The Fermentation of Different Dietary Fibers Is Associated with Fecal Clostridia Levels in Men[1]

Daisuke Chinda, Shigeyuki Nakaji,[2] Shinsaku Fukuda,* Juichi Sakamoto,* Tadashi Shimoyama,* Teruo Nakamura,† Tomohiko Fujisawa,** Atsushi Terada,** and Kazuo Sugawara

*Department of Hygiene, Hirosaki University School of Medicine, Hirosaki, Japan; *First Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki, Japan; †Department of Health, Hirosaki University School of Health Sciences, Hirosaki, Japan; and **Department of Food Hygiene, Division of Food Science and Technology, Nippon Veterinary and Animal Science University, Tokyo, Japan*

ABSTRACT Only a few reports have compared the fermentation of pectin and cellulose using the hydrogen-breath test, and no studies have examined the relation between the hydrogen breathing pattern and colonic microflora. Using breath-hydrogen measurements, we investigated whether different dietary fibers (DFs) were fermented differently and whether there were individual differences after ingestion of the same DF; we also examined the relation between individual fecal microflora and the fermentation of DF. Results of hydrogen tests in 14 men were compared after they had ingested 20 g of pectin, 20 g of cellulose, or 6 g of lactulose (a DF-like substance). We examined the relation between the breath hydrogen results and the subjects' fecal microflora. We defined significant fermentation (i.e., positive cases) as a continuous rise in hydrogen in the expiratory air of >19 ppm. The subjects were divided into 3 groups according to their hydrogen breath test pattern, i.e., positive for lactulose and pectin (Group LP, $n = 4$); positive for lactulose alone (Group L, $n = 7$); and negative for pectin, cellulose, and lactulose (Group N, $n = 3$). Individual differences were noted in subjects from Group LP and Group L. The detection frequency of lecithinase-negative clostridia was higher in Group LP than in the other groups ($P < 0.05$), and the detection frequency and the number of lecithinase-positive clostridia were higher in Groups LP and L than in Group N ($P < 0.05$). These findings suggest that the *Clostridium* species are associated with hydrogen production. The hydrogen breath test results of DFs depend on both the type of DF and the individual colonic microflora. The amount and constitution of colonic microflora might be predicted by the hydrogen-breath test using different DFs. J. Nutr. 134: 1881–1886, 2004.

KEY WORDS: • *pectin* • *cellulose* • *lactulose* • *microflora* • *Clostridium species*

Dietary fiber (DF)[3] is carbohydrate that in principle is not digested in the small intestine (1,2), but which on entering the colon is fermented by commensal bacteria, with the production of SCFAs (acetate, butyrate and propionate) and gases (e.g., hydrogen, methane, or carbon dioxide) (3–9). SCFAs are used as potential energy sources and are the major fuel for enterocytes (5,6,10–13). They also decrease gut pH (14,15). The gases produced by fermentation are absorbed into the circulation and diffused into the pulmonary alveolus; ~10% of the volume produced by the colonic flora is present in the breath on exhalation (16). Therefore, measurement of hydrogen in the breath after DF has been ingested reflects the anaerobic bacterial fermentation of DF in the colon (17,18). Several reports compared the fermentation of DF using the measurement of end-expiratory hydrogen concentrations (4–7,19,20) and suggested that they depend on the type of DF. Moreover, some researchers reported a number of factors that can affect gas production including the rate of gastrointestinal transit (21), colonic pH (22), and menstrual cycle (23). However, no studies have examined the relation between gas production and colonic/fecal microflora in vivo. To clarify this relation, it is important not only to know that a certain DF is fermented by a certain bacterium, but also to evaluate the constitution and volume of colonic bacteria.

