokine-immunoreactive mononuclear cell density (~200-450/mm^2) than UK controls (30-80/mm^2).
				
				Among subjects with elevated cytokines, similar densities were seen for both pro-inflammatory
					All Gambian subjects had evidence of enteropathy with crypt-hyperplasia and villous atrophy, and mean IELs >2 SD above UK norms, independent of nutritional status.
					
					Elevation of cell-mediated intestinal markers and mucosal proinflammatory cytokines was present across the 3 Gambian groups, variably correlated with nutritional status.
					
					L:M ratios were elevated in all Gambian groups, without apparent correlation to host nutritional status.
						Statistical methodology was not sufficiently detailed to determine what was compared (e.g. type of central tendency measure and variance calculations for L:M not stated)
						
						Duration of diarrhea not specified, but assumed to be persistent.
						
						Mucosal lymphocyte densities, cytokine immunoreactivity, and L:M results not stratified by history of diarrhea.

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] These figures are presumed to represent IEL means, however, this was not explicitly stated.

Evidence Table 7. Markers of non-specific intestinal injury.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
	were also studied. * UK subjects are presented in this table due to comparisons of interest made in the review. However we do not include these subjects in the sample size for this review.		villous height, crypt depth, villous:crypt ratio, and intraepithelial lymphocyte (IEL) density (per 100 epithelial cells).	(IFN-γ and TNF-α) and putative regulatory (IL-10 and TGF-β) cytokines. Epithelial expression of TGF-β was also enhanced compared to UK controls, but subjects with poorer nutritional status had lower densities of mucosal TGF-β+ cells, with median densities of 420 and 250 cells/mm^2 in the grade I and grade II PEM groups, respectively. L:M values[1]: • Group 1: 0.53 (0.4-1.3) • Group 2: 0.47 (0.02-2.20) • Group 3: 0.73 (0.14-2.2) • Not assessed among the UK controls Nutritional status was not associated with L:M, recoveries of lactulose or mannitol. L:M was correlated with mucosal B lymphocyte density (r=0.57, p<0.05), IEL (r=0.51, p<0.02), and perforin+ IEL (r=-0.64, p<0.03).		
2006 El Mouzan MI et al. Endoscopic duodenal biopsy in children Duodenal biopsy among children with suspected intestinal disease	Riyadh, Saudi Arabia 1.5 mo-18 yr olds referred to hospital for endoscopy with duodenal biopsy. 78% of subjects were <12 yr old; results not presented by age.	Retrospective case-series n=241 cases: • 102 with PD • 116 with unexplained short stature • 11 with refractory rickets • 12 with other conditions	<u>Endoscopic duodenal biopsy:</u> • **Gross endoscopic visualization** • **Histopathology**	14% had abnormalities on endoscopic visualization: • 1% had esophagitis • 6% had gastritis, 7 (47%) of which were *H. pylori* positive • 7% had duodenitis Biopsy results: • PD: • 26% normal • 29% chronic non-specific duodenitis	Villous atrophy was identified not only among 40% of children with PD, but also among 22%, 9%, and 17% of those with short stature, rickets, and other conditions, respectively. Authors argue that	Specific results for the 2 patients with protein losing enteropathy were not reported. For 27% of cases, the only histopathology finding was chronic non-specific duodenitis; the

[1] Not clearly indicated if these figures represent mean (CI) or another measure of central tendency.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
		(including 2 with protein losing enteropathy)		• 40% villous atrophy • 5% other* • Short stature: • 56% normal • 22% chronic non-specific duodenitis • 22% villous atrophy • Rickets: • 55% normal • 36% chronic non-specific duodenitis • 9% villous atrophy • Other: • 25% normal • 50% chronic non-specific duodenitis • 17% villous atrophy • 8% other* * 3 lymphangiectasia, 2 *Giardia*, 1 *Mycobacterium avium intracellulare.* Findings were reported according to presenting symptoms.	endoscopic biopsy is superior to "blind" capsule biopsy in developing country settings and allows for visualization of the intestine. Endoscopic visualization results were not reported by condition nor in relation to histopathology results; it is difficult to assess the value added compared to biopsy alone.	diagnostic, prognostic, and therapeutic utility of identification is unclear.
2000 Fagundes-Neto U et al. Studies of the small bowel surface by scanning electron microscopy in infants with persistent diarrhea Scanning electron microscope (SEM) and light microscope (LM) analyses of	Sao Paulo, Brazil 2-10 mo olds with PD and protein calorie malnutrition consecutively admitted to Sao Paulo Hospital.	Case-series n=16	<u>Jejunal secretions aspirate:</u> Bacterial concentrations <u>Jejunal tethered capsule biopsy:</u> **Histopathology by LM and SEM** <u>Rectal tethered capsule biopsy:</u> Histopathology	68.7% had bacterial overgrowth (concentration >10^4 colonies/mL): 3 had enteropathogenic *E. coli* while the rest had colonic microflora. All small intestine specimens had morphological abnormalities on LM: • 43.7% moderate villous atrophy • 56.3% subtotal villous atrophy SEM revealed abnormalities of varying intensity: • Among the 11 with SBBO,	Histological abnormalities were noted in all subjects by LM and SEM. Degree of villous atrophy noted on SEM seemed to be correlated with SBBO (no statistical tests were reported). Authors speculate that the mucous-fibrinoid pseudo-	Inconsistent reporting of proportions of histopathologic findings among all subjects and by SBBO status; assessment of potential relationship with SBBO between different histologic findings was not possible.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
small intestinal biopsy among infants with PD with and without SBBO				villous atrophy ranged from Grade II (n=4), Grade III (n=2), to Grade IV (n=3). • For the 5 subjects without SBBO, villous atrophy ranged from Grade I (n=1) to Grade 2 (n=4). • A mucous-fibrinoid pseudo-membrane over enterocytes was noted in 7 of the 11 with SBBO and none of the others. Other abnormalities noted on SEM included: • Mucus and debris covered large areas of the villous surface • Derangement of the enterocytes (in some cases cell borders were not clearly defined) • Reduced height and number (or absence in some places) of microvilli • Lymphocytes and fat droplets were observed over the surface of enterocytes (18%)[1] 10 subjects had colitis on rectal biopsy; this was not associated with SBBO or degree of small intestinal pathology on SEM.	membrane partially covering enterocytes is consistent with a malabsorptive process, with the findings of fat droplets on enterocytes surfaces, and with the state of malnutrition of the subjects.	
2001 Gandolfi L et al. Antiendomysial antibody test reliability in children with frequent	Brasilia, Brazil 6 mo-13 yr olds with acute, persistent or chronic diarrhea, and/or malnutrition being seen at the pediatric	Cross-sectional n=31	<u>Jejunal capsule biopsy:</u> **Histopathology**	30/31 (96.8%) had abnormal histopathology: • Suggesting non-specific inflammatory abnormalities in 27 (87.1%) subjects. • Demonstrating grade 3 mucosal abnormalities in all malnourished 1 yr olds	The vast majority of children with clinically severe diarrhea and/or malnutrition had some degree of abnormality on jejunal biopsy.	Biopsies of interest were not provided in subject-specific detail (e.g. characteristics of the 27 children with non-specific inflammation were

[1] These SEM results were not presented separately for those with and without SBBO.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
diarrhea and malnutrition: is it celiac disease Jejunal biopsy among children with PD and/or malnutrition	gastroenterology service of a university hospital and determined to have disease severity warranting biopsy. Subjects negative for CD-specific antibodies were of interest for this review.			negative for enteric parasites.		not detailed (e.g. presence of parasites, degree of malnutrition and/or diarrhea).
2000 Hafeez A et al. An audit of pediatric upper gastrointestinal endoscopies Duodenal biopsy among children with PD or growth problems	Islamabad, Pakistan 2 mo-12 yr olds referred from various hospitals to KRL Hospital Islamabad for abdominal pain, PD, short stature, FTT, GI bleeding, or anemia. The subjects of interest for this review were those with PD or growth problems.	Case-series n=41; • 28 with PD • 9 with FTT • 4 with short stature	<u>Endoscopic duodenal Biopsy:</u> • **Gross endoscopic visualization** • **Histopathology**	Positive histopathologic findings were identified in: • 21/28 with PD • 7/9 with FTT • 3/4 with short stature More abnormalities were found via histology than visualization, and findings did not necessarily correlate.	75% of the PD and 77% and the short stature/FTT patients had abnormalities by endoscopy. Authors assert the importance of biopsies among children with indications for endoscopy, due to lack of correlation between them and increased identification of abnormalities by biopsy.	There was possible bias in the manner of selection for endoscopy. 14 biopsies were unable to be analyzed (from 100 endoscopies). Authors did not report the endoscopic appearance of the mucosa. Histology findings were reported by specimen (with multiple specimens from some patients), not by condition or by patient, so specific results could not be interpreted in regards to this review.
2007 Jain S et al.	Delhi, India Children (ages	Case-control n=80;	<u>Stool Test</u>: **Occult blood**	Fecal occult blood test was positive in 30/50 (60%) cases and 0/30 controls.	A high proportion of severely malnourished	Among cases, half had a presenting complaint of

