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Respiratory Methane Excretion in Children with Lactose Intolerance

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To evaluate the relationship between colonic methane production and carbohydrate malabsorption, we measured end-expiratory methane levels in 70 normal and 40 lactose-intolerant children. Time-dependent excretion of hydrogen and methane was determined every 30 min for 120 min following a fasting oral lactose challenge (2 g/kg). Mean breath hydrogen levels in normals (lactose-tolerant) equaled 3.7 parts per million (ppm) throughout the study, but increased to >10 ppm by 60 min and remained elevated in lactose-intolerant subjects. Breath methane in normal children averaged 1.6 ppm from 0 to 120 min. In contrast, CH_4 excretion by lactose-intolerant children averaged 5.1 ppm at 90 min; and, by 120 min levels increased significantly compared with control. Breath methane levels in lactose-intolerant subjects following a lactose load continued to increase, however, despite the coingestion of exogenous lactase in amounts calculated to result in complete hydrolysis of the disaccharide. These data demonstrate that lactase-deficient children manifest significant increases in breath methane excretion following lactose ingestion and that enhanced methane production may be a consequence of several factors, including altered fecal pH and increased methanogenic substrates provided by colonic lactose fermentation. Further studies are required to determine the clinical significance of elevated methane production in lactose intolerance.

KEY WORDS: methanogenesis; carbohydrate malabsorption; pediatrics.

In the mammalian colon, methanogenesis results from the metabolic activity of anaerobic bacteria on available intraluminal substrates, such as carbohydrates, proteins, and glycoproteins (1, 2). Methane is excreted both in flatus and, following diffusion across the colonic epithelia into the bloodstream, is excreted in expired air (3). In adults, methane producers comprise 30–65% of subjects evaluated by random breath analysis (3–5). This wide range in the incidence of significant methanogenesis may be a consequence of varying gastrointestinal transit times and ethnic backgrounds (5). During early childhood, available evidence demonstrates that methane excretion is not as prevalent as reported in the adult population. Methane is rarely identified in the breath of subjects less than three years of age; nevertheless, adult patterns of excretion have been documented by age 11 (3, 6). The reasons for these age-related differences in methane production are unclear, but likely involve, at least in part, a late developmental acquisition of methanogenic flora (3).

The significance of methanogenesis, both in children and in adults, remains controversial. In patients with a variety of gastrointestinal diseases, such as diverticulosis, colon carcinoma, adenomatous polyps, and inflammatory bowel disease, dif-

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ferences in methane production, compared to control populations, have been identified (5, 7–10). However, the relationship(s) between intestinal methane exposure and mucosal dysfunction have not yet been described.

The relationship between methane gas generation and the availability of methanogenic substrates in the colon, such as malabsorbed carbohydrate, has not yet been investigated in the pediatric population. The most prevalent carbohydrate malabsorption syndrome in man is late-onset, primary lactose-intolerance, which results from a developmental decrease or absence of intestinal β-galactosidase (lactase) activity (11, 12). We have recently shown that administration of exogenous lactase to children with lactose intolerance significantly reduces the production of hydrogen gas and gastrointestinal symptoms following a lactose challenge (13).

Methane is produced from colonic bacterial reduction of CO_2 by hydrogen, which is derived from the dissimilation of nonabsorbed carbohydrates (2). Accordingly, to determine whether malabsorbed lactose can serve as a substrate for methane production, we evaluated the temporal relationship between methane and hydrogen production in children with late-onset, primary lactose intolerance.

MATERIALS AND METHODS

Patient Selection. Study subjects were chosen from those referred to the Division of Pediatric Gastroenterology and Nutrition, New York Medical College, for complaints of chronic, nonspecific abdominal pain, with or without diarrhea. Routine patient screening included: physical examination, CBC with differential, platelet count, erythrocyte sedimentation rate, serum transaminase, bilirubin and amylase concentrations, urinalysis, and urine culture. Children with primary underlying gastrointestinal disease (eg, inflammatory bowel disease, acute gastroenteritis, celiac disease) or manifesting acute and/or chronic malnutrition or growth failure (14), were excluded from this study. Those with normal screening studies subsequently underwent breath hydrogen analysis for diagnosis of lactose intolerance.

