# **Reassessment of the Role of Methane Production between Irritable Bowel Syndrome and Functional Constipation**

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#### Abstract

Background & Aims: Modifications of intestinal gas due to changes in microbiota may produce different symptoms. Our aim was to assess whether different patterns of hydrogen  $(H_2)$  and methane  $(CH_4)$  excretion were related to some intestinal disturbances. Methods. Six hundred and twentynine consecutive patients underwent a 50g-glucose breath test (GBT) on account of intestinal symptoms, which were evaluated by means of a questionnaire. "H2-producers" and "CH<sub>4</sub>-producers" were defined as with the presence of H<sub>2</sub> peak >12ppm more than the basal sample and mean CH<sub>4</sub> excretion of 2ppm, respectively. Forty healthy subjects were studied as controls. Results. A small intestinal bacterial overgrowth was found in 45 cases (7.2%) and was associated with older age (p=0.0122). Methane production occurred in 32.3% of the study population. Methane excretion was strictly related to chronic constipation (p<0.001). Median CH<sub>4</sub> excretion was higher in constipated patients compared with patients with normal daily stools (p=0.0406) and even more with patients complaining of diarrhea (p=0.0011). Different criteria for defining "methane-producers" provided similar results. Mean methane excretion of "methane producers" was 30.3ppm in functional constipation and 21.5ppm in constipation-irritable bowel syndrome (C-IBS) (p=0.0458). Conclusions. Methane excretion is clearly associated with alterations in intestinal motility, particularly favouring those with constipation. Mean methane excretion was higher in subjects suffering from functional constipation than C-IBS. Mean methane excretion  $\geq 2ppm$  appears to be an appropriate term to define "methane-producers".

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#### Key words

Methane – breath test – constipation – functional gastrointestinal disorders.

# Introduction

The prevalence of gastrointestinal manifestations is very high in the worldwide population in relation to the many causes that can determine them. Among these, functional gastrointestinal disorders (FGIDs) are predominant: a heterogeneous group of chronic diseases that are believed not to have structural or biochemical alterations that account for the symptoms [1]. However, this definition has started to become clearer because new information is being continuously discovered.

One of the emerging hypotheses suggests the relationship between dysmicrobism and intestinal dysfunctions, in which a low grade inflammation seems to be involved and central stimuli could exert important influence. Moreover, although difficult to quantify, intestinal gas seems to participate in the pathophysiology of these disorders. In fact, qualitative rather than quantitative modifications of the gas composition reflect changes in intestinal microbiota with the development of various gases which can produce different symptoms.

The glucose (GBT) or lactulose breath tests (LHBT) are simple tools to measure intestinal gases, but only hydrogen is commonly considered in clinical practice. Fasting breath hydrogen levels are higher in patients with IBS than in healthy controls, and a relevant increase of hydrogen excretion has been recognized as suggestive of a diagnosis of small intestinal bacterial overgrowth (SIBO) [2-6]. Moreover, many studies have proven that there is a considerable improvement of abdominal symptoms after antibiotic treatment [7-11]. Also, the diagnosis of lactose intolerance is based on hydrogen testing [12, 13]. Another gas, methane, is suspected of promoting constipation, but its influence on intestinal functions, as that of other gases such as hydrogen sulfide, is still controversial [14-18].

We have hypothesized that different gas compositions of the expired air are associated with different gastrointestinal symptoms. Thus, the purpose of this observational study was to further investigate the relationship between gastrointestinal complaints and gas excretion after GBTs in a large population, with particular attention to methane production. The second aim was to define the best cut-off level for increased GBT methane excretion, which better fits the symptom manifestations.

