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The effects of diets, supplemented with either whole persimmon or phenol-free persimmon, on rats fed cholesterol

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Abstract

The purpose of this study was to compare the hypolipidemic and antioxidant effects of two diets in rats fed cholesterol (NOC). The experiment was performed on 3 groups of growing male Wistar rats. In each group there were 12 animals and during 4 weeks they were fed different diets: the control group (CG) fed semipurified diet with 1% of NOC, and two experimental groups (EG1) and (EG2) fed the same diet fortified with 7% of whole dry persimmon and 7% of phenol-free dry persimmon, respectively. Before and after the 4 week trial period, in rats of all groups, total cholesterol (TC), LDL-C, HDL-C, tryglicerides (TG) and lipid peroxides (LP) were studied. The results of the experiment showed a statistically significant increase in plasma TC and LDL-C only in CG ($P < 0.0005$) and EG2 ($P < 0.0025$). The increase of TC and LDL-C in the EG-1 fed diet, supplemented with whole persimmon, was statistically not significant (P for both < 0.1). Only in EG1 was a statistically not significant increase in HDL-C registered ($P < 0.1$). The decrease in HDL-C/TC ratio in this group of rats was minimal (from 0.57 to 0.54). A significant increase of LP was found in all three groups. An increase of LP with the EG2-fed diet, supplemented with phenol-free persimmon, was equal to the increase of LP in CG. For the EG1-fed diet supplemented with whole persimmon, a statistically less significant increase of LP was found ($P < 0.05$ versus both EG2 and CG). The results of this experiment show that both diets fortified with 7% of whole dry persimmon and with 7% of phenol-free dry persimmon improve lipid levels. But only diet supplemented with whole persimmon exerts an antioxidant effect. Therefore, the antioxidant effect of this fruit is associated mainly with persimmon phenols and not with the persimmon fibre.

Keywords: Persimmon; Polyphenol free persimmon; Plasma lipids; Lipid peroxides; Antioxidant effect; Rats

1. Introduction

It has been shown that nutritional fibre influences lipid metabolism (Kiryama, Okazaki & Joshida, 1969; Kirby et al., 1981; Turnbull & Leeds, 1987; Davidson, Dugan, Burns, Bova, Story & Drennan, 1991). Kiryama et al. (1969) conducted experiments on rats and found that dietary fibre leads to a decrease in the level of total cholesterol. Davidson et al. (1991), Kirby et al. (1981) and Turnbull and Leeds (1989) used oat preparation in their studies in humans. All of them registered a statistically significant decrease in the level of LDL-C. There are conflicting reports about antioxidant properties of dietary fibre (Lin, 1994). According to Lin (1994), certain components of this fibre do exert an antioxidant effect. In our previous studies on rats we found that diet supplemented with persimmon positively influences lipid levels and antioxidant activity (Gorinstein et al., 1998). But we used whole persimmon fruit, which contains not only dietary fibre but also polyphenols (Gorinstein et al., 1994). It is known that phenolic compounds can be divided into soluble or extractable [EPP] and non-extractable [NEPP] (Bravo, Abia & Saura-Calixto, 1994). The EPP are substances with low or intermediate molecular mass. They can be extracted by different solvents. The NEPP are mainly condensed tannins of high molecular mass. Part of the tannins is in free form and part is bound to protein or fibre (Saura-Galixto, Goni, Manas & Abia, 1991; Terril, Rowan, Douglas & Barry,

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Nomenclature

HDL-C	high density lipoprotein cholesterol
LDL-C	low density lipoprotein cholesterol
LP	lipid peroxides
TC	total cholesterol
TG	triglycerides
VLDL-C	very low density lipoprotein cholesterol

1992). EPP are absorbed from the digestive tract and affect the metabolic utilization of amino acids (Barry, Allsop & Redekopp, 1986). NEPP are not absorbed in the intestine and can be recovered quantitatively in feces (Bravo, Saura-Calixto & Goni, 1992; Bravo, Manas & Saura-Calixto, 1993). It was decided to extract EPP from persimmon fruit and to explore the influence of diet supplemented with whole persimmon versus phenol-free persimmon on rats fed cholesterol. We have supposed that exclusion by extraction of EPP would significantly affect the antioxidant properties of this fruit. Persimmon is marketable throughout Europe but is not available all year round. Even at the time of picking it is not immediately edible and must be appropriately stored until the persimmon is completely ripe and loses its astringency, depending on its high tannins content. It is necessary to mention that this fruit is very delicate and the amount of mechanically damaged or bruised fruits is considerable. Therefore, it would be interesting to know whether addition also of dry whole persimmon to the diet of rats would be effective. As far as we know there have been no such investigations.

