In Vivo Digestibility of Carbohydrate Rich in Isomaltomegalosaccharide Produced from Starch by Dextrin Dextranase

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Abstract: Slowly digestible carbohydrates are needed for nutritional support in diabetic patients with malnutrition. They are a good source of energy and have the advantage that their consumption produces a low postprandial peak in blood glucose levels because they are slowly and completely digested in the small intestine. A high-amount isomaltomegalosaccharide containing carbohydrate (H-IMS), made from starch by dextrin dextranase, is a mixture of glucose polymers which has a continuous linear structure of α-1,6-glucosidic bonds and a small number of α-1,4-glucosidic bonds at the reducing ends. It has a broad degree of polymerization (DP) distribution with glucans of DP 10–30 as the major component. In our previous study, H-IMS has been shown to exhibit slow digestibility in vitro and not to raise postprandial blood glucose to such levels as that raised by dextrin in vivo. This marks it out as a potentially useful slowly digestible carbohydrate, and this study aimed to evaluate its in vivo digestibility. The amount of breath hydrogen emitted following oral administration of H-IMS was measured to determine whether any indigestible fraction passed through to and was fermented in the large intestine. Total carbohydrate in the feces was also measured. H-IMS, like glucose and dextrin, did not result in breath hydrogen excretion. Carbohydrate excretion with dietary H-IMS was no different from that of glucose or water. These results show that the H-IMS is completely digested and absorbed in the small intestine, indicating its potential as a slowly digestible carbohydrate in the diet of diabetic patients.

Key words: α-1,6-glucan, slowly digestible carbohydrate, digestibility, breath hydrogen, fecal excretion, isomaltomegalosaccharide

INTRODUCTION

The number of older patients with diabetes is increasing in developed countries.1,2 As these patients are at risk of malnutrition and sarcopenia, it is recommended that they maintain adequate energy intake while maintaining control of blood glucose levels.3 Oral nutrient supplements are a useful way to improve malnutrition in these patients. These typically consist of liquid diets containing the starch degradation product dextrin as a carbohydrate source. As dextrin is rapidly digested and absorbed in the human digestive tract, it is a useful energy source for the older people with malnutrition, but it tends to cause high blood glucose levels after ingestion in diabetics. Carbohydrates that are digested more slowly, but still completely, produce low blood glucose peaks and would be ideal for older patients with diabetes.

Such carbohydrates are called “slowly digestible carbohydrates” (SDCs).4,5 SDCs have mild resistance to digestive enzymes in the gastrointestinal tract and are slowly absorbed in the small intestine. Nevertheless, they are eventually completely absorbed in the small intestine and are expended as energy in the body (approximately 4 kcal/g) at a level similar to that of “rapidly digestible carbohydrates” (RDCs) such as dextrin and starch.6 Carbohydrates that have strong resistance to digestive enzymes are termed “indigestible carbohydrates” (IDCs). IDCs pass through the small intestine and are fermented by intestinal bacteria in the large intestine or excreted in the feces.7,8 Although IDCs have nutritional roles, acting as dietary fiber and prebiotics, they have lower energy value than RDCs and SDCs.7

In a previous study, we developed a new carbohydrate material by treating starch hydrolysate with dextrin dextranase (DDase), α-amylase, isoamylase, and pullulanase.8 This material is a mixture of glucose polymers whose degree of
polymerization (DP) covers a wide range. The main structural component of the glucans is α-1,6-glucan, which has a continuous linear structure of α-1,6-glucosidic bonds and a small number of α-1,4-glucosidic bonds at the reducing ends. Since carbohydrates with size range between DP 10 and 100 (or 200) are defined as megalosaccharides, α-1,6-glucans in this DP range are isomalto-oligosaccharides (IMS). In the newly developed material, about half of it were α-glucans in the range of DP 10 to 150, and the main component was α-1,6-glucan in DP 10 to 30.\(^6\) Therefore, the material is high-amount IMS containing carbohydrate (H-IMS). The basic structure of H-IMS is similar to that of low-molecular-weight dextran, except that it has α-1,4-bonds at the reducing end. In addition, we reported that H-IMS has a slower digestion rate in vitro and produces a lower increase in blood glucose in rats than dextran. However, it is unclear whether the result is because the H-IMS contains IDCs or because it contains SDCs that are slowly and completely digested and absorbed. Dextran, a very long-chain α-1,6-glucan, is known to be indigestible,\(^1\) and isomalto-oligosaccharide, an α-1,6-glucan (DP 2 to 5), is known to be completely digested in the small intestine.\(^2\) However, there have been no studies investigating the in vivo digestibility of an α-1,6-glucan whose DP is intermediate between dextran and isomalto-oligosaccharide. The purpose of this study was to evaluate the digestibility of H-IMS in the small intestine to determine whether it could be classified as a SDC.

