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Pistachio Intake Increases High Density Lipoprotein Levels and Inhibits Low-Density Lipoprotein Oxidation in Rats

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Aksoy, N., Aksoy, M., Bagci, C., Gergerlioglu, H.S., Celik, H., Herken, E., Yaman, A., Tarakcioglu, M., Soydinc, S., Sari, I. and Davutoglu, V. *Pistachio Intake Increases High Density Lipoprotein Levels and Inhibits Low-Density Lipoprotein Oxidation in Rats*. Tohoku J. Exp. Med., 2007, **212** (1), 43-48 —— There is increasing evidence that nuts have protective effects against coronary artery disease by improving lipid profile and inhibiting lipid oxidation. However, data about pistachio nuts are limited, and to our knowledge, there is no study investigating the effects of pitachio intake on lipid oxidation and serum antioxidant levels. This study, therefore, sought to determine the effects of pistachio intake on serum lipids and determine whether consumption of pistachio would alter serum antioxidant levels. Rats were randomly divided into three groups (n = 12 for each): control group fed basic diet for 10 weeks and treated groups fed basic diet plus pistachio which constituted 20% and 40% of daily caloric intake, respectively. Consumption of pistachio as 20% of daily caloric intake increased high-density lipoprotein (HDL) levels and decreased total cholesterol (TC)/HDL ratio, compared with those not taking pistachio. However, TC, low-density lipoprotein (LDL) cholesterol and triglyceride levels were unaffected by pistachio consumption. Consumption of pistachio as 20% of daily caloric intake increased serum paraoxonase activity by 35% and arylesterase activity by 60%, which are known to inhibit LDL cholesterol oxidation, compared with the control group. However, increased antioxidant activity was blunted when pistachio intake was increased to 40% of daily caloric intake. In conclusion, the present results show that consumption of pistachio as 20% of daily caloric intake leads to significant improvement in HDL and TC/HDL ratio and inhibits LDL cholesterol oxidation. These results suggest that pistachio may be beneficial for both prevention and treatment of coronary artery disease. ———— pistachio, nut, lipid, paraoxonase, arylesterase.

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Coronary artery disease is the leading cause of morbidity and mortality throughout the world. Dyslipidemia is among one of the most important risk factors for coronary artery disease, in which greater interest have been focused on the increased levels of low density lipoprotein (LDL) and low levels of high density lipoprotein (HDL) by researchers and clinicians. Epidemiologic studies revealed that high monounsaturated fat to saturated fat ratio and polyunsaturated fat to saturated fat ratio diet improve lipid profile and reduce the incidence of coronary artery disease (Mattson and Grundy 1985). Several studies have reported that nuts, especially almonds and walnuts, have beneficial effects on serum lipid levels and subsequent coronary artery disease (Fraser et al. 1992; Spiller et al. 1992; Sabate et al. 1993; Abbey et al. 1994). The specific mechanism or mechanisms for the favourable effects of nuts on lipid profiles and coronary artery disease are unknown, but may be related to the unsaturated fat content (Mattson and Grundy 1985; Mensink and Katan 1989; Reaven et al. 1991).

Paraoxonase (PON1) is known as a calcium-dependent protein and has a molecular mass of 43 kDa. PON1 is synthesized and secreted by liver and mostly located on HDL surface (Mackness et al. 1996). LDL oxidation has an important role in development and progression of coronary artery disease (Itabe 2003). PON1 inhibits LDL oxidation (Mackness et al. 1996). PON1 activity is altered under various conditions such as diet, diseases and clinical conditions, and medications including menopause and hormone replacement therapy (Topçuoğlu et al. 2005). Arylesterase activity, one of the three functions of the paraoxonase enzyme, is thought to protect LDL and HDL from oxidation and facilitate reverse cholesterol transport.

Among nut species, especially walnut and almond have been studied with respect to favourable effects on lipids and cardiovascular system (Spiller et al. 1992; Sabate et al. 1993; Hu and Stampfer 1999; Kris-Etherton et al. 2001). However, there has been very little investigation regarding the effect of pistachio on serum lipids, and to our knowledge, there is no study investigating the contribution of pitachio on antioxidant protection. This study, therefore, sought to determine the effect of different amounts of pistachio intake on serum lipids and determine whether consumption of pistachio would alter circulating levels of antioxidants (PON1 and arylesterase) in rats fed pistachio.

Methods

Animal preparation

The animals were housed in an animal laboratory with a 12:12-hr light-dark cycle, room temperature of 22-24°C, and had free access to diet and drinking water but were fasted overnight except for drinking water before the experiment. Pistachios and mixed standart diet were supplied by the Ministry of Agriculture, Pistachio Investigating Institute.

Basic animal diet

All animals were fed standard basic diet (#07-03-237, by Korkutelim rodent chow, Antalya, Turkey) for 10 days before the study as adaptation period. This diet chow powder contained protein (23%), starch (52%), sucrose (10%), fat (4%), fiber (5%), mineral mixture (3.5%), vitamin mixture (1%), methionine (0.5%) and lysine (1%).