2. Materials and methods

2.1. Animals and diets

All treatments and diets were formally approved by Warsaw Agricultural University Animal Ethics Committee.

The experiment was conducted on 36 male Wistar rats with a standard initial weight of 120 g. They were housed individually in stainless steel metabolic cages and were randomly assigned to three numerically equal groups, each of 12 rats: two experimental (EG1 and EG2) and one control (CG). All were fed, during a 4 week period, basal diet (BD) which included wheat starch, casein, soy bean oil, mineral mixture and vitamin mixture and cholesterol (NOC). The BD of the animals of the CG was supplemented with 7% of cellulose, of the EG1, with 7% of whole dry persimmon fruit and, of the EG2, with 7% of phenol-free dry persimmon. All above-mentioned components of the different diets are presented in the Table 1.

The NOC used was of analytical grade (USP) and was bought from "Sigma Chemical" just before the beginning of this study. The NOC batches were mixed carefully in a proportion of 1 g of NOC to 99 g of diets just before these diets were offered to the rats. The phenol-free persimmon was prepared as follows: the crude phenols were extracted from all parts of the persimmon fruit with ethyl acetate during 21 days at room temperature in ratio material: solvent 1:1.5 as described previously (Gorinstein et al., 1994). The extracted total phenols constituted 0.14% of the total weight of the whole persimmon fruit. All animals were fed ad libitum and the intake of the diets was monitored daily. The diets were offered once daily at 10 a.m. as accepted in Warsaw Agricultural University. Before the experiment, and after 4 weeks of feeding, blood samples were drawn from the tail vein. Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and lipid peroxides (LP) were determined as previously described (Gorinstein et al., 1998). TC, HDL-C, and TG were determined enzymatically. TC and TG were measured as described by Trinder & Webster (1984) with kits (PAP 100, No. 6.122.4 and 6.123.6, respectively); HDL-C was 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 a kit (No. 6.159.1) from Bio Merieux (Marcy l'Etoile, France). Lipid peroxide (LP) was determined colorimetrically (Tateishi et al., 1987) by the direct reaction with the methylene blue derivative, MCDP, (10-*N*-methyscarbamoyl-3, 7-dimethylamino-10H-phenothiazine) catalyzed by hemoglobin using a kit (9 No. CC-004) from the Kamiya Biomedical Company. LDL-C was calculated according to the Friedewald formula (Friedewald et al., 1972).

Table 1
Composition of the diets (%)

Ingredients	EG1	EG2	CG
Wheat starch	61	61	61
Casein	15	15	15
Soybean oil	11	11	11
Mineral mixture[a]	4	4	4
Vitamin mixture[b]	1	1	1
Whole dry persimmon	7	–	–
Phenol-free dry persimmon	–	7	–
Cellulose	–	–	7
NOC	1	1	1

[a] Minerals (per kg of diet): $CaHPO_4$, 15 g; K_2HPO_4, 2.5 g; KCl, 5 g; NaCl, 5 g; $MgCl_2$, 2.5 g; Fe_2O_3, 2.5 mg; $MnSO_4$, 125 mg; $CuSO_4 \cdot 7H_2O$, 0.2 mg; $ZnSO_4 \cdot 7H_2O$, 100 mg; KIO_3, 0.4 mg.

[b] Vitamins (per kg of diet): thiamin, 20 mg; riboflavin, 15 mg; pyridoxin, 10 mg; nicotinamide, 100 mg; calcium panthothenate, 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; choline, 1.36 g, carrier wheat starch.

2.2. Statistics

Values are given as means and ± SD. Data for the three dietary groups were tested by one-way ANOVA. Differences of $P < 0.05$ were considered significant.