**MATERIALS AND METHODS**

*Preparation of H-IMS.* DDase was prepared from the culture supernatant of *Glucobacter oxydans* ATCC 11894 by methods previously reported.\(^5\) One unit of DDase enzyme activity was defined as the amount of the enzyme producing 1 μmol of maltotriose per min in a reaction solution containing 0.5 mL of DDase solution and 0.5 mL of 2 % (w/v) maltotetraose dissolved in 50 mM acetate buffer (pH 4.2) kept at 35 °C. H-IMS was prepared as previously described,\(^6\) with some modifications. Corn starch was industrially hydrolyzed by the general liquefaction method using α-amylase in the factory (Nihon Shokuhin Kako Co., Ltd., Shizuoka, Japan). Its dextrose equivalent value, as estimated by the Somogyi modified method, was 6.5. DDase (5 units/g substrate), α-amylase (0.1 units/g substrate, Kleistase L-1; Amano Enzyme Co., Ltd., Aichi, Japan), isomaltase (200 units/g substrate, GODO-FIA; GODO SHUSEI CO., LTD., Tokyo, Japan), and pullulanase (0.6 units/g substrate, Pullulanae ‘Amano’ 3; Amano Enzyme Co., Ltd.) were added to 24 % (w/w) starch hydrolysate solution. The enzyme activity values for α-amylase, isomaltase, and pullulanas were as reported by their manufacturers. The reaction solution was kept at 50 °C, pH 5.0 for 120 h. The reaction was stopped by adjusting the pH to 4.0 and the mixture was then maintained at 80 °C for 12 h. The obtained product was desalted by an ion exchange resin (Amberlite MB4, Organo Corporation, Tokyo, Japan), filtered, and lyophilized. The DP distribution of the product was determined by high-performance liquid chromatography as follows: two OHpak SB-806M HQ columns (8.0 × 300 mm; Showa Denko K.K., Tokyo, Japan) connected in series; column temperature, 50 °C; flow rate, 0.3 mL/min; eluent, 50 mM sodium nitrate solution; detector, refractive index detector. A pullulan standard (Shodex Standard P-82, Showa Denko K. K.) was used to detect the elution time. The content of DP 1–9, DP 10–30, DP 31–150, and DP 151 or higher, which was judged based on the elution time, was 36, 48, 8, and 8 %, respectively (Fig. 1). Glycosidic bond formulation of the product was calculated from the peak area in H-NMR. NMR was measured with a Bruker 400 MHz NMR spectrometer (Bruker Japan K.K., Kanagawa, Japan) at room temperature in deuterated water. Proportion of linkage was determined from the area ratio of the H-1 peaks at 4.9 ppm for α-1,6-glucosidic bonds and 5.3 ppm for α-1,4-glucosidic bonds. The proportion of α-1,4 linkage and α-1,6 linkage was 24 and 76 %, respectively. This proportion is similar to IMS prepared by Lang et al.\(^5\) rather than dextran (molecular mass, 1,270 kDa) prepared by using DDase.\(^3\)

