

## Human Intestinal Disaccharidase Activities: Correlations with Age, Biopsy Technique, and Degree of Villus Atrophy

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**Summary:** The relationship between intestinal morphology, disaccharidase activity, and disaccharide absorption is controversial. A retrospective study of 798 consecutive biopsies was performed to determine whether disaccharidase activities varied by subject age, biopsy technique, and degree of villus atrophy. Lactase activity was inversely correlated with age in the absence or presence of villus atrophy; sucrase, maltase, and palatinase activities did not correlate with age. Biopsies obtained by capsule or endoscopy had similar disaccharidase activities. In

subjects 24 months of age or younger, the degree of mucosal injury was inversely correlated with lactase activity. In subjects older than 24 months, the degree of mucosal injury was inversely correlated with maltase and, to a lesser extent, lactase activities. The data suggest that disaccharidase activities in mucosal biopsies, whether obtained by endoscopy or capsule, are diminished in the presence of mucosal injury and correlate inversely with the degree of injury. **Key Words:** Lactase—Sucrase—Maltase—Villus atrophy.

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The relationship between intestinal morphology, disaccharidase activity, and disaccharide absorption is controversial. Several studies have indicated a significant correlation between villus height, degree of mucosal injury, and mucosal disaccharidase activities (1-4), while others have shown no correlation (5-9). Individuals with primary defects such as glucose-galactose malabsorption, sucrase-isomaltase deficiency, and adult-type hypolactasia have carbohydrate malabsorption without alterations in intestinal histologic appearance (10). Infants with protracted diarrhea, acquired monosaccharide intolerance (11), secondary disaccharidase deficiency in protracted diarrhea of infancy (12), or active celiac disease exhibit malabsorption of carbohydrates in the setting of significant villus atrophy.

The utility of disaccharidase assays in predicting the histologic appearance of the jejunum remains controversial. Traditionally, lactase, due to its location, has been thought to be the most vulnerable to injury, and therefore assessment of its activity by noninvasive means has been considered a reasonable indicator of mucosal functional integrity. However, the most commonly used noninvasive technique of assessing lactase activity, the breath hydrogen tolerance test, is semiquantitative and of inadequate specificity to select subjects for biopsy (13).

Because of the decline in lactase activity in a significant percentage of the population after the age of 3-5 years (14), we hypothesized that the activity of an alpha-glucosidase or of a combination of enzymes less dependent on age would be a superior predictor(s) of mucosal integrity. Therefore, a retrospective analysis of disaccharidase concentrations in mucosal biopsies obtained at the Children's Hospital of Buffalo from 1979 to 1987 was undertaken to test this hypothesis.

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This evaluation was undertaken to gain evidence about disaccharidase concentrations in mucosal injury. Secondary aims were to determine whether any developmental changes other than a decline in lactase would be present, to determine whether disaccharidase assays performed in endoscopic and capsule biopsies were equally predictive of the presence of mucosal injury, and to see if one or more enzyme assays will predict the degree of mucosal injury when histologic evidence is inconclusive.

## METHODS

### Subjects

The results of all small intestinal mucosal biopsies performed at the Children's Hospital of Buffalo in which histologic examination and disaccharidase determinations had been performed were included in the study regardless of the clinical indication for the biopsy. Subject age and pathology reports were obtained from a retrospective review of the hospital records.

### Assays

Lactase, sucrase, maltase, and palatinase (iso-maltose analog) were assayed by the method of Dahlqvist (15). Protein was assayed by the method of Lowry et al. (16).

### Histologic Grading

Estimation of the degree of atrophy was retrospectively obtained from pathology reports by a single individual (L.A.H.) who was blinded as to the results of the disaccharidase assays. Specimens with normal morphologic appearance were scored as 0; mild villus atrophy, duodenitis, or other inflammation as 1; moderate atrophy as 2; severe atrophy as 3; and complete villus atrophy, as in active celiac disease, as 4.

