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Enzymatically Produced Maltodextrin with Different Linkage Mode and Its Effect on Blood Glucose Elevation

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Abstract: We have prepared a new type of maltodextrin which yielded by simultaneous combination of enzymes. The enzymes are transglucosidase and maltose-forming amylase at the enzyme unit ratio of 1:20. This maltodextrin was found to be equivalent to standard one in terms of physicochemical properties including dextrose equivalent (DE), viscosity, osmotic pressure and stability against aging. Methylation analysis revealed that this maltodextrin had around 9% of “→6)-Glc-(1→” linkage mode, it was not present in standard maltodextrin. Enzymatic analysis showed that molecular weight of this “→6)-Glc-(1→” maltodextrin has Mw 1,000 to 3,000. Furthermore, we compared digestibility differences between “→6)-Glc-(1→” maltodextrin with standard maltodextrin, and “→4,6)-Glc-(1→” maltodextrin (this was prepared by isolating and collecting a component with starch intrinsic branched structure) with standard maltodextrin. In vitro studies revealed that both “→6)-Glc-(1→” and “→4,6)-Glc-(1→” maltodextrin were digested slower than standard maltodextrin. Whereas in humans, “→6)-Glc-(1→” maltodextrin was digested slower than standard one. The blood glucose level elevation after 60 min ingestion was significantly lower, suggesting that this “→6)-Glc-(1→” maltodextrin was slowly digested and absorbed. Additionally, results from the expired gas analysis in humans suggest that the energy coefficient of “→6)-Glc-(1→” maltodextrin is 4 kcal/g, similar to that of standard one. Considering these findings, “→6)-Glc-(1→” maltodextrin is likely a promising source of carbohydrates for diabetic patients and elderly people with poor glucose tolerance.

Key words: “→6)-Glc-(1→” maltodextrin, transglucosidase, blood glucose level, digestion, diabetic

INTRODUCTION

Maltodextrins, yielded by hydrolysis of starch with acids or enzymes, have been extensively used as a source of carbohydrates for nutrients such as medical foods by virtue of the low osmotic pressure and slightly sweet taste of maltodextrins, because such nutrients contain a lot of carbohydrates. In fact, carbohydrates with high osmotic pressure such as glucose and starch syrup are likely to induce osmotic diarrhea and abdominal flatulence, and those with strong sweet taste are unsuitable for daily ingestion as staple food. For these reasons, maltodextrins with low osmotic pressure and slightly sweet state are often used for such nutrients.

However, maltodextrins are rapidly digested/absorbed and have the potential of causing rapid elevation in blood glucose level after ingestion. Foods with such features are unfavorable for diabetic patients whose elevation in blood glucose level needs to be avoided as much as possible and for elderly patients who are often glucose-intolerant. Therefore, it is desirable to develop maltodextrins which are slowly digested and absorbed.1,2) Such maltodextrins can be utilized also in diet foods, beverages and food supplements as a source of carbohydrate. Hence, their development is quite valuable.

Research and development of carbohydrates of various molecular weights, ranging from starch to disaccharides, with slow digestion/absorption characteristics have been conducted.

For example, branched starch (e.g., yielded by enzymatic treatment of starch) and highly branched dextrin (e.g., yielded by collection of branched components of starch) were reported that they were slowly digested.5–7) However, these types of starch are not suitable for commercialization because of their complex manufacturing processes and low yields.

Isomalto-oligosaccharide and isomaltulose were reported that they were slowly digested.8,9) However, these substances have high osmotic pressure and their use as a source of carbohydrate for nutrients is sometimes limited.

In this juncture, this article will present our new findings about the slowly digestible maltodextrin which having a low osmotic pressure, as well as its efficient production method.

