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Prune suppresses ovariectomy-induced hypercholesterolemia in rats

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Elevated cholesterol among women who have experienced natural or surgical menopause has been linked to ovarian hormone deficiency. The purpose of this study was to investigate the efficacy of prune, a good source of dietary fiber and phytochemicals, on lowering cholesterol in an ovariectomized (ovx) rat model. Forty-eight 90-day-old female Sprague-Dawley rats were randomly assigned to four groups: sham-operated (sham) + control diet, ovx + control diet, ovx + low-dose (LD; 5%) prune, and ovx + high-dose (HD; 25%) prune. After 45 days of treatment, rats were euthanized and tissues were collected for analyses. Ovariectomy elevated serum total cholesterol by 22% compared with sham, and HD prune diet prevented this increase without affecting high density lipoprotein cholesterol concentrations. Animals fed the HD prune diet had 13% lower liver total lipids compared with ovx animals. The findings of this study showed that prune exhibits hypocholesterolemic properties in ovarian hormone deficiency. Dose-response studies should be conducted to establish the effectiveness of prune in prevention of hypercholesterolemia in postmenopausal women who are not on estrogen replacement therapy and seek dietary alternatives. Mechanistic studies also are needed to establish its mode of action. (J. Nutr. Biochem. 11:255–259, 2000)

Keywords: cholesterol; lipids; fiber

Introduction

At the onset of menopause, the risk for coronary heart disease (CHD) in women increases drastically due to ovarian hormone deficiency.[1,2] Increased blood cholesterol concentrations have been implicated as a major risk factor for CHD.[3] Estrogen replacement therapy (ERT) has been shown to be effective in preventing the rise in serum total and low density lipoprotein (LDL) cholesterol concentrations[4] in women who have experienced natural or surgically-induced menopause. The benefits of ERT have been exhibited primarily through favorable alterations in lipid and lipoprotein metabolism.[5] However, ERT may be accompanied by increased risk for endometrial and breast cancers[6,7] and therefore is recommended only for women who have no contraindications. Other pharmacologic means by which cholesterol can be lowered are also often associated with side effects[8] and may not be suitable for individuals who are taking other forms of medication. Thus, if possible, nonpharmacologic means of reducing cholesterol and CHD risks are preferred.

One alternative for lowering cholesterol is the incorporation of fiber-rich dried fruits such as prune in the diet. Although a number of studies have shown the effectiveness of fiber in reducing serum cholesterol, these studies have used purified fibers, which may act differently when consumed in their intact forms. Dietary fibers, particularly soluble dietary fibers such as pectin, psyllium, and oat bran, have been shown to be effective in reducing serum cholesterol concentrations in both humans[9,10] and animals.[11–13] Dietary fibers lower cholesterol by several mechanisms including enhanced fecal excretion of sterols and increased conversion of cholesterol to bile acids.[14]

Limited studies have investigated the cholesterol-lowering effects of prune. The only published animal study[15] showed that when 6% isolated prune fiber was incorporated into the diet of hyperlipidemic rats, it lowered serum and liver cholesterol concentrations as effectively as 3% pure pectin. The same investigators[16] reported that daily consumption of 100 g whole prune was effective in lowering serum total cholesterol in men with mild hypercholesterolemia. However, there are no studies investigating the role of prune in ovarian hormone deficiency-associated rise in serum cholesterol.

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Table 1 Major components of prune powder*

Component	Amount per 100 gram prune powder
Proximate analyses (g)	
Protein	3.0
Fat	0.5
Total carbohydrate	80.0
Total dietary fiber	9.0
Minerals (mg)	
Calcium	72.0
Iron	3.0
Potassium	1,050.0
Sodium	5.0
Phosphorus	108.0
Boron	3.4
Anti-oxidants (mg)	
Vitamin C	0.63
Carotenoids	0.60
Phenolic and flavonoid compounds	22.4
Quinones	0.058
ORAC units	5,770

*These values are reported by the California Prune Board (Pleasanton, CA USA) for 100 grams of dried prunes and can vary from batch to batch.
†ORAC–oxygen radical absorbance from McBride, J. (1999). Can foods forestall aging? *Agri. Res.* **47,** 14–17.

In addition to being a good source of dietary fiber, prune also contains a number of phenolic compounds as well as ascorbic acid and carotenoids[17] that may exert positive effects on lipid metabolism. The purpose of this study was to evaluate the effectiveness of prune in lowering ovariectomy-induced hypercholesterolemia using a rat model. The rationale for choosing the high-dose prune (25% w/w) used in this study was based on our preliminary observations that this dose can be readily tolerated by rats. Thus, the dose chosen in this study should not be translated to a minimum effective dose. Additional dose-response studies are necessary to establish prune levels between 5 to 25% that may be equally effective in lowering cholesterol in ovarian hormone deficiency.