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Fecal occult blood screening in children with severe malnutrition Fecal occult blood among severely malnourished children compared to healthy controls	unspecified) admitted with severe malnutrition and age-matched healthy controls recruited from an immunization clinic.	n=50 cases with severe malnutrition n=30 healthy controls	Blood Test: Hemoglobin	Among cases positive for fecal occult blood, 20 (66.7%) were found to have hemoglobin <8 g/dL. Enteric infections: • Parasitic infections were detected in 14/50 (28%) of cases, 12 (85.7%) of whom tested positive for fecal occult blood. • Bacterial infections were detected in 18/50 (36%) of cases, 13 (72.2%) of whom tested positive for fecal occult blood. • Of the remaining 18 for whom an enteric pathogen was not identified, 5 (27.8%) tested positive for fecal blood. Among the 30 cases with fecal occult blood, 16 were breastfed, 11 were fed cow's milk, and 3 were fed formula.	children had a positive fecal occult blood test, compared with no positives among healthy controls. Malnourished children with identifiable pathogens more often tested positive for fecal occult blood, although approximately 25% of those without an identifiable pathogen also tested positive. Presence of fecal blood did not appear to vary by feeding mode (e.g. breast milk, cow's milk, or formula), although data presented were limited.	diarrhea (duration not specified), but the authors did not report results stratified by diarrhea duration. Authors did not provide differences in proportions of occult blood among those with and without specific enteric pathogens. Statistical analysis was not provided; data were reported as proportions only. Matching scheme was not defined.
2002 Kapoor, S et al. Detecting protein losing enteropathy by Tc-99m dextran scintigraphy: A novel experience Tc-99m dextran scintigraphy as a marker of protein-	New Delhi, India 2-12 yr olds selected from hospitalized patients with symptoms suspicious for protein-losing enteropathy (hypoalbuminemia and edema).	Case-series n=3 <5 yr old	**Tc-99m dextran scintigraphy**	Abnormal Tc-99m dextran uptake was positive in one child found to have subtotal villous atrophy on biopsy and another thought to have abdominal tuberculosis. The child with the negative scan had marasmus and partial villous atrophy on biopsy.	Scintigraphy might be a useful, noninvasive method for detecting intestinal pathology.	This pilot study had a small sample size of 8 children, and only 3 were younger than 5 years.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
losing enteropathy or intestinal inflammation						
2004 Laadhar A et al. Determination of anti-transglutaminase antibodies in the diagnosis of celiac disease in children: results of a five year prospective study Duodenal biopsy among patients with suspected CD	Sfax, Tunisia Children admitted for endoscopic biopsy for symptoms of CD (not specified). Subjects of interest for this review were those who tested negative for CD-specific serology and who did not meet the study diagnostic criteria for CD--subtotal or total villous atrophy consistent with Marsh stages 3 or 4. Controls were aged 3 mo-17 yr (mean 4.5 yr).	Case-control n=99	<u>Endoscopic duodenal biopsy:</u> **Histopathology**	Of 99 subjects not meeting diagnostic criteria for CD, endoscopic biopsies revealed: • 76 had normal morphology of the intestinal mucosa • 7 had elevated densities of intraepithelial lymphocytes • 10 had partial villous atrophy (Marsh stage 2) • 6 had various other conditions such as giardiasis or gastritis	Among 169 children with symptoms of CD, 41% had subtotal or total villous atrophy, 10% had partial villous atrophy or inflammatory findings, and 45% had normal biopsies.	Article in French. Prevalence of CD antibodies did not clearly align with case/control designation. Because of the way the results were reported, we could not extract data on those who tested negative for CD-specific serology and who had Marsh stages 3 or 4 histopathology. Methods section described obtaining duodenal biopsies, while results and conclusion sections specify that jejunal specimens were obtained.

2006
 	
Leite CA et al.
 	
Functional, microbiological and morphological intestinal findings among human immunodeficiency virus infected children
	Sao Paulo, Brazil
	
	5 mo-12 yr old (median 24 mo) HIV-infected subjects recruited from a hospital and clinic.
	
	All subjects had some degree of protein-energy malnutrition.
		Cohort
		
		n=11;
		
		n=5 patients with current or recent episode of diarrhea
		
		n=6 patients with no diarrhea in the 30 days preceding enrollment
			<u>Blood Test</u>:
			D-xylose
			(9 tested)
			
			<u>Biopsy of small intestine by tethered capsule or endoscopy:</u>
			Histopathology
			(10 tested)
			
			<u>Rectal biopsy</u>:
				100% had low D-xylose absorption:
				• Mean: 15.6 mg/dL
				• SD: 5
				• Range: 8.9-24.4
				• Median: 14.2
				
				Small intestinal biopsy:
				• 100% had some degree of villous atrophy based on a I-IV grading system:
				  • Grade I: 3
				  • Grade I/II: 2
					There was a high prevalence (100%) of abnormal D-xylose results among HIV-infected children, regardless of diarrhea status.
					
					All patients also had cellular infiltration of the lamina propria and varying degrees of
						Portuguese language article.
						
						D-xylose <25 mg/dL was defined as indicative of malabsorption. This value is higher than what some references have noted as a cut-point [186].

Evidence Table 7. Markers of non-specific intestinal injury.
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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Small intestinal and rectal biopsy to assess morphology and D-xylose as a marker of malabsorption among HIV-infected children			Histopathology (6 tested)	• Grade II: 1 • Grade II/III: 1 • Grade III/IV: 1 • 2 samples were too superficial to assess • Intraepithelial lymphocytes were increased in half of the biopsies. • Lymphocytic and polymorphonuclear (PMN) infiltration of the lamina propria were present in 10/10 and 7/10 biopsies, respectively. Rectal biopsy: • 100% had normal architecture • Lymphocytic and PMN infiltration were present in 6/6 and 4/6, respectively.	villous atrophy. There was no correlation between D-xylose and degree of villous atrophy on biopsy.	Investigators used a well-articulated system of grading villous atrophy. Results were not presented by diarrhea status, perhaps due to small sample size.
2005 Lima AA et al. Intestinal barrier function and weight gain in malnourished children taking glutamine-supplemented enteral formula L:M as a marker of intestinal permeability and various stool tests among children with malnutrition or PD who received either glycine or glutamine	Fortaleza, Brazil 2-60 mo olds hospitalized with WAZ score <-2, ~70% of whom had PD.	RCT n=80; n=53 received supplemented formula • 27 with glycine • 26 with glutamine n=27 received nonsupplemented formula	<u>Urine Tests*</u>: • Lactulose[1] • Mannitol • L:M <u>Stool Tests**</u>: • Lactoferrin • Leukocytes • **Occult blood** • Reducing substances (RS) * n=80 tested at enrollment, n=65 tested at day 10. ** n=60 tested.	Mean[2] L:M (SE): • Glutamine group: • Baseline: 0.31 (0.10) (similar in all three groups) • Day 10: 0.10 (0.02); significant decrease, (p=0.01) • No significant decrease in L:M in glycine and nonsupplemented formula groups at day 10 Mean lactulose (SE): • Glutamine group: • Baseline: 0.97 (0.46) (similar in all three groups) • Day 10: NS decrease in all 3 groups	L:M significantly improved in the glutamine group only. >50% of subjects had intestinal inflammation by stool lactoferrin. Fecal leukocytes, RS, and occult blood were detected in fewer subjects than lactoferrin.	The relationship between stool markers and L:M was not reported. Data were not stratified by history of PD. Fecal fat was assessed, but results were not reported. Cut-off values for lactoferrin positivity were not described. Exclusively

[1] Lactulose and mannitol results were expressed as % of dose administered.
[2] Type of mean not specified.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments

supplemented formula or placebo				Mean mannitol (SE):
				• Glutamine group:
				  • Baseline: 3.42 (0.64) (similar in all three groups)
				  • Day 10: NS decrease in all 3 groups
				
				Proportion of stool markers at baseline among all subjects:
				• Lactoferrin: 53.3%
				• Leukocytes: 11.7%
				• RS: 3.3%
				• Occult blood: 5.0%
						breastfed children were excluded from study participation due to assessment of stool lactoferrin.

