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Inhibitory Effects of Psyllium on Rat Mineral Absorption Were Abolished by Reduction of Viscosity with Partial Hydrolysis

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Psyllium husk, a highly viscous fiber, has beneficial effects for health, but has been reported to inhibit absorption of calcium. The present study found the effects of fiber viscosity on calcium, magnesium, and zinc absorption with partially degraded psyllium preparations to be one fifth viscosity (LD-HP) and one seventieth viscosity (HD-HP) using normal and ovariectomized rats. Magnesium absorption was reduced with ingestion of intact psyllium (50 g/kg diet) for 4 weeks but this reduced absorption was increased with lower viscous psyllium preparations. Moreover, the absorption in the HD-HP group was higher than in the control group (100 g cellulose/kg diet) in ovariectomized rats. Changes in calcium and zinc absorption were similar to those in magnesium absorption. Cecal pH was reduced only in rats fed HD-HP in both normal and ovariectomized rats. These results indicate that reduction of psyllium viscosity tends to counter inhibitory effects on mineral absorption.

Key words: psyllium; partially hydrolyzed psyllium; viscosity; mineral absorption; ovariectomized rats

Many studies have reported that some dietary fibers improve lipid metabolism and mineral absorption in rats.[1–4] Psyllium is a source of natural and concentrated soluble fiber derived from the husks of *Plantago ovata*. It is approximately eight times more soluble fiber than oat bran on a per weight basis.[5] It increases stool weight and promotes laxation by increasing the moisture content of the stools.[6,7] It has been reported that psyllium decreased serum total cholesterol concentration with no effect on serum HDL-cholesterol.[8,9] In spite of these beneficial effects, Luccia and Kunkel found that psyllium decreased apparent absorption of calcium.[10]

Annison showed that dietary soluble non-starch polysaccharides inhibit nutrient absorption in broiler chickens by raising the viscosity of the digesta.[11] Van der Klis proposed that reduction of mineral absorption with increasing dietary concentrations of indigestible soluble polysaccharides (carboxymethylcellulose) was probably caused by the higher intraluminal viscosities in the small intestine.[12] Moreover, it has been reported that partially hydrolyzed guar gum, which has less viscosity than native guar gum, improves calcium absorption.[13] These previous findings suggest that viscosity is a factor affecting mineral absorption, but this has not been fully understood.

We examined the effects of psyllium viscosity on intestinal absorption of calcium, magnesium, and zinc. Calcium is the most abundant divalent cation and is responsible for regulatory functions such as neurotransmission, cellular secretion, and blood clotting.[14] It is obvious that a dietary deficiency in calcium leads to progressive bone loss.[15] Magnesium is the second most abundant intracellular cation in vertebrates, and an increasing number of clinical disorders such as diabetes and cardiovascular disease, have been found to be associated with magnesium deficiency.[16–18] Recently, magnesium deficiency has also been implicated as a risk factor for osteoporosis.[17]

Osteoporosis has recently become a disease of public concern especially apparent in post-menopausal women, and is thought to be due to estrogen deficiency.[19–21] In the present study, we used ovariectomized (OVX) rats as a model for postmenopausal osteoporosis with calcium malabsorption. Zinc absorption is also known to affect ingestion of fiber. Marginal zinc deficiency is associated with diets based on plant food, especially those diet rich in fiber.[22] But the effects of OVX on zinc absorption have not been reported. Zinc is an essential trace element involved in many important body functions.[23,24] Zinc deficiency results in retardation of growth,[25] neuropsychologic impairment,[26] lowering of gustatory and olfactory sensitivity, and immunological defects.[27]

The aim of the present study was to evaluate the effects of feeding psyllium and partially hydrolyzed psyllium with different viscosities on calcium, magne-

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Abbreviations: IP, intact psyllium; OVX, ovariectomy (ovariectomized); HD-HP, high degree hydrolyzed psyllium; LD-HP, low degree hydrolyzed psyllium

sium, and zinc absorption in normal and OVX rats. We focused on the effects of psyllium viscosity on mineral absorption.

Materials and Methods

This study was approved by the Hokkaido University Animal Committee. Animals were maintained in accordance with the guidelines of Hokkaido University for the care and use of laboratory animals.

