Influences of Different Types of Dietary Fibers to The Fermentation in The Intestinal Flora

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ABSTRACT

Purpose: One of the treatment of chronic constipation is intake of dietary fiber. This experimental study was designed to determine the effect of different type of dietary fibers on the microbiota in the large intestine.

Methods: Nine healthy volunteers participated to this study. To determine the fermentation of dietary fiber, breath hydrogen test was indicated. As hydrogen is not produced by the metabolism of mammalian cells, excretion of hydrogen from breath enhance activity of intestinal bacteria. Overnight fasted participants ate 200 gram of white bread with 10 gram of different type of dietary fiber. Tested dietary fibers were 1) cellulose, 2) soy fiber, 3) guar gum, and 4) control (without eating any dietary fiber). Before and every one hour after feeding for 8 hours, breath hydrogen samples were collected. Another test was compared the effect between cellulose and guar gum with a loaded food which activates the fermentation in the intestine. Breath hydrogen samples from same healthy volunteers were collected using same methods.

Results: During 8 hours of measurements, in the soy fiber group, the concentration of breath hydrogen were higher than the control group while they were not significantly different. The changes of the guar gum group were similar to the control group. In contrast with other group, the concentration of breath hydrogen in the cellulose group were not increased even after eating white bread which caused fermentation in the large intestine. The concentration after 8 hours of cellulose intake was $2.9 \pm 0.7$ ppm, and it was significantly lower than that of guar gum group ($7.4 \pm 1.7$ ppm, $p < 0.01$). In additional study with a well fermented food intake, cellulose reduced the increase of breath hydrogen concentration, while the difference with that of guar gum group did not reach statistically significant.
**Conclusion**: Cellulose might have suppressive effect on the fermentation in the large intestine. This compound may have possibility of the favorable benefit for the treatment of chronic constipation.

**INTRODUCTION**

Microorganisms in the lower gut ferment dietary fiber and produce hydrogen, methane and carbon dioxide gases. Some portion of these gases enters the blood stream and is excreted via the lungs\(^1\)\(^-\)\(^3\). The hydrogen breath test, which is based on the premise that hydrogen gas in humans is produced exclusively by colonic fermentation, uses levels of expired hydrogen as an indirect measure of disturbance of the intestinal flora\(^1\)\(^,\)\(^4\)\(^,\)\(^5\). The test is widely used to detect a battery of non-structural gastrointestinal disorders, particularly carbohydrate malabsorption, small intestinal bacterial overgrowth and irritable bowel syndrome. The breath test is also used in studies of food metabolism and various indicators of intestinal flora\(^6\)\(^-\)\(^18\).

For the treatment of constipation, dietary fiber is one of the most important tools, as they make feces bulky and add water\(^19\)\(^,\)\(^20\). However, some of the fibers cause excessive fermentation in the intestine and it is related to diarrhea or gas production\(^21\). While good dietary fiber for the treatment of constipation is less fermented substrate, it is difficult to determine which fiber is the best. The purpose of this study is to demonstrate the comparison of dietary fibers and determine the suitable to resolve constipation.

**PATIENTS AND METHODS**

*Basal analysis: fasting breath hydrogen data on Japanese subjects*

Thirty-five volunteers (21 men and 15 women, aged 21-65 years) fasted after their
usual dinner until the following morning (~0800) when hydrogen breath tests were
conducted at Hiroshima University School of Medicine. End-alveolar breath samples
were obtained by having the subjects exhale end-expiratory samples into 500-ml plastic
bags fitted with stopcocks. Samples were analyzed for hydrogen concentration with a
HCMA-T1™ Gas Chromatograph (Abilit Corporation, Osaka, Japan). The data were
presented as normalized breath-hydrogen concentrations in parts per million (ppm).

**Effect of dietary fiber intake on breath hydrogen**

Nine healthy volunteers (5 males and 4 females) were recruited to this study. Average age of the subjects was 35.4 years old. All participants fasted more than 12 hours after their evening meal until the following morning. At 7 am, the subject ate 200 gram of white bread with 10 gram of dietary fiber. Tested dietary fibers were 1) cellulose, 2) soy fiber, 3) guar gum, and 4) control (without eating any dietary fiber). These dietary fibers were purified powder without any impurities (provided by Ajinomoto Co. Inc. Japan). All subjects tested three kinds of dietary fibers and control. Each test was carried out at least 7 days interval. Before and every one hour after feeding for 8 hours, breath hydrogen samples were collected in following methods.

