

Effect of Red Wine Consumption on Serum Oxidation and Adiponectin Levels in Overweight and Healthy Individuals

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Obesity is a well-known independent risk factor for CVD and metabolic syndrome. It has been shown that moderate red wine consumption might reduce the risk of cardiovascular disease (CVD). The aim of this study was to investigate whether red wine has anti-inflammatory and antioxidative effect in overweight subjects.

Ten overweight and 14 healthy subjects drank 200 mL/day of red wine for one month. While the Cabernet Sauvignon wine caused a significant increase in paraoxonase activity ($p < 0.05$), it led to a decrease in basal and stimulated LDL diene levels in both of the groups ($p < 0.05$). Total antioxidant activity increased in the two groups following wine consumption.

While wine consumption had no effect on IL-6, TNF- α and CRP levels of overweight subjects, it led to an increase in adiponectin levels.

Current data consider that regular red wine consumption may be beneficial to improve the antioxidant potential of LDL oxidation in overweight patients. In addition to the prevention of LDL oxidation, moderate wine consumption might delay the onset of atherosclerosis through an increase in paraoxonase and adiponectin levels.

INTRODUCTION

Obesity is a well-known risk factor of many diseases including coronary heart disease, diabetes, metabolic syndrome *etc.* It has been shown that there was a direct relation between the markers of oxidative stress, susceptibility of LDL to oxidation and general obesity [Dandona *et al.*, 2001; Olusi, 2002; Mutlu Turkoglu *et al.*, 2003; Myara *et al.*, 2003; Njajou *et al.*, 2009].

Recently it has been shown that obesity and being overweight is strongly associated with elevated LDL oxidation and chronic low grade inflammation in humans [Wang *et al.*, 2010]. Several factors such as leptin, IL-1, TNF- α , IL-6 and resistin are overproduced by adipocytes as well as macrophages in adipose tissue [Das, 2002]. On the other hand, plasma levels of adiponectin, as an insulin-sensitizer, are down-regulated in obese patients [Hung *et al.*, 2008]. Many studies have demonstrated that a moderate red wine consumption decreased the risk and mortality from coronary heart disease [Gaziano *et al.*, 1993; Kerry & Abbey, 1997; Kondo *et al.*, 1994; Natella *et al.*, 2001; Iriti & Vitalini, 2012]. Previously, our data provided evidence for the acute antioxidant effect of red wine, especially the organic types, but there

were no statistically significant effects on the LDL-oxidation in humans. From our data, it can be concluded that the long-term consumption of red wine might have more potent antioxidant activity rather than a single dose, possibly due to the storage ability of flavonoids in body tissues [Akçay *et al.*, 2004] as suggested by Luzzi & Maiani [1999]. We hypothesized that wine flavonoids might be stored in adipose tissue and exert their anti-oxidant / anti-inflammatory effect in overweight subjects who have adipocytes where there was no accumulation of macrophages.

We investigated the effects of long-term (1 month) wine consumption on the antioxidant-oxidant system and inflammatory markers in both healthy and overweight subjects at risk of atherosclerosis and metabolic syndrome due to insulin resistance and adipocyte mass. We aimed to study whether red wine has an anti-inflammatory and antioxidative effect in overweight subjects.

SUBJECTS AND METHODS

Subjects

Ten overweight (6 men, 4 women) and 14 age-matched (7 men, 7 women) healthy subjects were enrolled in this study.

At the baseline, detailed clinical history of ethanol intake, smoking and dietary habits were collected by a structured

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questionnaire. All subjects have been examined by an endocrinologist. Before and after the intervention period, we measured body weight and anthropometric parameters and obtained blood samples after overnight fasting. Participants had no history of coronary artery disease, diabetes mellitus, hypertension, smoking, any inflammatory or liver disease, and they were not receiving any regular medications or antioxidant supplements. All the subjects included in the study followed an isocaloric diet, which was designed according to their personal preferences. Subjects with regular alcohol consumption were excluded.

All participants were asked to drink 200 mL/day of wine before dinner for 4 weeks. Since our previous data [Yildirim *et al.*, 2004] showed that Merlot and Cabernet sauvignon type wines showed a great antioxidant activity (AOA) and inhibitory effect against LDL-oxidation as well as high total phenol levels, all participants drank Cabernet Sauvignon type wine in this study.

