The effects of Corylus Avellana on Serum Lipid Profile and Oxidative Stress in Hyperlipidemic-diabetic Rats

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Abstract

There is limited evidence suggesting that nuts improve plasma antioxidant potential. The aim of this study is to investigate the effects of the hazelnut consumption on serum lipids, atherogenic indexes (AI) and oxidant-antioxidant status in hyperlipidemic-diabetic rats. Wistar-albino rats of both sexes, weighing 200-250 g were used in this study. The group of animals used in this study, which consisted of 32 rats, were divided into four groups: Control, control + hazelnut, hyperlipidemic-diabetic, hyperlipidemic-diabetic + hazelnut. Each group was fed with control or hyperlipidemic diets with the same amount of hazelnut (0.63%) added for 12 weeks. Diabetes has been induced by streptozotocin injection in diabetic groups. Blood glucose, serum lipids, glutathione (GSH), lipid peroxidation (LPO), and AI and atherogenic index of plasma (AIP) have been evaluated after the experiment was over. In hyperlipidemic-diabetic group, AI, LPO, and serum lipid levels were found to have increased significantly, whereas high-density lipoprotein-cholesterol and GSH levels were found to have decreased significantly in comparison with the control group (p<0.01). Hyperglycemia was also seen to have increased in this group. Impaired antioxidant-oxidant balance was noted to have improved, GSH increased while triglyceride decreased significantly (p<0.01). Hazelnut consumption also increased blood GSH levels and AI levels in the control group. Consumption of hazelnut at this dose (0.63%) may improve oxidant-antioxidant balance in healthy and hyperlipidemic-diabetic status without increasing blood lipids.

Introduction

Diabetes mellitus is associated with serum lipid and lipoprotein abnormalities; it is also thought to be related to diabetic atherosclerosis. It has been reported that rats with streptozotocin (STZ)-induced diabetes show marked hyperlipoproteinaemia after exogenous cholesterol loading (1-5). The dietary approach to prevent hypercholesterolemia and coronary heart disease (CHD) in Western countries generally focusses on the replacement of saturated fatty acids (SFAs) in the place of polyunsaturated fatty acids (PUFAs) and/or carbohydrates (6-11). Nuts are an important part of the mediterranean diet and are rich in fat but have a fatty acid profile that might be beneficial in reducing the risk of CHD (12-15). The composition and amount of lipids in the diet are effective factors in managing the balance of prooxidant-antioxidant status in living organisms. It has been observed that although SFAs have a tendency to increase atherosclerosis by increasing serum cholesterol levels. PUFAs have been found to reduce the effects of serum lipid levels (10,16,17). However, PUFAs increase susceptibility to lipid peroxidation (LPO) and disturb the prooxidant-antioxidant balance in favor of

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prooxidation (6,16,18). Nonetheless, monounsaturated fatty acids (MUFAs) like PUFAs have been reported to have antilipidemic effects (10,16). Since MUFAs are resistant to oxidation, they might lower the susceptibility of the organism to LPO and low-density lipoprotein (LDL) oxidation. Olive oil, which is rich in MUFAs, and is also known as an antiatherogenic oil, has been gaining importance in nutrition (16,18).

It has been found that hazelnut like olive oil is rich in MUFAs (16,18,19). Turkey is the main hazelnut producer in the world, contributing approximately 70% of the total global production, followed by Italy, the U.S and Spain (18-20). Hazelnut contains high amounts of vitamin E and quercetin, a flavonoid antioxidant, and luteolin, (19-21) and has a higher level of total radical scavenger capacity and resistance to sunlight than olive oil (16). Hazelnut has also been reported to serve as a good source of essential minerals, amino acids, B complex vitamins, and dietary fibers (19-22).

The present study aims principally to investigate the possible effects of the hazelnut consumption on serum lipid profile, glutathione (GSH), and LPO levels, as indices of antioxidant-oxidant status in hyperlipidemic-diabetic rats.

Materials and Methods

Animals and diets

All experimental protocols have been approved by the Marmara University School of Medicine Animal Care and Use Committee. In this study, 32 Wistar-Albino rats, aged 8 weeks at the beginning of the experiment (initial body weight, 200-250 g), have been used. The animals have been obtained from the Marmara University Animal Laboratory (Istanbul, Turkey). Animals have been housed in cages in an environment-controlled room (room temperature, 22±2°C; relative humidity, light/dark cycle, 12 h/12 h) with free access to food and water and all were observed daily for clinical signs of the disease.

The rats were divided into 4 groups of 8 rats: Control group, control + hazelnut group, hyperlipidemic-diabetic group, and hyperlipidemic-diabetic + hazelnut group. Each group had an equal number of male and female rats and was fed on one of the following diets for 12 weeks: Standard chow (control group) or the standard chow supplemented with 0.63g % hazelnut (control + hazelnut group), or the hyperlipidemic diet that had the composition as follows: cholesterol (1.63 g%), cholic acid (0.41 g%), sunflower oil (16.3 g%) and laboratory chow (81.6 g%) (hyperlipidemic-diabetic group), or the hyperlipidemic diet supplemented with 0.63 g% hazelnut (hyperlipidemic-diabetic + hazelnut group). The diets were prepared once a week in the laboratory and stored under the temperature of +4°C. Food consumption of each group was observed everyday throughout the experiment period.

