

Assessment of Intestinal Permeability with a Two-Hour Urine Collection

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The differential urinary excretion of orally administered lactulose and mannitol is used to evaluate intestinal permeability. This test usually involves a 5- to 6-hr urine collection. We hypothesized that a shorter collection time would give an equivalent result. Forty-three patients with a variety of gastrointestinal symptoms and diagnoses (group 1) and 42 patients with Crohn's disease (group 2) had a standard lactulose/mannitol permeability test. The lactulose and mannitol urinary excretion was calculated using the first urine (group 1) or the 1-hr and 2-hr urine (group 2) and was compared to the values calculated from the routine 5- or 6-hr collection. Lactulose excretion kinetics, expressed as the percent of the total urinary excretion within a given time period, were as follows: 21% in first hour (group 2), 29% in second hour (group 2), and 46% in first 2.5 hr (group 1). Mannitol urinary excretion kinetics were 16%, 31%, and 44%, respectively. The lactulose/mannitol ratio based on a standard urine collection correlated well with the ratio based on just the first urine produced by the patient ($R^2 = 0.94$; $P < 0.001$; group 1) and the 2-hr urine ($R^2 = 0.464$; $P < 0.001$; group 2). Future use of the lactulose/mannitol ratio to assess intestinal permeability may be able to be simplified by shortening the urine collection time.

KEY WORDS: intestinal permeability; lactulose; mannitol; small intestine; mucosal permeability.

The assessment of intestinal permeability using the differential excretion of lactulose and mannitol (or comparable dual sugar markers) is performed to evaluate small intestinal disease, such as celiac sprue (1) or Crohn's disease (2). The test has also been used in clinical research as a quantitative marker of small intestinal injury due to a variety of diseases such as HIV infection (3), Crohn's disease (4), and protozoal infections (5). The theoretical and practical issues regarding the performance of this test have been reviewed (6). The test is safe, noninvasive, and quan-

titative. However, urine is routinely collected for 5 or 6 hr after ingestion of the test sugars. This prolonged urine collection mandates either a home collection with storage and transportation of urine or a prolonged office or laboratory visit. We hypothesized that since it is the ratio of the sugars that is reported, a shortened collection time might not significantly alter the result. We therefore designed a study to answer the following questions: How much lactulose and mannitol are excreted in the first few hours as compared to 5 or 6 hr? How would a 1- or 2-hr test compare to a 5- or 6-hr test?

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MATERIALS AND METHODS

Patients. All patients met the following criteria for inclusion in the study: age 18–70, able to ingest the test solutions and comply with urine collections, and no evidence of renal impairment. All patients gave informed

TABLE 1. SUGAR EXCRETION AT DIFFERENT TIME INTERVALS AFTER INGESTION

	<i>First urine</i> (mean = 2.5 hr)*		<i>Second urine</i> (mean = 4 hr)*		<i>Total (6 hr)</i>	
	<i>Amount</i> (mg)	<i>Percent</i> <i>of total</i>	<i>Amount</i> (mg)	<i>Percent</i> <i>of total</i>	<i>Amount</i> (mg)	<i>Percent</i> <i>of total</i>
Group 1. Endoscopy (N = 43)						
Lactulose	0.04 ± 0.04	46	0.03 ± 0.04	34	0.09 ± 0.1	100
Mannitol	0.25 ± 0.22	44	0.22 ± 0.17	35	0.60 ± 0.38	100
	<i>First hour</i>		<i>Second hour</i>		<i>Total (5 hr)</i>	
	<i>Amount</i> (mg)	<i>Percent</i> <i>of total</i>	<i>Amount</i> (mg)	<i>Percent</i> <i>of total</i>	<i>Amount</i> (mg)	<i>Percent</i> <i>of total</i>
Group 2. Crohn's disease (N = 42)						
Lactulose	0.02 ± 0.03	21	0.03 ± 0.03	29	0.11 ± 0.12	100
Mannitol	0.13 ± 0.1	16	0.3 ± 0.17	31	0.87 ± 0.48	100

* In group 1 the patients passed urine when they felt the urge and not according to times as in group 2. The average time for first urine in group 1 was 2.5 hr; for second urine, it was 4 hr.

