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Fecal Hydrogen Production and Consumption Measurements

Response to Daily Lactose Ingestion by Lactose Maldigesters

STEVEN R. HERTZLER, PhD, RD, DENNIS A. SAVAIANO, PhD, and MICHAEL D. LEVITT, MD

The alteration of hydrogen (H_2) metabolism, which accounts for the large decrease in breath H_2 excretion following prolonged ingestion of malabsorbed carbohydrate (lactulose, lactose in lactose maldigesters) was studied in six lactose-maldigesting adults. Metabolic inhibitors of the three main H_2-consuming reactions (methanogenesis, sulfate reduction, and acetogenesis) were used to independently measure H_2 production and consumption in fecal samples obtained after 10 days of either dextrose or lactose feeding. Absolute fecal H_2 production (net of production minus consumption) after 3 hr of incubation with lactose was approximately threefold lower after lactose adaptation (242 ± 54 μl) compared to dextrose adaptation (680 ± 79 μl, $P = 0.006$). Fecal H_2 consumption was not affected by either feeding period. We conclude that decreased absolute H_2 production, rather than increased H_2 consumption, is responsible for the decrease in breath H_2 observed with lactose feeding.

KEY WORDS: colonic adaptation; hydrogen production; lactose maldigestion.

Fermentation of malabsorbed carbohydrate by the colonic bacteria results in the production of short-chain fatty acids and gases [H_2, carbon dioxide (CO_2) and methane (CH_4)]. The stoichiometry of a major fermentation reaction indicates that about 13,600 ml of hydrogen should be produced from the approximately 40 g of carbohydrate that has been estimated to reach the colon each day (1, 2). The actual release of such volumes of hydrogen obviously would have important implications for intestinal distension, rectal gas problems, and explosions. Fortunately, the vast majority of this H_2 is consumed by other colonic bacteria and only a minor fraction, the net of absolute production minus consumption, actually escapes from the feces and is available for excretion via the anus or the lungs.

A number of previous studies have shown that the breath H_2 response to ingestion of a malabsorbed sugar [lactulose (3–5) or lactose (6, 7)] is markedly reduced following ingestion of the sugars for one to two weeks. The mechanism for the decrease in breath hydrogen following colonic adaptation to carbohydrate is unclear. Some authors have hypothesized that adaptation results in greater fermentation by organisms that can metabolize lactose/lactulose without the evolution of H_2, such as bifiodobacteria or other lactic acid bacteria (3, 8). It is also possible that bacterial

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From the University of Minnesota, Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, Minnesota 55108; Purdue University, College of Family and Consumer Sciences, 1260 Stone Hall, Room 110, West Lafayette, Indiana 47907-1260; and Veterans Affairs Medical Center, 1 Veterans Drive, Minneapolis, Minnesota 55417.

Steven R. Hertzler's present address is: Kent State University, 130 Nixson Hall, Kent, Ohio 44242-0001.

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Address for reprint requests: Dr. Michael D. Levitt, Veterans Affairs Medical Center, 1 Veterans Drive, Minneapolis, Minnesota 55417.

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TABLE 1. TREATMENT SCHEME FOR MEASUREMENT OF FECAL HYDROGEN PRODUCTION AND CONSUMPTION

Treatment	*Fecal homogenate volume (ml)*	*Headspace gas volume (ml)*	*Headspace gas composition*	*Inhibitors of H_2 consumption**	*Addition of lactose†*	*Measurement*
1	5	25	10% CO_2 90% argon	N	N	Net H_2 production, no substrate
2	5	25	10% CO_2 90% argon	Y	Y	Absolute H_2 production from lactose
3	5	25	10% CO_2 90% argon	N	Y	Net H_2 production from lactose
4	5	25	10% H_2	N	N	H_2 consumption

* Inhibitors added were 20 mmol/liter homogenate BES (inhibits methanogenesis), 20 mmol/liter Mo (inhibits sulfate reduction), and 50 μmol/liter choloroform (inhibits acetogenesis).
† Lactose (5.5 mmol/liter homogenate) was added as fermentable substrate in treatments 2 and 3.

consumption of H_2 is up-regulated. To distinguish between these two possibilities, it is necessary to independently measure either absolute H_2 production or H_2 consumption. Recently, we described a technique to measure absolute H_2 production via the use of metabolic inhibitors of the major H_2-catabolizing reactions utilized by human colonic bacteria (9). In the present study, this technique was employed to determine if decreased absolute H_2 production and/or increased H_2 consumption accounts for the decreased H_2 excretion observed after colonic adaptation to ingestion of nonabsorbed carbohydrate.

