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Ingestion of Water-Soluble Soybean Fiber Prevents Osteopenia and Hypercholesterolemia Induced by Ovariectomy in Rats

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This study examined the effects of feeding water-soluble soybean fiber (WSSF), a highly fermentable dietary fiber with low viscosity, on intestinal calcium (Ca) absorption, bone mineral content, and serum total cholesterol levels using female Sprague−Dawley rats (5 weeks old) with or without ovariectomy (OVX). The rats in each group were fed a WSSF (50 g/kg of diet) or WSSF-free diet for 4 weeks. Ca absorption was higher in OVX rats fed the WSSF diet than in OVX rats fed the WSSF-free diet. Femoral Ca content in both sham and OVX rats fed the WSSF diet was higher than that in rats fed the WSSF-free diet. The serum total cholesterol levels were elevated after OVX compared with that in the sham rats, and this elevation was prevented by the WSSF diet. In conclusion, ingestion of WSSF prevented osteopenia and hypercholesterolemia induced by ovariectomy in rats.

KEYWORDS: Water-soluble soybean fiber; osteopenia; hypercholesterolemia; ovariectomy; rats

INTRODUCTION

Ovarian hormone deficiency is involved in osteoporosis and coronary heart disease (CHD) in aged women and animals (*1*−*4*). Some studies have shown that ovarian hormone deficiency impairs intestinal calcium (Ca) absorption and decreases bone mineral density in postmenopausal or oophorectomized women (*5*, *6*), and other studies have reported that estrogen deficiency induced increases in serum total cholesterol levels, which have been implicated as a major risk factor for CHD (*2*, *7*). Ovariectomized (OVX) rats are a widely used model for postmenopausal osteoporosis and CHD, because OVX induces intestinal Ca malabsorption, loss of bone mineral (*8*−*11*), and hypercholesterolemia (*12*, *13*).

Many studies have reported that dietary fiber improves lipid metabolism and mineral absorption in rats (*14*−*16*). Dietary fiber has been classified into two fractions, that is, soluble-viscous-fermentable fiber (soluble) and insoluble-less fermentable fiber (insoluble). It is generally accepted that the viscous water-soluble fraction is beneficial in lowering serum cholesterol concentrations (*17*). Soluble fibers are usually fermented by colonic microorganisms and produce short-chain fatty acids (SCFA), which are associated with the cholesterol-lowering effect (*16*, *18*). Furthermore, several studies have indicated that the ingestion of soluble dietary fiber increases Ca absorption in rats and that the large intestine was involved in this beneficial effect (*19*, *20*). Microbial fermentation in the large intestine has been proposed as a mechanism responsible for the increase in Ca absorption (*21*, *22*).

It is known that soybean consumption improves several aspects of postmenopausal health such as loss of bone and hypercholesterolemia (*23*−*25*). Soybean and soybean products are popular foods in Japan, and the lower incidence of osteoporosis in Japanese women may be attributable to the high consumption of soybean products (*26*, *27*). Although numerous studies have shown that soybean contains many compounds, such as isoflavones and soy protein, that have a positive effect on bone metabolism and CHD (*23*−*27*), little is known about the benefits of soybean fiber. Water-soluble soybean fiber (WSSF; SOYAFIBE-S, Fuji Oil Co. Ltd., Tokyo, Japan) is produced as a byproduct of soybean oil. This fiber contains many galacturonic acids in the core chain structure and has high fermentability and low viscosity. We previously reported that WSSF lowered serum and liver cholesterol levels in normal rats (*28*) and that WSSF improved gastrectomy-induced Ca malabsorption and osteopenia in rats (*29*). However, it has not been clarified whether WSSF improves ovariectomy-induced Ca malabsorption, bone loss, and hypercholesterolemia in rats.

The aims of this study were to examine the effects of feeding WSSF on Ca absorption, bone mineral contents, and serum cholesterol levels in OVX rats.

