

IBS Subjects with Methane on Lactulose Breath Test Have Lower Postprandial Serotonin Levels Than Subjects with Hydrogen

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We have previously shown that methane on lactulose breath test (LBT) is highly associated with constipation in IBS and that methane gas itself slows small bowel transit in dogs. Previous studies suggest that serotonin may have a role in the control of transit in IBS. In this study, we aim to evaluate the role of serotonin in methane producing IBS subjects. Rome I-positive IBS subjects were recruited into the study after exclusion criteria were met. A fasting LBT was performed after subjects filled out a questionnaire rating the degree of constipation and diarrhea. Within 7 days of this test, subjects returned fasting for determination of serotonin before and after a 75-g oral glucose meal. The serotonin response was compared between hydrogen and methane producing IBS subjects. After 2 subjects were excluded for inadequate blood samples, 18 subjects completed the study. Four of 18 subjects produced methane. The postprandial serotonin level in methane producing IBS subjects was lower than in hydrogen producers ($P < 0.05$). Methane producers had a reduction in serotonin after glucose. Methane producing IBS subjects have reduced postprandial serotonin. Whether methane is a surrogate marker of constipation or contributing to the reduced serotonin remains to be determined.

KEY WORDS: irritable bowel syndrome; lactulose breath test; methane; serotonin.

Irritable bowel syndrome (IBS) is a chronic gastrointestinal problem with no known etiology. Due to the enigmatic nature of this condition, researchers subcategorize IBS into symptom specific groups (1) to help explain some of the recent objective findings in this condition.

One objective finding in IBS is the relationship to serum serotonin. Almost 95% of all serotonin in the body is found in the gastrointestinal tract (2–4), pointing to its importance in this organ system. Serotonin is a neurotransmitter predominantly secreted by enterochromaffin cells of the gut located in the crypts of the intestinal villi (5). After release, the predominant function of this product is peri-

staltic stimulation of the gut (6–9). Recent findings reveal that IBS subjects with predominantly diarrhea have elevated postprandial serotonin compared to controls (10). Though there was a small number of subjects in this paper, the finding has a significant impact on the direction of research and treatment in IBS. Specifically, this has led to new approved therapeutic agents acting as an agonist (11) and antagonist (12, 13) on serotonin receptors.

Another recent objective finding in IBS is the high prevalence of abnormal lactulose breath tests (LBTs). In an uncontrolled (14) and now a controlled study (15), we find that up to 84% of subjects with IBS have an abnormal LBT, suggesting the presence of bacterial overgrowth. While there is still some debate as to whether the LBT test represents overgrowth or rapid transit, further data from two separate studies now suggest that methane on LBT in IBS subjects is almost universally associated with constipation-predominant IBS (15, 16). Follow-up work also shows that methane gas itself slows small intestinal

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transit (17). The mechanism by which methane alters intestinal motility is unknown. However, research in pulmonary circulation suggests that methane has an effect on smooth muscle through a serotonergic mechanism (18).

In this study, we investigate the role of methane on serotonin response and bowel symptoms in subjects with irritable bowel syndrome.

MATERIALS AND METHODS

Patient Population. Subjects meeting Rome I criteria (19) for IBS were recruited through newspaper advertising in the Los Angeles area. Subjects in this study were new and not included in any other previous protocol. Subjects were between 18 and 65 years old and were excluded if they had a history of bowel disease or risk factors for bacterial overgrowth. Exclusion criteria included a history of bowel resection, adhesions, cirrhosis, narcotic use, diabetes, connective tissue disease, proton pump inhibitor use, and IBD. The study was approved by the Cedars-Sinai Medical Center Institutional Review Board and all patients were consented prior to participation.