Hydrogen Breath Test. All patients fasted for a minimum of 8 hr prior to breath testing and had not received antibiotics for at least two weeks before study. Participants were placed on a lactose-free diet 24 hr prior to testing to minimize basal hydrogen (H_2) production. Each patient received lactose (2 g/kg body weight to a maximum of 50 g) and had serial breath samples (0, 30, 60, 90, 120 min following lactose ingestion) tested for the presence of hydrogen. Patients who produced hydrogen >10 parts per million (ppm) above baseline and who exhibited symptoms such as abdominal pain and diarrhea within 2 hr of lactose ingestion were considered to be lactose-intolerant (15). Patients who were not hydrogen producers, or those exhibiting baseline (0 time) breath hydrogen levels >10 ppm, were excluded from this study.

Lactose-intolerant patients who agreed to participate received two additional breath tests at least two weeks apart, during which time either lactase-containing tablets or placebo were administered in a single-blind, crossover fashion. The investigator determined which tablet to administer initially in a randomized fashion by lot. Both lactase (β-galactosidase produced from *Aspergillus oryzae*) -containing tablets and placebo of inert excepient were directly supplied by the manufacturer (Lactaid Inc, Pleasantville, New Jersey). Each active tablet contained 3000 Food Chemical Codex Units of β-galactosidase, pH optimum ~4.5. Participants chewed and swallowed one tablet per 5 g of lactose immediately prior to carbohydrate challenge. There were no sensory differences (ie, taste or texture) between the active or placebo tablets. Children who were found to be lactose-intolerant were asked to participate in an evaluation of the effectiveness of exogenous lactase in reducing breath hydrogen and methane. The protocol was approved by the Committee for the Protection of Human Subjects (Institutional Review Board), New York Medical College.

End-expiratory breath samples were collected in a gastight syringe according to the method of Metz et al (16). Breath hydrogen was measured following gas chromatographic separation, using a Carle model AGC 111 gas chromatograph (Hache Inc., Loveland, Colorado) equipped with a 9-ft, 5Å molecular sieve column, 45–50 mesh, and with a thermal conductivity detector. The system was standardized before each use with a reference gas mixture of 100 ppm hydrogen in nitrogen (MG Scientific Gases, North Branch, New Jersey), and the sensitivity of this method was to less than 0.1 ppm. Breath methane was measured following isothermal separation (125° C) using a Shimadzu model 9A gas chromatograph (Shimadzu Inc, Columbia, Maryland) fitted with a 2-m Carbosieve S-II column, 60–80 mesh (Supelco Inc, Bellefonte, Pennsylvania) and a flame ionization detector. The system was standardized before each use with a reference gas consisting of 99 ppm methane in nitrogen (Scott Specialty Gases, Plumsteadville, Pennsylvania). All methane values were corrected for ambient methane concentration, and the sensitivity of this method was to less than 0.1 ppm. Breath samples were analyzed for hydrogen and methane within 1 hr of collection and were quantitated using a Shimadzu CR3A computing integrator.

Statistical Analysis. Comparisons between normal and lactose-intolerant patients were made utilizing both chi-square analysis and Student's *t* test for unpaired samples (two-tailed). Differences in hydrogen and methane excretion associated with placebo and lactase treatment were assessed using Student's *t* test for paired data (two-tailed) (17).

RESULTS

Evaluation of 110 children referred for chronic recurrent abdominal pain showed that 40 children, or 36%, were lactose-intolerant, as diagnosed by

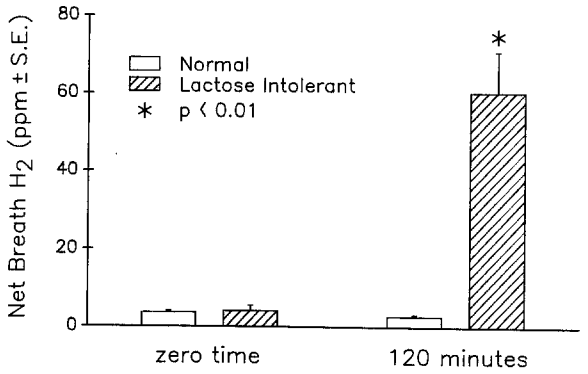

Fig 1. Net breath hydrogen excretion by 70 normal (□) and 40 lactose-intolerant (▨) patients measured immediately after (zero time), and 120 min following ingestion of a standard lactose load. Data shown are the mean (± SEM) breath hydrogen excretion values.

