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Dietary Fibers from Mushroom Sclerotia. 4. In Vivo Mineral Absorption Using Ovariectomized Rat Model

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The effect of three novel dietary fibers (DFs) prepared from mushroom sclerotia, namely, *Pleurotus tuber-regium*, *Polyporus rhinocerus*, and *Wolfiporia cocos*, on calcium and magnesium absorption was evaluated in ovariectomized (OVX) rats fed with sclerotial DF based and low Ca (0.3%) diets for 14 days. The animals in the *W. cocos* DF diet group possessed significantly ($p < 0.05$) higher levels of cecal total short-chain fatty acids (204 μmol/g of cecal content) and had an acidic pH (5.88) in their cecum when compared with those of the cellulose control group. Such an acidic environment was found to promote the ionization of the unabsorbed Ca and Mg in their cecum, which in turn significantly ($p < 0.05$) increased the concentrations of cecal soluble Ca (2.56-fold) and Mg (1.22-fold). Besides, the apparent Ca and Mg absorptions of the *W. cocos* DF group were also significantly ($p < 0.05$) enhanced (Ca, 16.5%; Mg, 15.3%) together with significantly ($p < 0.05$) higher serum Ca (3.61 mmol/L) and Mg (1.07 mmol/L) levels when compared with those of the cellulose control group. These data suggest that ingestion of *W. cocos* DF could improve the overall Ca and Mg absorptions of the OVX rats fed a low Ca diet. The potential use of sclerotial DFs as a functional food ingredient for enhancing mineral absorption is also discussed.

KEYWORDS: Calcium; magnesium; dietary fiber; mushroom sclerotia; ovariectomized rats

INTRODUCTION

Interest in dietary calcium has intensified in recent years as a result of scientific evidence linking it to osteoporosis (*1*). Apart from inadequate Ca intake, poor Ca absorption is another risk factor for osteoporosis (*2*). Previous studies showed that dietary fiber (DF), especially the insoluble fraction, could strongly bind to Ca and form unabsorbable complexes owing to its anionic nature (*3*). As a result, it has been proposed that DF might impair Ca absorption. However, during the past decade, substantial evidence has indicated that Ca absorption is not affected by fiber component per se (*4*, *5*). Besides, many recent studies have shown that fermentable DFs including oligosaccharides (e.g., fructo-oligosaccharides and inulin) as well as polysaccharides (e.g., resistant starch) even improved the overall Ca absorption in both humans (*6*, *7*) and rats (*8*, *9*).

According to Campbell (*10*), the beneficial effect of DF on overall Ca absorption depended on their fermentability and the dosage used as well as the duration of the animal experiments. Although the detailed mechanisms concerning the enhancing effect of the fermentable DF on overall Ca absorption remain unclear, it is widely accepted that microbial degradation of the fermentable DF in the large intestine would be the most important factor (*11*, *12*). Besides, the fermentation byproducts, short-chain fatty acids (SCFAs), were believed to be the major contributor (*13*, *14*) to increasing the concentration of ionized Ca and promoting its absorption in the large intestine (*15*, *16*). Apart from possessing desirable physicochemical and functional properties (*17*), our previous study (*18*) has found that three novel DFs prepared from mushroom sclerotia, namely, *Pleurotus tuber-regium* (Fr.) Sing., *Polyporus rhinocerus* Cooke, and *Wolfiporia cocos* (Schw.) Ryv. et Gilbn., had a remarkably low in vitro Ca binding capacity (4.79−5.91% binding) under a simulated physiological conditions of the small intestine (pH 6.8; 37 °C; ionic strength, 100 mM KCl) and that the bound Ca in the three sclerotial DFs were also readily released (percent released ranged from 34.2 to 72.3) at pH 5.8. Therefore, it would be valuable to find out whether the SCFAs generated from the fermentation of these three novel sclerotial DFs could lower the colonic pH to a similar acidic level and promote the Ca absorption in the large intestine in vivo.

The similarities found in the physiology between rats and humans in regard to Ca metabolism have made this animal an appropriate model for studying bone and Ca homeostasis as well as in the preclinical evaluation of pharmacological agents that may alter bone remodeling and Ca bioavailabilty (*19*). Ovariectomy performed on rats not only induces severe bone loss

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Table 1. Composition of the Experimental Diets (Grams per Kilogram of Diet)

ingredient	diet			
	control	*P. tuber-regium*	*P. rhinocerus*	*W. cocos*
casein	200	200	200	200
corn starch	150	150	150	150
sucrose	483.5	483.5	483.5	483.5
corn oil	50.0	50.0	50.0	50.0
mineral mixture[a]	35.0	35.0	35.0	35.0
$CaCO_3$	7.50	7.50	7.50	7.50
KH_2PO_4	9.00	9.00	9.00	9.00
vitamin mixture[b]	10.0	10.0	10.0	10.0
choline bitartrate	2.00	2.00	2.00	2.00
DL-methionine	3.00	3.00	3.00	3.00
cellulose	50.0			
P. tuber-regium DF		50.0		
P. rhinocerus DF			50.0	
W. cocos DF				50.0

[a] Modified AIN-76 mineral mixture without Ca and P. [b] AIN-76 vitamin mixture.

in the animals but also impairs their intestinal active Ca absorption (*20*, *21*). In the present study, an experimental design using young ovariectomized (OVX) rat model and low Ca diets was performed. Owing to the inhibition of active intestinal Ca absorption in OVX rats, the proposed enhancing effect of fermentable DF on Ca absorption in the large intestine of these treated animals could then be evaluated more specifically. Besides, the low Ca level (0.3%) used in all experimental diets [the minimum Ca level being that needed for a weanling rat to grow (*12*)], together with the high Ca requirement of the young growing rats, would make the effect of the three sclerotial DFs on Ca metabolism in the animals, if any, more easily observed. Furthermore, it had been reported previously that microbial degradation of the fermentable DFs (such as resistant starch, inulin, and fructo-oligosaccharides) promoted cecal absorption of both Ca and Mg in humans and rats (*6*, *9*, *11*). Therefore, parameters concerning the Mg absorption were also investigated in this study.

