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Bioavailability and Laxative Threshold of 1-kestose in Human Adults

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ABSTRACT

1-kestose is a trisaccharide and is one of the components of fructooligosaccharide (FOS). Although FOS is a typical non-digestible oligosaccharide with several beneficial health effects that have been clarified, the detailed properties of 1-kestose itself remain unknown. We first determined the digestibility of 1-kestose using rat and human small intestinal homogenates and the inhibition of 1-kestose to intestinal disaccharidases activity using rat small intestinal brush border membrane vesicles (BBMV). Thereafter, we estimated the bioavailability of 1-kestose based on the results from incremental blood glucose and insulin levels and breath hydrogen excretion after the oral ingestion of 5 and 30 g of 1-kestose in healthy human subjects. 1-kestose was hardly hydrolyzed by the rat and human intestinal disaccharidases, and competitively inhibited trehalase. When human subjects ingested 1-kestose, the blood glucose and insulin did not respond, and the excretion of breath hydrogen in a dose-dependent manner was markedly observed. The permissive dose for transitory diarrhea was estimated to be 0.24 g/kg of body weight in male subjects and 0.34 g/kg of body weight in female subjects. These results demonstrate that 1-kestose is a candidate to be the prebiotic agent in addition to FOS, and that the available energy of 1-kestose was estimated at 2 kcal/g based on the calculation method of the Health Promotion Act in Japan.

Keywords: 1-kestose, bioavailability, fructooligosaccharide, breath hydrogen, inhibition to disaccharidases, human
Abbreviations: BBMV, brush border membrane vesicles; **B.W.**, body weight; **FOS**, fructooligosaccharide; **O.D.**, optical density

INTRODUCTION

Fructooligosaccharide (FOS) is a mixture of 1-kestose, nystose, fructofrunosyl-nystose, sucrose, glucose, and fructose. Although FOS is a typical non-digestible oligosaccharide and has several beneficial health effects that have been clarified, the detailed properties of 1-kestose itself, especially in humans, remain unknown. The metabolic pathway of non-digestible oligosaccharides is as follows: it is hardly hydrolyzed by intestinal enzymes, reaches the large intestine without decomposition, is fermented by intestinal microbes and metabolites to carbon dioxide, methane, hydrogen, and other gases, and short chain fatty acids, such as acetic acid, propionic acid, and butyric acid. The beneficial health effects are demonstrated throughout the transition in the gastrointestinal tract.

In the case of FOS, the beneficial health effects that have been reported are not stimulating the response of blood glucose and insulin (Yamada *et al* 1990; Oku *et al*. 1984), to improve intestinal microflora and maintain gastrointestinal function (Hidaka *et al.* 1986; Tokunaga *et al.* 1986), to prevent diarrhea and improve immunity (Nakamura *et al*. 2006; Watanabe *et al*. 2008), and so on. Oku *et al*. (1984) and colleagues (Hosoya *et al*. 1988; Tokunaga *et al*. 1989) have already clarified that FOS is not digested by the small intestinal enzymes, is transferred into the large intestine, and is completely fermented by microbes in rat experiments using ^{14}C-FOS. Furthermore, Oku *et al.* (2003) clarified that the breath hydrogen gas was markedly excreted due to the ingestion of FOS in humans. It has already been determined that the available energy of these non-digestible and/or non-absorbable saccharides was classified into 0, 1, or 2 kcal/g according to the Health Promotion Act in Japan (Oku *et al.* 2002; Oku 2005).

The estimation of energy value depends on the rate of absorption in the small intestine, the rate of fermentation in the large intestine, and the value of the maximum permissive dose for transitory osmotic diarrhea in healthy Japanese people. In terms of the absorption, when the oligosaccharides are partly digested by the small intestinal disaccharidases, blood glucose level is increased by the released glucose. The breath hydrogen is excreted only when the non-digestible saccharide escapes the digestion and is fermented by the microbes. The concentration of breath hydrogen depends on the amount of ingested non-digestible oligosaccharides (Oku *et al.* 2003).

We first determined the digestibility of 1-kestose using rat and human small intestinal homogenates and the inhibition of 1-kestose to intestinal disaccharidases activity using rat small intestinal brush border membrane vesicles (BBMV). Thereafter, we estimated the bioavailability of 1-kestose based on incremental blood glucose and insulin levels and breath hydrogen excretion after the ingestion of 1-kestose in healthy human subjects.

