

Nutrient Requirements and Interactions

Dietary Persimmon Improves Lipid Metabolism in Rats Fed Diets Containing Cholesterol¹

Shela Gorinstein,² Elzbieta Bartnikowska,* Gustaw Kulasek,[†] Marina Zemser and Simon Trakhtenberg^{**}

Department of Pharmaceutical Chemistry, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel; *Department of Dietetics, Faculty of Human Nutrition, Warsaw Agricultural University, Warsaw, 02-787 Poland; [†]Department of Animal Physiology, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, 02-787 Poland; and ^{**}Institute of Cardiology, Kaplan Medical Center, Rehovot, Israel

ABSTRACT The effect of dietary persimmon (Pers, 7.0%) on lipid metabolism and antioxidant activity was investigated in 40 male Wistar rats adapted to cholesterol-free or 1% cholesterol diets. The rats were divided in four groups of 10. The basal diet contained wheat starch, casein, soybean oil, and mineral and vitamin mixtures. The control group (C) consumed the basal diet. To the basal diet were added 7 g/100 g dry persimmon (Pers), 1 g/100 g cholesterol (Chol), or both (Chol/Pers). The experiment lasted 4 wk. Plasma total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), total phospholipids (TPH), HDL phospholipids (HDL-PH), lipid peroxides (LP) and liver TC concentrations were measured. Groups did not differ before the experiment. In the Chol/Pers vs. Chol group, the persimmon-supplemented diet significantly ($P < 0.05$) lessened the rise in plasma lipids due to dietary cholesterol: TC (3.88 vs. 4.88 mmol/L; -20%), LDL-C (2.24 vs. 3.27 mmol/L; -31%), TG (0.72 vs. 0.89 mmol/L; -19%), LP (2.20 vs. 3.25 mmol/L; -32%) and TC in liver (32.8 vs. 49.9 μ mol/g; -34%), ($P < 0.001$). The Chol/Pers diet significantly reduced the decrease in HDL-PH due to dietary cholesterol (0.73 vs. 0.58 mmol/L; -25.8%, $P < 0.001$) and decreased the level of TPH (1.32 vs. 1.73 mmol/L; -23%, $P < 0.001$). Persimmon in rats fed the basal diet without cholesterol did not significantly affect the variables measured. These results demonstrate that persimmon possesses hypolipidemic and antioxidant properties that are evident when persimmon is added to the diet of rats fed cholesterol. These properties are attributed to its water-soluble dietary fiber, carotenoids and polyphenols. J. Nutr. 128: 2023-2027, 1998.

KEY WORDS: • persimmon • lipoproteins • phospholipids • lipid peroxides • rats

Atherosclerosis is the basis of the coronary artery disease (CAD),³ one of the most serious diseases in humans. Since the research of Anitschkow (1913), cholesterol became known as the "building material" for atherosclerotic plaques (Castelli et al. 1986, Freedman et al. 1988, Kwiterovich et al. 1992). In recent years, factors that influence the level of the lipids were investigated extensively. It was found that some components of dietary fiber, particularly those soluble in water, might influence lipid metabolism (Eastwood and Kay 1979, Shinnick et al. 1991). Kiryama et al. (1969) found that dietary fiber affects the level of lipids in rats fed cholesterol. A decrease in the levels of LDL cholesterol (LDL-C) in human studies using oat preparation was shown (Davidson et al. 1991, Kirby et al. 1981, Turnbull and Leeds 1987). Anderson et al. (1994) found

a positive influence of dietary fiber on lipid concentration. Significantly different effects were observed for serum and liver lipids in rats fed cholesterol. Jackson et al. (1994) found higher activity in rats fed malted barley with cholesterol than in rats fed malted barley without cholesterol. Moundras et al. (1997) reported the potent plasma cholesterol-lowering effect of guar gum, especially in rats fed cholesterol-containing diets. According to Lin (1994), certain components of dietary fiber also exert an antioxidant effect. The scientific community continues to search for new sources of dietary fiber. In recent years, the attention of some researchers has been focused on persimmon fruit (Achiva et al. 1997, Gorinstein et al. 1993 and 1994, Uchida et al. 1989). Persimmon (*Diospyros kaki* L.) is one of the more important fruit crops in Israel. There are two persimmon varieties: Fuio with seeds and seedless Triumph. Triumph, which is considered the most useful, was investigated. In this study, the interest was in investigating the whole fruit rather than a particular component or part. Persimmon contains the following (g/100 g of fresh fruit): water, 80.3 g; protein, 0.58 g; total lipids, 0.19 g; total carbohydrates, 18.6 g; total dietary fiber, 1.48 g; and some minerals, i.e., magnesium, iron, zinc, copper and manganese (Cerutti and Zappavigna

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²To whom correspondence should be addressed.