MATERIALS AND METHODS

Sample Preparation. Sclerotia of *P. tuber-regium*, *P. rhinocerus*, and *W. cocos* were obtained from the Sanming Mycological Institute in the Fujian Province of China. The sclerotia produced by the three species had a similar yield of ~50% biomass conversion (i.e., 2 kg of compost material produced ~1 kg of sclerotium). All sclerotia were cleaned, dried, and pulverized as previously described (*22*). DFs of the *P. tuber-regium*, *P. rhinocerus*, and *W. cocos* sclerotia were prepared according to a modified AOAC procedure using industrial enzymes as reported in our previous studies (*17*, *23*), and their yields were 81.7, 88.4, and 96.3 g/100 g of sample DM, respectively (*17*).

Diets. Four experimental diets (a cellulose-based control and three sclerotial DF based) were prepared according to the composition of the AIN-76 diet (*24*, *25*) with a reduction of Ca level from 0.50 to 0.30% in the mineral mixture (**Table 1**). The fiber source of the three sclerotial DF based diets came from their corresponding sclerotial DFs, whereas purified cellulose was used as the sole fiber source in the control group. Ca and Mg concentrations in all diets were measured by atomic absorption spectrophotometry (AAS) (Shimadzu AAS 640-13), described later (**Table 3**).

Animals and Experimental Design. Twenty-four 6-week-old female Sprague−Dawley rats with initial body weights of 130−150 g were obtained from Clea Japan (Tokyo, Japan). Animals were weighed when received and housed in individual stainless steel wire-mesh cages located in a temperature- and humidity-controlled room (22 ± 1 °C; 60−65% relative humidity) with a 12-h light/dark cycle (dark period from 8:00 p.m. to 8:00 a.m.). All rats were fed the original AIN-76 formulated diet containing 0.5% Ca (*24*, *25*) for 1 week before ovariectomy. At 7 weeks of age, all rats were bilaterally ovariectomized and were given a low-calcium control diet (0.3% Ca) for a total of 2 weeks to increase a demand for calcium before the metabolic period. After the phase of postoperative acclimatization, the OVX rats were randomly assigned into four subgroups ($n = 6$ per group) with similar mean body weights and housed individually in metabolic cages (day 1). Three groups of animals were fed the sclerotial DF based diets, and one group of rats was fed the cellulose control diet for a total of 14 days. During the feeding period, the body weight and food intake were recorded every 2 days. Feces samples collected daily for 4 days during the metabolic period were dried and ground into powder prior to subsequent mineral analysis. All OVX rats had free access to the test diets and distilled water throughout the experiment. The animals were maintained in accordance with the guidelines of the Tokyo University of Agriculture Animal Use Committee.

Serum Parathyroid Hormone (PTH), Ca, and Mg Concentrations. At the end of the experiment, the OVX rats were sacrificed by exsanguination after the dark period (between 8:00 and 10:00 a.m.), during which cecal fermentation was still very active (*9*). Blood samples were centrifuged (3000 rpm, 4 °C, 10 min) to separate serum, and serum Ca and Mg levels were determined in the presence of 0.1% LaCl by using an atomic absorption spectrophotometer (AAS, Shimadzu 640-13) according to the method of Gimblet et al. (*26*). The serum level of PTH was measured by a Rat Intact PTH ELISA Kit (Immutopics, Inc., San Clemente, CA).

Cecal and Fecal Ca and Mg Levels. After blood sampling, the whole cecum (including the undigested materials) of individual OVX rats was rapidly excised and weighed. The pH of cecal content was measured with a semiconducting electrode (ISFET pH sensor 0010-15C, Horiba Ltd., Kyoto, Japan), and samples of the cecal content were separately (~0.3 g each) weighed into three Eppendorf tubes. Two of the cecal content samples were then centrifuged (20000 rpm, 4 °C, 10 min), and the resulting supernatants were frozen (−20 °C) prior to analysis for their soluble Ca and Mg concentrations (i.e., the soluble Ca and Mg pool in the cecal supernatant) as well as the SCFA levels. The remaining cecal content sample was used to determine the amount of total Ca and Mg levels in the cecum (i.e., the total Ca or Mg pool in the cecal content). The empty cecum was flushed clean with cold 0.9% NaCl saline and weighed. The weight of cecal content was evaluated as the weight difference between the whole cecum (with undigested materials) and its corresponding cecal tissue. For mineral analysis, the cecal content and cecal supernatant samples were wet-ashed sequentially with nitric acid (65%) and hydrogen peroxide (30%) followed by appropriate dilution with 1 M HCl. The Ca and Mg concentrations in the solution were then measured by the AAS in the presence of 0.1% LaCl, like the serum samples, whereas the SCFAs including mainly acetic, propionic, and butyric acids in the other cecal supernatant sample were quantified by gas chromatography as previously described (*27*, *28*). The soluble Ca or Mg (%) in the cecum content was estimated by the following equation:

$$\text{soluble Ca or Mg in cecum (\%)} = \frac{\text{soluble Ca or Mg pool } (\mu\text{mol/g of cecal content})}{\text{total Ca or Mg pool } (\mu\text{mol/g of cecal content})} \times 100\% \quad \text{(I)}$$

