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INCAP Publication C–114

Sensitivity and Specificity of the Hydrogen Breath-Analysis Test for Detecting Malabsorption of Physiological Doses of Lactose

Jorge Luis Rosado and Noel W. Solomons[1]

We examined the changes in sensitivity and specificity that would occur with alterations in the sample-collection schedule and (or) cutoff criterion for the increase in hydrogen concentration in breath after administration of doses of lactose in the dietary range. In a breath-analysis test to classify individuals as lactose-absorbers or lactose-malabsorbers, 41 subjects drank 360 mL of intact cow's milk, containing 18 g of lactose, and breath samples were collected and analyzed at 30-min intervals for 5 h. An increase in H_2 concentration of ≥ 20 $\mu L/L$ above basal values at any of the 10 intervals was diagnostic of malabsorption. Increases of ≥ 18 or ≥ 15 $\mu L/L$ were only 85% as specific in classifying the same individuals. Reduction in the number of samples tested per subject uniformly reduced the sensitivity. However, a simplified procedure suitable for field studies (in which four samples—at 0, 2, 3, and 4 h—are collected and analyzed with ≥ 20 $\mu L/L$ as the cutoff value) gives 80% sensitivity and 100% specificity, as compared with the 11-sample procedure.

Additional Keyphrases: *lactose malabsorption · screening · cutoff value*

The principle that the colonic fermentation of incompletely absorbed carbohydrates results in increased excretion of breath hydrogen (H_2) has led to the development of noninvasive procedures for diagnosing carbohydrate malabsorption. As originally applied to the diagnosis of lactose malabsorption, the conventional dose of substrate was 50 g of lactose (1–4). However, because the technique is sufficiently sensitive to detect the malabsorption of as little as 2 g of incompletely absorbed carbohydrate (5), and because questions regarding carbohydrate tolerance usually involve *dietary* issues, the use of physiological doses of carbohydrate in their natural food forms has been advocated (6, 7).

Breath excretion of H_2 can be monitored by a continuous, closed collection system (8) or by determining the concentration of H_2 in expired air at fixed intervals (1, 3, 4, 9). With the latter approach, the criterion for determining malabsorption is usually based on the size of the increase from the fasting, basal concentrations of H_2. An increase of ≥ 20 $\mu L/L$ 2 h after an aqueous, pharmacological dose of lactose is a common cutoff value (3, 4). This simple approach, requiring only a single pre-dose and a single post-dose collection, has a sensitivity and specificity of 100% as an index of lactase deficiency in adult subjects (3). As the administered dose of substrate is decreased into the range of quantities encountered physiologically, the magnitude of breath H_2 production will also decrease. Thus, the frequency and timing of breath collections becomes an important determinant of the sensitivity and specificity of H_2 breath tests involving physiological amounts of lactose. This is particularly important in field studies, in which it is desirable to limit both the number of collection intervals and the time that the subjects must remain fasting. We have analyzed the results from a series of 41 adult subjects receiving 18 g of lactose from whole milk and report here our findings and conclusions.

Materials and Methods

The subjects were 41 healthy adults who participated in a screening test to select volunteers for a study to evaluate the effectiveness of in vivo hydrolysis of lactose (ms. submitted for publication). The protocol was approved by the MIT Committee on the Use of Humans as Experimental Subjects, and all subjects signed consent forms after the nature, purposes, and risks of the test had been explained.

Mixed expired air was collected from fasting subjects by having them breathe normally through a low-resistance two-way Hans Rudolph valve connected to a 5-L anesthesia gas-bag with nipple. A sample of the breath was aspirated

into a 60-mL plastic syringe fitted with a three-way stopcock. The air was maintained in the syringe until analysis within 8 h. The subject subsequently drank 360 mL of intact cow's milk, containing 18 g of lactose. The breath-sampling procedure was repeated at 30-min intervals over the next 5 h. During this time, the subjects were ambulatory and were allowed to drink water, but not to smoke or eat.

Breath H_2 concentration was measured with a Micro-Lyzer (Quintron Instruments, Co., Milwaukee, WI), a thermal conductivity detector-based gas-solid chromatographic system in which aspirated room-air is the propulsion gas (10). Once the instrument is calibrated with a compressed-gas standard of known H_2 concentration (Scotty Gas II; Supelco, Bellefonte, PA), the peak height of an unknown breath sample is read from a digital panel meter.

