Hydrogen and Methane Breath Test in the Diagnosis of Lactose Intolerance

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Abstract: The hydrogen (H\textsubscript{2}) breath test is a non-invasive investigation used to diagnose lactose intolerance (LI). Patients with LI may also expire increased amounts of methane (CH\textsubscript{4}) during a lactose test. The aim of this study is to evaluate the contribution of CH\textsubscript{4} measurements. We tested 209 children (1–17 years old) with symptoms suggesting LI with lactose H\textsubscript{2} and CH\textsubscript{4} breath tests. The result was positive when the H\textsubscript{2} excretion exceeded 20 parts per million (ppm) and the CH\textsubscript{4} was 10 ppm above the baseline. A clinician, blinded for the results of the breath test, registered the symptoms. Of the patient population, 101/209 (48%) were negative for both H\textsubscript{2} and CH\textsubscript{4}; 96/209 (46%) had a positive H\textsubscript{2} breath test result; 31/96 (32%) were also positive for CH\textsubscript{4}; 12/209 (6%) patients were only positive for CH\textsubscript{4}. The majority of hydrogen producers showed symptoms, whereas this was only the case in half of the H\textsubscript{2}-negative CH\textsubscript{4} producers. Almost all patients treated with a lactose-poor diet reported significant symptom improvement. These results indicate that CH\textsubscript{4} measurements may possibly be of additional value for the diagnosis of LI, since 5.7% of patients were negative for H\textsubscript{2} and positive for CH\textsubscript{4}, and half of them experienced symptoms during the test.

Keywords: lactose intolerance; hydrogen breath test; methane

1. Introduction

Lactose intolerance is defined as the occurrence of symptoms such as abdominal pain, bloating, and diarrhea after the ingestion of lactose in patients with lactose malabsorption. Although congenital and secondary lactase deficiencies occur, most children suffer from a “primary late onset” [1] or “adult type” lactase deficiency due to the non-persistence of the enzyme lactase-phlorizin hydrolase after childhood [2].

Adequate amounts of lactase, present in the villi of the small intestinal brush border, are required for the digestion of lactose. However, if the lactase activity is insufficient, undigested lactose is fermented in the colon by anaerobic bacteria. In that case, lactose is metabolized into carbon dioxide (leading to bloating), methane (CH\textsubscript{4}), propionic and butyric acids (short-chain fatty acids, leading to osmotic diarrhea), and hydrogen (H\textsubscript{2}). Gasses such as H\textsubscript{2} and CH\textsubscript{4} accumulate in the intestine, but also pass through the intestinal wall into the bloodstream and are transported to the lungs, where they are excreted in the exhaled breath [3]. In humans, the only source of H\textsubscript{2} and CH\textsubscript{4} is their production by bacterial metabolism of carbohydrates [4].

When anamnesis is suggestive for lactose intolerance, a H\textsubscript{2} breath test is considered the golden standard for the diagnosis, as it is a safe, cost-effective, and non-invasive investigative tool [5] with a high sensitivity (70–100%) and specificity (69–100%) [6]. A meta-analysis by Marton, Xue, and Szilagyi [7] comparing the diagnostic accuracy of lactose H\textsubscript{2} breath tests found an overall sensitivity of 0.88 (CI 0.85–0.90) and specificity of 0.85 (CI 0.82–0.87). A H\textsubscript{2} increase of 20 ppm above the baseline level is considered a positive test result, as suggested by the Rome Consensus Conference. In pediatric populations, a test duration of 3 h is advised, with samples taken at intervals of at least 30 min [8].
However, 2.5–15% of the H₂ breath test results are false-negative due to a variety of causes, such as non-producers of H₂ or increased production of CH₄ [9–11]. It is known that the production of one mole of CH₄ consumes four moles of H₂ and one mole of carbon dioxide, thereby also reducing the intracolonic gas content [12]. From this perspective, multiple studies examined the concomitant determination of CH₄ next to H₂ in order to improve the diagnostic accuracy of the H₂ breath test. A study by Vernia et al. [13] reported higher rates of false negative results in patients with predominant CH₄ production, with a CH₄ cut-off value of 10 ppm above the baseline, compared to patients with low CH₄ production. In other studies, significantly increased breath CH₄ levels were found in both lactose intolerant children [14] and adults [15], and the combined breath test for H₂ and CH₄ is considered more appropriate for the diagnosis of lactose malabsorption [16]. However, other authors did not find any advantage of the concomitant measuring of CH₄ [17]. Due to a lack of conclusive results in literature, the routine measurement of breath CH₄ excretion is not currently advised, although it is suggested in the Rome Consensus that measuring CH₄ excretion might improve diagnostic accuracy [8].

