

Ingestion of sugar-beet fiber prevents hypercholesterolemia induced by ovariectomy in rats

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Abstract

This study examined the effects of feeding sugar-beet fiber (SBF), a highly fermentable dietary fiber, on serum total cholesterol levels and liver cholesterol contents using female Sprague-Dawley rats (8 weeks old) with or without ovariectomy (OVX). The rats in each group were fed a SBF (100 g/kg diet) or SBF-free diet for 5 weeks. The serum total cholesterol levels were elevated after OVX compared with that in the intact rats, and this elevation was partially prevented by the SBF diet. There were no significant differences in the relative liver weight among the groups. The hepatic total cholesterol contents in the SBF group were significantly lower than that in the control group in OVX rats. In conclusion, ingestion of SBF lowered the hepatic cholesterol contents and partially prevented increasing in the serum total cholesterol levels in OVX rats.

1. INTRODUCTION

Ovarian hormone deficiency is involved in coronary heart disease (CHD) in aged women and animals (1-3). Some studies have shown that ovarian hormone deficiency induced increases in serum total cholesterol levels, which have been implicated as a major risk factor for CHD (1, 4). Ovariectomized (OVX) rats are a widely used model for postmenopausal women, because OVX induces hypercholesterolemia (5, 6).

Post-menopausal estrogen replacement therapy (ERT) has shown potential for prevention of CHD (1). However, ERT may be accompanied by unacceptable side effects such as endometrial and breast cancer in some women. Therefore, it would be most helpful to discover a natural and safe dietary substance that improves lipid metabolism in postmenopausal

women.

Many studies have reported that dietary fiber improves lipid metabolism in rats (7-9). Dietary fiber has been classified into two fractions; i.e., 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 (8). Soluble fibers are usually fermented by colonic microorganisms and produces short-chain fatty acids, which are associated with the cholesterol-lowering effect (7, 9). Some possible mechanisms are proposed for decrease in serum cholesterol. Fermentation product of dietary fiber stimulate large intestine, and modify cholesterol metabolism. Propionate, a fermentation product of fiber, can modify cholesterol synthesis and acetate is involved in the serum cholesterol-lowering effect (10-12). Besides, it

has been reported that dietary fiber lowered the serum cholesterol level by enhancement of the hepatic LDL receptor mRNA (13).

Feeding of sugar-beet fiber (SBF), a highly fermentable dietary fiber, was lowered serum and liver cholesterol levels in normal rats (9, 13, 14), and the effect requires the large intestine (15). Hara et al. reported that the fermentation products of SBF with cecal bacteria reduced plasma cholesterol concentration in rats (9). However, it has not been clarified whether SBF improves ovariectomy-induced hypercholesterolemia in rats.

The present study was conducted to examine the effects of feeding SBF on body weight gain, uterine atrophy, serum and hepatic cholesterol levels in OVX rats.

2. MATERIALS AND METHODS

Animals and diets.

Female Sprague-Dawley rats (8 weeks old; Japan Clea, Tokyo, Japan) weighing about 240 g 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 semi-purified stock diet shown in Table 1 for an acclimation period of 3 days. The rats were divided into two groups; one group of rats underwent bilateral ovariectomy (OVX) and the other group did not operation (intact). All rats had free access to water and the stock diet for 7 days to recover from surgical damage.

After the recovery period, the rats in each group were divided into two subgroups of 7–8 rats on the basis of serum cholesterol concentration and body weight, and then given either of two experimental diets, the control or SBF (100 g/kg diet, Nippon Sugar Beet Manufacturing, Obihiro, Japan) diet shown in Table 1, for 5 weeks.

The body weight and food intake were

Table 1 Composition of stock and experiment diets^a

Component	Amount (g/kg diet)
Casein ^b	250
Corn oil	50
Mineral mixture ^c	35
Vitamin mixture ^d	10
Choline bitartrate	2.5
Cellulose ^e	100
Sucrose	to make 1 kg

^aSugar-beet fiber (100 g/kg diet) was added to the above-diet instead of cellulose.

^bNew Zealand Dairy Board, Wellington, New Zealand.