MATERIALS AND EXPERIMENTAL METHODS

Test materials

1-kestose (M.W., 504, purity>99 %, Meiji Seika Kaisha Co., Ltd., Tokyo, Japan), fructooligosaccharide (a mixture, purity >98%, Meiji Seika Kaisha Co., Ltd., Tokyo, Japan), lactulose (M.W., 342, purity >98%, Morinaga Milk Industry Co., Ltd., Tokyo, Japan), cellobiose (M.W., 342, Matsutani Chemical Industry Co., Ltd., Hyogo, Japan), trehalose (M.W., 342, purity >99%, Hayashibara Biochemical Laboratories, Okayama, Japan), sucrose for *in vitro* experiments (M.W., 342, Wako Pure Industries Co., Ltd., Osaka, Japan), and sucrose for human experiments (M.W., 342, refined sugar, purity >99%, Dai-Nippon Meiji Sugar Co., Ltd., Tokyo, Japan) were used in this study. All materials were of analytical grade.

Received: 1 May, 2009. Accepted: 27 October, 2009.

Original Research Paper

Digestibility and inhibition to disaccharidases in *in vitro* experiments

1) Preparation of human intestinal mucosal homogenate

Fragments of human small intestine were obtained from five patients in a hospital. They had undergone surgery, with a region of the gastrointestinal tract having been dissected. The dissected regions varied from patient to patient. The malignant tissues were not determined in any of the small intestinal fragments used in this experiment. After the fragments were washed with ice-cold physiological saline, the mucosa was scraped off with slide glasses and homogenized in ice-cold physiological saline (5% wet w/v), using a homogenizer (Polytron, Kinematica Inc., Switzerland).

2) Preparation of rat intestinal brush border membrane vesicles (BBMV)

Ten male Wistar rats (250 g body weight; Clea Japan Inc., Tokyo, Japan) were killed by decapitation and the small intestines were immediately removed, and BBMV was prepared according to Kessler's method (Kesseler *et al*. 1978).

3) Measurement of the hydrolyzing activity for1-kestose and inhibition of 1-kestose to disaccharidases

The substrates were 1-kestose, sucrose, and trehalose dissolved in 0.1 M sodium maleic acid buffer (pH 6.0) and diluted to adequate concentrations. The hydrolyzing activity was determined by Oku's method (Oku *et al*. 1982) which is a modified version of Dalqvist's methods (Dahlqvist 1964) using Tris-glucose oxidase. Released glucose was measured using a spectrophotometer (UV-mini1240, Shimadzu Corp., Kyoto, Japan) at O.D. 500 nm. The concentration of protein was measured by the Bradford method (Bradford 1976). The enzymatic activity was calculated as specific activity (S.A.), expressed in terms of μM of substrate hydrolyzed per mg of protein for 1 h. The inhibitory constants and mode were determined by a Lineweaver-Burk plot using BBMV. To confirm whether 1-kestose inhibits disaccharidases in human small intestine, a mixed solution of equal molarity of 1-kestose and sucrose or trehalose was used as a substrate.

Estimation of bioavailability of 1-kestose in healthy human subjects

1) Subjects

The subjects who participated in this study included a total of 37 healthy males (20.1±1.5 y; 58.8±4.1 kg of body weight; BMI, 19.9±1.3) and 52 healthy females (21.0±2.2 y; 50.8±5.2 kg of body weight; BMI, 20.3±1.9). They had no history of diabetes, carbohydrate malabsorption, and pulmonary diseases.

2) Test solutions

To evaluate the digestibility of 1-kestose, 30 g of 1-kestose was ingested and compared to the same dose of sucrose. Furthermore, to evaluate its fermentability, 5 g of 1-kestose was ingested and compared to the same dose of FOS. All of the test materials were dissolved in 100 mL of warmed tap water.

3) Experimental protocol of the evaluation of the digestibility and fermentability of 1-kestose

The experimental protocol for estimating the bioavailability of 1-kestose in healthy humans was performed as in our previous studies (Oku *et al.* 2003; Nakamura *et al*. 2004). After overnight fasting, the health status of 10 subjects was checked, and their basal blood and end-expiratory gas were collected. After the ingestion of test solution, blood was collected at 30 min intervals for 3 h and end-expiratory gas was collected at 1 h intervals for 8 h after the ingestion. The experimental meal, the ingestion of which, it had been clarified, produced no hydrogen, was ingested after the collection of blood to avoid the sensation of starvation. Sera were separated by centrifugation, and the concentrations of serum glucose and insulin were analyzed by Trinder's method (Trinder 1969) and ELISA (Liversy *et al*. 1980), respectively. The concentration of breath hydrogen was determined by simple gas chromatography using Breath Gas Analyzer BGA1000 (Laboratory for expiration biochemistry nourishment metabolism, Co., Ltd., Nara, Japan). The fermentability was estimated in terms of its ratio to the fermentability of the same dose of FOS as a positive control.