2001 Mishra OP et al. Endoscopic and histopathological evaluation of preschool children with chronic diarrhea Duodenal biopsy among patients with chronic diarrhea	Varanasi, India 1-5 yr olds with PD selected randomly from an outpatient population in a urban setting. A high proportion of the children had varying degrees of protein energy malnutrition.	Case-series n=30 with endoscopy performed	<u>Endoscopic duodenal biopsy:</u> • **Gross endoscopic visualization** • **Histopathology**	7 (23.3%) had grossly abnormal endoscopic findings: • 5 (16.7%) with chronic duodenitis • 1 with duodenitis with multiple erosions • 1 with duodenitis with hemorrhagic gastritis 22 (73.3%) had abnormal histopathology: 1. 17 (56.7%) with villous atrophy with mononuclear cell infiltration • 1 (3.3%) with villous atrophy and eosinophilic infiltration • 2 (6.7%) with villous atrophy and mononuclear and eosinophilic infiltration • 2 (6.7%) with only mononuclear cell infiltration Mean duration of diarrhea (SD) was not associated with gross endoscopic findings but did vary by histopathology: • Normal histopathology: 9.0 wk (6.0), n=8 • Mononuclear cell infiltration: 9.0 wk (6.0), n=2	Grossly abnormal endoscopic appearance was found in one-quarter of children with chronic diarrhea assessed by endoscopy. Three-quarters had abnormal histology. More than half had villous atrophy with mononuclear cell infiltration; these patients had >1 month longer duration of diarrhea than those with either normal histology or mononuclear cell infiltration without villous atrophy. Age, enteropathogen recovery, and degree of malnutrition were not associated with	Authors do not report assessing relationship between gross endoscopic findings and histopathology. Number of patients with villous atrophy and both mononuclear and eosinophilic infiltration was very small (n=2), yet authors report a significant difference in their duration of diarrhea relative to those with normal histopathology.

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				• Villous atrophy with mononuclear cell infiltration: 14.9 wk (5.3), n=17, p<0.02 compared to normal histopathology • Villous atrophy with mononuclear and eosinophilic infiltration: 21.5 wk (2.1), n=2, p<0.02 compared to normal histopathology • Villous atrophy with eosinophilic infiltration: 6.0 wk, n=1 No consistent pattern was observed between gross endoscopic findings or histopathological lesions and age, WFA, or type of enteropathogen recovered in stools.	gross endoscopic or histopathological findings.	
2001 Mittal SK et al. Tropical sprue in north Indian children D-xylose and duodenal biopsy as markers of TS	New Delhi, India 0-15 yr old gastroenterology clinic patients with PD. Those with abnormal morphology on biopsy, abnormal D-xylose test, and clinical response to antibiotics were diagnosed as having TS. Those with abnormal morphology and response to gluten-	Case-series n=94; (38 with repeat biopsies) <5 yr old: n=44	<u>Duodenal biopsy, method not specified:</u> **Histopathology** <u>Blood Tests</u>: • Hemoglobin • D-xylose***** * Not specified whether from urine or serum, and units of measurement not provided.	36 (38.3%) were diagnosed with TS including 14/44 (31.8%) who were under 5 years of age. 18 (19.1%) were diagnosed with CD. Degree of villous atrophy among TS vs. CD patients: • Mild in 8/36 (22.2%) vs. 0 • Moderate in 23/36 (63.9%) vs. 4/18 (22.2%) • Severe in 5/36 (13.9%) vs. 14/18 (77.8%) Mean hemoglobin concentration (range) among TS patients was 8.3 g/dL (5.5-11) and did not differ from values of those with CD. Among the 22 TS patients,	More than half of the GI clinic patients with PD had some degree of villous atrophy. More than one-third and almost one-fifth of subjects were diagnosed with TS and CD, respectively. By study diagnostic definition, all TS patients improved with treatment. Among those who had repeat biopsies, almost three-quarters showed	Biopsy results were not provided for patients without TS or CD. It was unclear if there were patients with abnormal D-xylose and histology who did not respond to antibiotic therapy and therefore were not diagnosed with TS. Cut-off points used to define abnormal D-xylose tests were not provided.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
	free diet were diagnosed with CD. We include data on these subjects for comparative reasons.			repeat biopsies showed: • 16 with normalization • 5 with improvement • 1 worsened despite marked clinical improvement The D-xylose test was abnormal in all TS patients by diagnostic definition.	normalization of histology, while 23% had partial improvement and 1 patient had worsened pathology.	

2000
 	
Nichols B et al.
 	
Contribution of villous atrophy to reduced intestinal maltase in infants with malnutrition
 	
Jejunal biopsy, maltase activity, and enzyme messenger RNAs among malnourished and well-nourished children. Assessed association between maltase and villous atrophy and other mucosal intestinal markers indicative of loss of enterocytes and enterocytic function.
	Sao Paulo, Brazil
	
	Cases were children (mean age 9.9 mo, SD 8.1) hospitalized with malnutrition refractory to dietary rehabilitation.
	
	Controls were children (mean age 3.6 mo, SD 1.0) with HAZ and WAZ scores >-2 and normal intestinal mucosa on biopsy, hospitalized for Kasai procedure for biliary atresia.
		Case-control
		
		n=33;
		
		n=24 cases
		
		n=9 controls
		
		Subjects were matched on height and weight; ages differed within matched sets.
			<u>Jejunal capsule biopsy:</u>
			• **Histopathology***
			• Maltase activity
			• Intestinal messenger RNA (mRNA) abundances:
			  • Maltase-glucoamylase (MGA)
			  • Sucrase-isomaltase (SI)
			  • Villin, a structural protein expressed only in enterocytes
			  • Sodium-activated luminal glucose-galactose transporter 1 (SGLT), a functional protein expressed only in enterocytes
			  • β-actin
			
			* Mucosal atrophy was scored on a scale of 1 (absence of atrophy compared to an organ donor) to 4 (similar to children with active CD).
			
			Histology among controls was on surgically resected tissue.
				Mean villous atrophy score (SD):
				• Cases: 2.6 (0.8)
				• Controls: 1.2 (0.5), p=0.006)
				
				WAZ score was correlated with villous atrophy (r=0.65, p- value not reported).
				
				13/25 [sic] cases and 0/5 controls had subnormal (defined as <94 U/g protein) of maltase activity; mean maltase was 34% lower among cases (p=0.11). Maltase activity did not appear to decrease with WAZ score (further details not provided).
				
				However, in sub-analyses among those samples with an adequate β-actin, a housekeeping gene message, (n=10 cases, n=9 controls), cases' findings expressed as a mean percent of controls' (SD) included:
				• Villous length (reciprocal of atrophy score): 38.9 (41.6), p=0.004
				• Maltase activity: 37.1 (23.2), p=0.001
				• MGA mRNA: 45.1 (36.4), p=0.016
				• Villin mRNA: 52.5 (22.6), p=0.003
					The malnourished children had significantly greater villous atrophy than the younger controls.
					
					Among the subset tested for mRNA messages, maltase activity as well as the mRNA abundances for MGA, villin and SGLT were significantly correlated with case status and were correlated with villous atrophy.
					