MATERIALS AND METHODS

Materials. Standard maltodextrin (brand name: GlystarP) was prepared in our R&D center. Branched dextrin (brand name: CES), obtained by isolation and collection of branched components of starch, was obtained from Sanmatsu Kogyo Co., Ltd. (Tokyo, Japan). Fructo-oligosac-
charide (brand name: Meioligo) was obtained from Meiji Co., Ltd (Tokyo, Japan). Maltose-forming amylase (brand name: Biozyme L) and α-glucosidase (brand name: Transglucosidase L Amano) were obtained from Amano Enzyme Inc. (Nagoya, Japan). Pullulanase (EC 3.2.1.41, derived from Enterobacter aerogenes) was obtained from Sigma-Aldrich, Inc. (St. Louis, USA). Isoamylase (brand name: GODO-FIA) was obtained from Godo Shusei Co., Ltd. (Tokyo, Japan). β-amylase, from sweet potato was obtained from Sigma-Aldrich, Inc. Porcine pancreas amylase was obtained from F. Hoffmann-La Roche Ltd. (Basel, Switzerland).

Rat small bowel acetone powder was obtained from Sigma-Aldrich, Inc. Glucose CII Test Wako was obtained from Wako Pure Chemical Industries (Osaka, Japan).

“\( \alpha \rightarrow 6 \)-Glc\( p \)-(1\( \rightarrow \alpha \)) and “\( \alpha \rightarrow 4,6 \)-Glc\( p \)-(1\( \rightarrow \alpha \)) maltodextrin."

In this paper, “\( \alpha \rightarrow 6 \)-Glc\( p \)-(1\( \rightarrow \alpha \)) maltodextrin (hereinafter called EHBdex) means maltodextrin having glucosyl residues of “\( \alpha \rightarrow 6 \)-Glc\( p \)-(1\( \rightarrow \alpha \)) linkage mode. “\( \alpha \rightarrow 4,6 \)-Glc\( p \)-(1\( \rightarrow \alpha \)) maltodextrin (hereinafter called SHBdex) means maltodextrin with a percentage of glucosyl residues of “\( \alpha \rightarrow 4,6 \)-Glc\( p \)-(1\( \rightarrow \alpha \)) linkage mode higher than that of standard maltodextrin.

Preparation of EHBdex by enzymatic rearrangement.

Starch suspension in water (30%, w/w) was combined with oxalic acid to adjust pH at 1.9, followed by hydrolysis at 125°C for 1 h to yield maltodextrin (dextrose equivalent [DE] = 12. DE is defined by the equation: weight of direct-reducing sugar (expressed as glucose)/weight of solid \[ \text{DE} = \frac{\text{weight of direct-reducing sugar}}{\text{weight of solid}} \]. It was analyzed with the Willstaetter-Schuel method.\(^{[6]}\)) Obtained maltodextrin was adjusted to 30% (w/w) concentration and pH 5.6. To this maltodextrin \( 1 : 20 \) ratio of 150 U Transglucosidase L Amano (a type of α-glucosidase) and 2,968 U Biozyme L (maltose-forming amylase) per weight (kg) of maltodextrin solid added, followed by enzymatic reaction at 55°C for 3 h. One unit of Transglucosidase L Amano was defined as the enzymatic activity capable of forming 1 μmol/min of glucose when the reaction is conducted at pH 5.5 and 55°C with 1% (w/w) aqueous solution of methyl-α-D-glucopyranoside serving as the substrate. One unit of Biozyme L was defined as the enzymatic activity capable of forming 1 μmol/min of maltose when the reaction is conducted at pH 5.5 and 55°C with 5% (w/w) aqueous solution of dextrin (DE = 11, mean molecular weight = 1,700, mean polymerization degree = 10; a product of Matsutani Chemical Industry Co., Ltd.) serving as the substrate. Reaction was stopped by thermally inactivated enzyme with incubation at 95°C for 30 min. Reaction mixture was down-stream processed with active carbon treatment, diatomaceous earth filtration and decoloration/desalting with ion exchange resin, followed by concentration, spray-drying to yield a powder and is designated as “EHBdex”.

SHBdex prepared by isolation and collection of the branched structure originally possessed by starch.

CES\(^{[5]}\) (Sanmatsu Kogyo Co., Ltd.), prepared by isolating and collecting a component with starch intrinsic branched structure,\(^{[9]}\) was used as “\( \alpha \rightarrow 4,6 \)-Glc\( p \)-(1\( \rightarrow \alpha \)) maltodextrin and is designated as “SHBdex”.