We arbitrarily interpreted the tests as "positive," that is, as signifying a biologically significant failure to complete lactose absorption, when the maximum increase above the fasting, basal breath H_2 concentration was $\geqslant 25$ $\mu L/L$ at any of the 30-min intervals after the milk was drunk. This more stringent criterion, 5 $\mu L/L$ higher than the conventional cutoff criterion (3, 4), was chosen to provide for a clear separation of malabsorbers for inclusion into the longitudinal study of in vivo hydrolysis by exogenous lactases (ms. submitted for publication).

Sensitivity, an estimate of the efficiency with which a diagnostic test obtains truly positive results, is defined as the number of positive tests divided by the number of individuals affected, e.g., with lactose malabsorption. Specificity, an estimate of the efficiency with which truly negative results are obtained, is defined here as the number of negative tests divided by the number of individuals without lactose malabsorption. As a starting point, we have defined the results of the complete 11-sample standard collection procedure as the "true" diagnosis.

Results

Diagnosis of lactose absorption status. Of the 41 subjects, 20 had an increase in breath H_2 concentration of $\geqslant 25$ $\mu L/L$ at some interval during the 5-h collection period, while the maximum increase of the others was <25 $\mu L/L$. The pattern of change in breath H_2 is shown in Figure 1 for the lactose-malabsorbers (LM) and the lactose-absorbers (LA). In addition to carbohydrate, two other constituents of milk—fat and protein—retard gastric emptying. Thus, as shown in Figure 2, most of the breath H_2 concentration maxima occurred after the third hour, later than the 2-h post-dose sampling interval used in the aqueous lactose studies (3, 4).

Effect of cutoff value on sensitivity and specificity. We examined the sensitivity and specificity for various cutoff values for the maximum increase in breath H_2 concentration. At $\geqslant 20$ $\mu L/L$, none among the 20 individuals diagnosed as a LM would not also have been so diagnosed with this lower standard; moreover, none of the 21 LA subjects exceeded this concentration limit at any point. In other words, the 20 $\mu L/L$ criterion provided both a sensitivity and a specificity of 100%. At $\geqslant 18$ and $\geqslant 15$ $\mu L/L$, the sensitivity remained 100%, but the specificity fell to 86%; three of the 21 LA subjects would have been misclassified with either of the lower cutoff criteria.

Effect of sampling intervals on sensitivity and specificity. The cutoff value of $\geqslant 20$ $\mu L/L$ gives 100% specificity for the H_2 breath test with any selection of intervals, but the sensitivity decreases with the reduction in the number of sampling intervals (Table 1). If the data from only half of the post-dose intervals are used and the $\geqslant 25$ $\mu L/L$ criterion is applied, the sensitivity decreases by 10 to 20%. Sensitivity is also lost with the $\geqslant 20$ $\mu L/L$ criterion if only 60-min inter-
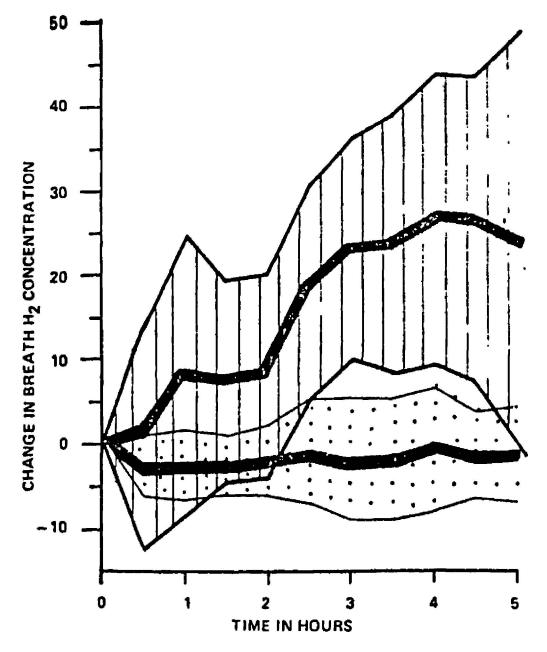