Rats in the hyperlipidemic-diabetic group and the hyperlipidemic-diabetic + hazelnut group were made to be diabetic by intraperitoneal (i.p.) injection of a single dose of 35 mg/kg STZ freshly dissolved in citrate buffer (0.05 M, pH 4.5). Controls were injected with citrate buffer. The diabetic state was characterized by the measurement of plasma glucose concentration 2 days after the STZ injection. The rats that became mildly diabetic when they were characterized by slight basal hyperglycemia.

Blood sample collection

At the end of 12 weeks, the animals were starved overnight and anesthetized with urethane (1.25 g/kg, i.p.). Blood samples were collected by tail snip and cardiac puncture for glucose, lipids, LPO and GSH determinations. Serum samples were obtained by centrifugation and stored at −20°C until analysis was done. GSH analysis was done on the same day without delay.

Biochemical analysis

Commercial assay kits (Human, Wiesbaden-Germany) with code numbers 10 028, 10 720P, 10 094, 10 084 were used for fasting serum total cholesterol (TC), triglyceride (TG), LDL-Cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) levels, respectively, whereas commercial assay kit (Randox) with code GL 2614 was used for the determination of fasting serum glucose levels.

Serum total lipid, LPO, and blood GSH levels were assessed using sulpho-phospho-vanilin (23), Ledwozyw thiobarbutiric acid (24), and Ellman (25) methods, respectively. In addition to evaluate changes in lipoprotein profile induced by hazelnut consumption, atherogenic indices were calculated using the following equations:

Atherogenic index (AI) = TC-HDL-C/HDL-C
TC and HDL-C were expressed as mg% (26).

Atherogenic index of plasma (AIP) = Log (TG/HDL-C)
TG and HDL-C were expressed as molar concentrations (27).

Statistical analysis

The results were expressed as mean ± standard deviation. The differences between the values of the
groups were tested by Student’s t-test and ANOVA. Differences with p=0.05 or less were considered statistically significant. All statistical analyses were performed with the Statistical Package for the Social Sciences software for Windows, Version 11 (SPSS Inc., Chicago, IL).

Results
Prior to the experiment, the groups were checked in terms of the differences in weight and no significant differences were found (p_{ANOVA}>0.1). Bodyweights (g) of the rats in control, control + hazelnut, hyperlipidemic-diabetic, and hyperlipidemic-diabetic + hazelnut groups were found to be 311.63±80.59, 277.75±81.26, 289.63±76.37, 282.50±86.46, respectively. They were not significantly changed over the 12 weeks experimental period (p>0.1). Food intake (16.75, 15.42, 16.21, 12.34 g/day/rat, respectively), was found to be decreased in the hazelnut and/or hyperlipidemic diet given groups in comparison with the control group.

Serum glucose levels, serum lipid parameters, and the atherogenic indices of the rats in all groups are shown in Table 1. Blood GSH and serum LPO levels of the groups are shown in Table 2.

Blood GSH and AIP values increased significantly in the control + hazelnut group compared to the control group (Tables 1 and 2). Hazelnut consumption did not change serum lipids, LPO, and AI levels significantly (Tables 1 and 2).

In the hyperlipidemic-diabetic group, serum glucose, TG, TC, LDL-C, LPO levels, and AI increased significantly, whereas HDL-C and GSH levels decreased significantly compared to the control group (p<0.01) (Tables 1 and 2).

The addition of hazelnut in the hyperlipidemic-diabetic group led to significantly higher GSH levels and lower TG levels compared to hyperlipidemic-diabetic group (p<0.01) (Tables 1 and 2).

Discussion
Diabetes mellitus is often accompanied by hypercholesterolemia, hypertriglyceridemia, and low HDL-C levels, and the incidence of arteriosclerosis is higher in diabetic patients than in non-diabetic individuals. Assuming that diabetic complications are caused by arteriosclerosis-associated vascular disease or damage, it is possible that hyperlipidemia, is a known risk factor for arteriosclerosis (28,29).

Kris-Etherton et al. (30) reviewed epidemiological and clinical studies that have demonstrated the effects of nuts on cardiovascular health and concluded that overall, the results suggest an independent beneficial effect of nut consumption on CHD risk. The exact mechanism by which nuts exert this protective effect is not clear. In addition to their highly unsaturated fatty acid profile, nuts contain other constituents that could also offer protection. These constituents include antioxidants (e.g. tocopherols), squalene, and plant sterols (6,12,31,32).

In this study, the effect of hazelnut supplementation on serum lipid profile and oxidative status in cholesterol-fed diabetic rats was examined. The dosage of hazelnut (5 oz/week) which decreased Type 2 diabetes risk in women in comparison with women with no diabetes history in the Nurses’ Health Study (33), was adapted to hyperlipidemic-diabetic rats.