MATERIALS AND METHODS

Material. We obtained WSSF from Fuji Oil Co. (Osaka, Japan). As reported previously, the WSSF was extracted from defatted soybeans under the following conditions: pH 5 and 120 °C for 1.5 h (*30*). The composition of WSSF is 2.9% moisture, 6.8% crude protein, 4.8% ash,

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Table 1. Composition of Stock and Experiment Diets[a]

component	amount (g/kg of diet)
casein[b]	250
corn oil	50
mineral mixture[c]	35
vitamin mixture[d]	10
choline bitartrate	2.5
cellulose[e]	50
sucrose	to make 1 kg

[a] Water-soluble soybean fiber (50 g/kg of diet, Fuji Oil Co. Ltd., Tokyo, Japan) was added to 95% of the above diet. [b] New Zealand Dairy Board, Wellington, New Zealand. [c] AIN-93G mixture except for Ca. It provided the following (mg/kg of diet): Ca, 2000; P, 1561; K, 3600; S, 300; Na, 1019; Cl, 1571; Mg, 507; Fe, 35.0; Zn, 30.0; Mn, 10.0; Cu, 6.0; I, 0.2; Mo, 0.15; Se, 0.15; Si, 5.0; Cr, 1.0; F, 1.0; Ni, 0.5; B, 0.5; Li, 0.1; V, 0.1. [d] AIN-93 mixture. It provided the following (units/kg of diet): nicotinic acid, 30 mg; pantothenate, 15 mg; pyridoxine, 6 mg; thiamin, 5 mg; riboflavin, 6 mg; folic acid, 2 mg; vitamin K, 750 μg; D-biotin, 200 μg; vitamin B_{12}, 25 μg; vitamin A, 4000 IU; vitamin D_3, 1000 IU; vitamin E, 75 IU. [e] Asahi Chemical Industry Co. Ltd., Tokyo, Japan.

and 75.1% soluble dietary fiber (*28*). This WSSF is a polysaccharide consisting mainly of galactose, arabinose, and galacturonic acids, and the average molecular weight is ~500000.

Animals and Diets. Female Sprague−Dawley rats (5 weeks old; Japan Clea, Tokyo, Japan) weighing ~100 g each were housed in individual stainless steel cages with wire-mesh bottoms. The cages were placed in a room with controlled temperature (22−24 °C), relative humidity (40−60%), and lighting (light from 8:00 a.m. to 8:00 p.m.). The rats had free access to water and the semipurified stock diet shown in **Table 1** for an acclimation period of 7 days. The rats were divided into two groups; one group of rats underwent bilateral ovariectomy (OVX), and the other group underwent bilateral laparotomy (sham). All rats had free access to deionized water and the stock diet for 5 days to recover from surgical damage.

After the recovery period, the rats in each group were divided into two subgroups of 10 rats and then given either of two experimental diets, the control or WSSF (50 g/kg of diet) diet shown in **Table 1**, for 4 weeks. The mineral and vitamin mixture of the experiment diets was prepared in accordance with the AIN-93 (*31*) except for Ca. In both experimental diets the Ca level was 2.0 g of Ca/kg of diet. To prevent hyperphagia associated with ovariectomy, the OVX rats were given the average amount of each diet ingested by sham rats during the experimental period.

Body weight and food intake were measured every day. Feces were collected from day 13 to day 15 (2 weeks) or from day 25 to day 27 (4 weeks) after feeding of the experiment diets. At the end of the experiment, the rats were anesthetized (Nembutal: sodium pentobarbital, 50 mg/kg of body weight, Abbott Laboratories, North Chicago, IL) and then killed after aortic blood was taken. Blood was centrifuged (1300*g* for 10 min at 4 °C) to obtain the serum. The uterus was removed from each rat and weighed to confirm the success of the ovariectomy. The cecum was removed with its contents and weighed. The contents were collected and stored at −40 °C until subsequent analyses. The femur was removed from each rat, carefully cleaned of adherent tissue, and freeze-dried for measurement of mineral contents.

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

Analyses. Ca concentrations in the experimental diets, feces, cecal contents, and femurs were measured by atomic absorption spectrophotometry (AA-6400F; Shimadzu Corp., Kyoto, Japan) after wet-ashing with an acid mixture (16 mol/L HNO_3: 9 mol/L $HClO_4$ = 3:1) without drying. Phosphate (P) was determined in the femoral solutions by using the molybdovanadate method (*32*). The cecal contents were homogenized with 9 volumes of deionized water. The total Ca concentration in the homogenate was measured according to the same method as that of other samples. The supernatant was obtained from the homogenate by centrifugation at 30000*g* for 20 min at 4 °C to evaluate the soluble Ca concentration in the cecal contents. The pH values of these homogenates were measured with a semiconducting electrode (ISFET pH sensor 0010-15C, Horiba, Ltd., Kyoto, Japan). The organic acids were measured by high-performance liquid chromatography (organic acid analysis system, Shimadzu Corp.) as previously described (*33*).

