Supplement Article

Use of the Lactulose to Mannitol Ratio to Evaluate Childhood Environmental Enteric Dysfunction: A Systematic Review

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Childhood gut dysfunction (enteropathy) is common in resource-poor environments. Stunting is its presumed major consequence. Identification of biomarkers of gut dysfunction could identify the presence of, and, ideally, assess interventions for, enteropathy. Classically, enteropathy has been identified histopathologically. However, less invasive assays may be more sensitive for detecting earlier perturbations reflecting specific functional derangements. The most commonly used test has been the urinary lactulose to mannitol ratio (L:M), which primarily assesses gut leakiness, and which also measures absorption. We systematically reviewed the L:M literature published from 2000 to 2010 pertinent to children in developing country settings, and identified 25 relevant publications representing heterogeneous studies. We conclude that the L:M test has many attributes, including reflecting 2 physiologic processes (absorption and permeability) and likely correlation with growth failure consequent to child gut dysfunction. However, improved test technical performance, data reporting, and correlation with host phenotypes are needed to maximize the utility of this test.

Keywords. biological markers; environment; intestinal diseases/diagnosis.

Poor growth is a major problem in developing countries. Its most consequential manifestation, stunting, is often evident within year 1 of life and is largely irreversible by year 3 [1]. Small intestinal dysfunction is common among residents, especially children, of tropical regions and is thought to be an important contributor to stunting [2]. The first descriptions of “tropical enteropathy” focused on expatriates [3–5], and engendered the concept of gut lesions caused by “environmental” factors. The term “tropical enteropathy” has been replaced by the term “environmental enteropathy,” and more recently by “environmental enteric dysfunction” (EED) (as used in this article), because it encompasses the functional effects of this disorder [6]. Shortened villi, elongated crypts, and increased lamina propria lymphocytic density are the histopathologic hallmarks of EED [7, 8].

The ideal biomarker for EED would have multiple attributes. It should signal a process that precedes the outcome of consequence and is measurable at a point where interventions are effective. A biomarker should also reflect processes responsive to intervention (ie, treatment or prevention), and thereby serve as a surrogate outcome in trials. Theoretically, small bowel histology could provide insight. However, procurement of small bowel tissue poses challenges, including the practicalities of gut access (eg, cost, safety), no basis for determining the number of samples required to obtain sufficient information, the challenge of sampling error (ie, sampling adjacent “normal tissue”), and risk of variable interpretation of findings. Furthermore, we do not know if functional abnormalities precede, overlap, or occur subsequent to histologic changes. Last, reliance on biopsies skews data toward adults who more frequently undergo endoscopy [9].

Tissue-independent assessments of enteropathy have included tests of stool, urine, or blood and have focused
on microbes, inflammation, immune activation, digestion, absorption, and permeability of the gut. However, the most extensive experience involves tests of gut permeability, a subset of which also assess gut absorptive capacity.

The lactulose to mannitol ratio (L:M) has been the most commonly used marker of mucosal intestinal function and has much appeal. This noninvasive test involves oral administration of a dose of both sugars (ie, lactulose and mannitol) followed by a timed urine collection. Lactulose is a large sugar that is minimally absorbed from an intact small intestine. However, if permeability is altered, this disaccharide traverses intercellular spaces, is then cleared by glomerular filtration without renal tubular reabsorption, and is easily measured in urine. The co-administered sugar alcohol, mannitol, is absorbed (via transcellular pathways) proportional to small bowel absorptive capacity (ie, surface area). Shortened microvilli diminish uptake and subsequent urinary excretion of mannitol, which, like lactulose, is filtered and not reabsorbed.

Here, we systematically and comprehensively review how the L:M test has been used to measure small intestinal function in children <5 years of age in developing country settings, and its association with other measures of gut function and growth in these populations.

METHODS

This work was part of a wider systematic review to address the following question: What biomarkers or diagnostic tests have been used to identify or have been shown to be associated with mucosal dysfunction of the small intestine or host inflammation in children <5 years of age from developing country settings (per World Bank classification) or among marginalized or indigenous populations in a developed country? The scope of the umbrella review question coupled with the lack of index terms in health sciences databases specific to both biomarkers and diagnostic tests as well the condition of interest (EED) necessitated a broad search strategy.

First, we interrogated the Cochrane Database of Systematic Reviews, but found none related to childhood tropical sprue; enteric dysfunction; malabsorption syndromes; or tropical, environmental, or any other form of enteropathy in resource-limited settings. We then constructed search strategies specific to PubMed, Embase, Global Health, and each of the World Health Organization (WHO) regional databases. We further identified relevant literature by the “snowball” technique, that is, reviewing bibliographies of all references meeting our review inclusion criteria. Articles were limited to those written in English, French, German, Italian, Portuguese, or Spanish.