#### Methods

#### Subjects and protocol

We reviewed a large sample of consecutive patients prospectively collected and assessed in the Department of Internal Medicine, Gastroenterology Unit, University of Genoa, Italy from January 2008 to January 2011. The number of subjects referred to our Centre during the study period determined the sample size. A GBT was taken according to gastrointestinal symptoms, such as modification in stool frequency, abdominal pain, bloating and flatulence in absence of any organic digestive disease, which was excluded by Xray or lower endoscopy in the past five years. Also laboratory examinations, such as hemocrome, PCR, ionogram, renal and liver function and coagulation, were normal. Factors able to impair intestinal motility such as hypothyroidism, diabetes mellitus, antidepressant agents and major abdominal surgery were considered as exclusion criteria. All patients were asked to complete a validated questionnaire to register their symptoms and to define the stool output by using the Bristol Stool Scale. We also evaluated 40 healthy volunteers as the control group. The study was approved by our Ethics Committee.

#### Questionnaire

All patients completed an interview questionnaire, already used in previous studies performed by our group and other groups [9, 19-22]. It is based on 9 variables (diarrhea, constipation, abdominal pain/discomfort, bloating, flatulence, weight loss, nausea, vomiting, tenesmus) which are scored from 0 (no symptoms) to 3 (severe) and provides a global symptomatic score (GSS), calculated as the sum of all symptom scores, with a range from 0 to 30.

#### **Glucose breath test**

On the evening before GBT, patients were asked to follow a diet containing boiled rice, meat and water alone. Then, they fasted until the beginning of the test. Glucose was given at a dose of 50 g dissolved in 250 mL of water. Breath hydrogen concentration, in parts per million (ppm), was measured by gas-chromatography (Quintron MicroLyzer model DP plus, QuinTron Instrument Company, Milwaukee, WI, USA) on samples of end expiratory air collected every 15 min for 2 hours. A basal sample was taken before glucose intake. Patients were asked to avoid smoking, food intake and physical exercise during the test. Baseline  $H_2$  values lower than 10 ppm were considered acceptable to perform the test. A single peak of hydrogen excretion higher than 12 ppm was the cut-off value for SIBO positive test. A mean methane excretion of 2 ppm was used to define patients as "methane producers". Any baseline  $CH_4$  value was considered acceptable. Thus, according to these criteria, patients were subdivided into 4 categories: "H<sub>2</sub>nonproducer-CH<sub>4</sub>nonproducer" (H-M-), "H<sub>2</sub>nonproducer-CH<sub>4</sub>producer" (H-M+), "H<sub>2</sub>producer-CH<sub>4</sub>nonproducer" (H+M-), H<sub>2</sub>producer-CH<sub>4</sub>producer" (H+M+). Two validated criteria used in previous studies to define methane excretion were considered for comparison with the value we employed in our investigation: any detection of methane > 5 ppm [16], and baseline methane value  $\geq$  3 ppm [23].

#### Data analysis

Demographic and clinical characteristics of the study population were shown as mean or median values, ranges and rates. These data were compared to hydrogen and methane productions and were analyzed by means of Logistic Regression. In addition, the Mann-Whitney test was used to evaluate the relation existing between mean  $H_2$  and  $CH_4$ ppm produced by each patient and daily stools. Box and Whisker plots were used for graphic representation. A ROC curve was used to evaluate the sensitivity and specificity of methane and hydrogen ppm produced during GBT to diagnose constipation.

The statistical analysis was performed with GRAPHPAD Software (QuickCalcs, San Diego, CA, USA) and with GNU Software (PSPP, Boston, MA, USA).

#### Results

The study population consisted of 629 patients. The control group comprised 40 healthy volunteers. Table I shows the main demographic characteristics and the principal complaints of all subjects referred to our centre. Prevalent symptoms were related to excessive intestinal gas (bloating 78.8%, abdominal pain 61.0%, flatulence 44.2%).

 Table I. Main demographic and clinical characteristics of the study population.