The aim of this pilot study is to evaluate the importance of the combined measurement of H₂ and CH₄ in the diagnosis of lactose intolerance.

2. Materials and Methods

Data were collected from November 2019 to July 2020 in the pediatric gastroenterology daycare unit of the KidZ Health Castle. Subjects were patients <18 years old, referred by a pediatrician for a lactose breath test because of reported symptoms suggestive of lactose intolerance. Parents and/or children (when old enough) gave permission to use the test results for this analysis. The history of the included patients was checked for the absence of evidence of small intestinal bacterial overgrowth, celiac disease, non-celiac gluten hypersensitivity, food allergy, or gastrointestinal infectious disease. Celiac disease was excluded because of the normal transglutaminase IgA antibodies in IgA sufficient children. The anti-gliadin IgG was within normal ranges in all children. The specific IgE for the common food allergies (egg white, cow milk, soy, fish, wheat, and gluten) was within normal ranges. The stool cultures, including parasites, were negative.

The tests were performed in the pediatric gastroenterology day-clinic after an overnight fasting time of at least 6 h. A basal H₂ measurement below 10 ppm was set as a control of the fasting state. The pre-test conditions, according to the 2009 Rome Consensus Conference [8], were respected. Exercise and smoking before and during the test were avoided. The exclusion criteria were children who were not presumed healthy, or had a history of probiotic or antibiotic intake during the two weeks preceding the H₂ breath test [18].

The lactose was administered orally (a dose of 2 g/kg, maximum 50 g) and diluted in a maximum of 250 mL of water. The expired air was collected in specific gas-tight syringes with a capacity of 60 mL. One breath sample was taken before the intake of lactose, and subsequent breath samples were collected in a sitting position every 15 min in a 3 h period after the ingestion of lactose. The patients were asked to remain in the waiting room and to avoid physical activity. The expired H₂ and CH₄ were measured with a specific analyzer (Microlyzer DP; Quintron Instruments, Milwaukee, WI, USA). The system was calibrated daily.

The result was considered positive when a H₂ peak exceeded 20 ppm above the baseline. The CH₄ excretion was considered positive when reaching 10 ppm above the baseline.

Gastro-intestinal symptoms such as abdominal pain, bloating, diarrhea, as well as extra-intestinal symptoms such as fatigue, headache, and dizziness, were registered every 15 min when a breath sample was collected. This was performed by a nurse, blinded for the results of the breath test.

The analyses were performed using SPSS version 26. The statistical significance of the differences between the percentage of participants who were CH₄ producers in various groups was determined by chi² analysis. The significance of the correlations between
the CH₄ and H₂ breath concentrations of producers vs. age was determined using the Spearman nonparametric correlation coefficient, because breath concentrations were not distributed normally.

3. Results

We tested 209 children with symptoms suggesting lactose intolerance with a lactose H₂ and CH₄ breath test. The mean age of our patients was 8.3 years (range 1.1–17.3 years, median 8.4 years, SD ± 3.78), including 56% girls and 44% boys. Over 90% reported gastrointestinal complaints, predominantly cramps or abdominal pain, flatulence, bloating, and diarrhea. In addition to the gastrointestinal complaints, a small proportion (~5%) also reported systemic complaints such as headaches.

In this group, 96 children (46%) had a positive H₂ breath test, of which 58% were girls. Of the female population, 65 of them (67.7%) tested positive for H₂, but showed no increased production of CH₄. On the other hand, 31 out of 96 (32.3%) patients had a positive test result for both H₂ and CH₄ excretion. In the group of CH₄ producers, 48% were girls. It is interesting to note that 28% (12/43) of the CH₄ producers had a negative result for H₂ production. Children were classified with malabsorption when the breath test was positive but the child continued to show no symptoms. The rise in H₂ or CH₄ within the first hour did not occur in any of the children, which may have been indicative of small intestinal bacterial overgrowth [19].