^cAIN-93G mixture (22). It provided (mg/kg diet): Ca 5000, 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.

^dAIN-93 mixture (22). It provided (U/kg 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 B₁₂ 25 µg, vitamin A 4000 IU, vitamin D₃ 1000 IU, vitamin E 75 IU.

^eAsahi Chemical Industry Co. Ltd., Tokyo, Japan.

measured every day. Tail blood was sampled before and 1, 3 and 5 weeks after feeding of the experiment diets, and serum was separated to measure total cholesterol concentration. At the end of experiment, the rats were anesthetized (Nembutal: sodium pentobarbital, 50 mg/kg body weight, Abbott Laboratories, North Chicago, IL, USA), and then killed after aortic blood was taken. Blood was centrifuged (1,300 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 liver was removed, weighed and stored at –40°C until subsequent analyses.

Analyses.

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). Cholesterol contents in the liver were estimated by enzymatic procedures (Cholesterol-C Test

Table 2 Initial body weight, final body weight, food intake, uterine and liver wet weights of intact and ovariectomized (OVX) rats fed the control diet or sugar-beet fiber (SBF) diet for 5 weeks^a

	Intact		OVX		ANOVA (<i>P</i> values) ^b		
	Control	SBF	Control	SBF	Treatment (T)	Diet (D)	T × D
Initial body weight (g)	242±3.3	241±2.2	240±5.4	241±5.2	NS ^c	NS	NS
Final body weight (g)	335±12.1 ^b	327±6.2 ^b	388±16.1 ^a	381±13.4 ^a	<0.001	NS	NS
Food intake (g/day)	20.1±0.88 ^{bc}	18.3±0.39 ^c	23.4±1.38 ^a	21.9±0.66 ^{ab}	<0.001	NS	NS
Uterine weight (mg/100g body weight)	198±20.7 ^a	196±8.0 ^a	51.7±14.0 ^b	64.3±10.9 ^b	<0.001	NS	NS
Liver weight (g/100g body weight)	3.42±0.10	3.48±0.13	3.39±0.13	3.16±0.09	NS	NS	NS

^a Values are means±SEM for 7-8 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.

Wako, Wako Pure Chemical Industries, Osaka, Japan) after Folch's extraction (16).

Statistical analyses.

Values shown represent the means ± SEM. Statistical analyses were performed by two-way ANOVA (treatment × diet). Duncan's multiple-range test (17) 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).

3. RESULTS

The mean final body weight and food intake were higher in OVX rats than in intact rats (Table 2). The uterine weights of all OVX rats were much lower than the average weight of the intact rats, indicating the success of the surgical procedure in all rats in the OVX group. Relative liver weight was not significantly affected by diet and operation.

The results of two-way ANOVA showed that the serum total cholesterol levels were affected by OVX (Fig. 1). The serum cholesterol levels were significantly higher in OVX rats fed control diet than in intact rats at 3 and 5 weeks after feeding of the experiment diets. The serum total cholesterol levels of OVX rats fed the SBF diet were lower than those of OVX

rats fed the control diet, and this cholesterol levels were almost same as that of intact rats at 3 weeks after feeding the experiment diet. There were no differences in serum total cholesterol levels among intact rats in all periods.

The results of two-way ANOVA showed that the serum total and HDL-cholesterol levels were again affected by OVX (Fig. 2). These levels of the OVX rats fed the control diet were higher than those of the intact rats. The total and HDL-cholesterol levels tended to be lower

