Original Article

Consumption of Pistachio Nuts Beneficially Affected Blood Lipids and Total Antioxidant Activity in Rats Fed a High-Cholesterol Diet

(pistachio / antioxidants / oxidative stress / sialic acid / hyperlipidaemia)

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Abstract. Although nuts are typically high in dietary fat, novel studies have shown that regular consumption of these heart-healthy foods might confer a beneficial effect on cardiovascular disease risk. In the present study, we aimed to analyse the effects of pistachio consumption on blood lipids, antioxidant activity, oxidative stress and sialic acid levels in high-fat-fed rats for 8 weeks. The oxidant-antioxidant status was evaluated by the determination of lipid peroxidation (thiobarbituric acid-reactive substances), total antioxidant activity, reduced glutathione content, activity of superoxide dismutase and total thiol levels. Furthermore, tissue damage was evaluated by total sialic acid levels in serum. Total cholesterol, triglycerides, sialic acid and thiobarbituric acid-reactive substances significantly increased whereas total antioxidant activity, reduced glutathione, total thiol levels significantly decreased in the hyperlipidaemic group compared to the control group. Pistachio consumption significantly decreased triglycerides and thiobarbituric acid-reactive substance levels and significantly increased total antioxidant activity in the hyperlipidaemic group. In conclusion, pistachio supplementation may improve blood lipids and ameliorate oxidative stress in experimental hyperlipidaemia, which may have beneficial applications in the prevention of cardiovascular diseases. However, its antioxidant mechanisms remain to be investigated.

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Abbreviations: CHD – coronary heart disease, CVD – cardiovascular disease, DTNB – 5,5'-dithiobis-2-nitrobenzoic acid, FDA – Food and Drug Administration, GSH – glutathione, MDA – malondialdehyde, NBT – nitroblue tetrazolium, ROS – reactive oxygen species, SA – sialic acid, SOD – superoxide dismutase, TAA – total antioxidant activity, TBARS – thiobarbituric acid-reactive substances, TC – total cholesterol, TG – triglycerides, TT – total thiol.

Introduction

Plant-derived products contain a wide range of phytochemicals and phenolic compounds that possess substantial antioxidant and antiradical activities. These phytochemicals and phenolics provide protection against harmful effects of free radicals and are known to reduce the risk of coronary heart disease (CHD), cardiovascular disease (CVD), stroke, atherosclerosis and inflammation associated with oxidative stress (Shahidi et al., 2007).

Nuts are typically high in fat. On the other hand, despite their fat content, in the past years research has indicated that nuts and pistachio are in fact beneficial to our health. Recent recognition of nuts as "heart-healthy" foods by the U.S. Food and Drug Administration (FDA) has provided a major boost to the image of nuts (Kocyigit et al., 2006). Most of the studies have focused on the lipid-lowering effect of nuts. Also, there is limited information about the antioxidant effects of nuts and pistachios (Matthäus and Ozcan, 2006).

Pistachio (*Pistacia vera* L.), a member of the Anacardiaceae family, is native of arid zones of Central and West Asia and distributed throughout the Mediterranean basin. The genus *Pistacia* contains only 11 species among which pistachio, cultivated for its edible nuts, is by far the most important economically. In Turkey, the pistachio is grown mainly in Gaziantep (Gentile et al., 2007).

In human body, a number of biochemical reactions involve generation of reactive oxygen species (ROS). Excessive ROS can attack lipids, carbohydrates, proteins, DNA, and result in oxidative stress. Compared to other cellular components, biomembranes rich in lipids are especially prone to oxidative damage. ROS attack the polyunsaturated fatty acid side chains of lipids and initiate a series of free radical-mediated chain reactions

termed as lipid peroxidation. During lipid peroxidation, malondialdehyde (MDA) is generated (Koracevic et al., 2001). Under normal conditions, the balance between the generation and diminution of ROS is controlled by the antioxidant defence system, which includes superoxide dismutase (SOD), glutathione (GSH), thiols, antioxidant vitamins and other dietary micronutrients such as flavonoids and polyphenols (Tapiero et al., 2002). Moreover, in body fluids total antioxidant activity (TAA) plays an important role against free radical attack and has been used for scientific purposes to examine the medical importance of free oxygen radicals and antioxidative defence (Koracevic et al., 2001).

