Hydrogen breath test for the diagnosis of lactose intolerance, is the routine sugar load the best one?

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INTRODUCTION

The enzyme lactase phlorizin hydrolase, located at the intestinal brush border, is necessary for the hydrolysis of lactose, the main sugar in milk. Due to the genetically programmed decrease in intestinal lactase activity that occurs post-weaning (lactase non-persistence), a large proportion of the human population loses, in adult age, the possibility to digest and absorb lactose. In Europe, its prevalence increases with a north-south and west-east gradient. Thus, about 50% of adult Italians cannot digest and absorb lactose normally.

Lactose malabsorption (LM) may be asymptomatic or induce symptoms similar to those of functional bowel disorders and irritable bowel syndrome, consisting of abdominal pain, gaseousness, flatulence and diarrhea. LM is not necessarily a predictor of the occurrence of symptoms, and the term “lactose intolerance (LI)” refers to a condition in which abdominal symptoms are experienced after the ingestion of lactose, in milk or dairy food.

The test for identifying the genotype responsible for lactase deficiency is not widely available and its use for the diagnosis of LM is debatable. Thus, the diagnosis of LM is usually based on a positive hydrogen breath test (HBT) with an oral load of 25 g lactose (HBT-25) and is often followed by the institution of a lactose-free diet, also in those patients who do not experience abdominal symptoms. This approach is debatable, as a reduction of calcium intake below the recommended daily allowance (RDA) may ensue. Moreover, also in those cases in which...
symptoms are triggered by the 25 g of lactose ingested during the test, such a strict reduction of milk and dairy products is often unnecessary, as the amount of lactose administered during the test considerably exceeds the amount of lactose ingested daily, by most adults.

The present study was aimed at evaluating whether a reduction in the daily intake of milk and lactose-containing food is really necessary in subjects with LM during a standard HBT-25. To this end, we performed the HBT with an oral load of 12.5 g lactose (HBT-12.5) in a group of patients with marked LM documented by means of HBT-25. Positivity of the test, occurrence and type of symptoms during the two tests were compared.

MATERIALS AND METHODS
During the period January, 2001 to May, 2004, 913 outpatients underwent a lactose tolerance test in our laboratory. The HBT was performed after 24 h on a low-fiber diet and a 12-h fasting period with an oral load of lactose at a dose of 0.5 g/kg body weight, up to a maximum of 25 g. End-alveolar air samples were collected in syringes using a modified Haldane-Priestly tube\(^3\), prior to the administration of lactose, and thereafter every 30 min for 4 h. Hydrogen (H\(_2\)) and methane (CH\(_4\)) concentrations were measured in parts per million (ppm) by means of a Quintron Model DP Microlyzer gas chromatograph (Quintron Instruments, Milwaukee, WI, USA). The test was defined as “positive” when a H\(_2\) peak exceeding 20 ppm over baseline values was observed in two or more samples. Tests not fulfilling the above-mentioned criteria were defined as negative. Those patients with a negative HBT, who did not excrete increased amounts of H\(_2\) after oral administration of 20 g lactulose in a subsequent HBT (24 patients), were defined as hydrogen non-producers. A positive test identified patients with LM, irrespective of the presence or absence of abdominal symptoms. Positivity of LM was arbitrarily defined as “high-grade” when H\(_2\) excretion exceeded 70 ppm in at least two samples, and “low-grade” in all other instances.

Of the 353 patients with positive HBT-25, 147 fulfilled the above-mentioned criteria for high-grade LM. Of these, 50 were excluded from the study due to the presence of small bowel diseases, such as Crohn’s disease and celiac disease, in which medical treatment or dietary modifications could result in variations in lactase activity. The remaining 97 patients were considered eligible for entry to the study and were required to undergo a further lactose tolerance test, with a lactose load of 12.5 g. Only seven refused to enter the study (compliance 92.78%) and seven had had experience with the lactose tolerance test, with a lactose load of 12.5 g. Only one patient had a negative test after the lactose administration, and thereafter every 30 min for 4 h. Hydrogen (H\(_2\)) and methane (CH\(_4\)) concentrations were measured in parts per million (ppm) by means of a Quintron Model DP Microlyzer gas chromatograph (Quintron Instruments, Milwaukee, WI, USA). The test was defined as “positive” when a H\(_2\) peak exceeding 20 ppm over baseline values was observed in two or more samples. Tests not fulfilling the above-mentioned criteria were defined as negative. Those patients with a negative HBT, who did not excrete increased amounts of H\(_2\) after oral administration of 20 g lactulose in a subsequent HBT (24 patients), were defined as hydrogen non-producers. A positive test identified patients with LM, irrespective of the presence or absence of abdominal symptoms. Positivity of LM was arbitrarily defined as “high-grade” when H\(_2\) excretion exceeded 70 ppm in at least two samples, and “low-grade” in all other instances.