Materials and methods

Animals and diets

Forty-eight 90-day-old female Sprague-Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN USA). Upon arrival at our institution, animals were individually housed and kept in an environmentally controlled laboratory. Guidelines for the ethical care and treatment of animals from the Animal Care and Use Committee at Oklahoma State University were strictly followed. After 3 days of acclimation, rats were divided into four groups of 12 rats each as follows: sham-operated (sham) + control diet, ovariectomized (ovx) + control diet, ovx + 5% low-dose (LD) prune, and ovx + 25% high-dose (HD) prune. Ovariectomy was performed on the ovx groups and the sham group went through the stress of surgery without removal of their ovaries. Rats in the control diet were fed a powdered casein-based diet (modification of AIN-93M[18]). The prune-fed groups received similar diets in which 5% and 25% of diets (w/w) were replaced with dried-powdered prune with known composition (*Table 1*). The diets were appropriately adjusted for total energy, carbohydrate, protein, fat, and total dietary fiber (*Table 2*). Additionally, the diets were adjusted for calcium and phosphorus concentrations to account for prune composition. Rats were fed ad libitum and had free access to deionized water. Food intake was determined by measuring the amounts of food given and of food left over.

Table 2. Composition of the experimental diets

Ingredients	Control	LD	HD
	g/kg diet		
Carbohydrate			
Total	720.7	720.7	720.7
Cornstarch	465.7	425.7	265.7
Maltodextrin	155	155	155
Sucrose	100	100	100
Prune*	—	40	200
Protein			
Total	140	140	140
Casein	140	138.5	132.5
Prune*	—	1.5	7.5
Fat			
Total	40	40	40
Soybean oil	40	39.75	38.75
Prune*	—	0.25	1.25
Fiber			
Total	50	50	50
Cellulose	50	45.5	27.5
Prune*	—	4.5	22.5
Vitamin mix†	10	10	10
Mineral Mix			
Total	35	35	35
Mineral mix (Ca-P deficient)‡	13.4	13.4	13.4
Calcium carbonate	9.88	9.79	9.43
Calcium from prune*	—	0.036	0.18
Potassium phosphate, monobasic	5.6	5.48	5.0
Sodium phosphate, monobasic	3.44	3.32	2.84
Phosphorus from prune*	—	0.054	0.27
Potassium citrate, monobasic	0.9	0.9	0.9
Sucrose	1.78	2.02	2.98
Choline bitartrate	2.5	2.5	2.5
L-cysteine	1.8	1.8	1.8
Caloric density§ (kJ/g diet)	17.6	17.5	17.4

*Dried powdered prunes obtained from Sunsweet Growers Inc. (Yuba City, CA USA). Composition of prune is shown in *Table 1*.
†Vitamin mixture (TD #94047) obtained from Harlan Teklad (Madison, WI USA). Vitamin mix consisted of (g/kg): nicotinic acid, 3.0; calcium pantothenate, 1.6; pyridoxine HCl, 0.7; thiamin HCl, 0.6; riboflavin, 0.6; folic acid, 0.2; D-biotin, 0.02; vitamin B_{12} (0.1% in mannitol), 2.5; DL-α-tocopheryl acetate (500 IU/g), 15; vitamin A palmitate (500,000 IU/g), 0.8; cholecalciferol (500,000 IU/g), 0.2; phylloquinone, 0.075; sucrose, 974.705.
‡Mineral mixture (TD #79055) obtained from Harlan Teklad. This mineral mix is a modification of the AIN76 lacking calcium and phosphorus with sucrose as diluent.
§Caloric density obtained by bomb calorimetry (Parr Instrument 1261 Calorimeter, Moline, Il.)

Forty-five days after surgery, nonfasted animals were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg body weight) and xylazine (5 mg/kg body weight) and exsanguinated via the abdominal aorta. Blood samples were collected and serum was separated by centrifugation at 1,500 × g for 20 min at 4°C. Aliquots of serum were frozen and kept at −20°C for later analyses. The liver was immediately removed, rinsed with ice-cold physiologic saline solution, weighed, put in sealed containers, and stored at −20°C until analyzed. Spleen, uterus, kidneys, heart, and adrenal glands were also collected, blotted, and weighed.