4) Estimation of the permissive dose for transitory diarrhea

The maximum non-effective dose level at which the test material does not induce osmotic diarrhea was estimated according to the method used in our previous studies (Oku *et al*. 2005, 2007). Subjects ingested several doses of 1-kestose and FOS (as a positive control), increasing stepwise from 10 to 40 g in 10 g increments. The administration was stopped at the dose level at which osmotic diarrhea was experienced or the largest dose levels set in this study. The ingested dose (g) per kg of body weight at which diarrhea was induced and the cumulative incidence of diarrhea were calculated, and the dose at which the incidence of diarrhea was 0% was calculated using a single linear regression model.

The prohibitions for the subjects included the intake of fermented foods, foods and beverages containing non-digestible and/or non-absorbable saccharides for 3 days before the experimental day. The ingestion time of the test materials was 2 or 3 h after the ingestion of breakfast or lunch; thereafter, the subjects avoided drink and intake except for water for 2 h after the ingestion of the test materials.

The subjects were required to observe and report the ingestion time, defecation time after the ingestion of the test substance, the constituents of stool, abdominal symptoms, and other side effects using formatted questionnaires. The shape and color of stool were determined according to a colored photocopy showing 6 categories of consistency shape, namely very hard, hard, normal, soft, muddy, and watery and color, which is categorized into 6 colors (Nakamura *et al*. 2002). The defecation of muddy and watery stool was defined as diarrhea in this study.

Statistical analysis and calculation

The normally distributed data were compared using the paired Student's *t*-test between the test materials and the control in terms of blood glucose, insulin, and breath hydrogen. The data were expressed as means and standard deviations with the significance level considered to be less than 0.05 using SPSS 12.0 for Windows, Japan.

Ethics

The study protocol was approved by the Ethical Committees of Siebold University of Nagasaki and Juzenkai Hospital. The rat experiments were carried out under the guidelines on the care and use of laboratory animals of Siebold University of Nagasaki.

RESULTS AND DISCUSSION

Digestibility of 1-kestose and inhibition to disaccharidases in *in vitro* experiments

1) Digestibility of 1-kestose using human homogenates

Fig. 1 shows the hydrolyzing activities using 5 human intestinal mucosal homogenates toward sucrose, trehalose, and 1-kestose. The hydrolyzing activity varied from sample to sample, because the region of the intestinal fragment was different depending on the disease condition. The specific activity of sucrase ranged from 31.0 to 4.5, and that of 1-kestose ranged from 0.3 to 0.1.

2) 1nhibition of 1-kestose to sucrase and trehalase using human intestinal homogenates

Fig. 2 shows the percentage of the inhibition to sucrase or trehalase induced by the addition of 1-kestose to sucrose or trehalose solutions. Sucrase activity was not inhibited when

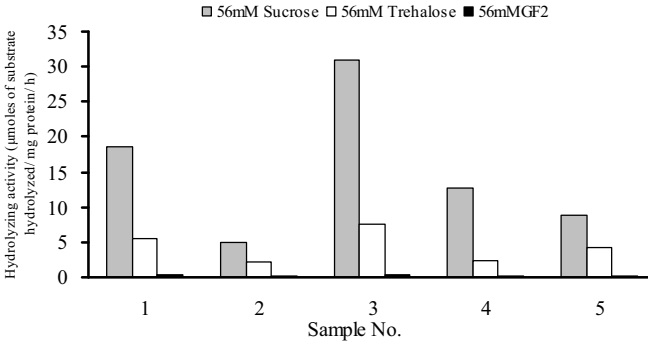

Fig. 1 Hydrolyzing activity of disaccharidase to sucrose, trehalose, and 1-kestose using 5 homogenates of human small intestine.

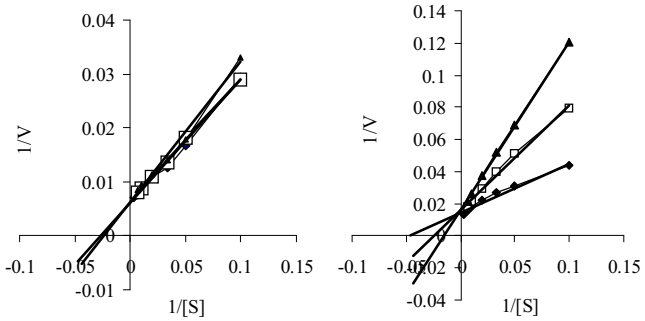

Fig. 3 Inhibitory mode of 1-kestose versus sucrase and trehalase using Lineweaver-Burk plot using brush border membrane vesicles of rat small intestine. For trehalase, *K*m, 20.6 mM; *V*max 68.0 μmol of substrate hydrolyzed/ mg protein/h; *K*i, 8.4 mM.

