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Effects of red wine consumption on serum paraoxonase/arylesterase activities and on lipoprotein oxidizability in healthy-men

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Abstract

Although there is a general consensus concerning the lower risk for cardiovascular disease in moderate drinkers, the mechanisms responsible for the cardioprotective effect of red wine remain unknown. It has been proposed that increased serum paraoxonase activity may be a mechanism of action underlying reduced cardiovascular disease risk in moderate drinkers, since paraoxonase inhibits lipoprotein oxidation. The aim of this study was to investigate the effects of red wine consumption on serum paraoxonase/arylesterase activities and on lipoprotein oxidizability in healthy-men. Fourteen healthy-men were included in the study. The subjects consumed 0.375 g alcohol / kg body weight for 3 weeks. Paraoxonase and arylesterase activities were studied spectrophotometrically. Oxidizability of apolipoprotein B-containing lipoproteins were determined, after separating them with precipitation method, by incubating with copper-sulfate. Paraoxonase activity did not change, however arylesterase activity significantly decreased after red wine consumption ($P < 0.01$). There was a reduced susceptibility of apolipoprotein B-containing lipoproteins to copper-sulfate induced oxidation after red wine consumption ($P < 0.01$). Our results support that red wine protects lipoproteins against oxidation, however there was not any significant change in serum paraoxonase activity after red wine consumption.

Key words: Red wine; Paraoxonase; Arylesterase; Lipoprotein oxidation

"Red Wine Consumption and Serum Paraoxonase/ Arylesterase Activities"

1. Introduction

It is widely agreed that moderate alcohol, in particular, red wine intake would improve the cardiovascular health of populations [1,2]. Red wine has been postulated to be beneficial, in part related to its natural antioxidant compounds and/or its association with enhancement in serum antioxidant activity [3,4]. Red wine contains high concentrations of polyphenolic substances, particularly flavanoids [5–7], that have been found to posses different biological properties, such as antiinflamatory responses, antihypertensive, antithrombotic effects and prevention of low density lipoprotein (LDL) oxidation [6]. Oxidative modification of LDL plays a key role in early atherogenesis [8,9] and it is reasonable to hypothesize that agents, which could slow or prevent this oxidative process, may be beneficial in lowering incidence of atherosclerosis and cardiovascular disease (CVD).

Different mechanisms have been proposed for the protective action of red wine consumption. Most effect claims to be explained by favorable effects on high-density lipoprotein (HDL) metabolism [10,11]. Since HDL has been shown to retard the accumulation of lipid peroxides on LDL [12], anti-atherogenic effects of HDL are not only attributed to its effects on reverse cholesterol transport but on lipid peroxidation as well. The antioxidant effect of HDL is apparently due to paraoxonase (PON) which is an enzyme located on HDL particles. PON has been shown to protect or inhibit LDL and HDL oxidation and has also been proposed to stimulate cholesterol efflux [12–14], the first step in reverse cholesterol transport. PON exerts paraoxonase and arylesterase activities, since the enzyme hydrolyzes organophosphates (such as paraoxon) and aromatic esters (such as phenyl acetate) [15,16]. The enzyme's paraoxon-hydrolysis activity varies widely among individuals, partly related to polymorphisms. The PON gene has two common coding

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region polymorphisms (M/L 55 and R/Q 192). The concentration and the activity of the enzyme and its ability to prevent lipid oxidation are regulated by these polymorphisms. Shih et al. had stated that PON gene expression is determined by both dietary and genetic factors [17]. Arylesterase activity born by PON is not affected by either polymorphism and can be considered as an index of actual protein concentration [16]. Paraoxonase activity was reduced in several groups of patients, such as individuals with diabetes, hypercholesterolemia and CVD [18,19]. Paraoxonase and arylesterase activities may be affected by lifestyle factors, such as diet and smoking that are accepted as controllable risk factors for atherosclerosis [20–23].

There are many studies investigating effects of red wine on LDL oxidation that have produced contrasting results [4,24–32]. In the present study, oxidizability of apolipoprotein B-containing lipoproteins (LDL + VLDL) were investigated, since oxidation of LDL and VLDL has been demonstrated to be involved in the pathogenesis of atherogenesis [33] and oxidized LDL and VLDL exert several biological common effects that may contribute to the initiation and progression of atheroscleosis [8,9,33–36]. In addition, Gugliucci et al. have shown that apolipoprotein B-containing lipoproteins have same oxidizability pattern as compared to pure LDL when incubated with copper ions [37].

The number of studies investigating effects of red wine on paraoxonase activity is limited [20,29] To our knowledge this is the first study investigating effects of red wine consumption on serum paraoxonase/arylesterase activities and lipoprotein oxidizability in human subjects.