3. Results

Addition of persimmon to the diets did not affect diet intake, its efficiency or body weight gains of rats. At baseline, all three groups did not differ from one another in the plasma lipid concentration or in the level of lipid peroxides (therefore, data not shown). Figs. 1–5 graphically reflect changes in the levels of lipids and in the antitoxidant activity. Fig. 1 summarizes the results of the changes in TC level in all 3 groups after the experimental period. After the trial, an increase in TC in all 3 groups was registered. But only in EG2-fed BD, NOC and phenol-free persimmon and in CG-fed BD and NOC was this increase statistically significant ($P < 0.0005$ for both groups). The increase in the level of TC in the EG1 was statistically not significant ($P < 0.1$). It was also found that the increase in TC in CG was statistically significantly greater than in EG2 ($P < 0.0125$). Fig. 2 shows the LDL-C level. The changes in LDL-C level were very similar to the changes in TC. An increase in the level of LDL-C, after 4 weeks of feeding in all 3 groups, was

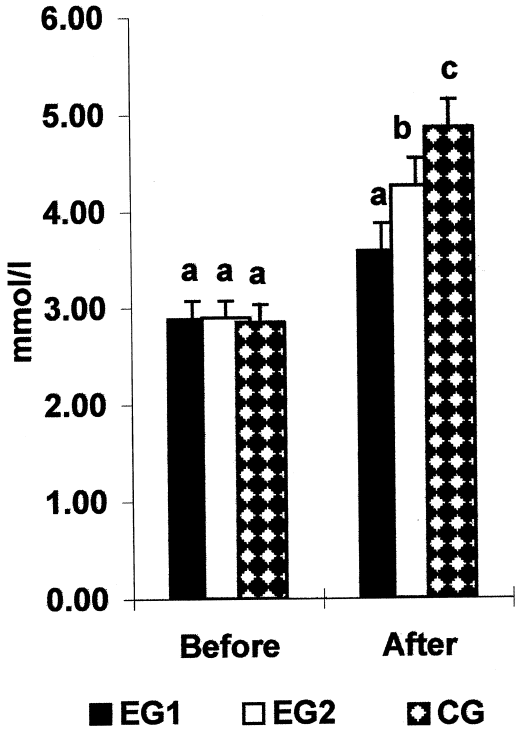

Fig. 1. Changes in total cholesterol level after the experiment. Means±S.D. (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

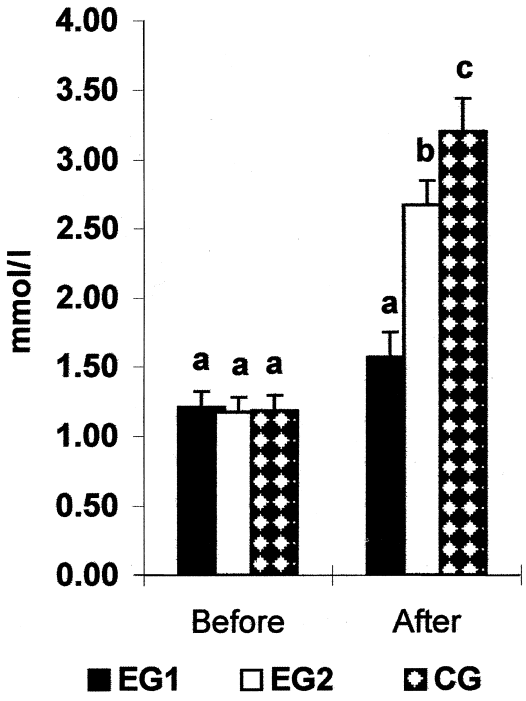

Fig. 2. Changes in LDL-C level after the experiment. Means±S.D. (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