Osmotic pressure. Aqueous maltodextrin solutions (Brix 10%) were used to measure the osmotic pressure with a VOGEL OM802-D osmotic pressure meter (Model OM 802-D; Vogel GmbH & Co. KG., Giessen, Germany).

Viscosity. Aqueous maltodextrin solutions (Brix 30%) were used to measure the viscosity with a Viscometer model BM (Tokyo Keiki Inc., Japan) at 30°C and 60 rpm.

Stability against aging. Aqueous maltodextrin solutions (Brix 50%) were frozen at -20°C, thawed at room temperature, and diluted to give Brix 30%. Then, turbidity was measured with a spectrophotometer (OD at 720 nm, converted on a 10-cm cell basis). This sequence of manipulations was repeated either until the turbidity increased or to a maximum of 5 cycles.

Methylation analysis. This method is used to determine polysaccharide linkage modes by modifying binding or non-binding linkages with different chemicals. The analysis was conducted according to the method reported by Ciucan et al.\(^{[10]}\)

Enzymatic analysis (debranching enzyme degradation and β-amylase degradation). The sample (200 mg) was dissolved in 8 mL of 100 mM acetate buffer (pH 5.5). The solution was combined with 10 μL of isoamylase (2,000 U) and 10 μL of pullulanase (0.625 U), followed by incubation at 55°C for 3 h. Then, the enzyme was thermally inactivated by incubation at 95°C for 15 min, followed by desalting with ion exchange chromatography and concentration by rotary evaporator. Pure water was added to the residue to a final volume of 6.4 mL, and the solution was then combined with 1.6 mL of 500 mM acetate buffer (pH 3.9), followed by addition of 10 μL of β-amylase (200 U) and 1 h incubation at 60°C. After thermal inactivation at 95°C for 15 min, desalting was performed with ion exchange chromatography, followed by concentration by rotary evaporator.

Digestion study in vitro. The digestion study in vitro was carried out to simulate carbohydrate digestion in the living body, by the method reported by Englyst et al.\(^{[11]}\) with minor modifications.

Porcine pancreas amylase was a product of F. Hoffmann-La Roche Ltd. (19,230 U/mL). Rat small bowel mucosal enzymes were prepared from rat small bowel acetone powder (Sigma-Aldrich, Inc.) as follows. Rat small bowel acetone powder (1.2 g) was suspended in 15 mL of 45 mM Bis-Tris-HCl Buffer (pH 6.6) containing 0.9 mM CaCl\(_2\). After homogenization and centrifugation at 3,000 rpm for 10 min, the supernatant was harvested as a crude enzyme solution containing rat small bowel mucosal enzymes. The activity of the crude enzyme solution (1 U) corresponded to the degradation of 1 mmol/min of maltose in 26 mM maltose solution.

The test substance was dissolved in buffer (45 mM Bis-Tris-HCl Buffer, pH 6.6)/0.9 mM CaCl\(_2\)) to yield a 0.24% (w/w) test substance solution. GlystarP, EHBdex and SHBdex served as test substances. An aliquot (25 mL) of the
solution of each test substance was transferred into a test tube and incubated for 10 min in a thermostat (37°C). Then, 0.5 mL of mixed enzyme solution (50 µL of porcine pancreas amylase [384.6 U/mL] + 140 µL rat small bowel mucosal enzymes [6.0 U/mL] + 310 µL buffer) was added to each solution and agitated well. After 15, 30 and 45 min and 1, 1.5, 2, 3, 4 and 6 h following the start of the reaction, 50 µL of 0.5 M perchloric acid was added to 200 µL of each reaction system to stop the reaction. The glucose level in the reaction system was then measured with Glucose CII Test Wako (Wako Pure Chemical Industries).