The aim of this study was to clarify whether different DFs are fermented differently, and whether there are individual

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[2] To whom correspondence should be addressed.
E-mail: nakaji@cc.hirosaki-u.ac.jp.
[3] Abbreviations used: AUC, the area under the curve; BL, glucose blood liver agar; BS, bifidobacteria selective agar; DF, dietary fiber; DHL, deoxycholate hydrogen sulfide lactose agar; EG, modified Eggerth-Gagnon agar; ES, eubacteria selective agar; L, group positive for lactulose alone; LBS, modified lactobacilli selective agar; LP, group positive for lactulose and pectin; N, group negative for pectin, cellulose, and lactulose; NAC, nalixidic acid cetrimide agar; NBGT, neomycin-brilliant green-taurocholate-blood agar; NN, neomycin-nagler agar; PD, potato dextrose agar; PEES, staphylococcus 110 agar; RS, resistant starch; TATAC, triphenyltetrazolium chloride-acridine orange-thallous sulfate aesculin crystal violet agar; TS, trypticase soy blood agar; VS, modified *Veillonella* selective agar.

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differences if the same DF is ingested in vivo, using the hydrogen-breath test. Furthermore, we examined the relation between individual fecal flora and the fermentation of DF.

SUBJECTS AND METHODS

Subjects. Healthy male volunteers [n = 14; 26.7 ± 3.2 y (mean ± SD), range 21–34 y; height, 172.0 ± 4.1 cm; body mass, 73.4 ± 10.8 kg] participated in this study. In all subjects, the end-expiratory hydrogen concentrations were measured after they had ingested pectin, cellulose, or lactulose, and colonic microflora were measured. Subjects were then placed in subgroups depending on the reaction to each material ingested, as described in the Results section. The interval from one experiment to another was a minimum of 7 d to allow the previous DF or lactulose to be completely eliminated from the gut, thus preventing any influence on the colonic microflora. None of the subjects took any antibiotics or laxatives during the 2 wk before the experiment and the experiment itself, and they had no past or present history of chronic gastrointestinal or pulmonary disease. During the measurement of end-expiratory hydrogen concentrations, all subjects refrained from smoking, sleeping, or exercising. Informed consent was obtained from all subjects. The protocol was approved by the Human Subjects Review Committee of Hirosaki University School of Medicine.

Measurement of end-expiratory hydrogen concentrations. On each occasion for the measurement of end-expiratory hydrogen concentrations, after a 12-h overnight fast following the ingestion of an 81-g balanced meal,[4] 500 mL of water was consumed with 1 of the 3 test substances. In addition, 200 mL of a liquid diet[5] was served 3 times as follows: immediately after, and 5 and 10 h after ingestion of the test meal. The men were allowed to drink water ad libitum throughout the day. All subjects rinsed out their mouths with a 0.1% chlorhexidine solution before consuming the test meals and gargled with a glass of water after consuming the test meals to remove the oropharyngeal flora, which were shown to interfere with the measurement of end-expiratory hydrogen concentrations (24,25).

On two occasions, the test substance consisted of either 20 g of pectin (Sigma Chemical) or 20 g of cellulose (Asahi Kasei). Pectin consists mainly of galacturonan, which is composed of α-1,4 linked D-galacturonic acids (acidic polysaccharides) (26). In this study, we used dried apple pectin of 81% purity. Cellulose is a polysaccharide that combines D-glucoses by α-1,4 linkages. In the present study, we used microcrystalline cellulose (Avicel PH-M06, Asahi Kasei) of 93–100% purity made from pulpwood. On one occasion, the test substance consisted of 10 mL of lactulose (600 g/L; Nikken Chemicals), a synthetic nonabsorbable disaccharide, which was used as a comparative study (27). It was shown in vitro that the rate of hydrogen formation from lactulose is similar to the hydrogen formation from normally ingested mono- or disaccharides (28).

Breath samples were collected before test meal ingestion and every 30 min for 15 h after consumption of the test meal. The exhaled hydrogen concentration was analyzed by GC (TGA-2000, TERAMECS) and expressed as parts per million (ppm).