Lactulose Breath Test. After inclusion and exclusion criteria were met, subjects were asked to present to the GI Motility Program office at Cedars-Sinai Medical Center having fasted from 7 PM the previous evening. Subjects were instructed to eat a light meal the previous evening that did not include legumes or a heavy fat or protein content. On the morning of testing subjects were asked to brush their teeth and not smoke prior to arriving for the research study. They were then given a questionnaire as previously described (15), which provided demographic information and bowel symptom scores. Following completion of the questionnaire, subjects were asked to provide a baseline breath sample. The breath was collected using a Quintron gas collection bag (Quintron Instrument Co., Milwaukee, WI) designed to maximize alveolar and minimize pulmonary dead space sampling. Subjects were then asked to ingest 10 g of lactulose (Constulose; Alpha USA USP Inc., Baltimore MD), after which repeat breath samples were collected every 15 min for 3 hr. Samples were analyzed immediately after acquisition to ensure quality measurements using a Model SC Quintron Gas Chromatograph (Quintron Instrument Co.). Both hydrogen and methane were quantitatively determined during the analysis. The results of the breath test were blinded to both the subjects and the investigators until completion of the study.

Determination of Serotonin. On a separate visit that was scheduled within 7 days of the LBT, subjects returned having fasted and prepared identically to what was described for the LBT. Subjects then had an intravenous placed in a large antecubital vein to facilitate blood draws. A baseline blood sample was obtained for determination of serotonin. To induce serotonin in a postprandial fashion, subjects were given a solution containing 75 g of glucose (Allegiance Healthcare Corp., McGaw Park, IL). The glucose meal was selected as per a previously validated technique (20, 21). The glucose solution, having a preset caloric load and predictable delivery to the small intestine, was less likely to be influenced by gastric emptying compared to a solid meal (10). Furthermore, a previous study had demonstrated that an oral glucose challenge is a potent stimulator of serotonin release by the gut (21). One hour after the ingestion of glucose, another blood sample was taken to determine serotonin levels.

Serotonin Analysis. Whole blood was collected in blood collection tubes containing 7.2 mg of EDTA and mixed. The blood was then transferred into a collection tube containing 35 mg of ascorbic acid and was immediately frozen. Frozen samples were then analyzed for serotonin using HPLC (HPLC 717+; Waters Corp., Milford, MD) through Quest Diagnostics (Van Nuys, CA).

Data Analysis. Serotonin levels were compared between hydrogen and methane producing IBS subjects using Student's *t*-test. Comparisons of nonparametric data utilized Fisher's exact test. Data are expressed as mean \pm SD.

RESULTS

Demographics. After inclusion and exclusion criteria were met, 20 Rome I-positive IBS subjects were enrolled in the study. In one subject, the intravenous did not provide adequate samples for both baseline and 60 min after glucose administration. In a second subject, the samples were lost. Of the remaining 18 subjects, 8 (44%) were women. The baseline and postprandial serotonin levels were not different between male and female subjects.

Comparison Between Hydrogen and Methane Producers. Of the 18 subjects, 4 (22%) produced methane. Baseline serotonin levels were not different between methane and non-methane producing subjects. The baseline serotonin level in hydrogen and methane producers was 79.5 ± 33.1 ng/ml ($n = 13$; one hydrogen producing subject did not have a baseline serotonin value) and 54.3 ± 29.5 ng/ml ($n = 4$), respectively ($P = 0.19$). However, 60 min after glucose administration, there was a significantly lower serum serotonin concentration in methane producing IBS subjects compared to hydrogen (Figure 1).

Methane was also associated with a drop in serotonin after the glucose load. Specifically, 4 of 4 methane producing subjects had no increase ($n = 1$) or a reduction ($n = 3$) in serotonin after glucose compared to 5 of 13 subjects in the hydrogen group ($P = 0.08$). The percentage change in serotonin level after glucose was $8.7 \pm 28.7\%$ among hydrogen producers and $-31.2 \pm 33.5\%$ in methane producers ($P < 0.05$) (Figures 2 and 3).

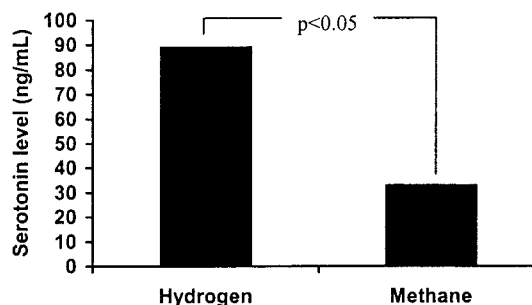


Fig 1. Postprandial serotonin levels after a glucose meal among IBS subjects with and without methane on lactulose breath test.