**Digestion studies in humans.** Each human study involved healthy adult males and females voluntarily desiring to participate in the study after having been well-informed about the objectives, methods, and anticipated adverse reactions. A written consent was obtained from everyone. The study was carried out in compliance with the principles set forth in the Declaration of Helsinki after approval by the Matsutani Chemical Industry Ethics Committee. Such study involved 12 healthy adults (6 males and 6 females) free of gastrointestinal and respiratory diseases. The mean age of the subjects was 34.3 ± 3.7 years (male: 33.8 ± 3.7 years; female: 34.7 ± 3.4 years). The mean body weight was 57.4 ± 10.7 kg (male: 65.7 ± 7.4 kg; female: 49.1 ± 5.5 kg). Fifty grams of each test substance (GlystarP, EHBdex and SHBdex) was dissolved in 200 mL of water. To perform the study under a uniform setting, the subjects ingested one of the following products of Ezaki Glico Co., Ltd., including Sukiyaki Donburi (Beef rice bowl) or curry rice by 9:00 pm of the previous evening and took no food or beverage other than water thereafter. At 9:00 am of the following morning, on fasting, the expired gas of each subject was collected into a 100-mL collection bag. Then, each subject ingested an aqueous solution of one of the test substances (200 mL). The expired gas of each subject was collected into a 100-mL collection bag 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h after ingestion. After 4 and 7 h following the ingestion of the test substance, a test diet (2 pieces of cheese, 1 boiled egg, 1 tuna can, and 500 mL refreshing beverage) not affecting breath hydrogen gas content was ingested by each subject in two divided portions. During the study period, each subject remained stationary in the room. Breath hydrogen gas concentration was measured with a TGA-2000H Breath Analyzer (TERAMECS CO., LTD., Kyoto, Japan, Laboratory for Expiration Biochemistry Nourishment Metabolism, Ltd.), and the area under the curve (AUC) was calculated by the trapezoidal method from breath hydrogen gas concentrations with the pre-experiment value taken as the baseline. The study was carried out on 3 test substances in a single-blind crossover manner at an interval of one week or longer.

**Satiety evaluation in humans.** The subjects of this study were identical to those of the digestion study in humans. Fifty grams of each test substance (GlystarP and EHBdex) was dissolved in 200 mL of water. To perform the study under a uniform setting, the subjects ingested one of the following products of Ezaki Glico Co., Ltd. (Osaka, Japan), including Sukiyaki Donburi (Beef rice bowl) or curry rice by 9:00 pm of the previous evening and took no food or beverage other than water thereafter. At 9:00 am of the following morning, on fasting, the subjects ingested the test substance. Each subject rated his/her satiety (suppression of hunger sensation) on a five-score scale (given below) immediately before and every 30 min during the 3-h post-ingestion period. The study was carried out on two test substances in a single-blind crossover manner at an interval of one week or longer.

Score 1: Hunger hard to endure
Score 2: Strong hunger
Score 3: Hunger
Score 4: Slight hunger
Score 5: No hunger

**Study of breath hydrogen gas in humans.** This study was carried out in accordance with the method reported by Oku et al.12 The subjects of this study were identical to those of the previous studies. Three test substances (GlystarP, EHBdex and fructo-oligosaccharide (FOS)) were used. EHBdex and GlystarP were loaded in a quantity of 50 and 100 g respectively. FOS was loaded in a quantity of 20 g. To perform the study under a uniform setting, the subjects ingested one of the following products of Ezaki Glico Co., Ltd., including Sukiyaki Donburi (Beef rice bowl) or curry rice by 9:00 pm of the previous evening and took no food or beverage other than water thereafter. At 9:00 am of the following morning, on fasting, the expired gas of each subject was collected into a 100-mL collection bag. Then, each subject ingested an aqueous solution of one of the test substances (200 mL). The expired gas of each subject was collected into a 100-mL collection bag 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h after ingestion. After 4 and 7 h following the ingestion of the test substance, a test diet (2 pieces of cheese, 1 boiled egg, 1 tuna can, and 500 mL refreshing beverage) not affecting breath hydrogen gas content was ingested by each subject in two divided portions. During the study period, each subject remained stationary in the room. Breath hydrogen gas concentration was measured with a TGA-2000H Breath Analyzer (TERAMECS CO., LTD., Kyoto, Japan, Laboratory for Expiration Biochemistry Nourishment Metabolism, Ltd.), and the area under the curve (AUC) was calculated by the trapezoidal method from breath hydrogen gas concentrations with the pre-experiment value taken as the baseline. The study was carried out on 3 test substances in a single-blind crossover manner at an interval of one week or longer.

