Metabolism of $^{13}$C-Isomaltooligosaccharides in Healthy Men

Takanobu KOHMOTO, Keisuke TSUJI,* Toshiyuki KANEKO, Masao SHIOTA,** Fumio FUKUI, Hajime TAKAKU,** Yasue NAKAGAWA,*** Tomio ICHIKAWA,* and Syuuhei KOBAYASHI**

Research and Development Center, Showa Sangyo Co., Ltd., Hinode, Funabashi, Chiba 273, Japan
*National Institute of Health and Nutrition, Toyama, Shinjuku-ku, Tokyo 162, Japan
**Technical Division, Showa Sangyo Co., Ltd., Kashima, Ibaraki 314-01, Japan
***Department of Food, Faculty of Home Economics, Jissen Women’s University, Hino, Tokyo 191, Japan
Received December 5, 1991

Isomaltooligosaccharides (IMO), sweeteners derived from corn-starch, selectively promote the growth of bifidobacteria in the human intestine. The metabolic fate of IMO in healthy men was investigated. The expiration rates of excess $^{13}$CO$_2$ and hydrogen of six men were measured while sedentary and while taking physical exercise after the $^{13}$C-labeled IMO intakes. The breath H$_2$ excretion kept at a constant state after IMO ingestion in the sedentary test and increased in the exercise test. The serum glucose and serum insulin increased 30 min after IMO ingestion. The $^{13}$CO$_2$ recoveries were 28.7% in the sedentary test and 60.9% in the exercise test. These recoveries were 70–80% compared those of maltose. These results indicated that a part of IMO was digested and the residual IMO was fermented by intestinal flora. The energy value of IMO might be about 75% of that of maltose.

Isomaltooligosaccharides (IMO) have a1–6 glucosidic linkage like isomaltose and exist in fermented foods such as miso, soy sauce, and sake. Isomaltose is one of the sugars in honey. The administration of IMO to healthy adult men and senile persons induced a significant increase in the number of fecal bifidobacteria. The minimum dosage of IMO for increasing bifidobacteria was 8–10 g. On the other hand, isomaltose was digested by isomaltase in the jejunum. This may demonstrate that part of IMO is digested by isomaltase in the jejunum and the residual IMO is used by bifidobacteria in the large intestine.

In this report, we studied the metabolic fate of IMO. The $^{13}$C-labeled IMO (13-C-IMO) were ingested by healthy men. The expiration rate of excess $^{13}$CO$_2$ was measured, concomitantly with that of hydrogen derived from intestinal fermentation. It was reported that physical exercise stimulated the metabolism and increased expiration rate of $^{13}$CO$_2$. After the labeled IMO intake, the excretory gas was measured during physical exercise.

Methods

1) Subjects. The volunteers consisted of six healthy men from 25 to 29 years old. None of them had been on antibiotic treatment or other therapy for at least one month before the first examination. This study was done according to the Helsinki Declaration as updated in Tokyo, Japan, 1975. Informed consent was obtained from all subjects, and the study protocol was approved by the ethical committee of the National Institute of Health and Nutrition.

2) Substrates. The $^{13}$C-IMO was prepared from the uniformly labeled $^{13}$C-glucose (Atom %, 99%, CEA, France) by the action of glucoamylase (AMG Novo, Sweden) and was purified with gel filtration (Toyopearl HW-40S Tosoh Co., Ltd., Tokyo), lyophilization. IMO used in this study was Isomalto-900® (Showa Sangyo Co., Ltd., Tokyo). The composition of the $^{13}$C-IMO (Atom %, 99%) and IMO analyzed by high-performance liquid chromatography (HPLC) is shown in Table I. These compositions were very similar.