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Results

Positivity of the HBT-12.5 g
Only 16 (17.7%) of the 90 patients enrolled still had a positive test during the HBT-12.5 while the remaining 74 (82.3%) were negative. The difference between HBT-12.5 and HBT-25, evaluated by means of the inference between proportions, was highly significant (P < 0.001). Of the 65 LI patients, only five experienced symptoms during the HBT-12.5, while another seven had a positive test, but reported no symptoms. Of the 25 patients with LM, only four had a positive test after an oral load of 12.5 g lactose. None reported symptoms.

Peak H\(_2\) excretion
Considering all 90 patients together, the mean value of peak H\(_2\) excretion during HBT-12.5 was 21.55 ppm ± 29.54 SD, whereas in HBT-25, the mean peak H\(_2\) excretion was 99.43 ppm ± 40.01 SD. As expected, the difference from HBT-25 was highly significant (P < 0.001). Considering only the data from the 16 patients who proved positive in both tests, the peak H\(_2\) excretion was 97.68 ppm ± 27.37 during HBT-25 and 69 ppm ± 36.53 SD during HBT-12.5. Thus, even in those patients who had LM during HBT-12.5, the amount of hydrogen excretion was significantly lower as compared to the first test (P < 0.01). No difference was found between patients who had symptoms during the test (LI) and those who were LM, but did not experience symptoms, as far as concerning the peak H\(_2\) excretion.

Amount of H\(_2\) excreted (AUC)
The amount of H\(_2\) excreted by the entire population of 90 patients was 18 ppm/h ± 27.12 SD in the HBT-12.5 compared to 97.08 ppm/h ± 40.56 SD in the HBT-25 (P < 0.001). Again, taking into account only data from the 16 patients who proved positive in both tests, the amount of H\(_2\) excreted during HBT-12.5, the AUC was significantly lower compared to that of the HBT-25 (54.29 ppm/h ± 41.23 SD vs 99.21 ppm/h ± 35.58 SD, respectively; P < 0.01). Again no difference was observed between LI and LM.

Symptoms
During the test with 25 g lactose in the 65 patients with LI, gaseousness was present in 36 (55.3%), abdominal pain in 34 (52.3%), flatulence in 16 (24.6%) and diarrhea in eight (13.8%), with some patients reporting more than one symptom (Figure 1). Only five patients with a positive HBT-12.5 experienced symptoms, namely gaseousness in three, flatulence in three and abdominal pain in one. None experienced diarrhea. Eight patients with a negative HBT-12.5 reported experiencing minor symptoms, consisting of gaseousness in five and abdominal pain in five. The relationship between
In the present series, some patients reported more than one symptom. It should be noted that, in HBT-25, minor symptoms, such as mild abdominal discomfort and abdominal distension, were reported not only by five patients with positive tests, but also by eight patients with a negative one.

**Probability of predicting a positive HBT-12.5**

Due to the small number of patients who proved positive during the low-dose test, positivity of HBT-25 does not help predict a positive HBT-12.5 (positive predictive value: 0.17). The occurrence of any symptom during HBT-25 showed only a slightly better positive predictive value for a positive HBT-12.5 (positive predictive value: 0.41). Taking into consideration the occurrence of individual symptoms during HBT-25, the positive predictive value was 0.06 for abdominal pain, 0.25 for gaseousness, 0.31 for flatulence and 0.12 for diarrhea. On the other hand, the absence of abdominal symptoms during HBT-25 had a negative predictive value of 0.84.

