Transitory Laxative Threshold of Trehalose and Lactulose in Healthy Women

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Summary The transitory laxative threshold of a partially digestible disaccharide, trehalose, and an undigestible disaccharide, lactulose, was estimated by the dose-response relation between the test substance and the prevalence of diarrhea in 20 healthy female subjects. The subjects ingested several indicated amounts of trehalose or lactulose once daily 2 to 3 h after a meal. The intake of the test substance was stopped at the dose level that caused diarrhea or when the dose reached the maximal level. A record of physical conditions, gastrointestinal symptoms, and fecal conditions was made by all subjects before and after each ingestion of the test substance. Half the subjects experienced no diarrhea even with the ingestion of the maximal dose level (60 g) of trehalose in this study, and the ingestion of up to 40 g of lactulose caused diarrhea in 75% of all subjects. Abdominal symptoms such as flatus, distension, and borborygmus appeared at high prevalence with lactulose and trehalose ingestion, and the effect of lactulose was significantly stronger than that of trehalose at the same dose level (p < 0.05). The quantity of trehalose and lactulose that induced diarrhea differed greatly from person to person. The transitory laxative threshold was estimated as 0.65 g/kg body weight for trehalose and 0.26 g/kg body weight for lactulose by using the regression equation between the dose levels of the test substances and the cumulative incidence of diarrhea. These results suggest that it would be quite acceptable to administer trehalose up to 33 g and lactulose up to 13 g in a person weighing 50 kg.

Key Words trehalose, lactulose, laxative threshold, diarrhea, maximal noneffective dose

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Oligosaccharides and sugar alcohols have been newly developed as bulking sucrose substitutes with beneficial health effects such as the reduction of available energy, the saving of insulin, the reduction of cariogenicity, and the improvement of intestinal environment (1, 2). Sugar substitutes with these beneficial health effects have been used in such things as soft drinks, chocolates, candies, cookies, pudding, and chewing gum. However, a sufficiently high ingestion of nondigestible sugar substitutes with beneficial health effects causes overt diarrhea, and minor symptoms such as abdominal distension and flatus often arise in animals and humans (3–5). This diarrhea is due to the osmogenic retention of fluid in the small and large intestines and to lactose intolerance. Trehalose and lactulose are disaccharides with beneficial effects, but with sufficient intake they will cause transitory diarrhea.

Trehalose, a nonreducing disaccharide, is found in mushrooms, honey, and baker’s yeast and also in numerous insects (6, 7). Recently, a new method has made possible the low-cost manufacture of trehalose from starch (8). It is hydrolyzed to glucose by the specific enzyme, trehalase, which is localized in the brush border membrane of the small intestine (9, 10). However, the hydrolyzing activity is lower than that of lactase, which is the lowest among disaccharidases (11). A small intake of trehalose from natural foods might be completely digested and absorbed in the small intestine. However, when large-enough quantities of trehalose are taken from trehalose-enriched processed foods, they may cause high osmotic diarrhea because of the large amount of undigested trehalose reaching the large intestine. Trehalose will be widely used in food products because it has unusual beneficial properties, including stability to heat and acid, nonreducing characteristics, prevention to retrogradation of starch, and excellent restitution from frozen and dry foods (12, 13).

Lactulose, which is produced from lactose by alkaline isomerization, is not hydrolyzed by any intestinal enzymes for hydrolysis of disaccharides (14, 15). Thus the ingested lactulose reaches the large intestine where it is fermented completely by intestinal bacteria, and it improves intestinal microflora. The functional property has already been applied in a medicine that improves the symptoms of hepatic encephalopathy (16). Also, in Japan lactulose has been added to some processed foods such as soft drinks that claim improvement of the colonic environment (17). But if lactulose is ingested in large-enough quantities, it frequently causes diarrhea.