3) Breath tests.

i) The sedentary test. After an overnight fast for approximately 12 hr, breath samples were obtained from the subjects in basal conditions. The subjects received an oral load of 50 mg $^{13}$C-IMO and 25 g IMO. 1.79 mmol as total $^{13}$C, in 150 ml of water. Breath samples were obtained at 15, 30, 60, 90, 120, 180, 240, 360, 420, and 480 min. The subjects exhaled into 250-liter gas bags, Douglas type, with a two-way stop cock, for ten minutes. The breath volume was measured with a wet gas meter (W-NK5 Shinagawa Seiki Co., Ltd., Tokyo). A breath sample was taken into a storage bag, 2-liter aluminum coated low density polyethylene bag. The subjects were kept sedentary during the test.

ii) The exercise test. The subjects were given the $^{13}$C-IMO as for the sedentary test. Physical exercises were arranged in the jogging style. The subjects’ pulses were controlled to about two times that of rests. One exercise cycle consisted of 5 min of physical exercise, 25 min of rest, and at each period breath samples were obtained every 5 or 10 min. The exercise cycles were carried out at 5, 45, 90, 135, 180, 225, 285, 360, 420, 480, 540, 600, 660, and 720 min. The exercise test was done 2 weeks after the sedentary test.

4) Measurement of breath hydrogen and carbon dioxide. The H$_2$ concentrations of each sample were analyzed in a Hitachi 263-50 gas chromatograph equipped with a photo-ionization detector as previously described.

A 1-ml sample of gas was put in a glass column, 2 m by 3 mm, packed with active carbon (60 to 80 mesh). The oven temperature was 90°C. Helium (50 ml per minute) served as a carrier gas. The $^{13}$CO$_2$ and CO$_2$ concentrations were measured with a $^{13}$CO$_2$ Analyzer EX-130 (Japan Spectroscopic Co., Ltd., Tokyo).

5) Measurement of serum biochemical values

Blood samples were obtained at 15 min before ingestion, and 30, 60, 90, 120, and 180 min (until 240 min in the exercise test) after the $^{13}$C-IMO ingestion.

Table I. Sugar Composition of $^{13}$C-IMO and IMO

<table>
<thead>
<tr>
<th>Sugar</th>
<th>$^{13}$C-IMO</th>
<th>IMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP$_1$ Glucose</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>DP$_2$ Maltose</td>
<td>2.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Isomaltose</td>
<td>32.6</td>
<td>32.3</td>
</tr>
<tr>
<td>Others$^a$</td>
<td>6.9</td>
<td>9.1</td>
</tr>
<tr>
<td>DP$_3$ Panose</td>
<td>13.4</td>
<td>12.3</td>
</tr>
<tr>
<td>Isomaltooliote</td>
<td>16.9</td>
<td>14.8</td>
</tr>
<tr>
<td>Isomaltotriose</td>
<td>15.5</td>
<td>15.5</td>
</tr>
<tr>
<td>DP$_5$ Isomaltohexaose and others</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>DP$_6$ Isomaltohexaose and others</td>
<td>4.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

DP, degree of polymerization.
$^a$ Nigerose and Kojibiose.
intake. Serum was prepared by centrifugation to measure biochemical values. Total cholesterol (TC) and HDL-cholesterol (HDL-C) were measured by the enzymatic method with the Determiner TC555 (Kyowa Medex Co., Ltd., Tokyo). Phospholipid (PL), triglyceride (TG), nonesterified fatty acid (NEFA), and lactic acid (LA) were measured by the enzymatic method with the Determiner PL, Determiner TG-555, Determiner NEFA, and Determiner LA (Kyowa Medex Co., Ltd., Tokyo), respectively. Serum glucose (SG) was analyzed by a glucose oxidase method with the Glucose B-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka). Insulin (IRI) and glucagon (IRG) were measured by the radioimmunoassay technique with Phadebas insulin RIA (Pharmacia K.K., Tokyo) and Glucagon kit Dauichi (Daiichi Radio-isotope Co., Ltd., Tokyo).

6) Excretion of IMO with feces. After three subjects received an oral load of 25 g IMO, their fresh feces were collected for three days. The feces were dried with lyophilization and were homogenized by ultradispersion in 50% ethanol. Isomalto, isomaltotriose, and panose were measured by HPLC. Significance was assessed by Student’s t-test and p values of less than 0.05% were considered statistically significant.