We identified 67,903 citations potentially relevant to EED generally that were published between January 2000 and April 2010. Titles, keywords, and abstracts were reviewed by pairs of research assistants to confirm relevance to EED per predetermined criteria (Supplementary Figure 1). The research assistant pairs “tagged” the confirmed citations to facilitate future retrieval by EED subtopic, including with a specific tag for diagnostic test/biomarker information. Inclusion/exclusion or tagging discrepancies within research assistant pairs were resolved by an author (D. M. D., Z. C. N., P. I. T., or K. V.). These were then examined against inclusion/exclusion criteria for the diagnostics/biomarkers review question (Supplementary Table 1).

RESULTS

Of the 3,815 articles published between January 2000 and April 2010 relevant to EED, we identified 374 (9.8%) and 77 (2.0%) of potential and confirmed relevance, respectively, to the umbrella diagnostics/biomarkers question. Of these 77 studies, 25 assessed L:M in children in 8 countries on 3 continents (Supplementary Table 2).

Community-based recruitment was utilized in 15 of the 77 studies, primarily of apparently healthy children [10–24] including infants of human immunodeficiency virus (HIV)–infected mothers [19, 21, 22]. In one study, subjects included children with various illnesses, including respiratory or enteric infections [25]. Sixty-three studies recruited children with varying degrees of malnutrition [26–31] and diarrhea [32–34], respectively. L:M data and details from the 25 studies are presented in the evidence table (Supplementary Table 2).

Range of L:M Values Reported

Most studies reported elevated (ie, abnormal) L:M values, but comparisons across studies were difficult because of differences in the way values were reported (Supplementary Table 3). For example, portrayals of central tendencies widely differed: 3 studies reported no central tendency values, 2 reported medians, and 20 reported means (2 as arithmetic means, 10 as geometric means [a mathematically preferred expression of a mean for ratios], and 8 reported means with type not specified).

The lowest (ie, the least abnormal) results were geometric means for HIV-negative South African infants <4 months of age born to HIV-infected mothers [21] (Supplementary Table 2). The highest values were geometric means reported by the same investigators in South African children aged 6–60 months hospitalized with severe diarrhea [32]. Indeed, these means were an order of magnitude higher than in other studies reviewed, prompting 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.

Mean L:M values from Asia and West Africa exceeded 0.12, which was cited as a reference mean for healthy 3- to 15-month-olds in the United Kingdom. However, this reference is
Table 1. Studies Testing Associations Between Anthropometric Indicators and Lactulose to Mannitol Ratio, Lactulose, and Mannitol

<table>
<thead>
<tr>
<th>Anthropometric Index</th>
<th>Studies Reporting on Relationship With L:M, No.</th>
<th>Studies Reporting on Relationship With Lactulose, No.</th>
<th>Studies Reporting on Relationship With Mannitol, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inverse Association</td>
<td>No Association</td>
<td>Inverse Association</td>
</tr>
<tr>
<td>ΔHAZ</td>
<td>2 [13, 18]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ΔWAZ</td>
<td>2 [16, 18]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WHZ</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ΔWHZ</td>
<td>1 [16]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BMI Z</td>
<td>0</td>
<td>1 [14]</td>
<td>0</td>
</tr>
</tbody>
</table>

Where not represented in L:M, lactulose, or mannitol columns, the studies did not report statistical evaluation of the biomarker and the growth parameter. Numbers in boxes represent number of papers addressing the indicated index and associations with test results, with references in brackets.

Abbreviations: Δ, change in corresponding metric; BMI Z, body mass index z score; HAZ, height-for-age z score; L:M, lactulose to mannitol ratio; WAZ, weight-for-age z score; WHZ, weight-for-height z score.

* Although no association was found between mannitol excretion and these growth parameters in the control group, an association was found in the group of subjects receiving alanyl-glutamine–supplemented enteral formula. The association was reported as inverse (ie, improved absorption was noted to have a negative relationship with growth).

problems; although it is mentioned in a review article [35] and a Gambian study [36] (both published by the same group in 1991), the data are not described, nor are primary citations provided. Additionally, these 2 publications do not specify if the 0.12 reference mean is a geometric or arithmetic calculation. Whereas the studies we reviewed used widely varying cut-points for “normal” values (mostly based on Western populations), we found that the 0.12 reference value was the one most frequently utilized (Supplementary Table 3).

In contrast to the Asian and West African studies, some South American [27, 28] and South African [19, 21] studies found L:M ratios that were normal (ie, not elevated) (Supplementary Table 2). For example, the median L:M values among children <10 years of age with growth attainment less than the median for their impoverished Brazilian urban community was 0.09 [27] and 0.03–0.04 [28]. These values are within the confidence intervals for those of healthy children in the community, but again, reference values for the healthy children were neither reported nor cited.