All participants were informed about the study and treatment protocol and gave written informed consent. The study was approved by the local Ethics Committee (protocol no:06–1/31) and conducted in accordance with the 1975 Helsinki Declaration, as amended in 1983).

Methods

Venous blood samples were taken at baseline and on the 30th day of wine consumption. Following centrifugation at 3500 rpm at 4°C for 15 min, plasma or serum was collected and stored at –80°C for a few weeks, until they were assayed. Serum concentrations of glucose, total cholesterol, HDL-cholesterol, triglycerides were measured in a chemical autoanalyzer with routine methods. LDL-cholesterol levels were calculated according to Friedman's formula.

TAO – Total antioxidant activity [Yildirim *et al.*, 2005]

The solution of 0.1 mmol/L DPPH (1,1-diphenyl-2-picrylhydrazin) was rapidly mixed well with serum sample (1/100; v/v). The decline in absorbance was recorded at 550 nm against ethanol blank over a period of 20 min in 5-min intervals in microplate reader. Trolox was used as standard.

FRAP – Ferric Reducing Antioxidant Power [Pulido *et al.*, 2000]

Mixing solution (10:1:1, v/v/v) of acetate buffer (10 mmol/L, pH=3.6), TPTZ (2,4,6-tripyridyl-s-triazine) (10 nM/LM) and FeCl₃ (20 mmol/L) were added into serum sample and stored at room temperature for 30 min. Readings were done at 620 nm in a microplate reader.

Total phenols [Singleton & Rossi, 1965; Stratil *et al.*, 2006]

Total phenolic content was determined by Folin-Ciocalteu method in the following modification: 0.200 mL of sample and 1.0 mL of Folin-Ciocalteu reagent diluted with water (1/10) were mixed. After 2 min; 0.8 mL of saturated sodium carbonate was added. After mixing on a shaker and heating at 50°C for 5 min, the reading was performed at 760 nm in a spectrophotometer against blank. The results were expressed as gallic acid equivalents (GAE) using calibration curve against gallic acid (Merck) standard (100 mg/L).

Serum paraoxonase and arylesterase activities

Serum paraoxonase and arylesterase activities were measured as described previously [Mackness *et al.*, 1991]. Briefly, paraoxon (5.5 mmol/L) and arylesterase were used as the substrates and the rates of their hydrolysis were determined in 100 mmol/L Tris/HCl buffer containing 2 mmol/L CaCl₂ at pH 8.0. P-nitrophenol in various concentrations (between 0.5 and 10.0 nmol/mL) that is product of paraoxonase reaction, was used as standard. Arylesterase activity was calculated using extinction coefficient.

In vitro serum oxidation [Stupans *et al.*, 2002]

Following dilution of serum with phosphate buffered saline (1/200; v/v), *in vitro* oxidation of serum was induced by incubating with 5 mmol/L CuSO₄ at 30°C for 120 min. Before (basal diene) and after (stimulated diene) incubation period, the absorbance was measured at 234 nm with LKB spectrophotometer and the calculations were performed using the extinction coefficient of 29,500 L/mol-cm.

Preparation of hemolysates

After separation of plasma, the packed erythrocytes were washed two times with 9 g/L NaCl solution and hemolysed with ice-cold water (1/5, v/v). The hemoglobin values were measured with the Drabkin's method.

Erythrocyte SOD (eSOD) activities

The eSOD activities were measured based on the inhibition of epinephrine autoxidation by SOD at 480 nm, with a Shimadzu spectrophotometer [Sozmen *et al.*, 2001]. The assay was calibrated by using purified SOD and one unit of enzyme was defined as the amount of enzyme, which inhibits 50% of autoxidation of epinephrine.

Erythrocyte catalase (eCAT) activities

The eCAT activities were determined as described by Sozmen *et al.* [2001] in which the degradation of hydrogen peroxide is recorded spectrophotometrically at 240 nm. One unit of CAT was defined as the amount of enzyme, which decomposes 1 μmol H₂O₂/min under specific conditions.

Enzyme linked immunosorbent assays (ELISA) were used for the determination of plasma levels of high sensitive-C-reactive protein (hsCRP) (DRG Diagnostics, EIA 3954, USA), interleukin-6 (IL-6) (Biosource, KHC0061, USA), tumor necrosis factor alpha (TNF-α) (Biosource, Cat.: KHC3011, USA), interleukin-10 (IL-10) (Biosource, Cat.: KHC0101, USA) and adiponectine (LINCO Research, Cat.: EZHADP-61K, USA).

