

## Studies on absorption and metabolism of palatinose (isomaltulose) in rats

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### Abstract

We evaluated the absorption and metabolism of palatinose in rats by the carbohydrate load test and the <sup>13</sup>C- and H<sub>2</sub>-breath tests. We compared the results of these tests with those of sucrose, since sucrose is an isomer of palatinose and generally known to be degraded and absorbed from the small intestine. In the carbohydrate load test, blood glucose and plasma insulin levels after oral administration of palatinose rose more gradually and reached a maximum that was lower than that after sucrose administration. In the <sup>13</sup>C-breath test, rats were orally administered [1-<sup>13</sup>C]sucrose or [1-<sup>13</sup>C]palatinose and housed in a chamber. The expired air in the chamber was collected, and the level of <sup>13</sup>CO<sub>2</sub> in the expired air was measured at appropriate intervals for 360 min. The value of time taken to reach the maximum concentration for expired <sup>13</sup>CO<sub>2</sub> from [1-<sup>13</sup>C<sup>glucose</sup>] ([1-<sup>13</sup>C<sup>glc</sup>]) and [1-<sup>13</sup>C<sup>fructose</sup>] ([1-<sup>13</sup>C<sup>fru</sup>]) palatinose was significantly longer than that from [1-<sup>13</sup>C<sup>glc</sup>] and [1-<sup>13</sup>C<sup>fru</sup>]sucrose, respectively. The value of area under the curve (AUC) for [1-<sup>13</sup>C<sup>glc</sup>]palatinose was larger than that for [1-<sup>13</sup>C<sup>glc</sup>]sucrose, but AUC for [1-<sup>13</sup>C<sup>fru</sup>] showed no difference between palatinose and sucrose. In the H<sub>2</sub>-breath test, the concentration of H<sub>2</sub> in the expired air was measured for 420 min. H<sub>2</sub> was hardly detected with both palatinose and sucrose and no significant difference was observed between the two groups. These results suggest that palatinose is utilised *in vivo* at a rate equal to that of sucrose.

**Key words:** Palatinose; <sup>13</sup>C-Breath test; H<sub>2</sub>-Breath test; Blood glucose

Palatinose (isomaltulose, 6-O- $\alpha$ -D-glucopyranosyl-D-fructose) is a sucrose isomer originally found in honey and sugarcane, where glucose and fructose are linked via a  $\alpha$ -1,6-glycosidic bond (cf. sucrose via  $\alpha$ -1,2). Palatinose can be produced by enzymatic modification of sucrose using micro-organisms. Palatinose produced by such a method is accepted as a generally recognised safe carbohydrate (GRAS notice no. GRN 000184).

When ingested, palatinose is enzymatically hydrolysed into glucose and fructose in the small intestine at a considerably slower rate than sucrose<sup>(1)</sup>. Because of the slower rate of its hydrolysis, the absorption of liberated glucose and fructose is delayed, resulting in a slower rate of increase and lower level of blood glucose<sup>(2,3)</sup>. Such characteristics of palatinose suggest that this disaccharide is suitable for application to nutritive diets that require delayed absorption of glucose to prevent a rapid increase in postprandial blood glucose levels.

However, in spite of its favourable characteristics, one question regarding its nutritional mechanism remains

unanswered: does palatinose fully digested and absorbed in the small intestine provide the amount of energy equivalent to that calculated based on the amount of ingested palatinose? If this question is adequately answered, palatinose will be regarded as a proper source of carbohydrate energy for nutrition in that it provides the necessary amount of energy and also prevents postprandial hyperglycaemia.

In the present report, we present results principally obtained from an expiratory air analysis in rats, in which palatinose labelled with a non-radioactive isotope was applied. Based on the analysis of the results so obtained, we discuss the metabolic fate of ingested palatinose and present lines of evidence that palatinose can be fully digested and absorbed in the small intestine.

### Materials and methods

#### Samples

[1-<sup>13</sup>C<sup>glucose</sup>] ([1-<sup>13</sup>C<sup>glc</sup>]) and [1-<sup>13</sup>C<sup>fructose</sup>] ([1-<sup>13</sup>C<sup>fru</sup>]) Sucrose were purchased from Omicron Biochemicals, Inc. (South

**Abbreviations:** 1-<sup>13</sup>C<sup>fru</sup>, 1-<sup>13</sup>C<sup>fructose</sup>; 1-<sup>13</sup>C<sup>glc</sup>, 1-<sup>13</sup>C<sup>glucose</sup>; AUC, area under the curve.

