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The influence of persimmon peel and persimmon pulp on the lipid metabolism and antioxidant activity of rats fed cholesterol

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The aim of this study was to compare the hypocholesterolemic and antioxidant effects of two diets supplemented with dry persimmon in rats fed cholesterol (C). Three groups of male Wistar rats each of 13 animals during 4 weeks were fed different diets: the control group (CG)—semipurified diet with 1% of C and two experimental groups (EG1) and (EG2)—the same diet fortified with 7% of dry persimmon peel and dry persimmon pulp, respectively. In animals of all three groups before and after the 4-week trial period total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG), and lipid peroxides (LP) were examined. After the completion of the experiment a statistically significant increase in plasma TC and LDL-C in all three groups was found. In the animals of EG1 this increase was statistically less significant than in CG ($P < 0.05$ and $P < 0.025$ for TC and LDL-C, respectively). A statistically significant increase in the level of HDL-C was observed. The smallest one was in EG1. But only in the EG1 the HDL-C/TC ratio was increased more significantly (from 0.56 to 0.59). In EG1 a statistically less significant increase in LP than in CG ($P < 0.01$) was registered.

The present results demonstrate that persimmon fruit exercises a hypocholesterolemic and antioxidant effects and therefore is considered for an antiatherosclerotic diet. A diet fortified with dry persimmon peel is more efficient than the same diet fortified with dry persimmon pulp. Therefore the persimmon peel showing the effectiveness of its antioxidant activity can be used by individual consumers and in industrial processing. (J. Nutr. Biochem. 9:223–227, 1998) © *Elsevier Science Inc. 1998*

Keywords: persimmon; pulp; peel; hypocholesterolemic; antioxidant effects; rats

Introduction

Atherosclerosis is the pathanatomic basis of coronary artery disease (CAD)—the most dangerous disease in the Western industrialized countries. CAD is responsible for one of every three deathes in men as well as in women.[1,2] In the last years some authors show a great importance of diets rich in vegetables and fruits for prevention of atherosclerosis.[3,4] According to experimental and clinical investigations the natural antioxidants of plants such as vitamins A, C, and E are able to reduce the morbidity and mortality from CAD by preventing oxidation of LDL-C.[5,6] Similar characteristics are attributed to polyphenols. The recent studies in vitro and in vivo revealed that phenolic compounds posses antioxidant properties.[7–10] A high intake of polyphenols (flavonoids) can significantly reduce the risk of mortality from cardiovascular diseases.[11] In the last years among other fruits some authors propose persimmon as a good source of nutritional antioxidant vitamins, polyphenols, and dietary fiber.[12,13] The concentration of these important components inter alia carotenoids and polyphenols is higher in the peel than in the pulp.[12,13] These facts are relevant to all three

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Table 1 The composition of the diets (%)

Components	EG1	EG2	CG
Corn starch	60	60	60
Casein	16	16	16
Soy bean oil	11	11	11
Mineral mixture[1]	4	4	4
Vitamin mixture[2]	1	1	1
Dry persimmon peel	7	—	—
Dry persimmon pulp	—	7	—
Cellulose	—	—	7
NOC	1	1	1

[1]Mineral supplied (per kg of diet): $CaHPO_4$, 15g; K_2HPO_4, 2.5g; KCl, 5g; NaCl, 5g; $MgCl_2$, 2.5g; Fe_2O_3, 2.5mg; $MnSO_4$, 125mg; $CuSO_4.7H_2O$, 0.2mg; $ZnSO_4.7H_2O$, 100mg.
[2]Vitamins supplied (per kg of diet): thiamin, 20 mg; riboflavin, 15 mg; pyrodoxin, 10 mg; nicotinamide, 100 mg; calcium panthotenate, 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 corn starch.

major groups of carotenoids (β-carotene, α-carotene, and zeaxanthin) and the two groups of polyphenols (ethanol and methanol soluble).[12,13] But most individuals do not use the peel of the persimmon. The same is true in some cases for industrial processing. In order to examine if such practice is justified we decided to study the influence of different diets (one, supplemented with persimmon peel and the second, supplemented with persimmon pulp) on lipid metabolism and antioxidant activity of rats. According to the results of this investigation we will come to a conclusion if to recommend the use of persimmon peel to human beings.

Methods and materials

Rats

In these series of the experiment we used 39 male Wistar rats with a standard weight of 120 g. They were housed individually in stainless steel metabolic cages and were divided into three groups: two experimental (EG1 and EG2) and one control (CG) each 1 of 13 rats.