MATERIALS AND METHODS

Subjects. Six free-living subjects (five men, one woman; $\bar{X}$ = 68 kg and 35 years of age) were studied. All subjects were in good health and had not used antibiotics in the preceding two months. Subjects were identified as lactose maldigesters based on a rise in breath hydrogen of >20 parts per million (0.9 μmol H_2/liter of air) following an aqueous lactose dose (0.35 g/kg body weight) administered after an overnight fast (10). One subject was a large methane producer (breath CH_4 > 20 ppm above the atmospheric concentration), while the breath methane of the remainder of the subjects was <1 ppm above atmospheric. Informed consent was obtained and the protocol for the study was approved by the Institutional Review Board Human Subjects Committee at the University of Minnesota.

Experimental Design. This study was a 22-day blinded, randomized feeding trial. Subjects were instructed to avoid all products high in lactose (milk, ice cream, yogurt) but otherwise were allowed to eat their normal diet. This diet was supplemented with either lactose (Land O'Lakes, Arden Hills, Minnesota) or dextrose (control) for days 1–11 and crossed over to the other treatment for days 12–22. The initial dose of sugar was 0.6 g/kg body wt/day and was increased by 0.2 g/kg increments every other day, up to a maximum of 1.0 g/kg/day by the end of the feeding period. The sugar supplements were ingested in three equal doses in 250 ml of tap water with breakfast, lunch, and dinner. At the end of each feeding period (days 11 and 22), subjects collected a single fecal sample for analysis.

Fecal Sample Collection. Fecal samples were collected in the morning on days 11 and 22. Subjects were provided with a stool collection apparatus (Commode Specimen Collection System, Sage, Inc., Crystal Lake, Illinois) and a polyethylene bag was used to line the inside of the container. Subjects defecated into the bag and were instructed to immediately squeeze residual air out of the bag before sealing it tightly with a rubber band. Fecal samples were transported to the laboratory within 1 hr of defecation and were processed within 3–4 hr.

Measurement of Fecal Hydrogen Production and Consumption. A weighed sample of feces was placed in a blender vessel fitted with a gas-tight lid. After exhaustively flushing the vessel with argon, deoxygenated buffer (0.2 M PO_4 buffered saline), which was warmed to 37°C, was added (1:20 w/v). The feces were then mixed for the minimal period (seconds) required to produce a smooth homogenate. A 5-ml aliquot of homogenate was anaerobically aspirated into each of eight plastic 60-ml syringes that had been previously purged with argon. The syringes were sealed with stopcocks, and four different measurements of H_2 metabolism were then carried out in duplicate: (1) net H_2 production from substrates endogenous to feces, (2) absolute H_2 production from lactose fermentation, (3) net H_2 production from lactose fermentation, and (4) H_2 consumption. The headspace gases and the additions to the homogenate for each of these four measurements are shown in Table 1.

The four sets of syringes were incubated in an orbital shaker at 37°C. At time 0 (syringe containing 10% H_2) and hours 1, 3, and 24 (all syringes), a 0.5-ml gas sample was removed for analysis using a 0.5-ml gas-tight syringe.

Gas samples were analyzed for H_2 and CH_4 content via a gas chromatograph equipped with a molecular sieve column and a reduction detector for H_2 and a hydrogen flame detector for CH_4 (10). The coefficient of variation for the duplicate measurements averaged 8.5%.

After the last gas sample was drawn at 24 hr of incubation, the pH of the fecal homogenates was obtained using a pH meter (Fisher Scientific, Pittsburgh, Pennsylvania).