Animal and Experimental protocol. Psyllium was partially hydrolyzed by treatment with 0.05 mol/l HCl (100 g intact psyllium/5 liter of the HCl solution) at room temperature for 30 min (low degree hydrolysis, LD-HP), or by treatment with 0.50 mol/l HCl (100 g intact psyllium/5 liter of the HCl solution) at 60 °C for 30 min (high degree hydrolysis, HD-HP). These partially hydrolyzed psylliums were extracted by the addition of 4 volumes of 99% EtOH (final concentration, 80% v/v), precipitated overnight and filtered. The precipitated fibers were washed with 99% EtOH until the pH of the washout solution reached pH6, and then air-dried at room temperature. These partially hydrolyzed psyllium preparations were powdered with a Willey mill equipped with a 0.5 mm sieve. Intact psyllium and partially hydrolyzed psyllium preparations were measured for viscosity by viscometer (BL viscometer, Tokyo Keiki, Co., Ltd., Tokyo, Japan). The recovery rates for LD-HP and HD-HP were 87.6% and 86.9% respectively.

Sixty-eight female Sprague-Dawley rats (6 weeks old, Clea Japan, Tokyo) weighing about 150 g were housed in individual cages in a room with controlled temperature (22 ± 2 °C), relative humidity (40% to 60%), and lighting (lights from 8:00 AM to 8:00 PM) throughout the study. The rats had free access to deionized water and a sucrose-based semi-purified stock diet, shown in Table 1, before operation. The rats were divided into two groups. One group underwent bilateral ovariectomy (OVX), and the other underwent bilateral laparotomy (sham). Both operations were performed under the same anesthetic procedure (Nembutal: sodium pentobarbital 40 mg/kg of body weight, Abbott Laboratories, North Chicago, Illinois, U.S.A). All animals had free access to deionized water and the stock diet for 6 d to recover from surgical damage.

After postoperative recovery, the sham and OVX rats were assigned randomly to four subgroups of 8–9 rats each. One subgroup (the control diet group) was fed a test diet based on AIN-93G formulation,[28] containing 100 g cellulose/kg diet (Table 1). The other three subgroups (IP, LD-HP, and HD-HP) were fed test diets added 50 g each of intact psyllium or partially hydrolyzed psyllium preparations replacing the cellulose of the control diet. Rats were fed the assigned test diets for 4 weeks. During the feeding period, food intakes were adjusted based on the lowest food intake group. All animals were allowed free access to deionized water throughout the test period. Body weight and food intake were measured every day.

Feces were collected during the last 5 d of the test period and freeze-dried to evaluate absorption of calcium, magnesium, and zinc. On the last day of the experiment, all the rats were killed under pentobarbital anesthesia (Nembutal: sodium pentobarbital 50 mg/kg of body weight, Abbott Laboratories). The cecum was removed with the contents, weighed, frozen immediately with liquid nitrogen, and stored at −40 °C for subsequent analysis. The cecal contents were collected by cutting open the frozen cecal wall, and the cecal wall was washed with saline and weighed. The content weight was evaluated as the difference between the cecum with and without contents.

Analyses. Freeze-dried feces were milled to a fine powder and the powdered feces (approximately 1.5 g) were dry-ashed at temperatures elevated linearly to 550 °C for 6 h, and then at 550 °C for 18 h with an electric furnace (Eyela TMF-3200, Tokyo Rikakikai, Tokyo, Japan). The ashed samples were treated with 5.49 mol/l HCl at 200 °C for 30 min and dissolved in 0.82 mol/l HCl. Calcium, magnesium, and zinc concentrations in the solutions of ashed samples were measured by polarizing Zeeman-effect atomic absorption spectrometry (Z-5310, Hitachi, Tokyo, Japan) after suitable dilution.

The cecal contents were diluted with 4 volumes of deionized water and homogenized with a Teflon homogenizer. The pHs of these homogenates were measured with a semiconducting electrode (ISFET pH sensor 0015-15C, Horiba, Ltd., Kyoto, Japan) as the pH of the cecum contents.