Results

Figure 1 shows the mean breath 13CO2 excretion curve of six volunteers kept at rest after the 13C-IMO ingestion. The maximum excretion of 13CO2 was from the second hour to the third hour after ingestion. The cumulative excess 13CO2 excretion for 8 hr after ingestion was 513 ± 15 μmol (mean ± SEM), and the 13CO2 recovery was 28.7 ± 0.8%. The mean breath H2 excretion curve is shown in Fig. 2 (A). The breath H2 excretion was constant after the 13C-IMO ingestion.

Table II shows the serum biochemical values of six volunteers in the sedentary condition. The serum glucose (SG) and serum insulin (IRI) increased 30 min after ingestion. The nonesterified fatty acid (NEFA) decreased until 90 min after ingestion and from then increased. Other

![Fig. 1. Breath 13CO2 Excretion of 6 Human Volunteers at Rest after Oral Intake of 13C Labeled Isomaltooligosaccharides. Each point shows mean ± SEM.](image1)

![Fig. 2. Breath H2 Excretion of 6 Human Volunteers at Rest (A) and Taking Exercise (B) after Oral Intake of 13C Labeled Isomaltooligosaccharides. Each point shows mean ± SEM.](image2)

![Fig. 3. Breath 13CO2 Excretion of 6 Human Volunteers Taking Intermittent Exercise after Oral of 13C Labeled Isomaltooligosaccharides. Each point shows mean ± SEM.](image3)

Table II. Serum Biochemical Values of Human Volunteers Keeping Sedentary Rested after Oral Intake of 13C Labeled Isomaltooligosaccharides

<table>
<thead>
<tr>
<th></th>
<th>0 hr</th>
<th>0.5 hr</th>
<th>1.0 hr</th>
<th>1.5 hr</th>
<th>2.0 hr</th>
<th>3.0 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>180±10</td>
<td>171±8</td>
<td>168±9</td>
<td>167±10</td>
<td>167±9</td>
<td>165±9</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>72.5±11.4</td>
<td>68.9±10.1</td>
<td>65.8±10.4</td>
<td>66.3±10.5</td>
<td>67.4±10.2</td>
<td>69.2±10.7</td>
</tr>
<tr>
<td>PL (mg/dl)</td>
<td>214±11</td>
<td>210±12</td>
<td>205±12</td>
<td>201±12</td>
<td>198±10</td>
<td>196±11</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>114±18</td>
<td>84±18</td>
<td>84±18</td>
<td>71±13</td>
<td>68±11</td>
<td>73±14</td>
</tr>
<tr>
<td>NEFA (μEq/l)</td>
<td>524±63</td>
<td>227±33*</td>
<td>93±11*</td>
<td>113±39*</td>
<td>258±87</td>
<td>460±54</td>
</tr>
<tr>
<td>SG (mg/dl)</td>
<td>109±2</td>
<td>136±5*</td>
<td>113±3</td>
<td>96±8</td>
<td>99±6</td>
<td>103±3</td>
</tr>
<tr>
<td>LA (mg/dl)</td>
<td>15.3±0.7</td>
<td>15.5±0.8</td>
<td>22.0±1.6</td>
<td>17.4±2.1</td>
<td>17.0±4.1</td>
<td>14.8±1.7</td>
</tr>
<tr>
<td>IRI (μU/ml)</td>
<td>4.8±0.4</td>
<td>32.0±2.7*</td>
<td>16.4±4.3</td>
<td>6.0±0.9</td>
<td>4.0±0.5</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>IRG (pg/ml)</td>
<td>51.8±18.3</td>
<td>33.8±3.4</td>
<td>35.4±4.9</td>
<td>36.0±4.1</td>
<td>42.8±4.6</td>
<td>35.2±4.0</td>
</tr>
</tbody>
</table>

Values are mean ±S.E. for 6 human volunteers.
TC, total cholesterol; HDL-C, HDL-cholesterol; PL, phospholipid; TG, triglyceride; NEFA, nonesterified fatty acid; SG, serum glucose, LA, lactic acid; IRI, insulin; IRG, glucagon. Significant difference from the values of 0 hr: *p<0.01 by paired Student’s t test.
values did not change significantly and were normal throughout each period.