	Study population	Healthy contols
Patients (n)	629	40
Gender (male/female)	164/465	9/31
Mean age (range) (years)	48 [16-88]	47 [18-72]
BMI (kg/m <sup>2</sup> )	23 [16-35]	23 [17-34]
Symptoms n (%):		
- diarrhoea (d)	178 (28.3%)	-
- constipation (c)	120 (19.1%)	-
- alternating d/c	87 (13.8%)	-
- abdominal pain	384 (61.0%)	-
- bloating	496 (78.8%)	-
- flatulence	278 (44.2%)	-
- nausea	152 (24.1%)	-
- vomit	32 (5.1%)	-
- tenesmus	79 (12.5%)	-

Mean hydrogen production in our population was 4.1 ppm (0.0-46.5) and the presence of a H<sub>2</sub> peak higher at least 12 ppm more than the basal sample was found in 45 cases (7.2%), identifying patients with SIBO. Subjects with SIBO had a mean age of 53 (95%CI: 47.7-59.1 years), while patients with negative GBT had a mean age of 47 (95%CI: 45.7-48.3 years). Thus older age was associated with the presence of bacterial overgrowth (p=0.012).

Methane production occurred in 32.3% of the study population and the mean value excreted was 21.1 ppm (ranges 1.0-84.8).  $CH_4$  values did not increase or barely increased with respect to baseline value after the oral glucose intake. There was not a remarkable difference between the criterion we used in this study and the cut-off values employed in the studies above (Table II). According to the  $H_2$  and  $CH_4$  profiles on GBT, the prevalence of the different patterns was "H-M-" 62.1%, "H-M+" 30.7%, "H+M-" 5.6%, "H+M+" 1.6%.

Table III shows the frequency of symptoms in each group. Diarrhea was more frequent among "H+M-" producers (31.4% of patients), while constipation was predominant among "H-M+" producers (27.4% of patients; P<0.001). Fig. 1 shows that the incidence of methane production on GBT increased in parallel with the reduction of daily bowel movements. Although incidence of SIBO slightly decreased from diarrhea to constipation, its value did not significantly differ among the three groups.

Healthy subjects presented a mean  $H_2$  excretion of 3.5 ppm (0.0 – 8) and methane production occurred in 30.0% of them and the median methane excretion was 6.1 ppm. Therefore, the rate of methane producers was similar between the control group and the study population, but methane excretion reached higher values in the latter group (p= 0.045).



**Fig 1.** Prevalence of "negative GBT", "methane production" and "SIBO" in the study population stratified according to bowel movements. (D: diarrhea; N: normal; C: constipation)

Analysis of demographic and clinical characteristics of the study population by means of Logistic Regression analysis revealed that only methane excretion was strictly related to constipation (p<0.001). Median methane excretion was higher in constipated patients compared with subjects with normal daily stools (p=0.04) and even more with respect to patients complaining of diarrhea (p:0.002), as illustrated in Fig. 2. Comparing these data with those obtained in the control group, we observed that methane excretion of healthy volunteers resulted in being similar to that of symptomatic subjects with normal intestinal motility. Besides, patients suffering from diarrhea and constipation had significantly lower or higher CH<sub>4</sub> values than healthy controls, respectively (p=0.015 and 0.045). Similarly, baseline  $CH_4$  values in constipated subjects were higher than those in patients with normal bowel movements (p=0.042).



**Fig 2.** Median hydrogen  $(H_2)$  and methane  $(CH_4)$  excretion related to daily stools.

Table II. Stratification of the study population according to different criteria defining H<sub>2</sub> and CH<sub>4</sub> excretion on GBT

	SIBO (	H+)	GBT negative for SIBO			
	Methane not producers (C-)	Methane producers (C+)	Methane not producers (C-)	Methane producers (C+)		
Median $CH_4 \ge 2 ppm$	34	7	392	196		
Any $CH_4$ value > 5 ppm	34	7	397	191		
$CH_4$ Baseline $\geq 3$ ppm	33	8	400	188		

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	(n)%	f/m	BMI	age	D	С	А	Abd	В	F	Ν	V	Т
H-M-	(391) 62.1	237/82	23	47; 16-88	(125) 31.9%	(60) 15.3%	(56) 14.3%	(250) 63.9%	(309) 79.0%	(178) 45.5%	(101) 25.8%	(26) 6.6%	(48) 12.3%
H-M+	(193) 30.7	142/51	22	47; 16-80	(40) 20.7%	(53) 27.4%	(24) 12.4%	(109) 56.4%	(148) 76.6%	(84) 43.5%	(40) 20.7%	(4) 2.1%	(28) 14.5%
H+M-	(35) 5.6	25/10	23	53; 20-81	(11) 31.4%	(6) 17.1%	(9) 25.7%	(20) 57.1%	(30) 85.7%	(13) 37.1%	(10) 28.6%	(2) 5.7%	(2) 5.7%
H+M+	(10) 1.6	8/2	23	49; 23-80	(2) 20.0%	(1) 10.0%	(2) 20.0%	(5) 50.0%	(9) 90.0%	(3) 30.0%	(1) 10.0%	(0) 0.0%	(1) 10.0%