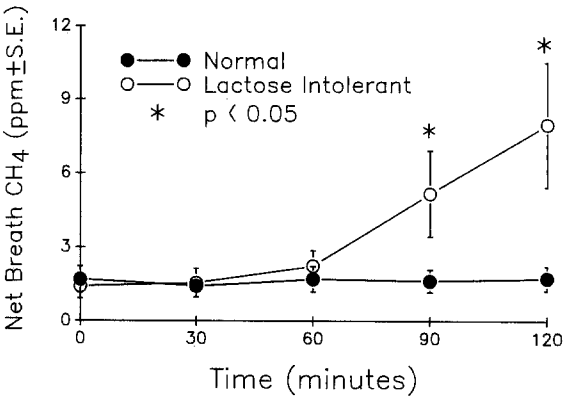

Fig 2. Time course of breath methane excretion by 70 normal (●) and 40 lactose-intolerant (○) patients. Data shown are the mean (± SEM) breath methane excretion values.

elevated breath hydrogen excretion and the presence of gastrointestinal symptoms following a standard lactose challenge. The mean ages (± SD) for all children studied and for those exhibiting lactose-intolerance were 9.6 ± 0.7 and 11.1 ± 3.7 years, respectively (P = NS). Subjects who remained asymptomatic and whose breath hydrogen was less than 10 ppm by 2 hr following a lactose challenge were considered to be lactose-tolerant, or "normal." The male-to-female ratio was the same in both lactose-tolerant and -intolerant groups.

Figure 1 shows that net breath hydrogen excretion immediately prior to ingestion of a lactose load (zero time) is the same in normal and lactose-intolerant children, averaging approximately 4 ppm. At 120 min following lactose ingestion, while breath hydrogen excretion by normal children remained at ~4 ppm, mean breath hydrogen levels in lactose-intolerant subjects increased to 61 ppm ($P < 0.01$, $N = 40$).

Analysis of methane content in expired air of these subjects showed that the percentage of methane producers (>1 ppm above ambient air) was similar in normal versus lactose-intolerant children (32% vs 45%, P = NS). To determine the temporal relationship(s) between carbohydrate malabsorption and methane production, the time course of breath methane excretion was measured in both study groups. As shown in Figure 2, methane levels in lactose-tolerant children remained constant throughout the 120-min study period following lactose ingestion, and averaged <2 ppm. A similar pattern of excretion was found in lactose-intolerant children for the first 60 min. By 90 min following the lactose load, however, methane excretion by lactose-intolerant children rose significantly to 5.1 ppm and, by 120 min, levels had increased above baseline ($P < 0.05$) to almost 8 ppm.

We have previously shown that coingestion of exogenous lactase-containing (β-galactosidase) tablets and lactose results in a significant reduction of both breath hydrogen and gastrointestinal symptoms in lactose-intolerant patients (13). We therefore examined the effects of exogenous lactase administration on net methane excretion in lactose-intolerant patients following lactose challenge. Figure 3 shows that breath methane levels in lactose-intolerant subjects following a lactose load continued to increase, despite the coingestion of exogenous lactase in amounts calculated to result in complete hydrolysis of the disaccharide. This is in contrast to breath hydrogen concentrations, which