Another test was conducted to determine the difference between guar gum and cellulose on fermentation in the intestine. Same healthy volunteers described above were fed 10 gram of guar gum or cellulose plus one hamburger. The hamburger was commercially available product, named “Cheese Burger” (vans, beef putty, sliced cheese, and baked onion were contained. McDonald’s, Japan). The hamburger was expected well fermented compared with white bread because not only bread but also beef, cheese, and onion were included. If guar gum or cellulose could reduce the
fermentation in the large intestine, changes of excretion of breath hydrogen could be different. Time schedule of fasting, breath sample collection, or interval of study was same with above examination.

**Hydrogen breath test**

End-alveolar breath samples were obtained into 500-ml plastic bags fitted with stopcocks. The bags were used the GaSampler System (Quintron Instruments, Milwaukee, WI) as described previously\(^2\). The subjects were instructed to exhale as deeply as possible, to obtain alveolar air, directly into the apparatus via a mouthpiece. A 5-ml aliquot of each breath sample was transferred to a silicone-greased plastic syringe fitted with a three-way plastic stopcock. Samples were analyzed for H\(_2\) concentration with a HCMA-T1\(^\text{TM}\) Gas Chromatograph (Abilit Corporation, Osaka, Japan). The data were presented as normalized breath-H\(_2\) concentrations in parts per million (ppm). During eight-hour study period, any food or drink containing sugar was not allowed.

**Statistical Analysis**

All measured results were expressed as means concentration. The data were analyzed using the Student's t-test, with \(p < 0.05\) used to indicate a significant difference.

**Ethical considerations**

This study was approved by the Medical Ethics Committee of Hiroshima University School of Medicine and signed informed consent was obtained from all participants. The study was carried out in accordance with the Declaration of Helsinki.
RESULTS

Basal analysis: fasting breath hydrogen data on Japanese subjects

The breath hydrogen concentrations of 35 subjects were determined after overnight fasting (Fig. 1). The average of breath hydrogen concentration in fasting status was 7.2 ± 8.7 ppm. The 5 subjects with an increase in hydrogen concentration of more than 20 ppm were classified as having diabetes (HgA1c was more than 6.0%). The 23 subjects with an increase of less than 10 ppm were classified as normal metabolizers. These results indicated that fasting breath hydrogen concentration of healthy subjects was stable within 10 ppm.

Effect of dietary fiber intake on breath hydrogen

Changes of breath hydrogen concentration after intake of each dietary fiber were demonstrated in Fig. 2. Control group, which was only white bread intake without any additional dietary fiber showed increased concentration of breath hydrogen 5 hours after intake. This changes means intestinal contents (eaten white bread) were fermented when it reached to the large intestine. In the soy fiber group, the concentration were higher than the control group while they were not significantly different. The changes of the guar gum group were similar to the control group. Interestingly, the concentration of breath hydrogen in the cellulose group were not increased even after eating white bread which caused fermentation in the large intestine. The concentration after 8 hours of cellulose intake was 2.9 ± 0.7 ppm, and it was significantly lower than that of guar gum group (7.4 ± 1.7 ppm, p < 0.01). This result suggested that cellulose itself was less
fermented in the large intestine, and moreover, it might suppress fermentation of eaten dietary fiber.

Another study using additional food also demonstrated difference between guar gum and cellulose (Fig. 3). The concentrations of breath hydrogen after 8 hours in cellulose group (2.9 ± 1.2 ppm) were lower than that in guar gum group (6.0 ± 2.1 ppm), although the difference did not reach statistically significant (p = 0.06). In these studies, none of the subjects reported adverse events or withdrawal.

**DISCUSSION**

The health benefits of dietary fiber have been appreciated. For the treatment of constipation, dietary advice is based on intake of dietary fiber. Dietary fibers are resistant to hydrolysis by enzymes in the small intestine and unabsorbed to the large intestine. The fibers retain water and make feces bulky, and also reduce the intestinal transit time. As side effect of dietary fibers, they sometimes cause gas production and diarrhea resulting from excessive fermentation. As each individual have different intestinal flora, compatibility of dietary fibers is variable. Ideal fibers are water-soluble, unabsorbed, and less fermented to avoid such side effect. However, few number of studies were available to determine the effect of different type of dietary fibers. Thus, in present study, we investigated bacterial reaction after eating dietary fiber using breath hydrogen test.

Hydrogen is not produced by the metabolism of mammalian cells. It is only formed in the body by bacterial fermentation of carbohydrates in the intestine. The anaerobic fermentation of carbohydrates results in the production of carbon dioxide, methane, and
hydrogen. These gases are consumed by bacteria or are quickly absorbed into the bloodstream\textsuperscript{1,2,4,5}. Individual hydrogen production can be studied by means of a breath test using lactulose (4-\textit{O}-\textit{b}-\text{D}-galactopyranosyl-\text{D}-fructose) as a substrate\textsuperscript{3, 26-28}. This synthetic carbohydrate is not absorbed in the small intestine and is fermented in the large intestine. The fermentation process and subsequent metabolic processes result in the production of gasses which are absorbed by the colonic mucosa and exhaled. Therefore, breath hydrogen measurements provide a semi-quantitative assessment of the quantity of soluble carbohydrate reaching the large intestine\textsuperscript{29, 30}.