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Bend, IN, USA). [1-<sup>13</sup>C<sup>glc</sup>] and [1-<sup>13</sup>C<sup>fru</sup>]palatinose were provided by Mitsui Sugar Company Limited (Tokyo, Japan). Unlabelled sucrose, palatinose and lactulose were purchased from Wako Pure Chemical Industries Limited (Tokyo, Japan). Distilled water was purchased from Ohtsuka Pharmaceutical Factory, Inc. (Tokushima, Japan).

### Animals

The experimental protocols were approved by the Animal Care Committee of the Division of Research and Development, Meiji Dairies Corporation.

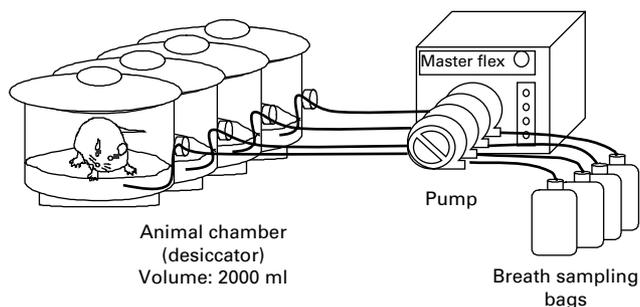
Six-week-old male Sprague–Dawley rats were purchased from Shizuoka Laboratory Animal Center (SLC, Shizuoka, Japan). The rats were housed in plastic cages on a cycle of 12 h light and 12 h darkness. Temperature and humidity in the cages were controlled at 22 ± 3°C and 55 (SD 15) %, respectively. The rats were fed *ad libitum* with a standard laboratory chow (CRF-1; Oriental Yeast Company Limited, Tokyo, Japan) and distilled water. The rats were fasted in meshed cages for 16 h to prevent coprophagy before each experiment, but had free access to drinking water.

### Blood glucose and plasma insulin levels

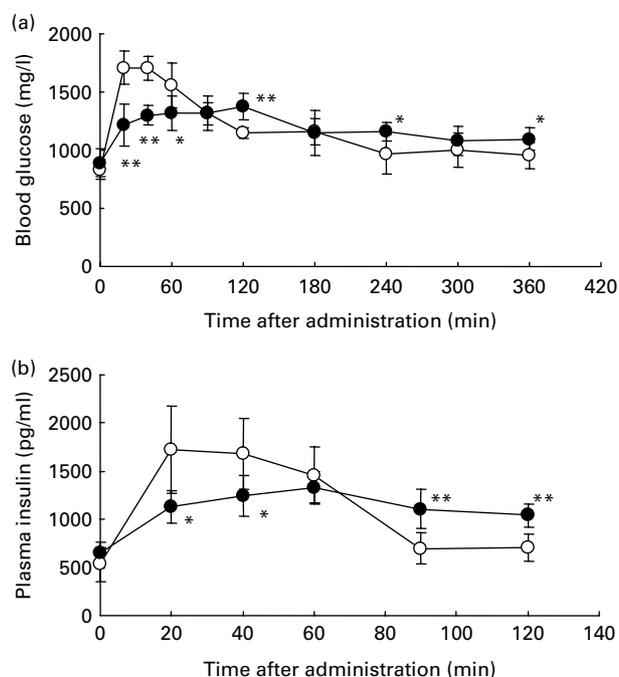
After the rats were fasted, preload blood samples were obtained from the tail vein at 0 min, and then the rats were orally administered sucrose or palatinose at a dose of 2 g/kg in a volume of 10 ml/kg. Postload blood samples were obtained at 20, 40, 60, 90, 120, 180, 240, 300 and 360 min. Blood glucose was measured using Dexter ZII (Bayer Company Limited, Osaka, Japan). Plasma insulin was measured using an ELISA kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan).

### Breath test system

A schematic illustration of the system used for collecting expired air from Sprague–Dawley rats reported by Uchida *et al.*<sup>(4)</sup> is shown in Fig. 1. In brief, this system is composed of an animal chamber (desiccator; 2000 ml),



**Fig. 1.** Schematic illustration of the system used to collect expired air from rats. Briefly, this system composed of a desiccator that was used as an animal chamber, a pump and breath sampling bags. Aspirating the expired air caused fresh air to automatically flow into the desiccator to replace it through a hole in the side of the chamber<sup>(4)</sup>.