1-kestose, the molarity ratio of which to sucrose was 1:1, 2:1, and 4:1, was added to the sucrose solution. On the other hand, when an equal molar solution of 1-kestose and trehalose was incubated with human intestinal homogenate, trehalase activity decreased to 62.9±19.3% and was markedly inhibited.

3) Inhibition constants (Ki) and mode of 1-kestose to sucrase and trehalase using rat BBMV

Fig. 3 shows a Lineweaver-Burk plot of sucrase and trehalase using rat BBMV. Trehalase activity was competitively inhibited by the addition of 1-kestose, and the *K*i of 1-kestose to trehalase was 8.4 mM, while the inhibition to sucrase due to the addition of 1-kestose was not detected.

These results clarified that 1-kestose is hardly hydrolyzed in human and rat intestinal disaccharidases, and that 1-kestose competitively inhibits small intestinal trehalase activity in humans and rats. However, the reason that 1-kestose inhibits trehalase is still unknown. These results demonstrate that it is possible for 1-kestose to be utilized through fermentation by the intestinal microbes. Although Yoshida *et al.* (2006) reported that 1-kestose and nystose had the effect of improving the intestinal microflora and the immunity in an experiment in mice, the beneficial health effect in humans has not been clarified.

Bioavailability of 1-kestose in healthy human subjects

1) Response of serum glucose and insulin, and the breath hydrogen excretion after ingestion of 1-kestose

Ten out of 52 female subjects participated in this experiment. They were tolerant of the transitory osmotic diarrhea induced by the ingestion of 1-kestose. The responses of serum glucose and insulin induced by the ingestion of 30 g of 1-kestose were not observed, while they were significantly increased by the ingestion of 30 g of sucrose, as shown in **Fig. 4**. On the other hand, the excretion of breath hydrogen was markedly increased by the ingestion of 30 g of 1-kestose, and it was scarcely detected in response to the ingestion of 30 g of sucrose, as shown in **Fig. 5**.

The areas under the curve for 8 h after the ingestion of 5 g of 1-kestose or FOS are shown in **Fig. 5**. The amount of hydrogen by 1-kestose was not significantly different from that of FOS. These results strongly suggested that 1-kestose is not digested by the human intestinal enzymes and is transferred into the large intestine without degradation. They demonstrate that most 1-kestose is spontaneously fermented by the intestinal microbes in the large intestine, and that the metabolites supply energy to the host through the absorption of short chain fatty acids. As a result, the available energy of 1-kestose estimated based on the response of serum glucose, insulin, and breath hydrogen was 2 kcal/g.

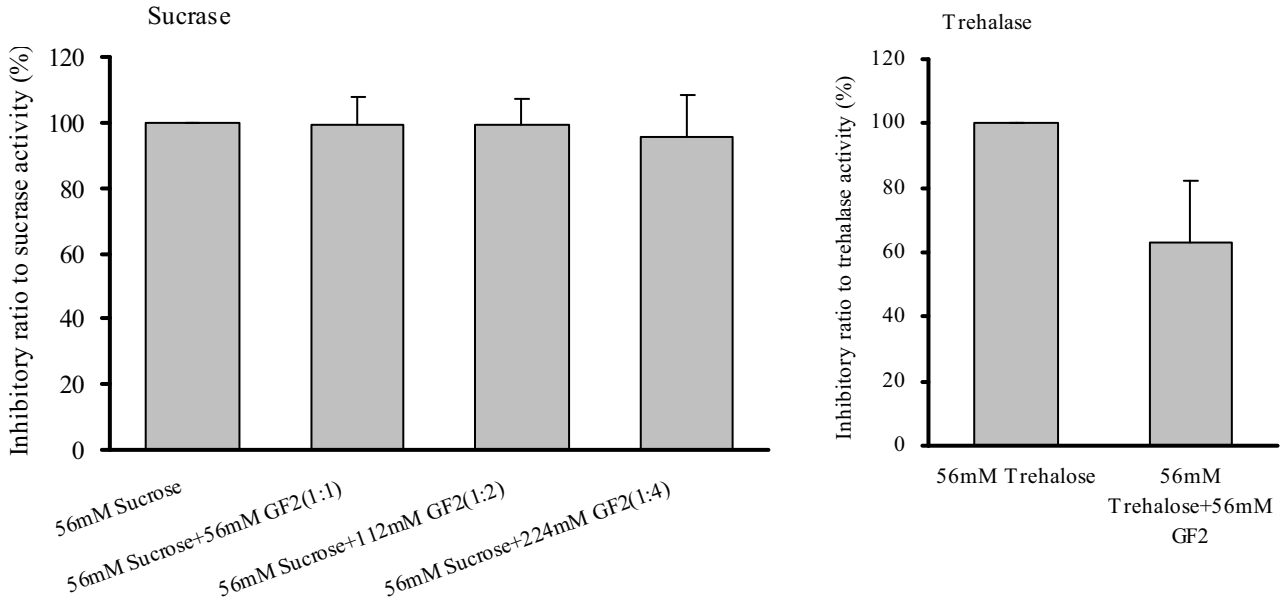

Fig. 2 Effect of different concentrations of added 1-kestose to sucrose or trehalose solution on sucrase or trehalase activities using homogenates of human small intestine. Data were expressed as means and S.D. of 5 human small intestinal samples.