Human sialic acids (SA) are N-acetylated derivates of neuraminic acid that are abundant in terminal monosaccharides of glycoconjugates. SA levels increase rapidly following the inflammatory and injury processes. Therefore, detection of total SA concentrations may be a valuable indicator of tissue damage, inflammation and tissue proliferation (Lindberg et al., 1993). Effects of pistachio nut consumption on serum SA levels have not been investigated before.

There is limited data on the antioxidant effects of pistachio nuts and only little data have been reported about the antioxidant content of preparations from the pistachio species (Kocyigit et al., 2006; Matthäus and Ozcan, 2006; Gentile et al., 2007; Shahidi et al., 2007). Moreover, to our knowledge, the effects of pistachio consumption on the oxidant-antioxidant status have not been investigated in the hyperlipidaemic rat model. Therefore, the aim of our study was to explore the effects of pistachio consumption on blood lipids, antioxidant activity, oxidative stress and SA levels of rats fed a high-cholesterol diet for eight weeks.

Material and Methods

Animals and diet

All animal protocols were approved by the committee on the use of live animals in teaching and research, The University of Istanbul. Thirty-two male Wistar Albino rats aged 8 weeks at the beginning of the experiment (initial body weight 220–260 g), were used. The animals were obtained from Istanbul University Animal Laboratory. Animals were housed in cages in an environment-controlled room (room temperature, 22 ± 2 °C; relative humidity; light/dark cycle 12h/12h) with free access to food and water.

Rats were divided into four groups of 8 rats each: 1 – control group, 2 – control+pistachio, 3 – hyperlipidaemic group, 4 – hyperlipidaemic+pistachio group. Each group was fed one of the following diets for 8 weeks: control diet (control group), control diet supplemented with 1.26 % g pistachio, control diet supplemented with 1.63 % g cholesterol, 0.41 % g cholic acid, 16.3 % g sunflower oil (hyperlipidaemic group), or hyperlipidaemic diet supplemented with 1.26 % g pistachio (hyperlipidaemic+pistachio group). The composition of the diets is shown in Table 1.

The diets were prepared once a week in the laboratory (except for the control diet, which was purchased from Denizeri Feed Manufacturer, Kocaeli, Turkey) and stored at a temperature of + 4 °C.

Blood collection

At the end of 8 weeks the rats were killed under urethane anaesthesia (1.25 g/kg) after overnight fasting. To minimize diurnal variations the rats were routinely sacrificed between 07.00 and 08.00 h. Blood samples were removed from the heart and collected into tubes. Serum samples were separated by centrifugation.

Assay of total cholesterol and triglycerides

Commercial assay kits (Human, Wiesbaden, Germany) with code numbers 10 028 and 10 720P were used for fasting serum total cholesterol and triglycerides. A spectrophotometer was used for measuring the absorbance (Shimadzu, Kyoto, Japan).

Assay of plasma total antioxidant activity

Plasma antioxidant activities were determined by the method of Koracevic et al. (2001): a standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals (OH˙). These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid-reactive substances (TBARS). Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. Finally, absorbance values were measured by a spectrophotometer at 532 nm.

Table 1. Composition of diets fed to rats for 8 weeks

	Groups			
Dietary Component [(g/g)%]	**Control**	**Control + Pistachio**	**Hyperlipidaemic**	**Hyperlipidaemic + Pistachio**
Cholic Acid	-	-	0.4	0.4
Cholesterol	-	-	1.63	1.63
Sunflower Oil	-	-	16.3	16.3
Pistachio	-	1.26	-	1.26
Standard Chow*	100	98.74	81.67	80.41

* Standard commercial chow content: 20 % crude protein, 2.85 % crude oil, 5.96 % cellulose, 8 % crude ash, 0.97 % calcium, 0.5 % phosphorus, 1.03 % lysine, 0.33 % methionine, 0.65 % methionine+cysteine, 0.14 % sodium, 1.13 % linoleic acid, vitamin A, 9000 IU/kg; vitamin D3, 2000 IU; vitamin E, 60 IU.
Ingredients used include: 45 % wheat, 20 % soybean bagasse, 6 % corn, 3 % sunflower bagasse, 10 % bran flour.