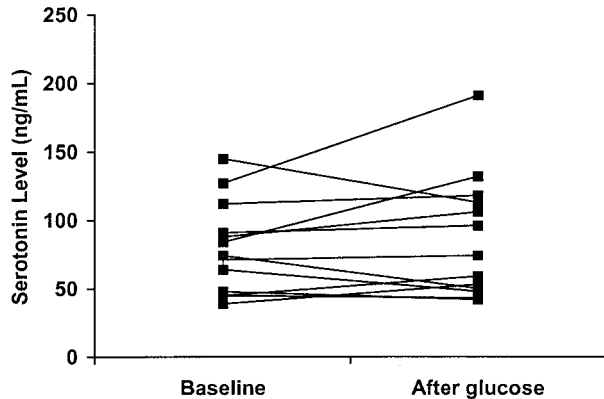


Fig 2. Individual serotonin responses after a glucose meal among IBS subjects producing only hydrogen.

DISCUSSION

In this study we tried to determine the role of serotonin in IBS subjects with methane on LBT. It appears that methane on LBT portends a lower postprandial serotonin response compared to that of hydrogen producing IBS subjects and may be linked to the finding of constipation among methane producing IBS subjects (15, 16).

Methane is one of a number of gases produced in the digestive tract by enteric flora. The two main gases produced by enteric organisms that allow differentiation from the human host are hydrogen and methane (22–24). Methane producing organisms are extremely fastidious, yet up to 70% of normal subjects have detectable methane during study of colonic gas production (25–30). What makes methane production interesting is its symbiotic metabolic dependence on the presence of hydrogen. In culture, methane producing bacteria are capable of producing said gas only in the presence of hydrogen (31). As such, when methane is seen on breath test there is usually no hydrogen detectable on the test (30).

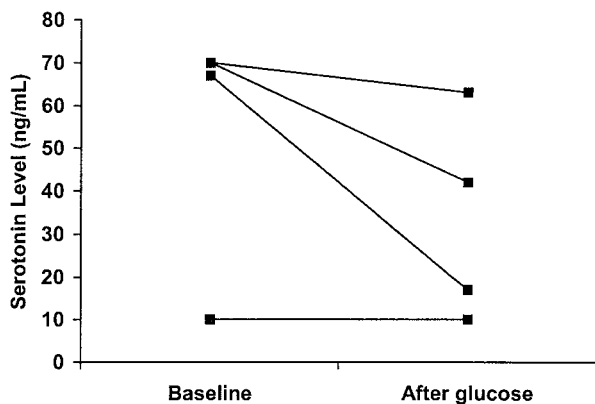


Fig 3. Individual serotonin responses after a glucose meal among IBS subjects producing only methane.

The relationship between endogenously produced methane gas and constipation was largely unstudied until recently. In the literature, two reports lend support to the notion of methane in constipation. In one report, methane is found in children with encopresis (32). Another study identified diverticulosis as a risk factor for methane production (33). However, the literature provides no understanding as to whether methane is simply associated with or contributing to the constipation in these subjects.

In a recently completed double-blind study of antibiotic treatment in IBS, we found that methane gas on LBT was always associated with constipation-predominant IBS (15). The significance of this result prompted an evaluation of a prospectively collected database for the same effect (16). This database included all subjects referred for lactulose breath testing, and among referred IBS subjects, all those with methane also declared constipation as their predominant symptom.

The suggestion that methane gas on LBT is in some way linked to constipation is interesting but lacks explanation as to whether the culprit is methane gas itself, the methanogenic bacteria, or a host response. Since methane gas is readily available, we recently determined that intraluminal infusion of methane into the canine small intestine slows transit (17). The means by which bacteriologically produced methane gas slows transit remains unknown.