The Ca and Mg contents of individual experimental diets were also determined in the same way as the cecal content using AAS to find out the actual Ca and Mg intake of the OVX rats. For determining fecal Ca and Mg contents, dried fecal powder (100 mg) was dry-ashed in a muffle furnace at 550 °C for 48 h followed by digestion and solubilization in 1 M HCl. After appropriate dilution with ultrapure water, the fecal Ca and Mg concentrations were measured by AAS as described earlier. Apparent absorption of Ca and Mg was calculated from the following equation:

$$\text{apparernt mineral absorption (\%)} = \frac{[\text{dietary mineral intake} - \text{fecal mineral excretion}]\ (\text{mg/rat/day})}{\text{dietary mineral intake (mg/rat/day)}} \times 100\% \quad \text{(II)}$$

Table 2. Food Intake, Body Weight Gain, and Food Efficiency of Ovariectomized Rats Fed the Four Experimental Diets[a]

diet group	food intake (g/rat/day)	body wt gain (g/rat/day)	food efficiency[b] (%)
control	16.7 ± 3.83	3.43 ± 1.08	20.5 ± 5.54
P. tuber-regium	18.1 ± 4.53	4.28 ± 0.89	23.6 ± 3.91
P. rhinocerus	20.0 ± 1.38	4.48 ± 0.59	22.4 ± 2.14
W. cocos	18.8 ± 1.86	4.80 ± 1.18	25.5 ± 6.51

[a] Data are mean values of six determinations ± SD. [b] Food efficiency = (body wt gain/food intake) × 100%.

Statistical Analysis. All data are presented as mean values of six determinations ± standard deviation (SD) and analyzed by nonparametric one-way ANOVA using the Kruskal−Wallis test ($p < 0.05$). When the ANOVA indicated a significant effect, multiple comparisons among the samples were also performed by the Mann−Whitney U test with Bonferroni correction ($p < 0.025$) to detect significant differences among groups. Bivariate correlation between variables was also determined nonparametrically using Spearman's test at $p < 0.05$ (*29*).

RESULTS AND DISCUSSION

Dietary Intake and Apparent Absorption of Ca and Mg. No adverse effects such as postoperative infection of the wound or diarrhea were observed in all OVX rats throughout the experiment. **Table 2** shows that the incorporation of the three sclerotial DFs into the diets did not significantly alter the food intake, body weight gain, and food efficiency of the sclerotial DF based diet groups when compared with those of the control group. Similar findings on other DFs such as resistant starch, inulin, and polydextrose had been reported previously (*9*, *30*). Because all experimental diets contained similar levels of Ca (3.16−3.27 mg/g) and Mg (0.48−0.53 mg/g), the Ca and Mg intake of all diet groups would be proportional to their corresponding food intake and did not differ significantly (**Table 3**). Among all diet groups, the *W. cocos* DF group exhibited the lowest ($p < 0.025$) fecal Ca and Mg excretion as well as the highest ($p < 0.025$) apparent Ca and Mg absorption (with 16.5 and 15.3% increases for Ca and Mg absorption, respectively) among all of the other diet groups including the control group (**Table 3**).

Cecal pH and SCFAs. The cecum of rat is the main site of degrading fermentable nondigestible carbohydrate, and the major product of cecal fermentation is the SCFAs (*9*, *12*). As shown in **Table 4**, all three sclerotial DF based diet groups possessed significantly ($p < 0.025$) higher molar concentrations of total SCFAs (ranged from 80.3 to 204 μmol/g of cecal content) in their cecum, with the *W. cocos* DF group being the highest ($p < 0.025$). However, only the cecal pH (∼5.88) and cecal content (1.15 g) of the *W. cocos* DF group were significantly ($p < 0.025$) lower than those of the cellulose control group (**Table 4**). These findings suggested that all three sclerotial DFs had higher fermentability than cellulose (*9*), but only that of the *W. cocos* DF was high enough to produce an amount of SCFAs sufficient to lower the cecal pH to an acidic level (*16*). For individual cecal SCFAs, fermentation of all three sclerotial DFs produced significantly ($p < 0.025$) higher concentration of acetate (ranging from 50.2 to 112 μmol/g of cecal content) and propionate (ranging from 12 to 51.6 μmol/g of cecal content) when compared with those of the cellulose group (**Table 4**). Although the *P. rhinocerus* DF group exhibited the lowest ($p < 0.025$) level of butyrate (10.3 μmol/g of cecal content), both *P. tuber-regium* and *W. cocos* DF groups possessed significantly ($p < 0.025$) higher levels of butyrate when compared with the control group (**Table 4**). It is worth noting that the *W. cocos* DF group

Table 3. Mineral Content in Diet as Well as Mineral Intake, Fecal Mineral Excretion, and Apparent Mineral Absorption of Ovariectomized Rats Fed the Four Experimental Diets[a]

	mineral content in diet (mg/g of diet)	mineral intake[b] (mg/rat/day)	fecal mineral excretion (mg/rat/day)	apparent mineral absorption[c] (%)
calcium				
control	3.16	52.8 ± 12.1	15.7 ± 2.55a	70.3 ± 4.32a
P. tuber-regium	3.25	58.8 ± 14.7	18.9 ± 3.28a	67.9 ± 7.16a
P. rhinocerus	3.20	64.0 ± 4.42	20.1 ± 2.90a	68.6 ± 6.65a
W. cocos	3.27	61.5 ± 6.08	11.1 ± 3.17b	81.9 ± 4.14b
magnesium				
control	0.50	8.27 ± 1.90	1.93 ± 0.51a	76.6 ± 4.06a
P. tuber-regium	0.53	9.54 ± 2.39	3.03 ± 0.77a	68.2 ± 6.98a
P. rhinocerus	0.48	9.56 ± 0.66	2.36 ± 0.31a	75.3 ± 5.46a
W. cocos	0.48	9.01 ± 0.89	1.05 ± 0.32b	88.3 ± 4.31b