Fig. 1. Mean post-ingestion changes in breath H_2 concentration ($\mu L/L$) after ingestion of 360 mL of intact milk (18 g of lactose) by 21 lactose-absorbers (LA) (*lower curve*) and 20 lactose-malabsorbers (LM) (*upper curve*) serially over 5 h

The shaded areas bracketing the curves represent ±1 SD of the means for each 30-min sampling interval for the respective cohorts: LM (*vertical stripes*); LA (*dotted area*). See text for diagnostic criteria

Table 1. Effect of Various Increments in Breath H_2 and Post-Dose Testing Intervals on Test Sensitivity

Increase in breath H_2, $\mu L/L$	Post-dose Interval, h	Sensitivity
25	1, 2, 3, 4, 5	90% (18/20)
20	1, 2, 3, 4, 5	90% (18/20)
25	0.5, 1.5, 2.5, 3.5, 4.5	90% (18/20)
20	0.5, 1.5, 2.5, 3.5, 4.5	100% (20/20)
25	2	15% (3/20)
25	3, 4	75% (15/20)
25	2, 3, 4	80% (16/20)
25	3, 4, 5	80% (16/20)

Specificity = 100% for all the above combinations.

vals "on the hour" are used; however, if the intervals "on the half hour" are used, sensitivity is 100%. If the $\geqslant 25$ $\mu L/L$ criterion is applied to just the 2-h breath sample, as done by some early investigators with a pharmacological dose of lactose (3, 4), the sensitivity with administration of 18 g of lactose in milk to LM is only 15%. Using the data for the third and fourth hours gives 75% sensitivity. Adding to these the data from either the second or fifth hour increases the sensitivity of diagnosis to 80%.

Discussion

Because of its simplicity, its noninvasive nature, and the low cost of analyses, the use of H_2 breath analysis for evaluating carbohydrate absorption has become increasing-
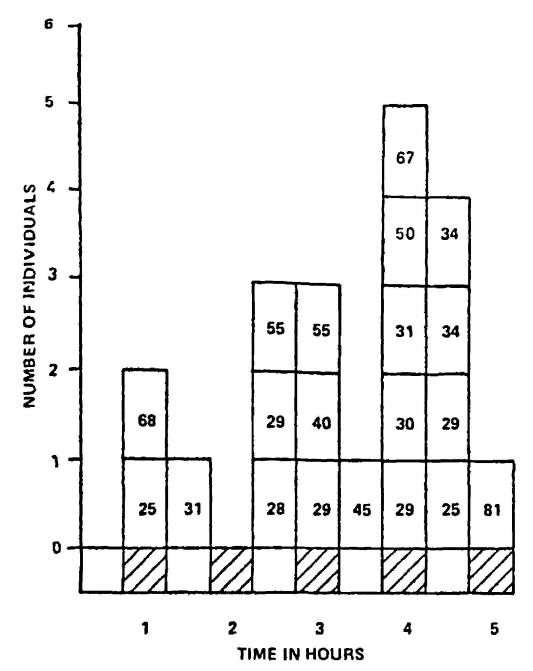
Fig. 2. Frequency distribution of the time of the maximum increase in breath H_2 concentration over the ten 30-min sampling intervals for each of the 20 individuals classified as LMs

The number inside the rectangle represents the magnitude of the maximum breath H_2 increments ($\mu L/L$)

ly popular over the past decade. As originally applied to the diagnosis of lactose malabsorption, the conventional, *pharmacological* dosages of carbohydrate, 2 g/kg of body weight up to a total dose of 50 g, derived from traditional "lactose tolerance tests," were used (1–4). Results of the H_2 breath test after this dose of lactose were found to be the most reliable indirect index of lactase deficiency (3); however, the original basis for the use of pharmacological doses of lactose was to allow the detection and discrimination of increments in *blood glucose* sufficient to separate LM from LA on the basis of post-lactose glycemia. The more responsive breath H_2 analysis—as little as 2 g of carbohydrate not absorbed provides a detectable increase in the excretion of breath H_2—permitted the application of oral doses of lactose in the physiological (dietary) range (7, 10). In recent years, the trend has been toward the use of the amount of lactose in 8- to 12-oz. (240- to 360-mL) glasses of whole milk, i.e., doses of 12 to 18 g of lactose (6, 12–21).