Table III. Frequencies of symptoms related to the gas pattern excretion.

"H-/+"=absence/presence of small intestinal bacterial overgrowth; "M-/+"=absence/presence of methane excretion. f/m=female/male, D=diarrhea, C=constipation, A=alternating stool pattern, Abd= abdominal pain, B=bloating, F=flatulence, N=nausea, V=vomiting, T=tenesmus.

Patients suffering from constipation were further distinguished into subjects affected by functional constipation and subjects affected by constipation-variant IBS (C-IBS) the frequency of methane excretion of the two groups was 52.5% and 40.5%, respectively (n.s.), while mean methane excretion of "methane producers" subjects was 30.3 ppm in the functional constipation group and 21.5 ppm in patients affected by C-IBS with a statistically significant difference (p=0.046).

Finally, we evaluated which cut-off value of methane excretion better correlated with presence or absence of constipation. Therefore, three ROC curves were obtained for  $CH_4$  values ranging from 1 to 50 ppm and according to the following criteria: mean  $CH_4$  excretion over the 2-hour test;  $CH_4$  increment from the baseline value; peak value reached in the 2-hours test.

The relation between constipation and methane excretion on GBT was such that the highest sensitivity (56.6%) corresponded to a mean  $CH_4$  excretion of 1 ppm which had a specificity of 60.4%. the cut-off value of 2 ppm had a sensitivity and specificity of 54.0% and 71.0%, respectively. In relation to all criteria considered, progressively higher cut-off values were associated with a marked increase of specificity up to 98.4%, but this corresponded to a decrease of sensitivity up to 10.8%, as illustrated in Fig. 3. AUC of mean, peak and increment value were 0.618 (IC95: 0.575-0.659), 0.607 (IC95: 0.565-0.649) and 0.563 (IC95: 0.520-0.606), respectively. Although the criteria considered in this study did not significantly differ between each other, it is noteworthy that the mean methane excretion reached the highest values of sensitivity and specificity.

### Discussion

The intestinal gas is mainly produced by bacterial metabolism through breaking-down carbohydrates to obtain energy. Therefore, the composition of the luminal gas reflects, to a large extent, the distribution and composition of intestinal microbiota. It is similar to the air breathed into the stomach, and it is enriched with by-products such as  $H_2$ ,  $CH_4$ ,  $CO_2$  and  $H_2S$ , losing  $O_2$  throughout its way to the rectum [24, 25]. In contrast with the other gases,  $H_2$  and  $CH_4$  once they are generated by bacterial fermentation, pass rapidly into the systemic circulation and are eliminated only



**Fig 3.** Association between constipation and different criteria for analyzing methane excretion: mean excretion, peak of excretion and increment of excretion relative to the basal sample.

through the lungs. Thus, their concentrations in breathed out air samples are proportionally correlated with those present in the intestinal lumen [26, 27].

In this study, the rate of bacterial overgrowth assessed by GBT was 7.2% and the older age was significantly associated with SIBO. Thus, it might be supposed that the elderly are more likely to have predisposing conditions for bacterial overgrowth, such as diverticulosis, reduction in intestinal motility, achlorhydria and higher use of medication, which are able to modify gastrointestinal motor and secretory functions.