Diets

The rats of all three groups were fed during a 4-week experimental period a basic diet (BD). The exact composition of the basic diet is presented in the *Table 1*. As is shown in this table the diets for all three groups include cholesterol. The nonoxidized cholesterol (NOC) of analytical grade was obtained from Sigma Chemical Co. (St. Louis, MO USA) The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the animals. The BD was supplemented with 7% of dry persimmon peel and 7% of dry persimmon pulp for EG1 and EG2, respectively. Instead of dry persimmon peel or pulp in BD for CG was included cellulose (7%). The energy of the diets was from 393.7 to 401.7 kcal/100g and this difference was not significant statistically. All rats were fed ad libitum and the intake of the diets was monitored daily. The diets were given once daily at 10 AM.

Laboratory tests

Before and after the experiment the blood samples were drawn from tail vein and then a wide range of laboratory tests was performed. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) by enzymatic methods using Bio Merieux kits (Marcy l'Etoile, France) and the lipid peroxides (LP)-colorimetrically (Manuel of Laboratory Operations, 1974)[14] according to Liebermann and Burchard (LRCP) were determined.

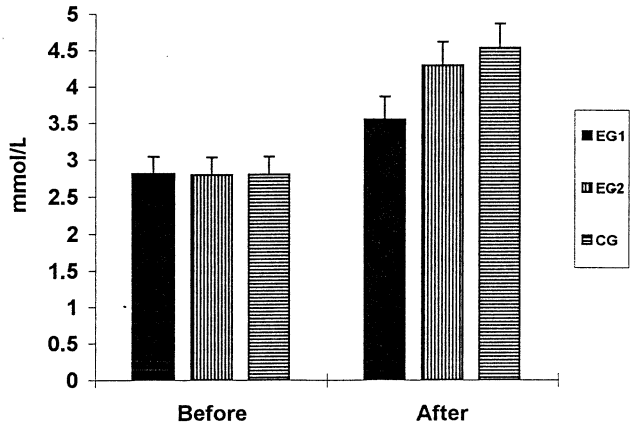

Figure 1 Changes in TC metabolism before and after the experiment.

Statistics

For definition of the statistical significance of the changes in various parameters the standard Student-Fisher tp test was used. For this purpose means and standard deviations ($M \pm m$) were defined. All laboratory parameters not only the value of means but also their 95% confidence intervals (CI) were calculated. The significance of t was obtained by equation: $t = (M_2 - M_1)/\sqrt{(m_1^2 + m_2^2)}$. Then according to the t value the significance of P from the table "Significance Limits of the Student Distribution" was found. The P values of <0.05 were adopted as statistically significant.

Results

Total cholesterol

The mean values of TC for the EG1, EG2, and CG before the investigation were 2.81, 2.79, 2.80 and their CI: 2.31–3.31, 2.27–3.31, and 2.28–3.32 mmol/L, respectively. After 4 weeks of feeding the mean values of TC for EG1, EG2, and CG were 3.55, 4.29, 4.53 and their CI: 2.88–4.22, 3.60–4.98, and 3.82–5.24 mmol/L, respectively. *Figure 1*, which is based on the $M \pm m$ values, reflects the changes in the TC metabolism after completion of the investigation. According to these data in all three groups of animals a statistically significant increase in the level of TC was recorded. This increase was significantly lower in EG1 (animals fed a diet supplemented with dry persimmon peel) than in CG ($P < 0.05$). The level of TC in EG2 was also higher than in EG1, but this difference was statistically not significant ($P < 0.1$).

LDL-C

The mean values of LDL-C for EG1, EG2 and CG before the investigation were 1.19, 1.21, 1.20 and their CI: 0.91–1.47, 0.91–1.51, and 0.92–1.48 mmol/L, respectively. After

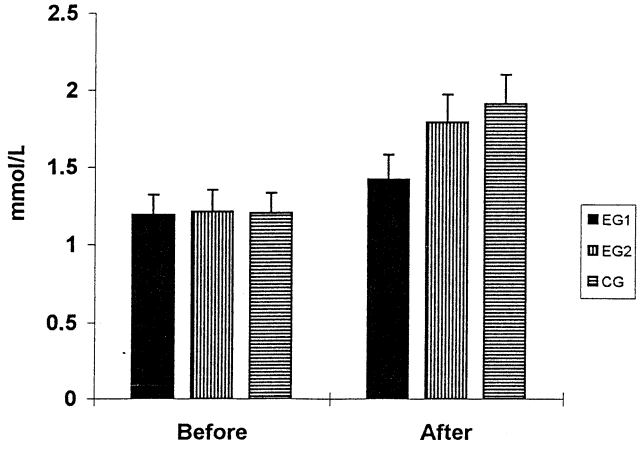

Figure 2 Changes in LDL-C metabolism before and after the experiment.