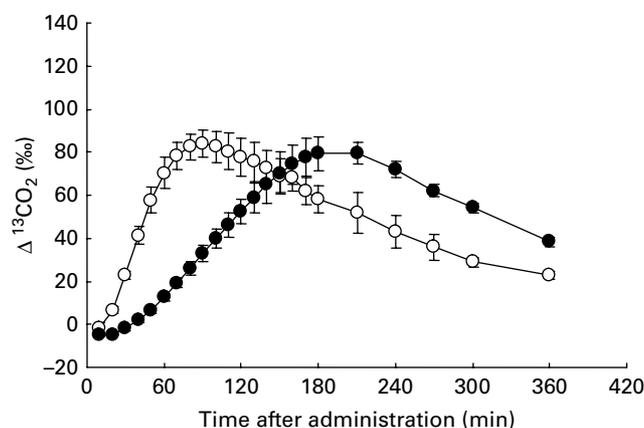


**Fig. 2.** Time course of blood glucose (a) and plasma insulin (b) response after a single oral administration of sucrose (O) or palatinose (●) in rats. Values are given as means and standard deviations represented by vertical bars (*n* 6). Mean values of palatinose were significantly different from those of sucrose: \**P* < 0.05, \*\**P* < 0.01.

pump (Masterflex L/S; Cole-Palmer Instrument Company, Barrington, IL, USA) and breath sampling bag (Ohtsuka Pharmaceutical Company Limited, Tokyo, Japan). The air in the chamber was continuously aspirated during the experimental period. The aspirated air was discharged outside this breath system except for the period collecting expired air in the breath sampling bag.

### <sup>13</sup>C-breath test

The rats were placed in the chamber immediately after the oral administration of [1-<sup>13</sup>C]sucrose or [1-<sup>13</sup>C]palatinose at a dose of 2 g/kg (mixture of 0.175 g of [1-<sup>13</sup>C]carbohydrate:



**Fig. 3.** Time course of Δ<sup>13</sup>CO<sub>2</sub> in expired air from rats after a single oral administration of [1-<sup>13</sup>C<sup>glucose</sup>] ([1-<sup>13</sup>C<sup>9glc</sup>]) sucrose (O) or [1-<sup>13</sup>C<sup>glc</sup>] palatinose (●). Values are given as means and standard deviations represented by vertical bars (*n* 4).

**Table 1.** Pharmacokinetics parameters after oral administration of [ $1\text{-}^{13}\text{C}^{\text{glucose}}$ ] ( $[1\text{-}^{13}\text{C}^{\text{glc}}]$ ) sucrose or [ $1\text{-}^{13}\text{C}^{\text{glc}}$ ] palatinose(Mean values and standard deviations,  $n$  4)

	$C_{\text{max}}$ (‰)			$T_{\text{max}}$ (min)			AUC (‰ min)		
	Mean	SD	$P^*$	Mean	SD	$P^*$	Mean	SD	$P^*$
[ $1\text{-}^{13}\text{C}^{\text{glc}}$ ]Sucrose	84	6	NS	93	13	<0.01	21 791	2027	<0.05
[ $1\text{-}^{13}\text{C}^{\text{glc}}$ ]Palatinose	81	7		195	17		24 617	1046	

 $C_{\text{max}}$ , maximum  $^{13}\text{CO}_2$  concentration;  $T_{\text{max}}$ , time taken to reach the maximum concentration; AUC, area under the curve.

\* Mean values of palatinose were significantly different from the value of sucrose.

1.825 g of unlabelled carbohydrate) in a volume of 10 ml/kg. The expired air was collected at 10 min intervals until 180 min and at 30 min intervals from 180 to 300 min with additional measurements at 360 min after the samples administration. The  $^{13}\text{CO}_2$  level in the breath sampling bag was measured with UBiT IR-300 (Ohtsuka Electronics Company Limited, Osaka, Japan). The measured values were presented as the  $\Delta^{13}\text{CO}_2$  (‰). The maximum  $^{13}\text{CO}_2$  concentration (‰), the time taken to reach the maximum concentration (min) and the area under the curve (AUC; ‰min) were calculated using the  $\Delta^{13}\text{CO}_2$  values. The AUC from the start of administration to the time when the speculated value of  $\Delta^{13}\text{CO}_2$  is zero was calculated using a software for pharmacokinetic, pharmacodynamic and non-compartment analysis (WinNonlin<sup>®</sup>, version 1.5; Pharsight Corp., Mountain View, CA, USA) by applying the linear trapezoidal rule. AUC reflects the absorption of labelled materials. Expired  $^{13}\text{CO}_2$  air was collected for 1.5 min each time. Ventilation volume was 150 ml/min.