³Abbreviations used: C, Control; CAD, coronary artery disease; Chol, nonoxidized cholesterol; HDL-C, HDL cholesterol; HDL-PH, HDL phospholipids; LDL-C, LDL cholesterol; LP, lipid peroxides; Pers, Persimmon; TC, total cholesterol; TG, triglycerides; TPH, total phospholipids; VLDL-C, VLDL cholesterol.

1977, Young and How 1986). A high concentration of antioxidants such as ascorbic acid (up to 7.5 mg), carotenoids (particularly β -cryptoxanthin, zeaxanthin and β -carotene), polyphenols and a specific group of polyphenols (tannins) were found in persimmon (Gorinstein et al. 1994, Gross 1987, Uchida et al. 1989). The dry residue of persimmon includes the following (g/100 g): polyphenols, 0.16–0.25; carotenoids, 0.002; and soluble and nonsoluble proteins, 0.64–1.3 (Daood et al. 1992, Gorinstein et al. 1994, Gross 1987, Pirretti 1991). Some components of persimmon show special activities (Uchida et al. 1989). Persimmon tannins prolonged life and reduced the incidence of stroke in hypertensive rats (Uchida et al. 1989, Uchida et al. 1990). This effect was attributed to the fact that persimmon tannins are 20 times more potent than antioxidant vitamin E. However, the effect of a persimmon-supplemented diet on lipid metabolism has not been examined.

The aim of this study, therefore, was to investigate the possible influence of this diet on lipid metabolism and antioxidant activity in rats fed cholesterol-containing and cholesterol-free diets.

MATERIALS AND METHODS

Rats and diets. The Animal Care Committee of Warsaw Agricultural University approved this study. The Institute of Animal Physiology and Nutrition of Polish Academy of Sciences (Jablonna, Poland) provided 6-mo-old male Wistar rats ($n = 40$) with a mean weight of 120 g. They were housed individually in stainless steel metabolic cages and were divided into four groups of 10. All four groups were fed a basal diet that included wheat starch, casein, soybean oil, and mineral and vitamin mixtures. The rats of the control (C) group were fed only the basal diet. The persimmon (Pers) diet group received the basal diet supplemented with 7 g/100 g dry persimmon (Pers). The cholesterol (Chol) group received the basal diet plus 1% nonoxidized cholesterol, whereas the cholesterol/persimmon (Chol/Pers) group received the basal diet supplemented with 1% nonoxidized cholesterol and 7 g/100 g dry persimmon. The experiment lasted 4 wk. Total cholesterol (TC), LDL-C, HDL cholesterol (HDL-C), triglycerides (TG), total phospholipids (TPH), HDL phospholipids (HDL-PH), lipid peroxides (LP) in plasma and TC concentration in liver were measured.

To prepare persimmon for use in this experiment, whole fruits of the seedless Triumph variety were dried at 40°C, powdered and mixed with the basal diet before the rats were fed. Cholesterol of analytical grade (USP) was obtained from Sigma Chemical, St. Louis, MO. The cholesterol batches were mixed carefully with the basal diet (1:99) just before the diets were offered to the rats. The dietary cholesterol was checked according to the HPLC method of Ansari et al. (1979) and was found not to contain cholesterol oxides. Total dietary fiber was determined according to Prosky et al. (1992). We have determined that persimmon contains (g/100 g of dry persimmon) 3.50 g of total dietary fiber, 1.75 g of soluble dietary fiber and 1.70 g of insoluble dietary fiber. The fiber concentration of persimmon-containing diets was 2.20 and 5.51 g/100 g soluble and insoluble dietary fiber, respectively. The diets contained, as percentage of energy, 62% carbohydrate, 24% fat and 14% protein. The calculated energy values of all diets were not significantly different. The exact compositions of the diets are presented in Table 1.

