Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status

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Abstract

In order to assess the relationship between methane (CH_4) producing status and the breath excretion of hydrogen (H_2) in healthy subjects, breath CH_4 and H_2 were simultaneously measured for 14 hours after oral ingestion of 10 g lactulose in 65 young volunteers. Forty were breath CH_4 producers and 25 were not. Statistically significant differences were observed between both groups, with lower values for CH_4 producers recorded for the following parameters: fasting basal value of breath H_2 (8·1 (4·9) v 5·2 (3·7) ppm, $p<0·05$), mouth-to-caecum transit time (68 (24) v 111 (52) min, $p<0·005$), and breath H_2 production measured as area under the curve 13·1 (6·9) v 8·8 (3·8) 10^3 ppm/min, $p<0·02$). There was no significant correlation between individual production of breath H_2 and CH_4. These results indicate that the response to lactulose depends on breath CH_4 producing status. In clinical practice, defining normal values of mouth-to-caecum transit time without knowledge of breath CH_4 producing status may lead to misinterpretation of the H_2 breath test.

The hydrogen (H_2) and methane (CH_4) produced in the human body derive entirely from colonic anaerobic bacterial fermentation. While most of these gases are consumed on site or excreted in flatus, the part expelled by the lungs can be easily collected and measured by end-expiratory sampling.[1,2] H_2 production increases when a fermentable carbohydrate is incompletely absorbed in the small intestine, forming the basis for the use of the H_2 breath test. This non-invasive procedure has been extensively used in clinical practice[3-11] and pharmacological studies[12] to measure mouth-to-caecum transit time. It has also been proposed as a semi quantitative method for evaluating intestinal malabsorption of carbohydrates.[13,14] The recent development of a simple gas analyser not only offers the opportunity to measure breath H_2 but breath CH_4 as well. Although the substrates for CH_4 production are not yet fully identified,[2,15] it has been shown that in Caucasian adults, only 30%–50% are breath CH_4 producers, whereas 90%–98% excrete breath H_2.[16-22] In most previously published investigations, however, little attention has been paid to the relationship between breath CH_4 producing status and the H_2 excretion profile after lactulose administration. In a previous study evaluating starch malabsorption of pasta,[23] we observed different patterns of H_2 production according to breath CH_4 producing status, a finding which has also been described in preliminary studies by other authors.[16,20,24] This prompted us to prospectively

assess the relationship between breath CH_4 producing status and the breath excretion of H_2 in healthy subjects.

Methods

SUBJECTS

Sixty five subjects (32 men, 33 women) ranging from 19 to 30 years (mean 22·4 (2)) were selected from a population of healthy volunteers without known disease and free from gastrointestinal symptoms as previously tested in our laboratory. In addition, their breath CH_4 producing status was already known. Forty were breath CH_4 producers as defined below. Their breath samples were compared with those of 25 healthy controls who were breath CH_4 non-producers. All volunteers were French born Caucasians of similar ethnic origin. As enemas, laxatives, and antibiotics can affect the colonic microflora and, hence, the production of intestinal gas,[25] any individual receiving these treatments within three months before the study was excluded. All subjects gave their informed consent to the study protocol which had been approved by the Ethics Committee of our hospital.

STUDY DESIGN AND BREATH ANALYSIS

The effect of a single orally administered of 10 g lactulose (Duphalac®, Duphar Laboratories, Villeurbanne, France) dissolved in 100 ml cold water was studied for 14 hours in the 65 selected subjects for breath H_2 and CH_4 production. The evening meal preceding the morning lactulose test was standardised to contain a low level of indigestible material[26,27] and consisted of fish, white rice, and a soft cheese; water was freely permitted. After a 12 hour fasting period, the lactulose test was performed at 09 00 hours after suitable oral hygiene (careful mouthwashing with a 1% chlorhexidine solution; Givalex®, Norgan Laboratories, Paris, France) to reduce oropharyngeal microfloral activity.[28-30]

