Journal of Pediatric Gastroenterology and Nutrition
28:282–290 © March 1999 Lippincott Williams & Wilkins, Inc., Philadelphia

INCAP Publication PCI/088

Epidemiology of Altered Intestinal Permeability to Lactulose and Mannitol in Guatemalan Infants

Keiko Goto, *Francisco Chew, *Benjamin Torún, Janet M. Peerson, and Kenneth H. Brown

*Program in International Nutrition, Department of Nutrition, University of California, Davis, California, U.S.A.; and *Instituto de Nutrición de Centro America y Panama, Guatemala City, Guatemala*

ABSTRACT
Background: Subclinical alterations of small intestinal function have been reported frequently in tropical countries. Studies of small intestinal permeability to lactulose and mannitol were therefore completed in Guatemalan infants from a low-income, periurban community to assess the prevalence of altered intestinal function and the factors associated with this condition.
Methods: Two hundred studies were successfully completed in 158 infants who had been free of diarrhea for at least 1 week before the day of study. Urinary concentrations of lactulose and mannitol during the 5-hour period after ingestion of 400 mg/kg body weight of lactulose and 100 mg/kg body weight of mannitol were measured by gas–liquid chromatography and compared by age group, feeding practices, anthropometric indexes, and serum iron and zinc concentrations.
Results: The overall prevalence of altered intestinal permeability (defined as a ratio of urinary recovery of lactulose to mannitol [L/M] $\geq$0.07) was 30%. The urinary L/M recovery ratio was positively associated with age; low weight for age; and, in infants less than 6 months of age, non–breast-feeding. Children with serum iron concentrations less than 7.16 µM/l (40 µg/dl) had higher median L/M ratios (L/M = 0.068; 95% confidence interval [CI], 0.054, 0.085) than those with iron levels higher than this cutoff (L/M = 0.052; CI = 0.046, 0.058; p = 0.038). The median urinary L/M recovery ratio in 10 currently asymptomatic infants who had diarrhea during the week before testing (0.087; CI = 0.49, 0.154) was higher than that in children who had been free from diarrhea for at least 1 week (0.052; CI = 0.048, 0.056; p = 0.01).
Conclusion: Age, feeding practices, low weight-for-age, low serum iron concentration, and recent diarrhea were all associated with altered intestinal function in this group of Guatemalan infants. *JPGN 28:282–290, 1999.* **Key Words:** Breast-feeding—Diarrhea—Intestinal permeability—Iron deficiency—Lactulose—Malnutrition—Mannitol.

The subclinical alterations of small bowel morphology and function that occur frequently in low-income tropical countries have been termed tropical enteropathy (1). The intestinal mucosa of affected people is characterized by shortened, leaf-shaped villi, increased crypt depth, and infiltration of the lamina propria by inflammatory cells. Diagnosis of this condition has traditionally relied on morphologic and functional tests of the small intestinal mucosa, such as mucosal biopsies and studies of absorption of xylose, fat, and vitamin B_{12} (1–3).

Recently developed tests of intestinal permeability can provide an alternative indicator of intestinal mucosal function (4,5). These tests examine urinary concentrations of selected probe molecules that permeate through the small intestinal mucosa and pass unchanged into the urine (6). Two sugar probes of different molecular weight (MWt), such as mannitol (MWt 183) and lactulose (MWt 342), are commonly chosen because of their differential uptake by the normal intestine (7). Mannitol, a small monosaccharide, can pass through numerous small water-filled pores in the cell membranes of the enterocytes (the so-called transcellular pathway), whereas the larger disaccharide, lactulose, can pass only very slowly through large intercellular spaces (the paracellular pathway). When the small bowel mucosa is damaged, the permeation of smaller substances is decreased because of the reduced mucosal surface area, whereas the uptake of larger molecules increases because mucosal damage creates leakier paracellular channels and/or an increased number of these channels (6). Therefore, permeability tests using two test substances can assess the overall integrity of the intestinal mucosa. Moreover, by using the ratio between two probes, it is possible to control for extraneous factors such as gastric retention, liver disease, and partial intraintestinal or urinary bacterial degradation of the probes that may otherwise confound the test results (8).

It has been known for some years that tropical enter-

Received March 3, 1998; revised September 16, 1998; accepted September 29, 1998.

Address correspondence and reprint requests to Dr. K. H. Brown, Department of Nutrition, University of California, Davis, CA 95616-8669, U.S.A.

opathy occurs as early as the first year of life (9,10), especially after the introduction of complementary foods to breast-fed infants. Moreover, a recent study in The Gambia found a significant relationship between abnormal intestinal permeability and faltering growth in infants, suggesting that altered permeability may have contributed to malnutrition or poor nutritional status may have induced changes in intestinal function (10). These combined sets of results suggest that age, feeding practices, and nutritional status may each independently influence small intestinal function. However, because these risk factors often change simultaneously during the first year of life, it is still unknown which of them is mainly responsible for altered intestinal function.