consent to participate in the study and the study was approved by the appropriate local review boards for human subject research. Two groups of patients were studied. Group 1 was composed of 43 patients who were scheduled for upper gastrointestinal endoscopy. Many of them were selected because of a clinical suspicion that they would have abnormal intestinal permeability. At the time of endoscopy, they were informed of the details of the lactulose/mannitol test and were asked to volunteer to have the test performed after the endoscopy. The indications for endoscopy included gastrointestinal bleeding, dyspepsia/epigastric pain, odynophagia/dysphagia, HIV-related diarrhea, Crohn's-related diarrhea, peptic ulcer, diarrhea and abdominal pain, mid-abdominal pain, gastroesophageal reflux disease (GERD), and postcholecystectomy syndrome. The mean age was 43 years, with 30 males and 15 females. There were 15 white Americans, 18 black Americans, 10 Latin Americans, and 2 Asians. Thirty were inpatients; 15 were outpatients. Thirty-five of these patients had abnormal findings at endoscopy but only one had an endoscopic abnormality in the small intestine (jejunal ulcer). Group 2 was composed of 42 patients with Crohn's disease who were part of a treatment trial. The location of disease was evenly divided between large bowel only, small bowel only, and mixed large and small bowel. One patient had duodenal Crohn's. Active disease, based on a Harvey-Bradshaw Index of greater than 5, was present in 16 of 42. The mean age was 38, with 24 male and 18 female. There were 32 white Americans, 3 black Americans, 2 of Middle Eastern origin, and 5 Latin Americans.

Lactulose/Mannitol Test. The test was performed as previously described (5). Briefly, the test solution was made of 5 g mannitol, 10 g lactulose, and 20 g sucrose in 150 ml of water. These proportions were chosen because of a project designed to reproduce the findings of a study on patients with Crohn's disease (2). The test solution was freshly prepared and refrigerated. The patients fasted overnight. After voiding and discarding the urine, they drank the entire 150 ml of the test solution. In group 1 the patients were instructed to void when they felt the urge but to collect the first and second specimen in separate containers which had been prelabeled. All remaining urine for a total of 6 hr after ingestion was placed in a third container. For group 2

the patients were instructed to void at 1 and 2 hr after ingestion of the solution and place the urine in prelabeled containers. The rest of the urine was collected in a third container to complete a 5-hr collection. The urine volumes were measured and aliquots were stored at 4°C. The concentration of sugars in each container were measured using high-pressure liquid chromatography (7). Amounts of the two sugars in each container were calculated. Before these data were analyzed, we had determined to use 0.03 as the upper limit of normal for the permeability ratio, based on prior practice and consistency with the previously cited study (2).

Data Analysis. The total amount of the sugars excreted during the entire collection period was compared to the amounts excreted during the first or second collection, expressed as percent of total excreted. The standard lactulose/mannitol excretion ratio (percent of ingested lactulose excreted during the entire urine collection divided by the percent of ingested mannitol excreted during the entire urine collection) was compared to a result using only the first or first and second urine collection. The correlation of the different results was evaluated by the Pearson correlation coefficient.

RESULTS

All patients complied with test instructions. Table 1 shows the amount of sugar excreted in each of the time intervals for both groups. In group 1 the average time to the first urine collection was 2.5 hr (range 15 min to 5 hr; 2 in the first hour, 8 in the second, 17 in the third, 6 in the fourth, and 9 in the fifth). Therefore, the first urine for group 1 is roughly comparable to the combination of the first and second urine of group 2. Lactulose excretion kinetics, expressed as the percent of the total urinary excretion within a given time period, were as follows: 21% in the first hour (group 2), 29% in the second hour (group 2), and 46% in the first 2.5 hr (group 1). Mannitol urinary

