Fermentation of endogenous substrates is responsible for increased fasting breath hydrogen levels in celiac disease

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Fasting breath hydrogen (FBH) levels are frequently increased in celiac disease (CD). In this study we sought to determine whether the unknown source of the fermented substrates is endogenous glycoproteins shed or exuded through the damaged mucosa. To test the role of nonabsorbable exogenous substrates, we subjected 39 untreated and 23 treated CD patients and 37 healthy volunteers to the H₂ breath test after administration of lactulose after both an unrestricted and a restricted pretest meal. To test the relevance of endogenous substrates, we measured breath H₂ excretion during a 9-hour fast and after the administration of lactulose solution. To determine whether the luminal content of CD patients contains an increased amount of fermentable substrates, we incubated samples of jejunal juice from 7 untreated CD patients, 6 healthy volunteers, and 6 dyspeptic patients in vitro with a fecal homogenate obtained from a healthy H₂-producer volunteer and measured the cumulative H₂ production. Untreated CD patients showed higher FBH levels than did treated patients and healthy volunteers. Only in untreated CD did FBH levels show no difference if a restricted or an unrestricted dinner was eaten the evening before the test. Nine-hour FBH levels were significantly higher in untreated than in healthy volunteers, whereas no difference was found after administration of lactulose. In vitro H₂ production was significantly higher in untreated CD patients than in controls. Increased FBH levels in CD do not depend on fermentation of malabsorbed exogenous substrates; endogenous substrates are increased in the lumens of CD patients and may be responsible for increased FBH levels. (J Lab Clin Med 2004;143:163-8)

Abbreviations: CD = celiac disease; FBH = fasting breath hydrogen; SIBO = small-intestine bacterial overgrowth

The measurement of breath hydrogen (H₂) excretion is considered a useful, noninvasive method for detecting carbohydrate malabsorption.1-4

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Breath H₂ excretion, in fact, greatly increases when a small amount of carbohydrates is supplied to the colonic bacteria, and the assessment of carbohydrate malabsorption is currently based on the comparison of fasting and postload breath H₂ levels.

Increased FBH excretion has been shown in a subgroup of patient with the presence of SIBO.5 Although this finding is characterized by low sensitivity,6 it is considered strongly suggestive of SIBO if H₂ breath excretion is found after a pretest dinner of carbohydrate-free foods, which rules out the persistent nonabsorbed carbohydrates in the colon.5-9 However, it seems that increased FBH levels are not present only in this condition. For instance, untreated patients with CD
often demonstrate high levels of breath H₂ excretion during fasting, and a 1-year period of therapy with a gluten-free diet permits normalization of these FBH levels.¹⁰

On pathophysiologic grounds, high FBH levels in CD are not probably the result of coexisting SIBO; the prevalence of SIBO in CD is about 20%,¹¹ whereas high FBH levels were found in about 60% of our CD patients. Moreover, FBH levels were still a frequent finding after a 1-year period of therapy with a gluten-free diet permits normalization of these FBH levels.¹¹

In this study we sought to test the hypothesis that in CD the prolonged fermentation of endogenous glycoproteins, continuously shed or exuded through the damaged mucosa, is involved in this phenomenon. Other possible mechanisms (eg, persistence of exogenous nonabsorbed carbohydrates in the colon, differences in mouth-to-cecum transit time and colonic fermentation capacity) were ruled out.

METHODS

Patients. Thirty-nine untreated CD patients (20 of them women; mean age 33 ± 5 years, range 22-45) and 23 treated ones (12 women; mean age 35 ± 5 years, range 23-47) took part in the study. In 25 untreated patients CD was suspected on the basis of the presence of the classical symptoms of malabsorption (diarrhea, steatorrhea, weight loss); in the remaining 14, CD was diagnosed on the basis of the following conditions: iron-deficiency anemia (n = 7), first-degree kinship with a CD patient (n = 3), dermatitis herpetiformis (n = 2), alopecia areata (n = 1), and recurrent aphthous stomatitis (n = 1). Diagnosis of CD was established by the demonstration of villous atrophy on duodenal biopsy; in all untreated patients, normalization of duodenal histologic findings was evident after 1 year of a gluten-free diet.

None of the patients had taken antibiotics or any drug that might modulate gut flora for at least 1 month before the study.

Methane production was found in none of the patients, so 37 healthy non-methane-producing volunteers (mean age 33 ± 5 years, range 22-48 years) were recruited from the medical staff to serve as a control group.

Our research was carried out in accordance with the Declaration of Helsinki and has been approved by our hospital’s ethics committee. All participants gave informed consent.