**DISCUSSION**

The diagnosis of LM is usually based upon the positivity of HBT after an oral load of lactose. The most commonly used load of lactose is 20-25 g, corresponding to an intake of 400-500 mL of milk, which is rarely ingested in a single dose. Indeed, 400-500 mL of milk exceeds in most instances, the total daily intake of milk and diary products. As HBT has been found to correlate with lactase activity in duodenal biopsies, the HBT-25 is, indeed, useful for population studies. Conversely, small amounts of the sugar were usually well tolerated. The present data indicates that the absence of abdominal symptoms during an HBT-25 is, in most instances, associated with a negative HBT-12.5. Unexpectedly, the presence of symptoms during HBT-25 was not useful for predicting a positive HBT-12.5. Less than 50% of the patients with abdominal symptoms (LI) display malabsorption of lactose in detectable amounts when the sugar load is reduced and the occurrence of symptoms are relatively rare. Thus, a moderate intake of lactose during a standard HBT-25 may prove harmless in the large majority of patients diagnosed as LI or LM.

Interestingly, during the HBT-12.5, eight patients reported symptoms despite a negative test with prevalence similar to that observed in a previous study performed in normal subjects and in patients with irritable bowel syndrome. In the present series, symptoms consisted of gaseousness and mild abdominal pain, whereas none of the patients had diarrhea. As patients were asked to report even minor symptoms, a “nocebo”, or “inverse placebo”, effect may have been elicited by the investigators.

Finally, false-negative results cannot be completely ruled out in these patients, due to a better sensitivity of late (> 240 min) increases in hydrogen excretion, as suggested by Di Stefano et al. These data, however, are debatable as these authors, using different hydrogen cutoff levels, considered definitely as lactose intolerant with a false-negative breath test those patients reporting symptoms during the HBT, irrespective of the test results. This is unlikely, as negative expectations often induce non-specific abdominal symptoms not only during lactose HBT, but also after a sham lactose load (personal unpublished data).

In conclusion, these data reaffirm that LI is dose-dependent. Considering the daily mean lactose intake in the general population, 50 or 25 g lactose tolerance breath tests may prove useful for epidemiological studies, looking for lactose deficiency. The widespread availability of genetic testing for lactase polymorphism may render obsolete this technique. Conversely, in the clinical setting, the use of the 12.5 g lactose tolerance test should be probably preferred to the 25 g test, at least in Caucasians and in the populations of the Mediterranean basin, as it may help to identify those patients who would profit from dietary restriction of lactose-containing food, minimizing the risk of inappropriately reducing calcium intake to those who do not need it.
COMMENTS

Background
Hydrogen breath test (HBT), after a lactase load of 25-50 g is widely used in the clinical setting for diagnosing lactose malabsorption (LM) and, when abdominal symptoms are present, of lactose intolerance (LI). The positivity of the test often induces dietary modifications, leading to the reduction of calcium intake.

Research frontiers
The authors confirmed in a large series of patients previous findings suggesting that most patients with LM, documented by HBT, tolerate well small amounts of lactose.

Innovations and breakthroughs
The present data indicates that an oral load of 12.5 g of lactose, corresponding to about 250 mL milk, is well tolerated by the majority of patients unable to completely digest and absorb 25 g lactose. Moreover, in the majority of them, an increased excretion of hydrogen was not documented after ingesting an isosmolar solution of 12.5 g of lactose, indicating that they can normally digest lactose at least up to a dose corresponding to 250 mL milk.

Applications
The authors suggest that 12.5 g lactose HBT should be preferred to the usual oral load of 25-50 g, in order to identify those patients who could really profit from a reduction of lactose-containing food, and minimize the risk of unnecessary reductions of calcium intake.

Terminology
HBTs are indicated by HBT. The positivity of the test defines a subject’s LM, irrespective of the occurrence of abdominal symptoms. The coincident occurrence of symptoms is required for defining LI patients.

Peer review
This is an interesting study in which the authors argue that the use of 25 g of lactose to test for LM may be inappropriate, as this is higher than the average dietary intake, and the removal of lactose from the diet may have other deleterious consequences such as reduced calcium intake.

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