Acidic colonic microclimate – possible reason for false negative hydrogen breath tests

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SUMMARY

About 5% of normal subjects fail to produce increased hydrogen breath concentration after ingestion of the non-digestible carbohydrate lactulose (low hydrogen producers). The existence of low hydrogen producers limits the diagnostic use of hydrogen (H₂) breath tests. We studied the effects of lactulose and of magnesium sulphate (MgSO₄) pretreatment on stool-pH and on hydrogen exhalation after oral loading with lactulose or lactose in 17 hydrogen producers and 12 low hydrogen producers. In seven hydrogen producers acidification of stool pH by lactulose pretreatment (20 g tid) decreased hydrogen exhalation and three of seven (43%) became low hydrogen producers. In contrast, after pretreatment of eight low hydrogen producers with magnesium sulphate (5 g twice daily) all eight produced hydrogen after a lactulose load. Similarly four lactose intolerant low hydrogen producers had abnormal lactose hydrogen breath tests after MgSO₄ pretreatment. MgSO₄ pretreatment neither resulted in false positive lactose hydrogen breath tests in five lactose tolerant hydrogen producers, nor increased the hydrogen exhalation in five additional hydrogen producing controls after ingestion of lactulose. The results of these studies confirm that hydrogen production from lactulose decreases when the colonic pH is lower (lactulose pretreatment), and increases when colonic pH is higher (MgSO₄ pretreatment). In low hydrogen producers the lacking increase of H₂ exhalation after ingestion of non-digestible carbohydrates can be overcome by MgSO₄ pretreatment, thus increasing the sensitivity of the test by avoiding false negative hydrogen breath tests in low hydrogen producers with disaccharide malabsorption or maldigestion. The underlying mechanism of this remarkable effect of MgSO₄ pretreatment warrants further investigation.

The hydrogen breath test is a valuable non-invasive diagnostic tool to detect carbohydrate malabsorption and bacterial overgrowth in the small bowel and to determine the gastrointestinal transit time. Metabolism of non-absorbed carbohydrates by gut bacteria results in the production of short chain fatty acids, CO₂ and H₂. About 20% of the formed H₂ is absorbed from the gut and exhaled by the lungs. The H₂ concentration in breath can be measured by gas chromatography or electrochemically. The magnitude of the H₂ excretion in the breath is influenced by a variety of factors such as smoking, diet, and antibiotic treatment. The number of colonic bacteria and the composition of colonic flora may change the H₂ excretion as well as the metabolic pathways mainly used by the colonic flora for degradation of carbohydrates. About 5% of normal subjects fail to show an increase of more than 20 ppm in H₂ excretion in breath after the ingestion of lactulose. Therefore, the H₂ breath test cannot be generally accepted as a noninvasive screening test for carbohydrate malabsorption. The reason for the lack of H₂ production by gut bacteria in these patients is unclear. The H₂ production from glucose by faecal incubates is pH dependent in vitro, the pH-optimum being at pH 7-0-7-45. In vivo, oral administration of lactulose – a non-digestible carbohydrate – produces a marked acidification of proximal colonic contents and results in a decrease of H₂ excretion in breath after oral lactulose loading. It was concluded, therefore, that the bacterial metabolism of lactulose and of other malabsorbed or maldigested carbohydrates and the H₂ production may be inhibited at low pH. In lactose intolerant patients or in patients treated with non-digestible disaccharides, repeated ingestion of such carbohydrates may lead to a low colonic pH because of bacterial degradation of carbohydrates to...
organic acids in the colon. We therefore tested the possibility of overcoming the low colonic pH by oral MgSO₄ application and investigated the effect of oral MgSO₄ pretreatment on faecal pH and on hydrogen breath tests in general and particularly in low hydrogen producers.