Serum total and HDL cholesterol levels were assayed using an enzymatic method by commercial kits (cholesterol-C Test Wako and HDL-cholesterol Test Wako, Wako Pure Chemical Industries, Osaka, Japan). The LDL + VLDL cholesterol level was calculated by subtracting HDL from total cholesterol.

Calculations and Statistical Analyses. Apparent Ca absorption rate was calculated using the following equation:

$$\text{Ca absorption rate (\%)} = 100 - (\text{total Ca intake} - \text{Ca excretion in feces})/\text{total Ca intake}$$

Values shown represent the means ± SEM. Statistical analyses were performed by two-way ANOVA (treatment − diet). Duncan's multiple-range test (*34*) was used to determine whether mean values were significantly different between groups ($P < 0.05$). All statistical analyses were done using SPSS for Windows, version 10.0 J (SPSS, Chicago, IL).

RESULTS

The mean final body weight was higher in OVX rats than in sham-operated rats with no differences in food intake between the groups (**Table 2**). The uterine weights of all OVX rats (36 mg/100 g of body weight) were much lower than the average uterine weight of the sham rats (188 mg/100 g of body weight), indicating the success of the surgical procedure in all rats in the OVX group (**Table 2**).

The absorption rate of Ca was lower in the OVX rats than in the sham rats (**Figure 1**). During the 2 weeks after the start of test feeding (**Figure 1A**), the absorption rate of Ca in OVX rats fed the WSSF diet was higher than that in rats fed the control diet. Moreover, the Ca absorption rate in OVX rats fed the WSSF diet was very similar to that in the sham rats. As for the 4 weeks after feeding (**Figure 1B**), the absorption rates of Ca in the WSSF groups were higher than those in the control groups in both OVX and sham rats.

The femoral Ca content was higher in the WSSF groups than in the control groups of both sham and OVX rats (**Table 3**). The femoral Ca contents in the WSSF groups were 15 and 20% higher than those in the control group in sham and OVX rats,

Table 2. Final Body Weight, Food Intake, and Uterine Weight of Sham and Ovariectomized Rats Fed the Control Diet or Water-Soluble Soybean Fiber Diet for 4 Weeks[a]

	sham		OVX		ANOVA (*P* values)[b]		
	control	WSSF	control	WSSF	treatment (T)	diet (D)	T − D
final body wt (g)	269 ± 8.3 b	276 ± 10.6 b	296 ± 3.2 a	300 ± 2.7 a	<0.001	NS[c]	NS
food intake (g/day)	18.5 ± 0.52	19.4 ± 0.50	18.9 ± 0.02	18.8 ± 0.05	NS	NS	NS
uterine wt (mg/100 g of body wt)	191 ± 23.7 a	184 ± 16.4 a	31.5 ± 2.35 b	40.4 ± 3.57 b	<0.001	NS	NS

[a] Values are means ±SEM for 10 rats. Values in a row not sharing a common letter differ significantly, $P < 0.05$, by Duncan's multiple-range test. [b] Significant as determined by two-way ANOVA. [c] NS, not significant, $P > 0.05$.

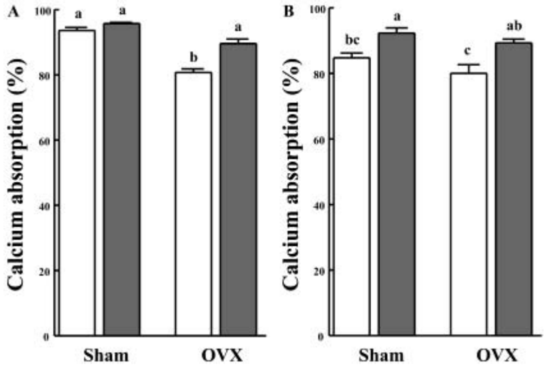

Figure 1. Apparent Ca absorption rate in sham and ovariectomized rats fed the control (□) or water-soluble soybean fiber (■) diet at 2 weeks (A) or 4 weeks (B) after the start of the experimental period. Values are means ± SEM, $n = 10$. Means not sharing a common letter differ, $P < 0.05$.

respectively. The amounts of Mg and P in the femur in OVX rats were lower than those in sham rats. The femoral Mg and P contents in rats fed the WSSF diet tended to be higher, although not significantly, than those in rats fed the control diet.