Levine et al.\textsuperscript{12} measured breath hydrogen concentration to determine the association between individual fecal microflora and the fermentation of dietary fibers. They were able to associate anaerobic species with hydrogen production, suggesting that breath hydrogen concentration reflects the activity of anaerobes in the large intestine. Previous measurements regarding the activity of colonic anaerobes had been based on bacterial counts in the feces or mucosal tissues. However, a fecal sample from a patient is not easy to collect and the costs of the counts are high. Moreover, bacterial counts do not always reflect the activity of the flora.

The volunteers in this study had breath hydrogen concentrations of 0-40 ppm. As most cases were within 10 ppm, the baseline concentration was stable in many subjects. However, high hydrogen concentrations (more than 25 ppm) were observed in some cases. Those patients were classified as diabetic (HbA1c was more than 6.0%), since glucose metabolic abnormalities exert a great influence on the concentration of fasting breath hydrogen\textsuperscript{29}. In other words, breath hydrogen concentration in healthy subjects is a reliable tool to know the anaerobic activity of intestinal flora.

In this study, we compared three kinds of dietary fiber those were commonly used
to resolve constipation. Guar gum is a polysaccharide composed of the galactose and mannose as a typical fermentable fiber, while the amount of guar gum has added to foods or supplements as natural dietary fiber. Soy bean is also used as dietary fiber contains various kinds of carbohydrates, such as disaccharide, trisaccharide, and tetrasaccharide. Cellulose was selected as a difficult to degrade dietary fiber\textsuperscript{12}. These dietary fibers were commercially available and commonly used in various food, but the difference of influences to intestinal bacteria had not been investigated.

The different effect of these dietary fibers on the fermentation in the colon were demonstrated in this study. Interestingly, breath hydrogen concentration in soy fiber group was higher than other group from early period after intake. While the difference was not significant, soy fiber might have stimulus effect on intestinal bacteria. Cellulose significantly reduced the fermentation in the intestine, compared with other dietary fibers and even without eating any dietary fiber. Additional study using additional food intake still demonstrated reduced effect on the fermentation compared with guar gum, although the difference did not reach significance. Now, there are two questions. At first, why could cellulose reduce the fermentation in the large intestine? And second, was this reduction beneficial for the colonic metabolism?

Even in recent meta-analysis, it remained unclear which type of fiber was the most effective on chronic constipation\textsuperscript{20}. Some study attempted to show the difference of fiber from the point of view of microbiota, but potential relationship between cecal microbiota and dietary fiber was still unclear\textsuperscript{31}. The one of the reasons of this issue was heterogeneity of colonic microbiota. As the heterogeneity may influence the results, each personal demonstrates different effect of dietary fiber on constipation, especially side effect of the fibers such as diarrhea or excessive gases.
Our results indicated the reduction of the fermentation in the large intestine with small variation between study subjects. This results may suggest that cellulose was not affected by different colonic microbiota as it was difficult to degrade. It was also beneficial because cellulose was not absorbed by the intestine that means less side effect. Moreover, as fermentation lead to produce intestinal gas and water, reduction of fermentation could avoid diarrhea. In conclusion, although further investigation is needed, cellulose may have possibility of the favorable tool for the treatment of chronic constipation without any harmful effect.

REFERENCES

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**Figure Legends**

**Fig. 1  Fasting breath hydrogen data on healthy Japanese subjects**

The average of breath hydrogen concentration in fasting status was 7.2 ± 8.7 ppm. In all subjects except five with diabetes, the concentration of breath hydrogen in fasted status was stable with less than 15 ppm.

**Fig. 2  Changes of breath hydrogen concentration after intake of each dietary fiber**

In the soy fiber group, the concentration were higher than the control group while they were not significantly different. The changes of the guar gum group were similar to the control group. The concentration of the cellulose group were not increased even after eating white bread. The concentration after 8 hours of cellulose intake was 2.9 ± 0.7 ppm, and it was significantly lower than that of guar gum group (7.4 ± 1.7 ppm, p < 0.01, Student's t-test).

**Fig. 3  Comparison between cellulose and guar gum with well fermented food intake**

The concentrations of breath hydrogen in cellulose group were lower than that in guar gum group, although the difference did not reach statistically significant (p = 0.06, Student's t-test).