We used interval sampling of the end-expiratory hydrogen concentrations, which were collected in a collection bag fitted with a T-piece. One side of the T-piece connected with a mouthpiece attached to the discard bag (400 mL) and the other side was attached to the collection bag (500 mL). First, air in the dead space was gathered into the discard bag. After that, expiratory air was taken into the collection bag using the 1-way valve. Gas (35 mL) was drawn from the collection bag with a syringe, and the end-expiratory hydrogen concentration was measured.

A previous study showed that hydrogen levels in expiratory air in 95% of ~900 fasting Japanese subjects did not exceed 19 ppm (29); thus, we defined significant fermentation (i.e., positive cases) as a continuous rise in hydrogen in the expiratory air of >19 ppm. In addition, we used the lowest hydrogen concentration as the basal value, and calculated the total hydrogen released as the area under the curve (AUC), which was the triangulated areas under the hydrogen concentrations vs. the time curves (27). The AUC was calculated as follows:

$$\begin{aligned}\text{AUC (ppm} \cdot \text{min)} = {} & [(C_1 - C_b) + (C_2 - C_b)] \\ & \times \tfrac{1}{2} \times t_1 + [(C_2 - C_b) + (C_3 - C_b)] \\ & \times \tfrac{1}{2} \times t_2 \cdots [(C_{n-1} - C_b) + (C_n - C_b)] \times \tfrac{1}{2} \times t_{n-1}\end{aligned}$$

which, if the intervals are of equal length ($t_1 = t_2 = \ \ldots \ = t_{n-1}$ = 30 min) can be reduced to the following:

$$\begin{aligned}\text{AUC (ppm} \cdot \text{min)} = {} & (\tfrac{1}{2} \times C_1 + C_2 + \ \cdots \ + C_{n-1} \\ & + \tfrac{1}{2} \times C_n - (n - 1) \times C_b) \times 30\end{aligned}$$

where $C_1, C_2, \ \ldots \ , C_{n-1}, C_n$ = hydrogen concentrations in ppm at the beginning and end of each interval, $n-1$ = number of intervals, n = total number of hydrogen concentration measurements, and C_b = basal hydrogen concentration.

Bacteriological analysis. We analyzed fecal flora as colonic bacteria, because Moore et al. (30) demonstrated that the intestinal microflora did not differ among any parts of the colon. The first fecal sample was taken from the first daily defecation. The specimens were immediately stored at 4°C in the laboratory until analysis. Fecal sampling was performed for 2 d before the beginning of the first experiment, twice for each subject, and each collection was separated by at least 7 d.

The fecal flora were analyzed using the processes reported by Mitsuoka et al. (31,32) and with the heat treatment from the report by of Terada et al. (33). Briefly, the specimens were weighed and homogenized with 10- or 100-fold volumes of sterile anaerobic diluent under a CO_2 atmosphere, and a series of 10-fold dilutions (10^{-2}–10^{-8}) were made in the same diluents. From appropriate dilutions, 0.05-mL aliquots were spread over 4 nonselective agar plates for fastidious anaerobes (modified medium 10); anaerobes (modified Eggerth-Gagnon agar: EG and glucose blood liver agar: BL); and aerobes (trypticase soy blood agar: TS) (31,32). Aliquots (0.05 mL) were also spread on 11 selective agar media for bifidobacteria (bifidobacteria selective agar: BS); eubacteria (eubacteria selective agar; ES); bacteroides (neomycin-brilliant green-taurocholate-blood agar: NBGT); lecithinase-positive clostridia (neomycin-nagler agar: NN); *Veillonella* and megasphaera (modified *Veillonella* selective agar: VS); lactobacilli (modified lactobacilli selective agar: LBS); enterococci and streptococci (triphenyltetrazolium chloride-acridine orange-thallous sulfate aesculin crystal violet agar: TATAC); staphylococci (staphylococcus 110 agar: PEES); Enterobacteriaceae (deoxycholate hydrogen sulfide lactose agar: DHL); *Pseudomonas aeruginosa* (nalixidic acid cetrimide agar: NAC); and yeasts and molds (potato dextrose agar: PD) (31,32).

