Everyday Breath Hydrogen Excretion Profile in Japanese Young Female Students

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Abstract A breath hydrogen test has been used widely as a noninvasive and simple method of detecting carbohydrate malabsorption as well as estimation of the small intestinal and orocecal transit time. By means of this method, we have examined the change in breath hydrogen concentration of young female students in their everyday life in order to reveal the breath hydrogen excretion profile under normal circumstances. In this survey, we have asked them to collect their own breath samples every one-hour as regularly as possible during one day from awakening until bedtime. We also asked them to complete the questionnaire concerning their dietary habit, dietary record and physical activities. Among the 43 subjects who gave the breath hydrogen records, 37 subjects excreted detectable hydrogen into their alveolar air. By comparing the changes in breath hydrogen concentration during the time of day, breath hydrogen excretions could be classified into two distinct patterns; more than half of the total hydrogen excretion occurred in the first half of the waking hours (designated as “pattern A”, 18 cases) and in the latter half (designated as “pattern B”, 19 cases). Taking into consideration the subjects’ records of diets and physical activities, the early-pronounced breath hydrogen excretion observed among 18 “pattern A” students was probably resulted from the malabsorption of the dietary carbohydrate in the breakfast meals. *J Physiol Anthropol* 19 (5): 229-237, 2000 <http://www.jstage.jst.go.jp/en/>

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Introduction

The function of the gastrointestinal system is to provide nutrients for the body. In this sense, it is the most essential function for human beings. However, recent changes in the lifestyle seem to influence the gut function

to develop some gastrointestinal disorders. Recently, for example, many young women complain about chronic constipation and many people suffer from peptic ulcer probably partially due to the deviation from normal dietary habits including irregular mealtimes and stressful eating behaviour. Thus, we have been studying the effects of the lifestyle factors on the function of the gastrointestinal system in terms of the transition and digestion of foods in the gut tract by means of a breath hydrogen test.

Breath hydrogen test has been used widely as a noninvasive and simple method of detecting carbohydrate malabsorption (Levitt et al., 1970) as well as determining small intestinal and orocecal transit time (Hirakawa et al., 1988). It is based on the ability of the anaerobic microorganisms inhabiting in the colon to ferment carbohydrate that has traveled unabsorbed through the small intestine, and to produce hydrogen as a metabolic by-product. This hydrogen traverses the gut wall, is transported to the lungs and is expelled through breathing. Therefore, a rise in concentration of hydrogen in serially collected exhaled breath samples after a carbohydrate-containing meal can be used to indicate its arrival at the cecum, and an increase in the rate of breath hydrogen excretion can be used to indicate the carbohydrate malabsorption at the small intestine.

Concerning the dietary carbohydrate in the meals, starchy foods generally (but not always) yield so-called “resistant starch” which can escape into the large bowel without being digested (Annison et al., 1994). Thus, even under physiological conditions, most starches are not completely digested by the gastrointestinal tract, and a substantial proportion, up to 20%, may remain undigested and reach the cecum to be fermented by the colonic microorganisms (Stephen et al. 1983). Among the dietary carbohydrates in the normal diet, Wolever et al. (1992) showed that the fermentable and non-fermentable dietary fibers have no effect on breath hydrogen levels over 12 hour in subjects previously consuming a normal diet.

Thus, we could suspect that the breath hydrogen levels reflect the degree of the digestion and the absorption of dietary starch in diet.

The breath hydrogen measurements have been widely applied in clinical medicine for the detection of carbohydrate malabsorption, in which most studies are carried out under controlled conditions including diets and activities (Perman et al, 1991; Read et al, 1980). Thus, the number of investigations about breath hydrogen excretion under normal circumstances is limited (Tadesse et al., 1980; Kneepkens et al., 1985). Prior to the study concerning the effects of after-meal behaviors and skin pressure on the small intestinal transit time of a meal (Sone et al., 1999 and 2000, respectively), we have surveyed the everyday change in breath hydrogen concentration of young female students who had been candidates for the study subjects. The aim of the preliminary survey was to evaluate the fasting levels of breath hydrogen that are important for the estimation of small intestinal transit time. In this survey, we became aware that there are two daytime breath hydrogen patterns, which could be distinguished by the occurrence or absence of the pronounced excretion of breath hydrogen around noon. This finding prompted us to carry out further study on everyday breath hydrogen excretion patterns of young female students from the viewpoint of their lifestyle.