[a] Except for mineral content in diet, data are mean values of six determinations ± SD. Means in columns with different letters (a, b) are significantly different (one-way ANOVA using Kruskal–Wallis test, $p < 0.05$; multiple comparisons using Mann–Whitney U test with Bonferroni correction, $p < 0.025$). [b] Mineral intake (mg/rat/day) = food intake in **Table 2** (g/rat/day) × mineral content in diet (mg/g). [c] Apparent mineral absorption (%) = [(mineral intake − fecal mineral excretion)/mineral intake] × 100%

Table 4. Cecal Short-Chain Fatty Acids and pH as Well as Weight of Cecal Tissue and Cecal Content of Ovariectomized Rats Fed the Four Experimental Diets[a]

diet group	total SCFAs[b] (μmol/g of cecal content)	acetic acid (μmol/g of cecal content)	propionic acid (μmol/g of cecal content)	buytric acid (μmol/g of cecal content)	cecal tissue (g)	cecal content (g)	cecal pH
control	55.7 ± 6.26a	33.4 ± 0.11a	8.88 ± 1.72a	13.4 ± 0.85a	0.82 ± 0.21a	2.07 ± 0.35a	7.25 ± 0.29a
P. tuber-regium	80.3 ± 0.70b	50.2 ± 1.42b	12.0 ± 0.55b	18.1 ± 0.02b	0.73 ± 0.24a	1.83 ± 0.25a	7.15 ± 0.43a
P. rhinocerus	82.1 ± 6.26b	50.9 ± 3.86b	20.9 ± 1.09c	10.3 ± 0.57c	0.73 ± 0.42a	1.85 ± 0.31a	6.99 ± 0.42a
W. cocos	202.6 ± 25.8c	112 ± 11.2c	51.6 ± 5.19d	39.0 ± 0.54d	1.25 ± 0.22b	1.15 ± 0.21b	5.88 ± 0.24b

[a] Data are mean values of six determinations ± SD. Means in columns with different letters (a–d) are significantly different (one-way ANOVA using Kruskal–Wallis test, $p < 0.05$; multiple comparisons using Mann–Whitney U test with Bonferroni correction, $p < 0.025$). [b] Total SCFAs = total short-chain fatty acids (acetate + propionate + butyrate).

Table 5. Total and Soluble Mineral Pool as Well as Soluble Mineral in the Cecum of Ovariectomized Rats Fed the Four Experimental Diets[a]

	total pool in cecal content (μmol/g of cecal content)	soluble pool in cecal supernatant (μmol/g of cecal content)	soluble mineral in cecum[b] (%)
calcium			
control	399 ± 51.5a	17.0 ± 1.09a	4.30 ± 0.28a
P. tuber-regium	492 ± 48.9b	25.1 ± 1.55b	5.11 ± 0.19b
P. rhinocerus	501 ± 67.9b	29.3 ± 2.71b	5.87 ± 0.25b
W. cocos	995 ± 151c	110 ± 14.3c	11.0 ± 0.23c
magnesium			
control	54.8 ± 1.45a	9.78 ± 0.64a	17.9 ± 0.69a
P. tuber-regium	91.7 ± 5.31b	15.6 ± 0.40b	17.0 ± 0.55a
P. rhinocerus	105 ± 2.30c	20.3 ± 2.04c	19.3 ± 1.52a
W. cocos	227 ± 28.6d	49.8 ± 5.90d	21.9 ± 0.17b

[a] Data are mean values of six determinations ± S. D. Means in columns with different letters (a–d) are significantly different (one-way ANOVA using Kruskal-Wallis Test, $p < 0.05$; Multiple comparisons using Mann–Whitney U Test with Bonferroni correction, $p < 0.025$). [b] Soluble mineral in cecum (%) = (Soluble pool in cecal supernatant/total pool in cecal content) × 100%

exhibited the highest ($p < 0.025$) molar concentration of all three individual SCFAs (**Table 4**).

Total and Soluble Mineral Pool in Cecum. It is shown in **Table 5** that both total and soluble Ca as well as Mg pool in the cecum of OVX rats fed the three sclerotial DF based diets were significantly ($p < 0.025$) higher than those of the control group, and the values of the *W. cocos* DF group were found to be the highest ($p < 0.025$). In both humans and rats, Ca and Mg are absorbed throughout the intestinal tract; however, their absorptions are not efficient [Ca, only 25−60% (*31*); Mg, only 35−70% (*32*)]. As a result, large amounts of dietary Ca and Mg would normally enter the cecum. In the case of OVX rats fed the *W. cocos* DF diet, a significant cecal enlargement (34.4% increase in weight of cecal tissue, $p < 0.025$, **Table 4**) was observed. This would further lead to accumulation of unabsorbed Ca and Mg in the cecum in addition to the impairment of active Ca absorption induced by ovariectomy. As a result, significantly ($p < 0.025$) higher levels of total Ca (2.39 times) and Mg (4.14 times) pool in the cecum of the *W. cocos* DF group were found relative to that of the cellulose control (**Table 5**). **Table 5** also shows that by comparison with the cecal soluble Ca and Mg of the control group, all three sclerotial DF based diet groups showed significantly ($p < 0.025$) higher soluble Ca in their cecum [1.19−2.56-fold, the *W. cocos* DF diet group being the highest ($p < 0.025$)], but only the *W. cocos* DF group exhibited a significantly ($p < 0.025$) higher level of cecal soluble Mg (1.22-fold).

