*Journal of Pediatric Gastroenterology and Nutrition*
6:109–113 © 1987 Raven Press, New York

# Variance in Fasting Breath Hydrogen Concentrations in Guatemalan Preschool Children

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**Summary:** The concentration of hydrogen ($H_2$) in expired air after an overnight fast is receiving interest as a diagnostic indicator in itself. We analyzed 319 fasting samples collected from 90 healthy, well-nourished preschool children aged 29–72 months in two institutional settings in Guatemala City. The overall range of fasting $H_2$ concentration was 0–40 ppm, with an arithmetic mean of 4.4 ± 5.4 ppm (± SD) and a geometric mean of 3.2 ppm. No differences between boys and girls was found, but there was a progressive increase in the mean levels and an increase in the number of samples with $H_2$ concentrations >10 ppm with decreasing chronological age. One child with three of six samples having $H_2$ concentrations >40 ppm was found to have intestinal multiple parasitism and hence was excluded from the sample. As compared with a previous report from the United States of fasting breath $H_2$ concentrations in older children, the mean and distribution of values for Guatemalan preschoolers is identical. Intraindividual coefficients of variation in 48 children studied on four occasions had a mean of 62 ± 31% (range 0–143%). **Key Words:** Fasting breath $H_2$ concentration—Bacterial overgrowth—Protozoa—Intraindividual variation—Dietary fiber.

The production of $H_2$ by microflora of the human colon during the fermentation of carbohydrate substrate is the basis for a series of tests of absorption and intestinal transit time (1–4). Although the original procedure involved confinement in a continuous-collection rebreathing system (5), modern procedures generally involve internal sampling of breath $H_2$; the assessment of the response is in terms of the *change* in concentration over baseline fasting levels (6). Thus, the level of the basal, reference concentration of pulmonary $H_2$ in the fasting state at the commencement of a clinical breath test is an important consideration in the conduct of such diagnostic procedures (7,8).

Moreover, the absolute level of $H_2$ in alveolar air in the preprandial state has been introduced as a potential index of certain pathological conditions including bacterial overgrowth (9–11), pneumatosis cystoides intestinalis (12), and necrotizing enterocolitis (13). An extensive series of pediatric patients' fasting breath $H_2$ concentrations was reported by Perman and co-workers (10). Their report covered a heterogeneous population of both healthy and diseased children of widely ranging areas. In the context of studies of lactose maldigestion and its treatment in Guatemalan preschool children, we assembled an experience with 319 samples of fasting breath $H_2$. The inter- and intraindividual variance in this index in these young children in a preindustrialized setting is the subject of the present report.

## SUBJECTS AND METHODS

The study included 90 preschool children, ranging in age from 29 to 72 months* in two centers: The Casa Guatemala orphanage, in which

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* The exact birth date of some of the orphaned children was not known, and estimations of their age have been made for classification into age groups.

children are 24-h residents (n = 20) and the Pamplona Day-Care Center, to which children were brought from their own homes daily, remaining from 7:30 a.m. until 5:30 p.m. (n = 70). There were 44 girls and 46 boys in the sample. Children were healthy, with adequate nutritional status, having a weight for height of at least 90% of the expected standard in all cases. If a child had an episode of gastroenteritis or was treated with antibiotics, studies were postponed until at least 4 weeks postrecovery or posttherapy. The protocol was approved by the Human Subjects Committee of the Institute of Nutrition of Central America and Panama. Informed consent for participation of the child was obtained from the parent or guardian of the day-care center children, and from the orphanage director, the courtappointed custodian for the children under her care.

### Collection of Expired Air

Children arrived fasting on the day of study. Samples of expired air were collected between 7:30 and 8:30 a.m. by having a child breathe through a low-resistance, one-way Hans Rudolph valve into a 5-L rubber anesthesia gas-bag. A subsample of gas was transferred into a plastic 60-ml syringe fitted with a three-way stopcock.

### Breath $H_2$ Determination

The concentration of $H_2$ in the fasting breath samples was determined on a Microlyzer CM external column chromatographic breath $H_2$ analyzer (Quintron Instruments Co., Milwaukee, WI, U.S.A.) (14), calibrated with a standard reference gas mixture containing 97 ppm $H_2$ in room air (Ideal Gas, Edison, NJ, U.S.A.). The concentrations registered on the digital display for the unknown breath samples were recorded in parts per million.