					While maltase deficiency has been reported in malnutrition in other studies, authors assert that these are the first results that directly support the hypothesis that reductions in maltase activity are due to villous atrophy. This study
						Tissue from patients requiring intestinal resection as part of their biliary atresia management provides an opportunity to assess presumably "normal" intestinal architecture. However, unless they mocked up *ex vivo* mucosal biopsies in these controls, resections will have lower proportions of villous to submucosa tissue compared to cases' samples derived from mucosal biopsies. While this probably doesn't affect histology, it might affect enterocyte functional assays and mRNA determination, as transmural tissue will bring in more diverse populations

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				• SGLT mRNA: 66.6 (23.1), p=0.057 • β-actin: 88.2 (15.8), p=0.189 Both villous length and maltase activity in a subset of cases were less than 40% of control values. MGA, villin, and SGLT mRNA abundances were correlated with villous atrophy score (r=0.73), (r=0.76), and (r=0.54), respectively (p-values not reported)[1]. MGA mRNA abundance was correlated with maltase activity (r=0.32).	also nicely correlates mRNA relative abundance with function.	of cells; only some of them might have transcripts of interest. However, the bias is likely in a direction that would reduce effect size. It was unclear if control inclusion criteria included absence of atrophy or if all potential controls lacked atrophy. Statistical methods might not have adequately taken into account the small sample size and matching scheme. Subsets of subjects were investigated for various tests. For example, 10 cases had mRNA analyses based on β-actin adequacy. Another instance of selected testing was the subset of 22 and 15 cases that had WAZ score to histology and mRNA correlation analyses,

[1] Villin and SGLT1 were assessed as a ratio with housekeeper gene β-actin.

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						respectively. Rationale for subset selection was not thoroughly described.

2003
 	
Pires AL et al.
 	
Digital morphometric and stereologic analysis of small intestinal mucosa in well-nourished and malnourished children with persistent diarrhea
 	
Computerized morphometric and stereologic assessments in proximal small intestine biopsy in subjects with PD
	Porto Alegre, Brazil
	
	6 mo-5 yr old inpatients from an urban setting who underwent biopsy as part of a work-up for PD.
	
	There was a high proportion of children with malnutrition in this study population.
		Cross-sectional, retrospective
		
		n=65
			<u>Small intestinal biopsies, site and method not specified*:</u>
			• Mucosal morphometric assessment by computer analysis (62 tested):
			  • **Villous height**
			  • **Crypt depth**
			  • **Villous:crypt ratio**
			  • **Mucosal thickness**
			• Digital assessment (500x magnification) (65 tested):
			  • **Enterocyte height**
			  • **Enterocyte nucleus height**
			  • **Brush border height**
			• **Stereological analysis to assess mucosal surface area** (62 tested)
			
			* From stored specimens previously assessed by micrometer.
				Computerized mucosal measures were similar to those by micrometer and were not associated with nutritional status.
				
				Digitally assessed enterocyte height, enterocyte brush border, and enterocyte nucleus height correlations:
				• WAZ score: r=0.25 (p=0.038), r=0.26 (p=0.03), and r=0.24, (p=0.05), respectively
				• WHZ score: r=0.29 (p=0.02), r=0.27 (p=0.03), and r=0.16 (p=0.19), respectively
				• HAZ score: r=0.16 (0.18), r=0.23 (p=0.06), r=0.23 (0.06)
				
				There was no correlation between mucosal surface area and growth parameters.
					Enterocyte measures show some correlation with WAZ and WHZ scores, but not with HAZ score. However, surface area and villous:crypt ratios were not correlated with any growth parameter.	

2008 Poddar U et al. Is tissue transglutaminase autoantibody the best for diagnosing celiac disease in children of developing countries	Chandigarh, India <14 yr (mean age 6.9 yr) presenting with symptoms consistent with CD (PD, FTT, and/or pallor) Subjects negative for CD were of interest for this	Case-control n=28 controls; • 22 with giardiasis • 1 with TS • 5 with SBBO	<u>Endoscopic duodenal biopsy</u>: **Histopathology**	Duodenal biopsy of those with giardiasis showed nonspecific chronic inflammation of lamina propria; there was no evidence of villous atrophy.	Duodenal biopsy demonstrated histological changes accompanying *Giardia* infection.	Authors did not report the biopsy findings in the TS or SBBO patients.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
Duodenal biopsies in controls with giardiasis, TS, and SBBO	review.					
2002 Poddar U et al. Celiac disease in India: Are they true cases of celiac disease Duodenal biopsy, D-xylose, and fecal fat in children with symptoms of CD but normal mucosal biopsy results	Chandigarh, India 18 mo-14 yr olds with PD, FTT, or pallor from a hospital pediatric gastroenterology unit. Subjects with normal crypt:villous ratio on biopsy were of interest for this review.	Case-control n=47	<u>Endoscopic duodenal biopsy</u>: **Histopathology** <u>Stool Tests</u>: • Fecal fat* • D-xylose** * In 72-hour stool collection. ** Not specified whether from urine or serum, and units of measurement not provided.	38% had chronic inflammatory cell infiltrates in the lamina propria. 55% had abnormal D-xylose concentrations. 20% had abnormal fecal fat test. No results beyond proportion positive were reported for any of the above markers.	Among controls with normal mucosal architecture by biopsy, more than one-third had PD. D-xylose and fecal fat might not correlate well with duodenal biopsy results.	Relationships between fecal fat, D-xylose and biopsy results were not reported. While 38% of controls had PD, results for the markers studied were not stratified by PD for this group. Seven children with biopsies consistent with CD did not respond to gluten-free diet and were excluded from the study. Cut-off points used to define abnormal D-xylose tests were not provided.
2008 Sherwani K et al. Prevalence of iron deficiency anemia in chronic diarrhoea and celiac disease - A western UP experience Duodenal biopsy in	Aligarh, India 1-12 yr olds (mean age 51.2 mo) from an urban setting with PD recruited from pediatric outpatient and inpatient units. Those with negative CD work-up were	Cross-sectional n=19	<u>Duodenal biopsy, method not specified:</u> **Histopathology**	Six patients had partial villous atrophy and non-specific duodenitis by biopsy.	Biopsy identified abnormal histopathology in approximately 1/3 of patients who did not have CD, but did not identify PD etiology in the remainder who did not have CD.	The 6 children with partial villous atrophy were thought to have SBBO as they recovered after treatment with broad spectrum antibiotics.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
patients with PD	subjects of interest for this review.					
2003 Tassara O et al. Gastrointestinal diseases in children infected with the human immunodeficiency virus Endoscopy and biopsy among HIV-infected children	Santiago, Chile 0-12 yr old (median 9 mo) HIV-infected children treated in hospital. A high proportion had PD.	Cross-sectional n=11	Endoscopic upper GI biopsy including esophagus, stomach, and/or duodenum: • **Gross endoscopic visualization** • **Histopathology**	Macroscopic inflammatory changes were observed on endoscopy in the esophagus, stomach or duodenum in 2 subjects. Biopsies of esophagus, stomach or duodenum showed inflammatory changes of varying degree in all 11 subjects.	Biopsy results might show inflammatory damage in cases with no macroscopic damage visible.	Spanish language article. Inflammatory changes identified in the digestive system were not specified by site (i.e. esophagus, stomach or duodenum). Results were not stratified by presenting symptoms, including PD.

Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting.

Abbreviations: AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, Δ=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)

Our findings indicate a profound lack of enteric histologic data on pediatric populations in resource-limited settings. Specifically, only 18 of the 77 studies that we reviewed contained any biopsy materials, and only three of these 18 publications also included any non-intestinal tissue candidate biomarkers [111, 136, 155]. Furthermore, only two [111, 136] of these three studies attempted to statistically relate the less invasive, non-intestinal markers to histologic findings; the only significant association reported was between L:M and intestinal tissue inflammation on histopathology. Moreover, only 718 subjects aged 0-18 years were represented among the 18 biopsy studies. Ages were mixed in many studies, such that it was impossible to discern which subjects were in the age range of maximal interest in this review (i.e., under five years of age), and indications for the biopsies were quite diffuse (e.g., diarrhea, poorly specified abdominal symptoms). Therefore, the subject population and data availability were suboptimal for the purposes of this review. In addition, none of the studies that included biopsies related the findings to the outcome of most considerable interest, i.e., stunting.