In addition, dilutions (10^{-1}–10^{-5}) of the fecal specimens were heated at 80°C for 10 min to select clostridial spores, and 0.1 mL of each dilution was inoculated onto BL agar and *Clostridium welchii* agar (Nissui Seiyaku). Medium 10 was used in the "Plate-in-bottle" method with incubation at 37°C for 4 d. Each of the EG, BL, BS, NBGT, ES, NN, VS, and LBS agar plates was incubated at 37°C for 3 d in an anaerobic "steel-wool" jar filled with an atmosphere of oxygen-free CO_2. Each of the TATAC, PEES, and PD agars, and TS and DHL agar plates was incubated aerobically at 37°C for 48 and 24 h, respectively. After incubation, each plate was examined for

[4] The balanced meal contained 1674 kJ: carbohydrates, 41.7 g; lipid, 22.2 g; protein, 8.2 g; sodium, 370 mg; potassium, 100 mg; calcium, 200mg; iron, 3.4 mg; magnesium, 50 mg; phosphate, 100 mg; retinal, 270 μg; thiamine, 0.6 mg; riboflavin, 0.6 mg; vitamin B-6, 0.8 mg; vitamin B-12, 1.2 μg; niacin, 8.5 mg; pantothenic acid, 3 mg; folic acid, 100 μg; vitamin C, 50 mg; calciferol, 1.25 μg; tocopherol nicotinate, 4 mg; resistant starch NOVELOSE240J, 2 g; Calorie Mate BLOCK, Otsuka Pharmaceuticals, Tokyo, Japan

[5] The liquid diet contained 837 kJ: glucide, 33.35 g; lipid, 4.45 g; protein, 6.65 g; sodium, 281 mg; potassium, 152 mg; chlorine, 310 mg; calcium, 78 mg; iron, 1.3 mg; magnesium, 29.5 mg; phosphate, 105 mg; retinal, 66.6 μg; thiamine, 0.11 mg; riboflavin, 0.13 mg; vitamin B-6, 0.23 mg; vitamin B-12, 0.56 μg; niacin, 1.65 mg; pantothenic acid, 1.11 mg; folic acid, 60 μg; vitamin C, 5.55 mg; calciferol, 0.56 μg; tocopherol nicotinate, 3 mg; no DF; HINEX-R, Otsuka Pharmaceuticals.

bacterial colonies. The identification of bacterial groups and yeasts and molds was performed with colonial and cellular morphologies, Gram reaction, spore formation, and aerobic growth. The bacterial counts per gram of wet feces were calculated and converted into a logarithmic equivalent for each bacterial species identified. The total viable count was calculated from the sum of the counts of each bacterial species.

Statistical analysis. The breath hydrogen data were classified into patterns, and subjects were grouped according to their pattern (as described in the Results section). ANOVA with a Bonferroni-type multiple comparison test was used to identify differences in bacterial counts. The chi-square test was used to assess differences in the frequency of occurrence among the three groups. The differences were considered significant at $P < 0.05$.

RESULTS

End-expiratory hydrogen concentrations. Subjects were classified into the following three main patterns (**Table 1**) on the basis of their hydrogen breathing patterns: positive cases after ingestion of lactulose and pectin (Group LP, $n = 4$); positive cases after ingestion of lactulose alone (Group L, $n = 7$); and negative cases after ingestion of pectin, cellulose, and lactulose (Group N, $n = 3$).

For example, in 4 of 14 subjects (29%), breath hydrogen levels after ingestion of pectin were positive, and 10 subjects (71%) were negative. Furthermore, individual differences in hydrogen concentration were found. End-expiratory hydrogen maximal values range from 58 to 130 ppm and AUCs from 15000 to 30000 (ppm · min) (Table 1). Similarly, there was large variation among these variables after ingestion of lactulose (data not shown).