Serum CuZn SOD activity measurement

Serum CuZn SOD activity was determined by the method of Sun et al. (1988) based on the inhibition of nitroblue tetrazolium (NBT) reduction. The absorbance of the reduction product was read at 560 nm in a spectrophotometer. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50 %.

GSH measurement

Serum GSH concentration was determined according to Beutler et al. (1963) using metaphosphoric acid for protein precipitation and 5'5'-dithiobis-2-nitro-benzoic acid (DTNB) for colour development.

Plasma protein sulphhydryls

Sulphhydryl concentrations were measured spectrophotometrically using DTNB as described by Ellman (1959).

Serum SA concentrations

SA levels were assayed using Warren's (1959) thiobarbituric acid assay. Samples were incubated with 0.9 ml 0.1N H_2SO_4 at 80 ºC for 1 h and total SA were determined in hydrolysate.

Determination of lipid peroxidation

Lipid peroxidation was assayed by measuring MDA levels in serum. MDA levels were determined as TBARS according to the method of Yagi (1984).

Statistical analyses

Friedman's test was performed to assess statistical differences between the groups of the study. Differences were considered significant when P values were < 0.05. All statistical analyses were performed with the SPSS software for Windows, Version 11.5.

Results

At the end of the experiment, the mean levels of body weight of the rats in control, control+pistachio, hyperlipidaemic and hyperlipidaemic+pistachio groups were found to be 280 ± 10; 276 ± 12; 310 ± 11; 307 ± 12 g, respectively. Pistachio consumption decreased body weight non-significantly in the control+pistachio and hyperlipidaemic+pistachio groups when compared with the control group and the hyperlipidaemic group (P > 0.05).

Pistachio administration to control rats non-significantly decreased TC, TG, TBARS and SA levels (P > 0.05) and non-significantly increased TAA, SOD, GSH and TT levels (P > 0.05) (Table 2).

In the hyperlipidaemic rat group, significant increases were observed in TC, TG, TBARS and SA levels (P < 0.001, P < 0.01, P < 0.05, P < 0.05, respectively). On the other hand, TAA, GSH and TT levels significantly decreased compared to the control group (P < 0.01, P < 0.001, P < 0.01, respectively) (Table 2).

Pistachio consumption significantly decreased TG and TBARS levels (P < 0.05, P < 0.01, respectively) and significantly increased TAA (P < 0.05) in the hyperlipidaemic group. Moreover, although statistically not significant, pistachio consumption increased SOD, GSH and TT levels and decreased TC and SA levels in the control+pistachio-administered group (Table 2).

Discussion

Hyperlipidaemia with increased concentrations of cholesterol and triacylglycerol-carrying lipoproteins is considered to be the cause of atherosclerosis, with its dual sequel of thrombosis and infarction. Traditionally nuts have been perceived by the general public as undesirable because of their high fat content. However, the high fat content of nuts represents largely unsaturated fats, which have favourable effects on blood lipids (Arafa, 2005). On the other hand, health professionals are still concerned whether hypercholesterolaemic patients advised to eat nuts will have increased serum lipid levels.