Figure 3 shows the mean breath $^{13}$CO$_2$ excretion curve of six volunteers taking intermittent exercise. The breath volume in exercise time increased about 2 times that of sedentary time, and the CO$_2$ concentration in exercise also increased. The maximum excretion of $^{13}$CO$_2$ was from the second hour to the third hour after ingestion as in the sedentary condition. The cumulative excess $^{13}$CO$_2$ excretion for 12 hr after ingestion was 1090 ± 58 mmol, and the $^{13}$CO$_2$ recovery was 60.9 ± 3.2%. The H$_2$ excretion increased in exercise time and a gentle peak excretion of H$_2$ was shown from the third hour to the fifth hour after ingestion (Fig. 2 (B)).

Table III shows the serum biochemical values of six volunteers in the exercise test. SG and IRI increased 30 min after ingestion. On the other hand, NEFA decreased until 60 min after ingestion and then increased. Other values did not change significantly and were normal throughout each period.

The feces of three subjects after an oral load of IMO were collected. IMO in the feces were measured by HPLC. IMO was not detected in the feces.

Discussion

Indigestible saccharides are fermented by intestinal microbes in the large intestine, and CO$_2$, H$_2$, and CH$_4$ are produced. A part of these gases is absorbed and expired into breath. Because the human cell can not produce H$_2$, and CH$_4$, the breath H$_2$ excretion is derived from intestinal fermentation. The breath H$_2$ excretion increased after ingestion of non-absorbable carbohydrate such as maltitol, lactulose, and transgalactosylated oligosaccharides. In this study, the breath H$_2$ excretion kept at a constant state after IMO ingestion in the sedentary test and increased in the exercise test. It was reported that glucose and maltose intake decreased expired H$_2$ to an almost undetectable level within about 2 hr. Furthermore, we reported that the administration of IMO to men induced a significant increase in the number of fecal bifidobacteria. It is considered that this breath H$_2$ excretion was caused by IMO ingestion. This result indicated that IMO was fermented by intestinal microbes in the large intestine.

SG and IRI increased 30 min after IMO ingestion as with glucose and maltose ingestion. This indicated that part of the IMO was absorbed. These suggested that part of IMO was digested and the residual IMO was fermented by intestinal microbes. The cumulative H$_2$ excretion 6 hr after ingestion calculated 13.0 ± 0.8 mmol (mean ± SEM) in the sedentary test and 15.2 ± 1.0 mmol in the exercise test. Tsuji et al. reported that the cumulative H$_2$ excretion 6 hr after maltitol ingestion calculated 52.9 ± 6.5 mmol in the sedentary test. If one considers only these figures, it was supposed that IMO was fermented by intestinal microbes about 25% compared maltitol.

Digestible saccharides are used as an energy source and are accumulated as glycogen or lipid. Indigestible saccharides are used by intestinal flora and residual are excreted with feces. IMO was not excreted with feces. After the labeled IMO intake, the $^{13}$CO$_2$ recoveries were 28.7% in the sedentary test and 60.9% in the exercise test. Compared with maltose whose CO$_2$ recovered were 34.5% and 88.4% respectively, the recoveries of the IMO were 83 and 69% of those of maltose. This result indicated that some parts of IMO were indigestible and were used by intestinal microbes.

Recently the energy evaluation of indigestible saccharides such as fructooligosaccharides and maltitol have been reported. Tsuji et al. reported that the energy use of sugars may be measured from the recovery rate of $^{13}$CO$_2$. In this study, it is estimated that the energy value of IMO may be about 70—80% of that of maltose. The physiological combustion energy of IMO may be 3.3 kcal/g (3.947 × 83%) in the sedentary test and 2.7 kcal/g (3.947 × 69%) in the exercise test from the proportional calculation to that of maltose, 3.947 kcal/g. The physical exercise enhanced the usage efficiencies of IMO. The physical exercise method after $^{13}$C-labeled sugar ingestion is superior to evaluate the energy resources of sugars. Further improvement in this method is expected to clarify the energy value of IMO and other indigestible sugars.

Acknowledgment. We thank Mr. Iijima of Towa Kasei Kogyo Co., Ltd., for providing a $^{13}$CO$_2$ Analyzer EX-130 and a wet gas meter.

References