#### $\text{H}_2$ -breath test

The rats were placed in the chamber immediately after the oral administration of the unlabelled sucrose, palatinose and lactulose at a dose of 2 g/kg in a volume of 10 ml/kg. The sample volume was 10 ml/kg. The expired air was collected at 120, 180, 240, 300, 360 and 420 min after the samples were administered. The  $\text{H}_2$  concentration in the breath sampling bag was measured using Breath Gas Analyzer TGA-2000 (Teramex Company Limited, Kyoto, Japan). Lactulose, an indigestible disaccharide, was used as a positive control. Expired  $\text{H}_2$  was collected for 1.5 min each time. Ventilation volume was 150 ml/min.

#### Statistical analysis

The results were represented as the means and standard deviations for the animals used. Statistical analysis was performed by Student's  $t$  test, and values of  $P < 0.05$  were regarded as significant.

## Results

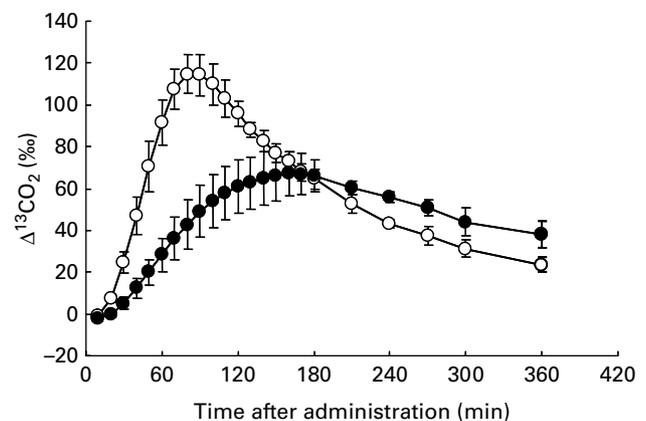
### Blood glucose and plasma insulin levels

Fig. 2(a) shows the time course of changes in blood glucose levels after an oral administration of sucrose or

palatinose. Basal glucose level in the palatinose and sucrose groups was 833 (SD 87) and 887 (SD 120) mg/l, respectively. When compared with the sucrose group, the blood glucose level in the palatinose group was significantly lower at 20, 40 and 60 min after administration, but significantly higher at 120, 240 and 360 min after administration. The time course of changes in plasma insulin level after the oral administration of sucrose or palatinose was also measured (Fig. 2(b)). When compared with the sucrose group, the plasma insulin level in the palatinose group was significantly lower at 20 and 40 min after administration, but significantly higher at 90 and 120 min after administration.

### $^{13}\text{C}$ -breath test

As shown in Fig. 3,  $\Delta^{13}\text{CO}_2$  increased rapidly in the rats given [ $1\text{-}^{13}\text{C}^{\text{glc}}$ ]sucrose and increased gradually after [ $1\text{-}^{13}\text{C}^{\text{glc}}$ ] palatinose administration. The maximum  $^{13}\text{CO}_2$  concentration value showed no significant difference between the sucrose and palatinose groups, whereas the time taken to reach the maximum concentration value was long and the AUC value was high in the palatinose group compared with the sucrose group (Table 1). Fig. 4 shows  $\Delta^{13}\text{CO}_2$  in the rats given [ $1\text{-}^{13}\text{C}^{\text{fru}}$ ]sucrose or [ $1\text{-}^{13}\text{C}^{\text{fru}}$ ]palatinose. The time taken to reach the maximum concentration in the palatinose group was significantly



**Fig. 4.** Time course of  $\Delta^{13}\text{CO}_2$  in expired air from rats after a single oral administration of [ $1\text{-}^{13}\text{C}^{\text{fructose}}$ ] ( $[1\text{-}^{13}\text{C}^{\text{fru}}]$ ) sucrose (○) or [ $1\text{-}^{13}\text{C}^{\text{fru}}$ ] palatinose (●). Values are given as means and standard deviations represented by vertical bars ( $n$  4).