Calculations and Statistical Analyses. Calcium, magnesium, and zinc absorption were calculated using the following equation:

Table 1. Composition of Stock[1] and Test Diets[2]

ELEMENT	g/kg diet
Casein[3]	250.0
Corn oil	50.0
Mineral mixture[4]	35.0
Vitamin mixture[4]	10.0
Choline bitartate	2.5
Fiber source	100.0
Sucrose	To make 1 kg

[1]Crystallized cellulose (100 g/kg diet; Avicel PH102, Asahi Chemical Industry Co., Ltd., Tokyo, Japan) was added to the stock diet.
[2]Crystallized cellulose (50 g/kg diet) was replaced with intact psyllium (50 g/kg diet; Psyllium powder S, Iwaki Mizutori, Tokyo, Japan), or two partially hydrolyzed psyllium preparations: LD-HP or HD-HP (50 g/kg diet).
[3]Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).
[4]Mineral and vitamin mixture were prepared according to the AIN-93G formulation. The calcium, magnesium, and zinc levels in test diets were adjusted to the level as the AIN-93G by accounting of these mineral contents of the fiber sources.

1) Net Ca, Mg, or Zn absorption (mmol/5 d or μmol/5 d)

= total Ca, Mg, or Zn intake − Ca, Mg, or Zn excretion in feces.

2) Ca, Mg, or Zn absorption rate (%)

$$= 100 \times \frac{\text{(total Ca, Mg, or Zn intake} - \text{Ca, Mg, or Zn excretion in feces)}}{\text{total Ca, Mg or Zn intake}}$$

Each value represents the mean ± standard error of the mean. The effects of operation and diet were analyzed by two-way analysis of variance (ANOVA). Duncan's multiple-range test was used to determine whether mean values were significantly different between groups ($P < 0.05$).[29)]

Results

The viscosity of LD-HP was reduced to approximately 20% of that of intact psyllium, and that of HD-HP was very low (Table 2).

Body weight gain was higher in OVX rats than in sham rats ($P < 0.001$, Table 3). Food intakes ($P = 0.003$) and fecal dry weights ($P = 0.017$) were also influenced by OVX. Intakes of both hydrolyzed psyllium diets in sham rats were lower than those of control and intact psyllium diets. Fecal dry weights excreted for 5 d were higher in the intact psyllium group than in the control group, even though the diets had the same fiber levels. The high fecal outputs were gradually reduced by lowering the viscosities of the psyllium preparations in both the sham and the OVX rats. The outputs in the HD-HP groups were lower than those in the control groups of both rats. Uterine weights were much lower in OVX rats (0.030 ± 0.004 g/100 g body weight) than in sham rats (0.246 ± 0.055 g/100 g body weight, n = 68, $P < 0.001$), indicating the success of the surgical procedure in all rats in the OVX group.

Calcium absorption rates were lower in the OVX groups than in the sham groups as a result of two-way ANOVA ($P < 0.05$, Table 4). Diet tended to influence the absorption rate ($P = 0.073$). The highest average values of absorption rates were in the HD-HP groups, and the lowest average values were in the IP groups in both sham and OVX rats. Net calcium absorption and absorption rates, however, were not significantly differ-

Table 2. Viscosity of Intact Psyllium and Partially Hydrolyzed Psylliums[1]

DIETARY FIBER	VISCOSITY (mPa.s)
IP	163.2
LD-HP	35.7
HD-HP	2.37

[1]Viscosity unit; mPa.s (millipascal second).
IP, Intact psyllium.
LD-HP, Low degree hydrolyzed psyllium.
HD-HP, High degree hydrolyzed psyllium.

Table 3. Body Weight Gain, Food Intake, and Fecal Output of Sham-operated and Ovariectomized Rats Fed the Control or Test Diets for 4 Weeks[1,2]