**In vitro digestibility.** The in vitro digestibility of the H-IMS was evaluated as previously described.\(^8\) Briefly, rat small intestine acetone powder (Sigma-Aldrich Japan Co., Ltd., Tokyo, Japan) was suspended in 45 mM maleate buffer (pH 6.6) at a concentration of 10 % (w/v) and centrifuged at 19,000 × G for 10 min. One unit of activity of the supernatant (rat intestinal crude enzyme) was defined as the amount of the enzyme hydrolyzing 1 μmol of maltose per min at 37 °C in a reaction solution containing 15 μL of the rat intestinal crude enzyme and 85 μL of 1.1 % (w/v) maltose dissolved in 45 mM acetate buffer (pH 6.0). Dextrin (Pinedex#:2, Matsu- tani Chemical Industry Co., Ltd., Hyogo, Japan) or H-IMS was dissolved to a final concentration of 0.45 % (w/v) in 45 mM maleate buffer (pH 6.6). Porcine pancreatic α-amylase (Type I-A, Sigma-Aldrich Japan) and the rat intestinal crude enzyme were added to the substrate solution at a concentration of 4,000 and 860 units/g substrate, respectively, and the solution was kept at 37 °C. One unit of activity of α-amylase was defined as the amount of the enzyme producing 1 mg of maltose per 3 min at 20 °C and pH 6.9. Then, 200 μL of 2 M Tris-HCl solution was added to 20 μL of the reaction solution to stop the reaction of rat α-glucosidase at each reaction period. The glucose concentration was measured by the glucose oxidase method using the Glucose C-II Test (Wako Pure Chemical Industries, Osaka, Japan).

*In vivo experiments.* Animals were treated in accordance
with the Standards Relating to the Care and Keeping and Reducing Pain of Laboratory Animals in Japan (Notice of the Ministry of the Environment of Japan, No. 84 of 2013) and the Laboratory Protocol of Animal Handling of Morinaga Milk Industry Co., Ltd. All animal experiments were approved by the Institutional Animal Care and Use Committee of Morinaga Milk Industry Co., Ltd. (approval numbers: 17-001 and 17-008) and were performed from May 2017 to June 2017. Male Sprague Dawley rats were obtained from Japan SLC Co., Inc. (Shizuoka, Japan). All rats were housed individually in plastic cages under conditions maintained at 22 ± 2 °C with a 12-h light/12-h dark cycle (light on from 8:00 until 20:00). The rats were acclimatized to the laboratory environment for at least 1 week before the tests started and were allowed free access to water and regular solid chow (MR stock, Nosan Co., Ltd., Kanagawa, Japan). A gas-free diet, a diet designed so that all the carbohydrates in AIN-93G are replaced by maltose, was used to suppress hydrogen production by fermentation of resistant carbohydrates (such as resistant starch or cellulose) by intestinal bacteria for evaluation of breath hydrogen gas excretion. A starch-free diet (a diet designed so that all the carbohydrates except cellulose in AIN-93G are replaced by maltose) was used to suppress the excretion of soluble resistant carbohydrate (such as resistant starch) to the stool. The compositions of the gas- and starch-free diets are shown in Table 1. The diets were prepared by Oriental Yeast Co., Ltd. (Tokyo, Japan).

**Postprandial breath hydrogen gas.** The measurement of breath hydrogen gas as an indicator of fermentation in the large intestine was performed according to previously published methods, with some modification. From two days prior to the test day, rats (n = 24, 8 weeks old) were housed on wire-mesh flooring and fed the gas-free diet. On the test day, each animal was assigned to one of four treatment groups: glucose, dextrin, lactulose, or H-IMS (n = 6 each). Chambers with two air vents and a capacity of 3.4 L (air-tight chamber, Muromachi Kikai Co., Ltd., Tokyo, Japan) were aspirated continuously at a flow rate of 200 mL/min from one vent with a pump connected to a tube (Pomp: Masterflex L/S, Tube: Noprene Tubing, Cole-Parmer Instrument Co., Ltd., Illinois, USA), while fresh air was allowed to enter from the other vent. For breath collection at 0 h, each rat was placed in the chamber for 20 min and then aspirated internal air was collected through the tip of the tube for 1 min into a bag (Collection bag, Laboratory for Expiration Biochemistry Nourishment Metabolism Co., Ltd., Nara, Japan). After the collection at 0 h, glucose (Nihon Shokuhin Kako Co., Ltd.), dextrin (TK-16, Matsutani Chemical Industry Co., Ltd.), lactulose (MLC-97, Morinaga Milk Industry Co., Ltd., Tokyo, Japan), or H-IMS was dissolved in distilled water well in advance at a concentration of 0.2 g/mL and was orally administered at a dose of 2 g/kg body weight (10 mL/kg body weight). Each rat was placed in the chamber for 20 min every hour until 9 h after administration and aspirated air samples were collected as described above. Feed was removed from the start of the 0-h collection to the end of 9-h collection. The hydrogen concentration in the bag was measured with a Breath Gas Analyzer (BGA-2000D, Laboratory for Expiration Biochemistry Nourishment Metabolism Co., Ltd.).