2. Methods and materials

2.1. Subjects

The subjects of this intervention study were 14 healthy male aged 25 to 38 years, who gave informed consent to participate. They had not been taking any medication or vitamin supplements. Subjects' body-mass indices were between 19 to 27 kg/m^2. The Human Ethics Committee of the Uludag University Medical Faculty approved the study protocol.

Subjects avoided drinking any alcoholic beverage for 3 weeks before red wine consumption period (washout period). All of the volunteers were asked to maintain their habitual diets and lifestyles for 3 weeks before the beginning of the study and during the 3 weeks of the study. Seven subjects were smokers and 7 were non-smokers. Subjects had been used to consuming not more than 3 cups of tea or coffee a day and they had a mild alcohol drinking habit. The red wine was consumed as 0.375 g alcohol / kg body weight in the evening during 3 weeks. The wine (alcohol content 12%) provided for the study was the only alcohol allowed during the study period. The wine was a generous gift from Kavaklıdere Ltd. (Ankara, Turkey, Cabernet Sauvignon).

2.2. Sample preparation

Following a washout period and at the end of 3 weeks of red wine consumption period blood samples were drawn following a 12 hr fast in the morning. To determine the susceptibility of apolipoprotein B-containing lipoproteins to oxidation, blood was collected into tubes containing EDTA (4.08 mM final concentration) and separated plasma was kept at +4°C and assayed within 24 hr.

Blood for measuring vitamin E and total carotenoids was covered with aluminum foil to protect from light. Serum aliquots separated for vitamin E, total carotenoids, apolipoprotein AI and B and plasma for malondialdehyde (MDA) determination were kept at – 20°C and assayed within 2 weeks. Serum total cholesterol, triglycerides, HDL-cholesterol levels and paraoxonase/arylesterase activities were measured the same day that the blood was collected.

2.2.1. Analysis.

Serum paraoxonase activity was studied in glycine buffer at pH = 10.5, according to Eckerson et al. [38] (within-day CV 3.9%). Serum arylesterase activity was measured using phenyl acetate as substrate in Tris HCL buffer at pH = 8.0, by the method of Haagen and Brock [39] (within-day CV 2.5%).

Vitamin E was measured according to the principle that tocopherols reduce ferric ions to ferrous ions and then the latter form a red complex with α,α' -dipyridyl [40] (within-day CV 6.5%). Serum total carotene was measured using the spectrophotometric method described by Neeld and Pearson [41] (within-day CV 5.4%). Vitamin E and total carotenoids were assayed in a dark room. Plasma MDA concentration was determined using colorimetric assay [42] (within-day CV 5.3%). In order to study lipid peroxidation in apolipoprotein B-containing lipoprotein fraction, this fraction was separated with precipitation method. Cholesterol content of this fraction was adjusted to 200 μg/mL with phosphate buffered saline [33]. Lipid peroxidation was assessed by measurement of thiobarbituric acid reactive substances (TBARS) [43]. TBARS were expressed as MDA equivalents content per milligram cholesterol (nmol/mg cholesterol) using 1,1′,3,3′ tetraethoxypropane as standard. Measurements were performed before (basal MDA) and after 3 hr of incubation with copper sulfate at 37°C. Basal MDA value was subtracted from the 3 hr value to obtain ΔMDA. Basal MDA represents the basal oxidative status of the apolipoprotein B-containing lipoprotein fraction, whereas ΔMDA represents the degree of oxidative modification (capacity for peroxidation) [44]. Total cholesterol, triglyceride, total protein, uric acid and HDL-cholesterol levels were studied on a Hitachi 902 autoanalyzer. Apolipoprotein AI and B levels were determined by nephelome-

Table 1
Lipid profile, uric acid, total protein, vitamin E and total carotene levels before and after red wine consumption.

Parameters	Before	After	P*
Total cholesterol (mmol/L)	4.61 ± 0.82	5.31 ± 0.88	<0.01
Triglycerides (mmol/L)	1.27 ± 0.66	1.38 ± 0.57	NS
HDL-cholesterol (mmol/L)	0.98 ± 0.18	1.04 ± 0.19	NS
LDL-cholesterol (mmol/L)	3.06 ± 0.67	3.63 ± 0.75	<0.01
Apolipoprotein AI (g/L)	1.40 ± 0.20	1.40 ± 0.20	NS
Apolipoprotein B (g/L)	0.97 ± 0.22	0.91 ± 0.16	NS
Uric acid (mmol/L)	0.31 ± 0.07	0.36 ± 0.06	<0.05
Total protein (g/L)	70.0 ± 4.0	77.0 ± 4.0	<0.01
Vitamin E (μmol/L)	24.6 ± 5.1	27.8 ± 6.7	<0.05
Total carotene (μmol/L)	2.96 ± 0.74	2.83 ± 0.87	NS

* Wilcoxon Signed Rank test was used to determine the differences among the data obtained before and after red wine consumption. Statistical significance was assigned at the 0.05 level. All data are expressed as mean ± SD.

