The Effects of Isomaltulose-Based Oligomers Feeding and Calcium Deficiency on Mineral Retention in Rats

Jun KASHIMURA,1,2 Mieko KIMURA,2 and Yoshinori ITOKAWA2

1 Chigasaki Laboratory, Mitsui Sugar Co., Ltd., Chigasaki 253, Japan
2 Department of Social and Preventive Medicine, Graduate School of Medicine, Kyoto University, Kyoto 606-01, Japan
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Summary We examined the effects of isomaltulose-based oligomers (IBOs) on the mineral content of the whole blood, kidney, liver and tibia in calcium deficient and calcium sufficient rats. Twenty-eight Wistar rats were divided equally into 4 groups and fed with the following diets ad libitum for 4 weeks: (1) calcium sufficient diet (Ca+, IBOs−), (2) calcium-sufficient-IBOs supply diet (Ca+, IBOs+), (3) calcium-deficient diet (Ca−, IBOs−), (4) calcium-deficient-IBOs supply diet (Ca−, IBOs+). There were no significant differences in final body weights among the groups. Food consumption in the calcium-deficient groups was higher than that in the calcium-sufficient groups. The tibia weight was significantly decreased, and the calcium, magnesium and phosphorous contents were significantly decreased, and iron content was significantly increased in the tibia of calcium-deficient rats. On the other hand, in IBOs feeding rats, tibia weight, and calcium, magnesium and phosphorous contents were significantly increased, and iron content was significantly decreased. These findings suggest that IBOs feeding improves mineral retention especially in a state of calcium deficiency.

Feeding of several oligosaccharides, such as lactose or some sugar alcohols, increase certain kinds of mineral absorption or retention (1–6). These effects were observed by the feeding of various kinds of sugars, even though their structures are quite different. The mechanisms of the effects are not clear, but recently the relationship between organic acids that are metabolized by intestinal bacteria and mineral absorption is gathering attention.

Isomaltulose (6-O-α-D-glucopyranosyl-D-fructofuranose) also known as palatinose, is used in various food products as a non-cariogenic nutritive sweetener (7). Isomaltulose-based oligomers (IBOs) are mixture of isomaltulose and its condensates and are obtained by heating isomaltulose under suitable conditions (8). The
condensates are classified as hetero-oligosaccharides composed of glucose- and fructose-residues. The IBOs used in this study consist of 50.5% isomaltulose, 6.2% octasaccharides, 12.9% hexasaccharides, 28.4% tetrasaccharides and 2% monosaccharides. Isomaltulose is digestible and the ratio of digestibility is estimated to be about 1/5 of sucrose (9), but the digestibility of condensates is not clear. IBOs feeding affects human fecal microflora, but isomaltulose feeding does not (10–13). This suggests that IBOs are not digested completely in the small intestine and that at least some of them reach the large intestine.

In our previous report (14, 15), it was noted that there were no significant differences in the mineral contents of various tissues between IBOs-fed rats and control rats. This study was undertaken to examine whether IBOs affect mineral metabolism in calcium-deficient rats that were fed the diet containing one-tenth the calcium level of the control diet.

METHODS

Diets and animals. Four-week-old Wistar male rats were used for this study (Japan Charles River Co., Ltd.). Twenty-eight rats were housed in individual wire cages in a room with the temperature controlled at 22–25°C and the humidity at 40–60%, and were fed a solid diet on the market and double-distilled water for 3 days. After pre-feeding, the rats were divided equally into the following 4 diet groups: (1) calcium-sufficient diet (Ca+, IBOs−: control diet), (2) calcium-sufficient–IBOs supply diet (Ca+, IBOs+), (3) calcium-deficient diet (Ca−, IBOs−) and (4) calcium-deficient–IBOs supply diet (Ca−, IBOs+).

The rats were fed the respective diets as shown in Table 1 and double-distilled

| Table 1. Composition of experimental diets (%) |
|-----------------------------------------------|
|                                  Ca-sufficient | Ca-sufficient | Ca-deficient | Ca-deficient |
|                                  IBOs(−)    | IBOs(+)      | IBOs(−)      | IBOs(+)      |
| Casein                           | 15.0         | 15.0         | 15.0         | 15.0         |
| Potato starch                    | 30.0         | 30.0         | 30.0         | 30.0         |
| Sucrose                          | 38.3         | 35.3         | 37.1         | 34.1         |
| IBOs                             | 0.0          | 3.0          | 0.0          | 3.0          |
| Olive oil                        | 10.0         | 10.0         | 10.0         | 10.0         |
| Salt mixture<sup>a</sup>         | 4.0          | 4.0          | 0.0          | 0.0          |
| Salt mixture<sup>b</sup>         | 0.0          | 0.0          | 2.0          | 2.0          |
| Phosphoric acid                  | 0.0          | 0.0          | 0.8          | 0.8          |
| Choline chloride                 | 2.0          | 2.0          | 2.0          | 2.0          |
| Cellulose                        | 2.0          | 2.0          | 2.0          | 2.0          |
| Vitamin mixture                  | 0.5          | 0.5          | 0.5          | 0.5          |