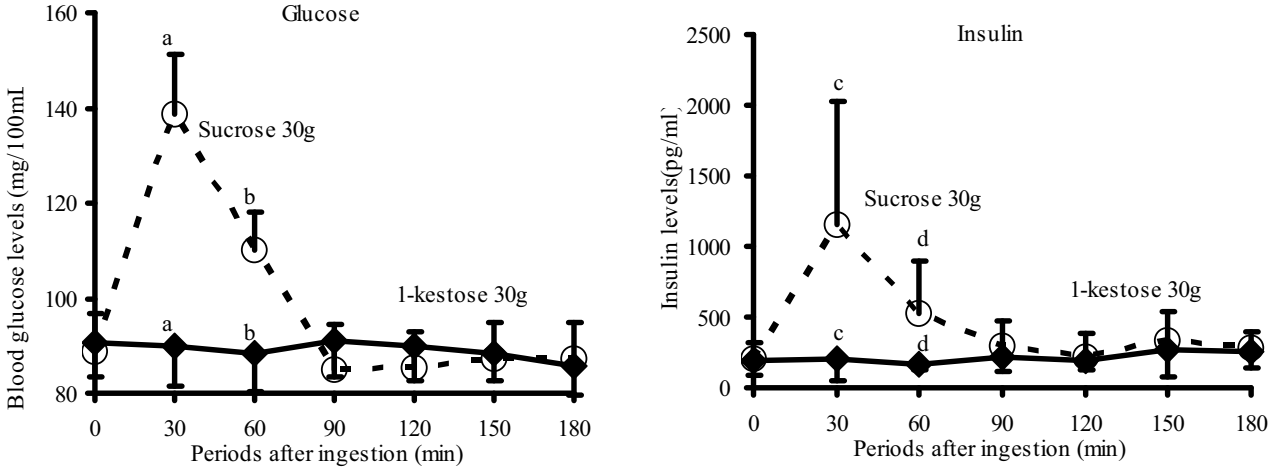

Fig. 4 Response of blood glucose and insulin levels after ingestion of 1-kestose and sucrose in human subjects. Data were expressed as means ±S.D. in 10 healthy subjects. a-d: Significant differences were observed between 30 g of sucrose and 30 g of 1-kestose, at $p<0.05$.

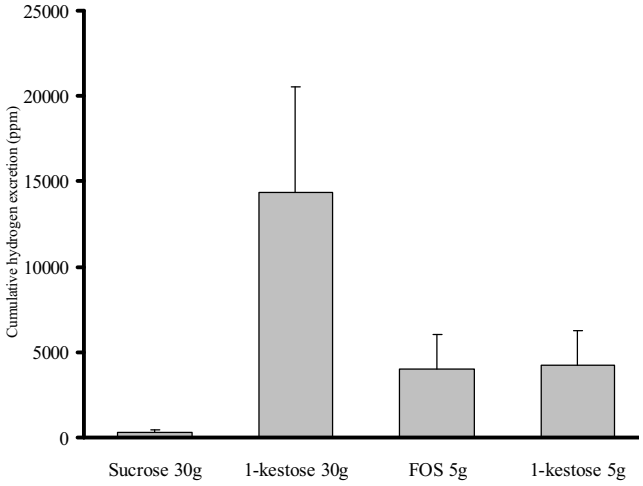

Fig. 5 Areas under the curve for 8 h of breath hydrogen excretion by ingestion of sucrose, 1-kestose, and FOS in human subjects. FOS, fructooligosaccharide; Data were expressed as means ±S.D. in 10 healthy subjects. a,b: There was a significant difference between 30 g of 1-kestose and 30 g of sucrose, and the concentration of hydrogen in 30 g of 1-kestose was significantly more than that in 5 g of 1-kestose, at $p<0.05$.