Methods

Patients
The H₂ production status was determined routinely by lactulose H₂ breath tests in patients with various gastrointestinal or hepatic diseases. After exclusion of patients with chronic inflammatory bowel diseases and with previous surgery of the gastrointestinal tract 17 hydrogen producers (seven with stable cirrhosis of the liver, five with duodenal ulcer, five healthy controls), and 12 low hydrogen producers (eight patients with stable cirrhosis of the liver, two with coeliac disease, two with isolated lactase deficiency) consented to participate in additional H₂ breath tests (study protocol, Table 1). Of these 29 patients 22 were men and seven women. They had not been on antibiotics during the last four weeks before and during the study. In seven hydrogen producers the lactulose H₂ breath test was repeated after pretreatment with lactulose (20 g tid for three days) and in five hydrogen producers after pretreatment with MgSO₄ (5 g dissolved in 200 ml tap water, given orally twice on the day before the second test). In eight low hydrogen producers the lactulose H₂ breath test was repeated after MgSO₄ pretreatment. In addition, lactose H₂ breath tests were carried out in five lactose tolerant hydrogen producers and in four lactose intolerant low hydrogen producers and the tests were repeated after MgSO₄ pretreatment. A fresh stool specimen was obtained for pH determination from each patient in the morning before each H₂ breath test and was worked up immediately.

LACTULOSE/LACTOSE H₂ BREATH TEST
After an overnight fast the patients received 20 g lactulose (Laevolac-Lactulose-Konzentrat, Fa Laevosan, Linz, Austria) dissolved in 250 ml herbal tea or 50 g of lactose in 500 ml herbal tea. Before and after the ingestion of the disaccharide endexpiratory air was collected with the ‘single breath technique’[2] in 15 minutes intervals for four hours. H₂ concentration in the breath was determined by an electrochemical cell (Stimotron, Wendelstein, FRG). Its accuracy is ±2%, the sensitivity is 2 ppm and it gives a linear response to breath hydrogen between 0 and 250 ppm. In addition, blood glucose was measured before and in 15 minutes intervals after lactose ingestion. A blood glucose rise of less than 20 mg% above the baseline was judged diagnostic for lactose malabsorption.[12]

MEASUREMENT OF THE FECAL pH
The faecal pH was determined in a smear of fresh stool by two indicator stripes (Merck, Darmstadt, FRG) as described.[1] The stripe used first had an accuracy of measurement of 1 in a range of pH 0–14, the second one had an accuracy of 0.2 in the range of pH 4.0–8.0.

Statistical analysis
The statistical calculations and the graphs were done by SAS (Statistical Analysis System of SAS Inst Inv, Cary, North Carolina) on the computer. The comparison of the means of both groups and tests was calculated by an unpaired twotailed Student’s t test. The maximal rise of H₂ excretion (max-H₂) in ppm (parts per million) above basal value and the area under the curve (AUC-H₂) in ppm×min – approximated by the rectangle rule – were calculated on the computer. AUC-H₂ is the area between the exhalation curve and the extrapolated basal value. If the maximal H₂ increase was less than 20 ppm after lactulose ingestion, the subject was defined as a low hydrogen producer.

Results

Effects of pH modulation of colonic contents on lactulose H₂ breath tests in hydrogen producers
A three day treatment with 20 g lactulose (tid) resulted in a markedly decreased production of H₂ after ingestion of 20 g lactulose (Fig. 1) with lower max-H₂ and smaller AUC-H₂ (Table 2). Three patients (43%) became low hydrogen producers by lactulose pretreatment. The stool pH was lower after lactulose pretreatment (5.1±0.2 vs 5.8±0.2; p<0.05). MgSO₄ pretreatment had no effect on H₂ production after ingestion of lactulose (Table 2) and did not change the stool pH (6.4±0.4 vs 6.6±0.3). The orocaeal transit time did not decrease significantly (111±44 vs 128±39 min; p=0.48).
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Effects of MgSO₄ treatment on lactulose H₂ breath tests in low hydrogen producers

The MgSO₄ pretreatment led to a steeper H₂ exhalation curve (Fig. 2) with higher max-H₂ and larger AUC-H₂ (Table 2) and to an increased stool pH (7.2±0.3 v 6.1±0.3; p<0.05). All eight low hydrogen producers became hydrogen producers after MgSO₄ pretreatment.