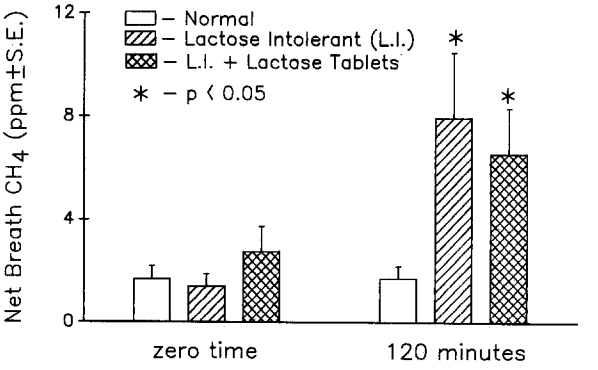

Fig 3. Net breath methane excretion by 70 normal (□), 40 lactose-intolerant (▨), and 18 lactose-intolerant patients (▩) who coingested lactose and lactase tablets (see Materials and Methods). Data shown are the mean (± SEM) of breath methane values measured immediately after (zero time) and 120 min following ingestion of a standard lactose load.

remained well within the normal range (<10 ppm above baseline) for up to 120 min (data not shown).

DISCUSSION

Gas production in the human colon arises, in part, from the intraluminal fermentation of carbohydrate and glycoprotein by anaerobic bacterial flora. In contrast to hydrogen, which is generated by bacterial metabolism and is measured in expired air in the majority of normal subjects, methane excretion has been identified in a significantly smaller segment of the population (18, 19). The excretion of methane in breath is dependent upon the colonic production of methane, as well as acquisition and delivery to the lungs via a competent peripheral vascular system (10). Most studies examining the incidence and significance of methane production have been conducted in adults. Data indicate that the presence of breath methane (above levels in ambient air) varies widely within populations, ranging from 33% to 65% of subjects evaluated; and gas generation is independent of gender, age, and the ingestion of substrates (ie, carbohydrates) known to increase hydrogen production (5).

In the pediatric population, methane excretion generally does not occur until at least 3 years of age, when an intraluminal methanogenic flora is established, presumably following nosocomial acquisition from older, methane-producing individuals (3). The data presented here demonstrate an incidence of methanogenesis in our normal pediatric controls (32%) that is similar to that previously reported (3, 6). While the percentage of methane producers in lactose-tolerant (32%) versus -intolerant (45%) subjects is not significantly different in our study groups, lactase-deficient subjects manifest a time-dependent, statistically significant increase in breath methane excretion following a lactose challenge. In these lactose-intolerant children, time-dependent methanogenesis would appear to be related to the delivery of malabsorbed carbohydrate to the distal small bowel and colon. Respiratory methane excretion increased significantly from baseline (and from control) levels by 90 min following lactose ingestion. This time course is similar to that shown for hydrogen generation by lactose-intolerant children, which shows a significant rise in breath hydrogen by 60–90 min after lactose challenge.

Hydrogen produced following lactose ingestion by lactose-intolerant children is likely preferentially oxidized by methanogenic bacteria present in the colon of methane-producing individuals. Methane generation then results from the reduction of CO_2 by H_2 through anaerobic metabolism, which is favored by an alkaline environment (1, 2). Breath hydrogen measured in methane-producing, lactose-intolerant subjects probably represents the difference between hydrogen generated by fermentation of the nonabsorbed carbohydrate and the hydrogen utilized in methanogenesis.

Another finding of this study is the inability of exogenous β-galactosidase, when administered in doses previously demonstrated to be effective in reducing both breath hydrogen and gastrointestinal symptoms following lactose challenge (13), to alter either the magnitude or time course of methane production in lactose-intolerant children. The quantity of enzyme used in the present study was calculated to result in complete lactose digestion by 5 min after ingestion. However, since samples of gastric and small intestinal contents were not obtained during the testing, complete hydrolysis could not be assured. Thus, the observed reduction in breath hydrogen may only reflect a decrease in intraluminal lactose to a concentration below the preestablished threshold level for malabsorption (10 ppm above baseline), as measured by standard techniques. Remaining unabsorbed carbohydrate could continue to serve as a substrate for methanogenic bacteria, or colonic hydrogen may remain high enough to result in significantly elevated breath methane excretion.

In addition, the increase in breath methane following lactose ingestion may reflect better mixing of colonic content in lactose intolerance, and the release of methane into the lumen, with subsequent absorption and excretion. These mechanical events may be independent of β-galactosidase administration.