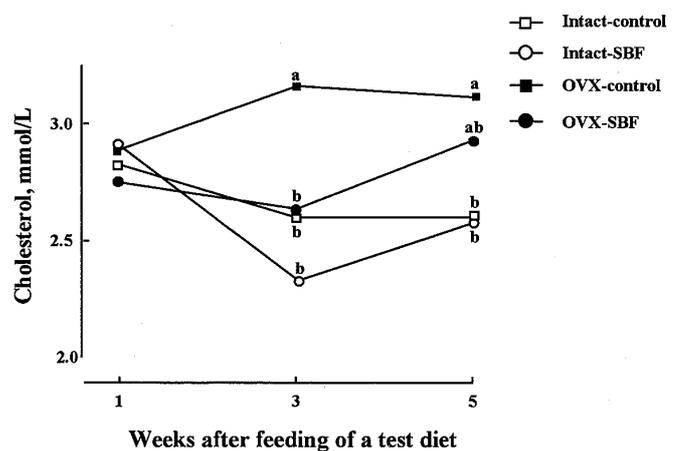


FIGURE 1

Serum total cholesterol levels in intact and ovariectomized rats fed the control or sugar beet fiber diet 1, 3 and 5 weeks after the start of the experimental period. Values are means ± SEM, n = 7-8. *P*-values estimated by two-way ANOVA were 0.474 (week 1), 0.002 (week 3), 0.004 (week 5) for treatment, 0.747 (week 1), 0.003 (week 3), 0.434 (week 5) for diet and 0.334 (week 1), 0.362 (week 3), 0.642 (week 5) for treatment × diet. Means not sharing a common letter differ, *P* < 0.05.

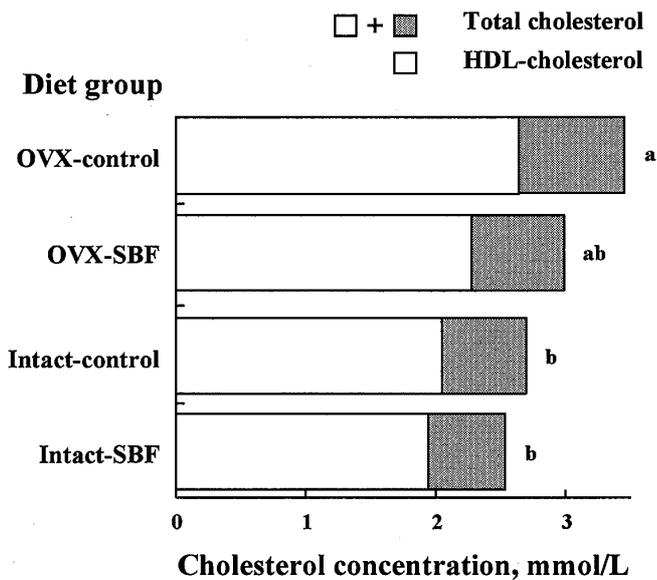


FIGURE 2

Serum total and HDL cholesterol levels in intact and ovariectomized rats fed the control or sugar beet fiber diet at 5 weeks after the start of the experimental period. Values are means \pm SEM, $n = 7-8$. P -values estimated by two-way ANOVA were 0.003 for treatment, 0.332 for diet and 0.655 for treatment \times diet. Means not sharing a common letter differ, $P < 0.05$.

in the SBF group than in the control group in OVX rats. In the intact rats, there were no differences in serum cholesterol levels between the SBF and control groups.

The results of two-way ANOVA showed that the liver total cholesterol contents were affected by diet (Fig. 3). In rats fed diet containing SBF, the liver total cholesterol contents were lower compared with rats fed the SBF-free diet. In OVX rats, the liver total cholesterol contents were lower in the SBF group than in the control group. In the case of intact rats, there were no significant differences in the liver total cholesterol contents among groups.

4. DISCUSSION

In this study, we examined the effects of SBF on serum cholesterol levels and hepatic cholesterol contents in OVX rats. We showed that OVX induced increases in serum cholesterol levels, and the serum total cholesterol