**Table 2.** Pharmacokinetics parameters after oral administration of [1-<sup>13</sup>C<sup>fructose</sup>] ([1-<sup>13</sup>C<sup>fru</sup>]) sucrose or [1-<sup>13</sup>C<sup>fru</sup>]palatinose(Mean values and standard deviations, *n* 4)

	C <sub>max</sub> (‰)			T <sub>max</sub> (min)			AUC (‰ min)		
	Mean	SD	P*	Mean	SD	P*	Mean	SD	P*
[1- <sup>13</sup> C <sup>fru</sup> ]Sucrose	116	10	<0.01	85	6	<0.01	24 508	959	NS
[1- <sup>13</sup> C <sup>fru</sup> ]Palatinose	69	12		167	15		29 483	4232	

C<sub>max</sub>, maximum <sup>13</sup>CO<sub>2</sub> concentration; T<sub>max</sub>, time taken to reach the maximum concentration; AUC, area under the curve.

\* Mean values of palatinose were significantly different from the value of sucrose.

longer than that in the sucrose group as seen in the [1-<sup>13</sup>C<sup>glc</sup>] examination. On the other hand, the maximum <sup>13</sup>CO<sub>2</sub> concentration value in the palatinose group was significantly lower than that in the sucrose group and the AUC value in the palatinose group was not significantly different from that in the sucrose group (Table 2).

### H<sub>2</sub>-breath test

Changes in molecular H<sub>2</sub> concentrations over time are shown in Fig. 5. H<sub>2</sub> concentration increased gradually in the lactulose group. In the case of lactose intolerance, the definition of a positive test has varied but a rise of 10–20 parts per million over basal values is usually considered to indicate malabsorption<sup>(5)</sup>. According to this definition, the H<sub>2</sub> concentration was significantly greater in the lactulose group compared with the concentration at baseline from 4 h and extending to the end of the experiment. On the other hand, we observed no increase in H<sub>2</sub> over baseline both in the sucrose and palatinose groups during the experiment. There was no significant difference between the sucrose and palatinose groups.

### Discussion

In 1983, Macdonald & Daniel<sup>(6)</sup> reported the bioavailability of palatinose in rats using [<sup>14</sup>C]palatinose and [<sup>14</sup>C]sucrose. They measured radioactivity excreted into faeces and urine and that expired into the air after the administration of labelled palatinose and sucrose. They found no significant difference between palatinose and sucrose, and they concluded that palatinose and sucrose were hydrolysed at comparable rates. In their study, however, the dosage of sucrose or palatinose was only 500 mg/kg body weight and the blood glucose levels after administration of these carbohydrates were not measured.

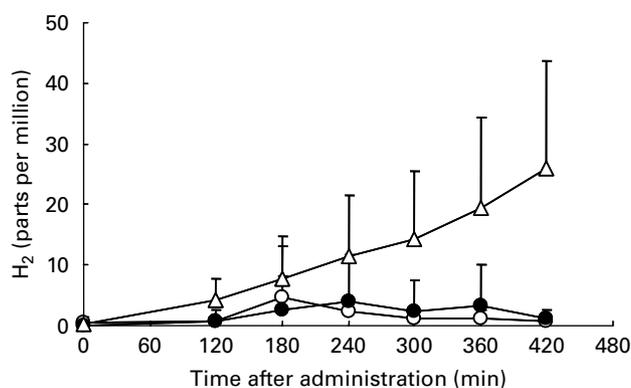
In the present report, we first confirmed doses where palatinose and sucrose show clearly different glycaemic responses, then proceeded to analyse their bioavailabilities. Palatinose and sucrose showed comparable glycaemic responses at doses <1 g/kg body weight, probably because palatinose and sucrose do not show glycaemic responses that are large enough to be compared against each other (data not shown). Therefore, we needed to identify the doses at which palatinose can clearly show a

lower glycaemic response compared with sucrose. To do so, we used the dose of 2 g/kg body weight.

When the dose of 2 g/kg body weight was administered, significant differences were observed in the levels of plasma glucose and insulin between the palatinose and sucrose groups. After 120 min, the blood glucose of the palatinose group was higher than that of the sucrose group. The rate of hydrolysis in the small intestine of palatinose is very slow compared with sucrose and the characteristic contributes to the transition of blood glucose.