Breath H₂ testing. To assess the role of prolonged intestinal H₂ production caused by the presence of nonabsorbable or slowly fermentable exogenous substrates in the colonic lumen, we performed H₂ breath testing on 2 different days. On the first, no dietary restrictions were imposed the evening before the test, whereas on the second a pretest meal consisting of rice, meat, and olive oil was administered the evening before testing.¹⁰ The ingestion of a dinner containing carbohydrate in the form of rice flour only on the evening preceding the test reliably reduces FBH levels in healthy volunteers.⁷-¹⁰ This meal was followed by a 12-hour fast before the test.

Breath samples were taken during the fast and every 15 minutes for 8 hours after ingestion of an isoosmotic solution containing 10 g of lactulose, a nonabsorbable disaccharide that is fully fermented by colonic flora. We recorded fasting and cumulative H₂ excretion—evaluated with the use of an area under the time-concentration curve calculation—for each patient. Oro-cecal transit time was defined as the presence of 3 sustained increments of H₂ breath excretion of at least 10 ppm over the baseline reading.¹⁴

To test the relevance of endogenous substrates, we measured breath H₂ excretion in 25 untreated CD patients and in 10 randomly chosen healthy volunteers during a 9-hour period of fasting and, on a separate day, after the ingestion of the isoosmotic solution containing 10 g of lactulose. Breath samples were taken every 15 minutes. Cumulative H₂ excretion for each subject was then recorded. In another portion of this evaluation, we administered a meal consisting only of rice flour on the evening before the test and, after subjects had fasted overnight, measured breath hydrogen.

Breath testing was started between 8:30 and 9:30 AM after thorough washing of each subject’s mouth with 40 mL of 1% chlorhexidine solution.¹⁵ Smoking and physical exercise were not permitted for 1 hour before or during the test.

We sampled alveolar air using a commercial device (Gasampler Quintron, Milwaukee, Wis) that allows the first 500 mL of dead-space air to be separated and discarded, after which the remaining 700 mL of end-alveolar air is collected in a gas-tight bag. Subjects were instructed to avoid deep inspiration and not to hyperventilate before exhalation. The variability of H₂ determinations with this collection method has been shown to be about 25%.¹⁸

A gas chromatograph dedicated to the detection of H₂ in air samples (Model DP; Quintron Instrument, Milwaukee, Wis) was used for breath-sample analysis. The accuracy of the detector was ±2 ppm of H₂, with a linear response range between 2 and 150 ppm of H₂.

In vitro studies of gas production. As a means of determining whether the luminal content of patients with untreated CD contains more fermentable substrates, we used an in vitro assessment system.¹⁹ In 7 CD patients a sample of juice from the first jejunal loop was taken by means of endoscopy while the patient was fasting, under sterile conditions. The aspiration site was checked with the use of fluoroscopy. One milliliter of juice was then incubated with a 1-mL aliquot of an anaerobically prepared homogenate of freshly passed stools obtained from a single healthy H₂-producing/CH₄-nonproducing volunteer at 37°C in a 500-mL glass flask (which had been flushed with argon) and fitted with a gas-tight lid on a rotating wheel. A gas mixture consisting of 10% CO₂ and 90% argon was added to the flask. We homogenized specimens with deoxygenated 0.1 mol/L PO₄-buffered saline solution (pH 7.0; 1:5 wt/vol) in a blender vessel that had been purged with argon. The H₂ production over an 8-hour period was measured. To correctly measure in vitro H₂ release, we also incubated 1 mL of saline solution with a 1-mL aliquot of
the fecal homogenate obtained from a healthy H\textsubscript{2}-producing/CH\textsubscript{4}-nonproducing volunteer. Next we calculated in vitro H\textsubscript{2} release on the basis of the difference between hydrogen release from the saline solution–augmented homogenate and that of the homogenate containing intestinal juice. We also obtained samples of jejunal juice from 6 healthy volunteers and 6 patients who underwent upper endoscopy for the presence of dyspeptic symptoms. These subjects served, respectively, as normal and a pathologic control groups for the comparison of in vitro results. The need for a normal control group in addition to dyspeptic patients is justified by a significantly reported prevalence figure of SIBO in dyspeptic patients.\textsuperscript{6}

**Data analysis.** All variables are expressed as mean ± SD. All parameters showed a nonparametric distribution on the Kolmogorov-Smirnov normality test. We performed nonparametric one-way analysis of variance, the Mann-Whitney U test for unpaired data, and Wilcoxon’s rank-sum test for paired data to compare 2 groups. Spearman’s rank correlation coefficient was used to estimate the level of association between 2 variables. We considered a 2-tailed \( P \) value of less than .05 statistically significant.

**RESULTS**

Table 1 confirms that FBH levels are significantly higher in patients with untreated CD than in patients with treated disease or in healthy volunteers. We noted no difference between the last 2 groups. Dividing the group of patients with untreated CD on the basis of the pattern of clinical presentation, we saw that patients with overt malabsorption at the time of diagnosis had FBH levels significantly higher than those in patients with minor symptoms (Fig 1).

