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Mannitol evaluated mineral absorption and bone retention in ovariectomized rats

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Abstract: Indigestible sugars are used in food production and pharmaceutical industry due to their desirable properties. The effects of mannitol on Ca and Mg absorption and retention in ovariectomized rats were evaluated. Five weeks old ovariectomized Wistar rats were given Ca, Mg-deficient diet for 28 days then fed a control diet or 8% mannitol diets for another 28 days. Feaces were collected twice for 72h to measure mineral absorption. On day 56, rats were slaughtered and cecal parameters were observed. Femurs were collected to measure mineral levels. Results were shown that mineral absorption and femoral mineral was significantly increased by mannitol feeding. Cecal parameters were significantly altered after mannitol consumption. In conclusion, Ca and Mg absorption and their retention were improved by cecal fermentation of mannitol in ovariectomized rats.

Keywords: absorption of Ca and Mg; femur; mannitol; ovariectomized rat

Mannitol included in Food Chemical Codex does not impact blood glucose level and insulin secretion (Song & Vieille 2009). Therefore, mannitol can be used as a low caloric sweeter additive, especially for the persons with obesity or diabetes. As an indigestible sugar alcohol mannitol pass through the upper gastrointestinal tract and become readily available for microbes in the hindgut where they are degraded through various metabolic pathways to short-chain fatty acids and gases, which was proved in our previous studies.

There have been a number of studies carried out on indigestible sugars and indigestible sugar alcohols, and found an increase in mineral bioavailability is one important properties of these sugars. Intestinal absorption of minerals including Ca, P, Mg, Zn, and Fe was reported to be enhanced by indigestible sugars such as lactosucrose, fructooligosaccharides, xylooligosaccharides, and galactooligosaccharides (Mattila *et al.* 2002; Kishino *et al.* 2006; Freitas *et al.* 2012; Takasugi *et al.* 2013). Bone mass loss is almost universal in postmenopausal women with dropped estrogen levels and a decline in bone mass. Osteoporosis is resulted by bone mass loss when it drops to a level where it is susceptible to fracture from minor trauma (Peck 1984). Medical and socioeconomic effects of postmenopausal osteoporosis are increasing daily because of a huge aging population. Therefore, to prevent bone mass loss by staples may be an effective action against osteoporosis in postmenopausal women. Dietary fructooligosacchaides and inulin were reported to prevent osteoporosis by raising bone mineral density through increasing calcium absorption and suppress bone resorption in ovariectomized rats (Zafar *et al.* 2004, Johnson *et al.* 2011, Nakata *et al.* 2014). In this study, as a model of postmenopausal bone loss, ovariectomized rats were used to study the effects of mannitol on mineral absorption and retention when suffering osteopenia.

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MATERIAL AND METHODS

Animals. Sixteen growing male (3 weeks old) Wistar rats (purchased from Japan SLC Inc., Japan) were housed individually, under conditions 23 ± 1°C with 50–60% relative humidity. The light was set as a constant 12 h light and 12 h dark cycle. All rats were ovariectomized. One week after ovariectomized surgery, the rats were weighed and randomly assigned to two treatment groups with 8 rats respectively, and fed Ca, Mg-deficient diet for 28 days. From 29th day of feeding trail, the rats in each group being fed one of the experimental diets (control diet and mannitol diets containing 8% mannitol) for 28 days. Diets and water were available ad libitum during the entire feeding trial.

Diets. The composition of the experimental diets (control diet, mannitol diets containing 8% mannitol and Ca, Mg deficient diet) was shown in Table 1. Control diet consisted of standard laboratory chow (AIN-93G) (Reeves *et al*. 1993). Ca, Mg-deficient diet had same compositions as control diet, except containing 4/7 $CaCO_3$ and MgO of mineral mix in standard laboratory chow (AIN-93G).

Sample collection and analysis. During feeding trial, the amount of food supplied, diet residues, and diet waste were measured daily. Feacal collection was carried out three times. Consequent 72-h feacal collection was in progress from days 35 to 37 and from days 51 to 53. On the last day of the feeding trial, the rats were anesthetized with diethyl ether after fasting for 3-h and sacrificed by exsanguinations from the celiac artery. Cecums with digesta and left femurs of rats were collected. Cecal digesta, femurs, diets and feaces were oven-dried for 24 h at 105°C and burned in a 550°C muffle furnace to obtain crude ash. The crude ash was dissolved in 50% sulfuric acid, and mineral levels in diets, feaces, cecal digesta and femurs were determined by flame atomic absorption spectroscopy (AAS180-30; Hitachi Ltd., Japan). The pH values of cacel digesta were measured with a pH meter (TWIN Horiba Ltd., Japan). Samples of the cecal digesta were used for the measurement of organic acids by high-performance liquid chromatography column detector Shim-pach SCR-102H (Shimadzu, Japan). Animal care and sacrificing were according to the experimental protocol (No. 51735) approved by the institutional ethics committee of Okayama University.