Bacteriological analysis. The detection frequency of lecithinase-negative clostridia was higher in Group LP than in Groups L and N ($P < 0.05$), and both the detection frequency and the number of lecithinase-positive clostridia were higher in Groups LP and L than in Group N ($P < 0.05$) (**Table 2**). The detection frequency of yeasts was also higher in Groups LP and L than in Group N ($P < 0.05$). However, the frequency of occurrence of *Veillonella* was lower in Groups LP and N than in Group L ($P < 0.05$). The groups did not differ in the frequency or counts of other organisms.

DISCUSSION

This study yielded two major findings concerning the hydrogen-breath test. One was that different types of DF are fermented to various extents in the large bowel; another finding was that patterns of fermentation differ among individuals despite ingestion of same amounts of the same DF.

Few studies have evaluated simultaneously the fermentation of both cellulose and pectin in the large bowel using the hydrogen-breath test. Wolever et al. (4) reported that 15 g of pectin or cellulose had no effect on breath hydrogen levels in 8 subjects. Tadesse et al. (19) measured breath hydrogen concentrations after single-dose feedings of DFs in 8 subjects, and 20 g of pectin or cellulose did not affect breath hydrogen. Such negative results are different from the results of other studies that used other DFs such as resistant starch (RS) (4–6,12,20,34). Ranganathan et al. (6) reported a significant rise after ingestion of 30 g of RS (lintner), and Hallfrisch et al. (34) reported that all 26 subjects had a significant rise in expiratory hydrogen levels after ingestion of breads made with white flour or whole wheat flours. Furthermore, these studies did not analyze individual colonic flora in the subjects.

In our study, only 4 of 14 volunteers had positive expiratory hydrogen levels after ingestion of 20 g of pectin, although no subject had a positive level after the ingestion of cellulose. The results suggested that pectin was more fermentable than cellulose in vivo as well as in vitro. Pectin is rapidly fermented by fecal bacteria, with maximum hydrogen production for pectin occurring at 4 h, whereas cellulose fermentation does not begin until after at least 12 h (35).

On the other hand, lactulose, a synthetic disaccharide (4-O-β-D-galactopyranosyl-D-fructofuranose), is given orally and passes through the small intestine into the large bowel, where it is rapidly fermented by bacteria with the formation of

TABLE 1

Comparison of fermentation of cellulose, pectin, and lactulose using the hydrogen breath test in 3 groups of men who were positive (+) for lactulose (L) or lactulose and pectin (LP) or negative (−) for cellulose, pectin, and lactulose (N)

Group	Subject	AUC[1] (ppm · min)	Cellulose H2-peak[2] (ppm)	Fermentation[3]	AUC (ppm · min)	Pectin H_2-peak (ppm)	Fermentation	Lactulose fermentation
LP	A	5235.0	28	(−)	15412.5	64	(+)	(+)
	B	5445.0	12	(−)	32985.0	130	(+)	(+)
	C	1440.0	7	(−)	21075.0	112	(+)	(+)
	D	1725.0	5	(−)	14940.0	58	(+)	(+)
L	E	7800.0	6	(−)	9450.0	26	(−)	(+)
	F	3840.0	12	(−)	6735.0	15	(−)	(+)
	G	2220.0	12	(−)	5505.0	14	(−)	(+)
	H	840.0	3	(−)	3375.0	12	(−)	(+)
	I	360.0	1	(−)	1950.0	8	(−)	(+)
	J	165.0	1	(−)	360.0	1	(−)	(+)
	K	870.0	1	(−)	180.0	1	(−)	(+)
N	L	585.0	3	(−)	330.0	2	(−)	(−)
	M	285.0	1	(−)	90.0	1	(−)	(−)
	N	3180.0	8	(−)	0.0	0	(−)	(−)

[1] AUC (ppm · min) = $\frac{1}{2} \times C_1 + C_2 + \cdots + C_{n-1} + \frac{1}{2} \times C_n - (n - 1) \times C_b) \times 30$ where $C_1, C_2, \ldots, C_{n-1}, C_n$ = hydrogen concentrations in ppm at the beginning and end of each interval, $n - 1$ = number of intervals, n = total number of hydrogen concentration measurements, and C_b = basal hydrogen concentration.