With regard to mouth-to-cecum transit time, in 4 patients with untreated CD we could not measure this parameter accurately. Of the remaining 35 patients in the untreated-CD group, no difference was found between the three groups studied (Table 1).

Fig 2 shows that FBH levels did not differ in patients with untreated CD depending on whether a restricted (18.3 ± 3.7 ppm) or an unrestricted pretest meal (19.1 ± 3.4 ppm) was prescribed the evening before the test. On the contrary, in treated CD patients (9.8 ± 2.2 vs 15.1 ± 4.5 ppm) and in healthy volunteers (6.6 ± 0.5 vs 14.5 ± 2.1 ppm), significantly lower FBH levels were evident after the restricted meal.

Fig 3 and Table II show the results of breath H\textsubscript{2}-excretion measurements during the 9-hour fast and after administration of lactulose in patients with untreated CD and healthy volunteers. Mean cumulative H\textsubscript{2} excretion during fasting was significantly higher in CD patients than in healthy volunteers, but we found no difference after the administration of lactulose.

In vitro incubation of jejunal juice from patients with untreated CD with a sample of fecal homogenate of a healthy H\textsubscript{2}-producing/CH\textsubscript{4}-nonproducing volunteer revealed H\textsubscript{2} production (11,555 ± 2050 ppm) signifi-

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**Table 1.** FBH levels and mouth-to-cecum transit time in patients with CD and healthy volunteers

<table>
<thead>
<tr>
<th>Study group</th>
<th>FBH (ppm)</th>
<th>Mouth-to-cecum transit time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated CD (( n = 39 ))</td>
<td>18.3 ± 3.7*</td>
<td>85 ± 10</td>
</tr>
<tr>
<td>Treated CD (( n = 23 ))</td>
<td>9.8 ± 2.2</td>
<td>78 ± 11</td>
</tr>
<tr>
<td>Healthy volunteers (( n = 37 ))</td>
<td>6.6 ± 0.5</td>
<td>80 ± 12</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD.

*\( P < 0.001 \) vs treated patients and healthy volunteers.
cantly greater than that in samples of juices from control patients (4855 ± 1550 ppm) or healthy volunteers (2055 ± 1050 ppm). No significant difference was evident among the last three groups (Fig 4).

**DISCUSSION**

Increased FBH excretion is a common feature in patients with untreated CD, but until now, the pathophysiological mechanism responsible for this finding was unknown. The prevalence of increased FBH levels was higher in patients with malabsorption symptoms (diarrhea, steatorrhea, weight loss) than in patients without overt malabsorption (iron-deficiency anemia, first-degree kinship with a CD patient, dermatitis herpetiformis, alopecia areata, recurrent aphthous stomatitis). This finding argues against a role for intestinal stasis in determining the increase in FBH level. Moreover, we detected no difference in mouth-to-cecum transit time among patients with untreated and treated CD and healthy volunteers.

Our results rule out a role for bacterial fermentation of persistent exogenous substrates in the colon. A restricted dinner, free of unabsorbable carbohydrates, the evening before the test represents a reliable way of reducing the FBH level on the morning of breath testing. Subjects were therefore first asked to avoid bread, pasta, and fibers, which are known to cause prolonged breath excretion of H\textsubscript{2}. On a separate day, another fasting H\textsubscript{2} excretion measurement was taken after subjects consumed an unrestricted meal. We detected no difference in terms of FBH level in the patients with untreated CD, suggesting that the presence or persistence of exogenous carbohydrates is not crucial in this condition.

It could be argued that in patients with celiac disease, characterized by a flat mucosa, malabsorption of rice starch may occur. The source of starch is important in the induction of starch malabsorption: it has been shown that healthy volunteers demonstrate intestinal malabsorption of an appreciable amount of wheat and potato starch, and the amount of unabsorbed starch is directly related both to the amount ingested and to intestinal transit time, generally being about 2.5% for wheat and potato in healthy volunteers.
trary, rice starch is completely absorbed by healthy volunteers. \textsuperscript{20} Our results show no difference in terms of intestinal transit time among the 3 study groups (Table I), suggesting that transit time has no effect on starch malabsorption in patients with untreated CD. It is possible that subjects with a flat mucosa experience malabsorption of rice starch; however, data on starch absorption capacity in patients with celiac disease are scant. At the time of this writing, just 1 paper has been published on rice-starch malabsorption in patients with small-bowel disease and malabsorption\textsuperscript{23}; in a group of 22 patients with small-bowel disease, three had CD but only one showed starch malabsorption.