As reported by numerous studies, SCFAs play a very important role in the enhancement of cecal mineral absorption when fermentable nondigestible carbohydrates are ingested (*9*, *14*, *16*) and the most widely accepted hypothesis for this enhancing effect is mainly via promoting ionization of unabsorbed minerals by the acidic environment generated by the SCFAs. In the present study, the significantly lower (acidic) cecal pH resulting from ingestion of the highly fermentable *W. cocos* DF was highly correlated with its notably higher level of total SCFA production ($r = -0.995$; $p < 0.05$; $n = 18$). Together with the significant cecal enlargement, this reduction on cecal pH also led to a remarkable augmentation of soluble Ca and Mg concentrations in the cecum ($r = -0.999$ and -0.939, respectively; $p < 0.05$; $n = 18$), which in turn promoted the apparent absorption (Ca, $r = 0.942$, and Mg, $r = 0.940$; $p < 0.05$; $n = 18$), probably by a passive diffusion mechanism via a paracellular pathway as suggested by other researchers (*9*, *16*). As reported in our previous study (*18*), this acidic cecal pH (5.88) would also promote the release of the *W. cocos* DF bound Ca (but not Mg), contributing to the soluble Ca pool in the cecum, which might also facilitate the cecal passive Ca absorption. Moreover, lowering of cecal pH in the *W. cocos* DF group was found to be strongly correlated with the amount of acetic ($r = -0.990$; $p < 0.05$; $n = 18$), propionic ($r = -0.995$; $p < 0.05$; $n = 18$), and buytric acids ($r = -0.933$; $p < 0.05$; $n = 18$) present in their cecum. Furthermore, the significantly ($p < 0.025$) higher molar concentration of butyric acid (**Table 4**) produced from the microbial breakdown of the *W. cocos* DF also played an essential role in the significantly ($p < 0.025$) higher weight of cecal tissue in the *W. cocos* DF diet group ($r = 0.949$; $p < 0.05$; $n = 18$). According to Levrat et al. (*33*) and Rémésy et al. (*34*), the significant cecal enlargement of the *W. cocos* DF group was due to a combined effect of both hypertrophy and hyperplasia (inducing thickening of cecal mucosa), which was attributed to the presence of SCFAs, especially the butyrate, acting as an energy source. Apart from an elevation of the weight of cecal tissue, the hypertrophy process would result in greater crypt column height and increase in cell number per crypt, thus leading to greater exchange surface area in the cecum (*34*). As a result, the absorption of cecal ionized Ca and Mg in the *W. cocos* DF group via the passive diffusion mechanism might be enhanced and the apparent absorption would then be improved. Similar observations in rats fed other fermentable DF (such as resistant starch, fructo-oligosacchardies, and inulin) have also been reported previously (*7*, *9*, *11*).

Another possible hypothesis for the role of the SCFAs is that they may contribute directly to the enhancement of Ca and Mg absorption via a cation exchange mechanism (*35*). SCFAs in a protonated form are absorbed across the apical membrane and undergo dissociation within the intracellular environment. These increased intracellular H^+ are then secreted from the cell into the lumen in exchange for Ca^{2+} or Mg^{2+}. Furthermore, Lutz et al. (*14*) and Trinidad et al. (*35*) proposed that SCFAs may even directly influence intestinal mineral absorption by forming a complex with less ionic charge [e.g., $(\text{calcium acetate})^+$], which could diffuse more readily across the cell membrane. Finally, SCFAs were reported to be responsible for the rise in cecal blood flow, thus increasing the overall cecal mineral absorption (*36*). On the basis of the aforesaid hypotheses, favorable concentration gradients for Ca and Mg absorption from luminal space toward the intestinal cell would likely be established in parallel with ingestion of the fermentable *W. cocos* DF diets.

Serum Ca, Mg, and PTH Levels. It is shown in **Table 6** that the improvement of apparent intestinal Ca and Mg absorption by the cecal fermentation of *W. cocos* DF accompanied not only a significant ($p < 0.025$) increase in both serum Ca (3.61 mmol/L) and Mg (1.07 mmol/L) concentrations

Table 6. Serum Ca, Mg, and Parathyroid Hormone (PTH) Concentrations of Ovariectomized Rats Fed the Four Experimental Diets[a]

diet group	serum Ca concn (mmol/L)	serum Mg concn (mmol/L)	serum PTH concn (pg/mL)
control	2.54 ± 0.20a	0.67 ± 0.11a	92.8 ± 11.8a
P. tuber-regium	2.73 ± 0.12a	0.77 ± 0.05a	71.2 ± 25.3ab
P. rhinocerus	2.62 ± 0.14a	0.79 ± 0.06a	89.8 ± 17.2a
W. cocos	3.61 ± 0.15b	1.07 ± 0.05b	57.1 ± 9.56b

[a] Data are mean values of six determinations ± SD. Means in columns with different letters (a, b) are significantly different (one-way ANOVA using Kruskal–Wallis test, $p < 0.05$; multiple comparisons using Mann–Whitney U test with Bonferroni correction, $p < 0.025$).

but also a significant ($p < 0.025$) reduction in serum PTH level (57.1 pg/mL).

Only the ionized Ca is physiologically important, and its concentration in serum is the target of its homeostatic control (*37*). To maintain homeostasis and supply the Ca requirements for the body, three processes, including Ca absorption by the intestine, reabsorption by the kidney, and resorption by the bone, are coordinately regulated by the two hormones, PTH and 1,25-$(OH)_2$D, to tightly control the serum ionized Ca concentration.