Calculations and Statistics. Data are expressed as the

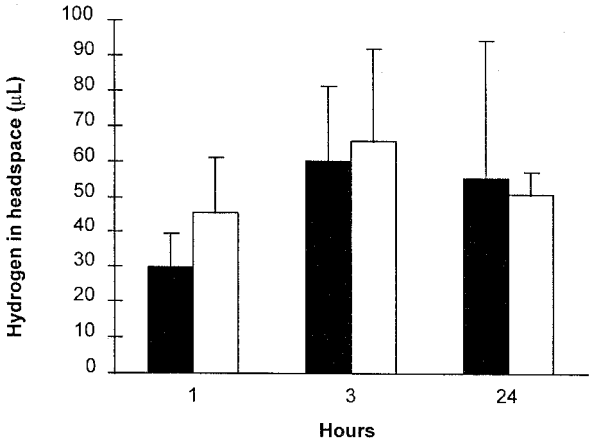

Fig 1. Net fecal H_2 production without additional substrate. Solid bars are after dextrose adaptation; open bars are after lactose adaptation. Bars represent the mean + SEM ($N = 6$ for hours 1 and 3, $N = 5$ for 24 hr).

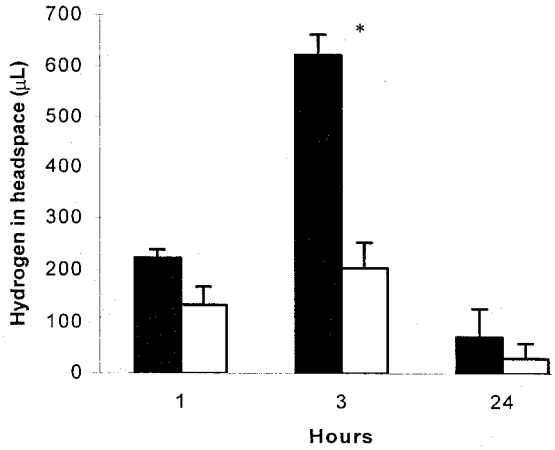

Fig 2. Net fecal H_2 production from lactose added *in vitro*. Solid bars are after dextrose adaptation; open bars are after lactose adaptation. Bars represent the mean + SEM ($N = 6$ for hours 1 and 3, $N = 5$ for 24 hr). * $P = 0.001$.

mean ± SEM ($N = 6$). The methane-producing subject was included in the analysis after calculation of hydrogen equivalents, using the stoichiometry of 4 mol H_2 consumed to yield 1 mol CH_4 (2). However, data were unavailable for the 24-hr time point for this subject due to a laboratory error, and thus, $N = 5$ for the 24-hr production calculations. Analysis of variance (ANOVA) was used to determine differences in H_2 production and consumption among the four types of measurements, the three time points, and between the lactose and dextrose feeding periods. Where significant differences were found, Tukey's HSD test was used for multiple comparisons (11, 12).

RESULTS

Net H_2 production by the fecal samples incubated without additional fermentable substrate is shown in Figure 1. There were no significant differences between the dextrose- and lactose-adapted periods at any time point. Figure 2 shows the net H_2 production resulting from the fermentation of lactose added to the homogenate (calculated from the difference between the H_2 production in the homogenate containing added lactose and that in the control syringe). Hydrogen production at 3 hr was significantly reduced ($P = 0.001$) by lactose adaptation (205 ± 50 μl H_2) compared to dextrose adaptation (623 ± 39 μl H_2). The quantity of H_2 in the syringes declined sharply between 3 and 24 hr as the rate of H_2 consumption exceeded the rate of production. By 24 hr, net H_2 production was not different between the lactose and dextrose periods. Figure 3 shows the absolute H_2 production by the fecal samples. There was no significant difference in H_2 production at the 1-hr time point. However, at 3 hr absolute H_2 production after the lactose feeding period (242 ± 54 μl, $P = 0.006$) was only about one third that after the dextrose period (680 ± 79 μl, $P = 0.006$) and a similar difference was maintained after 24 hr of incubation (289 ± 68 μl vs 703 ± 48 μl, $P = 0.003$).

