Comparative study of hydrogen and methane production in the human colon using caecal and faecal homogenates

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Abstract
Rates of hydrogen and methane production were compared in caecal and faecal homogenates in six male producers. Faecal homogenates produced hydrogen and methane in the absence of and after the addition of lactulose, whereas caecal homogenates produced hydrogen but little methane.

Hydrogen (H\textsubscript{2}) and methane (CH\textsubscript{4}) are produced during anaerobic bacterial activity in the human large intestine. Methane is absorbed in portal blood and excreted in breath, and pulmonary CH\textsubscript{4} excretion can be used as a simple indicator of CH\textsubscript{4} production in the colon.\textsuperscript{1} In general, the population can be divided into two groups on the basis of CH\textsubscript{4} excretion -- producers and non-producers of CH\textsubscript{4}.\textsuperscript{1}

In contrast to H\textsubscript{2} production, which rapidly fluctuates depending upon the availability of fermentable substrates, CH\textsubscript{4} excretion remains relatively constant throughout the day and is not influenced by meals.\textsuperscript{2,3} Similarly, ingestion of small doses (10 g) of lactulose, a non-absorbable disaccharide, does not increase breath CH\textsubscript{4} excretion.\textsuperscript{4} Larger amounts (20-33 g) of lactulose, however, can cause an appreciable increase in breath CH\textsubscript{4} excretion in CH\textsubscript{4} producers\textsuperscript{4} -- subjects harbouring a sufficient count of methanogenic bacteria, mainly Methanobrevibacter smithii, which uses H\textsubscript{2} to reduce CO\textsubscript{2} to CH\textsubscript{4}.\textsuperscript{7} Methanogenic bacteria are strict anaerobes whose growth is favoured by a high pH and low dilution rates.\textsuperscript{8} These environmental conditions probably exist in the distal but not in the proximal part of the human large intestine.\textsuperscript{9-11} Moreover, during experiments aimed at determining the site of colonic CH\textsubscript{4} production, Bond et al\textsuperscript{1} incidentally found that in one subject CH\textsubscript{4} production induced by in situ lactose infusion was occurring mainly distal to the colonic splenic flexure.

It was stated by Grimble in a recent issue of Gut\textsuperscript{12} that these data suggest that H\textsubscript{2} and CH\textsubscript{4} production may be separated by anatomy in the human large intestine and this prompted us to compare the rates of H\textsubscript{2} and CH\textsubscript{4} production from lactulose in human caecal and faecal samples.

Methods

**Subjects**
Studies were carried out on six healthy male volunteers (aged 22-45 years) who were CH\textsubscript{4} producers (defined as producing at least one part per million (ppm=\mu l/l) of CH\textsubscript{4} above room air concentration).\textsuperscript{1}

**Caecal and faecal sampling**
Caecal content was sampled in fasting subjects after intestinal intubation as described previously.\textsuperscript{13} Subjects were intubated with a double lumen tube led by a mercury bag that could be inflated with air to accelerate progression of the tube. When the bag reached the caecum, which was confirmed fluoroscopically, progression of the tube was stopped by deflating the bag. One lumen sealed with a three way stopcock was used to sample caecal content in a 50 ml sterile syringe after flushing the tube with 10 ml ultra-high purity nitrogen.

On the day elected for tube placement, stools were collected from each subject in a plastic bag containing an Anaerocult A (Merck, Darmstadt, Germany).

**In vitro incubation and analysis**
Caecal and faecal samples were processed immediately. Some 10 g were homogenised in 60 ml of 0.1 M anaerobic phosphate buffered saline (pH 7). Two 4 ml aliquots of caecal and faecal samples were then transferred to 50 ml sterile syringes. To one syringe each of treated caecal and faecal samples one ml of 1.25 g/100 ml solution of pure lactulose (Serva, Heidelberg, Germany) in 154 mM NaCl was added (experimental incubates), and the pH was measured with a pH meter (Radiometer, Copenhagen, Denmark). Then one ml of 154 mM NaCl was added to the other two syringes (control incubates). The contents of the syringes were mixed by shaking, flushed with 45 ml ultra-high purity nitrogen, and incubated at 37°C in a shaking water bath. Delays between all procedures were made as short as possible, and the total duration of handling time was about 15 minutes. After one hour of incubation the gas was recovered from both experimental and control incubates by displacement into another syringe that was equilibrated to room temperature before measurement of H\textsubscript{2} and CH\textsubscript{4}. The syringes were immersed in water at 100°C for five minutes, the final pH was determined, and the amounts of lactulose as fructose were measured by an enzymatic method (Boehringer, Mannheim, Germany). The H\textsubscript{2} and CH\textsubscript{4} concentrations were multiplied by the volume of gas in the syringes and expressed as \mu l. In the caecal and faecal incubates with lactulose, the volumes of H\textsubscript{2} and CH\textsubscript{4} were recorded as the changes in \mu l minus control values. The quantity of lactulose...
that disappeared after one hour of incubation was calculated by subtracting the postincubation value from the added amount of lactulose. Final results were expressed as μl H₂ and CH₄ per mg lactulose consumed.

Statistical analysis for significance was performed using Wilcoxon's signed rank test. Data are expressed as mean (SEM).

Results
In control incubates, the H₂ released by faecal homogenates tended to be lower than in caecal homogenates (0.717 (0.446) vs 3.560 (1.706) μl). Methane production occurred almost exclusively in faecal incubates at a rate significantly higher than in caecal homogenates (5.658 (1.858) vs 0.052 (0.008) μl, p<0.05). The initial pH (6.6 ± 7.2) and the final pH (6.7 ± 7.0) did not vary significantly in caecal and faecal control incubates.

The percentage of lactulose consumed during the one hour incubation period was not significantly different in caecal and faecal homogenates (80.5 (5.2) vs 97.5 (1.5) %). The H₂ liberated by faecal homogenates from lactulose tended to be higher than in caecal homogenates. Methane production occurred almost exclusively in faecal incubates at a rate significantly higher than in caecal homogenates (p<0.05) (Table). The initial pH (6.6 ± 7.2) and the final pH (5.9 ± 6.0) were not significantly different in caecal and faecal incubates.

Discussion
Although lactulose was extensively degraded in both caecal and faecal homogenates CH₄ production occurred only in faecal homogenates. The same result was obtained in control incubates. The extent to which the final pH of the incubates with lactulose changed did not explain the differences in CH₄ production between caecal and faecal homogenates. This finding seems to contrast with that of Pernan and Modler, who concluded that CH₄ production is sharply pH-dependent. In their incubation experiments, in which CH₄ formation was inhibited, however, the pH of faecal incubates declined to 6.0, whereas it averaged 6.0 in our experimental conditions. In any case, the important point is that the pH of caecal incubates was not significantly lower than that of the faecal ones.

We did not measure other fermentation products, such as organic acids and total viable anaerobic and methanogenic bacterial concentrations in the faecal and caecal samples. Nevertheless, our results confirm data from an in vitro model mimicking the human colon and provide further evidence that methanogenesis and probably the growth of methanogenic bacteria are taking place distal to the right part of the large intestine where suitable environmental conditions would be present. This could explain why large oral loads of lactulose able to induce diarrhoea with the presence of sugars in stools increase CH₄ excretion in CH₄ producers when a part of this sugar reaches the distal colon, whereas small doses of lactulose, which are completely degraded in the right colon, do not support CH₄ production. A few days later, it is not surprising that these substrates do not increase CH₄ excretion a short time after their ingestion, and this probably explains why breath CH₄ excretion is not influenced by meals, unlike H₂ which is produced when fermentable substrates from meals are delivered to the right colon.

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