To obtain more information on these and other possible risk factors for intestinal dysfunction, we conducted a cross-sectional study of Guatemalan infants to describe the epidemiology of altered small intestinal permeability and the factors associated with this condition. We used intestinal permeability to lactulose and mannitol to identify differences in small bowel function, both for the aforementioned reasons and because of the feasibility of using this method in field conditions. The permeability studies were completed in a sample of nonacutely ill infants stratified by age, feeding practice, and nutritional status to provide information on whether these factors alone or together were associated with small bowel function in this population. Serum iron and zinc status were also assessed because previous investigators had found a significant correlation between deficiencies of these nutrients and altered intestinal permeability (11–14). Finally, a small group of infants who had recovered clinically from diarrhea within the past week were examined, because other investigators have noted a relationship between enteric infections and altered intestinal permeability (5,15–17).

MATERIALS AND METHODS

Community and Subjects

The study was performed in a low-income, periurban community of Guatemala City, Guatemala. Approximately 8000 people resided in the neighborhood where the tests were conducted, of whom approximately 600 were infants less than 1 year of age. Two field workers from the community conducted a survey to identify infants who had not had diarrhea for at least 1 week. Mothers of 467 children in this age range were interviewed concerning their socioeconomic status and their infants' birth date, reported birth weight, gestational age, feeding practices, and health status. They were then invited to participate in further studies at the health center within the next few days.

The mothers and their infants were scheduled to arrive at the health center at 7:30 AM. The infants' current feeding practices (exclusively or predominantly breast-fed, mixed-fed, or non-breast-fed) were reconfirmed by interviewing the caregiver, and the children were then weighed and measured. Their nutritional status was classified by weight-for-age, length-for-age, and weight-for-length in relation to National Center for Health Statistics/World Health Organization reference data (18). The children were considered to be underweight if their z-score weight-for-age (WAZ) was less than −1.5.

Two hundred forty-six children participated in the permeability studies. Of these, 10 had had diarrhea within the past week and 5 had fever on the day of the test; their results were therefore excluded from the first set of analyses. Of the 231 nonacutely ill children who completed permeability tests, 38 were studied twice and 10 were studied three times, when either their age group or feeding practices changed, resulting in a total of 289 permeability studies in these children. Because several of these tests had to be eliminated because of incomplete urine collections or other problems described later, the final number of nonacutely ill subjects with adequate collections was 158, and the total number of acceptable studies was 200.

Small Intestinal Permeability Tests

Sugar solutions of 400 mg lactulose (kindly donated by Solvay Pharmaceuticals, Marietta, GA, U.S.A.) and 100 mg mannitol (Sigma, St. Louis, MO, U.S.A.) in 3 ml water were used for the permeability tests. Children were given the sugar solution (3 ml/kg body weight) by a dropper at least 2 hours after the previous meal. Thirty minutes after the dose, the children were allowed to drink cooled, previously boiled water. After 30 more minutes, they were allowed to consume milk, milk formula (Similac; Ross Laboratories, Columbus, OH, U.S.A.), eggs, or biscuits, according to their usual dietary practices.

A urine collection bag was placed shortly after the infants consumed the test dose of sugars, and all urine was collected for the next 5 hours. Every time a child voided, the urine volume was measured and recorded. One drop of 20% (wt/vol) chlorhexidine gluconate was added to each sample to prevent bacterial growth, and approximately 3 ml of the pooled urine sample was stored at −20°C until analysis. Urinary lactulose and mannitol concentrations were measured by gas–liquid chromatography, as described by Laker (19–20). Turanose (Sigma) and methyl-α-D-gluco-pyranoside (Aldrich, Milwaukee, WI, U.S.A.) were used as internal standards for lactulose and mannitol, respectively. Urinary creatinine concentration was measured colorimetrically, using the Jaffe reaction (21). Mannitol–creatinine and lactulose–creatinine ratios were calculated by using the urinary concentrations of each. Urinary lactulose–mannitol (L/M) recovery ratios were calculated as the ratio of the percentage recoveries of the oral doses of each test sugar.

Blood Collection and Mineral Analysis

One hour after the sugars were administered to the children, approximately 3 ml of venous blood was collected, and the serum was frozen for subsequent transport to Davis on dry ice. Hematocrit was measured at the health center, and iron supplements were administered to children with low hematocrits after completion of the testing procedures. Serum iron and zinc concentrations were analyzed by atomic absorption spectrophotometry after wet-ashing (22). Serum ferritin was measured by radioimmunoassay (Diagnostic Products Corporation; Los Angeles, CA, U.S.A.) and C-reactive protein was measured by radial immunoassay (The Binding Site; Birmingham, United Kingdom) in a subset of 64 children.

Statistics

Technical problems with urine collections (incomplete samples and/or contamination with feces) produced unacceptable studies in 89 instances. Thus, the main analyses were performed using the results of 200 tests in which the urine was collected uneventfully from 158 infants who were free of fever or recent diarrhea (group A). Data from the 10 children with recent diarrhea were analyzed separately, but the information from the 5 children with fever were not considered further because of the small sample size.