Our goal was to investigate the effect of pistachio consumption in a rat model of hyperlipidaemia. We aimed to adapt the dosage of pistachio (10 oz = 310 g) to rats, in order to examine the effect of pistachio con-

Table 2. TC, TG, TAA, SOD, GSH, TT, TBARS and SA levels of the rats in the control, hyperlipidaemic and hyperlipidaemic+pistachio group at the end of 8 weeks

	Control (N = 8)	Control+Pistachio (N = 8)	Hyperlipidaemic (N = 8)	Hyperlipidaemic+Pistachio (N = 8)
TC (mg/dl)	63.8 ± 10.3	62.9 ± 10.4	269.0 ± 23.9 *	237.7 ± 25.9
TG (mg/dl)	65.5 ± 5.7	64.7 ± 5.5	72.6 ± 5.9 **	66.4 ± 5.9†
TAA (mmol/l)	1.6 ± 0.3	2.1 ± 0.3	1.2 ± 0.3 **	1.6 ± 0.5†
SOD (U/ml)	5.5 ± 2.1	5.9 ± 2.3	6.1 ± 2.8	6.5 ± 3.1
GSH (% mg)	29.6 ± 3.8	31.0 ± 3.1	19.9 ± 2.5 ***	22.4 ± 3.3
TT (μM)	317.8 ± 35.9	321.5 ± 37.6	272.8 ± 27.0 **	297.7 ± 27.6
TBARS (nmol MDA/ml)	3.8 ± 1.4	3.8 ± 1.6	5.7 ± 1.4 ***	4.0 ± 1.0††
SA (mM)	16.5 ± 2.4	16.2 ± 2.2	21.3 ± 4.2 ***	19.4 ± 3.7

* P < 0.001 significantly different from the control group
** P < 0.01 significantly different from the control group
*** P < 0.05 significantly different from the control group
†P < 0.05 significantly different from the hyperlipidaemic group
††P < 0.01 significantly different from the hyperlipidaemic group

sumption on blood lipids, SA levels, and oxidant-antioxidant status in hyperlipidaemic rats. For this purpose, we have calculated the equivalent dose of pistachio for rats as 0.88 g pistachio/week, consuming human weight as 70 kg and rat weight as 200 g. Accordingly, pistachio-added diets that contain 1.26 % pistachio were prepared.

In our present study, TC and TG levels were significantly increased in the hyperlipidaemic group compared with the control group. However, pistachio consumption significantly decreased TG levels. Additionally, there was a trend for a decrease in TC levels in pistachio-treated hyperlipidaemic group when compared with the hyperlipidaemic group.

Clinical and epidemiological studies have reported the beneficial effects of tree nuts and peanuts on serum lipid levels (Hu et al., 1998; Aksoy et al., 2007; Emekli-Alturfan et al., 2007). On the other hand, there are controversial results about the effects of pistachio nut consumption on the lipid profile of patients with hypercholesterolaemia. Sheridan et al. (2007) studied the effects of 15 % of the daily caloric intake in the form of pistachio nuts on the lipid profiles of free-living human subjects with primary and moderate hypercholesterolaemia and found no significant differences in TC and TG levels. However, decreased TG and TC levels have been reported in patients with hypercholesterolaemia (Edwards et al., 1999). On the other hand, Kocyigit et al. (2006) observed non-significant decreases in TG levels in healthy volunteers.

Clinical studies have shown no associated increase in body weight when nuts are consumed (Edwards et al., 1999). Moreover, people who eat nuts may tend to engage in higher levels of physical activity than non-nut eaters. Hu et al. (1998) reported that nut consumption was associated with greater frequency of vigorous exercise among the Nurses' Health Study participants. The Physicians' Health Study also noted that men who ate nuts more frequently were more physically active. Additionally, nuts may also enhance satiety (Sabaté, 2003).

Oxidative stress, the disturbance of the delicate balance between oxidants and antioxidants, may result from increased production of free radicals and/or impaired antioxidant defence systems. TAA is a dynamic equilibrium that is influenced by the interactions between each plasma antioxidative constituent. The cooperation of antioxidants in human plasma provides greater protection against attacks by free radicals than any antioxidant alone. Plasma normally contains scavengers such as GSH, thiols and antioxidant vitamins to protect against free radical injury. GSH plays a unique role in the cellular defence system against toxic chemicals of endogenous and exogenous origin; as such, the depletion of GSH increases the vulnerability to free radical damage (Koracevic et al., 2001). Thiol groups are physiological free radical scavengers and may serve as antioxidants by several mechanisms. They may pre-emptively scavenge oxidants that initiate peroxidation, thus sparing vitamin E and/or lipids from peroxidative attack (Reed, 1990). SOD protects tissues from oxygen free radicals by catalysing the removal of superoxide radical anion formed by the one-electron reduction of the oxygen molecule (Koracevic et al., 2001).