2) Non-effective permissive dose at which does not induce transitory osmotic diarrhea of 1-kestose in healthy human subjects

Twenty-three males and 48 female subjects completely finished the experiments. The incidence of transitory osmotic diarrhea at each ingestion level is shown in **Table 1**. A total of 13 out of 23 males and 35 out of 48 females experienced osmotic diarrhea until the ingestion of 40 g of 1-kestose. In FOS ingestion, a total of 16 out of 23 males and 34 out of 48 females experienced osmotic diarrhea until the ingestion of 40 g of FOS (**Table 1**). To calculate the non-effective permissive dose which does not induce transitory osmotic diarrhea of 1-kestose and FOS in healthy human subjects, a linear regression of the dose (g/kg body weight) inducing diarrhea versus the cumulative incidence of diarrhea is shown in **Figs. 6** and **7**, respectively. The linear equations of 1-kestose for male and female subjects were as follows (shown in **Fig. 6**):

$y = -27.26+114.35x$ ($R^2 = 0.96$, n = 23 males)

$y = -48.84+143.88x$ ($R^2 = 0.99$, n = 48 females)

The calculated permissive dose for transitory osmotic diarrhea and the effective dose (ED_{50}) of 1-kestose were 0.24 and 0.68 g/kg B.W. for male subjects, and 0.34 and 0.69 g/kg B.W. for female subjects, respectively.

Table 1 Numbers of transitory diarrhea and cumulative incidence of diarrhea by the ingestion of 1-kestose in male and female subjects

	1-kestose			Fructooligosaccharide		
	20 g	**30 g**	**40 g**	**20 g**	**30 g**	**40 g**
Numbers of diarrhea incidence						
Male	5/23	4/18	4/14	2/23	9/21	5/13
Female	10/48	13/38	12/25	6/48	13/42	15/29
Cumulative incidence (%)						
Male	21.7	39.1	56.5	8.7	47.8	69.6
Female	20.8	47.9	72.9	12.5	39.6	70.8

The linear equations of FOS for male and female subjects were as follows (shown in **Fig. 7**):

$y = -73.32+202.88x$ ($R^2 = 0.95$, n = 23 males)

$y = -43.87+124.58x$ ($R^2 = 0.97$, n = 48 females).

The calculated permissive dose for transitory osmotic diarrhea and the effective dose (ED_{50}) of FOS were 0.36 and 0.61 g/kg B.W. for male subjects, and 0.35 and 0.75 g/kg B.W. for female subjects, respectively.

These results demonstrate that the laxative effect of 1-kestose, which is one of the components of FOS, is the same as that of FOS, although the calculated dose of 1-kestose was slightly different between males and females. However, the specific reason of for this has not been clarified yet. The levels of the maximum permissive dose for transitory osmotic diarrhea in males and females are within the ranges of the other non-digestible oligosaccharides that are difficult to digest. In **Table 2**, we summarized the maximum permissive doses of 1-kestose, FOS, lactulose and cellobiose obtained from our experiments using the same method. Lactulose and cellobiose are not digested by any human intestinal enzymes, and do not stimulate the elevation of blood glucose and insulin. The levels of the excretion of breath hydrogen of lactulose and cellobiose were the same as that of FOS. The maximum permissive dose of lactulose was 0.32 (0.26 in a previous study (Oku *et al.* 1998)) g/kg B.W., and that of cellobiose was 0.36 g/kg B.W. in female subjects.

As the maximum permissive doses were different between males and females in 1-kestose, the minimum doses at which the ingestion of 1-kestose and FOS caused transitory diarrhea were calculated and compared, as shown in **Table 3**. The minimum doses (g) of 1-kestose and FOS at which diarrhea was induced were not significantly different between males and females. However, the minimum doses (g) at which diarrhea were induced versus kg of body weight were significantly different between males and females for the ingestion of both 1-kestose and FOS. These results suggested that the difference between males and females was