To assess whether those children whose studies were acceptable could be considered representative of the community as a whole, their age, sex, feeding practice, reported birth weight, and selected indicators of socioeconomic status (housing quality, fuel source, and parental education and occupation) were compared separately with those who had unacceptable permeability studies (group B) and with those of all other children in the community who were surveyed initially. Analysis of variance (ANOVA) was used for comparison of continuous variables and the chi-square test of homogeneity for categorical variables (23).

The prevalence of abnormal intestinal mucosal function was defined on the basis of a urinary L/M recovery ratio of 0.07 or more, as has been suggested previously (15). However, because this cutoff may be considered somewhat arbitrary, we also examined possible risk factors for altered permeability using cutoffs of 0.05 or more and 0.03 or more and by comparing the L/M, lactulose–creatinine (L/C), and mannitol–creatinine (M/C) ratios as continuous rather than categorical variables for children with and without the particular characteristics of interest. To simplify the presentation of results, these are shown only for the cutoff of 0.07 or more and for the analyses of continuous variables. For the latter analyses, the permeability test results were compared by age group, feeding practice, and nutritional status, using both individual one-way ANOVAs, and three-way ANOVAs (23). The influence of non–breast-feeding on intestinal permeability was further examined by regressing the logarithm of urinary L/M recovery ratio on the age when breast-feeding was discontinued and on the amount of time since weaning had occurred.

Permeability test results were also separately regressed on the serum trace mineral concentrations. When the initial regression model was statistically significant, two-phase regression models were used to explore whether there was a threshold in the relationship between serum mineral concentration and the permeability test results (24). Serum ferritin values were only available for a subset of children. However, serum ferritin and serum iron concentrations were significantly correlated ($r = 0.45$; $p < 0.001$), and therefore only the relationships between serum iron and permeability test results are reported in detail because of the larger number of children with serum iron values. Logarithmic transformation of L/M, L/C, and M/C ratios, hematocrits and serum concentrations of zinc, iron, ferritin, and C-reactive protein were used for all analyses because of the skewed distributions of these data.

The L/M, L/C, and M/C ratios of children with recent diarrhea were compared with those of children who had been free from diarrhea, using both one-way ANOVA (with recent diarrhea as the main effect), and four-way ANOVA (with recent diarrhea, age, feeding practice, and nutritional status by WAZ as the main effects) to determine whether recent diarrhea was independently correlated with intestinal permeability.

ETHICAL CONSIDERATIONS

Children were admitted to the study with the written, informed consent of a parent or guardian. The study protocol was approved by the Human Subjects' Committees of the University of California, Davis, U.S.A. and the Instituto de Nutrición de Centro America y Panamá, Guatemala.

RESULTS

Characteristics of Study Children

The characteristics of children who successfully completed the permeability studies (group A, $n = 158$), of those whose permeability studies were unacceptable because of incomplete collections or fecal contamination (group B, $n = 73$), and of all other children surveyed in the study community ($n = 236$) are shown in Table 1. The three subsets of children were generally similar in age, reported birth weight, feeding practices, and selected indicators of socioeconomic status. A greater proportion of males completed the permeability studies successfully.

The anthropometric status and feeding practices of children in group A are shown by age group in Table 2. Younger children were more likely to be breast-fed and well nourished, whereas older children were more likely to be non–breast-fed, stunted, and underweight.

Prevalence of Elevated Lactulose–Mannitol Ratio

Using the previously stated definition of abnormal intestinal permeability (L/M recovery ratio $\geq$0.07), the overall prevalence of apparent intestinal dysfunction in the study subjects was 29.5%. The rate of altered permeability varied according to age, feeding practices, and nutritional status (weight-for-age), as shown in Table 3. Older infants were more likely to have elevated L/M ratios ($p = 0.042$), as were those who stopped breast-feeding ($p = 0.019$) and those whose WAZ was less than −1.5 ($p = 0.007$). Similar relationships between these risk factors and elevated L/M recovery ratios were present when cutoffs of 0.05 or more or 0.03 or more were used for these latter ratios, although the prevalences of altered permeability were, of course, even greater with the lower thresholds.

TABLE 1. *Characteristics of children in group A, children in group B, and all other children in the study community*

	Group A (n = 158)	Group B (n = 73)	Others in study community (n = 236)
Age (mo)	4.7 ± 3.4	5.7 ± 3.5	5.3 ± 3.3
Reported birth weight (kg)	3.08 ± 0.50	3.07 ± 0.53	3.12 ± 0.50
Sex (M), n (%)	94 (59.5)	30 (41.1)	111 (47.2)
Feeding Practice, n (%)			
Exclusively or predominantly breast-fed	76 (48.1)	33 (45.2)	118 (50.0)
Mixed fed	59 (37.3)	27 (37.0)	75 (31.8)
Non–breast-fed	23 (14.6)	13 (17.8)	43 (12.9)
Weight (kg)	6.22 ± 1.63	6.31 ± 1.75	—
Length (cm)	61.5 ± 7.6	62.1 ± 9.5	—
Length-for-age (z score)	−1.13 ± 1.12	−1.03 ± 1.18	—
Weight-for-age (z score)	−0.87 ± 1.20	−1.05 ± 1.22	—
Weight-for-length (z score)	−0.05 ± 0.91	−0.32 ± 1.25	—
Maternal education (yrs)	4.8 ± 3.2	4.4 ± 3.2	4.4 ± 3.3
Paternal education (yrs)	5.6 ± 3.2	6.2 ± 3.2	6.0 ± 3.4
Piped water in house, n (%)	117 (74.0)	57 (78.1)	183 (77.5)
Toilet in house, n (%)	53 (33.5)	26 (35.6)	74 (31.4)