Effects of MgSO₄ treatment on lactose H₂ breath test in lactose intolerant low hydrogen producers

The MgSO₄ pretreatment had no effect on the max-H₂ or AUC-H₂ in lactose intolerant hydrogen producers after lactose ingestion (Table 3, Fig. 3) or on stool pH (6.0±0.1 v 6.2±0.4). In contrast, MgSO₄ pretreatment resulted in increased max-H₂ and AUC-H₂ after lactose ingestion in lactose intolerant low hydrogen producers (Table 3, Fig. 4), thus all four patients became hydrogen producers and the lactose H₂ breath test was diagnostic for lactose malabsorption. The stool pH increased (5.9±0.2 v 5.2±0.1; p<0.05) after MgSO₄ pretreatment.

Discussion

The causes for a low hydrogen producer state are not clear at present. Several factors such as the amount of given disaccharides, the functional status of the small bowel, and the colonic microclimate may affect the

Table 2  Lactulose H₂ breath test before (baseline) and after lactulose or MgSO₄ pretreatment

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<thead>
<tr>
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<th>Baseline</th>
<th>After lactulose</th>
<th>After MgSO₄</th>
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<tr>
<td></td>
<td>n</td>
<td>Max-H₂ ppm</td>
<td>AUC-H₂ ppm x min</td>
</tr>
<tr>
<td>H₂ producers</td>
<td>7</td>
<td>69±12</td>
<td>8523±1646</td>
</tr>
<tr>
<td>H₂ producers</td>
<td>5</td>
<td>51±7</td>
<td>7385±917</td>
</tr>
<tr>
<td>Low H₂ producers</td>
<td>8</td>
<td>12±2</td>
<td>2347±369</td>
</tr>
</tbody>
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Every parameter as x±SEM; max-H₂ in ppm; AUC-H₂ in ppm x min; *p<0.05, t test, in comparison with the corresponding baseline.
magnitude of bacterial H\textsubscript{2} production in the colon. The incidence of low hydrogen producers in the normal population varies considerably from 0\% to 20\%.\textsuperscript{5,7,8,13} Regional or ethnical differences are mostly the result of different dietary habits and this may explain the different frequencies of low hydrogen producers in several studies. In addition, they can be attributed to different criteria applied to define low or non-hydrogen producers or flat H\textsubscript{2} breath tests by various authors. In this study low hydrogen producers were defined by a lack of an increase of H\textsubscript{2} concentration in the breath of greater than 20 ppm after an ingestion of 20 g lactulose. Using our rather strict criteria the incidence of low hydrogen producers in the Austrian population is less than 5\%.

As the H\textsubscript{2} production from disaccharides is a result of bacterial metabolism in the colon, the magnitude of H\textsubscript{2} production presumably is sensitive to changes in the composition of the colonic microflora and in the colonic microclimate which are interrelated. Faecal hydrogen production does not seem to be a good indicator for in vivo hydrogen producing
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Table 3  Lactose $H_2$ breath test before (baseline) and after MgSO$_4$ pretreatment

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<thead>
<tr>
<th></th>
<th>Baseline</th>
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<tr>
<td></td>
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<td>$n$</td>
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<tr>
<td>Lactose tolerant H$_2$ producers</td>
<td>5</td>
<td>Max-$H_2$</td>
</tr>
<tr>
<td>Lactose intolerant low H$_2$ producers</td>
<td>4</td>
<td>Max-$H_2$</td>
</tr>
</tbody>
</table>

Every parameter as $\bar{x} \pm$SEM; max-$H_2$ in ppm; AUC-$H_2$ in ppm x min; *p<0.05, in comparison with the corresponding baseline values.