Calculation and Statistics. Feed efficiency and the absorption of Ca and Mg were calculated by following Equations 1 and 2:

$$\text{Feed efficiency (\%)} = (\text{weight gain/feed intake}) \times 100 \quad (1)$$

$$\text{Mineral absorption (\%)} = (\text{intake} - \text{feacal excretion})/\text{intake} \times 100 \quad (2)$$

Data are shown as a mean ± s.d.; statistical difference of data was analysed by Student's t-test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group.

Table 1. Composition of experimental diets

Ingredients (g/kg)	Ca, Mg deficient*	Control	Mannitol
α-Corn starch	562	562	562
Casein	200	200	200
Sucrose	100	20	20
Corn oil	70	70	70
Cellulose powder	20	20	20
Vitamin mix	10	10	10
Mineral mix	35*	35	35
$CaCO_3$	7.14	12.495	12.495
MgO	1.39	2.435	2.435
L-Cystine	3	3	3
Mannitol	0	0	80
Gross energy (MJ/kg)	20.7 (calculation)	–	–

*same composition as control diet, except containing 4/7 $CaCO_3$ and MgO of mineral mix; sucrose was added to make up 3/7 $CaCO_3$ and MgO in mineral mix

RESULTS AND DISCUSSION

As shown in Table 2. The rats in control diet group and in mannitol diet group were fed Ca, Mg deficient diet from day 1 to day 29 of feeding trial. From day 29 to day 56, final body weight and daily feed intake in rats fed mannitol diet were significantly lower than those fed control diet. Daily weight gain and feed efficiency were similar. Dry matter digestibility of days 35–37 was significantly reduced by mannitol feeding. During days 51–53, there was no significant difference in dry matter digestibility between in rats in control group and in mannitol group. Mannitol is partly absorbed in small intestine, but is not converted to glucose (Dwivedi 1991). Indigestible sugar alcohols reach the hindgut, are fermented by the local microbial flora and produce hydrogen, methane,

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Table 2. Effects of dietary mannitol on body weight, feed intake, weight gain, feed efficiency, dry matter digestibility in ovariectomized rats

	Diet group	
	control	mannitol
Initial BW(g)	197.2 ± 8.4	198.7 ± 6.2
Final BW (g)	205.6 ± 10.2	195.4 ± 9.7*
Feed intake (g/day)	11.4 ± 0.2	10.9 ± 0.4**
Weight gain (g/day)	1.34 ± 0.21	1.26 ± 0.18
Feed efficiency (%)	12.3 ± 2.2	11.1 ± 1.6
Dry matter digestibility[a] (%)	95.6 ± 0.3	94.3 ± 1.1**
Dry matter digestibility[b] (%)	95.5 ± 0.5	95.1 ± 0.6

*$P < 0.05$; **$P < 0.01$; [a]days 35–37; [b]days 51–53

carbon dioxide, and short-chain fatty acids (SCFAs) (Bar 1990). Mannitol is a nutrient for gas-producing bacteria in large intestine (Keighley *et al*. 1981). Mannitol produces SCFAs and more gas than sucrose in hindgut. The gas produced is considered an energy loss. Short-chain fatty acids are used as a source of energy by the host (Roediger 1980), but available energy of SCFAs is 15–25% less than that of glucose. In addition, fat digestibility and fat accumulation in body was decreased by mannitol ingestion in rats (Nishiyama *et al*. 2009).

