Use of Breath Hydrogen and Methane as Markers of Colonic Fermentation in Epidemiologic Studies: Circadian Patterns of Excretion

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Introduction

Colonic fermentation, the process by which anaerobic bacteria break down dietary substrates in the colon to obtain energy for growth, may play a protective role in colorectal carcinogenesis (1). Possible mechanisms include a bulking effect, lowering of the colonic pH, and production of butyrate, which has been shown to act as a differentiating agent and tumor growth inhibitor (1). In order to test this hypothesis in epidemiologic studies, biomarkers of colonic fermentation must be developed.

Breath excretion of hydrogen has been shown to correlate strongly ($r = 0.9$) with hydrogen concentration in the colonic lumen (2). Consequently, breath hydrogen has been widely used by clinicians to test for carbohydrate malabsorption. Because of its validity, specificity, sensitivity, noninvasiveness, and low cost, breath hydrogen would constitute a good marker of fermentation for epidemiologic studies. Although data for subjects on regular diets are scant, the evidence to date suggests an important day-to-day variation and a circadian pattern related to meals for breath excretion of hydrogen (3,4).

Breath methane is also specific for colonic fermentation and is proportional to the intestinal production (5). Methane production appears to be dependent on the presence in the colon of a sufficiently large population of methanogenic bacteria (6); thus, it is usually detected in the breath of only a subset of the population [33–48% in western populations (6)]. Levels of methane excreted in the breath appear to be relatively constant and do not fluctuate with meals (3).

In this study, we explored the circadian excretory patterns of breath hydrogen and methane in order to develop a protocol for using these gases as markers of colonic fermentation in epidemiologic studies.

Methods

Twenty subjects (10 males, 10 females) of various ethnic backgrounds were studied. None had a history of gastrointestinal disease or recent use of antibiotics. They were instructed to collect end-expiratory samples at hourly intervals during waking hours for a minimum of two consecutive days. The mean number of measurements

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per person-day of observation was 14.5 (minimum = 10, maximum = 18). Breath samples were collected with gas samplers (Quintron Instrument Co., Milwaukee, WI). Experiments in our laboratory showed that these samples were stable for 14 days when stored at room temperature in the collection bags; longer periods of storage resulted in a change in the ratio of gases.

The breath samples were analyzed for hydrogen and methane using a Model DP Quintron Microlyzer and for carbon dioxide with a Model 24 Quintron Alveolyzer. End alveolar CO\textsubscript{2} partial pressure was assumed to be constant, and breath hydrogen and methane concentrations were corrected for possible atmospheric contamination by normalizing their values to the CO\textsubscript{2} concentration (4, 7). Instruments were calibrated using a standard gas mixture containing 93 ppm hydrogen, 22 ppm methane, and 4.62% CO\textsubscript{2} obtained from Quintron Instruments Co. The areas under curves plotting breath hydrogen and methane against time were used to represent the excretion of each gas during each 24-hr period, extrapolating during sleeping hours. The area under the curve was computed using a modification of the trapezoid rule (8).

Agreement between daily excretions based on all hourly measurements or a subset of these measurements was measured using the Spearman correlation coefficient \(r_s\) and the weighted-\(\kappa\) statistic for agreement among quartiles \([\kappa_w(9)]\).

**Results**

All subjects excreted hydrogen, whereas only six individuals (four males and two females) had breath methane levels over the 2-ppm sensitivity level of the system.

Figure 1 presents the hourly means and corresponding standard deviations for breath hydrogen, corrected for CO\textsubscript{2} and plotted against time. Each point represents the mean of measurements for that hour over all subjects and days of observation \(n = 48\). The curve shows moderately high values at the time of rising, followed by a decrease until mid-morning, and then a progressive increase during the afternoon and evening.

The area under each daily excretion curve for hydrogen/CO\textsubscript{2} was computed for each sex to estimate total daily excretion. For men, the mean area was 881 ppm\%/hr with a range of 4508-8952 ppm\%/hr and a standard deviation of 1425 ppm\%/hr. For women, the mean area was 8967 ppm\%/hr with a range of 4234-14,957 ppm\%/hr and a standard deviation of 4329 ppm\%/hr.

Table 1 shows a comparison of the area under the curve (daily excretion) obtained for hydrogen/CO\textsubscript{2} when all hourly measurements are used in its computation with the area under the curve when only 1, 2, 3, or 4 measurements at specified times are used. The estimate of total daily hydrogen/CO\textsubscript{2} excretion based on the 0600-hr sample correlates only moderately well with the observed excretion \((r_s = 0.6, \kappa_w = 0.5)\). A progressive improvement in agreement is observed when the number of measurements used increases. An excellent correlation \((r_s = 0.9)\) was found with four measurements. Varying somewhat the specified times for the four measurements had only a negligible effect on the level of agreement; therefore, we selected the most convenient times for our sampling protocol, i.e., at 0600, 1300, 1800, and 2200 hr.