**Statistical analysis.** Results of previous study in human were described as mean ± SE of the mean (SEM). In digestion studies, significance of differences between EHBdex with GlystarP, and SHBdex with GlystarP were assessed by the one-way analysis of variance (ANOVA), and the comparison between two groups were carried out by Dunnett’s multiple comparison test. In satiety study, significance of differences between groups were assessed by paired t-test. For all tests, p < 0.05 was used as critical level of significance.

**RESULTS AND DISCUSSION**

**Physicochemical characteristics.**

The most relevant physicochemical characteristics for the utilization of dextrin as food are viscosity, osmotic pressure and stability against aging. Viscosity affects the food taste and texture feeling. Osmotic pressure affects the ease in digestion and absorption. Stability against aging affects the taste and the external appearance of food. EHBdex differed slightly from GlystarP (standard dextrin)—in terms of viscosity and osmotic pressure—when used as a food, and
was rated equivalent to GlystarP (Table 1). In the evaluation of the stability against aging, there was no increase in turbidity (data not shown), thus endorsing a high stability. These results indicate that EHBdex and SHBdex can replace standard dextrin.

**Structure.**

**Methylation analysis.**

The percentage of \(\beta-(1\rightarrow6)\)-Glc is lower in EHBdex than in GlystarP (73.3%). SHBdex had a lower percentage (62.3%) of \(\beta-(1\rightarrow4)\)-Glc (Table 2). Thus, EHBdex had, compared with GlystarP, increased percentage of \(\beta-(1\rightarrow4)\)-Glc among \(\beta-(1\rightarrow6)\)-Glc and \(\beta-(1\rightarrow4,6)\)-Glc which are glucose having an \(\alpha,1,6\) linkage. On the other side, SHBdex had, compared with GlystarP, increased percentage of \(\beta-(1\rightarrow4,6)\)-Glc among \(\beta-(1\rightarrow6)\)-Glc and \(\beta-(1\rightarrow4,6)\)-Glc which are glucose having an \(\alpha,1,6\) linkage.

**Enzymatic analysis (debranching enzyme degradation and \(\beta\)-amylase degradation).**

Maltodextrin is composed of multiple glucans of varying molecular weights. Methylation analysis revealed that EHBdex is a glucan with developed \(\alpha,1,6\) linkage, the molecular weight of which is unknown. To answer this question, the amylopectin branching chain of maltodextrin was degraded with a debranching enzyme, followed by exposure to \(\beta\)-amylase that is capable of stopping the reaction at the point of \(\beta-(1\rightarrow6)\)-Glc and \(\beta-(1\rightarrow4,6)\)-Glc. The components remaining intact were subjected to gel filtration chromatography to analyze their molecular weights.

Figure 1 shows the overlapped molecular weight distributions after enzymatic degradation of EHBdex and GlystarP with the debranching enzyme and \(\beta\)-amylase. The component of the molecular weight distribution after the subtraction of GlystarP from EHBdex indicates the \(\beta-(1\rightarrow6)\)-Glc developed maltodextrin synthesized with \(\alpha\)-glucosidase rearrangement. As shown in Fig. 1, the predominant molecular weight of the synthesized \(\beta-(1\rightarrow6)\)-Glc maltodextrin had approximately \(M_n 1,000\) to 3,000. In addition, this result indicated that EHBdex is maltodextrin containing glucosyl residues of \(\beta-(1\rightarrow6)\)-Glc linkage mode at the non-reducing terminal.