[2] H_2-peak, hydrogen peak levels in the measurement of end-expiratory hydrogen concentrations.

TABLE 2

Fecal bacterial counts and the frequency of occurrence according to the species in 3 groups of men who were positive for lactulose (L) or lactulose and pectin (LP) or negative for cellulose, pectin, and lactulose (N)[1]

Organism	Group		
	LP (n = 4)	L (n = 7)	N (n = 3)
	log_{10} counts/g wet feces		
Total bacteria	10.87 ± 0.19	10.79 ± 0.22	10.92 ± 0.09
	log_{10} counts/g wet feces (% positive subjects)		
Enterobacteriaceae	6.77 ± 1.12 (100)	7.70 ± 0.69 (100)	7.63 ± 0.88 (100)
Streptococci	6.68 ± 0.88 (100)	7.40 ± 0.59 (100)	7.71 ± 0.37 (100)
Staphylococci	3.00 ± 0.27 (75)	2.99 ± 0.43 (71)	2.66 ± 0.28 (100)
Yeasts	3.45 ± 1.15 (50)[a]	4.43 ± 1.68 (57)[a]	0.00 ± 0.00 (0)[b]
Pseudomonas	4.20 ± 0.07 (75)	3.78 ± 0.98 (86)	3.86 ± 0.45 (67)
Lactobacilli	5.21 ± 1.10 (50)	6.18 ± 1.29 (86)	5.25 ± 0.05 (67)
Bifidobacteria	9.94 ± 0.34 (100)	9.97 ± 0.25 (100)	9.68 ± 0.92 (100)
Eubacteria	9.59 ± 0.25 (100)	10.13 ± 0.24 (100)	10.30 ± 0.15 (100)
Bacteroidaceae	10.58 ± 0.16 (100)	10.43 ± 0.33 (100)	10.65 ± 0.10 (100)
Peptococcaceae	9.61 ± 0.51 (100)	9.70 ± 0.27 (100)	9.46 ± 0.21 (100)
Clostridia (Lecithinase +)	6.75 ± 0.45[a] (50)[a]	5.69 ± 0.91[a] (29)[b]	0.00 ± 0.00[b] (0)[b]
Clostridia (Lecithinase −)	8.88 ± 0.79 (100)[a]	9.18 ± 0.44 (57)[b]	7.20 ± 0.00 (33)[b]
Veilonella	0.00 ± 0.00 (0)[b]	7.22 ± 1.31 (57)[a]	0.00 ± 0.00 (0)[b]

[1] Values are means ± SD. Means in a row without a common letter differ, $P < 0.05$.

SCFAs, lactic acid, hydrogen, and so on (36,37). In this sense, it should be called a "DF-like substance" according to the definition of DF, although it differs from the definitions suggested by Western researchers, by which DFs must be of "plant origin" (1,38–42). In the present study, fermentation of lactulose, as shown by the hydrogen-breath test, occurred in 11 of 14 volunteers. The results suggested that the disaccharide lactulose was more fermentable than the polysaccharide pectin or cellulose in the same subject. Therefore, all subjects were divided into 3 patterns according to the results of the hydrogen-breath test: Group LP, Group L, and Group N.

Considering the transit time from the mouth to the terminal ileum in previous studies (2,4,13,19,36,37,43), our experiment time allowed all of the cellulose, pectin, and lactulose sufficient time to enter the large intestine and be fermented. Therefore, this grouping was strongly affected by the difference in fecal microflora among the subjects. In an analysis of fecal microflora, the frequency of detection for clostridia differed significantly among individuals. These findings suggest that the distribution of the subjects into the three groups (Group LP, Group L and Group N) depended on the numbers of clostridia bacteria present in each subject.