In the present study, all rats were made estrogen-deficient after the ovariectomy operation. Previous studies had shown that the absence of estrogen promoted bone resorption and caused significant bone loss (*20*, *38*). Kanis (*39*) postulated that the bone resorption induced by the estrogen deficiency might elevate serum Ca levels, which in turn decreased the secretion of PTH and synthesis of 1,25-$(OH)_2$D, thus causing intestinal malabsorption of Ca. However, estrogen deficiency had also been found to impair intestinal Ca absorption either by decreasing the gene expression of vitamin D_1 α-hydroxylase [fewer/no binding of estrogen receptor (ER) in the kidney (*40*)], which in turn inhibited the synthesis of 1,25-$(OH)_2$D (*41*), or by increasing endogenous Ca secretion back into the intestine (*42*). Moreover, some studies had demonstrated that estrogen deficiency might be even directly responsible for the malabsorption of Ca because functional ER was also found in the intestine (*43*). As a result, in response to the long-term low Ca intake, the compensatory Ca concentration (effected only from bone resorption induced by the PTH and estrogen deficiency as well as PTH mediated renal reabsorption) would not likely be able to maintain the Ca homeostasis in the present OVX rat model and resulted in hypersecretion of PTH accompanied by low serum Ca level. In this study, the ingestion of the highly fermented *W. cocos* DF significantly ($p < 0.025$) improved the apparent Ca absorption, which attenuated the combined impact of the estrogen deficiency plus low Ca diets when compared with the cellulose control group. Consequently, a notable ($p < 0.025$) increase in serum Ca level (**Table 6**) followed by a significant ($p < 0.025$) decrease in plasma PTH concentration was observed (**Table 6**).

In contrast to Ca, the importance of active and passive processes in the intestinal absorption of Mg is unclear (*44*). Nevertheless, many recent studies have reported that the passive paracellular transport of Mg was the predominant mechanism of intestinal Mg absorption in both humans and rats (*44*, *45*), whereas active Mg absorption was important only under conditions of extremely low dietary Mg intake (*44*). Mg absorption occurs throughout the intestinal tract, with higher efficiency being found in the distal part of the intestine (*45*). Compared with Ca, Mg is less tightly controlled, and the kidney is the organ that possibly closely regulates the Mg homeostasis (*46*). According to Breslau (*47*), within the physiological range of human serum Mg level (2.2−2.5 mM), the serum Mg concentration possesses an effect on PTH secretion similar to that of Ca but to a lesser extent. In the present study, the bone resorption induced by the ovariectomy plus the significant ($p < 0.025$) increase of Mg absorption in the cecum (**Table 3**) collectively resulted in an elevation of serum Mg level (*6*, *9*) in the *W. cocos* DF diet group (**Table 6**), which might also lead to a suppression of the PTH secretion as reported by others (*48*, *49*). As elucidated by Coudray and his co-investigators (*44*), the endogenous excretion of Mg was nearly directly proportional to the dietary Mg intake in rats. Therefore, in the present study, the similar dietary Mg intake among all diet groups (**Table 3**) would likely be accompanied by a similar endogenous Mg excretion. However, with an aim to maintain the serum Mg level within its physiological range, a remarkably high urinary Mg excretion would be expected (*46*, *50*), although this parameter was not determined in this study.

In conclusion, the ingestion of *W. cocos* DF significantly ($p < 0.025$) improved the apparent absorption of both Ca and Mg in the OVX rats (**Table 3**) together with a significant ($p < 0.025$) elevation of their serum Ca and Mg concentrations but suppression of their serum PTH levels (**Table 6**). Substitution of cellulose by the *W. cocos* DF led to a greater cecal fermentation and produced significantly ($p < 0.025$) higher amounts of total SCFAs that not only lowered the cecal pH to a slightly acidic level but also caused significant ($p < 0.025$) enlargement of the cecum (**Table 4**). As a result, remarkable ($p < 0.025$) augmentation of the concentration of cecal soluble Ca and Mg was observed (**Table 5**), and the apparent Ca and Mg absorption of the *W. cocos* DF group was also significantly ($p < 0.025$) enhanced. These findings illustrated that the detrimental effect induced by both ovariectomy and low Ca diet was alleviated by ingestion of the *W. cocos* DF, and its enhancing effect on Ca and Mg absorptions in the cecum of OVX rats would be of particular interest for populations with inefficient active Ca absorption such as the elderly and postmenopausal women. Because fermentability is the main factor determining the enhancing effect of a nondigestible carbohydrate on mineral absorption, to explore the three novel sclerotial DFs as functional food ingredients for enhancing overall Ca and Mg absorptions, their fermentability should be improved (*51*), probably by isolating their main DF component, β-glucan-rich polysaccharides, or even preparing some novel β-glucose-based oligosaccharides (GOS) from the three sclerotial DFs using partial acid or enzymatic hydrolysis. Further investigation on the possible beneficial effect of isolated β-glucan-rich polysaccharides and GOS on Ca and Mg absorptions in the OVX rats will be carried out.

ABBREVIATIONS USED

DF, dietary fiber; ER, estrogen receptor; GOS, glucose-based oligosaccharides; OVX, ovariectomized; PTH, parathyroid hormone; SCFAs, short-chain fatty acids; TDF, total dietary fiber.

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LITERATURE CITED

(1) Kennefick, S.; Cashman, K. D. Investigation of an *in vitro* model for predicting the effect of food components on calcium availability from meals. *Int. J. Food Sci. Nutr.* **2000**, *51*, 45−54.

(2) Rodriguez-Martinez M. A.; Garcia-Cohen E. C. Role of Ca^{2+} and vitamin D in the prevention and treatment of osteoporosis. *Pharmacol. Ther.* **2002**, *93*, 37−49.