Methods. All rats were fed once a day at 1000 h. Diet intake was monitored daily. At the end of the experiment, the rats were anaesthetized using diethyl ether; blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests. After anaesthesia, the abdomen was opened to take samples of the liver for determination of TC. The weight gain of the rats was recorded on a weekly basis. TC, HDL-C, TPH, HDL-PH and TG were determined enzymatically. TC and TG were measured as described by Trinder and Webster (1984) with kits (PAP 100, #6.122.4 and #6.123.6, respectively); TPH was measured according to a combined enzymatic method using phospholipase D, choline oxidase and

TABLE 1

Composition of the diets

Ingredients	Control ¹	Chol ¹	Pers	Chol/Pers
	g/kg diet			
Casein	180	180	180	180
Soybean oil	60	60	60	60
Wheat starch	690	680	620	610
Dry persimmon ²	0	0	70	0
Cholesterol ³	0	10	0	10
Vitamin mixture ⁴	10	10	—	10
Mineral mixture ⁵	60	60	60	60
Total fiber	—	—	77.2	77.2
Soluble fiber	—	—	22.1	22.1
Insoluble fiber	—	—	55.1	55.1

¹ Control and cholesterol diets were fiber free.

² Dry persimmon was prepared and supplied by the Department of Pharmaceutical Chemistry (see Materials and Methods).

³ Sigma Chemical (St. Louis, MO).

⁴ Vitamins (per kg of diet): thiamin, 20 mg; riboflavin, 15 mg; pyridoxin, 10 mg; nicotinamide, 100 mg; calcium pantothenate, 70 mg; folic acid, 5 mg; biotin, 0.3 mg; cyanocobalamin, 0.05 mg; retinyl palmitate, 1.5 mg; *dl*- α -tocopheryl acetate, 125 mg; cholecalciferol, 0.15 mg; menadione, 1.5 mg; ascorbic acid, 50 mg; myo-inositol, 100 mg; carrier wheat starch; 1.36 g.

⁵ Minerals (per kg of diet): CaHPO₄, 15 g; K₂HPO₄, 2.5 g; KCl, 5 g; NaCl, 5 g; MgCl₂, 2.5 g; Fe₂O₃, 2.5 mg; MnSO₄, 125 mg; CuSO₄ · 7H₂O, 0.2 mg; ZnSO₄ · 7H₂O, 100 mg; KIO₃, 0.4 mg.

peroxidase (Takayama et al. 1977) with a kit (#6.149.1) from Bio Merieux (Marcy l'Etoile, France). HDL-C and HDL-PH were determined by the same enzymatic methods after the precipitation of LDL-C and VLDL cholesterol (VLDL-C) fractions with phosphotungstic acid in the presence of magnesium ions with kit (# 6.159.1) from Bio Merieux. LP was determined colorimetrically (Tateishi et al. 1987) in a direct reaction between methylene blue derivative (MCDP, 10-*N*-methyscarbonyl-3,7-dimethylamino-10H-phenothiazine) catalyzed by hemoglobin using kit (9#CC-004) from Kamiya Biomedical (Seattle, WA). LDL-C was calculated according to the Friedewald formula (Friedewald et al. 1972). TC in liver was analyzed according to Mazur et al. (1990).

Data analysis. Values are given as the means \pm SEM; where appropriate, data were tested by two-way ANOVA (Chol \times Pers) using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA) followed by Duncan's new multiple range test (Duncan 1955) to assess differences between group means. Differences of $P < 0.05$ were considered significant.

RESULTS

The addition of persimmon or cholesterol to the diets did not affect food intake, body weight gain or feed efficiencies (data not shown). At baseline, the four groups did not differ from one another in plasma lipid concentrations (data not shown). After the experiment, the concentrations of TC and LDL-C in both the Chol and Chol/Pers groups were greater than in the C and Pers groups (Table 2), and in Chol rats, TC and LDL-C were significantly greater than in the Chol/Pers group ($P < 0.001$). Therefore, the persimmon-supplemented diet significantly hindered the cholesterol-induced increase in plasma TC (20%, $P < 0.001$) and LDL-C (31%, $P < 0.001$). The HDL-C/TC ratio was lower in both the Chol/Pers and Chol groups than in the C and Pers groups (0.42 ± 0.03 and 0.32 ± 0.03 vs. 0.56 ± 0.04 and 0.56 ± 0.04 , respectively, and the ratio in the Chol/Pers group was significantly greater than that in the Chol group ($P < 0.025$). The TG concentration was greater in the Chol group than in the other three groups

TABLE 2

Plasma lipids and total cholesterol concentration in liver of rats fed diets with and without 1% cholesterol (Chol) and with and without 7% persimmon (Pers)^{1,2}