Prospectively collected small bowel biopsies may provide useful data, guide biomarker development and validation, and reveal information about the cause(s) and pathophysiology of EED. Appropriate conditions are necessary for biopsy studies to be illuminating. For example, the context in which subjects and controls are selected is vital. Regarding cases, a rigorous clinical case definition must be established that defines growth faltering and minimal extent of intestinal functional abnormalities. Exclusion criteria should also be established for disorders that might mimic EED, such as celiac disease or inflammatory bowel disease. The pathology associated with these disorders, however, could inform biomarker association with intestinal dysfunction not specific to, but nevertheless relevant to and sensitive for EED. Such markers could be part of a set of markers used to diagnose EED. However, the usefulness of a biopsy to guide biomarker development and validation depends on several factors. Ideally, the lesion should be from treatment-naïve hosts; this does bring up an ethical dilemma, however. For

example, placement of a patient on gluten free diet, even briefly, or treating a patient with possible inflammatory bowel disease, prior to biopsies can obscure findings and render the histopathologic evaluation less valuable. Second, biopsies obtained under protocol must take into account the distribution of the lesion, so as to confirm the negative predictive value of any set of biopsies. Two disorders provide some guidance in this realm. For celiac disease, the lesion is surprisingly non-uniform [276-279], and multiple biopsies must be sought to avoid beta error. A similar situation applies to gastric biopsies for *Helicobacter pylori* detection [280-286]. A comparison group is also necessary, and to date, most “normal” or “control” tissues were from children in developed country settings. Hence, the abnormalities noted in children’s biopsies cannot be attributed to the disease process (enteropathy), or its consequences (stunting, most particularly), rather than to residence in an area in which such disorders are common. In other words, biopsy association with disease is, based on the available literature, no more strong than EED association with geography.

A case could be made that biopsies are part of a routine evaluation of a child with failure to thrive, and a recent publication from North America suggests that this diagnostic modality is commonly employed in the evaluation of stunting disorders [287]. However, the precise diagnostic yield of a biopsy is not stated in this publication, nor are the data weighted by the symptoms or the age of the children. At this time, it remains uncertain how generalizable the utility of these recommendations is in settings where EED is common.

The safety of a biopsy also needs to be considered in pondering the value of tissue assessment, or of biomarker discovery or validation. If a biopsy is obtained as part of an evaluation of poor growth, then the small risk of the biopsy usually is less than the potential benefit. However, this calculus assumes that substantial pre-procedure and post-procedure care is available to mitigate the likelihood of complications. The use of anesthesia in inpatient or

outpatient settings is safe, but should conform to the highest grade of safety [288]. An additional concern is that endoscopy of the duodenum carries a risk of causing intramural hematomas, estimated at 0.08% of upper endoscopic procedures in the United States [289, 290]. This complication results in small bowel obstruction, severe pain, and Ampulla of Vater obstruction and requires management involving prolonged hospitalization and total parenteral nutrition and/or or naso-enteral feeds. Coagulopathies such as von Willebrand Disease, other platelet disorders, and vitamin K deficiency (the risk of which is increased in chronic diarrhea and malabsorption) [291, 292] are thought to contribute to approximately half of endoscopy-related duodenal hematomas [289, 290, 293-307]. Among von Willebrand Disease variants, only severe (type 3) disease can be readily identified by available screening tests (prothrombin time) although such assessments can be used to pre-endoscopically identify treatment-responsive vitamin D deficiency.

If biopsies are obtained as part of a research protocol to identify markers that predict clinically consequential EED (and even if biopsies are only used for clinical care), it is necessary to assemble a panel of individuals whose tissues are evaluated in parallel, but who do not have the most consequential of the complications of putative enteropathy, namely, stunting (assuming this remains the outcome of greatest concern). It would not be possible to recruit healthy control children for biopsies, because the procedure would offer limited benefit—especially in relation to potential harm. However, some surrogate controls might arise as adventitiously obtained tissue becomes available either during operations or other endoscopic procedures, and the use of small bowel obtained at time of portoenterostomy is a particularly inspired choice [53].

Caveats must be attached to any histologic assessment of the guts in children being evaluated for enteropathy as part of a care plan. It is very unlikely that intestinal biopsies would

be used as a diagnostic procedure at the beginning of an evaluation for malnutrition or poor growth. More likely, it would be a procedure of last resort, after less invasive evaluation failed to establish an etiology, and after attempts at nutritional and intestinal rehabilitation are undertaken and prove unsuccessful. However, as noted above, the empiric treatment of consequential intestinal dysfunction might change the pathology, and thereby diminish the diagnostic value of the procedure. Also, if biopsies are obtained, and subjected to analysis, it is critical that the materials be handled in a systematic manner, and processed per protocol, so as to maximize the data that they generate.

In summary, there is no evidence to date that biopsies have been used to define the entity of childhood EED, because inadequate controls have been studied (children without evidence of functional impairment of gut function living in the same environment). There is no evidence that the biopsies relate to the outcome of greatest concern, namely, stunting. The data obtained from biopsies could be falsely normal, because of attempts at intestinal rehabilitation that will presumably precede the endoscopy. This negative assessment does not mean that biopsies are without worth, only that their value as providing case-defining information, or guiding biomarker discovery or validation, has yet to be made. However, if biopsies are obtained, it is critical that they be performed in a rigorous, disciplined and ethical manner, and that the data to be obtained are maximized.

5.9 Markers of Extra-Small Intestinal Function

We included markers of non-small intestinal organ function as these might provide important indirect assessments of precursors to or resultants of small intestinal injury. We reviewed those markers that were examined among children with presentations potentially consistent with enteropathy, such as persistent diarrhea, or were examined in relationship to

markers of intestinal inflammation or dysfunction. The data relevant to this review are listed for each of these studies in Evidence Table 8.

Evidence Table 8. Markers of extra-small intestinal function.
Biomarkers in bold are primarily markers of extra-small-intestinal function.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
2010 Bukhari AS et al. DNA damage and plasma homocysteine concentrations are associated with serum metabolites and mineral constituents' profiles in children with persistent diarrhea Serum proteins, metabolites, and levels of DNA damage among children with and without PD	Faisalabad, Pakistan 3-6 yr olds admitted to hospital with PD and healthy controls.	Case-control n=72; n=36 cases with PD n=36 healthy controls	<u>Blood Tests</u>: Serum proteins and metabolites: • Albumin • Globulin • **Homocysteine** • Total protein • **Total cholesterol, HDL, LDL, triglycerides** • **AST, ALT** • **T3, T4** • Total oxidant status (TOS), Total anti-oxidant status (TAS), and thiobarbituric reactive substances (TBARS) DNA damage to lymphocytes	Mean values significantly higher among PD cases than in healthy controls: • LDL • Homocysteine • TOS • TBARs • DNA damage Mean values significantly lower among PD cases than in healthy controls: • Total protein • T4 • TAS	Multiple serum markers were associated with PD, especially DNA damage to lymphocytes (p=0.0001). The authors speculate that zinc deficiency, more commonly found in the children with PD, might be responsible for increased homocysteine concentrations and play an important role in mediating DNA damage.	Control recruitment strategy was not well described. TOS, TBARS and TAS were incompletely defined. Some values differed by gender in both the case and control groups: • Triglycerides • Total cholesterol • HDL • T3 Multiple markers studied; analyses did not appear to address potential confounding.
2000 Fagundes-Neto U et al. Studies of the small bowel surface by scanning electron microscopy in infants with persistent diarrhea Scanning electron microscope (SEM) and light microscope (LM) analyses of small intestinal biopsy among infants with PD with and without SBBO	Sao Paulo, Brazil 2-10 mo olds with PD and protein calorie malnutrition consecutively admitted to Sao Paulo Hospital.	Case-series n=16	<u>Jejunal secretions aspirate:</u> Bacterial concentrations <u>Jejunal tethered capsule biopsy:</u> Histopathology by LM and SEM <u>Rectal tethered capsule biopsy:</u> **Histopathology**	68.7% had bacterial overgrowth (concentration >10^4 colonies/mL): 3 had enteropathogenic *E. coli* while the rest had colonic microflora. All small intestine specimens had morphological abnormalities on LM: • 43.7% moderate villous atrophy • 56.3% subtotal villous atrophy SEM revealed abnormalities of varying intensity: • Among the 11 with SBBO, villous atrophy ranged from Grade II (n=4), Grade III (n=2), to Grade IV (n=3). • For the 5 subjects without SBBO, villous atrophy ranged from Grade I (n=1) to Grade 2 (n=4). • A mucous-fibrinoid pseudo-	Histological abnormalities were noted in all subjects by LM and SEM. Degree of villous atrophy noted on SEM seemed to be correlated with SBBO (no statistical tests were reported). Authors speculate that the mucous-fibrinoid pseudo-membrane partially covering enterocytes is consistent with a malabsorptive	Inconsistent reporting of proportions of histopathologic findings among all subjects and by SBBO status; assessment of potential relationship with SBBO between different histologic findings was not possible.