the investigation the LDL-C mean values for EG1, EG2, and CG were 1.42, 1.79, 1.91 and their CI: 1.08–1.76, 1.40–2.18 and 1.50–2.32 mmol/L, respectively. *Figure 2*, which is based on the $M \pm m$ values, shows the changes in LDL-C metabolism after 4 weeks of feeding. According to these data, in all three groups an increase in the level of LDL-C was found. But only in the EG1 this increase was statistically less significant than in CG ($P < 0.025$).

HDL-C

The mean values of HDL-C for EG1, EG2 and CG before the investigation were 1.60, 1.57, 1.58 and their CI: 1.23–1.97, 1.23–1.91, and 1.24–1.92 mmol/L, respectively. After the trial period the HDL-C means for EG1, EG2, and CG were 2.11, 2.49, 2.60 and their CI: 1.66–2.56, 2.02–2.96, and 2.1–3.1 mmol/L, respectively. *Figure 3*, which is based on the $M \pm m$ values, demonstrates the changes in the HDL-C metabolism after the completion of the experiment. In all three groups an increase in the level of HDL-C was observed. This increase was less in the EC1 but statistically not significant in comparison with EG2 and CG ($P < 0.15$

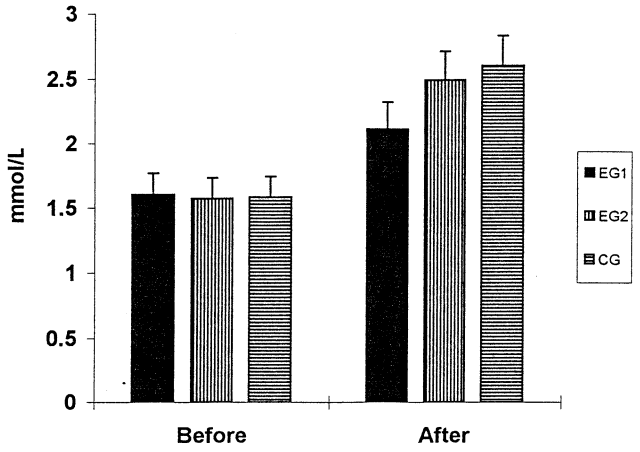

Figure 3 Changes in HDL-C metabolism before and after the experiment.

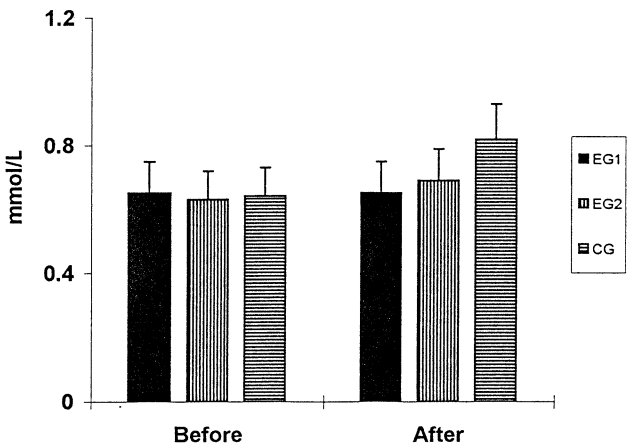

Figure 4 Changes in TG metabolism before and after the experiment.

and $P < 0.1$, respectively). But only in this group of animals a marked increase in the HDL-C/TC ratio (from 0.56 before to 0.59 after the investigation) was found.

Triglycerides

The mean values of TG for EG1, EG2 and CG before the investigation were 0.65, 0.63, 0.64 and their CI: 0.43–0.87, 0.41–0.85, and 0.45–0.83 mmol/L, respectively. After 4 weeks of feeding the mean values of TG were 0.65, 0.69, 0.82 and their CI: 0.43–0.87, 0.47–0.91, and 0.58–1.06 mmol/L, respectively. *Figure 4*, which is based on $M \pm m$ data, indicates that only in the EG1 any changes in TG metabolism after the trial period were indicated. An increase in the level of TG in the animals of the two other groups was registered. But this increase was statistically not significant ($P < 0.35$ and $P < 0.15$ for EG2 and CG, respectively).