### Statistical Analysis

Stepwise multiple regression analysis of disaccharidase concentrations by age, histologic grade, and biopsy technique were performed. The  $r^2$  and  $p$

values for all significant correlations ( $p < 0.05$ ) are reported in each section. A series of planned analyses for several age groups were performed to determine the suitability of the several assays in older subjects and the relative ability of the two biopsy techniques (capsule or endoscopy) to detect abnormal results.

Cross-tabulation was performed to permit the calculation of sensitivities and specificities for the several age groups and biopsy techniques; an injury score  $> 0.5$ , obtained from each regression equation, was considered abnormal in all calculations.

## RESULTS

Seven hundred ninety-eight subjects underwent small intestinal biopsy during the interval (613 by endoscopy, 178 by capsule, and seven by a technique not documented in the record).

### Effect of Subject Age

Among subjects with a normal histologic appearance, ( $n = 589$ ), lactase activities were inversely correlated with age ( $r^2 = 0.1133$ ,  $p < 0.001$ ). The developmental profile of mucosal lactase specific activity is presented in Fig. 1. The specific activity begins to decline in infancy and reaches a plateau during the preschool years. Among subjects with damaged mucosa ( $n = 202$ ), a marginally significant inverse correlation between subject age and lactase activities was obtained ( $r^2 = 0.0539$ ,  $p < 0.05$ ).

### Effects of Biopsy Technique

In subjects 24 months of age or younger ( $n = 225$ ), the age group in which endoscopic and cap-

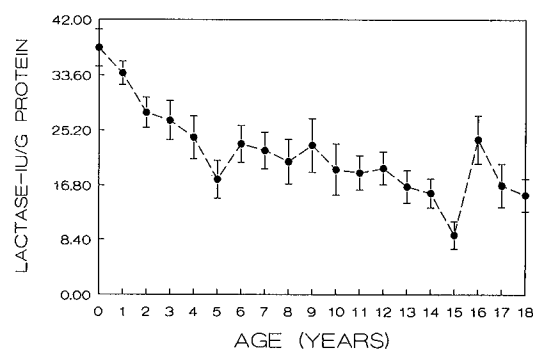


FIG. 1. Lactase-specific activity in subjects with normal histologic findings. Lactase-specific activity (mean  $\pm$  SEM; IU/g protein) declines with age in normal subjects ( $n = 589$ ).

sule biopsy techniques were performed in sufficient numbers to be compared, disaccharidase-specific activities were inversely correlated with histologic findings. Capsule biopsy specimens demonstrated more highly significant relationships between the degree of mucosal injury and disaccharidase-specific activities than endoscopic biopsy specimens ( $r^2 = 0.3462$  versus  $r^2 = 0.1849$ ). Posthoc tests revealed no difference in any of the enzyme-specific activities in tissues obtained by the two techniques; therefore, the groups were combined for presentation.

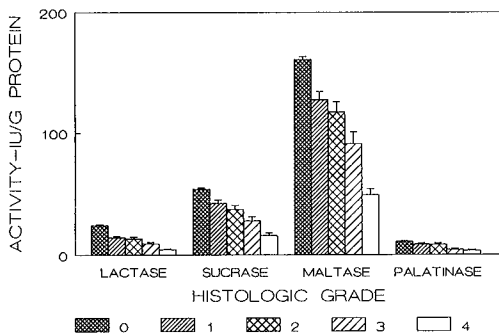
**Effects of Mucosal Injury on Disaccharidase-Specific Activities**

When age was removed as a variable, maltase and lactase activities were inversely correlated with the degree of mucosal injury (maltase:  $r^2 = 0.2120$ ,  $p < 0.0001$ ; lactase:  $r^2 = 0.1235$ ,  $p < 0.0001$ ). Lactase was the enzyme most susceptible to injury, with only 18% of the control value retained in biopsies with grade 4 injury; approximately 30% of control values of sucrase, maltase, and palatinase were retained in biopsies with a grade 4 injury (Fig. 2).