Evidence Table 8. Markers of extra-small intestinal function.
Biomarkers in bold are primarily markers of extra-small-intestinal function.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				membrane over enterocytes was noted in 7 of the 11 with SBBO and none of the others. Other abnormalities noted on SEM included: • Mucus and debris covered large areas of the villous surface • Derangement of the enterocytes (in some cases cell borders were not clearly defined) • Reduced height and number (or absence in some places) of microvilli • Lymphocytes and fat droplets were observed over the surface of enterocytes (18%)[1] 10 subjects had colitis on rectal biopsy; this was not associated with SBBO or degree of small intestinal pathology on SEM.	process, with the findings of fat droplets on enterocytes surfaces, and with the state of malnutrition of the subjects.	

2005
 	
Galpin L et al.
 	
Effect of *Lactobacillus* GG on intestinal integrity in Malawian children at risk of tropical enteropathy
 	
L:M and sucrose: lactulose ratio (SUC:L) as markers of intestinal and gastric permeability, respectively, in
	Mwenye, Malawi
	
	36-60 mo olds recruited from a rural community, excluding children with severe acute malnutrition or severe chronic illnesses.
	
	Subjects were considered at risk for EED due to residence in a location with high prevalence of EED.
		RCT
		
		n=164;
		
		n=81 received *Lactobacillus* GG (80 completed the study)
		
		n=83 received placebo (81 completed the study)
		
		Subjects received 30-days of
			<u>Urine Tests</u>:
			• Lactulose[2]
			• Mannitol
			• **Sucrose (SUC)**
			• L:M
			• **SUC:L**
				At enrollment:
				• 73% had L:M >0.10
				• 40% had L:M >0.20
				• Mean[3] L:M (SD):
				  • Treatment: 0.18 (0.16)
				  • Placebo: 0.22 (0.20)
				• Mean lactulose (SD) in treatment group: 0.25 (0.17)
				• Mean mannitol (SD) in treatment group: 8.0 (4.5)
				• Mean SUC:L (SD):
				  • Treatment: 0.58 (0.64)
				  • Placebo: 0.60 (0.64)
				
				Mean excretion of sucrose (SD) increased from 0.057 (0.042) to 0.078 (0.058) in the treatment group
					A high baseline prevalence of abnormal L:M was observed, with no change after intervention.
					
					High mannitol excretion (relative to UK norms) drove the abnormal L:M.
					
					There was little effect on SUC:L with intervention; sucrose excretion increased in both
						Difficult to interpret sucrose tests because there are limited data on laboratory values for these tests in young children.

[1] These SEM results were not presented separately for those with and without SBBO.
[2] Lactulose, mannitol, and sucrose results were expressed as % of dose administered.
[3] Arithmetic mean.

Evidence Table 8. Markers of extra-small intestinal function.
Biomarkers in bold are primarily markers of extra-small-intestinal function.

Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
asymptomatic children presumed at risk of EED	Presumed that if SBBO is etiology for EED, treatment with *Lactobacillus* will result in improved gut integrity.	*Lactobacillus* GG or placebo. Only the 161 subjects who completed the study had repeat testing.		(p=0.01), but similar results were observed in the placebo group. Otherwise there were no changes in lactulose, mannitol, L:M, or SUC:L after treatment or placebo.	treatment and control groups.	

2006
 	
Leite CA et al.
 	
Functional, microbiological and morphological intestinal findings among human immunodeficiency virus infected children
 	
Small intestinal and rectal biopsy to assess morphology and D-xylose as a marker of malabsorption among HIV-infected children
	Sao Paulo, Brazil
	
	5 mo-12 yr old (median 24 mo) HIV-infected subjects recruited from a hospital and clinic.
	
	All subjects had some degree of protein-energy malnutrition.
		Cohort
		
		n=11;
		
		n=5 patients with current or recent episode of diarrhea
		
		n=6 patients with no diarrhea in the 30 days preceding enrollment
			Blood Test:
			D-xylose
			(9 tested)
			
			Biopsy of small intestine by tethered capsule or endoscopy:
			Histopathology
			(10 tested)
			
			Rectal biopsy:
			Histopathology
			(6 tested)
				100% had low D-xylose absorption:
				• Mean: 15.6 mg/dL
				• SD: 5
				• Range: 8.9-24.4
				• Median: 14.2
				
				Small intestinal biopsy:
				• 100% had some degree of villous atrophy based on a I-IV grading system:
				  • Grade I: 3
				  • Grade I/II: 2
				  • Grade II: 1
				  • Grade II/III: 1
				  • Grade III/IV: 1
				  • 2 samples were too superficial to assess
				• Intraepithelial lymphocytes were increased in half of the biopsies.
				• Lymphocytic and polymorphonuclear (PMN) infiltration of the lamina propria were present in 10/10 and 7/10 biopsies, respectively.
				
				Rectal biopsy:
				• 100% had normal architecture
				Lymphocytic and PMN infiltration were present in 6/6 and 4/6, respectively.
					There was a high prevalence (100%) of abnormal D-xylose results among HIV-infected children, regardless of diarrhea status.
					
					All patients also had cellular infiltration of the lamina propria and varying degrees of villous atrophy.
					
					There was no correlation between D-xylose and degree of villous atrophy on biopsy.
						Portuguese language article.
						
						D-xylose <25 mg/dL was defined as indicative of malabsorption. This value is higher than what some references have noted as a cut-point [186].
						
						Investigators used a well-articulated system of grading villous atrophy.
						
						Results were not presented by diarrhea status, perhaps due to small sample size.

2003 Tassara O et al. Gastrointestinal diseases in children infected with the	Santiago, Chile 0-12 yr old (median 9 mo) HIV-infected children treated in hospital. A high proportion had PD.	Cross-sectional n=11	Endoscopic upper GI biopsy including esophagus, stomach, and/or duodenum: • **Gross endoscopic visualization**	Macroscopic inflammatory changes were observed on endoscopy in the esophagus, stomach or duodenum in 2 subjects. Biopsies of esophagus, stomach or duodenum showed inflammatory	Biopsy results might show inflammatory damage in cases with no macroscopic damage visible.	Spanish language article. Inflammatory changes identified in the digestive system were not

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human immunodeficiency virus Endoscopy and biopsy among HIV-infected children			• **Histopathology**	changes of varying degree in all 11 subjects.		specified by site (i.e. esophagus, stomach or duodenum). Results were not stratified by presenting symptoms, including PD.

2009
 	
Trehan I et al.
 	
A randomized, double-blind, placebo-controlled trial of rifaximin, a nonabsorbable antibiotic, in the treatment of tropical enteropathy
 	
L:M, sucrose:lactulose, and sucralose:lactulose as markers of small intestinal, gastric, and colonic permeability, respectively, among those receiving rifaximin or placebo
	Limela, Malawi
	
	All 3-5 yr olds from the village were recruited.
		RCT
		
		n=144;
		
		n=72 received rifaximin for 7 days
		
		n=72 received placebo
		
		It was presumed that if SBBO is the etiology for enteropathy, treatment with rifaximin would result in improved intestinal integrity.
			<u>Urine Tests</u>
			• Lactulose[1]
			• Mannitol
			• **Sucrose (SUC)**
			• **Sucralose (SCL)**
			• L:M
			• **Sucrose:lactulose ratio (SUC:L)**
			• **Sucralose:lactulose ratio (SCL:L)**
			
			Subjects were tested before and after treatment.
				At enrollment:
				• Mean mannitol (SD):
				  • Treatment: 9.57 (5.24)
				  • Placebo: 10.29 (6.62)
				• Mean lactulose (SD):
				  • Treatment: 0.30 (0.18)
				  • Placebo: 0.34 (0.25)
				• Mean SUC (SD):
				  • Treatment: 0.062 (0.04)
				  • Placebo: 0.074 (0.058)
				• Mean SCL (SD):
				  • Treatment: 0.51 (0.29)
				  • Placebo: 0.58 (0.53)
				• Mean L:M (SD):
				  • Treatment: 0.18 (0.12)
				  • Placebo: 0.17 (0.09)
				• Mean SUC:L (SD):
				  • Treatment: 0.50 (0.34)
				  • Placebo: 0.64 (0.90)
				• Mean SCL:L (SD):
				  • Treatment: 0.42 (0.32)
				  • Placebo: 0.39 (0.23)
				• For both groups combined:
				  • 76% had L:M >0.10
				  • 34% had L:M >0.20
				
				No significant post- intervention differences were observed in any fractional sugar excretion or dual sugar test, including among children
					There was a high proportion with elevated L:M which did not change with rifaximin treatment.
					