HDL: High-density lipoprotein, LDL: Low density lipoprotein, NS: Non-significant.

try (Dade Behring, BNProspec, Germany). LDL-cholesterol was calculated with the Friedewald's formula [45]. The total phenolics content was determined photometrically by using the Folin-Ciocalteu method [46]. The total phenolics content is calculated as gallic acid equivalents using gallic acid monohydrate (Sigma) as standard.

2.3. Statistical analysis

Statistical analyses were carried out by SPSS10 program for Windows. Wilcoxon Signed Rank test was used to determine the differences among the data obtained before and after red wine consumption. The study group was also analyzed according to their smoking habits. Mann-Whitney U test was used to compare baseline values (before red wine consumption) of the smoker and non-smoker subjects and to test the difference (% change) in the values determined before and after red wine consumption. The individual differences (% changes) between the data obtained before and after red wine were calculated by the formula: (After − Before / Before) x 100. All data are expressed as mean ± SD. Statistical significance was assigned at the 0.05 level.

3. Results

There were no significant differences in the body-mass indices before and after red wine consumption. Total cholesterol, LDL-cholesterol, total protein ($P < 0.01$), uric acid and vitamin E ($P < 0.05$) levels significantly increased after red wine consumption (Table 1). Arylesterase activity decreased by 22%, from 71 ± 17 IU/L to 60 ± 27 IU/L ($P < 0.01$, Fig. 1). Paraoxonase activity determined before (283 ± 82 IU/L) and after (290 ± 77 IU/L) red wine consumption were not significantly different. Basal (without incubation) MDA levels of the apolipoprotein B-containing lipoprotein fraction before (6.4 ± 0.7 nmol/mg cholesterol) and after (7.5 ± 2.3 nmol/mg cholesterol) red wine consumption were not significantly different, however ΔMDA levels of apolipoprotein B-containing lipoproteins decreased by 29%, from 72 ± 18 nmol/mg cholesterol to 55 ± 11 nmol/mg cholesterol ($P < 0.01$, Fig, 2) after red wine consumption. Plasma MDA levels did not show any difference before (9.7 ± 1.9 nmol/mL) and after (9.2 ± 1.0 nmol/mL) red wine consumption. In the present study smoking and non-smoking subjects were separately analyzed and data determined before red wine consumption and the difference (% change) before and after red wine consumption between the smoking and non-smoking subjects (except the lower HDL-cholesterol levels in the smoker group) were not significantly different (data not shown). The total phenolics content of the red wine was 2880 ± 69 mg/L, (n:5, X ± SD).

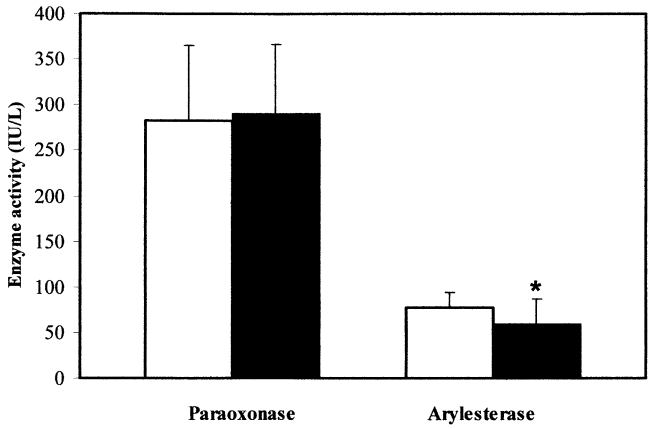

Fig. 1. Serum paraoxonase and arylesterase activities (IU/L) before (□) and after (■) red wine consumption. *Arylesterase activity decreased significantly ($p < 0.01$) after red wine consumption (Wilcoxon Signed Rank test). Data are expressed as mean ± SD.

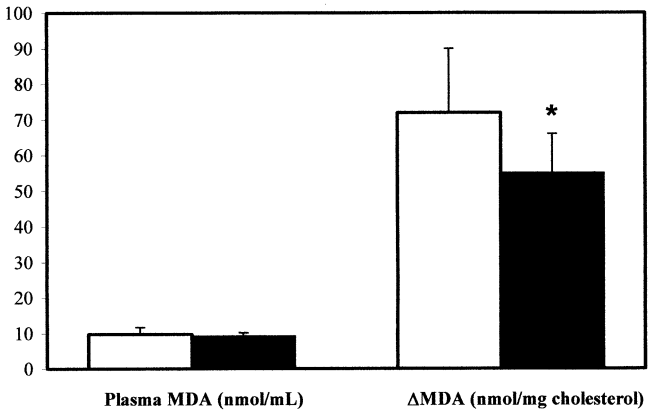

Fig. 2. Levels of plasma malondialdehyde (MDA) and ΔMDA levels of apolipoprotein B-containing lipoproteins before (□) and after (■) red wine consumption. Apolipoprotein B-containing lipoproteins were incubated with copper sulfate for 3 hours. ΔMDA was obtained by subtracting basal (without incubation) MDA value from the 3 hour value. *ΔMDA was significantly ($p < 0.01$) decreased after red wine consumption. Data are expressed as mean ± SD.