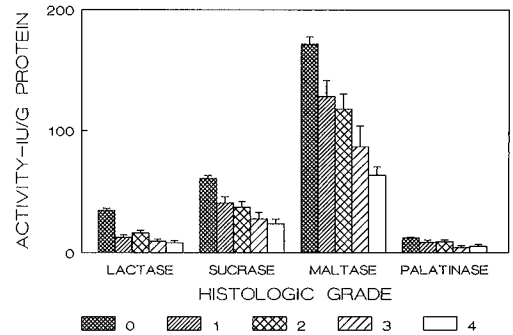
In subjects 24 months of age or younger ( $n = 223$ ), lactase activities were inversely correlated with the degree of mucosal injury ( $r^2 = 0.2322$ ,  $p < 0.0001$ ; Fig. 3).

In subjects older than 24 months ( $n = 575$ ), maltase and lactase activities were significantly inversely correlated with the degree of mucosal injury (maltase:  $r^2 = 0.2340$ ,  $p < 0.0001$ ; lactase:  $r^2 = 0.1098$ ,  $p < 0.001$ ; Fig. 4).

A separate analysis of disaccharidase-specific activities in mucosal injury was performed in subjects



**FIG. 2.** Enzyme activity by degree of mucosal injury. Specific activity (mean  $\pm$  SEM; IU/g protein) of disaccharidases declines with increasing degree of mucosal injury (0 = normal; 1-4 = increasing degrees of injury;  $n = 589, 60, 53, 32,$  and  $56,$  respectively).

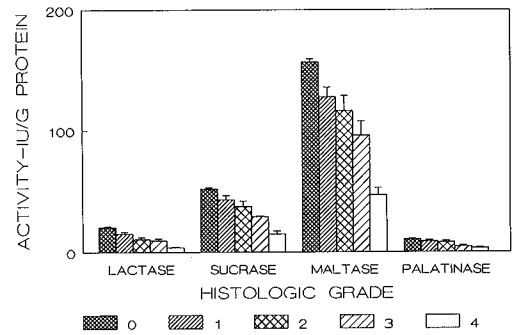


**FIG. 3.** Enzyme activity by degree of mucosal injury in subjects 24 months of age or younger. Specific activity (mean  $\pm$  SEM; IU/g protein) of disaccharidases declines with increasing degree of mucosal injury (0 = normal, 1-4 = increasing degrees of injury;  $n = 155, 16, 27, 14,$  and  $10,$  respectively).

60 months of age or older ( $n = 388$ ). Since a significant percentage of subjects with adult-type hypolactasia should be present by this age, one would suspect significant overlap between normal and villus atrophy groups. Maltase and lactase activities were inversely correlated with the degree of mucosal injury (data not shown).

**Sensitivity and Specificity of Disaccharidase Assays in Predicting Histologic Findings**

The potential utility of disaccharidase assays in the identification of subjects with injured mucosa can be inferred from calculation of sensitivity and specificity from the regression equations. Reduced models, employing only those enzyme(s) that were significantly correlated with the degree of mucosal injury, were utilized for these calculations. Among the subjects 24 months of age or younger, the sensitivity of the lactase assay was 98%; the specificity



**FIG. 4.** Enzyme activity by degree of mucosal injury in subjects older than 24 months of age. Specific activity (mean  $\pm$  SEM; IU/g protein) of disaccharidases declines with increasing degree of mucosal injury (0 = normal, 1-4 = increasing degrees of injury;  $n = 434, 44, 26, 18,$  and  $46,$  respectively).

was 43%. The sensitivity of the lactase assay in biopsies obtained by the capsule and endoscopic biopsy techniques were similar (100 versus 98%); the specificity of the capsule technique was higher than that of endoscopy but was not significantly different (48 versus 38%).