In both the sham and OVX rats, the cecal wall and contents weight in the WSSF groups were higher than those in the control groups (**Table 4**). Cecal pH values in rats fed WSSF diet were lower than those in rats fed the control diet. Ovariectomy did not influence these cecal parameters. The total Ca pool in the cecal contents of OVX rats was much higher than that of sham rats, especially in rats fed the control diet. In the OVX rats, the soluble Ca pool and solubility of the WSSF diet group were higher than those of the control group. Concentrations of SCFA and other organic acids in the cecal contents are shown in **Table 5**. Concentrations of acetic acid, propionic acid, and total SCFA (the sum of acetic, propionic, and butyric acids) in the cecal contents were higher in rats fed the WSSF diet than in rats fed the control diet in the case of OVX rats but not in sham rats. The concentrations of succinic and lactic acids did not differ among groups.

The serum total and HDL cholesterol levels of the OVX rats fed the control diet were higher than those of the sham rats (**Figure 2**). The LDL + VLDL cholesterol level was also higher, although not significantly, in the OVX rats fed the control diet compared with sham rats. The serum total and HDL cholesterol levels of OVX rats fed the WSSF diet were lower than those of OVX rats fed the control diet, and these cholesterol levels were almost same as that of sham rats. In the sham rats, there were no differences in serum cholesterol levels between the WSSF and control groups.

DISCUSSION

In the present study, we demonstrated that ingestion of WSSF, a highly fermentable fiber with low viscosity, increased the femoral Ca contents in OVX rats (**Table 3**). We also showed that the WSSF diet increased Ca absorption that had been previously reduced due to estrogen deficiency with a low-Ca diet (**Figure 1**). We adopted a 2.0 g of Ca/kg of diet feeding rate, which is a moderate restriction level in rats (*35*). Ovariectomy has been shown to induce osteopenia with an increase in bone turnover dominating bone resorption (*36*, *37*) and impairment of intestinal Ca absorption (*38*). Low dietary Ca levels also reduce femoral Ca contents (*35*). Our finding, in which bone mineralization was increased by WSSF, shows that the soluble fiber component in soybean may be effective in the prevention of osteoporosis after menopause.

The observed decrease in Ca absorption caused by ovariectomy (**Figure 1**) agrees with the results of previous studies (*9*, *38*). The total Ca pool in the cecum increased more in OVX rats than in sham rats (**Table 4**), which suggests an increase in nonabsorbed Ca in the small intestine and that OVX impaired Ca absorption in the small intestine. The ingestion of WSSF improved OVX-induced Ca malabsorption in rats (**Figure 1**). We previously reported that feeding WSSF increased Ca absorption in the large intestine in gastrectomized rats (*29*). In the case of gastrectomized rats, the improvement in Ca absorption as the result of feeding WSSF may be associated with cecal fermentation. In the present study, we also showed that feeding the WSSF diet led to a lower cecal pH, a greater pool of soluble Ca in the cecal contents (**Table 4**), and increased concentrations of total SCFA, particularly acetate and propionate (**Table 5**), in rats. These results suggest that feeding WSSF produces a decrease in pH through the production of organic acids through cecal fermentation and that this acidification of the cecal contents induces the solubilization of Ca. Moreover, SCFA is the promotive factor for Ca absorption in the large intestine (*22*). An increase in Ca absorption from the distal colon by acetate and propionate has been reported in humans (*21*). The present results also suggest that increases in acetate and propionate in the large intestine are responsible for the enhancement of Ca absorption associated with WSSF feeding.