Children in group A were those who successfully completed intestinal permeability studies and were free from diarrhea for at least 1 week. Children in group B were those whose permeability studies were incomplete or contaminated (see Statistics section in text). Anthropometric data were available only for those who were present at the test center. Data not marked otherwise are mean ± standard deviation. No significant differences were determined by subject category, except for the percentage of males, which was greater in group A ($p < 0.05$).

Type of Altered Permeability

The ratios L/M, M/C, and L/C were examined separately by age, feeding practices, and nutritional status to assess whether a decreased M/C ratio, increased L/C ratio, or both were responsible for the elevated L/M ratios that were observed in relation to these factors. The L/C ratio was not significantly correlated with any of these characteristics, whereas M/C and L/M ratios were significantly correlated with all of them, indicating that altered M/C ratios were responsible for the observed differences in L/M ratios by the risk factors of interest.

Comparison of Lactulose–Mannitol Ratios by Potential Risk Factors

The geometric mean L/M ratio was related to age, feeding practices, and nutritional status (Table 4). Older children had a higher mean L/M ratio than younger children (p = 0.03). Exclusively and predominantly breast-fed infants had the lowest L/M ratio among three feeding practice groups (p = 0.004). Underweight children had higher L/M ratios than those whose WAZ was more than −1.5 (p = 0.001).

Because these three possible risk factors correlated

TABLE 2. *Characteristics of children in group A, by age group*

	0–2 months (n = 43)	3–5 months (n = 68)	6–11 months (n = 89)	Significance
Weight (kg)	4.46 ± 1.06^{a}	6.00 ± 1.04^{b}	7.38 ± 1.19^{c}	p < 0.001
Length (cm)	54.1 ± 5.8^{a}	60.7 ± 3.5^{b}	66.4 ± 7.4^{c}	p < 0.001
Length-for-age (z score)	−0.62 ± 0.95^{a}	−1.00 ± 1.11^{ab}	−1.43 ± 1.09^{b}	p < 0.001
Weight-for-age (z score)	−0.36 ± 1.05^{a}	−0.57 ± 1.11^{a}	−1.32 ± 1.20^{b}	p < 0.001
Weight-for-length (z score)	0.08 ± 0.72^{ab}	0.12 ± 0.94^{a}	−0.32 ± 0.98^{b}	p = 0.007
Nutritional status, n (%)				
WAZ ≤ −1.5	5 (11.6)	16 (23.5)	40 (44.9)	p < 0.001
Feeding practice, n (%)				
Exclusively or predominantly breast-fed	35 (81.4)	26 (38.2)	2 (2.2)	p < 0.001
Mixed fed	6 (14.0)	37 (54.4)	62 (69.7)	
Non–breast-fed	2 (4.6)	5 (7.4)	25 (28.1)	

Significance of differences by age group (analysis of variance or chi-square). Means with different superscripts are significantly different from each other at α = 0.05 (Tukey's test). Data are mean ± standard deviation.

TABLE 3. *Prevalence of elevated urinary lactulose–mannitol (L/M) recovery ratio (≥0.07) by infant age, feeding practice, and nutritional status*

	Urinary L/M recovery ratio		
	≥0.07	<0.07	Significance[a]
Age group *n* (%)			
0–2 months	8 (18.6)	35 (81.4)	0.042
3–5 months	17 (25.0)	51 (75.0)	
6–11 months	34 (38.2)	55 (61.8)	
Feeding practice *n* (%)			
Exclusively or predominantly breast-fed	11 (17.5)	52 (82.5)	0.019
Mixed fed	34 (32.4)	71 (67.6)	
Non–breast-fed	14 (43.8)	18 (56.3)	
Nutritional status *n* (%)			
Weight-for-age *z* score >–1.5	33 (23.7)	106 (76.3)	0.007
Weight-for-age *z* score ≤–1.5	26 (42.6)	35 (57.4)	

[a]Significance of difference in the proportion of infants with low or high lactulose–mannitol ratio by individual characteristic of interest (age group, feeding practice, or weight-for-age category) by chi-square test.

with each other (Table 2), their independent relationships with intestinal permeability were explored using three-way ANOVA. Preliminary analyses indicated that the age categories could be collapsed to 0 to 5 months and 6 to 11 months, and the feeding practice groups could be reduced to breast-feeding or non–breast-feeding. Each of the factors explored was significantly associated with the urinary L/M ratios ($p < 0.001$). There was a significant interaction between age group and feeding practice (Table 5). The L/M ratio was significantly greater in the non–breast-fed group than in the breast-fed group in the younger children, but there was no relationship between L/M ratio and feeding practices in the older children.

