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Test–Retest Reproducibility of Hydrogen Breath Test for Lactose Maldigestion in Preschool Children

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Summary: The test–retest reproducibility of the H_2 breath test within the same individual has not been rigorously evaluated in preschool children. In the present study, 10 children—5 of whom were diagnosed as lactose-digesters on their first testing, and 5 of whom were diagnosed as lactose-maldigesters at first screening—were retested under identical conditions of a second opportunity. In each case, the same diagnostic classification was provided, for a reproducibility of 100%. Regression of the area under the curve of the change in breath H_2 concentration during the 3 h of the test had a Pearson's correlation coefficient of 0.59 ($p = 0.05$). The time-course of 3-h H_2 breath tests in 43 children with lactose maldigestion revealed a peaking of the concentration of H_2 most commonly 120 min following the oral dose of 240 ml whole milk. Seventy-seven percent of the children who eventually proved to be lactose maldigesters were so diagnosed by the end of the second hour of the breath test. Thus, even the abbreviated breath sampling schedule used in children is sensitive, and few maldigesters would go undetected because of a late rise in breath H_2 concentration. **Key Words:** Carbohydrate absorption—Lactose—Hydrogen breath test—Diagnostic reproducibility—Intestinal transit time.

The hydrogen (H_2) breath test is commonly used in children to assess lactose-digesting capacity (1–4). The approach is noninvasive and can be applied to the evaluation of the completeness of lactose digestion from usual dietary amounts of lactose in milk (5,6). Despite all the vast published literature in which *intra*individual or *inter*individual comparisons of carbohydrate malabsorption have been made, it is curious that the test–retest reproducibility of the H_2 breath test in the same person on two or more occasions has not been rigorously investigated.

Two factors—the stability of intestinal flora, and the time-course of a breath H_2 response—could theoretically have bearing on the reproducibility of lactose breath H_2 test results in children. Douwes and colleagues (7) in Amsterdam showed a 9.2% (9 of 98) rate of flat breath H_2 responses at 60 and 90 min after a standard dose of the nondigestible disaccharide, lactulose, in 6- to 12-year-old children. The stability of the H_2-producing colonic flora in schoolchildren was cast in doubt, however, by the return of H_2 responses in 7 of 9 initial nonresponders and the disappearance of this capacity in 2 of 11 children when reassessed with lactulose 6–8 months after the original study.

Because children do not tolerate prolonged periods of fasting, the duration of an H_2 breath test is commonly restricted to $\leq$3 h (with samples collected at 30- or 60-min intervals) (8–12), as compared with sampling periods generally of up to $\geq$5–6 h in adult subjects (13–15). Thus, the more abbreviated tests with fewer sampling intervals in children would, in theory, be more apt to lack diagnostic sensitivity, and hence intraindividual reproducibility as well.

The only publication relevant to the reproducibility of lactose absorption breath tests is that of Welsh and co-workers (16), who studied five *adult* subjects, all lactose-*mal*digesters, on four separate

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occasions, each with a test dose of low-fat milk 5 ml/kg body weight over a 3-h observation period. They concluded that the test was reproducible. A recent experience in preschool children in Guatemala (12) casts additional perspective on the issue of breath test reproducibility in pediatric patients.

MATERIALS AND METHODS

Participants were 44 girls and 46 boys, ranging in age from 29 to 72 months, studied at two centers in Guatemala City—the Pamplona Day-Care Center and the Casa Guatemala orphanage—participants in a larger study on in vivo use of β-galactosidases for lactose maldigestion (12). The children had adequate nutritional status, with all having a weight for height of ≥90% of that anticipated based on the 50% percentile of the NCHS standard. If a child had had an episode of gastroenteritis or was being treated with antibiotics, a 4-week interval was allowed before a test was performed. The protocol was approved by the Human Rights Committee of the Institute of Nutrition of Central America and Panama. Informed consent for participation by the child was obtained from the parent or legal guardian. The orphanage director was the court-appointed custodian for the orphaned children under her care.

Lactose Digestion Tests

Children arrived at the test site fasting on the morning of the study. A sample of basal pulmonary gas was collected between 7:30 and 8:30 a.m., by having the subject breathe through a low-resistance, one-way, Hans Rudolph valve into a 5-L rubber anesthesia gas-bag (3). The child then consumed a 240-ml volume of whole cow's milk, containing 12 g of lactose. Breath sampling was repeated at 60, 120, and 180 min after the draught of milk in an identical fashion.

Breath H_2 Determinations

The concentration of H_2 in the expired air was determined on a Microlyzer CM external column chromatographic breath H_2 analyzer, using a sintered-oxygen detector (Quintron Instruments Co., Milwaukee, WI, U.S.A.) (17,18), calibrated with a standard reference gas mixture containing 97 ppm of H_2 in room air (Lil' Squirt, Ideal Gas Co., Edison, NJ, U.S.A.). The concentration of H_2 registered in the digital display in parts per million was recorded.