Significantly more CH₄ producers were present in the group of H₂ producers (Table 1; 5.7 vs. 14.8%; CHI square < 0.001), which is in line with the theory that elevated amounts of H₂ are necessary for production of CH₄. In 10 children excreting high amounts of CH₄ (>20 ppm above baseline), 6 of them also tested positive for the H₂ test. Only 2 of these 10 children were younger than 8 years old, suggesting that age might play a significant role in methanogenesis. Furthermore, we found age to be correlated significantly with baseline CH₄ levels (Spearman.149, \( p = 0.031 \)) and with maximum CH₄ (Spearman.142, \( p = 0.040 \)), but not with H₂ values.

Table 1. Hydrogen (H₂) versus methane (CH₄) results.

|         | H₂ + | H₂ −  | Total |
|---------|------|-------|-------|
| CH₄ +   |      |       |       |
| M: 3    | 31 (14.8%) | 12 (5.7%) | 43 (20.6%) |
| I: 28   |      |       |       |
| 65 (31.1%) |      |       |       |
| CH₄ −   |      |       |       |
| M: 13   | 101 (48.3%) | 166 (79.4%) | 267 (126.7%) |
| I: 52   |      |       |       |
| Total   | 96 (46.0%) | 113 (54.0%) | 209 (100.0%) |

Legend: M: malabsorption; I: intolerance; H₂ positive: peak exceeded 20 parts per million (ppm) over the baseline; CH₄ positive: peak > 10 ppm above baseline.

Prior to the administration of lactose, we found a mean breath H₂ excretion of 19.7 ± 20.9 and a net CH₄ excretion of 18.2 ± 11.2 (Table 2). No significant differences were seen in the baseline values of H₂ or CH₄ between normal and lactose malabsorbing children. After the administration of lactose, high elevations of breath H₂ (Delta H₂ > 100) were seen, as expected in the lactose-intolerant children, but also in CH₄ producers.

Significant correlations were found between delta H₂ and delta CH₄ values (P.358, \( p < 0.001 \)), delta H₂ and max CH₄ values (P.191, \( p = 0.005 \)), and between delta CH₄ and max H₂ values (P.312, \( p < 0.001 \)). This suggests that the elevations in H₂ and elevations in CH₄ coexist; this supports the hypothesis that CH₄ production is dependent on a (minimum of) H₂ production by conversion.

Overall, the difference in timing between CH₄ and H₂ peaks did not reach significance (median of 105 H₂ vs. 120 CH₄ min, \( p = 0.058 \)). There was no influence of sex (\( p = 0.20 \)) or age (\( p = 1 \)).
In total, 82 patients were treated with a lactose-free diet, and 56 (68.3%) were re-evaluated after 4 to 6 weeks. All but three patients reported significant improvement with the diet. These three patients had CH\textsubscript{4} starting values between 16 and 34 ppm; one patient also had a H\textsubscript{2} baseline value >20 ppm. At the follow-up consultation, the lactose-free diet was changed to a lactose-poor diet according to individual tolerance, and further follow-up was done by the referring physicians.

Table 2. Baseline and maximal values of H\textsubscript{2} and CH\textsubscript{4} in different groups.

|                | H\textsubscript{2} + | H\textsubscript{2} – | CH\textsubscript{4} + | CH\textsubscript{4} – |
|----------------|----------------------|----------------------|----------------------|----------------------|
| Baseline H\textsubscript{2} | 21.3 (±22.1)         | 18.5 (±19.9)         | 15.1 (±12.5)         | 21.2 (±22.8)         |
| Max H\textsubscript{2} level | 142.4 (±101)         | 21.2 (±21.4)         | 120.0 (±106.6)       | 59.3 (±80.8)         |
| Delta H\textsubscript{2}   | 121 (±95.9)          | 2.7 (±14.7)          | 104.6 (±106.5)       | 38.1 (±72.9)         |
| Baseline CH\textsubscript{4} | 17.5 (±8.9)          | 18.8 (±12.7)         | 21.4 (±15.1)         | 17.2 (±9.3)          |
| Max CH\textsubscript{4}    | 26.4 (±13.6)         | 22.0 (±15.9)         | 36.4 (±20.8)         | 19.8 (±9.5)          |
| Delta CH\textsubscript{4}  | 8.9 (±8.9)           | 3.2 (±6.3)           | 15.0 (±10.7)         | 2.6 (±3.2)           |

Legend: max: maximal.