4. Discussion

The cardioprotective effect of red wine has been partly attributed to its favorable effects on the lipid profile. However interventional studies showed discrepant results, probably related to the differences in dosage or time course of red wine consumption. Lavy et al. found increment in triglyceride and HDL-cholesterol levels and did not find any change in total cholesterol or LDL-cholesterol levels after 2 weeks of 400 mL red wine (11% alcohol) consumption [11]. Sharpe et al. reported that there had been no change in triglyceride, total cholesterol and HDL-cholesterol levels, but found a decrement in LDL-cholesterol levels in volunteers that were given 200 mL of red wine per day for 10 days [24]. In this study total cholesterol and LDL-cholesterol levels were found to be increased after red wine consumption and there were not significant changes in HDL-cholesterol or triglyceride concentrations.

Total protein and uric acid are the two main antioxidant molecules in the human plasma and their levels increased significantly ($P < 0.01$ and 0.05, respectively) after red wine consumption. It has been hypothesized that antioxidant and cardioprotective effects of red wine might be mediated by increased uric acid levels [47].

In the present study it was found that, moderate red wine consumption significantly reduced susceptibility of apolipoprotein B-containing lipoproteins to in vitro copper mediated oxidation, however there were no significant differences between the basal (without incubation) MDA levels before and after red wine consumption. Increased resistance of apolipoprotein B-containing lipoproteins to oxidation might be attributed to increased levels of vitamin E detected after red wine consumption. Similar to our results, Frankel et al. had found elevated plasma levels of alpha-tocopherol in red wine drinkers [28]. However, Fuhrman et al. reported that there had been no change in plasma vitamin E and carotenoid levels after red wine consumption and antioxidant effect of red wine in vivo had not been related to carotenoid or vitamin E levels [4]. Hayek et al. had shown that LDL isolated from E^o mice after consumption of red wine were found to be less oxidized and serum PON activity to be significantly increased [29]. Van der Gaag et al. investigated effects of several alcoholic beverages, including red wine, on serum paraoxonase activity in healthy men and found that daily moderate alcohol consumption increased serum paraoxonase activity. These investigators suggested that increase in paraoxonase activity might be one of the mechanisms underlying the reduced CVD risk in moderate drinkers [20]. In the present study although paraoxonase activity did not change after red wine consumption, there was a significant decrement in arylesterase activity. The decrement observed in arylesterase activity may be an indicator of depression in enzyme synthesis, as it has been proposed that arylesterase activity reflects the mass of the enzyme [16]. Depression in enzyme synthesis may be related to lesser requirement for its activity because of a possible favorable antioxidant status, that may be provided by red wine polyphenols and/or total protein and uric acid enhancement. Although Van der Gaag et al. found increased paraoxonase activity after 3 weeks of red wine consumption, these authors did not observe any difference in enzyme concentrations [20]. However not much is known about the association between diet and paraoxonase activity [21] and little information is available on the relation between antioxidant status and paraoxonase activity or synthesis [48]. It has been shown that smoking has inhibitory effect on serum paraoxonase activity. Although paraoxonase activity was lower in the smoking subjects than those in the non-smoker subjects, the difference between the groups was not statistically significant. This could be because of the limited number of cases (n: 7) in each group.

Different types of red wines might exert varying effects due to their different antioxidant contents [6,49,50]. It should be taken into consideration that, the presence of natural wine compounds depends on the phenolic compounds in the grapes from which the wine is made, wine making techniques and numerous reactions during aging [6,46,47]. Discrepancies between the results of red wine studies may result from these factors.

Our results in part, supported the hypothesis that moderate red wine consumption protects lipoproteins against oxidation, as we demonstrated a decrement in susceptibility of apolipoprotein B-containing lipoproteins to in vitro oxidation. There were not any significant changes in serum paraoxonase activity, however, since the observed decrement in arylesterase activity may be the reflection of depression in PON synthesis, it may suggest a favorable antioxidant status after red wine consumption. Further studies are needed to clarify the relation of red wine consumption and atherogenesis, since red wine and/or some of the compounds it contains seem to be a promising preventive agent as a beverages or as a drug in the close future.

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