Among the subjects more than 24 months of age, the sensitivity of a model that utilized only the maltase- and lactase-specific activities was 85%; the specificity was 51%. A similar pattern was observed in subjects 60 months of age or older (data not shown).

### DISCUSSION

This study was performed to address these questions: Are any developmental changes other than a decline in lactase present in children undergoing biopsy? Are disaccharidase activities similar in endoscopic and capsule biopsies? Can one or more enzyme assay(s) predict the degree of mucosal injury when histologic findings are inconclusive?

Among subjects with normal histologic findings, lactase declined with age, as has been previously reported (10). Our data demonstrate that the decline begins in late infancy, with a plateau attained by the late preschool years; previous studies have reported that the developmental decline in enzyme concentration does not occur until 3–5 years (14,17).

Prior to the availability of newer fiberoptic endoscopes with narrower insertion diameters, the majority of small intestinal biopsies were performed by one of several capsule techniques. The performance of biopsy by these means is time consuming and is often performed with fluoroscopy. Endoscopic biopsy affords the opportunity to perform multiple biopsies in a short period without fluoroscopy. Unfortunately, the biopsies are commonly obtained from a more proximal site than are biopsies obtained by capsule; moreover, they are usually significantly smaller. Histological evaluation of these smaller samples is more difficult, and when few samples are obtained, a significant number may be inadequate for histologic analysis (18). In this study, both capsule and endoscopic biopsy techniques provided tissues in which disaccharidase-specific activities were reduced to a similar degree in the presence of mucosal injury. No interactions were observed between enzyme activities and biopsy technique when analyzed by analysis of variance with the degree of injury as the dependent

variable; the two techniques are equally predictive of mucosal injury, despite the differences in biopsy location, as evidenced by the high sensitivity of the regression models.

Disaccharidase-specific activities were reduced in mucosal injury, in agreement with previous reports (1–4). Previous studies that failed to demonstrate reduced activities have dealt with smaller samples than the current report and/or conditions in which villus atrophy may be patchy; these factors may have limited their ability to detect significant relationships between morphology and enzyme activities. The magnitude of reduction in severe atrophy was greater for lactase than for the alpha-glucosidases. In infants and toddlers, lactase activity was the most sensitive index of injury. In children older than 24 months, maltase and, to a lesser extent, lactase activities were the most sensitive indices of injury. Measurement of these enzyme activities in biopsy specimens correctly identified damaged mucosa in samples as evidenced by the high sensitivities in each group and biopsy technique. The low specificity of the disaccharidase assays, however, may limit their clinical utility when used to distinguish normal subjects from those with villus atrophy. Furthermore, villus atrophy is a syndrome with many causes; biopsy specimens insufficient for adequate histologic assessment clearly limit their utility in making a diagnosis (i.e., celiac disease).

The ability to detect lactose (19) and sucrose (20) malabsorption by measuring hydrogen concentrations in exhaled breath has become routine in recent years and has changed the approach to individuals with primary disaccharidase deficiencies (i.e., with normal mucosa). Newcomer et al. found breath hydrogen excretion to be the most sensitive and specific indirect method for the detection of lactase deficiency when compared with measurements of plasma glucose, plasma galactose, and  $^{14}\text{CO}_2$  concentration in breath (21). A correlation between milliliters of hydrogen excreted per minute and lactase-specific activity was not reported in that study. A low degree of correlation between jejunal histologic findings, jejunal lactase activity, breath hydrogen concentration following a lactose load, and blood glucose level rise following a lactose load was reported by Maffei et al. (22). Although the amount of carbohydrate malabsorbed and fermented by the colonic flora is directly proportional to the amount of hydrogen excreted per unit of time (23), intersubject variability is of a sufficient magnitude that the

hydrogen breath test is probably only a semiquantitative estimate of the degree of carbohydrate malabsorbed (13). It is likely, therefore, that mucosal biopsy will remain the "gold standard" in the diagnosis of primary lactase and sucrase-isomaltase deficiency.

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