In the case of gastrointestinal transit, serotonin is always one of the first targets for investigation of transit since it is the key mediator of the peristaltic reflex (6–9) and 95% of serotonin in the body is related to the gastrointestinal tract (34–36). Attention to serotonin in clinical IBS has been, in part, related to a finding of increased postprandial levels of serotonin in diarrhea-predominant IBS (10). These major findings have culminated in the development of pharmacologic therapy for IBS based on serotonin (11–13). Although elevated serotonin appears to be a rationale for diarrhea-predominant IBS, the understanding of constipation-predominant IBS has not been so obvious. If methane has a role in constipation-predominant IBS, the relationship between methane and serotonin needs to be determined.

Although there is no evidence in the literature regarding the influence of methane on serotonin in the gut, the anesthesia literature contains one clue. Most inhaled anesthetic agents are methane derivatives. In a recent study, methane gas and its derivatives appeared to have inhibiting effects on serotonin uptake in the pulmonary circulation (18). Perhaps the circulation of serotonin is in some way limited by methane exposure.

In conclusion, it appears that methane on LBT in IBS subjects has some relationship to serum serotonin levels as suggested by reduced serotonin responsiveness to meal

stimulation in these subjects. Based on previous work, there may be some effect of methane on transit (17). More work is needed to understand this intriguing interaction between gaseous bacterial by-products and the movements of the human small intestine.