Ovarian hormone deficiency induces hypercholesterolemia, which has been implicated as a major risk factor for CHD (*2*−*4*). In this study, we showed that OVX induced increases in serum cholesterol levels and that this elevation in serum cholesterol was prevented by the WSSF diet (**Figure 2**). LDL + VLDL cholesterol levels were decreased in OVX rats fed the WSSF diet, although not significantly. It has been reported that the increase in serum total cholesterol concentrations due to ovariectomy was attributed to a rise in non-HDL cholesterol fractions (*3*). It would be helpful to decrease the risk for CHD. Dietary fiber, particularly viscous dietary fiber, has been shown to be effective in reducing serum cholesterol levels in rats (*16*, *17*). However, WSSF has low viscosity. In previous reports, we demonstrated that fermentation products (SCFA) lower plasma cholesterol concentrations (*18*) and that SCFA suppresses the hepatic cholesterol synthesis rate in rats (*39*). In this study,

Table 3. Femoral Mineral (Ca, Mg, and P) Contents in Sham and Ovariectomized Rats Fed the Control Diet or Water-Soluble Soybean Fiber Diet for 4 Weeks[a]

	sham		OVX		ANOVA (P values)[b]		
	control	WSSF	control	WSSF	treatment (T)	diet (D)	T – D
Ca (mmol/femur)	2.11 ± 0.045 b	2.42 ± 0.114 a	2.00 ± 0.050 b	2.41 ± 0.042 a	NS[c]	<0.001	NS
Mg (μmol/femur)	69.5 ± 3.00 ab	72.4 ± 1.89 a	65.4 ± 1.69 b	67.1 ± 1.11 ab	0.020	NS	NS
P (mmol/femur)	1.47 ± 0.063 ab	1.60 ± 0.050 a	1.33 ± 0.052 b	1.45 ± 0.036 ab	<0.001	NS	NS

[a] Values are means ± SEM for 10 rats. Values in a row not sharing a common letter differ significantly, $P < 0.05$, by Duncan's multiple-range test. [b] Significant as determined by two-way ANOVA [c] NS, not significant, $P > 0.05$.

Table 4. Cecal Wall Weight, Contents Weight, pH, Total Calcium Pool, and Soluble Calcium Pool in Sham and Ovariectomized Rats Fed the Control Diet or Water-Soluble Soybean Fiber Diet for 4 Weeks[a]

	sham		OVX		ANOVA (*P* values)[b]		
	control	WSSF	control	SSF	treatment (T)	diet (D)	T – D
wall (g of wet wt)	2.03 ± 0.12 b	3.07 ± 0.16 a	1.97 ± 0.11 b	3.35 ± 0.18 a	NS[c]	<0.001	NS
contents (g of wet wt)	1.79 ± 0.177 b	3.63 ± 0.689 a	2.29 ± 0.162 b	4.04 ± 0.391 a	NS	<0.001	NS
pH	7.19 ± 0.089 a	6.62 ± 0.084 b	7.19 ± 0.130 a	6.32 ± 0.078 b	NS	<0.001	NS
total Ca pool (μmol)	49.9 ± 4.79 b	58.3 ± 11.5 ab	89.3 ± 19.2 a	72.6 ± 5.56 ab	0.023	NS	NS
soluble Ca pool (μmol)	5.61 ± 0.35 b	9.28 ± 1.05 b	6.46 ± 0.44 b	14.6 ± 2.83 a	0.050	0.001	NS
soluble Ca (%)	11.9 ± 1.04 b	19.4 ± 2.81 a	9.5 ± 1.59 b	19.8 ± 3.14 a	NS	0.001	NS

[a] Values are means ± SEM for 10 rats. Values in a row not sharing a common letter differ significantly, $P < 0.05$, by Duncan's multiple-range test. [b] Significant as determined by two-way ANOVA. [c] NS, not significant, $P > 0.05$.