When expired <sup>13</sup>CO<sub>2</sub> was measured in the animals administered either labelled palatinose or sucrose at this dose, a significant delay in time taken to reach the maximum concentration was observed in the palatinose group. The delay was observed irrespective of the difference in labelled positions in palatinose, [1-<sup>13</sup>C<sup>glc</sup>] or [1-<sup>13</sup>C<sup>fru</sup>]. These data evidently show that palatinose is hydrolysed in the intestine at a lower rate than sucrose, and therefore coincidentally absorbed at a lower rate than sucrose. Of note, the rate of hydrolysis of palatinose by brush-border membrane preparations from rat jejunum was actually measured and was 11–12 % as compared with the rate towards sucrose<sup>(7)</sup>.

When the values of AUC were compared, a significant difference was observed between the [1-<sup>13</sup>C<sup>glc</sup>]palatinose group, and the [1-<sup>13</sup>C<sup>glc</sup>]sucrose group; the AUC of the palatinose group was significantly larger than that of the sucrose group. One possible explanation is that palatinose is not only hydrolysed and absorbed more slowly than sucrose but it might also be oxidised in a pathway different

**Fig. 5.** Time course of H<sub>2</sub> in expired air from rats after a single oral administration of sucrose (○) or palatinose (●) or lactulose (△). Values are given as means and standard deviations represented by vertical bars (*n* 4).

from that of sucrose, resulting in the production of more labelled CO<sub>2</sub>. Such speculation is supported by the fact that significantly different levels of insulin secretion were observed between the two groups; in fact, insulin affects the metabolism of carbohydrates and lipids<sup>(8,9)</sup>. On the one hand, insulin stimulates the pathway of glycogen synthesis in hepatocytes<sup>(10)</sup> that do not generate CO<sub>2</sub>. On the other hand, insulin enhances the activity of lipogenic enzymes such as acetyl-CoA carboxylase and fatty acid synthase in rat hepatocytes<sup>(11)</sup>. There is a possibility that glucose of sucrose origin is easy to use for fatty acid synthesis compared with glucose of palatinose origin. In fatty acid synthesis, [1-<sup>13</sup>C]glucose and [1-<sup>13</sup>C] fructose do not become the substrate of labelled CO<sub>2</sub>.

There was a significant difference in the values for maximum <sup>13</sup>CO<sub>2</sub> concentration between the fructose-labelled palatinose and sucrose; however, no such difference was seen between the glucose-labelled ones. These data evidently indicate that fructose derived from palatinose and that from sucrose are metabolised in different pathways. This difference might also be explained by the different levels of insulin secretion. Fructose uptake into cells is not affected by insulin levels, whereas its metabolism in the glycolysis pathway is regulated by insulin<sup>(12)</sup>. Consequently, for the glucose and fructose of palatinose origin, the metabolism after absorption from the small intestine is different from the glucose and fructose of sucrose origin. The initial level of the insulin secretion might take part in it.

Carbohydrates, if not absorbed in the small intestine, are transferred into the colon, where they are utilised for fermentation by enterobacteria. Colonic carbohydrate fermentation produces molecules such as H<sub>2</sub>, CH<sub>4</sub> and SCFA<sup>(13)</sup>. The H<sub>2</sub> produced in fermentation is absorbed and expired into the air. Therefore, the transfer of carbohydrates into the colon can be assessed by the detection of H<sub>2</sub> in expired air. As shown in Fig. 5, the administration of lactulose, a disaccharide indigestible in the small intestine, produced a significant amount of H<sub>2</sub> in the expired air. Contrarily, when rats were administered palatinose or sucrose, levels of H<sub>2</sub> in the expired air stayed well below 10 parts per million, the level of H<sub>2</sub> above which carbohydrate malabsorption in the small intestine is diagnosed<sup>(5)</sup>, for 7 h, a period that is long enough for unabsorbed carbohydrates to pass through into the colon. These results indicate that palatinose is fully hydrolysed and absorbed in the small intestine as well as sucrose is, even if their metabolic pathways are different.

## Acknowledgements

[1-<sup>13</sup>C<sup>glc</sup>] and [1-<sup>13</sup>C<sup>fru</sup>]palatinose were provided by Mitsui Sugar Company, Limited (Tokyo, Japan). H. T. participated in data collection, data analysis, data interpretation and manuscript preparation. T. Y., M. U., Y. U. and H. S. participated in study design, data interpretation and review of the manuscript. M. K. and A. S. participated in data collection and data analysis. T. K., M. O., K. S. and J. K. participated in data interpretation and review of the manuscript. There are no conflicts of interest.

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