Seven studies examined the variability of L:M with age, either between or within individuals. Three studies found no association [15, 17, 19], whereas 4 did [10, 14, 20, 24]. Only 2 of these 7 studies examined and reported the separate impacts of lactulose and mannitol excretion on the relationship with age. The geometric mean L:M among a cohort of rural Gambian infants aged 2 months more than doubled between 3 and 12 months (r = 0.44, P < .001), secondary to divergent (ie, increased lactulose and decreased mannitol) excretion [10]. Campbell et al [14] found a small, nonsignificant improvement in mean L:M over 3.5 months driven by improved mannitol excretion without changed lactulose excretion.

L:M Association With Growth and Other Host Outcomes

Eight studies assessed the relationship between L:M and growth, and 5 found significant inverse relationships [10, 13, 14, 18, 24] (Table 1 and Supplementary Table 2). Among the few studies that assessed the individual roles of lactulose and mannitol excretion, higher lactulose excretion appeared to drive the correlation between L:M and height-for-age z score (HAZ) [10, 14]. One study [28] did not report L:M results in comparison to growth outcomes (only those for individual sugar excretion) and demonstrated that increased mannitol absorption was directly associated with weight-for-height and weight-for-age z score (WHZ and WAZ, respectively).

Four studies investigated the potential association between L:M and Giardia infection. Three found no [13, 24] or limited [18] association (Supplementary Table 2). In the fourth study, the geometric mean L:M among mildly stunted Nepalese children from urban squatter settlements aged <6 years was 0.26, but significantly differed between Giardia-infected and noninfected children (0.43 vs 0.25) [17]. There was no association between L:M and diarrhea history [17] or helminthiasis [17, 18]. Associations were found between L:M and infection with HIV [19, 21] and Cryptosporidium and rotavirus [33]. Two studies reported seasonal L:M variation in rural Bangladesh, with peaks during [16] or following [18] monsoon season.

L:M as an Endpoint in Intervention Trials

L:M was used as an endpoint in intervention studies of therapeutic diets [11, 28, 34], antihelminthic/Giardia treatments [16, 18], antibiotics [12], probiotics [23], and vitamin A supplementation [15, 19, 20, 25, 27]. Interestingly, one study demonstrated reduced lactulose as well as mannitol excretion, but an
unchanged overall ratio [27], thereby highlighting the value of reporting absorption of the sugars separately.

Power considerations were often subordinated to study realities. For example, Goto et al [16] recruited 410 children based on sample size calculations to observe changes in growth parameters. However, larger loss to follow-up than anticipated and other methodological issues reduced the final sample for analysis to 222 children, which was less than that needed for adequate power for projected HAZ and WAZ outcomes. Also, Northrop-Clewes et al [18] indicated that their target sample size was based on logistical reasons, before stating that "the null effect of deworming . . . could . . . have stemmed from an inadequate sample size or duration of follow-up" and concluded that "better outcomes were achieved in other studies with much smaller samples . . . and shorter duration . . . ." This comparison draws attention to the potential role of publication bias in any review of the literature.

**Association Between L:M and Other Markers**

Five studies related L:M to other potential markers of intestinal dysfunction. No correlation was identified with lactose to lactulose ratio urinary excretion [17] or stool neopterin [13]. Divergent relationships were found with systemic immunoglobulin concentrations [10, 24]. Only one study related L:M to histopathology [26], and demonstrated correlation with densities of mucosal B lymphocytes, intraepithelial lymphocytes (IEL), and perforin-positive IELs. However, it is not clear if other measured intestinal markers were either not correlated or not assessed for correlation.

**Test Technical Issues**

Performance of the L:M test can be compromised by technical issues, and there was a lack of standardization of procedures and reporting of certain details between studies. For example, only 11 studies reported if subjects were fasted before challenge, and when specified, the durations of fasting differed. Also, the sugar load was dosed according to weight in only 14 of the 25 studies we evaluated (Supplementary Table 3).

**DISCUSSION**

The L:M test appears to have utility in assessment of intestinal function, especially its relation to nonideal linear growth [10, 13, 14, 18, 24], which would, by itself, justify refinement of this test for investigative purposes. However, several issues diminish our enthusiasm for a blanket endorsement of this test without further validation (Supplementary Table 3). The concerns relate to variable subject preparation, inconsistent urine collection and assay methods, poor citation of primary methods, ill-defined purported reference values used as abnormal/normal cutpoints, and variability in how results are portrayed.

Our first concern is failure to dose the sugar load to body size. Lactulose and/or mannitol can cause diarrhea and occasionally vomiting; standardized dosing might avert this complication. The hyperosmolar challenge might also cause reverse solvent drag (retention of the solute in the gut), and prompted suggestions to coadminister a liquid meal [37].