	n	Body weight gain g	Food intake g/d	Fecal output dry g/5 d
Sham				
Control	8	74.7 ± 2.36^{b}	16.9 ± 0.16^{a}	11.0 ± 0.09^{d}
IP	8	80.1 ± 7.92^{b}	17.2 ± 0.22^{a}	13.5 ± 0.17^{a}
LD-HP	8	76.6 ± 8.93^{b}	16.6 ± 0.18^{b}	12.6 ± 0.32^{bc}
HD-HP	8	76.6 ± 4.20^{b}	16.5 ± 0.15^{b}	9.7 ± 0.25^{e}
Ovariectomized				
Control	9	98.4 ± 2.69^{a}	17.2 ± 0.15^{a}	12.0 ± 0.13^{cd}
IP	9	98.4 ± 1.47^{a}	17.1 ± 0.17^{a}	12.7 ± 0.17^{b}
LD-HP	9	103.0 ± 2.73^{a}	17.1 ± 0.17^{a}	12.4 ± 0.25^{bcd}
HD-HP	9	104.0 ± 2.04^{a}	17.2 ± 0.16^{a}	9.2 ± 0.18^{f}
ANOVA (P value):				
Operation		<0.001	0.003	0.017
Diet		0.846	0.128	<0.001
Operation × Diet		0.766	0.160	0.095

[1]Each value represents mean ± standard error of the mean.
[2]Values in a column not sharing a superscript letter differ significantly, $P < 0.05$.
IP, Intact psyllium.
LD-HP, Low degree hydrolyzed psyllium.
HD-HP, High degree hydrolyzed psyllium.

Table 4. Calcium Absorption in Sham-operated and Ovariectomized Rats Fed the Control or Test Diets for 4 Weeks[1,2]

	Fecal Ca output mmol/5 d	Net Ca absorption mmol/5 d	Ca absorption rate %
Sham			
Control	5.09 ± 0.20^{ab}	5.23 ± 0.28	50.5 ± 2.26^{ab}
IP	6.01 ± 0.19^{a}	4.38 ± 0.18	42.1 ± 1.78^{ab}
LD-HP	5.24 ± 0.14^{ab}	4.64 ± 0.18	46.9 ± 1.08^{ab}
HD-HP	4.90 ± 0.26^{b}	5.15 ± 0.28	51.3 ± 2.57^{a}
Ovariectomized			
Control	6.01 ± 0.41^{a}	4.37 ± 0.41	42.1 ± 3.91^{ab}
IP	5.96 ± 0.28^{a}	4.28 ± 0.28	41.8 ± 2.72^{b}
LD-HP	5.94 ± 0.21^{a}	4.47 ± 0.23	42.9 ± 2.13^{ab}
HD-HP	5.49 ± 0.45^{ab}	4.99 ± 0.45	47.6 ± 4.26^{ab}
ANOVA (P value):			
Operation	0.011	0.142	0.045
Diet	0.064	0.094	0.073
Operation × Diet	0.390	0.576	0.569

[1]Each value represents mean ± standard error of the mean.
[2]Values in a column not sharing a superscript letter differ significantly, $P < 0.05$.
IP, Intact psyllium.
LD-HP, Low degree hydrolyzed psyllium.
HD-HP, High degree hydrolyzed psyllium.

Table 5. Magnesium Absorption in Sham-operated and Ovariectomized Rats Fed the Control or Test Diets for 4 Weeks[1,2]

	Fecal Mg output mmol/5 d	Net Mg absorption mmol/5 d	Mg absorption rate %
Sham			
Control	0.52 ± 0.02^{cd}	1.21 ± 0.04^{b}	69.8 ± 1.54^{ab}
IP	0.87 ± 0.03^{a}	0.85 ± 0.03^{c}	49.4 ± 1.72^{c}
LD-HP	0.75 ± 0.03^{b}	0.91 ± 0.04^{c}	54.9 ± 1.75^{c}
HD-HP	0.44 ± 0.02^{d}	1.26 ± 0.04^{ab}	74.2 ± 1.62^{ab}
Ovariectomized			
Control	0.56 ± 0.03^{c}	1.18 ± 0.03^{b}	67.8 ± 1.89^{b}
IP	0.82 ± 0.05^{ab}	0.88 ± 0.05^{c}	51.8 ± 2.85^{c}
LD-HP	0.86 ± 0.05^{a}	0.88 ± 0.06^{c}	50.5 ± 3.10^{c}
HD-HP	0.42 ± 0.03^{d}	1.34 ± 0.03^{a}	76.0 ± 1.94^{a}
ANOVA (P value):			
Operation	0.373	0.644	0.717
Diet	<0.001	<0.001	<0.001
Operation × Diet	0.125	0.502	0.360

[1]Each value represents mean ± standard error of the mean.
[2]Values in a column not sharing a superscript letter differ significantly, $P < 0.05$.
IP, Intact psyllium.
LD-HP, Low degree hydrolyzed psyllium.
HD-HP, High degree hydrolyzed psyllium.