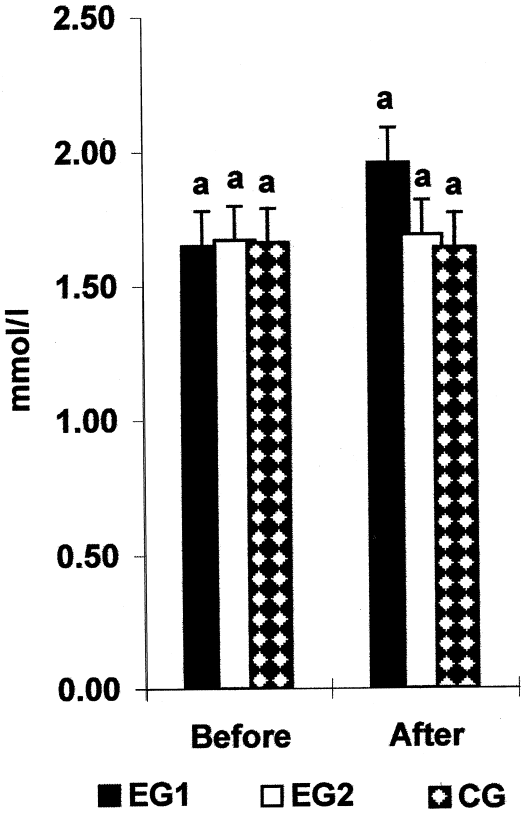

Fig. 3. Changes in HDL-C level after the experiment. Means±S.D. (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

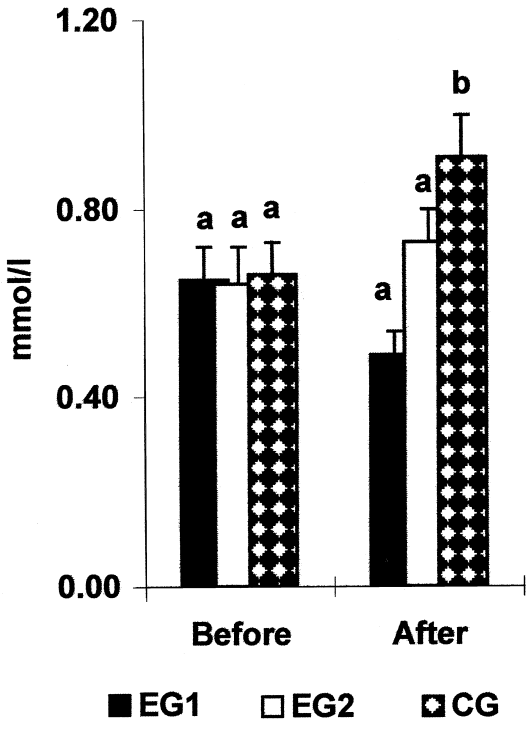

Fig. 4. Changes in TG level after the experiment. Means±S.D. (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

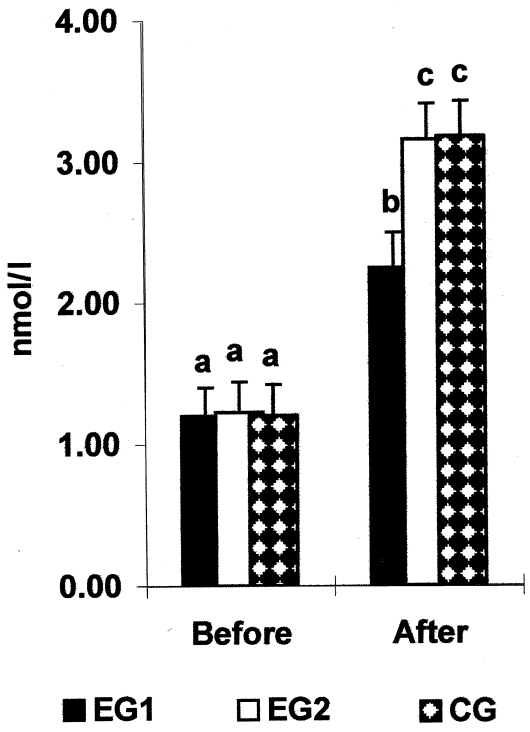

Fig. 5. Changes in the level of lipid peroxides after the experiment. Means±S.D. (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