Diet	Plasma lipids						Liver
	TC	LDL-C	HDL-C	TG	TPH	HDL-PH	TC
	mmol/L						μmol/g
Control	2.85 ± 0.14 ^c	1.25 ± 0.06 ^c	1.59 ± 0.08	0.69 ± 0.06 ^b	1.78 ± 0.18 ^a	1.06 ± 0.09 ^a	5.85 ± 0.15 ^c
Pers ³	2.73 ± 0.12 ^c	1.13 ± 0.05 ^c	1.60 ± 0.06	0.67 ± 0.05 ^b	1.63 ± 0.15 ^a	1.04 ± 0.07 ^a	5.70 ± 0.14 ^c
Chol	4.88 ± 0.24 ^a	3.27 ± 0.15 ^a	1.58 ± 0.09	0.89 ± 0.09 ^a	1.73 ± 0.21 ^a	0.58 ± 0.04 ^c	49.9 ± 0.51 ^a
Chol/Pers	3.88 ± 0.19 ^b	2.24 ± 0.11 ^b	1.62 ± 0.08	0.72 ± 0.06 ^b	1.32 ± 0.14 ^b	0.73 ± 0.06 ^b	32.8 ± 0.49 ^b
ANOVA (P-value)							
Pers	0.01	0.01	NS	0.01	0.01	0.01	0.01
Chol	<0.001	<0.001	NS	<0.001	0.01	<0.001	<0.001
Pers × Chol	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001

¹ Values are means ± SEM, *n* = 10.

² Means without letters in common differ significantly (*P* < 0.05).

³ Abbreviations used: Chol, nonoxidized cholesterol; HDL-C, HDL cholesterol, HDL-PH, HDL phospholipids; LDL-C, LDL cholesterol; NS, not significant (*P* ≥ 0.05); TC, total cholesterol; TG, triglycerides; TPH, total phospholipids.

(Table 2), indicating that the persimmon-supplemented diet significantly prevented the rise of TG in rats fed cholesterol (19% vs. Chol group, *P* < 0.001). Plasma concentration of TPH in the Chol/Pers group was less than that in the Chol group (Table 2, 23%, *P* < 0.001), indicating that the persimmon-supplemented diet significantly decreased TPH in rats fed cholesterol. The concentration of HDL-PH was less in both the Chol/Pers and Chol groups than in the C and Pers groups (Table 2). Its concentration in the Chol/Pers group was greater than that in the Chol group (25.8%, *P* < 0.001), indicating that the persimmon-supplemented diet lessened the decrease in HDL-PH due to dietary cholesterol. Liver weight was 4.33 g in all groups. Liver TC concentration in rats fed Chol/Pers and Chol were five and eight times higher than in those fed the C and Pers diets, respectively. The persimmon-supplemented diet significantly hindered the rise of liver TC due to dietary cholesterol (Table 2) (34% vs. Chol group, *P* < 0.001). Plasma LP concentrations were greater in both the Chol/Pers and Chol diet groups compared with the C and Pers groups (Fig. 1). This concentration was also significantly greater in Chol- than in Chol/Pers-fed rats (32%, *P* < 0.001); thus persimmon again reduced the cholesterol-induced increase in plasma LP.

DISCUSSION

Some authors have claimed that a diet rich in vegetables and fruits can prevent atherosclerosis (Lorgeril et al. 1994, Partiff et al. 1994). At present, the markets of Europe and North America offer many tropical fruits such as persimmon, guava, wax apple, lichi, rambutan, mango and pineapple (Brekke 1992). Based on recent investigations (Gorinstein et al. 1993 and 1994), the main object of this study was to evaluate persimmon's ability to prevent atherosclerosis. The major classical risk factor for atherosclerosis, hyperlipidemia, remains a foundation of this disease (Faggiotto et al. 1984, Faggiotto and Ross 1984). The fight against hyperlipidemia and other classical risk factors, including hypertension, cigarette smoking and diabetes mellitus, is the basis for the prevention of atherosclerosis. Indeed, intensive reduction of the above-mentioned risk factors has a beneficial effect, i.e., the disease regression in the risk reduction group is twice as fre-

quent as in control group (Haskell et al. 1995). Since the work of Anitschkow (1913), it has been known that a proper diet is a very important measure in preventing hyperlipidemia. Dietary fiber can exercise a favorable effect on lipid metabolism