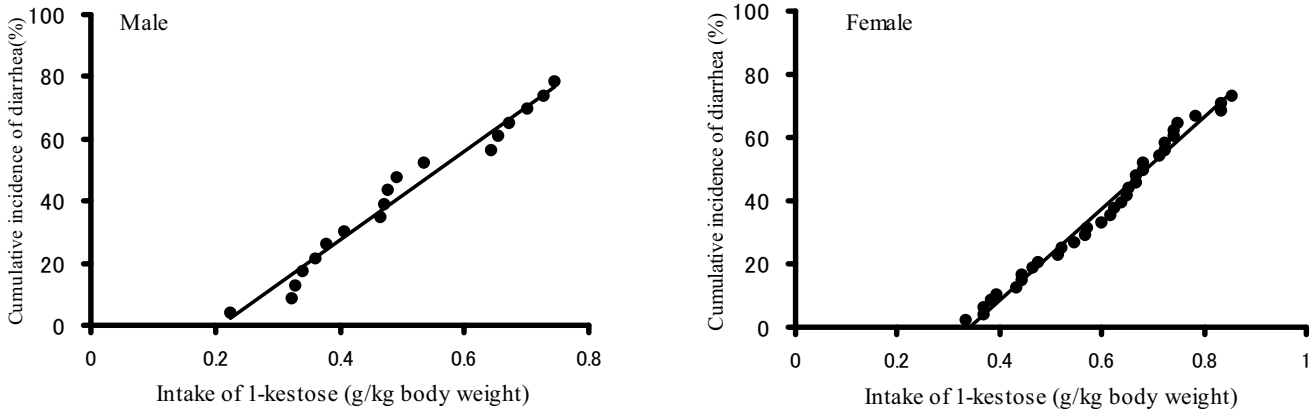

Fig. 6 Relationship between the amount of ingestion of 1-kestose and cumulative incidence of diarrhea in male and female subjects. Calculated maximum permissive dose and dose of ED_{50} in males were 0.24 and 0.68 g/kg body weight, respectively. y=-27.26+114.35x (R^2=0.96, n=23). Calculated maximum permissive dose and dose of ED_{50} in females were 0.34 and 0.69 g/kg body weight, respectively. y=-48.84+143.88x (R^2=0.99, n=48).

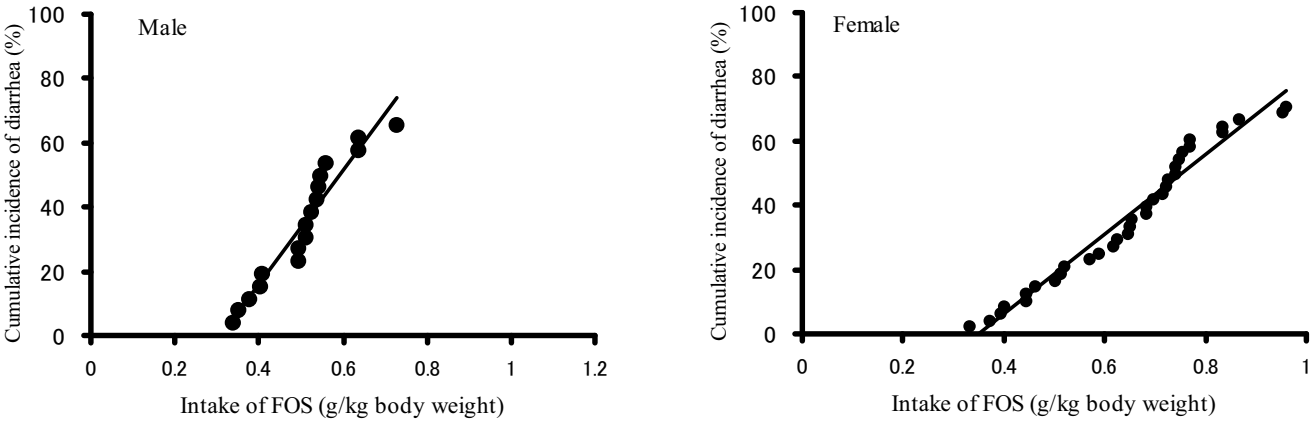

Fig. 7 Relationship between the amount of ingestion of fructooligosaccharide and cumulative incidence of diarrhea in male and female subjects. Calculated maximum permissive dose and dose of ED_{50} in males were 0.36 and 0.61 g/kg body weight, respectively. y=-73.32+202.88x (R^2=0.95, n=23). Calculated maximum permissive dose and dose of ED_{50} in females were 0.35 and 0.75 g/kg body weight, respectively. y=-43.87+124.58x (R^2=0.97, n=48).

observed due to the difference in body weight between males and females. Generally, since the body weight of males is greater than that of females, it is thought that the difference of the maximum permissive dose does not present a serious problem practically.

Table 2 Summary of maximum permissive dose and ED50 at which do not induce diarrhea by the ingestion of 1-kestose, fructooligosaccharide, lactulose, and cellobiose in male and female subjects.

	Maximum permissive dose (g/kg body weight)		Dose of ED50 (g/kg body weight)	
	Male	**Female**	**Male**	**Female**
1-kestose	0.24	0.34	0.68	0.69
Fructooligosaccharide	0.36	0.35	0.61	0.75
Lactulose		0.32		0.61
Cellobiose		0.36		0.62

Table 3 Minimum dose at which caused transitory diarrhea by the ingestion of 1-kestose in male and female subjects.