The permissive dose levels of trehalose and lactulose in dietary life have not been determined, even though their consumption is likely to increase. The safety for acute and subacute toxicities and carcinogenicity for them has been confirmed (18, 19). The maximal safety level of these saccharides becomes important in the design and processing of food production and in nutrition education. In the present study, we administered several different amounts of trehalose or lactulose to healthy female subjects and estimated their transitory laxative thresholds and their effects on abdominal symptoms.
Laxative Threshold of Trehalose

Table 1. Characteristics of healthy female subjects.

|       |           |
|-------|-----------|
| Age   | 21.8 ± 5.4 y |
| High  | 157.8 ± 4.6 cm |
| Body weight | 51.3 ± 5.9 kg |

Twenty healthy subjects from 22 female students participated in this experiment.

SUBJECTS, MATERIALS, AND METHODS

1. Subjects and materials. Before the experiments were started, 22 Japanese female students were questioned about their daily frequency of defecation, their past history of gastrointestinal diseases, and foods they consumed that easily induced diarrhea. Twenty healthy subjects who gave their informed consent were selected and participated in the study (Table 1).

The subjects in this study had no hard constipation or gastrointestinal diseases in their histories. The subjects with 4 or fewer defecation days per week were numbered 3 and those with 5 or more were numbered 17. One subject experienced diarrhea irregularly when she drank cold liquids such as milk and soft drinks.

Trehalose (purity more than 99.8%) was provided by Hayashibara Inc. (Okayama, Japan), and lactulose (purity more than 98%) was provided by Morinaga Milk Industry Inc. (Tokyo, Japan).

2. Administration of test substances and questionnaire. The dose levels of trehalose were 30 g, 40 g, 50 g, and 60 g, and of lactulose 10 g, 20 g, 30 g, and 40 g. The test substance was dissolved in about 200 mL of water and ingested quickly 2 to 3 h after a meal. The intake of test substances was carried out from small to large amounts and stopped at the dose level that caused diarrhea or at the maximal amount used in this study. The ingestion of the next test substance was carried out after the disappearance of abdominal symptoms caused by the intake of the former test substance.

From the day before administration, the subjects were required to avoid any ingestion of foods and beverages containing other sugar substitutes and of fermented foods that might cause diarrhea. On the day of administration, they were not permitted to eat or drink for at least 2 h after intake of the test substance.

After the administration, the time of onset of abdominal symptoms and diarrhea, the type of abdominal symptoms, the frequency of defecation, and the macroscopic finding of stools divided into 6 types were recorded by the subjects. The food intake was also recorded by each female subject on the day of administration, and the nutrient intake was calculated.

3. Observation of fecal shape and frequency, gastrointestinal symptoms, and physical condition. A questionnaire on diarrhea included an examination of stool shape, i.e., very hard (ball shaped, like rabbit stool); hard; normal (banana shaped); soft (paste); very soft (muddy); and watery (20). Pictures of stool shapes meeting
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the criteria for classification were handed to each subject to standardize their judgment of fecal condition. A muddy or watery stool was defined as diarrhea. The question on abdominal symptoms asked for information about upper and lower abdominal pain, vomiting, nausea, thirst, flatus, distension, borborygmus, and tenesmus.

4. **Statistical analysis and calculation of results.** The minimal dose level of the test substance that caused diarrhea was calculated for each subject as the test substance weight (g) ingested per body weight (kg), and the cumulative incidence of diarrhea for dose levels was then obtained. From the minimal dose level that induced diarrhea and the cumulative incidence of diarrhea, a regression equation was made and the maximal noneffective dose of the test substance that did not cause diarrhea was estimated (20). The maximal noneffective dose of trehalose and lactulose were compared by using one-way ANOVA; the side-effect symptoms by test substances were compared by the chi-square test using Stat View (ver. 4.0). Differences were considered with a significance level of \( p < 0.05 \).

5. **Ethical considerations.** This study was approved by the Ethical Committee of the Faculty of Medicine, the University of Tokyo.