4. Discussion

In our study population, 14.8% of the children with a positive H\textsubscript{2} breath test (>20 ppm above baseline) were also positive for CH\textsubscript{4} (>10 ppm above baseline). Moreover, we detected significant CH\textsubscript{4} production in 5.7% of the children with a negative H\textsubscript{2} test, which is in line with the amount of false negatives (2.5–15%) previously reported [9–11]. Houben et al. reported that the additional measurement of CH\textsubscript{4} (considering an increase with >5 ppm as positive) improved the accuracy of the test, as 16% of subjects (out of 1051 patients, with 178 children) with normal lactose digestion and no H\textsubscript{2} excretion were found to excrete CH\textsubscript{4} [20]. In this study, a rise in 13CO\textsubscript{2} excretion was used as a standard to diagnose lactase deficiency [20]. Based on a retrospective analysis of 282 breath tests, Peron et al. concluded that merging H\textsubscript{2} and CH\textsubscript{4} stoichiometric values resulted in an increased sensitivity [21].

Almost all (53/56) children in our study indicated fewer gastrointestinal complaints when on a lactose-free diet, suggesting that they likely did suffer from lactose intolerance. As excessive CH\textsubscript{4} production is thought to cause more health consequences than an excess production of H\textsubscript{2} [22], it is of high importance to detect these subjects. The CH\textsubscript{4} levels in expired air are suggested to be related to constipation in patients with irritable bowel syndrome [22]. This hypothesis is indirectly endorsed by our finding that only half of the CH\textsubscript{4} producers who did not produce H\textsubscript{2} showed symptoms suggesting lactose intolerance, whereas this was the case in 80–90% of the H\textsubscript{2} producers. However, some authors point out the difficulty of using reported symptoms during breath tests in the diagnosis of lactose intolerance, as they are often not reliable and/or poorly correlated [23,24].

Recently, instruments for the simultaneous measurement of CH\textsubscript{4} and H\textsubscript{2} have become more widely available for clinical use. Normally, patients produce either H\textsubscript{2} or CH\textsubscript{4}, and co-producers were rarely identified when using older instruments [25]. However, with more modern detection systems, co-production is detectable even at low levels [22]. Ruzsanyi et al. [23] reported elevated CH\textsubscript{4} levels in a majority of the children with a positive H\textsubscript{2} breath test. Furthermore, since it is suggested that excessive CH\textsubscript{4} production may cause more health consequences than an excess of H\textsubscript{2} [22], it becomes even more important to measure CH\textsubscript{4}. In our study, 46% of the children demonstrated lactose malabsorption with a positive H\textsubscript{2} breath test (of which 83% also reported symptoms compatible with LI). These data are comparable to other results in children [14,23], showing that 38% and 36%, respectively, of the tested children had a positive H\textsubscript{2} breath test. The ratio of boys and girls in the group with a positive H\textsubscript{2} test was identical to the ratio of the entire study group.
Background atmospheric CH₄ is about 1.7 ppm. The subjects excreting more than 1 ppm of CH₄ above the environmental value [26] are considered breath CH₄-producers. Therefore, a threshold of 3 ppm was proposed [27]. Others proposed a threshold of 10 ppm [19,23], and in a recent study by Hammer et al. [28], malabsorption was defined by a net increase of ≥5 to ≥12 ppm for CH₄, and ≥10 to ≥15 ppm for H₂ plus CH₄. In our group, 25% of the children showed elevated CH₄ excretion of more than 10 ppm. It was stated that patients usually produce either H₂ or CH₄, and only rarely produce both [25].