Fecal H_2 consumption is shown in Figure 4. There was no significant difference in the rate of H_2 consumption by feces collected after the dextrose versus lactose feeding periods. The CH_4-producing feces consumed H_2 far more rapidly (>99% consumed in 24 hr) than did the non-CH_4-producing feces (approximately 70% consumed in 24 hr), but there was no difference in the H_2 consumption rate after lactose or dextrose feeding in the methanogenic feces. Meth-

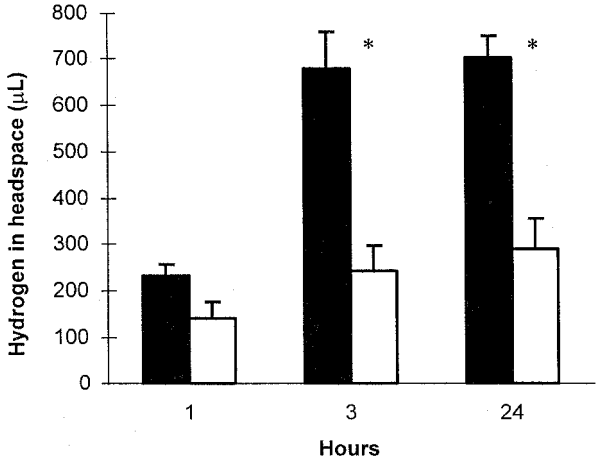

Fig 3. Absolute fecal H_2 production from lactose added *in vitro*. Solid bars are after dextrose adaptation; open bars are after lactose adaptation. Bars represent the mean + SEM ($N = 6$ for hours 1 and 3, $N = 5$ for 24 hr). * $P = 0.006$; ** $P = 0.003$.

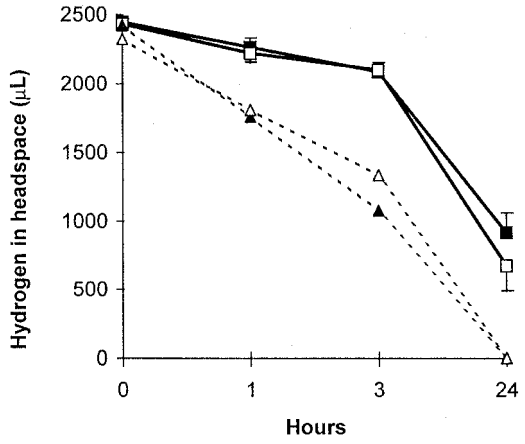

Fig 4. Fecal H_2 consumption from headspace containing 10% (2500 μl/25 ml) hydrogen. Solid lines are means of data for methane nonproducers ($N = 5$); dashed lines are for the methane producer ($N = 1$). Open symbols are after dextrose adaptation; closed symbols are after lactose adaptation. Values for methane nonproducers are the mean ± SEM.

ane production was always negligible in the feces of the non-CH_4 producing subjects.

The pH of the fecal homogenates ranged between 7.0 and 6.5 after 24 hr of incubation. However, pH was significantly lower ($P = 0.002$) in the homogenates to which lactose had been added (pH 6.69 ± 0.02) versus those to which no lactose had been added (pH 6.84 ± 0.92).

DISCUSSION

The goal of this study was to identify the alteration of fecal H_2 metabolism that accounts for the decreased H_2 excretion observed in individuals who have ingested nonabsorbable carbohydrates (eg, lactulose, lactose in lactose maldigesters) for one to two weeks (3–7). Clinical measurements of H_2 production, such as breath H_2 excretion, assess the net of absolute H_2 production minus consumption of this gas. While bacterial production of H_2 has received extensive study, the importance of fecal H_2 consumption has only been recently recognized. The bulk of H_2 consumption by human feces appears to be accounted for by three reactions—methanogenesis, sulfate reduction, and acetogenesis. The relative importance of the mechanisms appears to have a geographical or methodological variability. British studies suggest that virtually all H_2 catabolism can be accounted for by sulfate-reducing or methanogenic bacteria (13). In contrast, our study of H_2 consumption by feces obtained from 14 subjects in Minneapolis indicated that methanogenesis, sulfate reduction, and other reactions, presumably acetogenesis, were predominant in 35%, 30%, and 35% of subjects, respectively (9).

There is increasing evidence that abnormalities of H_2 consumption may play a role in colonic disease. The most clear-cut example is the recent demonstration that a lack of hydrogen consumption accounts for the enormous hydrogen excretion and presumably the intestinal cysts of patients with pneumatosis cystoides intestinalis (14). In addition, sulfate-reducing bacteria utilize hydrogen to reduce sulfate to sulfide, and it has been suggested that the highly toxic hydrogen sulfide produced might play an etiological role in ulcerative colitis (15). Lastly, while not yet clearly demonstrated, it appears likely than an imbalance between H_2 production and H_2 consumption plays an important role in excessive flatulence (16, 17).