**Excretion in stool.** The measurement of fecal excretion of total carbohydrate was performed according to methods previously described, with some modifications. From one week prior until four days after oral administration, rats (n = 24, 7 weeks old) were housed on wire-mesh flooring and fed the starch-free diet. On treatment day, each animal was assigned to one of four groups: water, glucose, polydextrose, or H-IMS (n = 6 each). For the water group, water was administered at 10 mL/kg body weight. For the other three groups, glucose, polydextrose (Litesse, Danisco Japan Co., Ltd., Tokyo, Japan), or H-IMS was dissolved in distilled water at a concentration of 0.2 g/mL and administrated orally at a dose of 2 g/kg body weight (10 mL/kg body weight). Stool was collected and weighed every day for 4 days. Total fecal wet weight and food intake during these 4 days were also measured. The amount of total carbohydrate in the stool samples was determined by the phenol–sulfuric acid method, with glucose used as the standard. The measured value quantified as glucose equivalent was converted to amount of polysaccharide by multiplying by a polysaccharide coefficient of 0.9. Fecal excretion rate for each animal was calculated by first subtracting the mean fecal carbohydrate excretion in the water group from the fecal carbohydrate excretion in the animal followed by dividing by the oral dose in each rat.

**Statistical analysis.** Data are reported as mean ± standard error of the mean (SEM). Statistical analyses were performed using JMP statistical software (version 13.2.1, SAS Institute Japan Inc., Tokyo, Japan). The non-parametric Steel–Dwass test was used for multiple comparisons of the differences at each time point in the breath hydrogen tests, as these values did not show a normal distribution. The Tukey–Kramer test was used for multiple comparisons in the fecal excretion test. Differences at p < 0.05 were considered significant.

## RESULTS

**In vitro digestibility of H-IMS.**

Dextrin was rapidly digested after the addition of the enzyme solution containing α-amylase and rat small intestine mucosa, with released glucose reaching saturation after 4 h (Fig. 2). In contrast, H-IMS was digested more slowly, with released glucose levels relative to dextrin after

### Table 1. Compositions (g/100 g) of the gas- and starch-free diet.

| Component          | AIN-93G diet | Gas-free diet for breath H₂ test | Starch-free diet for fecal excretion test |
|--------------------|--------------|-----------------------------------|------------------------------------------|
| Casin              | 20.0         | 20.0                              | 20.0                                     |
| Corn starch        | 39.7         |                                   |                                          |
| α-Corn starch      | 13.2         |                                   |                                          |
| Sucrose            | 10.0         |                                   |                                          |
| Maltose            |              | 67.9                              | 62.9                                     |
| Cellulose powder   | 5.0          | 5.0                               |                                          |
| Soybean oil        | 7.0          | 7.0                               | 7.0                                      |
| Mineral mix        | 3.5          | 3.5                               | 3.5                                      |
| Vitamin mix        | 1.0          | 1.0                               | 1.0                                      |
| Others¹            | 0.6          | 0.6                               | 0.6                                      |

¹ Others include L-cystine (0.30 %), choline bitrate (0.25 %), and tertiary butylhydroquinone (0.0014 %).
4 and 24 h reaching 76.2 and 93.5 %, respectively.

**Breath hydrogen gas.**

To investigate the fermentation of carbohydrates in the large intestine, we assessed the amount of hydrogen present in exhaled breath following treatment. Figure 3 shows the postprandial hydrogen concentration in the test chamber after oral administration of glucose, dextrin, H-IMS, or lactulose. With lactulose, hydrogen was detected from 2 h after administration, and the concentration continued to increase during the observation period, up to 9 h after administration. No increase in hydrogen concentration was observed after administration of H-IMS, glucose, or dextrin.

**Fecal carbohydrate excretion.**

The total amount of carbohydrate contained in the stool on the 4 days following administration of water, glucose, H-IMS, or polydextrose is shown in Fig. 4. Fecal carbohydrate excretion in the polydextrose group peaked on the first day after administration and then continued to decrease, only reaching a level not significantly different from that of the water group on day 4. In contrast, the amount of fecal carbohydrate excreted in the H-IMS, glucose, and water groups remained at the same low level for all 4 days, with no significant differences in values among them.