In this paper, we describe the categorization of two different breath hydrogen excretion patterns observed in young female students and discuss the possible cause for the occurrence of the two patterns from the viewpoints of digestion and absorption of dietary carbohydrate in the gastrointestinal tract.

Subjects and Methods

Subjects

The breath sampling examinations were carried out in November of 1998 and in July of 1999, volunteered by 23 and 20 female students, respectively. All were non-smoker and in good health at the time of investigation. Their bowel movements were suspected to be regular according to the answer to the corresponding question about the frequency of defecation in a day. They are aged 21 to 22 years and senior students majoring in nutrition. The mean body weight and height of '98 group and '99 group were 49.5 ± 3.9 kg (mean $\pm$ SD), 160.2 ± 4.9 cm and 49.5 ± 4.0 kg, 159.5 ± 5.0 cm, respectively.

Breath sampling

The subjects were allowed to follow their usual lifestyle, and asked to collect their own breath samples every one-hour as regularly as possible from awakening until bedtime. Therefore, we examined the everyday pattern of breath hydrogen excretion throughout the day under

non-challenge conditions. Breath samples were collected into commercially available collection bag system using a mouthpiece adaptor specially devised to collect end alveolar breath samples (Teramecs Co. Ltd., Kyoto, Japan). The breath samples were collected from the mouth in hermetic bags fitted with a three-way valve. One valve was attached to a disposable mouthpiece through which the subjects breathed. The other two valves were attached to air-discarding bags and air-collection bag. At the end of a forced breath (about after 10 seconds), the end-expiratory air was trapped in the hermetic bag by closing the valves to air-discarding bag and mouth. The breath-collection bags containing sample breath were brought to our laboratory the next day after the collection. The collected end-expiratory air sample (20 ml) was withdrawn by a syringe and then injected into the gas chromatography (MicroLyzer model 12i, Quintron Instruments, Milwaukee, WI, USA) which had been calibrated with a reference gas containing 99 ppm (parts per million) hydrogen in room air following its instruction manual. Thus the hydrogen concentration was expressed in parts per million (ppm).

Meanwhile, they kept a record of time-allocation using a question table for time-usage questionnaire described in a database book published by NHK (NHK, 1995). In addition, we asked the subjects to keep a record of all food items they consumed at six meals and between meals on the day before the breath-sampling day and on the breath-sampling day. We also asked them to fill in the questionnaire concerning their dietary habit using a question table issued by the Osaka City Bureau of Environment and Welfare.

All results are expressed as mean $\pm$ SE. Student's *t*-test and chi-square test were used for the test of significance in comparing the interval scales and the nominal scales, respectively.

Results

Among the 43 subjects who successfully completed the breath-sampling task, 6 subjects excreted less than 10-ppm hydrogen at the maximum (3 subjects in each year). These subjects who excreted less breath hydrogen were expected by the reports in the concerning literatures, and it is assumed that they lack hydrogen-producing bacteria in their colon. Therefore, we conducted further analysis using 37 breath hydrogen records and the accompanied questionnaires. All these 37 subjects proved to take breakfast on the breath-sampling day according to their answers on the questionnaires.