	Minimum dose for diarrhea	
	(g)	**(g/kg body weight)**
1-kestose		
Male	29.2 ± 8.3	0.51 ± 0.14 a
Female	30.6 ± 8.0	0.60 ± 0.15 a
Fructooligosaccharide		
Male	31.6 ± 6.5	0.55 ± 0.10 b
Female	32.5 ± 7.6	0.64 ± 0.10 b

3) Abdominal symptoms induced by the ingestion of 1-kestose and FOS

Fig. 8 shows the incidence of abdominal symptoms induced by several doses of 1-kestose and FOS. The onset of abdominal pain, distention, flatus, and borborygmus are commonly observed in the ingestion of non-digestible and/or non-absorbable saccharides (Sobajima *et al*. 1998; Brighenti *et al*. 2006). These symptoms were observed dose dependently, and the incidences were not different between 1-kestose and FOS. Serious symptoms and side effects were not observed throughout the experiments.

In conclusion, it was clarified that the available energy of 1-kestose was 2 kcal/g, and its properties were to be resistant to digestion, to be fermented by intestinal microbes, not to stimulate the response of blood glucose and insulin, and to induce transitory osmotic diarrhea via a sufficient single ingestion dose in humans. Furthermore, the inhibition of trehalase was observed in the *in vitro* experiment. These results strongly demonstrate that 1-kestose is a candidate to be a prebiotic agent and could contribute to human health.

ACKNOWLEDGEMENTS

The authors would like to thank the patients for donating their fragments of small intestine, and also Meiji Seika Kaisha Co., Ltd. (Tokyo, Japan) for providing 1-kestose and FOS, Morinaga Milk Industry Co., Ltd. (Tokyo, Japan) for providing lactulose, Hayashibara Biochemical Laboratories (Okayama, Japan) for providing trehalose, and Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan) for providing cellobiose, respectively.

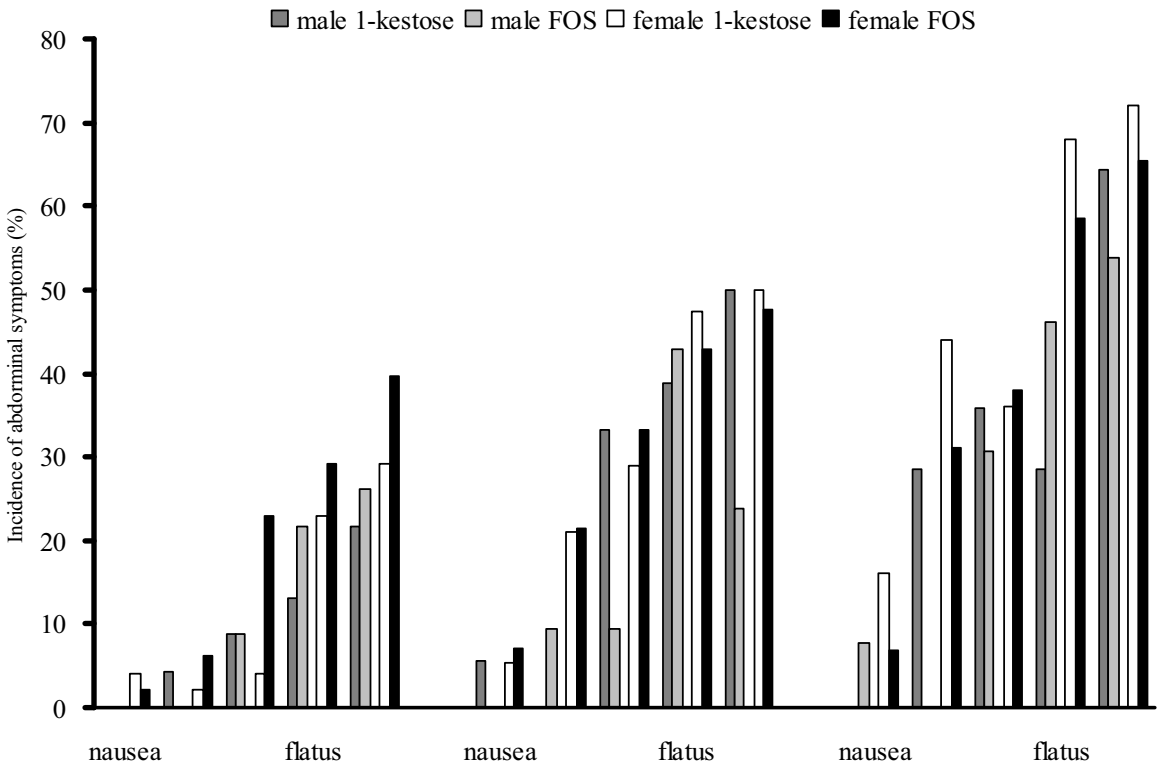

Fig. 8 Incidence of abdominal symptoms by ingestion of 1-kestose and fructooligosaccharide.

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