Hammer et al. [28] also found that the addition of CH₄ hardly influenced the results of malabsorption, and only under the use of specific cut-offs (the combined rise in H₂ and CH₄ should be less than 18) was a significant increase in the rate of malabsorbers seen. However, in our study, most of the CH₄-positive tests (72%) were found in the children that also demonstrated a positive H₂ breath test. Medow et al. [14] also reported significantly increased breath CH₄ levels in lactose intolerant children, although they already considered a H₂ test positive from 10 ppm above baseline. Schneider et al. [29] found a significant correlation between CH₄ and H₂ breath tests (Fisher’s exact test, \( p < 0.001 \)), with a strong relationship (Phi coefficient: \( \varphi = 0.84, p < 0.001 \)) and excellent agreement (Cohen’s \( \kappa = 0.837; 95\%\ CI 0.682–0.992; p < 0.001 \)). Furthermore, they stated that it might be possible to detect non-hydrogen producers using a lower CH₄ cut-off value (5 ppm), as it resulted in 26% more positive CH₄ breath tests. Ruzsanyi et al. [23] reported elevated CH₄ levels in a majority (83%) of the children with a positive H₂ breath test. It should be noted that in these studies, CH₄ levels were considered to be elevated when exceeding the baseline by only 1 ppm. They reported elevated CH₄ concentrations (>10 ppm above baseline) in 15% of the population, and an additional 28% of values greater than 1 ppm above the baseline were seen. However, it must be noted that most of them already showed elevated values before the ingestion of lactose, which suggests that other factors are contributing to methanogenesis (e.g., small intestinal bacterial overgrowth (SIBO) [22], constipation [30], or constipation-predominant IBS [31]). Recently, Gottlieb et al. [32] confirmed significantly elevated CH₄ levels in SIBO (confirmed with a lactulose breath test) and even proved a single-time-point CH₄ breath sample taken after an overnight fast (without administration of substrate) to be equally accurate in diagnosing SIBO.

There is also evidence that CH₄ producers experience slower transit times [33], and higher CH₄ excretion levels are seen in subjects suffering from constipation [34]. We found slightly later peak values in CH₄ (120 min) compared to H₂ peaks (105 min), but this difference was not significant (\( p = 0.058 \)). Possibly significant elevations of H₂ could still occur after the test registration (after 180 min).

The CH₄ producing status depends on many influencing factors, such as age [35], sex [36], ethnic background [37,38], exercise [39], and gastrointestinal diseases [22,38]. CH₄ is rarely identified in the breath of subjects less than three years of age [40–42], which is probably due to a later acquisition of a methanogenic microbiome [14]. CH₄ production is considered to increase with age in an approximately linear way [35] until the adult distribution is reached [26]. However, others did not find any correlation between age and exhaled maximum CH₄ concentration in children [23]. Furthermore, primary late onset lactose intolerance is characterized by a gradual reduction in lactase activity, and it generally manifests itself only after the age of 5 to 6 years of age in white populations, although it can occur earlier in predominantly non-white populations [1]. In general, it does not occur before the age of 2 years, which may explain why CH₄ production and excretion is rarely seen in this age category. We found age to be correlated significantly with the start CH₄ values (\( P = 0.149, p = 0.031 \)) and with the maximum CH₄ values (\( P = 0.142, p = 0.040 \)), but not with H₂ values. However, some authors reported CH₄ production already present in infants and toddlers [25,43].

Several authors mention a female dominance in CH₄ producers, certainly in young women [35,36], but other studies did not find any significant difference between male and female CH₄ producers [26,44], and little is known about sex differences in CH₄ excretion in children. No significant differences based on sex could be found in our study.
Ethnic differences were not taken into account in our study, which is a shortcoming, as it might influence the CH$_4$-producing status [37,38]. Furthermore, since the lactose-free diet intervention was open, and a blinded lactose challenge was not performed, a placebo effect cannot be excluded.

5. Conclusions

Although previous publications concluded that the addition of CH$_4$ determination to the standard H$_2$ measurement increases the sensitivity of a lactose breath test, the contribution of CH$_4$ is still debated and not routinely performed. Our results confirm that the concomitant measurement of CH$_4$ is of additional value for the diagnosis of LI, since 5.7% of the children showed only an elevation of expired CH$_4$ and half of these children were symptomatic during the breath test. Further research is needed before recommending systematic CH$_4$ measurements.

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