Breath samples were collected at 15 minute intervals for 30 minutes before lactulose ingestion, every 10 minutes for the first two hours, and then every 15 minutes for the next 12 hours thereafter. During the test, subjects were forbidden to eat, smoke, or take exercise.[28] Alveolar air samples were obtained after a normal inspiration, by having the subjects exhale through a mouthpiece into two bags connected by a three-way valve. When the first 500 ml expiratory air filled one plastic bag, the end alveolar air was then collected in a second bag (1·1 rubber anaesthesia bag adapted with a one way valve). The end alveolar air was then immediately transferred into 50 ml plastic

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TABLE CH_4 producing status and results of H_2 breath test in healthy subjects

	CH_4 non-producers (n=25)	CH_4 producers (n=40)	Total (n=65)
Age (yrs)	22·7 (0·9)	22·3 (1·7)	22·4 (1·7)
Sex (M/F)	15/10	17/23	32/33
Fasting basal H_2 concentration (ppm)	8·1 (4·9)*	5·2 (3·7)	6·3 (4·4)
H_2 non-producers (n)	0	4	4
Mouth-to-caecum transit time (min)	68 (24)†	111 (52)	94 (47)
Delta Max H_2 (ppm)	60 (41)	46 (23)	52 (32)
Time of H_2 peak (min)	180 (80)	209 (98)	197 (92)
H_2 production (10^3 ppm/min)	13·1 (6·9)‡	8·8 (3·8)	10·6 (5·7)
Time for H_2 return to basal concentration (min)	564 (178)	573 (137)	569 (154)
Duration of H_2 production (min)	495 (177)	462 (120)	476 (146)
H_2 concentration at 840 min (ppm)	4·9 (3·2)	3·0 (2·1)	3·8 (2·8)

Results are expressed as mean (SD). See Methods for definition of CH_4 producing status.
*p<0·05 v CH_4 producers; †p<0·005 v CH_4 producers; ‡p<0·02 v CH_4 producers.

syringes fitted with two way stopcocks, and analysed within a two hour period.

The H_2 and CH_4 concentration in breath samples were determined simultaneously with a Micro Lyzer DP gas chromatograph (Quintron Instrument Company, Milwaukee, WI) using a molecular sieve column (12′ Hysep Q, 60/80 mesh). Dry air was used as the carrier gas at a flow rate of 40 ml/minute. The chromatograph was calibrated with a H_2 and CH_4 reference mixture in compressed air (Quingas® 2). Results were expressed as parts per million (1 ppm= approximately 0·05 μmol/l for H_2 and CH_4). For both gases, the smallest detectable concentration was 2 ppm with a linear accuracy response range of 2–150 ppm.

Breath CH_4 concentrations were taken as the difference between the result of a breath sample and the room air concentration; breath CH_4 producing status was defined as a mean CH_4 breath sample concentration greater than 2 ppm above that in ambient air. This criterion was based on the sensitivity and reproducibility of the method used.

The fasting breath H_2 (FBH$_2$) and CH_4 (FBCH$_4$) concentrations used were the mean of the three samples before lactulose ingestion. Breath H_2 producing status was defined as the ability of a subject to produce an increase in breath H_2 of greater than 20 ppm above baseline values (ΔH_2) at one or more breath collections after lactulose ingestion.[3][31] An early H_2 peak was

defined as an increase of H_2 greater than 5 ppm above FBH$_2$ before the first 30 minutes.[30] Mouth-to-caecum transit time was defined as the time from the beginning of lactulose intake until the period just before the initial increase above fasting levels of 10 ppm or more H_2 where this increase was sustained.[+10] As the phase of the menstrual cycle (luteal v progestational phase) has been shown to modify the duration of the mouth-to-caecum transit time,[11] this variable was studied in each woman.

Breath CH_4 production was estimated as the area under the curve (AUC) between 0 and 840 minutes; in breath H_2 and CH_4 producers, the AUC for CH_4 were determined before the lactulose had reached the caecum at time (ti) (corresponding to the mouth-to-caecum transit time) and for the same time thereafter.

STATISTICAL ANALYSIS

Results were expressed as means (SD) with the exception of the figures (means (SEM)). Paired and unpaired variables were analysed with the Student's t test. Linear correlations between individual H_2 and CH_4 results were calculated using the least square method. Differences were considered to be significant at the p<0·05 level.