(3) Platt, S. R.; Clydesdale, F. M Mineral binding characteristics of lignin, guar gum, cellulose, pectin and NDF under simulated duodenal pH conditions. *J. Food Sci.* **1987**, *52*, 1414−1419.

(4) Harrington, M. E.; Flynn, A.; Cashman, K. D. Effects of dietary fiber extracts on calcium absorption in the rat. *Food Chem.* **2001**, *73*, 263−269.

(5) Kennefick, S.; Cashman, K. D. Inhibitory effect of wheat fibre extract on calcium absorption in Caco-2 cells: evidence for a role of associated phytate rather than fiber *per sec*. *Eur. J. Nutr.* **2000**, *39*, 12−17.

(6) Coudray, C.; Bellanger, J.; Castiglia-Delavaud, C.; Rémésy, C.; Vermorel, M.; Ravssignuir, Y. Effect of soluble or partly soluble dietary fibers supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *Eur. J. Clin. Nutr.* **1997**, *51*, 375−380.

(7) Tahiri, H. M.; Tressol, J. C.; Arnaud, J.; Bornet, F.; Bouteloup-Demange, C.; Feillet-Coudray, C.; Ducros, V.; Repin, D.; Brouns, F.; Roussel, A. N.; Rayssiguier, Y.; Coudray, C. Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. *J. Bone Miner. Res.* **2001**, *16*, 2152−2160.

(8) Morohashi, T.; Sano, T.; Ohta, A.; Yamada, S. True calcium absorption in the intestine is enhanced by fructo-oligosaccharide feeding in rats. *J. Nutr.* **1998**, *128*, 1815−1818.

(9) Younes, H.; Coudrays, C.; Bellanger, J.; Demigné, C.; Rayssiguier, Y.; Rémésy, C. Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. *J. Nutr.* **2001**, *86*, 479−485.

(10) Campbell, J. M.; Fahey, G. C.; Wolf, B. W. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short chain fatty acids, pH, and microflora in rats. *J. Nutr.* **1997**, *127*, 130−136.

(11) Ohta, A.; Ohtuki, M.; Baba, S.; Adachi, T.; Sakata, T.; Sakaguchi, E. I. Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructo-oligosaccharides. *J. Nutr.* **1995**, *125*, 2417−2424.

(12) Shiga, K.; Hara, H.; Kasai, T. The large intestine compensates for insufficient calcium absorption in the small intestine in rats. *J. Nutr. Sci. Vitaminol.* **1998**, *44*, 737−744.

(13) Kishi, M.; Fukaya, M.; Tsukammoto, Y.; Nagasawa, T.; Takehana, K.; Nishizawa, N. Enhancing effect of dietary vinegar on the intestinal absorption of calcium in ovariectomized rats. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 905−910.

(14) Lutz, T. The effect of SCFA on Ca absorption by rat colon. *Exp. Physiol.* **1991**, *76*, 615−618.

(15) Mineo, H.; Hara, H.; Tomita, F. Short chain fatty acids enhance diffusional Ca transport in the epithelium of the rat cecum and colon. *Life Sci.* **2001**, *69*, 517−526.

(16) Younes, H.; Demigné, C.; Rémésy, C. Acidic fermentation in the caecum increases absorption of calcium and magnesium in the intestine of the rats. *Br. J. Nutr.* **1996**, *75*, 301−314.

(17) Wong, K. H.; Cheung, P. C. K. Dietary fibers from mushroom sclerotia: 1. Preparation and physico-chemical and functional properties. *J. Agric. Food Chem.* **2005**, *53*, 9395−9400.

(18) Wong, K. H.; Cheung, P. C. K. Dietary fibers from mushroom sclerotia: 2. In vitro mineral binding capacity under sequential simulated physiological conditions of the human gastrointestinal tract. *J. Agric. Food Chem.* **2005**, *53*, 9401−9406.

(19) Walter, F. The rat. In *The Clinical Chemistry of Laboratory Animals*, 2nd ed.; Walter, F. L., Fred, W. Q., Eds.; Taylor and Francis: Philadelphia, PA, 1999; pp 33−48.

(20) Wronski, T. J.; Dann, L. M.; Scott, K. S.; Cintron, M. Long-term effects of ovariectomy and aging on the rat skeleton. *Calcif. Tissue Int.* **1989**, *45*, 360−366.

(21) Bolscher, M. T.; Netelenbos, J. C.; Barto, R.; Van Buuren, L. M.; Van der Vijgh, W. J. Estrogen regulation of intestinal calcium absorption in the intact and ovariectomized adult rats *J. Bone Miner. Res.* **1999**, *14*, 1197−1202.

(22) Wong, K. H.; Cheung, P. C. K.; Wu, J. Z. Biochemical and microstructural characteristics of insoluble and soluble dietary fiber prepared from mushroom sclerotia of *Pleurotus tuber-regium*, *Polyporus rhinocerus* and *Wolfiporia cocos. J. Agric. Food Chem.* **2003**, *51*, 7197−7202.

(23) Wong, K. H.; Cheung, P. C. K. Preparation of non-digestible carbohydrates from mushroom sclerotia using industrial enzymes. In *Abstract of Vahouny−ILSI Japan International Symposium on Non-digestible Carbohydrate*; Vahouny−ILSI Japan: Tokyo, Japan, 2004; p P-21.

(24) American Institute of Nutrition. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. *J. Nutr.* **1977**, *107*, 1340−1348.

(25) American Institute of Nutrition. Second report of the ad hoc committee on standards for nutritional studies. *J. Nutr.* **1980**, *110*, 1726.