**RESULTS**

1. **Relationship between trehalose or lactulose intake and fecal condition**

   Figure 1 shows the relationship between the ingestion of trehalose or lactulose and fecal condition. The macroscopic examination of feces was carried out on the basis of 6 types shown in the provided color print. When subjects \((n=20)\) ingested 30 g of trehalose, none had watery or muddy feces and most had feces of a normal shape. With the ingestion of trehalose 40 g, 5 of 20 subjects (25%) had watery or muddy feces, and the other 15 subjects had no diarrhea. The ingestion of trehalose 50 to 60 g caused watery or muddy feces in 5 of 15 subjects (33%). Half the total subjects in this study (50%) had no diarrhea, even with the ingestion of trehalose 60 g (Fig. 1).

   On the other hand, when lactulose 10 g was ingested by the same subjects \((n=20)\), none had watery or muddy feces, and most had feces of a normal shape. With the ingestion of lactulose 20 to 30 g, 10 of 20 subjects (50%) had watery or muddy feces, and the ingestion of lactulose up to 40 g caused diarrhea in 75% of the total \((n=20)\) (Fig. 1).

   These results suggest that the effect of trehalose and lactulose on the fecal shape differed greatly from person to person and that the effect of lactulose is evidently stronger than that of trehalose.

2. **Estimation of transitory laxative threshold of trehalose and lactulose**

   To estimate the transitory laxative threshold of trehalose and lactulose, the minimal amount (g/kg body weight) of ingestion of the test substance that caused diarrhea was calculated for the individual subjects. These values were used to
Fig. 1. Relationship between ingestion of trehalose or lactulose and fecal condition in healthy female subjects. The individual subject ingested the indicated amount of test substances. The subjects who experienced diarrhea did not ingest the next volume of test substance.
Fig. 2. Correlation between the ingestion of trehalose or lactulose and diarrhea incidence in healthy female subjects. The minimal amount (g/kg body weight) of test substance that caused diarrhea was calculated for each individual subject; then the cumulative frequency of diarrhea was calculated in order from the smaller value. From the correlation equation between test substance intake and diarrhea incidence, the maximal noneffective dosage for diarrhea was estimated.

calculate the cumulative incidence of diarrhea. The relationship between the several dose levels of trehalose (g/kg body weight) and diarrhea incidence in all subjects is shown in Fig. 2.

The cumulative incidence of diarrhea shows the ratio of subjects with diarrhea to the total subjects \((n=20)\) participating in this study. The point of lowest incidence \((1/20=5\%)\) shows a subject who had diarrhea with the lowest intake of test substance. The second lower point \((2/20=10\%)\) shows that 2 subjects had diarrhea in the second lower ingestion of the test substance. Each subsequent point shows the incidence with 3rd to 10th subject in the order. In this study, 50\% of subjects \((10/20=50\%)\) had diarrhea with an ingestion of up to 60 g (maximal dose) of trehalose, and the rest of the subjects \((50\%)\) had no diarrhea even with the maximal trehalose intake (60 g). Therefore the relationship between test substance ingestion and diarrhea incidence can be given by a certain linear relationship.

The correlation between trehalose intake and diarrhea incidence was expressed in an equation as follows:

\[
y = 79x - 51
\]  

\(y = 79x - 51\)  

The point at which this straight line crosses the \(x\) axis, namely, the \(x\) coordinate of 0\% of diarrhea incidence \((y=0)\), indicates the transitory laxative threshold. From Eq. (1), the maximal noneffective dosage of trehalose was estimated as 0.65 g/kg
Table 2. Maximal noneffective dosage and ED$_{50}$ of trehalose and lactulose in healthy female subjects.

|                          | Maximal noneffective dose (g/kg body weight) | ED$_{50}$ (g/kg body weight) |
|--------------------------|---------------------------------------------|------------------------------|
| Trehalose                | 0.65                                        | 1.28                         |
| Lactulose                | 0.26                                        | 0.67                         |

ED$_{50}$, amount which 50% of subjects do not occur diarrhea.

body weight. The dosage level (ED$_{50}$) at which 50% of subjects did not have diarrhea was 1.28 g/kg body weight.