Results

H_2 BREATH TEST

Demographic characteristics of the subjects and results for the H_2 breath test are summarised in the Table. Four subjects (6·1%) failed to produce significant amounts of breath H_2 after 10 g lactulose administration (ΔH_2<20 ppm). They were all breath CH_4 producers excreting large amounts of CH_4 (mean FBCH$_4$ 25·2 (12) ppm). Hence, breath H_2 production was analysed in only 61 subjects (25 CH_4 non-producers, 36 producers).

Mean breath H_2 concentrations in the control group and breath CH_4 producers are shown in Figure 1. No early H_2 peak was observed in the 61 subjects; all values returned to basal concentrations during the 14 hour test period.

Mouth-to-caecum transit times are shown in Figure 2. Although there was considerable overlap between the individual values measured in the two groups, the mean mouth-to-caecum transit time was significantly longer (p<0·005) in breath CH_4 producers. In this latter group, 17 subjects were above the highest value observed in the controls. No statistically significant difference was observed according to sex or phase of the menstrual cycle. H_2 production (AUC) was significantly lower in breath CH_4 producers (p<0·05) than in the control group (Table).
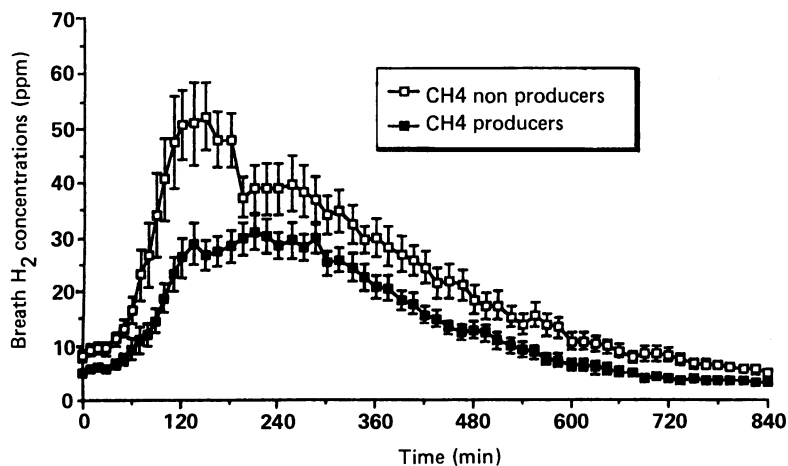

Figure 1: Breath H_2 concentrations (mean (SEM)) in CH_4 non-producers (n=25) and producers (n=36; 4 CH_4 producers not represented after failing to produce H_2) after oral administration of 10 g lactulose.

CH_4 PRODUCTION

In the CH_4 producers, the mean FBCH$_4$ concentration was 14·3 (8·3) ppm. The evolution of breath CH_4 concentration observed in the two groups is shown in Figure 3.

Among the 36 breath H_2 and CH_4 producers, 30 subjects (83%) showed an increase in CH_4 production after lactulose had reached the
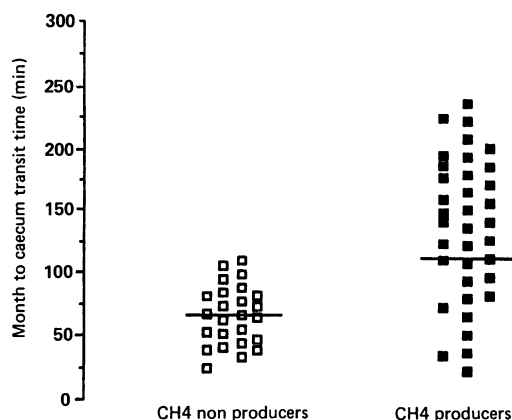
Figure 2: Individual values of mouth-to-caecum transit time in CH_4 non-producers (n=25) and producers (n=36; 4 CH_4 producers not represented after failing to produce H_2). Horizontal lines indicate mean of each group. The intergroup difference was statistically significant (p<0·005).

caecum at the time ti. In these 30 subjects, the CH_4 AUC was 0·4 (0·5) 10^3 ppm/min from lactulose administration (ti—mouth-to-caecum transit time) to ti and increased to 1·1 (0·9) 10^3 ppm/min from ti to ti+mouth-to-caecum transit time (p<0·0005).