Table 3 Effects of ovariectomy (ovx), low-dose prune (LD), and high-dose prune (HD) consumption on body and organ weights in rats*

Measures	Sham	Ovx	Ovx+LD	Ovx+HD	Pooled SE	ANOVA (*p*-value)
Food intake (g/rat/day)	12[a]	16[a]	16[a]	15[a,b]	0.90	=0.004
Body weights (g)						
Initial	228	228	226	231	2.0	=0.317
Final	266[b]	314[a]	328[a]	328[a]	4.3	<0.001
Organ weights (g/100 g body wt.)						
Uterus	0.265[a]	0.045[b]	0.044[b]	0.044[b]	0.01	<0.001
Liver	2.76	2.65	2.49	2.70	0.07	=0.076
Kidneys†	0.674[a]	0.575[b]	0.548[b]	0.554[b]	0.02	<0.001
Spleen	0.233	0.231	0.236	0.232	0.01	=0.975
Heart	0.368[a]	0.328[b]	0.322[b]	0.320[b]	0.01	<0.001
Adrenal†	0.038[a]	0.028[b]	0.029[b]	0.029[b]	0.00	<0.001

*Values are means, $n = 12$ in each group.
†The weight of both organs together.
[a,b]Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.
ANOVA–analysis of variance.

Serum triglycerides and total, and high density lipoprotein cholesterol

Serum triglycerides (TG) and total cholesterol concentrations were determined enzymatically using kits from Sigma Diagnostics (St. Louis, MO USA). Serum high density lipoprotein (HDL) cholesterol was determined by a modification[13] of the precipitation technique of Sjoblom and Elklund.[19] These tests were performed on a Cobas-Fara II Clinical Analyzer (Montclair, NJ USA) following the manufacturer's instructions and using commercially available calibrators and quality control samples. Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

Liver total lipids and cholesterol

Portions of livers were homogenized using a hand-held homogenizer (Biospec Products Inc., Bartlesville, OK USA) and then extracted with a 2:1 (v/v) chloroform:methanol mixture. After addition of 0.12 mol/L NaCl solution to the extraction solution and separation of phases, aliquots of the organic phase were analyzed for liver total cholesterol. Liver total cholesterol was determined using a color reagent of glacial acetic acid-$FeSO_4$-H_2SO_4.[20] Total liver lipids were determined using the Folch gravimetric method.[21] Liver total cholesterol and lipids were calculated and reported per gram of liver.

Statistical analyses

Data analyses involved computation of means and standard error for each of the treatment groups.[22] One-way analysis of variance (ANOVA) was performed to determine whether there were statistically significant ($P < 0.05$) differences by treatment groups. When an ANOVA indicated any significant differences among the means, the Tukey-Kramer follow-up multiple comparison tests was used to determine which means were significantly different. GraphPad Instat Software (version 2.0, 1993; San Diego, CA USA) was used for all statistical analysis.

Results

Food intake and body and organ weights

Data on food intake and body and organ weights are presented in *Table 3*. The average food intakes of the ovx groups did not differ significantly from each other, and as expected,[23] the sham animals did not consume as much as the ovx animals. The treatment groups started with similar mean body weights ranging from 226 to 231 g. All rats gained weight during the study, but the weight gains of rats in the ovx groups were significantly higher than those of the sham animals. Animals that were fed prune diets did not experience diarrhea as judged by fecal appearance and fecal wet and dry weights.

Ovariectomy caused atrophy of uterine tissue, indicating the success of the surgical procedure. Kidneys, heart, and adrenal gland weights (g/100 g body weight) were significantly higher in the sham animals compared with all ovx animals (*Table 3*). However, there were no differences in the liver and spleen weights among any of the treatment groups.

Serum total, HDL, and non-HDL cholesterol and triglycerides

Ovariectomy significantly elevated serum total cholesterol concentrations by 22% compared with sham animals (*Table 4*). This ovariectomy-induced elevation in serum total cholesterol was lowered somewhat by the LD prune diet but was prevented ($P < 0.01$) by the HD prune diet. The increase in serum total cholesterol concentrations due to ovariectomy was attributed to a 62% rise in non-HDL cholesterol fractions when compared with sham animals (*Table 4*). Importantly, the HD prune diet also prevented the increase in non-HDL cholesterol concentrations without significantly affecting the HDL cholesterol concentrations (*Table 4*).

Liver total cholesterol and total lipids

Liver total cholesterol was not significantly affected by any of the treatments including ovariectomy (*Table 4*). However, liver total lipids were approximately 18% higher ($P = 0.053$) in ovx animals than in sham animals. Animals fed the HD prune diet had 13% lower liver total lipids than did ovx animals.