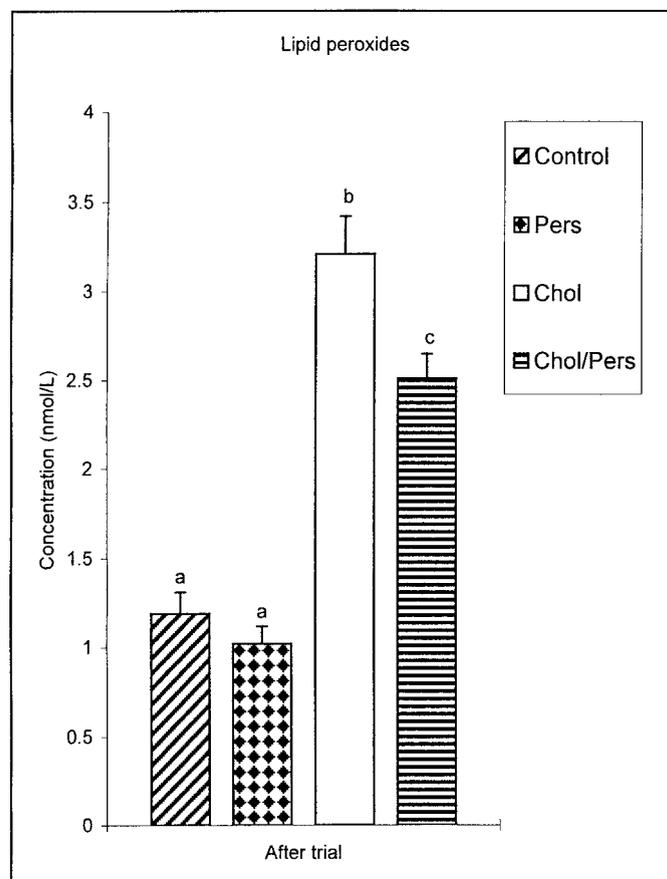


FIGURE 1 Lipid peroxide concentrations in plasma of rats fed diets with and without 1% cholesterol (Chol) and with and without 7% persimmon (Pers) for 4 wk. Values are means ± SEM (vertical lines), *n* = 10. Bars with different letters are significantly different (*P* < 0.05).

(Kritchevsky 1987, Moore 1967, Tsai et al. 1976). But could persimmon, which contains water-soluble components of dietary fiber, carotenoids and polyphenols positively influence lipid metabolism and antioxidant activity in rats? We have found that persimmon exerted positive effects on lipid metabolism; the TPH, TC and LDL-C concentrations in the Chol/Pers diet group were significantly lower than those in the Chol diet group. We had expected that the persimmon-supplemented diet, which contains soluble fiber, would positively influence lipid metabolism. It is important to emphasize that in the rats fed the Chol/Pers diet, the level of HDL-PH was lower than in controls and higher than in Chol diet group. Kunz et al. (1994) claimed that the amphiphilic properties of HDL-PH are essential for removal and transport of hydrophobic cholesterol, and therefore the level of HDL-PH is more important than the level of HDL-C for this function. Our results are consistent with those obtained by Kiryama et al. (1969), who conducted similar experiments in rats. Kirby et al. (1981), Turnbull and Leeds (1987) and Davidson et al. (1991), who used oat preparations in their human studies, also found a decrease in the levels of LDL-C.

As in this study, Tsai and Chen (1979) and Uysal (1986) observed that a cholesterol-containing diet increases plasma LP. To our knowledge, ours is the only study to reveal the positive influence of persimmon on the antioxidant status of rats. According to Lin (1994), certain components of dietary fiber also exert antioxidant effects. We found that the increase in plasma LP concentration due to dietary cholesterol was lower in the Chol/Pers than in the Chol group (fivefold vs. eightfold). This was predicted because of persimmon's high concentration of carotenoids, polyphenols and soluble fiber, which could positively influence antioxidant activity (Daood et al. 1992, Gorinstein et al. 1994, Gross 1987). The exact mechanism of the action of persimmon is not known and deserves further investigation. In conclusion, this study demonstrates that persimmon has a significant influence on rat lipid metabolism. The persimmon-supplemented diet significantly hindered cholesterol-induced increases in TC, TG and LDL-C levels. Persimmon possesses a certain antioxidant effect. Therefore, the addition of persimmon to a generally accepted diet may be beneficial in the prevention of atherosclerosis; however, further study is required.

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