Table 5. Concentrations of Short-Chain Fatty Acids and Other Organic Acids (Micromoles per Gram of Contents) in the Cecal Contents of Sham and Ovariectomized Rats Fed the Control Diet or Water-Soluble Soybean Fiber Diet for 4 Weeks[a]

	sham		OVX		ANOVA (*P* values)[b]		
	control	WSSF	control	WSSF	treatment (T)	diet (D)	T – D
acetate	23.2 ± 3.28 b	27.0 ± 1.37 ab	24.1 ± 2.12 b	34.8 ± 3.32 a	NS[c]	0.044	NS
propionate	5.28 ± 1.34 b	10.8 ± 0.53 b	7.25 ± 1.27 b	17.5 ± 2.43 a	0.038	0.001	NS
butyrate	4.32 ± 0.96	3.08 ± 0.48	4.59 ± 0.62	4.92 ± 0.63	NS	NS	NS
total SCFA[d]	32.8± 4.29 b	40.9 ± 1.68 b	35.9 ± 3.13 b	57.3 ± 5.23 a	NS	0.013	NS
succinate	2.35 ± 0.75	3.13 ± 3.03	3.68 ± 1.83	0.68 ± 0.25	NS	NS	NS
lactate	2.53 ± 0.74	0.86 ± 0.19	3.13 ± 1.22	1.61 ± 1.01	NS	NS	NS

[a] Values are means ± SEM for 10 rats. Values in a row not sharing a common letter differ significantly, $P < 0.05$, by Duncan's multiple-range test. [b] Significant as determined by two-way ANOVA. [c] NS, not significant, $P > 0.05$. [d] Total SCFA is the sum of acetic, propionic, and butyric acids.

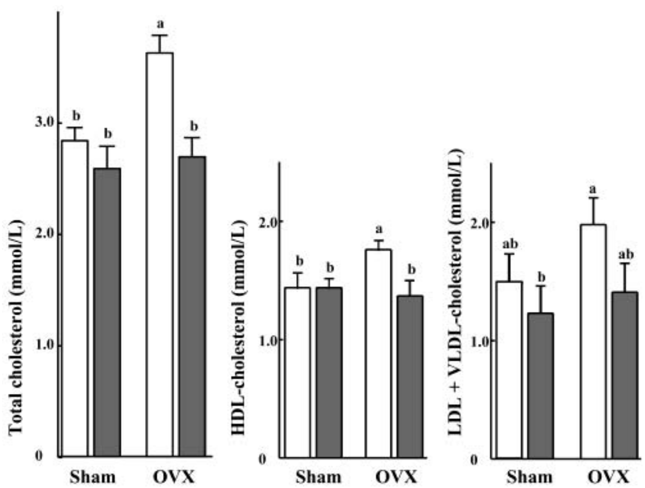

Figure 2. Serum total, HDL, and LDL + VLDL cholesterol levels in sham and ovariectomized rats fed the control (□) or water-soluble soybean fiber (■) diet at 4 weeks after the start of the experimental period. Values are means ± SEM, $n = 10$. Means not sharing a common letter differ, $P < 0.05$.

we showed that the feeding of WSSF led to higher levels of SCFA in the cecal contents of rats (**Table 5**). Our result suggests that the cecal fermentation caused by ingestion of WSSF may be involved in the cholesterol-lowering effects in OVX rats.

In the case of OVX rats, the final body weight was increased compared with that of sham-operated rats (**Table 2**), although we prevented hyperphagia associated with ovariectomy. Ovarian hormones have been shown to affect body composition independently of changes in food intake (*40*). Thus, OVX may affect energy metabolism.

Estrogen replacement therapy has been shown to be effective in preventing bone loss (*1*) and alterations in lipid metabolism (*2*, *3*); however this therapy may be accompanied by serious side effects (*3*). Therefore, nonpharmacologic therapy is preferred. Guidelines for dietary calcium intake have been proposed for minimizing the risk of osteoporosis; however, the calcium intake in Japan is lower than the dietary reference intake of calcium (*41*). In this situation, ingestion of WSSF may effectively promote calcium absorption and maintain favorable lipid metabolism in menopausal women.

In conclusion, ingestion of WSSF increased Ca absorption and femoral Ca content in both OVX and sham rats. Moreover, the ovariectomy-induced elevation in serum cholesterol was canceled by the WSSF diet. We also suggest that cecal fermentation is involved in the beneficial effects of WSSF.

ABBREVIATIONS USED

CHD, coronary heart disease; OVX, ovariectomy; SCFA, short-chain fatty acid; WSSF, water-soluble soybean fiber; LDL, low-density lipoprotein; VLDL, very low density lipoprotein; HDL, high-density lipoprotein.

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