Age of Termination of Breast-Feeding Versus Intestinal Permeability

Among children who had already discontinued breast-feeding ($n = 34$), the relationships between intestinal permeability and both the age of termination of breast-feeding and the number of months since weaning were explored, using regression analyses. The L/M ratio was negatively correlated with the age of termination of breast-feeding ($r = -0.38$; $p = 0.036$; Fig. 1). However, there was no significant correlation between L/M ratio and duration of the period of non–breast-feeding, suggesting that it was the early termination of breast-feeding rather than the amount of time since weaning that was associated most closely with altered small intestinal mucosal function.

Serum Iron and Zinc Concentrations and Intestinal Permeability

There was a significant association between intestinal permeability and serum iron concentration. Children with serum iron concentrations less than 7.16 μM/l (40 μg/dl) had higher geometric mean L/M ratios (L/M = 0.068; 95% CI = 0.054, 0.085) than those with iron levels higher than this cutoff (L/M = 0.052; CI = 0.046,

TABLE 4. *Median, 25th, and 75th percentiles of urinary lactulose–mannitol recovery ratio by age group, feeding practice, and nutritional status*

	Median	25%	75%	Significance
Age group				
0–2 months	0.041	0.028	0.069	$p = 0.025$
3–5 months	0.049	0.033	0.072	
6–11 months	0.054	0.038	0.087	
Feeding practice				
Exclusively or predominantly breast-fed	0.041	0.029	0.064	$p = 0.004$
Mix fed	0.054	0.035	0.081	
Non–breast-fed	0.060	0.043	0.097	
Weight-for-age				
Normal	0.045	0.032	0.069	$p < 0.001$
Underweight	0.065	0.044	0.098	

TABLE 5. *Geometric mean (and 95% confidence interval) urinary lactulose–mannitol recovery ratio by age group and feeding practice*

Age group (mo)	Feeding practice	
	Breast-fed	Non–breast-fed
0–5 months	0.046 (0.042, 0.050)[a] (n = 104)	0.094 (0.057, 0.155)[b] (n = 7)
6–11 months	0.058 (0.050, 0.068)[b] (n = 64)	0.056 (0.044, 0.073)[a,b] (n = 25)

Means with different superscripts are significantly different from each other at α = 0.05 (Tukey's test).

0.058; p = 0.038). As with the aforementioned risk factors, the altered L/M ratio associated with low serum iron concentrations could be explained primarily because of differences in the M/C ratio. Serum ferritin concentrations were also associated positively with the M/C ratios (r = 0.27; p = 0.032) in the subgroup of children for whom serum ferritin results were available. According to the two-phase regression model, the M/C ratio did not increase significantly with iron concentrations more than 10.8 μM/l (60 μg/dl), suggesting that the relationship between intestinal permeability and iron status may have been present only among children with iron deficiency. There were no significant relationships between serum zinc concentrations and any of the intestinal permeability results.

Diarrhea Group Versus Group A

The 10 diarrhea-free children who had had an episode of diarrhea within 1 week of the permeability studies were analyzed separately. The L/M ratios were higher in the diarrhea group than in the children in group A, primarily because of reduced M/C ratios in those with recent diarrhea (Table 6). A combined ANOVA model including independent variables of age, feeding practice, nutritional status (WAZ), diarrhea status, and all significant interactions, indicated that recent diarrhea was independently associated with the L/M ratios (p = 0.05).

DISCUSSION

The present studies were performed to define the rate of altered intestinal function, measured by permeability to test sugars, among infants in a poor, periurban community of Guatemala City and to explore possible risk factors associated with this condition. The children who were included in the analyses could be considered generally representative of all children less than 1 year of age in the study community, because there were no significant differences in age, reported birth weight, feeding practices, and socioeconomic status of the study group compared with the remaining children in the community. Not unexpectedly, because of the greater ease in collecting urine samples from boys, more of them successfully completed the studies than girls. However, none of the study results differed by the sex of the child, and this overrepresentation of boys should not have introduced any bias into the pooled results.

Interpretation of these tests of intestinal permeability is complicated by uncertainty regarding the appropriate cutoff to use for distinguishing between normal and abnormal intestinal function. Several investigators have published data on the range of urinary L/M recovery ratios in healthy European children who had no evidence of gastrointestinal disease. Noone et al. (17) reported the results of studies of 10 British children aged 3 to 18 months whose L/M ratios ranged from 0.018 to 0.05, with a median of 0.032. Hamilton et al. (25) found that the L/M urinary excretion ratios of 33 Scottish children 7 months to 14 years of age ranged from 0.005 to 0.09, with a median of 0.036. Isolauri et al. (16) presented results from 9 healthy Finnish children aged 2 to 23 months whose L/M ratios were all less than 0.05 and from 19 children aged 7 to 26 months who were febrile but free from intestinal symptoms and whose urinary recovery ratios ranged from approximately 0.005 to 0.12, with a median of approximately 0.02. Van Elburg et al. (26) found an upper limit of normal (mean + 2 standard deviations) of 0.09 among 30 healthy Dutch children whose ages ranged from 0 to 16 years. Ford et al. (15) reported an upper limit of normal of 0.07 for studies of children using lactulose and rhamnose (MWt = 164), which has a similar molecular weight as mannitol (MWt = 183).