When diabetic rats are subjected to a low-fat diet, their ad libitum food intake has increased. A reverse relationship between fat consumption and polyphagia was found, which suggested that diabetic rats that were with fed a low-fat diet increased their food consumption while ensuring adequate fat intake (1). This reverse relationship was confirmed by the present study. Food intake was found to have decreased in the hazelnut and/or hyperlipidemic diet given groups compared to control group.

Table 1. Serum glucose, lipids, and AI of the four groups.

| Group               | Control (n=8) | Control+hazelnut (n=8) | p      | Diabetic+hyperlipidemic (n=8) | Diabetic hyperlipidemic+hazelnut (n=8) | p     |
|---------------------|--------------|------------------------|--------|-----------------------------|----------------------------------------|------|
| Glucose (mg/dL)     | 101.00±14.04 | 101.13±11.81           | NS     | 194.38±22.99*               | 191.00±32.72                          | 1.000|
| TC (mg/dL)          | 66.00±11.38  | 63.75±10.36            | NS     | 309.00±33.88                | 312.88±33.19                          | NS   |
| LDL-C (mg/dL)       | 15.00±2.73   | 15.38±14.07            | NS     | 74.25±29.36                | 71.88±10.74                           | 0.995|
| HDL-C (mg/dL)       | 27.91±3.06   | 31.57±14.07            | NS     | 22.80±22.41                | 21.47±23.39                           | 0.948|
| TG (mg/dL)          | 62.57±5.10   | 60.95±3.74             | NS     | 86.45±25.70                | 72.19±25.68                           | 0.0001*|
| AI                  | 1.38±0.44    | 1.01±0.26              | NS     | 12.80±22.91*               | 13.78±22.00                           | 0.446|
| AIP                 | 0.22±0.053   | 0.17±0.053             | NS     | 0.22±0.053                 | 0.17±0.053                            | 0.081|

Values are mean±SD. *p<0.01 significantly different from control group. **p<0.05 significantly different from control group. n: Number of animals. NS: Nonsignificant (p>0.05). TC: Total cholesterol, TG: Triglyceride, LDL-C: Low-density lipoprotein-cholesterol, HDL-C: High-density lipoprotein-cholesterol, TL: Total lipid, AI: Atherogenic index, AIP: Atherogenic index of plasma, SD: Standard deviation
Dietary supplementation with hazelnuts at the end of 12 weeks did not cause significant changes in blood lipids in the control group. There was even a trend for lower concentrations of TC, TG, LPO, and Al levels and higher HDL-C levels in the same group. Similar to our findings, Kris-Etherton et al. (34) demonstrated beneficial effects of nuts on blood lipid concentrations with a high MUFA, low saturated fatty acids (SFA) diet in normocholesterolemic subjects.

Addition of cholesterol to the diet of hyperlipidemic-diabetic rats caused an increase in serum TC, TG, LDL-C, and TL levels. Cholesterol feeding is also associated with an increase in the activity of hepatic phosphatidate phosphohydrolase 1, which is a key enzyme in regulating TG synthesis (35). The increase in TG synthesis may result in increased very low-density lipoprotein (VLDL) secretion. In order to stabilize the hydrophobic TG core, it is also probable that there is an increase in the secretion of VLDL-C. Fungwe et al. (32) have demonstrated an absolute requirement of cholesterol for synthesis and secretion of VLDL. The increase in VLDL secretion might also contribute to increases in LDL-C levels and decrease plasma TG levels (14,22,40).

In the present study, hazelnuts were simply added into the diets of control and hyperlipidemic-diabetic rats to resemble the daily hazelnut consumption in individuals without any dietary restriction. Dietary supplementation with hazelnut at the end of 12 weeks did not increase serum lipid levels even decreased some of them in control and hyperlipidemic-diabetic groups. These results show that both healthy and hyperlipidemic subjects can safely consume hazelnuts at the dose investigated. Moreover, the increased GSH levels and decreased TG and AIP levels in hyperlipidemic-diabetic rats indicate that hazelnut consumption might have a protective effect against the development of cardiovascular and other hyperlipidemic and diabetic complications.

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Table 2. Oxidative stress markers of the four groups.

| Group                          | Control (n=8) | Control+hazelnut (n=8) | p        | Diabetic+hyperlipidemic (n=8) | Diabetic+hyperlipidemic+hazelnut (n=8) | p        |
|-------------------------------|--------------|-----------------------|----------|-----------------------------|--------------------------------------|----------|
| GSH (mg)                      | 25.84±3.42   | 37.40±3.01            | 0.0001** | 20.31±0.93                  | 25.36±1.84                            | 0.0001** |
| LPO (nmolMDA/mL)              | 3.11±0.41    | 2.93±0.42             | 0.774    | 5.78±0.08                   | 5.55±0.11                             | 0.722    |

Values are mean±SD. *p<0.01 significantly different from control group. **p<0.05 significantly different from the control group. n: Number of animals, NS: Nonsignificant (p>0.05), GSH: Glutathione, LPO: Lipid peroxidation, SD: Standard deviation
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