The other important trend is the use of the H_2 breath test in field studies and population surveys (18, 22–25). In a clinical setting, sampling breath for H_2 determinations as frequently as one to six times an hour is not unreasonable, but in population studies, a more limited sampling regimen must be imposed. With the 50-g dose of lactose, a pre-dose and a 2-h post-dose collection were the only samples obtained by Newcomer et al. (3) and were sufficiently sensitive. We have observed occasionally, however, that even at this high a dose in aqueous solution, the increase of breath H_2 by ≥ 20 $\mu L/L$ does not occur until the third hour post-dose or later (6). Thus, we were concerned for the potential loss of diagnostic sensitivity with reduced dosages of lactose, espe-

cially in combination with limited breath-sampling intervals.

We have found that, at least for a dose of intact cow's milk with a lactose content of approximately 18 g (360 mL of milk), the conventional criterion of ≥ 20 $\mu L/L$ is as efficient for detecting the same individuals as being LM as the more restrictive concentration of ≥ 25 $\mu L/L$ used for assigning follow-up treatments (ms. submitted for publication). At cutoff points with lower H_2 concentrations, specificity in the diagnosis of lactose malabsorption is progressively lost.

Given the option of reducing the oral dosages of lactose into the physiological range, it was logical to consider reducing the primary cutoff criterion for the increment in breath H_2 concentration that would be considered diagnostic of malabsorption as well. In fact, Barr (26) and Bayless (27) have both argued that "incomplete absorption" of orally administered carbohydrate is represented by an increment in breath H_2 concentration of ≥ 10 $\mu L/L$. Had this criterion been applied to our sample of 41 individuals, 24 would have been classified as malabsorbers and 17 as absorbers. However, a distinction must be made between "incomplete absorption" and "biologically significant malabsorption." Whether the carbohydrate that escapes small-intestinal removal and reaches the colon to produce symptoms and evolve H_2 derives from an original oral dose of 10 g or 50 g of lactose, it seems reasonable that the same *absolute* amount of incompletely absorbed substrate would be required to cause overt manifestations of intolerance in either situation. In an earlier study involving a dose of 12.5 g of lactose, an increase in breath H_2 concentrations of at least 20 $\mu L/L$ was generally required before a subject reported experiencing symptoms (6). The ± 1 SD limits around the mean of the interval changes in breath H_2 concentration in our present subjects (Figure 1) similarly indicate the limitations of using small increases in breath H_2 concentration as cutoff values.

The later appearance of the peak increase in breath H_2 concentration after drinking milk lactose than after aqueous lactose is consistent with a slower rate of gastric emptying of the food vehicle (28, 29), so that peak increments occur in the later hours of a 5-h collection period. Using only the results of the analyses of the breath samples from the third and fourth hour post-dose gave a test sensitivity of 75%. Although adding one additional collection improves the sensitivity slightly, including results from the fifth hour prolongs the overall period of fasting while increasing sensitivity by only 5%. This same increase in sensitivity can be achieved by measuring the 2-h post-dose expired air, thus decreasing the number of breath collections from 11 to four, but still achieving an 80% sensitivity and 100% specificity with a physiological dose of lactose.

In a previous report in which we measured breath H_2 at 30-min intervals in conjunction with a physiological (12.5 g) dose of lactose (6), we found the same (100%) sensitivity for samples obtained "on the hour" or "on the half hour" during a 6-h period. In the present series, using only half of the post-ingestion breath samples gave only 90% sensitivity. This underscores the need for prior determination of the effects on the diagnostic accuracy of breath test procedures when modifications in cutoff criteria or sampling intervals are introduced.

In summary, a reasonable approach for field studies involving physiological amounts of lactose, one that would have 80% sensitivity and 100% specificity, would involve the following criterion: an increase of ≥ 20 $\mu L/L$ in any one of the sampling intervals at 2, 3, and 4 h after ingestion of 360 mL (12 oz.) of whole milk. Examination of dietary issues

in lactose utilization is best performed with physiological amounts of carbohydrate (7), but a careful evaluation of the sensitivity and specificity of the diagnostic criteria is an essential prelude to any screening test.

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