In 11 breath CH_4 producers (27·5%) a transient disappearance in CH_4 production was observed, generally at the end of the test (mean 567 (180) min). This decline was generally associated with a low level of H_2 production.

Breath CH_4 concentrations were always below 3 ppm during the first 10 hours in control subjects; a delayed increase (Fig 3) occurred in 13 subjects (52%), with no return to basal values during the experimental period.

CORRELATION BETWEEN BREATH H_2 AND CH_4 PRODUCTION

No significant correlation was found between the H_2 AUC and either the whole CH_4 AUC (n=40) or the CH_4 AUC after lactulose had reached the caecum (n=36). Individual patterns of excretion curves for breath H_2 and CH_4 tended to be either that of a high H_2 and low CH_4 or that of a high CH_4 and low H_2. No subject was both a high H_2 and high CH_4 producer.

Discussion

While the definition of CH_4 producing status dates back to the introduction of the breath test method, the criteria used to define a subject as a CH_4 producer has varied considerably with time. Initially, Bond *et al*[2] arbitrarily proposed that only those subjects with breath CH_4 concentrations greater than 1 ppm above atmospheric CH_4 be designated as CH_4 producers. A single breath sampling, however, may fail to detect an average of 18% of the breath CH_4 producers in a given population.[17] More recently, McKay *et al*[19] have shown that all healthy subjects may produce CH_4, though production of the gas appears in breath only after reaching a certain threshold. These investigators defined a CH_4 producer as a subject emitting at least 2 ppm above room air concentration, based on the sensitivity and reproducibility of the method used. In our study, the smallest detectable CH_4 concentration was 2 ppm; thus, CH_4 producers were defined as subjects producing mean CH_4 concentrations after four breath samples of greater than 2 ppm above those in ambient air.

FBH_2 in our subjects was relatively low in comparison with levels found in a previous study.[30] These results are, however, in agreement with data obtained by other investigators who showed that an evening meal containing a low level of indigestible carbohydrates before breath testing led to reduced FBH_2 concentrations.[26 27] Like Bjorneklett and Jenssen,[16] our breath CH_4 producers showed lower FBH_2 values than breath CH_4 non-producers. Previous studies[32 33] have indicated that FBH_2 measurements may be useful for the diagnosis of bacterial overgrowth and coeliac disease, but the influence of CH_4 producing status was not examined.

Reports of the incidence of non-H_2 producers range from 0% to 27%[16 20 23] depending on the criteria used to define the absence of breath H_2 production. Failure to produce breath H_2 after the 10 g lactulose ingestion was infrequent in our population sample (6·1%). Here again, the definition of a breath H_2 producer could explain these variations. For instance, in one recent study[31] where ability to produce H_2 was defined as an increase in breath H_2 to greater than 20 ppm within four hours after ingestion of 10 g lactulose, 21% of subjects were found to be non-producers. Upon using similar criteria, 15% of our CH_4 producers would be non-H_2 producers. This indicates that the lactulose breath test must be extended by at least six hours (Fig 2) as H_2 increases of about 20 ppm can occur with a delay, especially in CH_4 producers. As in a previous study,[16] we found that our four H_2 non-producers all excreted large amounts of CH_4.

After ingestion of a meal, a so called early rise in breath H_2 concentrations has been described[34] with a subsequent return to basal level; this early peak thus precedes the actual peak caused by carbohydrate malabsorption. Various hypotheses have been proposed to explain this early increase. H_2 production may be enhanced by the passage into the caecum of either ileal secretions or carbohydrates retained in the terminal ileum from a previous meal.[34] Buccal fermentation, however, may well be a major determinant, with proper oral hygiene able to
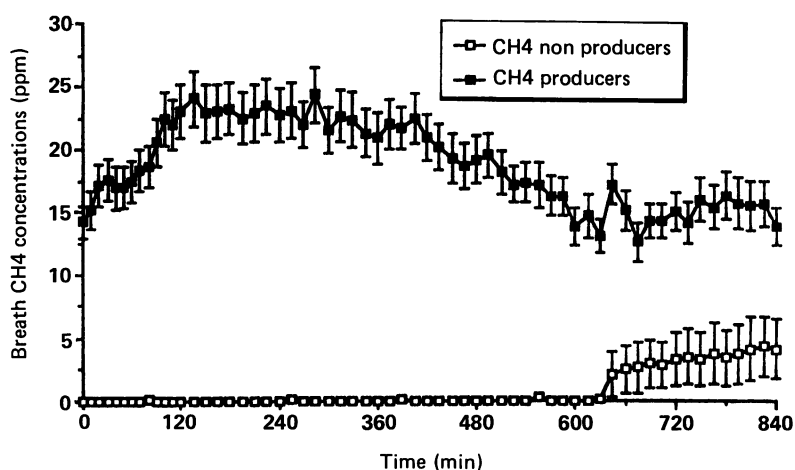