### Data Analysis

Arithmetic means and SD and the geometric means of grouped data were computed as descriptive statistics. Because the data showed similar distribution curves (although not Gaussian) for different age groups, intergroup comparisons were made using the Student's *t* test for unpaired data (15). Where several repeated measures existed for the same individual, the coefficient of variation was calculated.

## RESULTS

When 319 fasting breath $H_2$ concentrations analyzed in studies at both centers (1) were pooled without regard to age, sex, or the number of times an individual was represented in the sample, the arithmetic mean was 4.4 ± 5.2 ppm (geometric mean (GM) = 3.2 ppm).* The values ranged from 0 to 40 ppm. Of these, 156 breath samples were from girls, with a mean of 4.0 ± 3.9 ppm ($GM^2$ = 3.1 ppm); 163 were from boys, with a mean of 4.8 ± 6.3 ppm (GM = 3.2 ppm). No statistically significant difference was observed between boys and girls.

In one defined substudy at the Pamplona center, 48 individuals (24 boys and 24 girls, aged 30–72 months) were studied on four distinct occasions with an interval between any two successive studies ranging from 2 to 49 days. The individual with the widest range of breath $H_2$ concentration values was a 65-month-old boy who had levels of 40, 6, 4, and 1 ppm. The narrowest range was seen in a 32-month-old boy with successive values of 4, 4, 4, and 4 ppm. The coefficients of variation for the four individual breath $H_2$ analyses in the 48 children ranged from 0–143% (mean 62 ± 31%).

The wide range of ages in the group of 48 children allowed us to compare distributions of fasting breath $H_2$ concentration by age category. This included brackets of: (I) ≤41 months (n = 16); (II) 42–53 months (n = 8); (III) 54–65 months (n = 14); and (IV) ≥66 months (n = 10). The distributions and descriptive statistics for these data are shown in Fig. 1. The highest value recorded was 40 ppm. A progressive tendency for a narrower range of fasting $H_2$ concentration with increasing age is apparent.

Figure 2 uses the same age brackets for a similar analysis of all 319 breath samples comparing the global data for all studies in both centers with the exception of the one unusual case.† Despite the en-

* For calculating the geometric mean, the basal values of 0 ppm (n = 11) were excluded. We included numbers of n = 308 for the total sample, n = 152 for the female group, and n = 156 for the male group.

† One child of ~36 months of age at the Casa Guatemala orphanage, with 102% of the expected weight for height, had fasting $H_2$ values on six different occasions of 10, 64, 5, 2, 56, and 44 ppm. Three of the six were out of the range of all values of the other children. Shortly thereafter, he became symptomatic, and a diagnosis of giardiasis and amebiasis was confirmed in his stool examinations. His data were excluded from the pool of "normal" data.

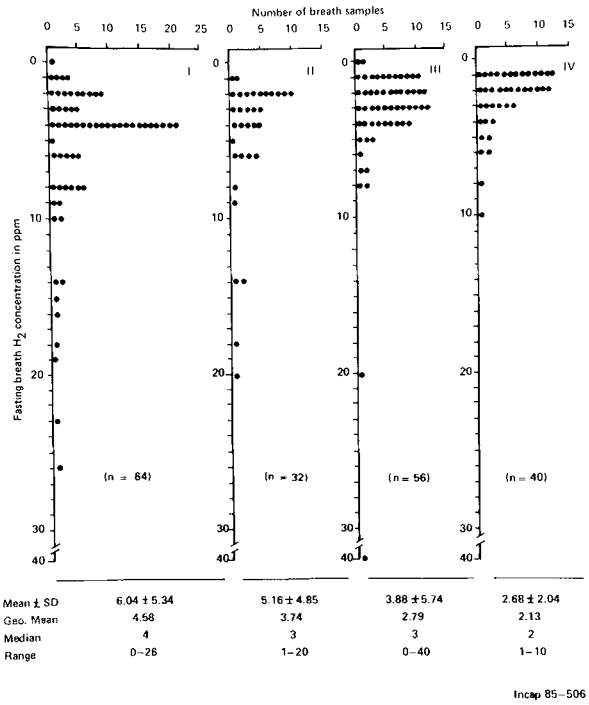


**FIG. 1.** The distribution of fasting breath $H_2$ concentrations in 48 children studied on four separate occasions divided by age groups into group I (n = 16, aged <41 months), group II (n = 8, aged 42–53 months), group III (n = 14, aged 54–65 months), and group IV (n = 10, aged >66 months). The number of samples for each age group is shown in parentheses. Descriptive statistics are provided below each panel.