<sup>a</sup>The mineral mixture of Mameesh and Johnson (J. Nutr., 65, 161 (1958)). <sup>b</sup>The mineral mixture containing one-tenth the Ca level of the mineral mixture of Mameesh and Johnson.
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water ad libitum for 4 weeks. The daily food consumption and the body weight of each rat were monitored weekly during the test period.

After the feeding, each rat was anesthetized and killed by exsanguination, and tissues (brain, heart, liver, kidneys, testis and tibia) were taken and weighed.

**Determination of minerals.** Each tissue and diet (approximately 1g) were weighed, and placed in a test tube and digested by 5ml nitric acid in a hot-block bath (TPB-62, Advantec, Tokyo, Japan). These contents were heated at 50°C for 1 h followed by at 85°C for 24 h and at 110°C for 24 h. The digested solutions were diluted to a suitable concentration to analyze minerals by double-distilled water. The concentrations of minerals in diets, blood, liver, kidneys and tibia were determined with an inductively coupled plasma-atomic emission spectrometer (ICPS-1000 II, Shimadzu, Co., Kyoto, Japan). These concentrations were measured by the internal standard method (using Yttrium as the internal substance).

**Statistical test.** The statistical analysis of all data obtained in this study was performed using two-way analysis of variance using a quality control assisting system (16).

**RESULTS**

There were no significant differences in final body weights among the groups as shown Fig. 1. Table 2 shows the effects of IBOs feeding and calcium deficiency on food consumption and on liver and tibia weights. Food consumption in the calcium-deficient groups was significantly higher than that in the calcium-sufficient groups. The rats fed calcium-deficient diets might try to compensate for the lack of calcium, and food consumption was increased. Food consumption was increased by IBOs feeding, but this effect was not statistically significant. Tibia weight was significantly lower in the calcium-deficient groups than the calcium-sufficient groups indicating that calcium-deficient rats were in a state of calcium deficiency. On the other hand, liver weight in calcium-deficient groups significantly increased as compared to those in calcium-sufficient groups. There were no significant differences in other tissue weights among the groups.

Table 3 shows the effects of calcium deficiency and IBOs feeding on calcium contents in the whole blood, liver, kidneys and tibia. The significant changes induced by calcium deficiency were as follows: calcium levels were decreased in whole blood, kidneys and tibia. On the other hand, the significant effects by IBOs feeding were increased calcium levels in all tissues of calcium-deficient rats, especially in the tibia, and increased calcium levels in tibia of calcium-sufficient rats.

The changes in magnesium and phosphorous levels were similar. No notable change was observed in magnesium and phosphorous levels of all tissues, except for the tibia. Magnesium and phosphorous levels were significantly decreased by calcium deficiency and were significantly increased in the tibia by IBOs feeding (Tables 4, 5).

The changes in the iron levels were opposite to those in calcium, magnesium
Fig. 1. Body weight curve.

Table 2. The effect of IBOs feeding and Ca deficiency on food consumption, liver and tibia weights.\(^1\)

| Treatment                     | Food consumption (amount g for 4 weeks) | Liver weights (g) | Tibia weights (g) |
|-------------------------------|----------------------------------------|-------------------|-------------------|
| Ca-sufficient IBOs(−)         | 395.2±6.8                              | 6.48±0.15         | 0.358±0.004       |
| Ca-sufficient IBOs(+)         | 424.4±11.5                             | 6.58±0.18         | 0.387±0.004       |
| Ca-deficient IBOs(−)          | 444.5±13.5                             | 7.11±0.20         | 0.243±0.004       |
| Ca-deficient IBOs(+)          | 479.3±16.6                             | 6.78±0.14         | 0.273±0.003       |

Analysis of variance-\(p\) values\(^2\)

| Effect         | Food consumption | Liver weights | Tibia weights |
|----------------|------------------|---------------|--------------|
| Ca effect      | <0.01            | <0.01         | <0.01        |
| IBOs effect    | N.S.             | N.S.          | <0.01        |
| Ca×IBOs        | N.S.             | N.S.          | N.S.         |