Based on these previous reports, we considered urinary L/M recovery ratios of 0.07 or more to be abnormal in the present study. This cutoff value is in an intermediate range compared with previous studies of presumably healthy children in industrialized countries. Many of the children in the earlier studies were older than those in the present one, and older children tend to have higher L/M ratios, as is noted later in the article. The results of the permeability tests indicate that the prevalence of elevated L/M ratios, using the stated cutoff, was common (30%), and this increased gradually during the first year of life. Elevated L/M ratios were caused mainly by low M/C ratios, suggesting that decreased mucosal surface area rather than dysfunction of mucosal tight junctions was the primary intestinal alteration responsible for the differences in permeability test results. Similar findings were reported by Lunn et al. (10) in Gambian children.

We also examined the relationships between possible risk factors of altered intestinal permeability and L/M ratios without applying specific cutoffs. Urinary L/M recovery ratios were positively correlated with age, feeding practice, and nutritional status. Non–breast-feeding was associated with decreased permeability to mannitol in infants less than 6 months of age, but not in older ones. This suggests that breast-feeding may affect small intestinal mucosal function only in younger infants. This con-

FIG. 1. Age of termination of breast-feeding versus urinary lactulose–mannitol recovery ratio ($p = 0.004$).

clusion is supported by the observations that L/M ratios were correlated with the age of termination of breast-feeding, but not with the period of time since weaning. Children who were breast-fed for at least 6 months may have been protected from subsequent changes in intestinal function after weaning.

Breast-feeding may prevent altered mucosal function in several ways. First, breast-feeding is associated with a decreased number of infections, because of specific immunologic and other nonspecific protective factors in human milk (27) and reduced exposure to contaminated foods. For example, investigators in studies in Peru and the Philippines found that the prevalences of diarrhea and other infections were significantly less in children who were exclusively breast-fed compared with those who received mixed diets or no breast milk (28,29). Second, recovery of the small bowel mucosa after infection may be faster in breast-fed infants because of factors in breast milk that promote intestinal mucosal proliferation (30). Finally, the additional foods given to children aged 0 to 5 months may not substitute for breast milk adequately in quantity and quality of nutrients provided. For example, nutrients such as iron and zinc are absorbed more efficiently from breast milk than from non–breast-milk sources (31,32). Thus, nutritional deficiencies in young non–breast-fed infants may contribute to altered intestinal permeability.

TABLE 6. *Results of permeability studies of children who had diarrhea within the past week and those in group A*

	Diarrhea ($n = 10$)	Group A ($n = 200$)	Significance
Median L/M ratio	0.090 (0.038, 0.121)	0.050 (0.033, 0.078)	$p = 0.008$
Median M/C ratio	3.703 (1.341, 5.509)	5.18 (3.53, 6.78)	$p = 0.28$
Median L/C ratio	1.012 (0.845, 1.332)	0.992 (0.704, 1.358)	$p = 0.37$

L/M, lactalose–mannitol; M/C, mannitol–creatinine; L/C, lactulose–creatinine.

Children with diarrhea during past week were asymptomatic at the time of the intestinal permeability studies. Children in group A were those who successfully completed intestinal permeability studies and were free from diarrhea for at least one week. Data in parentheses are ratios in the 25th and 75th percentiles.

Even among breast-fed children in the study community, the L/M ratio gradually increased with age. Likewise, in a previous study of Gambian children who were breast-fed for two years, L/M recovery ratios increased significantly with age up to 15 months (5). These age-related findings may be because of progressively worsening nutritional status after birth, increased incidence of infection, or normal maturational changes of the small intestinal mucosa. It is well known that the concentrations of protein and trace elements, such as iron and zinc, decline in human milk during the course of lactation (33). It is possible that breast milk is insufficient to maintain normal intestinal mucosal function of older infants unless sufficient amounts of nutritionally adequate complementary foods are added to the diet. Furthermore, the greater frequency of diarrhea and other infections that occur in older infants as more foods are added to the diet may contribute to alterations of small intestinal mucosal function. It is also possible that age-related change in intestinal permeability is a normal developmental phenomenon, as has been claimed by other investigators (34).

Nutritional status, assessed by WAZ, was significantly associated with intestinal permeability results, regardless of the child's age or feeding practice. It is uncertain, however, whether nutritional status was the cause or result of altered small intestinal function. It is well known that malnourished children are more susceptible to infections (35) and that their illnesses tend to be more prolonged (36). Moreover, studies in experimental animals have found that intestinal mucosal recovery after enteric infection is delayed in malnourished hosts (37). Thus, malnourished children may have had altered permeability because of more frequent prior infections and prolonged intestinal injury thereafter. However, aberrations of intestinal permeability may also be associated with malabsorption of macronutrients or micronutrients, thereby causing secondary malnutrition. Previous studies have found an inverse relationship between xylose absorption (also believed to be an indicator of small intestinal mucosal surface area) and absorption of macronutrients from mixed diets (3). Additionally, field studies in The Gambia found that children with abnormal intestinal permeability had slower growth during 1 year of observation (10). Thus, it is possible that abnormal intestinal function may have contributed to malnutrition in these children.