					Baseline L:M measurements in this study resembled those of another Malawian population in similar environmental conditions [120].
					
					SCL excretion in this population was similar to that found in healthy American children (0.4%), while SCL:L was comparatively lower (0.8) and driven by lactulose [210]. SCL:L might be a better marker of colonic permeability [211-213]. Results from this study potentially indicate that colonic
						Methodological differences in specimen collection and testing, in particular for SCL excretion, might account for some differences in values compared to other studies.
						
						This was the first use of SCL for site-specific absorption testing in a developing country setting.

[1] Lactulose, mannitol, SUC, and SCL results were expressed as % of dose administered.

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Reference and Study Outcomes of Diagnostic Interest	Location and Target Population	Design and Sample Size	Biomarker	Results	Conclusion	Comments
				with elevated pre-intervention L:M.	permeability was normal. Few data exist on SUC excretion. Results in this trial are similar to those found in another Malawian population (0.06% SUC excretion) [120] and high compared to healthy older children from developed country settings (0.02-0.03%) [210, 212].	

Notes: Some studies included subjects ≥5 yr of age. Where these studies provided data separately for children <5 yr, we present results for only those subjects. Where these studies did not stratify results by age, but did report the number of children <5 yr included in the study, we provide a breakdown of under-5s.
All studies reporting lactulose:rhamnose ratio results presented values multiplied by a factor of 100 for ease of reporting.

Abbreviations: AD=acute diarrhea, ALT=alanine aminotransferase, AST=aspartate aminotransferase, CBC=complete blood count, CD=celiac disease, CI=95% confidence interval, Cr=creatinine, Δ=change in, EED=environmental enteric dysfunction, FTT=failure to thrive, GI=gastrointestinal, HAZ=height-for-age Z-(score), HDL=high density lipoproteins, HIV=human immunodeficiency virus, HLA=human leukocyte antigen, IEL=intraepithelial lymphocytes, IgA=immunoglobulin A, IgE=immunoglobulin E, IgG=immunoglobulin G, IgM=immunoglobulin M, IL=interleukin, IFN=interferon, LDL=low density lipoproteins, L:M=lactulose:mannitol ratio, mo=month(s), NS=not statistically significant, PD=persistent diarrhea, RCT=randomized controlled trial, SBBO=small bowel bacterial overgrowth, SD=standard deviation, SE=standard error, SES=socioeconomic status, Tc-99m=technetium 99, T3=triiodothyronine, T4=thyroxine, TE=tropical enteropathy, TGF=transforming growth factor, TNF=tumor necrosis factor, TS=tropical sprue, WAZ=weight-for-age Z-(score), WBC=white blood cell count, WFA=weight-for-age, WHZ=weight-for-height Z-(score), wk=week(s), yr=year(s)

One study assessed markers of liver and thyroid function among hospitalized persistent diarrhea cases and healthy controls [107]. These authors found that thyroid hormones were significantly lower in children with diarrhea than in controls. Liver tests showed associations in conflicting directions.

The remaining five studies examined tests related to segments of the gastrointestinal tract outside of the small bowel [13, 118, 120, 136, 167]. Three of these performed endoscopy and/or biopsy on the esophagus, stomach, and/or rectum [118, 136, 167]. In a study of 11 HIV-infected malnourished children rectal biopsy tissue uniformly demonstrated normal architecture, but increased lymphocytic and neutrophilic infiltration. Duodenal biopsies were also infiltrated with these cells but all subjects had additionally some degree of abnormal architecture [136]. Another case series found macroscopic inflammatory changes in 18% of endoscopies and 100% of biopsies of the upper gastrointestinal tract in eight children with HIV infection, many of whom had persistent diarrhea [167]. However, the investigators did not report whether these findings were identified in the esophagus, stomach and/or duodenum. A third study in this category found that nearly two-thirds of subjects had evidence of colitis based on rectal biopsy; this was not associated with small bowel bacterial overgrowth or degree of small intestinal pathology observed by scanning electron microscope [118].

Tests of small intestinal permeability have been described in a previous section (5.3), but certain dual-sugar permeability tests are more specific for evaluating colonic or gastric function. These markers include urinary sucrose, urinary sucralose, the urinary sucrose:lactulose ratio (SUC:L), and the urinary sucralose:lactulose ratio (SCL:L). Sucrose excretion and sucrose:lactulose (SUC:L) were components of a panel of urinary excretion tests that comprised the clinical endpoints as measures of gastric permeability for two community-based intervention studies, one of which additionally tested sucralose excretion and sucralose:lactulose (SCL:L) as measures of colonic permeability. The first of these studies was

a randomized, controlled trial of *Lactobacillus* GG [120]. The mean excretion of sucrose increased in the treatment group following treatment, but similar results were observed in the placebo group. Following the intervention, there was no change in the mean SUC:L in either group. The second of these studies was a randomized, controlled trial that assessed L:M, SUC:L, and SCL:L responses to treatment with rifaximin, a nonabsorbable antibiotic. The investigators hypothesized that if small bowel bacterial overgrowth played an etiologic role in enteropathy, treatment with the antibiotic would result in improvements in measurements of permeability [13]. Sucralose excretion in this population was similar to that found in healthy American children while SCL:L was comparatively lower and driven by lactulose [210]. These results were similar to those found in another Malawian population [120] in where excretion values were 2-3 fold greater than those excreted by healthy older children from developed countries [210, 212]. No significant post-intervention differences were observed for any of these markers. The authors asserted that this was the first use of sucralose for site-specific absorption testing in a developing country setting. Few data exist on sucrose excretion, but overall values for sucrose excretion were similar across the two studies.

5.10 Relationships between Markers of EED, Including Histopathology

Biomarkers provide, by definition, “read-outs” or values that are related to one or more host processes. Here we review inter-marker relationships, independent of host outcomes (which are reviewed below).

Only four studies included assessments of “noninvasive” biomarkers (i.e., those not requiring the introduction of an endoscope or tube into the small bowel (e.g., for visualization, to obtain duodenal fluid or biopsy tissue) as well as intestinal histopathology [111, 136, 155]; only two of these studies compared histopathology to less invasive markers. Leite et al. assessed

the relationship of D-xylose and the degree of villous blunting and found no association [136]. Campbell et al. found that L:M was associated with various findings on scanning electronic microscopy (SEM) and was correlated with mucosal B lymphocyte density, intraepithelial lymphocyte (IEL) density, and the presence of perforin-positive IELs [111].

An additional three studies compared endoscopic visualization or markers in intestinal tissue obtained by biopsy to standard light microscopic histopathology [53, 118, 126]. Neither endoscopic [126] nor SEM [118] findings were associated with histopathology on light microscopy. However, intestinal maltase activity, as well as intestinal mRNA abundances for maltase-glucoamylase and the enterocyte-specific proteins villin and sodium-activated luminal glucose-galactose transporter 1, were correlated with villous atrophy [53].

Twelve studies [15, 43, 58, 110, 123-125, 132, 149, 151, 158, 159] compared 15 different non-intestinal tissue markers to each other. Four of these studies assessed serum L:R to other markers and found relationships with sucrose breath values [159], serum lactose [58], urinary nitrites [43], and urinary L:R [125], but did not demonstrate associations with stool reducing substances [58] or serum red blood cell markers [58]. Three other studies assessed urinary L:M to other markers and found no associations with fecal neopterin [15], urinary lactose or lactose:lactulose [124], or a variety of systemic immune/inflammatory markers including AGP, total IgG, albumin, and hemoglobin [123]. However, one additional study did find that L:M was associated with not only total serum IgM and IgA levels, but also IgG [110]. This study also found a correlation between L:M values and IgG endotoxin core antibody concentrations, and additionally found urinary lactulose excretion to correlate with circulating IgG endotoxin core antibody and endotoxin concentrations. Other studies found that urinary lactose:creatinine was inversely related to hemoglobin [151] and that fecal lactoferrin was related to TNF-α receptor I [132]. Others found no relationship between urinary nitrite and stool reducing substances [43] or between sucrose breath test and hemoglobin or C-reactive protein [159].