Table 6. Zinc Absorption in Sham-operated and Ovariectomized Rats Fed the Control or Test Diets for 4 Weeks[1,2]

	Fecal Zn output μmol/5 d	Net Zn absorption μmol/5 d	Zn absorption rate %
Sham			
Control	37.1 ± 0.45	8.76 ± 0.67^{ab}	19.0 ± 1.32^{ab}
IP	39.0 ± 1.44	6.19 ± 1.42^{b}	13.7 ± 3.13^{b}
LD-HP	38.7 ± 0.82	5.53 ± 1.01^{b}	12.3 ± 2.18^{b}
HD-HP	38.3 ± 1.13	7.00 ± 1.20^{b}	15.3 ± 2.47^{b}
Ovariectomized			
Control	38.2 ± 0.96	7.97 ± 0.96^{ab}	17.3 ± 2.08^{ab}
IP	38.4 ± 0.47	6.22 ± 0.47^{b}	13.9 ± 1.05^{b}
LD-HP	38.2 ± 1.08	8.39 ± 1.09^{ab}	18.0 ± 2.32^{ab}
HD-HP	36.5 ± 1.37	10.68 ± 1.36^{a}	22.7 ± 2.88^{a}
ANOVA (P value):			
Operation	0.534	0.061	0.079
Diet	0.527	0.057	0.085
Operation × Diet	0.592	0.117	0.157

[1]Each value represents mean ± standard error of the mean.
[2]Values in a column not sharing a superscript letter differ significantly, $P < 0.05$.
IP, Intact psyllium.
LD-HP, Low degree hydrolyzed psyllium.
HD-HP, High degree hydrolyzed psyllium.

ent among the diet groups.

Net magnesium absorption, the magnesium absorption rate, and fecal output of magnesium were influenced by diet ($P < 0.001$), but not by OVX, with no interaction between these factors. Both parameters of magnesium absorption in the IP and LD-HP diet groups were lower than those in the control group in both sham and OVX rats (Table 5). In contrast, both parameters in the HD-HP group were similar to those in the control group of sham rats, and were significantly higher in the OVX rats ($P < 0.05$). These parameters in the HD-HP groups were also higher than those in the IP or the LD-HP group. In both sham and OVX rats, fecal magnesium outputs in the IP and LD-HP groups were higher than those in the control and HD-HP groups.

As for the results of two-way ANOVA, both OVX and diet tended to affect net zinc absorption and the zinc absorption rate ($P < 0.1$). In the OVX rats, both variables of zinc absorption were higher in the HD-HP groups than in the IP groups (Table 6).

The cecal parameters shown in Table 7 were all influenced by OVX and diet ($P < 0.001$), with no significant interaction except for weight of contents. The wet weight of cecal wall (g/100 g body weight) was higher in sham and OVX rats fed three test fiber diets than in rats fed the control diet. The weights of cecal contents of the HD-HP groups were higher than those of the other three groups, but there was a significant interaction between OVX and diet ($P = 0.021$). The pH of the cecal content was lower in the HD-HP group than in the other three groups in both sham and OVX rats. There were not any changes in cecal parameters among the control, IP, and LD-HP groups.

Table 7. Weight of the Cecal Wall and Cecal Contents and pH of the Cecal Contents of Sham-operated and Ovariectomized Rats Fed the Control or Test Diets for 4 Weeks[1,2]