(26) Gimblet, E. G.; Marney, A. F.; Bonsnes, R. W. Determination of calcium and magnesium in serum, urine, diet and stool by atomic absorption spectrophotometry. *Clin. Chem.* **1967**, *13*, 204−214.

(27) Cheung, P. C. K.; Wong, K. H. Non-digestible carbohydrates from mushroom sclerotia: Relationship of fermentability and chemical structure. In *Abstract of Vahouny−ILSI Japan International Symposium on Non-digestible Carbohydrate*; Vahouny−ILSI Japan: Tokyo, Japan, 2004; p P-20.

(28) Wong, K. H.; Wong, K. Y.; Kwan, H. S.; Cheung, P. C. K. Dietary fibers from mushroom sclerotia: 3. In vitro fermentability using human fecal microflora. *J. Agric. Food Chem.* **2005**, *53*, 9407−9412.

(29) SPSS Inc. *SPSS Base 9.0 Application Guide*; SPSS Inc.: Chicago, IL, 1999.

(30) Hara, H.; Suzuki, T.; Aoyama, Y. Ingestion of the soluble dietary fiber, polydextrose, increases calcium absorption and bone mineralization in normal and total-gastrectomized rats. *Br. J. Nutr.* **2000**, *84*, 655−661.

(31) Wood, R. J. Calcium and phosphorus. In *Biochemical and Physiological Aspects of Human Nutrition*; Stipanuk, M. H., Ed.; Saunders: Philadelphia, PA, 2000; pp 643−670.

(32) Coudray, C.; Demigné, C.; Rayssiguier, Y. Effects of dietary fiber on magnesium absorption in animals and humans. *J. Nutr.* **2003**, *133*, 1−4.

(33) Levrat, M. A.; Behr, S. R.; Rémésy, C.; Demigné, C. Effects of soybean fiber on cecal digestion in rats previously adapted to a fiber-free diet. *J. Nutr.* **1991**, *121*, 672−678.

(34) Rémésy, C.; Behr, S. R.; Levrat, M. A.; Demigné, C. Fiber fermentation in the cecum and its physiological consequences. *Nutr. Res.* **1992**, *12*, 1235−1244.

(35) Trinidad, T. P.; Wolever, T. M.; Thomposon, L. U. Effects of calcium concentration, acetate, and propionate on calcium absorption in the distal colon. *Nutrition* **1999**, *15*, 529−533.

(36) Demigné, C.; Levrat, M. A.; Rémésy, C. Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between cecum and blood plasma in the rats. *J. Nutr.* **1989**, *119*, 1625−1630.

(37) Norman, A. W.; Litwack, G. *Hormones*, 2nd ed.; Academic Press: San Diego, CA, 1997; pp 251−280.

(38) Frost, H. M. On the estrogen−bone relationship and postmenopausal bone loss: a new model. *J. Bone Miner.* Res. **1999**, *14*, 1473−1477.

(39) Kanis, J. A. *Osteoporosis*; Blackwell Science: London, U.K., 1994.

(40) Bojar, H.; Dreyfurst, R.; Maar, K.; Stab, W. Distribution of oestradiol receptor molecules in human kidney. *J. Clin. Chem. Clin. Biochem.* **1977**, *15*, 319−321.

(41) Gallagher, J. C.; Riggs, B. L.; DeLuca, H. F. Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. *J. Clin. Endocrinol. Metab.* **1980**, *51*, 1359−1364.
(42) Draper, C. R.; Dick, I. M.; Prince, R. L. The effect of estrogen deficiency on calcium balance in mature rats. *Calcif. Tissue Int.* **1999**, *64*, 325−328.
(43) Arjmandi, B. H.; Salih, M. A.; Herbert, D. C.; Sims, S. H.; Kalu, D. N. Evidence for estrogen receptor-linked calcium transport in the intestine. *Bone Miner.* **1993**, *21*, 63−74.
(44) Coudray, C.; Coudray, C. F.; Grizard, D.; Tressol, J. C.; Gueux, E.; Ravssignuir, Y. Fractional intestinal absorption of magnesium is directly proportional to dietary magnesium intake in rats. *J. Nutr.* **2002**, *132*, 2043−2047.
(45) Schweigel, M.; Martens, H. Magnesium transport in the gastrointestinal tract. *Front. Biosci*. **2000**, *1*, 666−667.
(46) Quamme, G. A.; de Rouffignac, C. Epithelial magnesium transport and regulation by the kidney. *Front. Biosci.* **2000**, *1*, 694−711.
(47) Breslau, N. A. Calcium homeostatsis. In *Textbook of Endocrine Physiology*, 4th ed.; Griffin, J. E., Ojeda, S. R., Eds.; Oxford University Press: New York, 2000; pp 357−392.
(48) Toba, Y.; Kajita, Y.; Masuyama, R.; Takada, Y.; Suzuki, K.; Aoe, S. Dietary magnesium supplementation affects bone metabolism and dynamic strength of bone in ovariectomized rats. *J. Nutr.* **2000**, *130*, 216−220.
(49) Massrt, S. G.; Coburn, J. W.; Kleeman, C. R. Evidence for suppression of parathyroid gland activity by hypermagnesemia. *J. Clin. Invest.* **1970**, *49*, 1619−1629.
(50) Toba, Y.; Masuyama, R.; Kato, K.; Takada, Y.; Aoe, S.; Suzuki, K. Effects of dietary magnesium level on calcium absorption in growing rats. *Nutr. Res.* **1999**, *19*, 783−793.
(51) Cashman, K. Prebiotics and calcium bioavailability. *Curr. Issues Intest. Microbiol.* **2003**, *4*, 21−32.

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