Categorization of breath hydrogen pattern

Because of wide inter-subjects variations in breath hydrogen concentration and irregularity of breath sampling time, in this paper, we referred to the

Fig. 1 Mean hourly ratios of respiratory excretion of hydrogen in each time interval. Based on 37 subjects' breath hydrogen excretion records. Bars represent the SE

Fig. 2 Pie chart representation of a typical proportion of hydrogen excretion in three time ranges. Fig. 2-1 and Fig. 2-2 depict "pattern A" and "pattern B", respectively.

respiratory excretion of hydrogen as the hourly ratio of hydrogen excretion against the total breath hydrogen excreted in the waking hours starting from the time when the subject woke up (let awakening time be 0:00). Those breath hydrogen excretion values were calculated as the area under the corresponding hydrogen concentration vs time curve respectively (Levitt et al, 1987). Figure 1 shows the mean hourly ratios of respiratory excretion of hydrogen of 37 subjects in each time interval. It is obvious that there are two nadirs of hydrogen excretion, at 3:00–4:00 and 9:00–10:00 after waking up. Consequently, we divided the waking hours into 3 time ranges by these nadirs, 0:00 to 3:00, 3:00 to 9:00 and 9:00 to 18:00, and calculated the proportion of hydrogen excretion in each time range against total hydrogen excretion. Figure 2-1

and 2-2 show two typical pie charts depicting the proportion of hydrogen excretion in each time range. We categorized the hydrogen excretion pattern of Fig. 2-1 as "pattern A", where more than half of the total hydrogen was excreted in the first half (0 to 9:00) of the day, and that of Fig. 2-2 as "pattern B", where more than half of the total hydrogen was excreted in the latter half (9:00 to 18:00). According to this categorization, we classified the 37 hydrogen excretion profiles into two groups and found that 9 of 20 cases (1998) and 9 of 17 cases (1999) were categorized as "pattern A", and the remaining 11 and 8 subjects in 1998 and in 1999, respectively, were categorized as "pattern B". Figure 3 shows two typical changes in breath hydrogen levels (ppm) along the time of day (hour). In both patterns, the fasting breath hydrogen

Fig. 3 A typical breath hydrogen record of “pattern A” (●●, subject ID, 982) and that of “pattern B” (▲▲, subject ID, 984). Breath hydrogen levels were expressed as ppm along the time of day (hour).

Fig. 4 Changes in mean hourly ratios of respiratory excretion of hydrogen (%) in each time interval. Mean of “group A” (●●) and “group B” (◆◆). Bars represent the SE.

excretion ceased until 11 o'clock in the morning, however, they differ in the time of the afterward excretion peak; there were two excretion peaks at 1 o'clock and 8 o'clock in the afternoon giving “double peaks” profile in “pattern A”, while there was a small excretion peak around noon

and the breath hydrogen level increased gradually from 5 o'clock in the afternoon in “pattern B”. In these cases, the subjects took breakfast at 8 o'clock and lunch at noon. Figure 4 shows a line chart of the average hydrogen excretion ratios of 18 subjects of “pattern A” and 19

Fig. 5 Schematic comparison of each group's average evening meal time and bedtime on the day before breath-sampling day, and their awakening time, breakfast and noon meal time on breath-sampling day based on their time-usage records.

subjects of "pattern B" (designated each subject group as "group A" and "group B", respectively).

Comparison of time-allocation

Because breath hydrogen excretion could be influenced by the subjects' daily living activities (Cammack et al, 1982) and by its own circadian rhythm (Kagaya et al, 1998), we examined their time-allocation on the day before breath-sampling day and on the breath-sampling day. Figure 5 compares each group's average evening meal time and bedtime on the day before breath-sampling day, and their awakening time, breakfast and noon meal time on breath-sampling day based on their time-usage records. It is apparent that there are no significant difference in the evening meal time and bedtime on the day before breath-sampling day and that of taking noon meal on the breath-sampling day, however, "group A" got up and took breakfast earlier than "group B", consequently the lengths of sleeping and fasting time of the former group were significantly shorter than those of the latter group ($p < 0.01$ and $p < 0.05$, respectively). Figure 6 shows the comparison of whole day time-allocation between the two groups. There are no significant differences between time lengths for commuting, rest/leisure, study work, meals and personal chores, however, "group A" spent longer time for housework than those of "group B" in compensation for their shorter sleeping time.