	Cecal wall wet g/100 g body weight	Cecal contents wet g/100 g body weight	pH
Sham			
Control	0.20 ± 0.02^{c}	0.76 ± 0.05^{c}	7.40 ± 0.03^{b}
IP	0.26 ± 0.01^{ab}	0.95 ± 0.10^{c}	7.49 ± 0.07^{b}
LD-HP	0.27 ± 0.01^{a}	1.03 ± 0.09^{c}	7.52 ± 0.08^{b}
HD-HP	0.30 ± 0.03^{a}	1.35 ± 0.11^{b}	6.64 ± 0.08^{c}
Ovariectomized			
Control	0.21 ± 0.01^{bc}	0.75 ± 0.04^{c}	7.69 ± 0.09^{ab}
IP	0.30 ± 0.01^{a}	1.43 ± 0.12^{b}	7.96 ± 0.05^{a}
LD-HP	0.31 ± 0.02^{a}	1.40 ± 0.12^{b}	7.62 ± 0.10^{b}
HD-HP	0.31 ± 0.02^{a}	1.94 ± 0.11^{a}	6.86 ± 0.22^{c}
ANOVA (P value):			
Operation	0.037	<0.001	<0.001
Diet	<0.001	<0.001	<0.001
Operation × Diet	0.663	0.021	0.404

[1]Each value represents mean ± standard error of the mean.
[2]Values in a column not sharing a superscript letter differ significantly, $P < 0.05$.
IP, Intact psyllium.
LD-HP, Low degree hydrolyzed psyllium.
HD-HP, High degree hydrolyzed psyllium.

Discussion

Partial hydrolysis of psyllium by acid treatment caused reductions in viscosity dependent on degree of hydrolysis. Ingestion of these lower viscous psyllium preparations clearly increased magnesium absorption compared to that for intact psyllium, and there were

similar tendencies in the cases of calcium and zinc absorption. It has been proposed that higher intraluminal viscosities in the small intestine cause reduced absorption of minerals.[12] But psyllium is a xylan polymer rich in hydroxyl and carboxyl groups, and these chemical structures participate in the ion-exchange process and have chelating properties.[30] These properties of psyllium might also be responsible for the increased fecal output and reduced mineral absorption in the IP and LD-HP groups. In contrast, ingestion of HD-HP increased net magnesium absorption and the magnesium absorption rate compared not only with IP and LD-HP but also with the control diet. Zinc absorption showed a similar tendency to magnesium absorption. These results strongly suggest that the viscous property of psyllium is a major factor in reduced mineral absorption.

It has been found that promotion of intestinal fermentation is a mechanism for a stimulatory effect of dietary fiber and oligosaccharides on calcium and magnesium absorption.[31–35] Two mechanisms have been proposed for this stimulatory effect: expansion of the lumen surface due to enlargement of the cecum and an increase in soluble mineral concentration in contents of the large intestine as a result of a decrease in lumen pH. The result shown in Table 3 showed that IP and LD-HP were poorly fermentable types of fibers because fecal dry weight was high in these two groups as well as in the cellulose control group. But fecal output in the HD-HP group was lower than in the control group in both sham and OVX rats, which indicates that high degree hydrolysis of psyllium might increase its fermentability. Lowering cecum pH also supported an increase in fermentability in HD-HP. The reduction of viscosity and increase in cecal fermentation might be associated with the higher magnesium absorption in the HD-HP group.

In the present study, we found that ovarian hormone deficiency impaired calcium absorption in OVX rats as compared to sham rats (Table 4). It has been reported that calcium balance is affected in OVX rats by increases in intestinal calcium secretion and malabsorption with decreasing levels of estradiol.[36] Reduced calcium absorption by OVX was not lowered by feeding of intact psyllium. The calcium absorption rate in OVX rats fed HD-HP was similar in level to that in sham rats. This result and the increase in magnesium absorption in OVX rats suggests that feeding of HD-HP is beneficial for bone impaired by OVX because magnesium is involved in bone strength as well as calcium.[37] This must be investigated in the future.

Zinc absorption was higher in the HD-HP group than in the intact psyllium group of OVX rats, but not higher than that of sham rats. The reason for the higher zinc absorption in OVX rats fed HD-HP is not known. Feeding patterns may have been different between the sham and the OVX rats because food intakes were somewhat restricted in the OVX rats to adjust mineral intakes to the levels of those of the sham rats. This difference might have affected zinc absorption.

In conclusion, partial hydrolysis of psyllium abolished impairment of mineral absorption by intact psyllium, and this modified fiber increased magnesium absorption. A reduction of viscosity and an increase in the cecal fermentability of psyllium may be involved in the improvement of mineral absorption.

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