5.11 Relationships between EED Biomarkers and Growth or Other Outcomes of Interest

We sought to find biomarkers of processes relevant to consequential outcomes in the host. In the context of EED, the outcome of greatest proximate interest is growth failure, specifically linear growth failure, but other potential injuries (e.g., persistent diarrhea) and distal consequences (e.g., delayed cognitive development) were also considered.

Eight studies attempted to associate various markers with persistent diarrhea, six of which found such an association. In five of the six studies, these were systemic markers such as serum proteins or indices of serum white or red blood cells [106, 107, 142, 166, 308]. A sixth study found that concentrations of immunoglobulin in duodenal aspirates were associated with persistent diarrhea among children infected with *Giardia* [128]. A study of mannose-binding lectin (MBL) found that while a deficiency of the marker was associated with cryptosporidiosis, MBL concentrations were not significantly associated with either duration of diarrhea or persistent diarrhea [130]. While the eighth study did assess for hemoglobin association with persistent diarrhea, it was not clear from the reporting of their multivariate results if there was an association [114].

Fourteen studies [101, 103, 111, 113, 117, 121, 126, 131-133, 138, 154, 155, 167] included subjects with and without persistent diarrhea but did not statistically assess relationships between markers and this outcome, although five of these studies [101, 103, 117, 126, 155] did present data stratified by the outcome of persistent diarrhea.

While acute diarrhea is not a cardinal EED symptom, we included it as an outcome of interest when looking at studies that sought its association with biomarkers of enteric dysfunction. Our rationale was that intestinal damage or pathophysiology resulting from acute enteric processes might still provide meaningful insights into the utility of various markers in

representing pathologic gut processes. Eight studies [43, 58, 116, 124, 125, 159, 166, 308] investigated the relationship between markers and acute diarrhea. It should be noted that some of these studies included children with persistent diarrhea, but did not stratify results according to acute or persistent presentations; we include all of these studies under the heading “acute diarrhea”. Most of these studies found associations between acute diarrhea and the markers investigated. One of these studies [125] found a relationship between acute diarrhea and both urinary and serum L:R while another [58], a study limited to serum L:R testing, found associations between diarrhea and serum L:R and lactulose. However, Goto et al. [124] did not find an association between the L:M ratio and acute diarrhea.

Studies also found associations between acute diarrhea and other tests specific for intestinal function, such as the sucrose breath test [159], and fecal fat [116]. Association was also observed for two markers of innate immunity, neutrophil polarization in response to a chemotactic factor and monocyte spontaneous proliferation assays [104], as well as nitric oxide production [43]. While one study found associations between acute diarrhea and various red blood cell parameters [166], another [104] found a lack of association between acute diarrhea and other systemic markers such as white blood cells, transferrin, serum albumin, cytokines, and immunoglobulin subtypes.

Sixteen [15, 101, 108, 121, 130-134, 136, 140, 152, 158, 162, 168, 172] studies had subjects with and without acute diarrhea but did not statistically analyze the relationship between markers and acute diarrhea, although three [101, 152, 162] presented data stratified by acute diarrhea status. In two studies [101, 162], a high proportion of those with acute diarrhea were lactoferrin positive. One of these studies [101] also found that a low proportion of cases with acute diarrhea were positive for stool IL-8, but all of the individuals positive for stool IL-8 had acute diarrhea. The study [152] utilizing the D-xylose test found that of the eight children

with abnormal results, one had diarrhea and of 19 children with diarrhea, only one had an abnormal result for D-xylose.

Thirteen studies included children with symptomatic or clinically silent giardiasis [15, 105, 116, 117, 122-124, 128, 133, 137, 147, 150, 154]; diagnostic methods to identify *Giardia* varied between microscopy and antigen testing. Seven of these studies investigated the relationship between markers and giardiasis [15, 116, 123, 124, 128, 147, 150] and five found an association between at least one marker and the infection [116, 123, 124, 128, 147], although the studies commonly reported associations between one biomarker and not others. Some studies found serum total IgG and AGP [123], and lactose hydrogen breath test results [147] were significantly higher in asymptomatic, *Giardia*-infected children than in uninfected controls. Significantly higher mean concentrations of total IgM were found in duodenal aspirates of *Giardia* cases with persistent diarrhea compared to controls without diarrhea [128]. In a study that included subjects with and without diarrhea, giardiasis was associated with the presence of fecal fat, and this association held across four testing methods [116]. In contrast, a study of a fecal marker of inflammation, neopterin, did not find an association with *Giardia* infection [15]. The relationship between *Giardia* infection and a urinary marker of intestinal permeability differed across studies. While Goto et al. found an association between giardiasis and mean urinary L:M value in one study [124], they did not find this association in a subsequently published study [123]. Campbell et al. did not find an association between *Giardia* infection and urinary L:M [15], and another report found that urinary L:M was not consistently associated with giardiasis [150]. The relationship between circulating markers and *Giardia* infection varied by marker; specifically, an association was observed between infection and serum albumin but not hemoglobin concentrations [123]. Six studies [105, 117, 122, 133, 137, 154] that included both infected and uninfected subjects did not statistically investigate the relationship between *Giardia* infection and these markers, although two [105, 117] of these studies did present data stratified

by infection status and a third [154] provided a general description of the histopathology in infected subjects. These three studies investigated cases with intestinal symptoms consistent with celiac disease and referred for duodenal biopsy. Among the children that were not diagnosed as having celiac disease, the studies reported widely varying percentages of subjects with *Giardia* infection: 0.8% [117], 5.4% [105], and 78.6% [154].

Eight studies presented data on children with asymptomatic and/or symptomatic cryptosporidiosis. Five of these [101, 130-132, 172] tested the association of *Cryptosporidium* infection with various markers, three of which were conducted in Haiti by the same group [130-132]. Fecal lactoferrin [101, 132] serum mannose-binding lectin deficiency [130], and mean urinary L:M [172] were associated with cryptosporidiosis. A sixth study tested longitudinally collected stools of Brazilian children for *Cryptosporidium* and presented the results for the children with infection, finding that stool lactoferrin was strongly associated with symptomatic infection in children with *C. parvum* but not *C. hominis* [108].

In the three cryptosporidiosis studies that included testing for various fecal cytokines, results were mixed. For example, two studies found no association between infection and IL-8 [101, 131], IL-4 [131], or IL-10 [131], while associations were identified with each of these cytokines, as well as with IL-13, in one of the studies from Haiti [132]. One of the studies from Haiti found an association between fecal IFN-γ and control status rather than among those with *Cryptosporidium* infection [131], while the other did not detect IFN-γ in the stools of any subjects [132].

In two additional studies of subjects with and without *Cryptosporidium* infection, abnormal D-xylose results were found more often among infected children [152], while fecal lactoferrin did not appear to be related to infection status based on the numbers reported in the study, although statistical testing of the relationship was not described [162].

Sixteen studies assessed the association of markers of intestinal function with nutritional status outcomes [15, 43, 53, 108-112, 122-124, 139, 145, 150, 151, 153]. The anthropometric assessments used in these investigations varied, and the results for given measures were mixed. We describe the results of the eight studies that measured the relationship between L:M and anthropometrics above (see Table 15) [15, 110-112, 122-124, 150].

Urinary lactose:creatinine [151], stool neopterin [15], and intestinal lactase activity [109] were associated with all anthropometric indices investigated while stool lactoferrin [108] and intestinal maltase activity were not [53]. Results for urinary nitric oxide and WAZ correlation were of borderline statistical significance (effect ratio 0.88, p=0.05) [43].

Four studies examined the association between histopathology and growth parameters [53, 111, 145, 153]; two studies identified no relationship between weight-for-age measures [111, 145], while one reported that WAZ score was correlated with degree of villous atrophy [53]. Examination of tissue by digital morphometry in another study produced ambiguous results [153]. One of these studies also examined the relationship of weight-for-age with endoscopic visualization of the intestine and found no association [145]. Campbell et al. found varying results when assessing the relationship of different intestinal tissue cytokines and immune markers with WAZ score [111].