In contrast, lactose fermentation results in the formation of watery, acidic stools in addition to reduced gastrointestinal transit time. Both of these events should result in decreased methane generation in lactose-intolerant subjects. These observations suggest that methane production in lactose-intolerant subjects is, in part, a consequence of factors other than the presence of intraluminal, undigested carbohydrate, and confirms studies that showed methane production to be independent of diet (3, 4). Investigations comparing the intraluminal flora of normal and lactose-intolerant children, and their ability to produce methane from different

metabolic substrates and in response to a range of lactose concentrations, are required to explain these findings.

Although the significance of methane production in children is unclear, several reports in adults have correlated colonic methanogenesis with various gastrointestinal diseases, including Crohn's disease, ulcerative colitis, irritable bowel syndrome, diverticulosis, and polyposis (5, 9). Relationships between methanogenesis and cancer, peripheral vascular disease, and cystic fibrosis have also been described (7–10). Whether pediatric methane producers, and lactose-intolerant subjects in particular, are at greater risk for the subsequent development of gastrointestinal disease must be addressed in future studies.

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REFERENCES

1. Nottingham PM, Hungate RE: Isolation of methanogenic bacteria from faeces of man. J Bacteriol 96:2178–2179, 1968
2. Mah RA, Ward DM, Baresi L, Glass TL: Biogenesis of methane. Annu Rev Microbiol 31:309–341, 1977
3. Bond JH, Engel RR, Levitt MD: Factors influencing pulmonary methane excretion in man. J Exp Med 133:572–588, 1971
4. Pitt P, deBruijn KM, Beeching MF, Goldberg E, Blendis LM: Studies on breath methane: The effect of ethnic origins and lactulose. Gut 21:951–959, 1980
5. McKay LF, Eastwood MA, Brydon WG: Methane excretion in man—a study of breath, flatus and faeces. Gut 26:69–74, 1985
6. Peled Y, Gilat T, Liberman E, Bujanover Y: The development of methane production in childhood and adolescence. J Pediatr Gastroenterol Nutr 4:575–579, 1985
7. Segal I, Walker ARP, Lord S, Cummings JH: Breath methane and large bowel cancer risk in contrasting African populations. Gut 29:608–613, 1988
8. Pique JM, Pallares M, Cuso E, Vilar-Bonet J, Gassull MA: Methane production and colon cancer. Gastroenterology 87:601–605, 1984
9. Peled Y, Weinberg A, Hallak A, Gilat T: Factors affecting methane production in humans. Dig Dis Sci 32:267–271, 1987
10. McKay LF, Brydon WG, Eastwood MA, Housley E: The influence of peripheral vascular disease on methanogenesis in man. Atherosclerosis 47:77–81, 1983
11. Paige D, Bayless T (eds): Lactose Digestion: Clinical and Nutritional Implications. Baltimore, Maryland, The Johns Hopkins Institutions, 1981
12. Lebenthal E, Ross TM, Nord KS, Branski D: Recurrent abdominal pain and lactose absorption in children. Pediatrics 67:828–832, 1981
13. Medow MS, Thek KD, Newman LJ, Berezin S, Glassman MS, Schwarz SM: β-Galactosidase tablets in the treatment of lactose intolerance in pediatrics. Am J Dis Child 144:261–1264, 1990
14. Waterlow JC: Classification and definition of protein–calorie malnutrition. Br Med J 3:566–569, 1972
15. Biller JA, King S, Rosenthal A, Grand RJ: Efficacy of lactase-treated milk for lactose-intolerant pediatric patients. J Pediatr 111:91–94, 1987
16. Metz G, Gassull MA, Leeds AR, Blendis LM, Jenkins DJA: A simple method of measuring breath hydrogen and carbohydrate malabsorption by end-expiratory sampling. Clin Sci Mol Med 50:237–240, 1976
17. Zar JH: Biostatistical Analysis. Englewood Cliffs, New Jersey, Prentice-Hall International, 1974
18. Levitt MD, Ingelfinger FJ: Hydrogen and methane production in man. Ann NY Acad Sci 150:75–81, 1968
19. Calloway DH, Murphy EL: The use of expired air to measure intestinal gas formation. Ann NY Acad Sci 150:82–95, 1968