As shown in Fig. 2, the correlation between lactulose intake and diarrhea incidence was given in an equation as follows:

$$y = 122x - 32$$

From Eq. (2), the maximal noneffective dosage of lactulose was estimated as 0.26 g/kg body weight. The ED$_{50}$ for lactulose was 0.67 g/kg body weight (Table 2).

3. Appearance of abdominal symptoms by trehalose or lactulose intake

The appearance of abdominal symptoms associated with trehalose or lactulose intake is shown in Fig. 3. When the subjects experienced diarrhea in lower dose levels of the test substances, they did not ingest the test substance at the next higher dose level. In other words, the subjects who were not resistant to diarrhea caused by the ingestion of nondigestible saccharides were eliminated from the next experiment with higher dose levels. Therefore the number of subjects decreased as the dose levels increased.

Other main abdominal symptoms that occurred with greater frequency than diarrhea included borborygmus, flatus, and distension with the ingestion of trehalose or lactulose. Abdominal symptoms of lower frequency were distension and nausea. The appearance of upper and lower abdominal pain increased with the higher ingestions of lactulose and trehalose. The ingestion of trehalose 30 g, 40 g, 50 g, and 60 g caused flatus and borborygmus in more than 40% of all subjects, except for 40 g ingestion for flatus. Moreover, the ingestion of trehalose induced distension in more than 20% of the subjects. More than 20% of subjects experienced nausea with the ingestion of more than 40 g of trehalose. The appearance of upper and lower abdominal pains increased, depending on the amount of ingestion. But none complained of tenesmus with any ingestion of trehalose.

When 20 g of lactulose was ingested by 20 subjects, flatus, and borborygmus were found in more than 40% of them. When the ingestion of lactulose was increased to 40 g, more than 40% of the subjects experienced lower and upper abdominal pains. Furthermore, nausea appeared with the ingestion of lactulose at 20 g, 30 g, and 40 g in more than 30% of the subjects.
Fig. 3. Trehalose or lactulose ingestion and appearance of gastrointestinal symptoms in healthy female subjects. The ingestion of test substances was the same in Fig. 1. 1, vomiting; 2, nausea; 3, discomfort; 4, flatus; 5, distension; 6, borborygmus; 7, tenesmus; 8, upper abdominal pain; 9, lower abdominal pain; 10, thirst.

Figure 4a shows an occurrence of the three main abdominal symptoms with the ingestion of the same amount of trehalose and lactulose. With the same amount (40 g) of ingestion, the appearance of borborygmus, flatus, and distension was clearly higher with lactulose ingestion than with trehalose ingestion ($p < 0.05$). No significant difference, however, in the occurrence of the three main abdominal symptoms was seen between trehalose 40 g ingestion and lactulose 20 g ingestion (Fig. 4b). These results support the idea that trehalose is partially digested by trehalase and absorbed in the small intestine, and lactulose is not hydrolyzed by any digestive enzymes.

These results demonstrate that the frequent appearance of flatus, distension, and borborygmus was observed at any level of trehalose or lactulose ingestion, and that ingestion in large amounts causes lower and upper abdominal pains. Nausea was found more frequently with lactulose and larger doses of trehalose; it rarely appeared with the small-amount doses. The cause of the high incidence of nausea in high-level ingestion of trehalose seems to be the consequence of high osmotic pressure in the stomach induced by the ingested trehalose. The frequency of abdominal symptoms caused by trehalose ingestion was less in comparison with those induced by lactulose ingestion because the ingested trehalose is partially digested and absorbed in the small intestine.
The transitory laxative threshold (maximal noneffective dose) of trehalose and lactulose was calculated from a regression equation between the dose levels of the test substance and the cumulative incidence of diarrhea. The maximal noneffective dose was 0.65 g/kg body weight for trehalose and 0.26 g/kg body weight for lactulose in healthy female subjects. These results suggest that a single ingestion of 30 g of trehalose or 13 g of lactulose does not cause diarrhea in human beings weighing 50 kg or more.