A summary of this study’s results is shown in Table 2. There was no significant difference in total food intake or in total wet weight of stool between groups for the 4 days after treatment. However, total carbohydrate excretion was about seven times higher in the polydextrose group than in the other three groups. Incremental value of carbohydrate excretion in the polydextrose group was 199.9 ± 17.5 mg/4 days, an amount equivalent to 35.1 % of the mean oral dose. In contrast, the values for total amount of carbohydrate excreted in feces in the H-IMS and glucose groups were equivalent to those in the water group, indicating no increase in fecal carbohydrate amount due to the administration of these carbohydrates.

![Fig. 2. Digestion profiles of dextrin and the H-IMS.](image1)

Data are shown in free glucose concentration at each time point after the addition of the digestive enzymes. Data are presented as mean ± SEM of the results obtained in quadruplicate for each measurement time.

![Fig. 3. Changes in postprandial hydrogen concentration in the internal air of the test chambers after administration to rats of either glucose, dextrin, H-IMS, or lactulose.](image2)

Data are presented as mean ± SEM. The symbols indicate the significant differences at each time point. *, glucose group vs. lactulose group; †, dextrin group vs. lactulose group; ‡, H-IMS group vs. lactulose group. p < 0.05 was considered statistically significant.

![Fig. 4. Fecal carbohydrate excretion in rats on each day after administration of either water, glucose, H-IMS, or polydextrose.](image3)

Data are presented as mean ± SEM. Asterisks indicate a significant difference between two groups on each day. p < 0.05 was considered statistically significant.

| Substance  | Dose (mg) | Food intake (g/4 days) | Fecal weight (g/4 days) | Fecal carbohydrate excretion (mg/4 days) | Fecal excretion rate (%) |
|------------|-----------|------------------------|-------------------------|------------------------------------------|-------------------------|
| Water      | 0         | 75.7 ± 3.2             | 6.6 ± 0.4               | 39.8 ± 2.3‡                            | -0.6 ± 0.1              |
| Glucose    | 567 ± 9   | 72.3 ± 3.2             | 6.7 ± 0.3               | 36.1 ± 0.6§                            | -0.6 ± 0.4              |
| H-IMS      | 566 ± 9   | 69.4 ± 5.2             | 6.5 ± 0.5               | 36.4 ± 2.4‡                            | -0.6 ± 0.4              |
| Polydextrose | 569 ± 7  | 74.1 ± 1.8             | 7.4 ± 0.4               | 239.6 ± 7.1†                           | 35.1 ± 1.0              |

Data are presented as mean ± SEM. Different superscript lowercase letters indicate a significant difference at p < 0.05.
DISCUSSION

Earlier, we reported that H-IMS has a slower in vitro digestion rate and produces a lower increase in blood glucose in rats than dextrin.\(^9\) In this report, we evaluate the in vivo digestibility of H-IMS in rats. We have found that H-IMS is completely digested and absorbed in the small intestine. To the best of our knowledge, this is the first report on the in vivo digestibility of an \(\alpha\)-1,6-glucan mixture containing IMS as the major component.

Hydrogen gas is generated when carbohydrates escape digestion in the small intestine and reach the large intestine, where they are fermented by the intestinal anaerobic bacteria.\(^{18,19}\) This hydrogen diffuses into the blood and is then detectable in exhaled breath. Since approximately 70 % of gut bacteria are capable of producing hydrogen, detection of exhaled hydrogen is an effective indicator of carbohydrate fermentation in the large intestine.\(^{20,21}\) It has been reported that treatment with lactulose, an IDC, results in breath hydrogen levels of 10 and 20 ppm at 4 and 24 h post-treatment, respectively, at a dose of 2 g/kg body weight in rats.\(^{19}\) In this study, lactulose exhibited exhaled hydrogen at the same level as in the previous study (Fig. 3). The previous study also reported that both digestible sucrose (known to be a RDC) and isomaltulose (known to be a SDC\(^{22}\)) did not produce an increase in exhaled hydrogen after administration in rats.\(^{15}\) In this study, measurements were completed up to 9 h after the animals were fed with H-IMS. Detectability of breath hydrogen of fermentable IDC usually starts with a delay of 2 to 4 h after feeding and lasts for many hours.\(^{15,23,24}\) Thus, we considered, breath hydrogen should have been detected by 9 h after DDase ingestion at the latest. In the absence of detectable breath hydrogen, we conclude that fermentation in the large intestine does not occur after ingestion of H-IMS.