registered. But only in EG2 and in CG was the increase statistically significant ($P < 0.0005$ for both groups). The increase of LDL-C in EG1 was statistically not significant ($P < 0.1$). Fig. 3 summarizes the HDL-C in all 3 groups after 4 weeks of different feeding. Only in the CG was a decrease in the level of HDL-C found, but this decrease was statistically not significant ($P < 0.45$). In both EG1 and EG2 an increase in the level of HDL-C was registered, but this increase was statistically not significant ($P < 0.2$ and $P < 0.475$, respectively). It is important to underline that the decrease of the HDL-C/TC ratio was minimal only in the EG1 (from 0.57 to 0.54). Fig. 4 shows the changes in TG level. Only in CG was a statistically significant increase in the level of TG noted ($P < 0.025$). The increase in TG level in EG2 was statistically not significant ($P > 0.25$) and the TG level in EG1 remained unchanged. Fig. 5 shows the changes in the level of LP. After 4 weeks of feeding, in all 3 groups, a statistically significant increase in LP was found ($P < 0.0025$, 0.0005 and 0.0005 for EG1, EG2 and CG, respectively). The increase in LP in EG2 and CG was statistically significantly greater than in EG1 ($P < 0.05$).

4. Discussion

Recent studies suggest that one of the important mechanisms of atherosclerosis is the oxidation of cholesterol-rich LDL-C particles (Steinberg, Parthasarathy, Carew, Khoo & Witztum, 1989; Steinbrecher, Zhang & Londheed, 1990; Witztum & Steinberg, 1991; Aviram, 1993). The oxidation of LDL-C enhances its atherogenicity and facilitates penetration of lipids into arterial walls (Steinberg et al., 1989; Steinbrecher et al., 1990). It has been shown that phenolic substances possess antioxidant properties and inhibit the oxidation of LDL-C in vitro and in vivo (Harborne, 1989; Frankel, Kanner, German, Parks & Kinsella, 1993; Frankel, Waterhouse & Kinsella, 1993; Maxwell, 1993; Rankin et al., 1993; Morel, Lescoat, Cillard & Cillard, 1994). The natural sources of nutritional phenols are fruits and vegetables. Therefore, some authors propose diets rich in these compounds for prevention of atherosclerosis (Lorgeril et al., 1994; Partiff, Rubber, Boston, Marotta, Hartog & Mancini, 1994). In the modern world of extensive trade ties, some tropical fruits, such as pineapple, wax apple, rambutan, lichi, guava and mango are available in Europe and North America. As stated, the aim of this investigation was to assess the influence of whole persimmon versus phenol-free fruit and in particular to study the impact of diets, supplemented with whole or phenol-free persimmon fruit, on lipid levels and antitoxidant activity of rats fed cholesterol. As far as we know this is the first experiment of its kind concerning this fruit. Both diets, fortified with 7% of whole dry persimmon or with 7% of phenol-free dry persimmon, improved lipid levels but

the result was statistically significant only with addition of whole fruit.

According to Piretti (1991), the mean composition of persimmon fruit is: water, 78–84.6% and dry residue, 15.4–21.6%. The dry residue includes (in %): soluble and insoluble proteins (0.64–1.3), total sugars (14.7–19.6), reducing sugars (13.8–15.8), tannins (0.20–1.41), phenols (0.16–0.25). The phenolic components, after ripening of persimmon fruit, undergo prominent polymerization, resulting in formation of macromolecular substances, which are no longer water-soluble. Therefore, phenols were extracted by ethyl acetate. The four weeks of feeding different diets has shown that phenols in whole dry persimmon exercise a marked antioxidant effect, which was more statistically significant than the effects of phenol-free fruit. These results were expected; phenols of persimmon are 20 times more potent in vitro than the classical antioxidant vitamin E (Uchida et al., 1989, 1990). As was mentioned, the rise of LP in EG2 was equal to the rise of LP in CG. This fact indicates that phenol-free persimmon does not exercise an antioxidant effect.

5. Conclusion

1. Both diets fortified with 7% of whole dry persimmon or with 7% of phenol-free dry persimmon improved lipid level. This improvement was significant when whole dry persimmon was added. 2. The antioxidant effect of persimmon fruit was mainly associated with its phenols and not with its soluble fibre. 3. The antioxidant effect of persimmon was achieved by adding whole dry fruit to basal diet.

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