Figure 2 presents the hourly means and corresponding standard deviations for methane/CO\textsubscript{2}, summarized over the 17 person-days of observation for methane producers plotted against time. Excretion was constant during the day with, perhaps, an increase after dinner. The mean area under the curve (daily excretion) for methane/CO\textsubscript{2} was 8311 ppm\%/hr with a range of 1476-16,255 ppm\%/hr and a standard deviation of 4239 ppm\%/hr.

Table 2 shows a comparison of daily excretion of methane/CO\textsubscript{2} estimated from all hourly measurements or from 1, 2, 3, or 4 measurements at specified times. Because

**Table 1. Areas under the excretion curve for breath hydrogen/CO\textsubscript{2} (ppm\%/hr) computed with a variable number of hourly measurements.**

| No. of measurements | Time of day, hr | Mean area | SD  | \(r_s^a\) | \(\kappa_w^b\) (95% CI)\(^c\) |
|---------------------|----------------|-----------|-----|----------|---------------------|
| All                 | All            | 7240      | 3637| 1.0      | 1.0                 |
| 1                   | 0600           | 7222      | 5505| 0.56     | 0.52 (0.29-0.74)    |
| 2                   | 0600, 2200     | 7798      | 4859| 0.75     | 0.69 (0.50-0.87)    |
| 3                   | 0600, 1300, 2200| 7807     | 4293| 0.83     | 0.79 (0.67-0.91)    |
| 4                   | 0600, 1100, 1600, 2200| 7234       | 3729| 0.89     | 0.87 (0.78-0.95)    |
| 4                   | 0600, 1300, 1800, 2200| 8050     | 4099| 0.89     | 0.83 (0.73-0.94)    |

\(^a\)Spearman correlation comparing areas computed with 1, 2, 3, or 4 measurements with areas based on all measurements.  
\(^b\)Weighted \(\kappa\) statistic for agreement among quartiles for the same comparisons, using cut points for the area computed with all points.  
\(^c\)95% confidence interval.
of the absence of a circadian pattern for breath excretion of methane, relatively good estimates of daily excretion were obtained with as few as one measurement. Daily excretion of hydrogen/CO₂ was similar in methane and nonmethane producers.

**Discussion**

This study shows that breath excretion of hydrogen follows a well-defined circadian pattern, with a decrease during the early morning followed by a progressive increase during the rest of the day. A similar pattern was observed by Levitt et al. (4) in five subjects studied for 5 days. In contrast, breath methane excretion was relatively constant throughout the day in the present study; this is also consistent with past observations (3).

Breath hydrogen excretion is influenced by diet, and particularly by carbohydrate ingestion. The progressive increase in excretion during the day is consistent with the observation that, in our population, dinner is the largest meal and lunch the second largest. The relatively high levels that we observed at time of rising were probably present throughout the night and may be due to a decrease in colonic motility during sleep (10). Factors that influence methane excretion are thought to be more complex, and diet is probably not the sole determinant (6). We are now exploring the interindividual variation and lifestyle correlates for methane and hydrogen excretion among a large population sample in Hawaii.

Our data suggest that four samples distributed throughout the day are optimal for characterizing individuals correctly by their hydrogen excretion over a 1-day period. In contrast, two measurements appear to be sufficient for estimating breath methane excretion. Such protocols would be adequate for assessing mean excretion levels in population groups; however, for studies conducted at the individual level, day-to-day variation in excretion must also be considered. Data derived previously suggest a relatively large intradividual variation in breath hydrogen. We are now characterizing this other source of variation by collecting four breath samples a day over a 2-week period in a group of subjects.

To our knowledge, only one study has assessed breath hydrogen in individuals at increased risk for colon cancer while on their regular diet (11). That study used a single measurement. The present data indicate that that protocol was most certainly inadequate and resulted in extensive misclassification of exposure, although one measurement was probably satisfactory for measuring breath methane excretion, at least in studies conducted at the population level (12).

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**Table 2. Areas under the excretion curve for breath methan/CO₂ (ppm/%) computed with a variable number of hourly measurements.**

| No. of measurements | Time of day, hr | Mean area | SD | r₅ | a (95% CI) |
|---------------------|-----------------|-----------|----|----|---------|
| All                 | All             | 8311      | 4239 | 1.0 | 1.0     |
| 1                   | 0600, 2200      | 10,189    | 6062 | 0.71| 0.61 (0.29–0.92) |
| 2                   | 0600, 2200      | 9578      | 5550 | 0.88| 0.74 (0.49–0.98) |
| 3                   | 0600, 1300, 2200| 9218      | 5203 | 0.96| 0.89 (0.79–0.99) |
| 4                   | 0600, 1100, 1600, 2200 | 8750     | 4894 | 0.95| 0.88 (0.76–1.00) |
| 4                   | 0600, 1300, 1800, 2200 | 8923    | 4996 | 0.95| 0.91 (0.82–1.00) |

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**Note:**

* S Spearman correlation coefficient comparing areas computed with 1, 2, 3, or 4 measurements with areas based on all measurements.
* a Weighted κ statistic for agreement among quartiles for the same comparisons, using cutoffpoints for the area computed with all points.
* 95% confidence interval.
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