Comparison of food consumption pattern

As described in the principle of breath hydrogen test, the kind of food items included in the meals could influence the hydrogen exertion profiles (Levitt et al, 1987). Figure 7 shows the comparison of the kinds of cereals consumed by each group at the previous evening meal and at breakfast and noon meal on the breath-sampling day. In this figure and Fig. 8, frequency on y-axis means the total number of food items (Fig. 7) or food groups (Fig. 8) in each meal of each group, which had been written on the subjects' records of meals. This figure shows that there was no difference in the kinds of cereals consumed by each group. In addition, we categorized the food items reported in the dietary records into 18 food groups according to the standard tables of food composition in Japan and compared the food consumption pattern as depicted in Figure 8. These figures show that the kinds of food groups in the previous evening meal was almost the same in both groups, however "group A" took more vegetables than those of "group B" at the breakfast and noon meals on the breath-sampling day.

Statistical analysis showed that there was no significant difference in their everyday dietary habits between the two groups (data not shown).

Discussion

In this study, we found two clearly distinguishable breath hydrogen excretion patterns in everyday life of

Fig. 6 Schematic comparison of whole day time-allocation between the two groups based on their time-usage records.

Fig. 7 Comparison of the kinds of cereals consumed by each group at the previous evening meal and at breakfast and noon meal on the breath-sampling day. "A Dinner" and "B Dinner" mean the dinner of "group A" and "group B", respectively, and so on.

young female students. Among them, the change of "pattern B" was similar to the breath hydrogen excretion profiles previously reported as "normal pattern" (Tadesse et al., 1980; Kagaya et al., 1998), in which the breath hydrogen level fluctuated in a manner with a relatively high level of excretion early in the morning, a decrease in the level around mid-day and some rise early in the afternoon. It has been accepted that this fluctuation appears to be related to the diet taken during the previous hours. The early morning increase in the breath hydrogen (called as a fasting breath hydrogen) is probably due to the previous evening meal and the

afternoon rise is due to the morning meal. These facts are proved by diminished morning increase after long period of fasting or a light evening meal, and the abolishing of the afternoon rise by avoiding breakfast (Tadesse et al., 1980; Levitt et al. 1987). Thus, the change classified as "pattern B" (see Fig. 3) in this study could be regarded as a "normal" breath hydrogen fluctuation manner in everyday life of young female students.

In contrast to the "pattern B", the pronounced breath hydrogen excretion around noon as shown in Fig. 3 (in average ratio, between 3:00 and 9:00 after waking up in Fig. 4) is characteristic of the breath hydrogen excretion profile classified as "pattern A". What causes such a difference in breath hydrogen excretion profiles? Concerning the subjects' exercises/activities, the subjects' time-allocations were not different from each other in both groups except the time length for sleeping and fasting. This similarity in their time-allocations after awakening ruled out the influence of the exercises/activities on the passage of a solid meal through the stomach (Cammack et al., 1982) and on intestinal motility (Soffer et al., 1991).

As described in "Introduction" and the preceding part of this section, the primary breath hydrogen peak after the cease of fasting breath hydrogen excretion is due to the production of hydrogen by the microbial fermentation of the dietary carbohydrates that has been ingested at breakfast and remained undigested to reach the cecum. In the cecum, there are several ways in which sugar monomers can be fermented. Most intestinal nonsporing anaerobes, such as *Eubacterium aerofaciens*, *Eub. budayi*, and *Eub. ramilus*, use the Embden-Meyerhof-

Fig. 8 Comparison of food consumption patterns in each meal of the two groups in terms of food groups.