In vitro, clostridia convert substrates such as DF into hydrogen gas as efficiently as enterobacteriaceae and eubacterials (44,45). However, our results suggest that clostridia contributed to significant increases in hydrogen levels during the breath test results after ingestion of pectin. In other words, grouping by breath test after ingestion of polysaccharides and oligosaccharides, which are resistant to digestion and absorption in the small intestine, may enable us to predict the volume of clostridia in the large intestine.

Several of the *Clostridium* species are associated with a large number of diseases (46–50); the most commonly isolated species are C. *perfringens* (46–48) and C. *butyricum* (51–54). In addition, a decreased level of lecithinase-negative clostridia lowers intestinal putrefactive products and may lead to improvement in the intestinal environment (33,37,55). It would therefore seem that the intestinal environment was poorer in Group LP than in the other groups. Previous studies have reported that pectin enhanced 1,2-dimethylhydrazine–induced colon carcinogenesis in rats (14), and β-glucuronidase, which is plentiful in many *Clostridium* species, promotes colorectal carcinogenesis (56,57). Thus, there is a possibility that pectin might increase the numbers of clostridia and the level of β-glucuronidase activity, thereby promoting colorectal carcinogenesis.

The current research is based on the premise that increased concentrations of SCFAs, and butyrate in particular, reduction in pH, or a combination of these factors results in growth inhibition and diminished toxin production by several of the *Clostridium* species (i.e., C. *difficile*) in vitro (15). Therefore, long-term ingestion of DF in Group LP subjects might improve their intestinal environment because fermentation of DF yields SCFAs as major end-products.

In this study, the detection frequency of yeasts were also higher in Groups LP and L than in Group N ($P < 0.05$). However, yeasts had no influence on the hydrogen breath test because the number was very small ($\sim 10^3$–10^4). The detection frequency of *Veillonella* was higher in Group L than in Groups LP and N ($P < 0.05$). *Veillonella* produce acetate, propionate, CO_2, and hydrogen from lactate (58). Therefore, *Veillonella* may play a role in the fermentation of lactulose, although the role of *Veillonella* in colonic bacterial flora is unknown at present.

Not all fermentation in the large intestine produces hydrogen, and there should be other fermentation products such as CO_2, methane, and SCFAs because Van Soest (59) reported that half of ingested cellulose or hemicellulose disappears (i.e., is fermented) in the large intestine, and most ingested pectin disappears. We cannot know precisely what proportion of DF fermentation was evaluated by the hydrogen-breath test. Therefore, measurement of other variables, especially fermented substances from DF such as SCFAs in the large intestine, is warranted to evaluate DF fermentation comprehensively.

Another major finding was that there were noted individual differences in the pectin fermented group, with a 3.9-fold variation in both the AUC and peak value. Such individual variation was reported in previous studies; a 2.3-fold variation in the H_2-peak in a 20-g lactulose fermented group (n = 8) (27) and a 2.9-fold variation in a 50-g raw potato starch fermented group (n = 7) (5). Indeed, in our study, the 2 subjects who produced the most hydrogen had the highest detection frequency of clostridia. This finding suggested that

the individual differences in hydrogen production depend on the number of clostridia.

It is not known whether the ecosystem of colonic microflora is connected with the increase in clostridia, and it is therefore necessary to design further studies with larger subject populations. The comparatively small population in the present study might have imposed a limitation on the study. The advantage of the breath test is that it does not require a great deal of skill for measurement of hydrogen concentrations; many breath samples can be analyzed at once and results are available immediately. The hydrogen-breath test is a simple and noninvasive method compared with the measurement of the fermentation of carbohydrates by colonic microflora, making it a useful test for the evaluation of the intestinal fermentation of DF and intestinal bacteria, especially clostridia.

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