Cecum parameters in rats are shown in Table 3. Compared with control diet, cecal total weight, wall weight and content weight were significantly increased by mannitol feeding. The rats in mannitol diet group had significantly higher concentrations of succinic acid, formic acid, and butyric acid, and significantly lower concentrations of acetic acid, propionic acid and isovaleric acid, compared with control diet group. Mannitol is mostly fermented by local microbes to produce SCFAs in hindgut (Hongo *et al*. 2010). Intestinal gas and SCFAs are the main products of mannitol fermentation. Mannitol is used by several intestinal bacteria, such as *Lactobacillus plantarum*, some *Bifidobacteria*, *Escherichia coli*, and *Streptococcus mutans*, as a primary energy source for growth (Chakravorty 1964; De Vries & Stouthamer 1968; Maryanski & Wittenberger 1975; Neves *et al*. 2002). When the rats fed a 5% mannitol diet, the population of *Viridans streptococci* and *Bifidobacteria* were significantly decreased and the population of fusiform bacteria was significantly increased in cecum. These were concomitant with an increase in butyric acid concentration and a decrease in acetic acid concentration in cecum (Morishita 1994). An increase in butyric acid production due to mannitol is metabolized by butyric acid producing bacteria such as *Clostridium indolis* and *Lactobacilli* (Maekawa *et al*. 2005). Because the production and distribution of SCFAs depend on indigestible sugar fermented by different bacteria species in lumen, leading to a different SCFAs pattern. Cecal pH was reduced and cecal tissue weights and cecal content weights were increased by mannitol feeding in this study. Production of SCFAs from bacterial fermentation is concomitant with luminal acidification. The production of lactic acid and succinic acid contributes to the regulation of the luminal pH (Sakata *et al*. 1999). The higher production of lactic acid contributes to luminal acidification more efficiently than the production of SCFAs in the rat cecum (Hoshi 1994). Bacteria proliferation improved by mannitol in cecum was responsible for the increase in cecal content weight. The increase in cecal tissue weight can be explained by three ways. At first, the inverse correlation between luminal acidification and the epithelial cell proliferation of large intestine showed that lower luminal pH stimulated epithelial cell proliferation (Lupton *et al*. 1985). Secondly, SCFAs have been shown to stimulate intestinal cell proliferation in vivo and in vitro, especially butyric

Table 3. Effects of dietary mannitol on cecum parameters in the ovariectomized rats

	Diet group	
	control	mannitol
pH	7.5 ± 0.3	6.0 ± 0.3***
Weight (g)		
Total	2.45 ± 0.50	6.05 ± 1.75***
Wall	0.44 ± 0.07	1.13 ± 0.21***
Content	2.01 ± 0.44	4.92 ± 1.76***
Cecal organic acid concentrations (μmol/g)		
Succinic	4.62 ± 1.37	12.37 ± 13.51*
Lactic	4.45 ± 0.73	4.18 ± 1.01
Formic	0.36 ± 0.16	1.82 ± 1.83*
Acetic	46.42 ± 9.50	30.76 ± 7.03***
Propionic	13.44 ± 2.4	9.85 ± 2.96**
Isobutyric	2.27 ± 0.22	2.26 ± 0.69
Butyric	10.41 ± 2.33	29.32 ± 8.66***
Isovaleric	2.72 ± 0.36	2.15 ± 0.49**
Valeric	1.75 ± 0.36	1.84 ± 0.57
Total organic	85.11 ± 15.65	94.55 ± 22.55

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

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acid, accelerate epithelial cell proliferation, thereby increasing cecal tissue weight (Sakata 1986, 1987; Ichikawa & Sakata 1998, Raschka & Daniel 2005; Comalada & Bailón 2007). Secondly, butyric acid promoted intestinal cell proliferation through several processes such as the release of growth factors or gastrointestinal peptides or the modulation of mucosal blood flow (Blottière *et al*. 2003). Thirdly, osmotic pressure of SCFAs causes cecal development and cecal enlargement (Rémésy *et al*. 1993; Lopez *et al*. 2000).