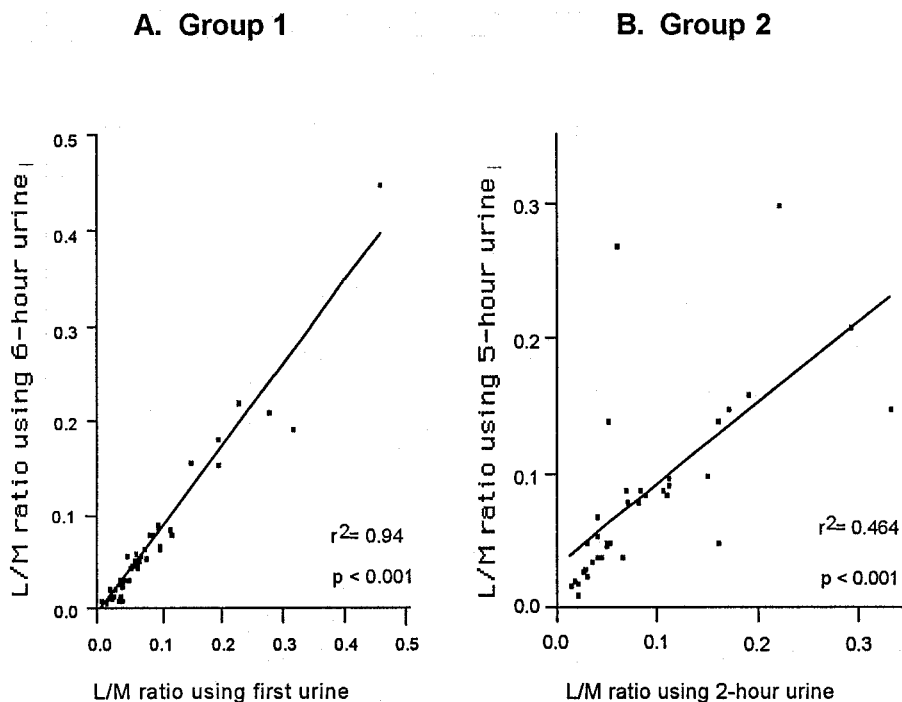


Fig 1. Correlation between standard lactulose/mannitol test and shortened urine collection: (A) A strong correlation was demonstrated between the lactulose/mannitol ratio derived from the first spontaneously passed urine specimen (mean time from ingestion of test sugars was 2.5 h) and a full 6-hr collection. Test subjects were 43 patients undergoing diagnostic upper endoscopy for gastrointestinal symptoms. (B) A strong correlation was demonstrated between the lactulose/mannitol ratio calculated from a 2-hr urine collection and a 5-hr urine collection. Test subjects were 42 patients with Crohn's disease.

excretion kinetics were 16%, 31%, and 44%, respectively.

The similarities in the excretion patterns of the lactulose and mannitol suggest that excretion ratios calculated on the basis of shortened collection intervals would be similar to the standard collection times. In fact, the lactulose/mannitol ratio based on a 6-hr urine collection in group 1 correlated well with the ratio based on just the first urine produced by the patient ($R^2 = 0.94$; $P < 0.001$; Figure 1A). Similarly, both the 1-hr ($R^2 = 0.438$; $P < 0.001$; data not shown) or 2-hr ($R^2 = 0.464$; $P < 0.001$; Figure 1B) excretion ratios based on shortened urine collections in group 2 correlated with the ratios calculated with the five hour collections. Figure 2 shows the relationship between the standard lactulose/mannitol excretion ratios determined from the total 5- or 6-hr collections and the shortened collection intervals in the two groups combined ($R^2 = 0.757$; $P < 0.001$).

The lactulose/mannitol excretion ratio results are often dichotomized into "normal" and "abnormal" based on a upper limit of normal cutoff point. In our studies we have used 0.03 as the cutoff. Table 2 shows

the accuracy of the shortened form of the test in predicting the results of the standard test using the longer collection times. In group 1, 31 of 43 patients (72%) had an abnormal test based on the 6-hr collection. All but one of these were identified by the shortened "first urine" version of the test, and none of the values in the normal range were found to be abnormal by the shortened test (98% accuracy). In group 2, 33 of 42 patients had an abnormal test using the total urine collection. Table 2 shows that the 1-hr test had an accuracy of 83% and the 2-hr test had an accuracy of 91%. Figure 2 shows that the lactulose/mannitol ratio calculated on the basis of the shortened urine collection time (2-hr or first urine) agrees with the long urine collection time (abnormal being >0.03) 94% of the time.