The transitory laxative threshold of trehalose was about three times that of lactulose, although they are both disaccharides. The difference in the laxative threshold between trehalose and lactulose is based on the difference in digestibility of the two disaccharides. Because trehalose is partially digested by trehalase (9), the quantity transferred to the large intestine is less than that of lactulose, which is essentially not digested by any disaccharidases (15) when the same amount is administered orally. Therefore the elevation of osmotic pressure in the colon by trehalose is weaker than that of lactulose with the ingestion of the same amount. Thus it is reasonable to predict that the laxative threshold of trehalose is much lower than that of lactulose.
larger than that of lactulose.

Trehalose will be widely applied to food products making use of its beneficial properties. The amount of trehalose used in one serving or in one package of food products seems to be less than 10 g in practice. Therefore the regular intake of trehalose in daily life cannot be expected to cause diarrhea. Lactulose is used to assist the growth of intestinal microflora and to improve the intestinal environment. Because the optimal amount of lactulose added to food products is less than 5 g per serving or per package (21), lactulose is also quite safe in terms of diarrhea. However, the combined intake of several foods that contains nondigestible oligosaccharides may exceed the maximal permissible dose and cause diarrhea.

Oligosaccharides, such as fructooligosaccharide, 4'-galactooligosaccharide, galactosyllactose, galactosylsucrose, and xylooligosaccharide, and sugar alcohols, such as lactitol, maltitol, and paratinit, are essentially not hydrolyzed by digestive enzymes (15, 22) and are fermented readily by colonic bacteria. A large-enough ingestion of these sugar substitutes causes diarrhea regularly. The maximal noneffective dose is from 0.28 g to 0.4 g/kg body weight (1, 21). These values are less than half those for trehalose (0.65 g/kg body weight), but they are similar to those (0.26 g/kg body weight) of lactulose, which is essentially not digested by the small intestinal enzymes. This value of lactulose obtained in the present study appears to be reasonable.

The maximal noneffective dose in this study is based on a case in which the test substance is ingested transitorily once daily. If it is separately ingested two or three times a day, the maximal noneffective dose per day appears to increase beyond the values obtained in this study. Patil et al have demonstrated that the laxative threshold of sorbitol was 1.18 g/kg body weight/d when 10 g of sorbitol was ingested twice daily by female subjects who were given 5 g twice daily for 9 d, though the estimation method is clearly different from our method (23). The value is fivefold higher than our results reported previously. The separate ingestion twice or more daily of the test substance and the adaptation to the test substance increases the maximal noneffective dose per day because colonic bacteria that can readily use them multiply within a few days.

A sex difference has been observed in the maximal noneffective dose of sorbitol and erythritol, and the value for females is greater than that for males (20). Furthermore, Koizumi et al demonstrated that female subjects are more tolerant than male subjects for diarrhea because of high osmotic pressure (24). Generally, females seem to be more resistant than males to diarrhea induced by the large ingestion of nondigestive saccharides, though the reasons are not clear. Therefore the maximal noneffective doses of trehalose and lactulose for males might be lower than those for females. If they are lower, trehalose and lactulose will be quite safe for diarrhea, because the body weight of male is heavier than that of female.

When nondigestible sugar substitutes are administered orally, flatus, distension, and/or borborygmus occur as the side-effects of fermentation that produces gases such as carbon dioxide, hydrogen, and methane (25). Because lactulose and trehalose...
readily produce gas in the large intestine, abdominal symptoms such as flatus, distension, and borborygmus increase. But after adaptation to these saccharides, gas production decreases readily because the number of intestinal bacteria that produce carbon dioxide and hydrogen gases declines.

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