Because gastric emptying might affect absorption kinetics, standard and adequate fasting should be employed. However, this recommendation may be difficult to implement when testing infants who cannot easily tolerate fasts, and was not consistently done in the studies that we reviewed. Ideally, this practice should be standardized, or studied to determine if it is, in fact, necessary. There are additional concerns regarding test function: bacteriuria (either from infection or contamination) could lower urinary sugar concentrations [38]; intestinal permeability might be altered by the solutes [39, 40]; humans produce and excrete mannitol [39]; and lactulose hastens orocecal transit [41]. Also, analytical processes can play an important role in urinary L:M determination. Specifically, many of the studies were performed in an era in which high-pressure liquid chromatography was the method of analysis; however, recently, mass spectrometry in combination with chromatography has been increasingly utilized because of its ease of use and accuracy [42].

Individual mannitol and lactulose components provide information unique to 2 distinct, important intestinal processes: permeability and absorption. However, only 18 studies provided separate results, and of these, only 11 reported excretion as a fraction of total sugar administered. 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 [28].

Five-hour “bagged” urine collections are considered standard, but are a considerable inconvenience to research staff and families. Collection times varied across the studies, and 2 did not report this information at all. Additionally, there was a paucity of test failure data in most studies. Kukuruzovic et al [43] published a study that employed 5-hour urinary collection, and although it did not meet this review’s inclusion criteria (it was published before 2000 and utilized the lactulose to rhamnose ratio to assess permeability), it exemplifies the portrayal of test failures. The authors reported that of 234 tests attempted among children under age 6 years, 37% failed, most often due to urine leakage or contamination by stool and most prominently in girls with acute diarrhea (47% failure). Other causes of failure, such as vomiting or refusal to drink the sugar solution, occurred in 9% of test attempts.

Another major concern is the absence of reference norms. Most studies reported elevated L:M in their populations based on Western (usually UK) childhood values [44–51] or to presumed norms for children in developing country settings.
Values may change with physiologic maturation, but age stratification or adjustment was rarely performed. Perhaps most vexingly, there was no standard reporting of central tendency measures, making comparison of results between studies very difficult. Clearly, despite the relative abundance of L:M data, variations in design and reporting hampered interstudy comparisons.

CONCLUSIONS

In summary, the L:M test has many attributes as a measure of gut dysfunction, including its safety, plausible correlation with gut pathophysiology, and longstanding use in research projects. However, the L:M literature is highly heterogeneous in terms of the assay method, specimen collection, and data reporting standards utilized. Moreover, normal values for children who have healthy small bowel mucosa but who reside in regions of risk for enteropathy are not established. However, L:M is currently the most commonly used standard against which other markers of small bowel permeability and overall function are applied. Even if perfected, the L:M as a stand-alone test is unlikely to suffice as a biomarker for all purposes (ie, screening populations, diagnosing dysfunction in individual cases, and monitoring this condition and its consequences). Biomarker discovery and validation efforts are needed to assess the utility of other gut function and host inflammation biomarkers. If a perfect biomarker, or even an adequate one, cannot be found, an alternative might be the development of an “enteropathy index” that integrates a constellation of clinical symptoms and signs and/or a set of markers. Variations of an “enteropathy index” might be needed to accommodate the processes of screening, diagnosis, and clinical monitoring. If so, the L:M test might play a useful role in the latter 2 scenarios.

Efforts to refine the L:M test are needed in parallel to novel biomarker discovery and scrutiny of other existing tests. Refinement should focus on methodological standardization as well as delineation of normal values in populations of interest by assessment of values among children from developing countries who are genetically similar to those at risk for enteropathy, but not residing in risky environments. A similarly daunting task was accomplished when the WHO growth standards were defined among children from multiple countries who were breastfed as infants and “lived in socio-economic conditions favorable to growth” [57]; these children were measured to derive normative growth standards. Finally, and perhaps most important, longitudinal assessments of at-risk children are needed to determine if a refined L:M, other markers, or an aggregative “enteropathy index” are correlated with the outcome of most consequential interest—the healthy growth of children in resource-limited settings.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Tomas Allen for assistance in building the systematic search terms and Ariana Jasarevic for manuscript preparation.

Financial support. This review was supported by the Tropical Enteropathy and Enteric Dysfunction: Opportunities to Advance Current Knowledge (grant number 5240) and the Enteric Dysfunction Biomarker Development and Coordination (grant number 1071928) to Washington University from the Bill & Melinda Gates Foundation. Dr Tarr is also supported by P30DK052574.

Supplement sponsorship. This article appeared as part of the supplement “The Malnutrition and Enteric Disease Study (MAL-ED): Understanding the Consequences for Child Health and Development,” which is sponsored by the National Institutes of Health and the Foundation for the National Institutes of Health.

Potential conflicts of interest. P. I. T. has an intellectual property interest in novel technology to measure gut permeability. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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