\(^1\)Data are expressed as M±SE for 7 rats per group. \(^2\)\(2\times2\) analysis of variance. A \(p\)-value of at least 0.05 was considered statistically significant and is given for main treatment effects and interactions. N.S.=not statistically significant.

and phosphorous levels, the iron level was significantly increased by calcium deficiency in the liver, kidneys and tibia, and were significantly decreased in the tibia by IBOs feeding (Table 6).
Table 3. The effect of IBOs feeding and Ca deficiency on calcium contents in whole blood, liver, kidneys and tibia.\(^1\)

|                | Whole blood (µg/wet g) | Liver (µg/wet g) | Kidneys (µg/wet g) | Tibia (mg/wet g) |
|----------------|------------------------|------------------|-------------------|------------------|
| Ca-sufficient IBOs (−) | 62.3 ± 8.6             | 38.6 ± 1.2       | 86.0 ± 11.7       | 64.6 ± 2.6       |
| Ca-sufficient IBOs (+)  | 61.9 ± 3.1             | 38.1 ± 5.8       | 79.9 ± 7.0        | 68.9 ± 3.8       |
| Ca-deficient IBOs (−)   | 54.2 ± 6.8             | 34.9 ± 3.8       | 61.7 ± 3.5        | 28.3 ± 2.2       |
| Ca-deficient IBOs (+)   | 56.6 ± 2.8             | 39.8 ± 1.7       | 64.3 ± 1.7        | 38.4 ± 1.8       |

Analysis of variance-p values

|                |                  |                  |                  |                  |
|----------------|------------------|------------------|------------------|------------------|
| Ca effect      | <0.01            | N.S.             | <0.01            | <0.01            |
| IBOs effect    | N.S.             | N.S.             | N.S.             | N.S.             |
| Ca × IBOs      | N.S.             | N.S.             | N.S.             | N.S.             |

\(^1\)See footnote for Table 2.

Table 4. The effect of IBOs feeding and Ca deficiency on magnesium contents in whole blood, liver, kidneys and tibia.\(^1\)

|                | Whole blood (µg/wet g) | Liver (µg/wet g) | Kidneys (µg/wet g) | Tibia (mg/wet g) |
|----------------|------------------------|------------------|-------------------|------------------|
| Ca-sufficient IBOs (−) | 44.1 ± 2.5             | 250.1 ± 5.0      | 232.4 ± 7.1       | 1.33 ± 0.06      |
| Ca-sufficient IBOs (+)  | 44.5 ± 1.5             | 243.9 ± 36.1     | 229.7 ± 7.8       | 1.40 ± 0.04      |
| Ca-deficient IBOs (−)   | 44.6 ± 2.2             | 253.1 ± 18.6     | 234.8 ± 6.6       | 0.72 ± 0.04      |
| Ca-deficient IBOs (+)   | 43.2 ± 4.1             | 261.9 ± 7.9      | 236.2 ± 11.2      | 0.83 ± 0.04      |

Analysis of variance-p values

|                |                  |                  |                  |                  |
|----------------|------------------|------------------|------------------|------------------|
| Ca effect      | N.S.             | N.S.             | N.S.             | <0.01            |
| IBOs effect    | N.S.             | N.S.             | N.S.             | <0.01            |
| Ca × IBOs      | N.S.             | N.S.             | N.S.             | N.S.             |

\(^1\)See footnote for Table 2.

Table 5. The effect of IBOs feeding and Ca deficiency on phosphorous contents in whole blood, liver, kidneys and tibia.\(^1\)

|                | Whole blood (µg/wet g) | Liver (mg/wet g) | Kidneys (mg/wet g) | Tibia (mg/wet g) |
|----------------|------------------------|------------------|-------------------|------------------|
| Ca-sufficient IBOs (−) | 497 ± 25               | 3.93 ± 0.20      | 3.35 ± 0.11       | 33.5 ± 2.0       |
| Ca-sufficient IBOs (+)  | 497 ± 18               | 3.73 ± 0.52      | 3.34 ± 0.15       | 35.2 ± 2.4       |
| Ca-deficient IBOs (−)   | 484 ± 30               | 3.83 ± 0.31      | 3.32 ± 0.09       | 17.4 ± 1.5       |
| Ca-deficient IBOs (+)   | 491 ± 27               | 3.98 ± 0.11      | 3.35 ± 0.10       | 22.4 ± 1.5       |

Analysis of variance-p values

|                |                  |                  |                  |                  |
|----------------|------------------|------------------|------------------|------------------|
| Ca effect      | N.S.             | N.S.             | N.S.             | <0.01            |
| IBOs effect    | N.S.             | N.S.             | N.S.             | <0.01            |
| Ca × IBOs      | N.S.             | N.S.             | N.S.             | <0.05            |