Figure 3: Breath CH_4 concentrations (mean (SEM)) in CH_4 non-producers (n=25) and producers (n=40) after oral administration of 10 g lactulose.

eliminate this phenomenon.[28-30] In our study, the evening meal contained little indigestible material and attention was paid to meticulous oral hygiene. These two precautions were sufficient to eliminate the 'early peak'.

The H_2 breath test is a simple, non-invasive method for measurement of mouth-to-caecum transit time, reflecting the arrival of the 'head' of the meal in the caecum. Although defined as the interval between meal ingestion and the detection of a significant and sustained rise in breath H_2 excretion,[4 11] there is no uniformly accepted recommendation concerning either the threshold increase in H_2 concentration or the dose of the lactulose load. Values of 10 ppm and 10 g, respectively, have been proposed by most investigators.[3 6 7 11] In one study, CH_4 production was associated with slow colonic transit,[35] but any apparent difference in mouth-to-caecum transit times between CH_4 producers and non-producers has previously not been reported. We are unable to explain this difference which may be related to lower H_2 production and/or different patterns in gut motility. In order to assess the impact of sex on the study parameters, the same percentage of women in luteal and progestational phases was studied in each group. No significant difference in mouth-to-caecum transit times was noted between these phases.

Previous studies[1 13 14] suggest that malabsorption of carbohydrates can be quantified with reference to lactulose. In agreement with Bjorneklett and Jenssen[16] using 33 g lactulose, our results showed a significant difference in breath H_2 production between CH_4 producers and non-producers after 10 g lactulose, but there was no correlation between breath H_2 and CH_4 production.

Breath CH_4 production has rarely been examined over an extended time period.[2 16] In this 14 hour trial, we observed that 83% of CH_4 producers were able to excrete additional CH_4 after ingestion of 10 g lactulose. This increase in CH_4 has been observed previously with larger doses of lactulose.[16 17 20]

The finding that breath CH_4 may almost disappear rapidly in breath CH_4 producers has been previously observed by Fritz and Siebert, though without explanation.[24] Our observation of late CH_4 production in breath CH_4 non-producers has never been reported and might reflect modifications in colonic flora after 24 hour fasting.

Irrespective of the reasons for the observed differences between CH_4 producers and non-producers, these results appear clinically relevant. Indeed, ignoring these facts could lead to misinterpretation of mouth-to-caecum transit time in clinical practice or in the pharmacological assessment of prokinetic drugs. In the future, it is possible that determination of different thresholds of H_2 concentrations for the calculation of mouth-to-caecum transit time may help solve the apparent discrepancies between CH_4 producers and non-producers. For instance, in this study, the arrival of lactulose in the caecum was defined as a rise in the H_2 concentration of at least 10 ppm. Hence, at 70 minutes (mouth-to-caecum transit time in controls), the mean H_2 concentration was 18 ppm

($FBH_2 = 8$ ppm + 10 ppm). The corresponding increase in H_2 for CH_4 producers was 5 ppm (10 ppm − $FBH_2 = 5$ ppm) (Fig 1). Therefore, it is possible that thresholds for the detection of lactulose in the caecum may have to be revised in accordance with CH_4 producing status. This approach would in fact require the use of a different method for the measurement of mouth-to-caecum transit time not based on H_2 production by colonic flora. Until new thresholds of H_2 concentrations are validated, we suggest limiting the use of the H_2 breath test to intra-individual comparisons (crossover design) after stratification of CH_4 producers and non-producers. The same restrictions apply to quantification of carbohydrate malabsorption in physiopathological studies.

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