Data Analysis

If the increment in breath H_2 concentration at one or more of the three post-milk collection intervals was ≥20 ppm, compared with the baseline sample, the test was considered to be *positive,* and the subject was classified as an incomplete digester of lactose; if the change in breath H_2 levels was consistently <20 ppm, it constituted a *negative* test, of a lactose-digester. The area under the discontinuous curve of the serial changes in H_2 concentration (the excess breath H_2 excretion volume) was computed in arbitrary units of parts per million per hour by a previously described triangulation method (19). Within-subject comparison of H_2 excretion volumes (in parts per million per hour) was performed using Pearson's product-moment linear regression analysis (20).

RESULTS

Of the 90 children at the two centers screened with a 240-ml oral challenge with intact milk, 43 (48%) were classified as incomplete lactose digesters. The time-course of the appearance of the diagnostic increment (≥20 ppm) for these 43 children is shown in Fig. 1. Seven (16%) of the children showed a diagnostic increase in H_2 concentration by 1 h; an additional 26 children manifested their diagnostic increase at the second postdose sampling interval, 2 h after the milk, for a cumulative diagnosis of 77% of the maldigesters by 120 min. The remaining 23% were diagnosed as lactose-maldigesters at the third hour. On the other hand, the highest recorded H_2 rise in 95% of our positive subjects was seen in the final 2 h of the 3-h breath test.

Of the 10 children studied on two occasions, 5 proved to be complete digesters of lactose, and 5 were incomplete digesters. As shown in Fig. 2, each subject received the same classification on both testings. The area under the breath H_2 curve in parts per million per hours for the two tests are paired for each individual in Fig. 3. The coefficient of correlation was 0.59 ($p = 0.05$).

DISCUSSION

The H_2 breath-analysis test is a noninvasive and sensitive tool for the assessment of intestinal car-

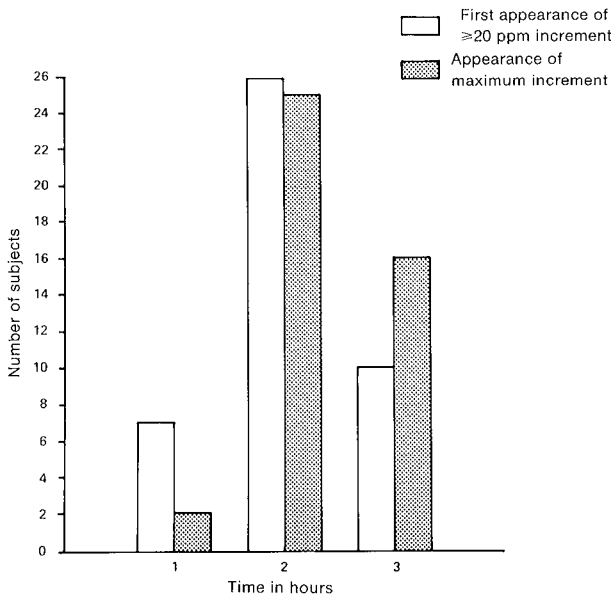

FIG. 1. Time-course of the rise in breath H_2 concentration in the 43 of 90 examined children who proved to be lactose-maldigesters after a 240-ml draught of intact cow milk. **Open bars:** number of children having the first appearance of a diagnostic rise in breath H_2 concentration of ≥20 ppm at the distinct sampling intervals. **Shaded bars:** number of children having their highest recorded breath H_2 increment at a given time interval.

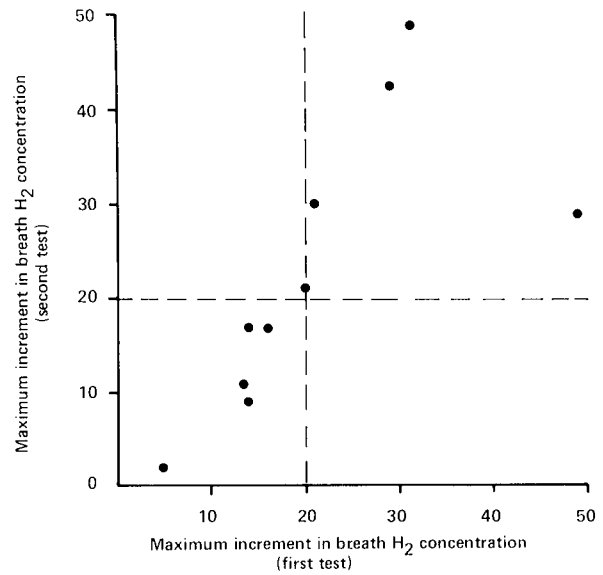

FIG. 2. Comparison of the maximum increment in breath H_2 concentration after 240 ml milk in 10 preschool children on two separate occasions. The units for both axes are H_2 concentration in ppm.

bohydrate handling in children. It can be applied with oral doses of lactose in its native, dietary form, that of milk. The comfort and endurance for children in the semifasting test conditions, however, dictates a shorter overall breath-collection period. The consequences of this for the diagnostic accuracy of classification, and for intraindividual reproducibility, are obvious. For a given child tested over 3 h on two occasions, there existed the possibility that a diagnostic rise in breath H_2 concentration would be registered on one test during 180 min of collection, but would occur later (and therefore be undetected) on a second occasion, due to variability in the rate of mouth-to-colon transit time of the undigested lactose. This would lead to false-negative test results.