**Table 2.** Percentage of glucosyl residues in different linkage mode in various kinds of maltodextrins.

<table>
<thead>
<tr>
<th>Glycosidic linkage</th>
<th>Linkage mode of glucosyl residue*</th>
<th>GlystarP</th>
<th>EHBdex</th>
<th>SHBdex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal</td>
<td>Glc-(1(\rightarrow))</td>
<td>20.0</td>
<td>19.5</td>
<td>18.4</td>
</tr>
<tr>
<td>1,4 linkage</td>
<td>(\beta-(1\rightarrow4))-Glc</td>
<td>73.3</td>
<td>62.7</td>
<td>62.3</td>
</tr>
<tr>
<td>1,6 linkage</td>
<td>(\beta-(1\rightarrow6))-Glc</td>
<td>0.0</td>
<td>8.9</td>
<td>2.0</td>
</tr>
<tr>
<td>1,3 linkage</td>
<td>(\beta-(1\rightarrow3))-Glc</td>
<td>4.8</td>
<td>5.2</td>
<td>13.7</td>
</tr>
<tr>
<td>1,2 linkage</td>
<td>(\beta-(1\rightarrow2))-Glc</td>
<td>9.0</td>
<td>3.2</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>(\beta-(1\rightarrow2))-Glc</td>
<td>0.9</td>
<td>0.6</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*For example, \(\beta-(1\rightarrow4)\)-Glc indicates glucosyl residue having a glucosidic linkage at 1,4 position. GlcP, glucopyranose.

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![Figure 1](image.png)

Fig. 1. Molecular weight distribution of EHBdex and GlystarP after reaction with branching enzyme and \(\beta\)-amylase.

The solid line expressed a molecular distribution of EHBdex, and the dotted line expressed a molecular distribution of GlystarP. x-axis, molecular weight (logarithmic expression; log\(M\)); y-axis, weight percent per log\(M\) (d W/d log\(M\)); ▼ indicated the number-average molecular weight (\(M_n\)).
Functional characteristics.

Digestion study in vitro.

Figure 2 (A) illustrates the time course of glucose level synthesized upon reaction with the digestive enzyme. In comparison to the digestion of GlystarP, the digestion of EHBdex and SHBdex resulted in lower glucose levels between 45 min and 2 h after the start of the reaction. Moreover, the glucose levels become identical to GlystarP during the period between 3 and 6 h after the start of the reaction. The final glucose concentration was approximately 200 mg/dL (identical to the theoretical level). Figure 2 (B) illustrates the glucose formation rate calculated based on the data shown in Fig. 2 (A). The glucose formation rate was lower with EHBdex and SHBdex than with GlystarP. The comparison between EHBdex and SHBdex revealed a lower glucose level and a lower glucose formation rate for the digestion of SHBdex. These results indicate that EHBdex and SHBdex are digested slower than the standard dextrin GlystarP but are eventually completely digested. Additionally it was shown that SHBdex is digested slower than EHBdex.

Digestion study in humans.

The blood glucose levels after the ingestion of EHBdex was lower than GlystarP or SHBdex at every time point, and the difference at 60 min was statistically significant. It is noteworthy that although the difference in the percentage of α-1,6 linkages between EHBdex and SHBdex is minimal (Table 2), the blood glucose level after the ingestion of SHBdex, which differs from EHBdex in terms of the linkage mode at the α-1,6 linkage, was similar to that after the digestion of GlystarP (Fig. 3 (A)). Furthermore, the AUC of the blood glucose change of EHBdex was significantly smaller than GlystarP. The relative value of AUC was 78 for EHBdex and 101 for SHBdex (GlystarP = 100) (Fig. 3 (B)). These results indicate that EHBdex is digested slower than the standard dextrin and has a lower glycemic index. In the analysis of the subjective symptoms carried out by interview-

![Graphs](image-url)

**Fig. 2.** Glucose levels induced by the reaction with digestive enzymes (A), glucose formation rate induced by the reaction with digestive enzymes (B). (A) The quantity of glucose which released over time after the reaction of test substances by a mixture of porcine pancreas amylase and rat small bowel mucosal enzymes was measured. (B) The glucose formation rate was calculated based on the data shown in Fig. 2(A). O, GlystarP; ■, EHBdex; ▲, SHBdex.