Lipid peroxides

The mean values of LP for EG1, EG2, and CG before the experiment were 1.2, 1.18, 1.19 and their CI: 0.88–1.52, 0.84–1.52, and 0.85–1.53 nmol/L, respectively. After the completion of the experiment the mean values of LP for EG1, EG2, and CG were 2.81, 3.49, 3.7 and their CI: 2.31–3.31, 2.84–4.14, and 3.03–4.37 nmol/L, respectively. *Figure 5*, which is based on the $M \pm m$ values, reflects the changes in the level of LP after completion of the investigation. In all three groups we noted a statistically significant increase in the level of LP ($P < 0.0005$ for all three groups). But the increase in the LP level in the EG1 and EG2 was statistically less significant than in CG ($P < 0.01$ and $P < 0.05$, respectively).

Discussion

In 1913 Anitschkow[15] created an animal model of atherosclerosis and since then hypercholesterolemia is firmly connected to the above mentioned pathological process. It was proved by epidemiological, experimental, and clinical investigations. In spite of the fact that scientists discovered some other risk factors for atherosclerosis like hypertention,[16,17] smoking,[18,19] diabetes mellitus,[20,21] and physical

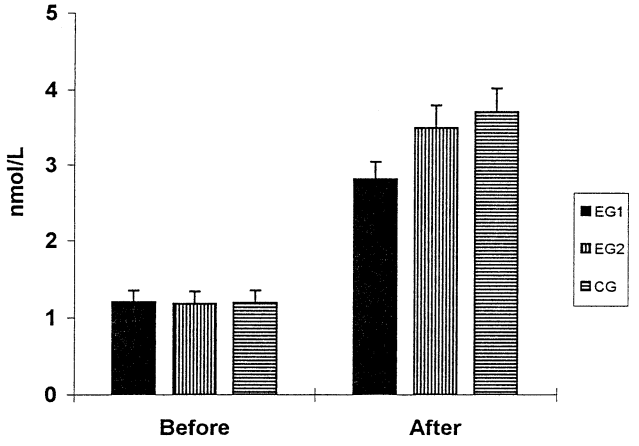

Figure 5 Changes in the level of LP before and after the experiment.

inactivity[22,23] hypercholesterolemia remains the major one. But in recent years the scientific community is looking beyond cholesterol.[24] It was shown that one of the important mechanisms predisposing to the development of atherosclerosis is oxidation of cholesterol-rich LDL particles.[25,26] The oxidation of LDL-C enhances its atherogenicity[24,27] and facilates penetration of lipids into arterial wall. In the last 10 years some authors have shown that nutritional antioxidants inhibit the oxidation of LDL-C and in this way prevent atherosclerosis.[5,28] This fact is the basis for the increased interest in new vegetables and fruits for atherosclerosis-preventing diets. Our attention attracted persimmon fruit. According to our previous investigations the concentration of antioxidants and polyphenols in persimmon was higher in peel than in pulp. Therefore, we decided to conduct a study to investigate hypocholesterolemic and antioxidant properties of persimmon with emphasis on the use of dry persimmon peel and pulp. The two EGs and CG were fed the same semipurified diet containing cholesterol. But the diets for EG1 and EG2 were fortified with 7% of dry persimmon peel and 7% of dry persimmon pulp, respectively. The control diet was containing 7% of cellulose in order to exclude the effect of the fiber content of persimmon on lipid metabolism. Regarding the numerous studies demonstrating that soluble and insoluble dietary fiber reduces cholesterol absorption from cholesterol supplemented diets the BD diet was containing similar components except persimmon. Therefore the results of this experiment demonstrated specific persimmon effect with greater antioxidant activity by persimmon peel as opposed to pulp.

After 4 weeks of feeding we recorded:

1. A statistically less significant increase in the level of total cholesterol in both EGs than in CG.
2. A statistically less significant increase in the level of LDL-C in EG1 than in CG.
3. A marked increase in the HDL-C/TC ratio only in EG1.
4. A statistically less significant increase in the level of lipid peroxides in both EGs than in the CG.

All these differences were more significant for EG1 fed a diet fortified with dry persimmon peel.

In conclusion, we were able to show that:

1. persimmon fruit exercises hypocholesterolemic and antioxidant effects,
2. the main source of these indices is the persimmon peel,
3. therefore not only the persimmon pulp but also the persimmon peel can be used by individual consumers and in industrial processing,
4. as a fruit with antilipidic and antioxidant properties persimmon can be recommended for antiatherosclerotic diets.

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