On the other hand, 32.3% of our population produced methane, thus confirming data from other studies [18, 27, 28]. This rate, however, does not necessarily reflect the prevalence of methanogenic microflora in the colon, as past studies have shown that this flora can be isolated by fecal incubation from some patients with negative methane excretion on GBT [29, 30]. In particular, in humans CH<sub>4</sub> is mostly produced by the *Methanobrevibacter smithii* strain as a result of the conversion of 4 mol H<sub>2</sub> and 1 mol CO<sub>2</sub> to 1 mol CH<sub>4</sub>, competing for H<sub>2</sub> with sulfate reducing bacteria. This process occurs mainly in the left colon [31, 32] and is an important reason for measuring both gases by GBT. In fact, considering H<sub>2</sub> excretion only, there is some loss

of information: for example, in cases of negative GBT, it would not be possible to distinguish normal subjects from patients with slow hydrogen excretion due to the absence of hydrogen-producing bacteria or because of increased  $H_2$  consumption by metabolic processes in the bowel lumen. Therefore, the combined analysis helps physicians in the interpretation of gas patterns excreted in relation to patients' clinical complaints.

Our analysis, in fact, demonstrated that methane production was strictly associated with constipation. Moreover, after subdividing the study population according to daily stool frequency, we found that mean  $CH_4$  excretion seemed to increase in parallel with the reduction of bowel movements. Similar findings were observed by other authors in patients with C-IBS by using LHBT [15], but many doubts remain as to whether methane is able to produce constipation or rather is a consequence of intestinal hypomotility.

Experiments in animal studies [33] suggested an active role for methane in affecting intestinal motility, while other human investigations have shown that slow transit may facilitate growth of methanogenic bacteria [34, 35]. It cannot be excluded, however, that methanogenic organisms lead to constipation indirectly through the modification of the luminal environment, by producing active substrates or by competing with other bacterial species [36-38].

In our study,  $H_2$  and  $CH_4$  excretion were also measured in healthy volunteers. Their concentrations in the air samples were as low as expected. In particular, median methane excretion resulted in being similar to that of the subgroup of symptomatic patients with regular bowel movements, while it differed significantly from those with diarrhea and constipation. Therefore, this seems to verify that methane production has a role in these symptoms due to modified intestinal motility without any effect on other intestinal symptoms.

In this study, the value used to define methane producers (> 2 ppm) did not show a significant difference from other published criteria, that is "any value > 5 ppm" [16] and "baseline  $\geq$  3 ppm" [23]. In fact, the cut-off value, set at values higher than the mean excretion of 2 ppm identified only 3 and 5 more methane-producer subjects than the criteria above-mentioned. However, we considered the mean CH<sub>4</sub> excretion as the most valid parameter, since it is less influenced by a potential sampling error and seems to be more representative of the whole excretion flow.

As to the correlation between methane producers and constipation, a comparison of various ROC curves was carried out by considering  $CH_4$  excretion from different aspects: mean excretion, peak of excretion and increment of excretion relative to the basal sample. Although AUC values were not significantly different from the 0.5 curve, the "mean excretion" and "peak" value showed very similar levels and they resulted higher than the curve representing the  $CH_4$  increment from the baseline value following the oral glucose intake. This could be explained on the basis that  $CH_4$  concentration remains constant during the test and is not commonly affected by oral glucose intake. Anyway, all

criteria employed showed that the test specificity increases in parallel with CH<sub>4</sub> levels, while progressively higher values induce a linear fall of sensitivity. In our study a mean CH<sub>4</sub> excretion of 2 ppm provides a reasonable compromise between the best sensitivity and specificity (54.0% and 71.0%). Therefore, this cut-off can be considered as a valid parameter to define methane producers, since higher values determine a marked lost of sensitivity. However, these findings are not sufficient to consider CH<sub>4</sub> excretion on GBT as a good diagnostic test. In fact, there is no doubt that these results must be interpreted with caution in relation to the diagnostic accuracy provided by hydrogen breath tests. As reported in the Rome I Consensus Conference, the global diagnostic accuracy of hydrogen GBT and LHBT compared to jejunal aspirate culture for diagnosing SIBO was 71.7% and 55.1%, respectively, with sensitivity and specificity of 62.5% and 81.8% for GBT, and 52.4% and 85.7% for LHBT [39]. Furthermore, our intention was to determine a reasonable value of methane excretion to define a subject as a "methane producer" that better fits with symptom manifestations and not to develop a new test.