**Fig. 3.** Changes in blood glucose level for 120 min after ingestion (A), area under the curve of blood glucose level for 120 min after ingestion (B). (A) Human postprandial blood glucose spike of GlystarP, EHBdex and SHBdex was measured. O, 50 g GlystarP; ■, 50 g EHBdex; ▲, 50 g SHBdex; n = 12; * p < 0.05 (Dunnett). (B) The area under the curve was calculated by the trapezoidal method from blood concentrations of Fig. 3 with the pre-experiment value taken as the baseline. n = 12; * p < 0.05 (Dunnett).
ing the study subjects, no adverse reactions possibly attributable to the ingestion of any of the test substances were detected.

SHBdex has a percentage of α-1,6 linkages close to that of EHBdex, and, according to the in vitro digestion study, its digestion was slow. However, the magnitude of the change in blood glucose level in humans following its digestion was close to that of GlystarP. These results suggest that the slow digestive linkage mode of glucan is (1→6)-Glc-(1→x), forming an α-1,6 linkage at the non-reducing terminal of the maltodextrin, rather than (1→6)-Glc-(1→x) that forms a branching structure in human. This discrepancy between the digestion study in vitro and the digestion study in humans seems to be attributable to a difference between the digestive enzymes used in vitro and the human digestive enzymes.

*Satietiy evaluation in humans.* Based on the analysis of the changes in the satiety score following the ingestion of EHBdex, the scores after 2 and 2.5 h following the ingestion were higher than those after the ingestion of the standard dextrin, GlystarP. Although none of these differences was statistically significant, the P value for the paired t-test in the score after 2 h was 0.05, indicating a tendency for high satiety (less hunger sensation) following the ingestion of EHBdex as compared to GlystarP (Fig. 4).

*Energy coefficient.*

*Breath hydrogen analysis in humans.* Breath hydrogen gas analysis enables the evaluation of the fermenting and degrading potentials in the large bowel for the carbohydrates that are indigestible and unabsorbed in the small bowel. In the present study, this analysis was carried out to examine whether or not EHBdex had reached the large bowel (i.e., whether or not it had been digested and absorbed completely in the small bowel). The study conducted by Iida et al. confirmed the relationship between the quantities of fructo-oligosaccharide (FOS) ingested and the concentration of breath hydrogen gas, demonstrating a positive correlation between the quantities ingested and the AUC of the breath hydrogen gas concentration. For example, when FOS was ingested in a quantity of 5, 10 and 20 g, the AUC of the breath hydrogen gas concentration was 117, 250 and 464 ppm, respectively. If the ingested quantity is lower than 5 g, the variation in the reading by the measuring device will be larger. Therefore, with this evaluation method, the lower detection limit is estimated at about 100 ppm (AUC of breath hydrogen gas concentration) when the quantity reaching the large bowel is 5 g.

When the breath hydrogen gas concentration following the digestion of EHBdex was compared with that following FOS digestion, the EHBdex ingestion group showed no marked changes in the breath hydrogen gas concentration and almost no hydrogen gas formation during the period between immediately after the ingestion and 10 h after at any of the two dose levels (50 and 100 g) (Fig. 5 (A)). Although the dose level was smaller (20 g) than the EHBdex dose, in the FOS ingestion group, the breath hydrogen gas concentration began to rise 1 h after the ingestion and reached 80 ppm at 8 h. Comparing the AUC of the breath hydrogen gas concentration, the AUC in the EHBdex ingestion group was below the lower detectable level at both dose levels (50 and 100 g). In the FOS ingestion group, AUC was 528 ppm.

By comparing GlystarP and FOS in a similar way, it is clear that the ingestion of GlystarP at dose level of 50 or 100
g resulted in the almost null formation of the breath hydrogen gas, similarly to the results obtained for EHBdex (Fig. 5 (B)). The AUC for the breath hydrogen gas concentration was below the lower detectable level at both dose level (50 and 100 g).

These results suggest that, similarly to the standard dextrin GlystarP, EHBdex is completely digested and absorbed in the small bowel and does not reach the large bowel. The energy coefficient for EHBdex seems to be 4 kcal/g, similar to that of standard maltodextrin.

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