To identify the role of H_2 production versus H_2 consumption in the genesis of an unusual net production of this gas, it is necessary to measure either absolute production or consumption or both. The conventional means of determining the rates of the simultaneously ongoing production and consumption reactions utilizes the disappearance rate of a different isotope of the compound whose production is being determined. However, this approach is not feasible in the case of H_2 metabolism because of the extremely rapid exchange of hydrogen atoms in H_2 with those in fecal water (18), and alternative methodologies must be employed.

When the supply of fermentable substrate is limited, the rate of H_2 consumption at high H_2 tensions far exceeds the rate of absolute H_2 production. Thus, the disappearance of H_2 from the headspace over fecal samples incubated without added substrate provides a measure of the H_2-consuming ability of the homogenate. We have studied two different methods of measuring absolute H_2 production. Since hydrogen consumption is proportional to H_2 tension in the homogenate, H_2 consumption can be minimized by maintenance of an extremely low fecal H_2 tension. This has been achieved by incubating thin films of fecal homogenate underneath a very large gas space (19). A simpler approach, that of the present study, involves the use of metabolic inhibitors of the H_2-consuming reactions. Methanogenesis, sulfate reduction, and acetogenesis are specifically inhibited by 2-bromoethanesulfonic acid, sodium molybdate, and chloroform, respectively (20–22). Thus, the H_2 released by a homogenate containing these three inhibitors provides a measurement of absolute H_2 production.

In the present study fecal samples were collected

from lactose-maldigesting subjects who had ingested large quantities of either lactose or dextrose for 10-day periods. The absolute production and consumption rates of H_2 by fecal homogenates were then determined using the above-described techniques. As shown in Figure 3, the absolute H_2 production rate during lactose fermentation was only about one third as rapid ($P = 0.006$) in feces collected after the lactose versus the dextrose feeding period. In contrast, there was no significant difference between the H_2 consumption rates observed after the two feeding periods. Thus, we conclude that the decreased H_2 response to lactose after lactose adaptation results from a decreased absolute production of H_2, whereas H_2 consumption is not altered. This finding has important implications for the development of stoichiometric equations that predict the amounts of SCFA and H_2 that could be generated from a given amount of fermentable carbohydrate. The existing equations do not take into account the effect of adaptation on H_2 production. It has been demonstrated that adaptation to lactulose (3, 4) results in a shift toward the generation of acetic and lactic acids, rather than H_2, as end products of fermentation.

While this study does not shed light on the mechanism by which lactose feeding reduces absolute H_2 production, previous work has shown marked increases in fecal β-galactosidase (3, 4, 6, 7) and reduced fecal hexose excretion (3, 4) after lactose or lactulose adaptation. Induction of increased levels of β-galactosidase in the existing flora would not be expected to reduce H_2 production. Rather, it seems necessary to postulate that lactose stimulates the proliferation of bacterial species such as bifidobacteria or other lactic acid bacteria that carry out non-H_2-producing, lactose-fermentation reactions (23). In support of this concept is the demonstration of Terada et al (24) that chronic administration of lactulose (3 g/day for 14 days) resulted in a fivefold increase in fecal bifidobacteria counts and a 2 log decrease in lecithinase-positive clostridia (which are H_2 producers). Bifidobacteria also have been shown to inhibit other H_2 producing bacteria, such as *Escherichia coli* (25, 26). Perman et al have proposed that the fermentation of malabsorbed lactose reduces fecal pH, and this low pH inhibits further lactose catabolism (5). This clearly cannot be the explanation for our findings, since the pH of the fecal homogenates was maintained between 7 and 6.5 in all instances.

Given the minimal symptoms that result from lactose malabsorption in the adapted colon (6, 7) and the many claims that high bifidobacteria populations are beneficial to health (23), it seems possible that a potential approach to lactose maldigestion is the regular ingestion of dairy products rather than lactose avoidance or the use of lactose digestive aids.

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