Dietary intervention and study protocol

All procedures were performed according to the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals of Gaziantep University, Gaziantep, Turkey. Rats were then divided randomaly into three groups (12 animals in each group). First group served as control and was fed only basic diet (2,600 kcal/kg energy intake daily). Second group was fed basic diet and pistachio which constituted 20% of daily caloric intake (2.5 g/day). Third group was fed basic diet and pistachio which constituted 40% of daily caloric intake (5 g/day). Daily pistachio intake was decided as 20% of daily caloric intake for the second group, because a subject eats pistachio of 100 g, which is about 570 kcal constituting 20% of daily caloric intake. Fourty percent was decided for the third group in order to see the effect of a higher amount of pistachio on serum lipids and antioxidant capacity. Rats were weighed at least three times a week to ensure that growth rates in all dietary groups were similar. After 10 weeks of feeding, blood samples were collected after overnight fasting.

Biochemical parameters

Blood samples (0.2 ml) were collected into heparinized microcentrifuge tubes. Samples were taken and plasma was separated from red blood cells. For measurement of serum levels of lipids following parameters were investigated: total cholesterol (TC), LDL cholesterol, HDL cholesterol, triglyceride, and TC/HDL ratio. Lipids were determined by spectrophotometric method. Serum PON1 and arylesterase activities were assayed according to Ruiz et al. (1995) and Juretic et al. (2001), respectively. Phenotypic distribution of PON1 activity was determined by the double substrate method. Briefly, the ratio of hydrolysis of paraoxon in the presence of 1M NaCl to hydrolysis of phenylacetate was used to classify rats into three possible phenotypes.

Statistical analysis

Data are expressed as mean ± S.D. Unpaired student *t*-test was used to compare differences between control-fed and pistachio-fed rats, $p < 0.05$ was considered statistically significant. All statistical studies were carried out with SPSS program (version 10.0, SPSS, Chicago, IL, USA).

Results

The effects of pistachio intake on serum lipids and antioxidant levels are shown in Table 1. No significant differences were observed in serum TC, LDL cholesterol and triglyceride levels between the groups.

The HDL cholesterol levels were significantly increased in both groups, which were fed pistachio as 20% and 40% of daily caloric intake in comparison to the control group. However, the difference in HDL cholesterol levels between the groups fed pistachio as 20% and 40% of daily caloric intake were not statistically significant. Thus, the degree of increase in HDL cholesterol in the pistachio-fed groups was not associated with the increased amount of pistachio intake. The TC/HDL ratio was significantly decreased in both groups, which were fed pistachio as 20% and 40% of daily caloric intake in comparison to the control group. However, there was no significant difference in TC/HDL ratio between the two groups, indicating that the degree of decrease in TC/HDL ratio in the pistachio-fed groups was not associated with the increased amount of pistachio intake.

Serum PON 1 activities were significantly increased in both groups fed pistachio as 20% and

Table 1. The effects of pistachio intake on serum lipids and paraoxonase/arylesterase activities.

	Control group (12 rats)	Pistachio as 20% of daily caloric intake (12 rats)	Pistachio as 40% of daily caloric intake (12 rats)
TC (mg/dl)	60 ± 5.2	60 ± 8.5	66 ± 10.3
LDL (mg/dl)	34 ± 4.1	32 ± 3.6	35 ± 7.4
HDL (mg/dl)	17 ± 2.7	21 ± 3.1*	22 ± 2.8*
TG (mg/dl)	42 ± 6.0	32 ± 5.4	39 ± 5.1
TC/HDL	3.9 ± 0.3	2.8 ± 0.4*	3.0 ± 0.3*
PON1 (U/l)	130 ± 17	208 ± 19*	195 ± 14*
Arylesterase (U/l)	210 ± 20	285 ± 24*	250 ± 21*

Comparison of consuming different amounts of pistachio in terms of serum lipids and antioxidant levels. Note that significant improvement in HDL and TC/HDL ratio and LDL cholesterol oxidation was blunted when pistachio intake was increased to 40% of daily caloric intake from 20% and the differences were not statistically significant between the groups consumed pistachio as 20% and 40% of daily caloric intake in terms of all biochemical parameters.

TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; PON, paraoxonase. Data are expressed as mean ± S.D.

*$p < 0.05$. Significant compared to the control group.

40% of daily caloric intake in comparison to the control group. On the other hand, pistachio-fed group as 40% of daily caloric intake was associated with lower PON 1 enzyme activity when compared with the group fed pistachio as 20% of daily caloric intake; thus, antioxidant effect of pistachio was blunted in rats when the amount of pistachio was increased from 20% to 40% of daily caloric intake, although the serum PON 1 activities between the groups were not statistically significant. Arylesterase activities showed the changes similar to those in PON 1 enzyme activities; serum arylesterase activities were significantly increased in both groups fed pistachio as 20% and 40% of daily caloric intake in comparison to the control group. On the other hand, the group fed pistachio as 40% of daily caloric intake is associated with lower serum arylesterase activity when compared with the group fed pistachio as 20% of daily caloric intake. Thus, positive effect of pistachio on arylesterase activity was also blunted in rats when the amount of pistachio was increased from 20% to 40% of daily caloric intake, although the serum arylesterase activities between the two groups were not statistically significant.