There was a significant correlation between serum iron concentration and L/M ratio, confirming the results of previous studies (13,14). This suggests that young children may require adequate iron reserves to maintain normal intestinal mucosal function. Alternatively, as discussed above, it is conceivable that those intestinal changes that are identified by permeability studies may interfere with iron absorption.

No significant relationship between zinc status and intestinal permeability was found, possibly indicating that zinc status did not affect intestinal permeability. However, it is well known that serum zinc concentration is a poor indicator of zinc status in individual subjects (38). Thus, the absence of a significant association between serum zinc concentrations and intestinal permeability study results may not necessarily rule out a functional relationship between zinc status and intestinal mucosal integrity.

Previous investigators have found that intestinal permeability may be altered in children with acute and persistent diarrhea (5,15–17). In our study, the significantly higher L/M ratio in the small number of children who had diarrhea during the previous week suggests that the recovery of the intestinal mucosa may be delayed by more than several days after diarrhea resolves. These findings are compatible with results reported by Noone et al., (17) who found that derangements of permeability may persist for several weeks after acute rotaviral enteritis (17). In the present study, the results of the four-way analysis of variance indicated that recent diarrhea was independently associated with elevated L/M ratios. Notably, three of the children with recent diarrhea had an L/M ratio of 0.07 or less, although the factors enabling their more rapid recovery are not known. Isolauri et al. (16) found that children who were fed continuously during illness had a more rapid return to normal intestinal permeability than those who were fasted (16). Unfortunately, the children's recent dietary histories were not recorded in the present study.

In conclusion, our study indicates that age, feeding practices, anthropometric status, serum iron concentration, and history of recent diarrhea were all associated with altered small intestinal mucosal permeability among infants in the study community. Prospective longitudinal studies comparing feeding practices, duration of diarrhea, enteric pathogens, small intestinal bacterial overgrowth, immune responses to foreign antigens, and intestinal permeability in young children should be helpful in providing further understanding of the causes of impaired small intestinal function and their association with young children's nutritional status.

Acknowledgment: The authors thank Prof. John Labavitch and Dr. Carl Greve of the University of California Davis and Dr. Omar Dary of the Institute of Nutrition of Central America and Panama (INCAP) for their assistance with the laboratory analyses; the INCAP field staff; and the mothers and children of the study community.

This research was supported by the University Development and Linkage Program of the United States Agency for International Development, Cooperative Agreement DAN-A-0091-00115-00, which provides funds for collaborative activities between the University of California, Davis and INCAP.

REFERENCES

1. Lindenbaum J. Progress in gastroenterology. Tropical enteropathy. *Gastroenterology* 1973;64:637–52.
2. Halsted CH, Sheir S, Sourial N, Patwardhan VN. Small intestinal structure and absorption in Egypt. *Am J Clin Nutr* 1969;22:744–54.
3. Brown KH, Khatun M, Ahmed M. Relationship of the xylose absorption status of children in Bangladesh to their absorption of macronutrients from local diets. *Am J Clin Nutr* 1981;34:1540–7.
4. Ukabam SO, Homeida MMA, Cooper BT. Small intestinal permeability in normal Sudanese subjects: Evidence of tropical enteropathy. *Trans R Soc Trop Med Hyg* 1986;80:204–7.
5. Behrens RH, Lunn PG, Northrop CA, Hanlon PW, Neale G. Factors affecting the integrity of the intestinal mucosa of Gambian children. *Am J Clin Nutr* 1987;45:1433–41.
6. Menzies IS. Intestinal absorption and secretion. *In: Transmucosal passage of inert molecules in health and disease.* London: MTP Press, 1984:527–42.
7. Elia M, Behrens R, Northrop C, Wraight P, Neale G. Evaluation of mannitol, lactulose and ^{51}Cr-labelled ethylene-diamine-tetra-acetate as markers of intestinal permeability in man. *Clin Sci* 1987; 73:197–204.
8. Cooper BT. Tests of small intestinal permeability in clinical practice. *J Clin Gastroenterol* 1984;6:499–501.
9. Lindenbaum J, Harmon JW, Gerson CD. Subclinical malabsorption in developing countries. *Am J Clin Nutr* 1972;25:1056–61.
10. Lunn PG, Northrop-Clewes CA, Downes RM. Intestinal permeability, mucosal injury, and growth faltering in Gambian infants. *Lancet* 1991;338:907–10.
11. Roy SK, Behrens RH, Haider SM, et al. Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhea and persistent diarrhea syndrome. *J Pediatr Gastroenterol Nutr* 1992;15:289–96.
12. Bates CJ, Evans PH, Dardenne M, et al. A trial of zinc supplementation in young rural Gambian children. *Br J Nutr* 1993;69: 243–55.
13. Berant M, Khourie M, Menzies IS. Effect of iron deficiency on small intestinal permeability in infants and young children. *J Pediatr Gastroenterol Nutr* 1992;14:17–20.