In agreement with a recent study performed with LHBT [40], no other symptoms showed a significant correlation with methane in our investigation. However, it should be considered that complaints different from diarrhea and constipation are strongly dependent on the patient's perception and are difficult to evaluate. Daily stool frequency, instead, is an objective variable that can be quantified by both patients and physicians. Moreover, the use of the Bristol Stool Scale and Rome II Criteria for defining daily bowel movements proved to be closely related to the oral-cecum transit time (OCTT) obtained by means of radiological tools such as abdominal X-ray, MRI and 99mTC scintigraphy [23, 41-42]. Thus, an accurate medical visit is useful for assigning patients to different groups according to stool frequency.

To the best of our knowledge, only one study regarding the association between methane production and constipation has been performed using GBT [23]. In this study, Attaluri and colleagues related methanogenic flora to chronic constipation and reported a quantitative correlation between the degree of methane production and colonic transit assessed by means of radio-opaque markers. Our analysis, based on clinical manifestations, confirmed these findings in a wider population, but without radiological assessment of intestinal transit.

Although LHBT may be more suitable for this aim as lactulose reaches the colon, we chose GBT for many reasons. First of all, it does not influence intestinal motility as LHBT can do. It has a higher diagnostic accuracy for SIBO and is not usually affected by hydrogen colonic production as it is rapidly absorbed in the proximal small bowel. For the same reason, hydrogen produced in the small bowel is not used by methanogenic organisms usually harbored in the left colon. Furthermore, a recent study [42] demonstrated that LHBT is more valuable at indicating OCTT than SIBO in patients with IBS, thus confirming the results of previous studies

It must also be stressed that the lines dividing FGIDs are often arbitrary, especially the pathophysiological distinction between C-IBS) and functional constipation is still unclear [1]. Nevertheless, we tried to distinguish subjects suffering from functional constipation from constipation-variant IBS, according to the Rome III criteria and we observed that the rate of methane producers was slightly higher in functional constipation and they had significantly greater mean methane excretion than patients suffering from C-IBS. Thus, it appears that methanogenic flora might have a major role in functional constipation rather than IBS, although there was no significant difference in symptom severity between these two groups. Nevertheless, the earlier onset and the more constant behaviour of functional constipation, which is at variance with the greater fluctuation of IBS clinical presentation, may account for different CH<sub>4</sub> levels in expired air.

These findings seem to be very interesting, but further studies are necessary to confirm them. Certainly, additional investigations such as stool cultures, validated methodology for accurate measurement of transit time and reassessment of patients' overtime after adequate treatment might be of help to clarify the role of methane in these disorders. In the future, testing constipated subjects for methane excretion could be helpful to tailor an appropriate treatment for these patients. However, it is not clear if methane-producer patients suffering from constipation may have different causes than those constipated who are not  $CH_4$  producers.

# Conclusion

Our data confirm that methane excretion assessed with GBT is strongly associated with constipation. A mean  $CH_4$  excretion of 2 ppm seems to be an appropriate cut-off value to define patients as "methane producers". Mean methane excretion seems to be the best index which is inversely related to the daily intestinal movements. Therefore, there is a qualitative as well as quantitative correlation between methane and intestinal motility. Moreover, mean methane excretion resulted in being higher in subjects suffering from functional constipation than in those with C-IBS.

# **Conflicts of interest**

All authors declare that they have no conflicts of interest and that the work is original.

# Author's contribution

Manuele Furnari was responsible for planning the study, analyzing data and drafting the manuscript. Edoardo Savarino participated in the statistical analysis and manuscript preparation. Luca Bruzzone, and Alessandro Moscatelli participated in the patient management and data collection. Lorenzo Gemignani and Elisa Giambruno were responsible for subject enrollment. Pietro Dulbecco and Vincenzo Savarino participated in the writing of the manuscript. All authors have seen and approved the final version of the manuscript. Guarantor of the article is Vincenzo Savarino.

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