Parnas pathway to form pyruvate from hexose monomers. Pyruvate may then be metabolized by one of the three main pathways: 1) oxidative decarboxylation to acetyl-

CoA 2) reduction to lactate, or 3) carboxylation to produce oxaloacetate which can subsequently be reduced to succinate, a precursor of propionate. The main pathway to hydrogen production is thought as the oxidative decarboxylation of pyruvate to acetyl-CoA. Ferridoxin is reduced in the process and is then itself oxidized by H^+ with the formation of hydrogen (Robb et al., 1991). Concerning the cereals that provide the colonic microorganisms with the starch, there was no difference in the kinds (see Fig. 7) and the amounts (according to the records of the meals, data not shown) of cereals in the breakfast meal between the “group A” and “group B”. This fact indicates that the breakfast meal of each group contained, in average, almost the same proportion of “resistant” and “digestible” starch. Therefore, the pronounced breath hydrogen excretion characteristic for the “pattern A” should be due to the malabsorption of the digestible starch in the morning meal. In other words, the moderate elevation of the breath hydrogen excretion at the early afternoon observed in “pattern B” (between 12:00 and 16:00 hr in Fig. 3 and 4-5 to 8-9 in Fig. 4) should reflect the fermentation of “resistant starch” by the intestinal microorganisms, which occurs in the physiological conditions (Stephen et al., 1983). While in “pattern A”, the additional breath hydrogen excretion occurred owing to the “unusual” fermentation of the digestible starch in addition to the “physiological” fermentation of “resistant starch”. As described in the result section and depicted in Fig. 8, “group A” consumed more vegetables than “group B”. But this higher consumption of vegetables that contains high dietary fiber does not directly relate to the pronounced breath hydrogen excretion because Levitt et al. (1987) and Wolever et al. (1992) showed that the dietary fiber was not the source of the hydrogen and had no effect on breath hydrogen levels over 12 hours in subjects previously hours consuming a normal diet. However, we cannot rule out the inhibitory effects of the higher ingestion of dietary fibers due to the higher ingestion of vegetables on the absorption of glucose and maltose produced by the digestion of starch on the small intestinal brush border membrane (Sone et al., 1992).

With the data obtained in this study, it is difficult for us to give a clear answer for the cause of this malabsorption that is suspected to occur in the subjects of “pattern A”. We can only indicate that the significantly shorter lengths of sleeping and fasting time of “group A” affected on the gastrointestinal functions through the unknown mechanism.

The following is a discussion on one of the possible causes for the pronounced excretion of breath hydrogen in “group A”. We suspect that the bacterial overgrowth in the small intestine occurred among the subjects of “pattern A”, because it results in a similar pronounced breath hydrogen excretion as that of “pattern A” in serial breath hydrogen

collection (Riordan et al., 1995). Breath hydrogen test has been widely applied for diagnosing small intestinal bacterial overgrowth as a noninvasive alternative to culture of small intestinal aspirate. In this application, it is currently accepted that the pronounced breath hydrogen excretion in serial breath hydrogen concentrations, which occurs at earlier stage than usual after ingestion of a test meal, results from unabsorbed carbohydrate fermentation by small intestinal overgrowth flora (Davidson et al., 1984). The reason for the small intestinal bacterial overgrowth is unsettled, although the decrease in gastric, biliary, pancreatic, and intestinal secretions, in addition, the reduction in the intestinal motility may result in the bacterial overgrowth of the upper intestine (Sherwood et al., 1992; Ganog, 1999). In this connection, our preliminary study showed that after-meal behaviors affected the small intestinal transit time of a meal (Sone et al., 1999), and skin pressure applied by a girdle suppressed the digestion and absorption of dietary carbohydrate in the small intestine (Sone et al., 2000). These recent results suggest that some everyday lifestyle factors including sleeping and fasting time, could affect the intestinal functions, probably through the autonomic nervous system, to result in the small intestinal bacterial overgrowth. Thus, here we would like to propose the hypothesis that the fluctuation in breath hydrogen as "pattern A" is due to bacterial overgrowth in the small intestine. We need further and well-designed experiments to prove this hypothesis. However, it will be a serious nutritional problem if our speculation turns to be true. This malabsorption could cause almost the same disadvantage as that of skipping breakfast, which can interfere with the learning ability of the young students (Pollit, 1995).

In conclusion, we observed two clearly distinguishable breath hydrogen excretion patterns in everyday life of young female students. One of which followed the similar changes to those previously reported as a "normal" breath hydrogen excretion pattern, while another pattern is characteristic of its earlier and pronounced breath hydrogen excretion after the morning meal.

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