Recently, Abramowitz and co-workers (21) in Israel examined 132 consecutive lactose breath H_2 tests in subjects with a mean age of 6 years, with sampling at 30-min intervals over 3 h. Of the 55 positive tests, *all* would have been diagnosed as abnormal if only the basal and 120-min sample had been recorded. Thus, Abramowitz and co-workers supported the notion that little diagnostic sensitivity is lost with the abbreviated collection schedules used in children. Their dose was a 2 g/kg lactose load in a 20% aqueous solution, and their cut-off criterion for maldigestion only a ≥10 ppm rise above basal H_2 concentration. In our study, we used a proportionately lower dosage of lactose in a full-fat milk form with a more stringent criterion for maldigestion in terms of H_2 excretion. Because we previously showed a slower rate of arrival of lac-

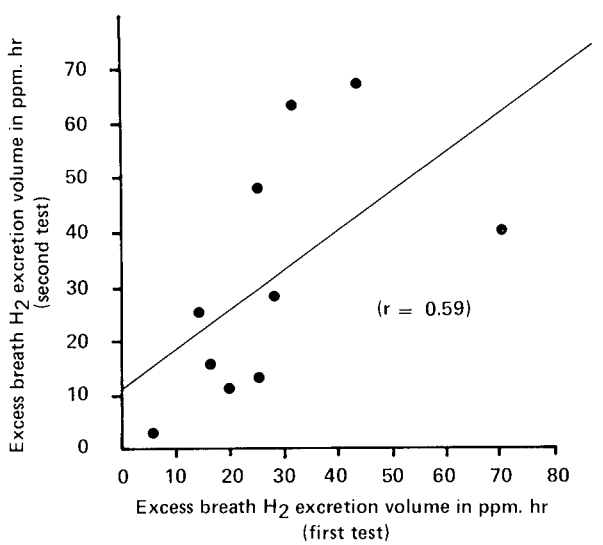

FIG. 3. Correlation between the excess breath H_2 excretion volume after 240 ml milk in 10 preschool children on two separate occasions.

tose to the colon from milk, as compared with lactose in water (5), we still had legitimate cause for concern about the effects of intestinal transit on sensitivity. The fact that our H_2 valus were generally at their zenith at 120 min even with the milk form of lactose reassures us of the probability that we captured all of the true positive tests within the time-window of three postdose samples up to 180 min.

Our sample of retested subjects was small (n = 10), but was more than twice the size of the only other reported study, that in adult subjects by Welsh and colleagues (16), in which test–retest reproducibility of the lactose digestion breath H_2 test was examined. Moreover, whereas all of Welsh's subjects were lactose-maldigesters, we showed reproducibility of results with both test-positive and test-negative individuals. In terms of the binary classification, the 3-h test in preschoolers was 100% reproducible over a 1- to 2-week intertest period.

The area under the discontinuous curve of the change in breath H_2 concentrations (5) is often used to quantify response to oral carbohydrate. We found a statistically significant correlation within subjects on the two occasions, but the results were well short of identity. The experience of Welsh and colleagues (7) in their four retested subjects is again instructive. As a quantitative index of the H_2 response, these Oklahoma researchers used the mean of the H_2 concentrations from the nine breath samples collected during the second and third hours of their 3-h test. The coefficient of variation within subjects in their study ranged from 6 to 40% for this index (mean 26 ± 13%).

Because of the important applications of the H_2 breath test in pediatric gastroenterology, it is curious that its intraindividual test–retest reproducibility has not heretofore been tested in children. It was disconcerting that Douwes and coworkers (7) found major shifts in the H_2-response capacity of colonic flora when schoolchildren were tested after 6-month intervals. This has obvious implications for longitudinal studies with long intertest intervals. At least over the short term, however, a high degree of reproducibility for the classification of both test-positive (maldigester) and test-negative (digester) results was achieved in our small sample of 10 restudied preschool subjects. It is reasonable, moreover, to conclude that the conventional 3-h observation period provides acceptable diagnostic sensitivity for pediatric carbohydrate breath H_2 tests, even when full milk is applied with a $\geq$20 ppm cut-off criterion.

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