14. Juby LD, Rothwell J, Axon ATR. Lactulose/mannitol test: An ideal screen for celiac disease. *Gastroenterology* 1989;96:79–85.
15. Ford RPK, Menzies IS, Philips AP, Walker–Smith JA, and Turner MW. Intestinal sugar permeability: Relationship to diarrheal disease and small bowel morphology. *J Pediatr Gastroenterol Nutr* 1985;4:468–74.
16. Isolauri E, Juntunen M, Wiren S, Vuorinen P, Koivula T. Intestinal permeability changes in acute gastroenteritis: Effects of clinical factors and nutritional management. *J Pediatr Gastroenterol Nutr* 1989;8:466–73.
17. Noone C, Menzies IS, Banatvala JE, Scopes JW. Intestinal permeability and lactose hydrolysis in human rotaviral gastroenteritis assessed simultaneously by non-invasive differential sugar permeation. *Eur J Clin Invest* 1986;16:217–25.
18. U.S. National Center for Health Statistics. *NCHS growth curves for children birth–18 years, United States.* Rockville MD: National Center for Health Statistics, Vital and Health Statistics Series 11, No. 165 Department of Health, Education and Welfare, 1977. Publication PHS 78-1650.
19. Laker MF. Estimation of disaccharides in plasma and urine by gas–liquid chromatography. *J Chromatogr* 1979;163:9–18.
20. Laker MF, Mount JN. Mannitol estimation in biological fluids by gas–liquid chromatography of trimethyl derivatives. *Clin Chem* 1980;2613:441–3.
21. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. *Scand J Clin Lab Invest* 1965;17:381–7.
22. Clegg MS, Keen CL, Lönnerdal B, Hurley LS. Influence of ashing techniques on the analysis of trace elements in animal tissue. 1: Wet Ashing. *Biol Trace Element Res* 1981;3:107–15.
23. Neter J, Wasserman W, Kutner MH. *Applied linear statistical models.* 2nd ed. Homewood, IL: Richard D. Irwin, 1985.
24. Hinkley DV. Inference in two-phase regression. *J Am Stat Assoc* 1971;66:736–43.
25. Hamilton I, Hill A, Bose B, Bouchier IAD, Forsyth JS. Small intestinal permeability in pediatric clinical practice. *J Pediatr Gastroenterol Nutr* 1987;6:697–701.
26. Van Elburg RM, Uil JJ, Kokke FTM, et al. Repeatability of the sugar-absorption test, using lactulose and mannitol, for measuring intestinal permeability for sugars. *J Pediatr Gastroenterol Nutr* 1995;20:184–8.
27. Hanson LA, Carlsson B, Jalil F, et al. Antiviral and antibacterial factors in human milk. In: Hanson LA, ed. *Biology of human milk.* New York: Nestle, Vevey/Raven Press, 1988:141–56.
28. Brown KH, Black RE, Lopez de Romaña G, Kanashiro HC. Infant-feeding practices and their relationship with diarrheal and other diseases in Huascar (Lima), Peru. *Pediatrics* 1989;83:31–40.
29. Popkin BM, Adair L, Akin JS, Black R, Briscoe J, Flieger W. Breast-feeding and diarrheal morbidity. *Pediatrics* 1990;86:874–82.
30. Menard D, Arsenault P. Epidermal and neural growth factor in milk: Effects of epidermal growth factor on the development of the gastrointestinal tract. In: Hanson LA, ed. *Biology of human milk.* New York: Nestle, Vevey/Raven Press, 1988:105–22.
31. McMillan JA, Landaw SA, Oski FA. Iron sufficiency in breast-fed infants and the availability of iron from human milk. *Pediatrics* 1976;58:686–91.
32. Sandström B, Cederblad Å, Lönnerdal B. Zinc absorption from human milk, cow's milk, and infant formulas. *Am J Dis Child* 1983;137:726–9.
33. World Health Organization. *The quantity and quality of breast milk. Report on the WHO collaborative study on breast-feeding.* Geneva: World Health Organization, 1985.
34. Weaver LT. The impact of milk and weaning diet on gastrointestinal permeability in English and Gambian infants. *Trans Roy Soc Trop Med Hyg* 1988;82:784–9.
35. Brown KH, Solomons NW. Nutritional problems of developing countries. *Infect Dis Clin North Am* 1991;5:297–317.
36. Black RE. Persistent diarrhea in children of developing countries. *Pediatr Infect Dis J* 1993;12:751–61.
37. Butzner JD, Butler DG, Mimiats OP, Hamilton JR. Impact of chronic protein-calorie malnutrition on small intestinal repair after acute viral enteritis: A study in gnotobiotic piglets. *Pediatr Res* 1985;19:476–81.
38. Aggett PJ. Malnutrition and trace element metabolism. In: *The malnourished child.* New York: Nestle, Vevey/Raven Press, 1990: 156–76.