Table 4 Effects of ovariectomy (ovx), and prune on serum and liver parameters*

Measures	Sham	Ovx	Ovx+LD	Ovx+HD	Pooled SE	ANOVA (*p*-value)
Serum						
Total cholesterol (mmol/L)	2.49[b]	3.04[a]	2.89[a,b]	2.51[b]	0.12	0.005
HDL cholesterol (mmol/L)	2.11	2.44	2.22	2.12	0.12	0.185
Non-HDL cholesterol (mmol/L)	0.37[b]	0.60[a]	0.67[a]	0.39[b]	0.06	0.012
Triglycerides (mmol/L)	1.04[a]	0.68[a,b]	0.57[b]	0.63[a,b]	0.10	0.022
Liver (mg/g liver)						
Total cholesterol	3.02	3.01	2.96	2.84	0.12	0.723
Total lipids	41.1[b]	48.5[a]	48.4[a]	42.2[b]	2.2	0.053

*Values are means, $n = 12$ in each group.
[a,b]Within a row, values that do not share the same superscript letters are significantly ($P < 0.05$) different from each other.
LD–low-dose prune diet. HD–high-dose prune diet.
ANOVA–analysis of variance. HDL–high density lipoprotein.

Discussion

In agreement with our previous findings[13] and those of other investigators,[24–26] ovariectomy significantly increased serum total cholesterol. The exact mechanisms by which ovariectomy causes hypercholesterolemia are not known. The higher rates of sterol synthesis cannot be suggested as a possibility because ovariectomy in rats has been reported[27,28] to significantly reduce the activity of hepatic HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis. Diminished LDL receptor activity is also unlikely to occur in ovariectomy.[29,30] Perhaps the most plausible explanation for the increased serum cholesterol is the reduced rate of cholesterol clearance or its hepatic conversion to bile acids. The removal of the ovaries per se and the substantially atrophied uterus eliminates sites of LDL clearance. For instance, the ovaries in small animal models such as hamsters are reported to be second to the liver in LDL clearance capacity on a per gram tissue basis.[31] Aside from having substantially reduced uterine size, the ovx animals also had lower kidney, heart, and adrenal weights. Although we cannot offer an explanation for these observations, the removal of ovaries and reduced combined mass of these tissues are likely involved in the impaired LDL clearance and elevation of serum cholesterol concentrations in ovariectomy.

Reduced hepatic 7α-hydroxylase activity may also, in part, explain the hypercholesterolemia observed in ovarian hormone deficiency. The findings of a recent study by our laboratory[32] have demonstrated that ovariectomy reduces hepatic 7α-hydroxylase mRNA levels and that estrogen administration significantly reverses this effect.[33] In support of these findings, a study by Colvin et al.[34] found that oral administration of conjugated equine estrogen in ovx cynomolgus monkeys significantly elevated liver 7α-hydroxylase activity. These and perhaps other mechanisms working alone or in combination appear to contribute to the elevated cholesterol that is associated with ovarian hormone deficiency.

In this study, liver total cholesterol concentrations were unaffected by either ovariectomy or prune diets. However, this is expected because hepatic cholesterol concentrations are usually sensitive to the presence of cholesterol in the diet. We previously observed that the addition of as low as 0.3% cholesterol in the diet causes a fourfold increase in liver cholesterol concentrations.[11]

In this study, the inclusion of 25% prune in the diet prevented ($P < 0.01$) the ovariectomy-induced rise in both serum total and non-HDL cholesterol concentrations. Although not significant, 5% prune in the diet also lowered serum cholesterol in ovx animals. Prune is considered a good source of dietary fiber,[35] containing approximately 9% total fiber, of which approximately 60% is pectin.[16] Our observations[11,12] as well as those of others[36–38] have shown that inclusion of 3 to 8% pectin in the diet of rats is necessary to detect a significant decrease in serum total cholesterol. In the present study, the fiber levels obtained from 5% and 25% prune in the diets provided approximately 0.3% and 1.4% pectin, respectively. These levels are far below those previously found effective, implying that the hypocholesterolemic effects of prune may not be solely due to its fiber contents. Phenolic and flavonoid compounds found in prune (*Table 1*) have been reported to lower serum levels of cholesterol[39] as well as prevent LDL cholesterol oxidation.[40] The extent to which the phenolic and flavonoid contents of prune contribute to its hypocholesterolemic effects and reducing LDL cholesterol oxidation must be investigated.

Additionally, the mechanisms by which prune counteracts the disturbances caused by lack of ovarian hormone must be examined in future studies. The findings of this study do not necessarily indicate that 25% prune in the diet is needed to lower cholesterol. Prune levels below 25% in the diet may be equally effective in lowering cholesterol in ovarian hormone deficiency. However, this is speculative and dose-response studies must be conducted to demonstrate this view.

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