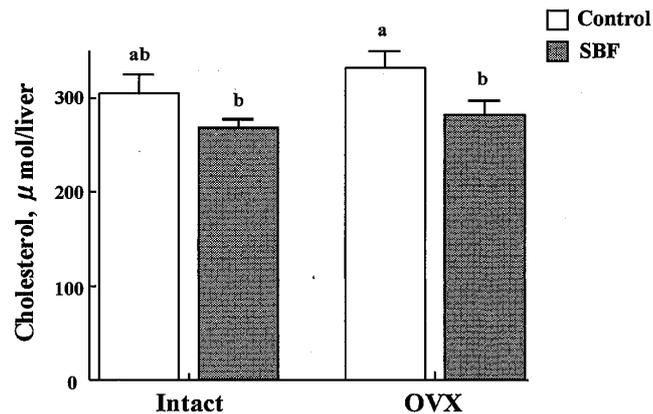


FIGURE 3

Hepatic total cholesterol contents in intact and ovariectomized rats fed the control or sugar beet fiber diet at 5 weeks after the start of the experimental period. Values are means \pm SEM, $n = 7-8$. P -values estimated by two-way ANOVA were 0.133 for treatment, 0.002 for diet and 0.699 for treatment \times diet. Means not sharing a common letter differ, $P < 0.05$.

concentrations in the SBF groups were reduced by 17 % compared with the control groups at 3-weeks after feeding of experiment diet, and reduced 6 % at 5-weeks (Fig. 1). Dietary fiber, particularly viscous dietary fiber, has been shown to be effective in reducing serum cholesterol levels in rats (7, 8). SBF is a highly fermentable dietary fiber, and previous study showed that the feeding of SBF decreased serum cholesterol concentration in rats (9). Short-chain fatty acids, particularly acetate and propionate, may be involved in lowering serum cholesterol concentration (10-12). Fukushima et al. also reported that SBF lowered serum total cholesterol levels by enhancement of the hepatic LDL receptor mRNA (13). Besides, it has been reported that estrogen decreased the serum cholesterol concentration and increased the LDL receptor gene expression or activity (18). Therefore, estrogen deficiency maybe reduces the LDL receptor gene expression and increases the serum cholesterol levels. The feeding of SBF maybe improves the LDL receptor gene expression. Activation of LDL receptor causes increase in LDL uptake by the liver, and decrease in the serum LDL cholesterol levels. This study did not measure

any fermentation products and can not elucidate changes in the LDL receptor gene expression, but we speculate that fermentation products of SBF and alteration of LDL receptor gene expression reduced serum cholesterol levels by feeding of SBF in OVX rats. Further investigation is needed into the hypocholesterolemic mechanism of SBF in OVX rats.

This study showed that the hepatic cholesterol contents were reduced by SBF (Fig. 3). The previous study demonstrated that feeding of the SBF decreased serum total cholesterol by enhancement of bile acid excretion, and this increase probably induced hepatic cholesterol synthesis activity (9, 10). Because bile acids are the main excretion route for cholesterol from the body, changes in bile acid metabolism may have been implicated in hypocholesterolemic action. Buhman et al. reported that dietary psyllium reduced liver cholesterol content and increased in fecal bile acid excretion and/or hepatic bile acid synthesis (19). Story also observed that soluble fibers increased fecal excretion of bile acids, and altered the percentage of bile acids excreted by the liver (20). Elucidating whether the hypocholesterolemic effect of SBF is caused by the bile acids excretion requires further investigation.

The food intake was higher in OVX rats than in intact rats (Table 2), and this result correlated with hypercholesterolemia ($r = 0.462$, $P < 0.05$). The increased food intake in OVX rats suggests a shift in energy metabolism due to ovarian hormone deficiency, and calorie intake affects the serum cholesterol concentration (21). In the present study, the food intake of OVX rats fed the SBF diet was lower tendency compared with rats fed the control diet. This result may be associated with the hypocholesterolemic effect of SBF feeding.

Estrogen replacement therapy has been shown to be effective in preventing the alterations in lipid metabolism, however this therapy may be accompanied by serious side effects (1). Therefore, nonpharmacologic therapy is prefer-

red. In this study, although SBF feeding reduced the serum and hepatic cholesterol levels, there were not significant differences in uterus tissue, body weight gain, the relative liver weight among the OVX group (Fig. 1, 3 & Table 2), which suggests that feeding of SBF does not induce side-effects on the uterus and liver weight. Ingestion of SBF may keep favorable lipid metabolism in menopausal women.

In conclusion, ingestion of SBF lowered the hepatic cholesterol contents and partially prevented increasing in the serum total cholesterol levels in OVX rats. Additional studies are needed to demonstrate SBF efficacy in humans and animals.

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