capability, but it was shown that the faecal $H_2$ production is pH dependent. In carbohydrate malabsorption or maldigestion or in subjects treated with non-digestible disaccharides the colonic microclimate will become acidic. Chronic ingestion of non-digestible disaccharides results in stimulation of acid producing bacterial enzymes such as β-galactosidases and caecal pH will decrease faster after disaccharide ingestion. At low pH the growth and metabolism of many microorganisms are inhibited and the portion of bacteria consuming or not producing $H_2$ such as Lactobacillus, Bifidobacterium and Eubacteria will increase which prefer acidic milieu. Thus, patients with carbohydrate malabsorption or maldigestion who consume a diet rich of non-digestible/non-absorbable carbohydrates could frequently be low hydrogen producers; this matter considerably limits the diagnostic value of $H_2$ breath tests in this interesting group of patients.

The results of this study show that the failure to produce $H_2$ from malabsorbed or non-digestible carbohydrates can be overcome by pretreatment with MgSO$_4$. Thereby, the low hydrogen producer state can be eliminated and the diagnostic value of the $H_2$ breath test for detecting carbohydrate malabsorption or for measuring orocecal transit time can be improved. The mechanism by which MgSO$_4$ pretreatment enhances or facilitates intestinal $H_2$ production and $H_2$ exhalation in low hydrogen producers, is not clear. Possible ways of action could involve effects on intestinal motility and transit time.

![Graph](http://gut.bmj.com/content/gut/29/1/21)

Fig. 4  Lactose $H_2$ breath test without (□—□; mean +SEM) and with (●—●; mean –SEM) MgSO$_4$ pretreatment (2×5 g) in four low hydrogen producers.
as well as on intestinal bicarbonate secretion resulting in alterations of the colonic microclimate in favour of bacterial H₂ production, absorption and exhalation. The pH increasing effect of MgSO₄ could be reached by its cathartie effect cleansing the caecum from carbohydrates which are the substrates for acid producing bacteria, without itself being substrate for bacterial metabolism in contrast to lactulose. Unfortunately our data do not permit a conclusive explanation of the enhancing effect of MgSO₄ pretreatment on H₂ exhalation which we observed particularly striking in lactose intolerant low hydrogen producers. As the stool pH increased after MgSO₄ pretreatment from pH 6.1 to pH 7.2 a pH-mediated mechanism appears likely to be operative. Additional studies including colonic pH monitoring are, however, needed to clarify definitely a possible pH mediated effect, as stool pH does not reflect the pH in different parts of the colon.⁹⁰ In addition, stool pH also depends on the caecal anal transit time. The colonic pH increases from the caecum to the rectum⁹⁰ and thus a shorter caecal anal transit time will be associated with lower stool pH. Therefore, in hydrogen producers without diarrhoea at the beginning of the study the stool pH is hardly comparable to that after treatment with cathartics such as MgSO₄ or lactulose. The pH in the colon ascendens can be accurately measured by radiotelemetry using pH detectors in capsules or by plastic tubes swallowed by the patient and their location controlled by radiography. Using this technique it was shown that lactulose treatment decreases pH in colon ascendens below 5.⁹¹ These techniques, however, cannot be used routinely in patients screened for carbohydrate malabsorption. Treatment with MgSO₄ was shown by radiotelemetry to increase colonic pH.⁹⁰ Thereby, the conditions for bacterial H₂ production can be optimised. In addition, there was a tendency to decrease the oro-caecal transit time by MgSO₄ pretreatment, thus more substrates for bacterial H₂ production could reach the colon and the magnitude of hydrogen production could be increased by a higher rate of entry of unabsorbed carbohydrates into the caecum;¹¹ but this may be only a side effect of MgSO₄ pretreatment, the main effect probably being a pH mediated change of the metabolism of colonic bacteria.

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References