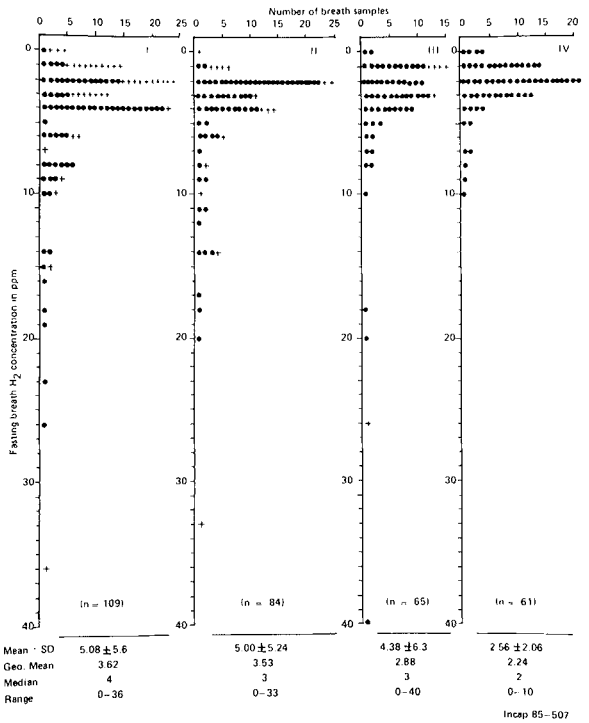


**FIG. 2.** The distribution of fasting breath $H_2$ concentrations in all 319 samples in the 90 preschool children in the entire population studied divided into age groups as described in the text and in Fig. 1. Children from the Pamplona Day-Care center (●); children in the Casa Guatemala orphanage (†). The number of samples for each age-group is show in parentheses. Descriptive statistics are provided below each panel.

largement in the sample number for the age group, group IV (>66 months) from 40 to 61 analyses, the highest fasting concentration was still only 10 ppm. With the *total* sample, the mean for group-IV subjects' samples was statistically different from those of all three other age groups.

## DISCUSSION

Our data shed new insights into the distribution of intraindividual variability of $H_2$ concentrations in preprandial samples from healthy preschool children. It is generally accepted that fermentation of glycoproteins from desquamated intestinal cells released in the process of normal villous turnover determines a basic, underlying colonic $H_2$ production (16). Because the traditional application of breath $H_2$ measurement has been to examine absorptive responses to carbohydrate, emphasis has generally been focused on *reducing* the fasting breath $H_2$ concentrations to maximize sensitivity. Only recently has an *intrinsic* diagnostic significance to the variance in the absolute level of $H_2$ in preprandial expired air become, in itself, clinical interest (9–13,17).

The determinants of this variance are complex. Presumably, one's peristaltic rhythm and the size of one's anaerobic fecal flora (18), along with the specific balance between $H_2$-producing and $H_2$-consuming microbes in the large bowel (4,19) constitute the *endogenous* factors conditioning fasting breath $H_2$ levels. Dietary factors related to the amount of carbohydrates, both digestible (7,8, 20,21) and nondigestible (14) in a previous day's intake are exogenous modulators of fasting breath $H_2$. In a pathological context, bacterial colonization of the superior gastrointestinal tract constitutes another major determinant of fasting levels of $H_2$ in pulmonary gas (9–11).