By comparing cumulative breath H\textsubscript{2}-excretion levels during a 9-hour period of fasting and after the administration of lactulose, we were able to test the pathophysiological role of endogenous substrates. Patients with untreated CD excreted significantly more H\textsubscript{2} than did healthy volunteers during fasting, whereas no significant difference was found between patients with untreated CD and healthy volunteers after the administration of lactulose. This latter finding suggests that intestinal fermentation capacity is similar between the two groups and therefore is not responsible for differences in FBH levels. On the other hand, the results of fasting evaluation indicate that the intestines of patients with untreated CD contain more fermentable substrates than do those of healthy volunteers. On the basis of the absence of significant difference between the groups studied, in terms of breath H\textsubscript{2}-excretion values measured after the restricted and the unrestricted pretest meals, it is conceivable that these substrates are of endogenous origin. Moreover, in CD patients the absence of a reduction of breath hydrogen excretion during 9 hours of fasting discounts the interference of starch malabsorption in this group of patients.

The results of the in vitro test confirm this interpretation. Even if the presence of higher amounts of fermentable substrates in the intestine of patients with untreated CD were demonstrated with certainty only on the basis of the direct measurement of glucidic residuals in the juice, an indirect evaluation has been performed in this study with the use of a fecal incubation system. Because it has been shown that the prevalence of SIBO in dyspeptic patients is as high as 44%, depending on the diagnostic criteria used,\textsuperscript{19} we also enrolled a subgroup of healthy volunteers so that we might obtain samples of normal jejunal juice. Samples obtained from patients with untreated CD produced more H\textsubscript{2} than did those obtained from dyspeptic patients and healthy volunteers after the samples were incubated with fecal bacteria.

Metabolism of mucin by human colonic flora has previously demonstrated,\textsuperscript{24} and glycoproteins of both dietary (exogenous) and endogenous origin have been shown in vitro to represent an effective substrate for H\textsubscript{2} production.\textsuperscript{25} Therefore the increased availability of endogenous substrates in the small intestines of CD patients enhances colonic H\textsubscript{2} production when these substrates reach the colon. Data on pH value at the level of the small bowel suggest that the site of H\textsubscript{2} production is the colon. In fact, H\textsubscript{2} production is a pH-dependent metabolic process; it was shown that it is maximal at neutral pH, whereas acidic pH values inhibited its production.\textsuperscript{26} It was also shown that the upper-jejunal content of patients with untreated CD is even more alkaline than that of normal subjects,\textsuperscript{27} suggesting that a more favorable pH value at this level contributes to H\textsubscript{2} production. However, if fermentation takes place, an acidic environment is produced as a result of fermentation products, suggesting the absence of fermentation at the small-bowel level in CD patients.

Studies in pediatric populations have yielded conflicting results in terms of increased FBH in CD patients. A preliminary evaluation, in fact, suggested that in pediatric CD the presence of increased FBH levels is strictly related to the presence of SIBO.\textsuperscript{28} However, in that study, the upper limit of the control range was higher than that reported\textsuperscript{6} and commonly used. It is therefore possible that the prevalence figure has been underestimated. However, another Italian study,\textsuperscript{29} confirms our observations.

Finally, FBH levels seem to be related strictly to intraluminal glycoprotein shedding or release and therefore cannot be minimized by the usual procedures adopted in preparation for testing. This makes it difficult to interpret the results of breath H\textsubscript{2} testing in a significant percentage of patients with active CD. Increased breath H\textsubscript{2} excretion after overnight fasting has been proposed as an insensitive but specific marker of SIBO.\textsuperscript{5,30,31} On the contrary, our results show that specificity may be low as well. High fasting breath H\textsubscript{2} excretion may also suggest the presence of an organic disease characterized by an intraluminal cellular shedding, resulting in release of glycoprotein and, therefore, increased intraluminal carbohydrate fermentation. This hypothesis is also bolstered by a recent finding indicating that in Crohn’s disease and ulcerative colitis, 10% to 20% of patients demonstrate high fasting breath H\textsubscript{2} excretion, regardless of disease activity or the presence of conditions predisposing a subject to SIBO (eg, stricture, fistula, surgery).\textsuperscript{32}

Finally, the authors of an earlier paper\textsuperscript{10} reported that FBH levels were significantly reduced after consumption of a gluten-free diet and that persistently high values were associated with the persistence of intestinal lesions. This abnormality was still present in patients who complied poorly with the diet. Even if this infor-
mation can only be acquired with a prospective longitudinal study, it is possible to hypothesize a role for FBH as a simple, repeatable, noninvasive, inexpensive tool for predicting the improvement of diet-induced intestinal lesions. Persistently high FBH levels argue for more careful clinical surveillance, suggesting poor dietary compliance.

REFERENCES