As shown in Figure 1 and 2, compared with control diet, Ca and Mg absorption and Ca and Mg amounts in femur were significantly increased by mannitol feeding. Ovariectomized rat was commonly used as experimental animal model of postmenopausal women. Postmenopausal women face to the risk of osteoporosis because the production and regulating effects of the related hormones including parathyroid hormone, calcitriol, estrogen were altered after menopause. Bone loss syndrome is mainly modulated by the decreased calcium absorption and bone resorption to an associated elevation in circulating parathyroid hormone and the decrease in calcitriol and estrogen (Nuti *et al.* 2000). In ovariectomized rats, estrogen deficiency caused bone mass loss (Egermann *et al*. 2005). Ca absorption decreased progressively and the decrease became statistically significant 8 and 12 weeks following ovariectomy (Kalu & Orhii 1999). Dietary inulin and fructooligosaccharides increased bone mineral density, breaking strength, Ca content of femur and Ca absorption in ovariectomized rats (Zafar *et al*. 2004). Galactooligosaccharides were fermented in the lower part of intestine and prevented bone loss by increasing Ca absorption in ovariectomized rats (Chonan *et al*. 1995). More recent studies have shown a positive effect of fermentable carbohydrates on Mg absorption in ovariectomized rats and postmenopausal women. Ca loss is caused by estrogen deficiency, and Mg is related to bone health as well. Dietary Mg supplementation promoted bone formation and prevents bone resorption in ovariectomized rats (Toba *et al*. 2000). Mg retention increased by short chain fructooligosaccharides in postmenopausal women (Tahiri *et al*. 2001). In this study, mannitol feeding induced a lower cecal pH which increased mineral cations concentration. An increased amount of soluble and ionized minerals was adequate for their absorption by epithelial cells (Raschka & Daniel 2005). The enlarged cecal wall tissue caused by mannitol feeding led a greater exchange surface area for mineral absorption, allowing a better absorption of Ca and Mg. In addition, SCFAs may raise the absorptive capacity of intestinal epithelium by increasing intestinal blood flow and fluid and electrolyte uptake to directly improve mineral absorption in hindgut (Topping 2001). Butyric acids supply energy to intestinal epithelial cells and regulate electrolyte exchange of minerals and hydrogen to facilitate mineral absorption (Lutz & Scharrer 1991).

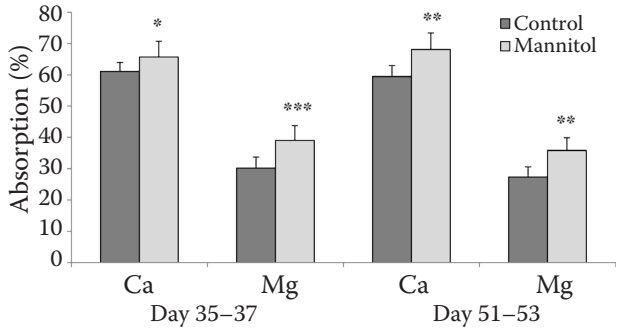

Figure 1. Effects of dietary mannitol on Ca and Mg absorption during feacal collection period in the ovariectomized rats

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

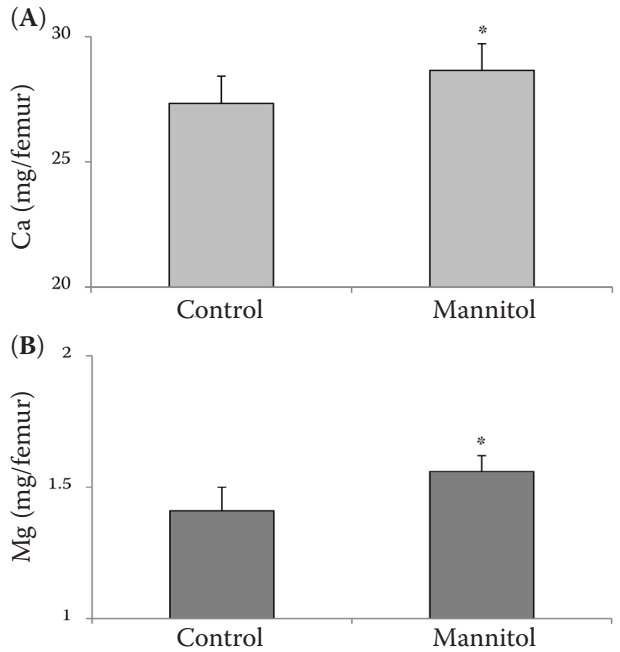

Figure 2. Effects of dietary mannitol on the amounts of Ca (**A**) and Mg (**B**) in femurs in the ovariectomized rats

*$P < 0.05$

CONCLUSION

The present study suggests that 8% dietary mannitol increased the absorption of Ca and Mg and the reten-

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tion Ca and Mg in femurs in the ovariectomized rats. When the overiectomized rats were used as a model of postmenopausal bone loss, dietary mannitol could increase the intestinal absorption of Mg and Ca through a greater exchange surface area for mineral absorption and a lower cecal pH which increased mineral cations concentration which were induce by the fermentation of mannitol in cecum. Therefore, mannitol might be developed as a low-calorie sweetener for the people who suffering bone loss in the further research.

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