\(^1\)See footnote for Table 2.
Table 6. The effect of IBOs feeding and Ca deficiency on iron contents in whole blood, liver, kidneys and tibia.1

|                      | Whole blood (µg/wet g) | Liver (µg/wet g) | Kidneys (µg/wet g) | Tibia (mg/wet g) |
|----------------------|------------------------|------------------|--------------------|------------------|
| Ca-sufficient IBOs(−)| 477±15                 | 202±33           | 52.9±4.5           | 18.1±1.3         |
| Ca-sufficient IBOs(+) | 468±20                 | 219±43           | 47.7±3.9           | 17.1±0.7         |
| Ca-deficient IBOs(−) | 491±26                 | 502±87           | 73.0±7.0           | 40.5±6.2         |
| Ca-deficient IBOs(+) | 481±32                 | 505±82           | 75.3±6.2           | 34.6±1.4         |

Analysis of variance- p values

|                      | Ca effect | IBOs effect | Ca×IBOs |
|----------------------|-----------|-------------|---------|
| Ca effect            | N.S.      | <0.01       | <0.05   |
| IBOs effect          | N.S.      | N.S.        | N.S.    |
| Ca×IBOs              | N.S.      | N.S.        | N.S.    |

1See footnote for Table 2.

DISCUSSION

In this study, the following findings were seen. (a) The effects of calcium deficiency were revealed especially in the tibia, and the changes related to calcium deficiency in the tibia were a significant decrease in tibia weight and in the calcium, magnesium and phosphorous levels, and a significant increase in the iron level. (b) The significant changes related with IBOs feeding in the tibia were an increase in tibia weight and in the calcium, magnesium and phosphorous levels, and a decrease in iron levels. (c) In the whole blood, liver and kidneys, no marked difference in mineral levels was observed between calcium-sufficient IBOs(+) and calcium-sufficient IBOs(−) rats. (d) The calcium, magnesium and phosphorous levels were increased and the iron level was decreased in the whole blood, liver and kidneys in the calcium-deficient groups by IBOs feeding.

Lengemann et al. (2) pointed out that absorption of calcium was enhanced by lactose feeding and that it increased in the presence of vitamin D. Wasserman and Comar (3) indicated that cellobiose, sorbose, ribose, xylose, lactose, raffinose, melibiose, glucosamine, mannitol and sorbitol enhanced absorption of calcium, and the effectiveness in the ability to enhance calcium absorption appeared to be positively correlated with the residence time of the carbohydrate in the gut or absorption of carbohydrate by a passive mechanism. Vaughan and Filer (4) also showed the enhancing action of carbohydrates and the effects of the presence of various ions on the intestinal absorption of calcium. It was suggested by Goda et al. (5, 6) that maltitol intake increased absorption of intestinal calcium in the rat. In our previous study (14, 15), the effects of IBOs feeding on various tissue mineral contents in rats fed IBOs for 8 weeks were examined; no marked change was observed in various tissue minerals (calcium, magnesium and phosphorous) contents or tissue weights in rat fed IBOs as compared with the control. The findings obtained in our present and previous studies show that IBOs feeding may increase...
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mineral retention only in the early stage or during a short period, if the rats were fed calcium-sufficient diets, or it may increase mineral retention only in a state of calcium deficiency. In this case, a mineral imbalance was induced by calcium deficiency, and minerals reached the hindgut and might be absorbed with IBOs products digested by the intestinal micoflora (10-13). That is, Kashimura et al. found that palatinose and its condensates, trehalulose, isomalt and isomaltulose-based oligomers were utilized by various intestinal bacterias such as *Bifidobacterium*, part of *Mitsuokella multiacida*, *Bateroides*, *Clostridium* and *Peptostreptococcus*, etc.

The mechanism of the favorable effect of IBOs or other saccharides that are difficult to digest with the host’s intestinal enzymes and to metabolize with intestinal bacteria, on mineral metabolism, especially in the hindgut, is not clear. In our previous studies (14, 15), phosphorus, zinc, copper and manganese levels in plasma, bone and various tissues changed by feeding of palatinose and its condensates. It is pointed out clearly in this study that IBOs feeding has a favorable effect on mineral retention in calcium-deficient rats, and on the relationship between mineral absorption and short-chain fatty acids that are produced from saccharides by intestinal bacteria in the hindgut.

To clarify the mechanism of the effects of indigestible saccharides on mineral absorption in the hindgut, additional studies are underway.

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