DISCUSSION

The lactulose/mannitol permeability test result is expressed as the percent recovery of an ingested dose of lactulose divided by the percent recovery of an ingested dose of mannitol. If the urine collection is

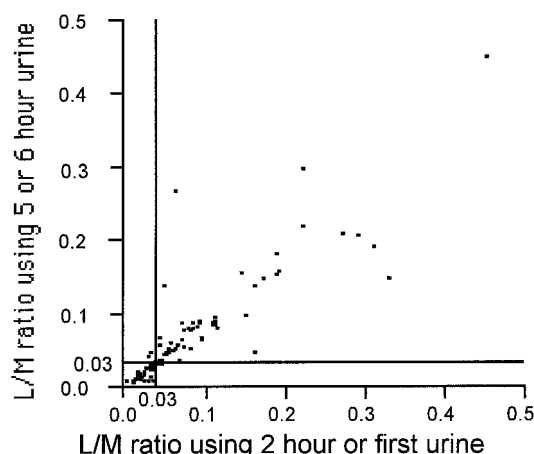


Fig 2. Correlation between standard lactulose/mannitol test and shortened test—groups 1 and 2 combined. The *x* axis shows the lactulose/mannitol ratio based on a 2-hr urine or first urine. The *y* axis is the lactulose/mannitol ratio based on the 5- or 6-hr urine collection. The upper limit of normal is 0.03. The figure demonstrates that there is agreement (normal versus abnormal) between the two tests in 80/85 patients.

shortened then the percentages will decrease, but the ratio will change only if there is a difference in the timing of the excretion of the two test sugars. One previous study showed that both controls and patients with celiac disease excreted about 50% of the total 5-hr collection of lactulose in the first 2.5 hr (8). About 60% of the total 5-hr urinary excretion of the monosaccharide, L-rhamnose, was excreted in the first 2.5 hr in both patients and controls. Even though the ratios in patients were much higher than in controls, both test sugars appeared to be excreted at a similar rate if expressed as percent of total per unit time. A related study showed that the percentage of both lactulose and L-rhamnose excreted in the first 2.5 hr of a 5-hr collection was similar, with only about a 10% variation in the multiple testing situations that they reported (9). Increased osmolarity of the test solution caused an increase in the percentage of 2.5-hr excretion, but it was similar for both the disaccharide and monosaccharide.

TABLE 2. AGREEMENT BETWEEN STANDARD AND SHORTENED URINE COLLECTION TO DETERMINE NORMAL OR ABNORMAL PERMEABILITY

	<i>Agreement with standard lactulose-mannitol: above or below 0.03</i>		
	<i>Agree</i>	<i>False high</i>	<i>False low</i>
First urine (group 1)	42/43	1/43	0/43
One hour (group 2)	35/42	3/42	4/42
Two hour (group 2)	38/42	1/42	3/42

We were able to demonstrate in this selected group of 85 patients that lactulose and mannitol, on average, are excreted in the urine at a similar rate. For both sugars, about 20% of a prolonged collection is excreted in the first hour, and 50% in the first 2 hr. This explains why lactulose/mannitol ratios calculated on the basis of shortened collection times are similar to the prolonged collections (Figures 1 and 2). The superior correlation in group 1 is probably due to the longer collection time. The first urine was longer than 2 hr in 32 of the 42 patients studied in this group. However, there may be specific patients whose disaccharide excretion rates differ based on disease type, severity, or distribution. Our data are not definitive regarding this possibility.

Only five patients' results shown in Figures 1 and 2 show a worrisome departure from the line of equivalence, and only three of these involve values in the "near normal" range. In none of these cases is either result below 0.03. All five of these patients had Crohn's disease, but there were no consistent features (ie, location of disease or disease activity) that were associated with the large deviation.

About three quarters of the 85 patients in our study had an abnormal permeability test based on the standard urine collection time. Using the prolonged collection as the gold standard and 0.03 as the upper limit of normal, the shortened collection time had a sensitivity of 96% and a specificity of 97% in identifying an abnormal result.

The assessment of intestinal permeability is used for clinical evaluation of disease presence or severity or for quantitative assessment of intestinal injury for clinical research. Based on the results presented here, we believe that future assessment of intestinal permeability may be simplified by using a shortened urine collection time.

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