Next, to determine whether the H-IMS constituted a biologically unavailable component that is excreted in feces without being absorbed in the small intestine or fermented by intestinal bacteria, we evaluated the fecal carbohydrate excretion after administration. By the same method, a previous study reported that indigestible maltodextrin had a fecal excretion rate of 38 %, which is the basis for setting the excretion level from the non-reducing end.\(^{27}\) Therefore, the digestibility of \(\alpha\)-1,6-glucans is considered to be affected by their DP. In fact, the higher the DP of samples, the lower the in vitro digestion rate, as we learned in the previous study in which we prepared \(\alpha\)-1,6-glucans samples adjusted to various DP ranges by hydrolysis and fractionation of dextran.\(^{8}\) We speculate that \(\alpha\)-1,6-glucans with DP below a certain level, such as the H-IMS, low-molecular-weight dextran in the same DP range as the H-IMS, as well as isomalt-oligosaccharide,\(^{15}\) are completely digested in the small intestine. On the other hand, \(\alpha\)-1,6-glucans with a high DP, such as high-molecular-weight dextran, are considered to be partially indigestible in vivo because they need too long time to be digested completely.\(^{15}\)

Generalization of our findings to human alimentation of H-IMS is not straightforward because we did not assess dose dependency. We evaluated in vivo digestibility by a single administration of H-IMS at a dose of 2 g/kg body weight, which was used in the previous study about digestibility of isomalto-oligosaccharide.\(^{15}\) Since our goal is to use H-IMS as a nutrition source, further investigations will be needed to evaluate its digestibility at high doses.

The animal model rather than humans are used because H-IMS is a newly developed carbohydrate. Previous investigations of the digestibility of other carbohydrates reported almost the same postprandial breath hydrogen pattern of resistant glucan\(^{31}\) and fecal excretion rate of resistant maltodextrin\(^{40,30}\) in rats as in humans. These findings suggest that the rat is a good animal model for human carbohydrate digestion and its use is an appropriate first step prior to human trials. In the future, to estimate the digestibility and energy value of the H-IMS in humans, breath hydrogen measurement,\(^{25}\) indirect calorimetry,\(^{26,27}\) and evaluation of breath carbon dioxide excretion using a tracer\(^{41,45}\) in humans would be needed.

This study suggests that the H-IMS is a SDC that would be useful for energy supplementation in diabetic patients. Although isomalto-oligosaccharide\(^{25}\) and trehalose\(^{30}\) have already been developed as SDCs, they are disaccharides, and isomalto-oligosaccharide contains fructose as one of its two constituent sugars. In
contrast, the H-IMS is a polysaccharide constructed only from glucose, as well as starch. Disaccharides tend to cause increased osmotic pressure in the gastrointestinal tract, which leads to diarrhea. The effects of chronic fructose intake on metabolism are still controversial. Considering daily intake, the advantage of the H-IMS is that its structure is similar to that of starch, the main nutrient source for humans, and it can be produced from starch, an inexpensive raw material.

**CONCLUSIONS**

The H-IMS, which is a linear α-1,6-glucan-based carbohydrate produced using Dβase, showed neither generation of breath hydrogen nor increased fecal sugar excretion after administration in vivo; therefore, it is considered that the H-IMS is almost completely digested and absorbed in the small intestine. The H-IMS could be a beneficial SDC as the nutrition source that provide energy while avoiding rapid postprandial blood glucose increase in diabetic patients.

**CONFLICTS OF INTEREST**

Eri Kokubo, Hirofumi Sonoki, Ayako Ito, Yuki Nakazato, Yasuhiro Takeda, and Kazuhiro Miyaji are employees of Morinaga Milk Industry Co., Ltd. Kenta Aizawa, Hiroki Takagi, and Masayasu Takada are employees of Nihon Shokuhin Kako Co., Ltd.

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