Discussion

In this 10-week dietary intervention trial in rats, we are able to show that consumption of pistachio as 20% of daily caloric intake leads to significant improvement in HDL and TC/HDL ratio and inhibits LDL cholesterol oxidation.

In one small study on moderate hypercholesterolemic subjects (four men and six women), substituting 20% of the daily caloric intake in the form of pistachio revealed beneficial effect on LDL cholesterol, TC, and TC/HDL cholesterol ratio (Edwards et al. 1999). The main weak point in that study is that pistachio was substituted as fat calories (Edwards et al. 1999). Thus, whether the results were due to the lower fat calories or the pistachio is unclear.

So far there is no study investigating the effect of pistachio on serum lipids and antioxidants. Our study is the first in literature that shed light on the relation between pistachio and serum antioxidant capacity, which was comparable with previous walnut and almond studies (Anderson et al. 2001; Halvorsen et al. 2002; Takeoka and Dao 2003; Tapsell et al. 2004; Wijeratne et al. 2006). In addition to the improvement in HDL cholesterol and TC/HDL cholesterol ratio, pistachio has beneficial effect on LDL cholesterol oxidation by means of increasing serum antioxidant capacities, suggesting potential role of pitachio on cardiovascular protection. TC, LDL cholesterol and triglycerides levels were unaffected by pistachio consumption but a study by Kinosian et al. (1995) showed that the changes in TC/HDL and LDL/HDL ratios were better predictors of coronary artery disease than the changes in LDL alone.

Antioxidant contents of nuts, except for the pistachio, were determined in one study (Halvorsen et al. 2002). And that systematic screening of total antioxidants in dietary plants revealed that wallnuts, almonds, and hazelnuts contain antioxidant activity. The contents of antioxidants in wallnuts were found much higher than other nuts. Although we did not compare the antioxidant properties of pistachio with wallnut, the antioxidant capacity of pistachio is clearly evident in our study.

The other most important finding of our study is that the favorable effect of pistachio on serum HDL cholesterol. Epidemiologic studies and studies in animals suggest that raising the levels of HDL cholesterol may retard the development of atherosclerosis. In humans, each increase in baseline HDL cholesterol of 1 mg per deciliter (0.03 mmol/l) is associated with 6 percent decrease in the risk of death from coronary disease or myocardial infarction (Gordon et al. 1986). Data from large-scale clinical trials with statins and fibrates indicate that observed clinical benefits are related, at least in part, to improvements in HDL cholesterol levels. Thus, there is increasing interest in HDL cholesterol as a secondary target of therapy for the prevention of coronary artery disease. According to our study results, pistachio as 20% of daily caloric intake can be added to diet for dyslipidemia especially for isolated low HDL cholesterol levels. Although it is early to propose a treatment modality for humans from our animal study, raising HDL cho-

lesterol by means of intake of pistachio might offer further improvements in coronary artery disease risk reduction. In the setting of isolated low HDL cholesterol levels, adding pistachio to diet with combination of lipid-lowering drugs, such as fibrates, niacin, and statins, can provide further improvements in HDL cholesterol since evidence suggests that raising low HDL cholesterol as a secondary target of therapy will have additional benefits for coronary artery disease risk reduction.

In the present study pistachio consumption was associated with a proportional increase in monounsaturated fatty acid intake and proportional reduction in polyunsaturated and saturated fatty acid consumption. These changes could contribute to the favourable efffects of pistachio nuts on serum lipids and antioxidant capacity in rats. The reason why the beneficial effects of pistachio are blunted with a higher dose is interesting. Although pistachio is rich in monounsaturated fatty acids, with increased doses the amount of saturated fatty acids is also increased which might have blunted the favourable effects. One other possible mechanism is that consuming higher amounts of pistachio (40% of the daily caloric intake) will inevitably increase the total caloric intake. Further studies should be designed to address this dose response relationship and potential mechanisms responsible from it.

Pistachio nut is, of course, a fatty food, which raises the question that additional calorie intake and consequent weight gain by eating more nuts might be harmful. However, adding different amounts of pistachio nuts did not cause weight difference among groups. We did not measure the serum levels of HDL, LDL, TC, triglyceride, PON1 and arylesterase at the beginning of the study, because all rats were genetically same and were fed same diet until the beginning of the study. Also consuming pistachio constituting 20% of daily caloric intake everyday may not be feasible and additional studies investigating the effect of lower doses would help in this context.

In conclusion, the present results show that consumption of pistachio as 20% of daily caloric intake leads to significant improvement in HDL cholesterol and TC/HDL ratio. Morover, consumption of pistachio as 20% of daily caloric intake appeares to increase PON1 activity by 35% and arylesterase activity by 60%, which inhibit oxidation of LDL cholesterol. These results suggest that pistachio may be beneficial for both prevention and treatment of coronary artery disease. Further studies will be required to determine the mechanism achieving this effect and to document that the improved serum lipid profile decreases the risk of coronary artery disease.

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