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REFERENCES

- Thompson WG, Longstreth GF, Drossman DA, et al.: Functional bowel disorders and functional abdominal pain. *Gut* 45:II43-II47, 1999
- Bertaccini G: Tissue 5-hydroxytryptamine and urinary 5-hydroxyindoleacetic acid after partial or total removal of the gastrointestinal tract in the rat. *J Physiol (Lond)* 153:239-249, 1960
- Espramer V, Testini A: Observations on the release and turnover rate of 5-hydroxytryptamine in the gastrointestinal tract. *J Pharm Pharmacol* 11:618-623, 1959
- Kim D-Y, Camilleri M. Serotonin: A mediator of the brain-gut connection. *Am J Gastroenterol* 95:2698-2709, 2000
- Inokuchi H, Kawai K, Takeuchi Y, Sano Y: Immunohistochemical demonstration of EC cells in rat gastrointestinal tract. *Histochemistry* 74:453-456, 1982
- Bülbring E, Lin RCY: The effect of intraluminal application of 5-hydroxytryptamine and 5-hydroxytryptophan on peristalsis; the local production of 5-HT and its release in relation to intraluminal pressure and propulsive activity. *J Physiol* 140:381-407, 1958
- Bülbring E, Crema A: Observations concerning the action of 5-hydroxytryptamine on the peristaltic reflex. *Br J Pharmacol* 13:444-457, 1958
- Bülbring E, Crema A: The action of 5-hydroxytryptamine, 5-hydroxytryptophan and reserpine on intestinal peristalsis in anaesthetized guinea pigs. *J Physiol* 146:29-53, 1959
- Costa M, Furness JB: The peristaltic reflex: An analysis of the nerve pathways and their pharmacology. *Naunyn-Schmiedelberg Arch Pharmacol* 294:47-60, 1976
- Bearcroft CP, Perrett D, Farthing MJG: Postprandial plasma 5-hydroxytryptamine in diarrhoea predominant irritable bowel syndrome: a pilot study. *Gut* 1998;42:42-46, 1998
- Müller-Lissner SA, Fumagalli I, Bardhan KD, et al.: Tegaserod, a 5-HT₄ receptor partial agonist, relieves symptoms in irritable bowel syndrome patients with abdominal pain, bloating and constipation. *Alim Pharmacol Ther* 15:1655-1666, 2001
- Camilleri M, Chey WY, Mayer EA, et al.: A randomized controlled clinical trial of the serotonin type 3 receptor antagonist alosetron in women with diarrhea-predominant irritable bowel syndrome. *Arch Intern Med* 161:1733-1740, 2001
- Camilleri M, Northcutt AR, Kong S, et al.: Efficacy and safety of alosetron in women with irritable bowel syndrome: a randomised, placebo-controlled trial. *Lancet* 355:1035-1040, 2000
- Pimentel M, Chow EJ, Lin HC: Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol* 95:3503-3506, 2000
- Pimentel M, Chow EJ, Lin HC: Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: A double-blind, randomized placebo controlled study. *Am J Gastroenterol* 98:412-419, 2003
- Pimentel M, Mayer AG, Park S, et al.: Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Dig Dis Sci* 48:86-92, 2003
- Lin HC, Pimentel M, Chen JH: Intestinal transit is slowed by luminal methane. *Neurogastroenterol Motil* 4:437, 2002
- Hede AR, Andersson L, Post C: Effect of homologous series of halogenated methanes on pulmonary uptake of 5-hydroxytryptamine in isolated perfused rat lung. *Acta Pharmacol Toxicol* 57:291-296, 1985
- Drossman DA, Richter JE, et al. (eds): Functional gastrointestinal disorders: Diagnosis, pathophysiology and treatment: a multinational consensus. Boston, Little, Brown, 1994
- O'Hara R, Fox RO, Cole JW: Serotonin release mediated by intraluminal sucrose solutions. *Surg Forum* 10:215, 1959
- Drapana T, McDonald JC, Stewart JD: Serotonin release following instillation of hypertonic glucose into the proximal intestine. *Ann Surg* 156:528-536, 1962
- Levitt MD: Production and excretion of hydrogen gas in man. *N Engl J Med* 281:122-127, 1969
- Levitt MD, French P, Donaldson RM Jr: Use of hydrogen and methane excretion in the study of the intestinal flora. *J Lab Clin Med* 72:988, 1968 (abstr)
- Bond JH Jr, Engel RR, Levitt MD: Factors influencing pulmonary methane excretion in man. An indirect method of studying the in situ metabolism of the methane-producing colonic bacteria. *J Exp Med* 133:572-588, 1971
- Levitt MD, Ingelfinger FJ: Hydrogen and methane production in man. *Ann NY Acad Sci* 150:75-81, 1968
- Levitt MD, Bond JH Jr: Volume, composition and source of intestinal gas. *Gastroenterology* 59:921-929, 1970
- Calloway DH, Murphy EL: The use of expired air to measure intestinal gas formation. *Ann NY Acad Sci* 150:82-95, 1968
- Bond JH Jr, Engel RR, Levitt MD: Factors influencing pulmonary methane excretion in man. An indirect method of studying the in situ metabolism of the methane-producing colonic bacteria. *J Exp Med* 133:572-588, 1971
- Pitt P, DeBrujin KM, Beeching MF, et al.: Studies on breath methane: the effect of ethnic origins and lactulose. *Gut* 21:951-959, 1980
- Bjornekleit A, Jenssen E: Relationship between hydrogen (H₂) and methane (CH₄) production in man. *Scand J Gastroenterol* 17:985-992, 1982
- Stadtman TC: Methane fermentation. *Annu Rev Microbiol* 21:121-142, 1967
- Fiedorek SC, Pumphrey CL, Casteel HB: Breath methane production in children with constipation and encoparesis. *J Pediatr Gastroenterol* 10:473-477, 1990
- Weaver GA, Krause JA, Miller TL, Wollin MJ: Incidence of methanogenic bacteria in a sigmoidoscopy population: an association of methanogenic bacteria and diverticulosis. *Gut* 27:698-704, 1986
- Bertaccini G: Tissue 5-hydroxytryptamine and urinary 5-hydroxyindoleacetic acid after partial or total removal of the gastrointestinal tract in the rat. *J Physiol (Lond)* 153:239-249, 1960
- Espramer V, Testini A: Observations on the release and turnover rate of 5-hydroxytryptamine in the gastrointestinal tract. *J Pharm Pharmacol* 11:618-623, 1959
- Kim D-Y, Camilleri M. Serotonin: A mediator of the brain-gut connection. *Am J Gastroenterol* 95:2698-2709, 2000