The only systematic information on fasting $H_2$ in normal children comes from a study of children in the United States. Perman and colleagues (10) analyzed 221 fasting breath samples taken between 8:00 a.m. and 9:00 a.m. in children without gastro-

intestinal pathology. Although they (14) used "end-expiratory" samples of alveolar air and we used mixed exhaled air collected through a one-way valve, we previously showed that the pulmonary "washout" effect of the conscious breathing effort negates the influence of bronchial anatomic dead space (22); thus, our absolute $H_2$ concentrations should be comparable with those of Perman and colleagues (10). Quite consistent with Perman's finding of 42 ppm as the upper limit of fasting $H_2$ concentration in the normal children aged 11–16 years, the upper curve of $H_2$ concentration for our preschool children <6 years of age was 40 ppm. In the study of Perman and colleagues (10), moreover, only 3% of patients with recurrent abdominal pain syndrome and 17% of patients with chronic diarrhea had fasting levels >42 ppm.

Mean $H_2$ concentration fasting for 221 U.S. children was 7.1 ± 5.0 ppm (10), whereas the global mean for all samples in 90 healthy, well-nourished preschool children in institutional settings in Guatemala was 4.4 ± 5.2 ppm. In children studies systematically on four disparate occasions, a coefficient of variation (cv) of 62%. This is similar to the cv of 63% found by Lopez and Solomons (17) in 35 individuals sampled immediately on awakening on three consecutive mornings.

In the comparative context of the U.S. and Guatemalan studies in children the role of diet is of note. It is well known that an immediate (within 4–8 h) response of breath $H_2$ to fiber and indigestible oligosaccharides in leguminous beans is quite common (23–25). Perman and co-workers (10) showed that a bean meal the night before a breath test produced a greater fasting breath $H_2$ concentration than did either wheat or rice, and the range of values was 0–38 ppm, with only three values exceeding 20 ppm. Most Guatemalan children in this study had beans in at least their midday or evening meal every day of the year, including the days preceding their study. It is curious, then, that the overall mean of fasting breath $H_2$ concentrations is numerically lower than that for the U.S. children and distinctly lower than the U.S. adults following their evening bean meals. This could suggest an ability of the colonic flora to "adapt" to a constant delivery of unabsorbed polysaccharides from the bean-rich diet of the Guatemalan children. The studies of O'Donnell and Fleming (26) in North American adults, however, cast doubt on the ability of the colonic flora to adapt to chronic exposure to legume oligosaccharides with a diminished $H_2$ response.

Perman and colleagues (10) did not comment on age differences in $H_2$ levels throughout the 6-year span of their population. An age effect on fasting breath $H_2$ is demonstrable in our data, with the oldest individuals—those >5.5 years of age—having fasting breath $H_2$ concentrations in a very narrow range, all <11 ppm. Younger children had numerous samples >10 ppm. One can speculate that the age-related differences are due to: (a) an age-specific difference in colonic floral mass; (b) a variability in nocturnal peristalsis; or (c) most likely, greater contact with soil and fecal material in younger children, leading to a *low-grade* bacterial colonization of their upper gastrointestinal tracts.

The ecological conditions of our children would be expected to differ from those of the U.S. sample studied by Perman and co-workers (10) both by virtue of age-related sanitary practices, but also by the environmental hazards of fecal contamination in day-care centers and orphanages in preindustrialized tropical countries. The diet is far richer in dietary fiber and undigestible oligosaccharides in Guatemala due to dietary reliance on corn tortillas and black beans as staples. Nonetheless, no overall differences in breath $H_2$ concentration in a preprandial morning sample is seen. The 40–42 ppm cut-off criterion for fasting samples for a normal child population seems to be confirmed in two studies of distinct populations. There is considerable intraindividual variance in fasting $H_2$ levels, and this must be taken into account in the design of any prospective study that uses preprandial $H_2$ levels as a discrete experimental variable.

**Acknowledgment:** The authors thank Ms. Angela Galmadez and the nursing staff of the Casa Guatemala orphanage and Ms. Beatriz Molina and the staff of the Pamplona Day-Care Center and the parents for their cooperation. Lic. Ricardo Sibrián and Dr. Oscar Pineda were generous with their advice and counsel in the analysis of data. The author (C.B.) was a research fellow sponsored by the LactAid Co. of Pleasantville, NJ, U.S.A.; their financial support is deeply appreciated.

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