In the present study TBARS levels significantly increased whereas TT, TAA, GSH levels significantly decreased in the hyperlipidaemic group. Decreased antioxidant levels are possibly due to their increased utilization combating excessive plasma oxidative stress in hypercholesterolaemic rats. Consequently, decreased TAA in the hyperlipidaemic group might be responsible for the increased peroxidation of the membrane lipids in this group since increased peroxidation of membrane lipids causes reduction in the activity of antioxidative enzymes. Disturbed balance between oxidants and antioxidants due to hyperlipidaemia has been shown before (Emekli-Alturfan et al., 2008).

On the other hand, there is an increasing but inconclusive body of evidence suggesting that nuts improve antioxidant levels (Kocyigit et al., 2006; Gentile et al., 2007; Emekli-Alturfan et al., 2008). Consequently, in the present study pistachio supplementation in the hyperlipidaemic group significantly decreased TBARS levels and increased TAA when compared with the hyperlipidaemic group.

The polyphenol phytoalexin trans-resveratrol was detected in the aqueous extracts from the edible nut of five Turkish cultivars of pistachio (Tokusoglu et al., 2005). Consequently, the antioxidant effects of pistachio against oxidative damage might originate from phytochemicals in its content since resveratrol and anthocyanins have strong free radical scavenging ability (Tapiero et al., 2002; Tokusoglu et al., 2005; Kocyigit et al., 2006). Polyphenols, including flavonoids, can exert their antioxidant activity by inhibiting the activities of enzymes, including lipooxygenase and cyclooxygenase, by chelating metal ions, and, most importantly, by scavenging free radicals. Generally, polyphenols are potent free radical scavengers because phenolic groups are excellent nucleophiles (Tapiero et al., 2002). Moreover, it may be assumed that polyphenols in pistachio reinforce the antioxidant system in this experimental model.

These results suggest that pistachio could be a useful compound to control hypercholesterolaemia by both improving the lipid profile and modulating oxidative stress. This modified balance between the antioxidative enzymes might be able to remove superoxides more efficiently. Consistent with our results, Emekli-Alturfan et al. (2007) reported increased plasma GSH levels in hyperlipidaemic rats fed peanuts.

Measurement of SA levels has been shown to provide a useful tool for evaluating the inflammatory status and tissue damage in chronic disease as well as to correlate with the presence of atherosclerosis (Lindberg et al., 1993; Eguchi et al., 2005). It has been reported that high levels of SA have been considered as a risk factor for cardiovascular disease (Lindberg et al., 1993). In our study, serum SA levels were found to be elevated in hyperlipidaemic rats. Probably, superoxide anion and the related

reactive oxygen species (ROS) are suggested to specifically cleave and liberate the SA residues (Eguchi et al., 2005). Yet, the exact mechanism is still not clearly understood. These data, together with the high lipid peroxidation, seem to provide a measure of low-grade inflammation and oxidative stress in hyperlipidaemic rats. Wakabayashi et al. (1994) found a significant relationship between the serum SA level and atherosclerotic risk factors such as serum uric acid, systolic and diastolic blood pressure, atherogenic index, and white blood cell count in patients with hyperlipidaemia. Similar results have also been reported by others (Oztürk et al., 2007). Finally, it is suggested that SA reflect the degree of atherosclerotic progress involving inflammation processes.

Consequently, high cholesterol diet causes reduction in the total antioxidant defence potential in plasma and lead to oxidative stress. The results obtained in this experimental study suggest that pistachio consumption may improve lipid profiles, suppress oxidative stress, inhibit peroxidation reactions and support the antioxidant system, accordingly reducing the risk of CHD and CVD associated with oxidative stress.

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