Statistical analysis

Statistical analysis was performed using SPSS 13.0 computer package. All descriptive data were expressed as mean ± SD. Comparisons of the baseline characteristics between the 2 groups were performed by Mann-Whitney-U Test and Student's T-test. The effect of wine in each group was assessed by Paired Wilcoxon Signed Ranks Test; *p*-values < 0.05 were considered as statistically significant.

TABLE 1. The baseline characteristics and serum lipid and antioxidant levels of subjects at baseline and after wine consumption. Data were given as mean \pm S.D.

	Healthy baseline	Healthy after wine consumption	Overweight baseline	Overweight after wine consumption
Age (years)	39 \pm 7	–	46 \pm 9	–
Weight (kg)	67 \pm 8.5	–	86 \pm 10	–
BMI (kg/m ²)	22.2 \pm 2.8	–	27.7\pm2.7 *	–
Total cholesterol (mg/dL)	186 \pm 35	179 \pm 29	206\pm26**	212 \pm 25
Triglyceride (mg/dL)	90 \pm 32	90 \pm 44	153\pm64*	163 \pm 60
HDL-cholesterol (mg/dL)	49 \pm 10	46 \pm 6	43 \pm 4	46 \pm 9
LDL-cholesterol (mg/dL)	118 \pm 33	113 \pm 25	132\pm26 **	134 \pm 19
ApoA1 (mg/dL)	1.4 \pm 3.5	1.4 \pm 4.0	1.3\pm3.3 **	1.4 \pm 4
Glucose (mg/dL)	81 \pm 10	80 \pm 13	92 \pm 15	86\pm8 ##
e-TBARS (nmol/gHb)	932 \pm 164	962 \pm 213	880 \pm 169	786 \pm 150
e-SOD (U/gHb)	390 \pm 205	305 \pm 126	333 \pm 185	137 \pm 38
e-Catalase (U/gHb)	15344 \pm 2863	16116 \pm 4815	15566 \pm 3869	13057 \pm 5524
Paraoxonase (U/L)	61 \pm 32	85\pm56 ##	76 \pm 32	62 \pm 28
Arylesterase (U/L)	114 \pm 35	121 \pm 29	102 \pm 31	123\pm31##
Phenol (mg/mL)	5.1 \pm 1.6	4.3 \pm 1.7	4.2 \pm 1.3	4.6 \pm 2.3
FRAP (mmol/L)	2028 \pm 333	2149\pm532 #	2202 \pm 252	2399 \pm 266
TAO activity (mmol/L Trolox eq.)	1.5 \pm 2.1	1.1 \pm 1.1	0.73 \pm 0.63	1.23 \pm 0.83

* $p < 0.01$ and ** $p < 0.05$ (Student's t Test) versus healthy controls; ## $p < 0.05$. # $p = 0.064$ (Wilcoxon Signed Ranks Test) versus to matched-baseline values.

RESULTS

The baseline characteristics and the serum lipid levels of subjects are summarized in Table 1. As expected, BMI and weight of overweight subjects were higher ($p < 0.001$) than these of the healthy controls. Total cholesterol ($p < 0.05$), triglyceride ($p < 0.001$), LDL-cholesterol ($p < 0.05$) levels of overweight subjects were higher than these of the healthy subjects. There were no significant differences in HDL-cholesterol levels between two groups. Following wine consumption glucose levels significantly ($p < 0.05$, Wilcoxon Signed Ranks Test) decreased in overweight participants, while lipid parameters did not show any change.

As it has been shown in Table 1, there were no statistically significant differences (Mann-Whitney-U test) in any of the parameters that show oxidant-antioxidant status between the groups at the beginning of the study. Although FRAP levels ($p = 0.064$) of controls following wine consumption were higher than the baseline levels, these differences were not statistically significant. Paraoxonase activities significantly increased upon wine consumption in healthy controls ($p < 0.016$). Arylesterase ($p = 0.047$) of overweight subjects on 30th day were higher than their baseline levels. FRAP ($p = 0.059$) and phenol levels ($p = 0.08$) also increased but not statistically significant. While eSOD activity ($p = 0.08$) was slightly decreasing, eCAT activity and eTBARS levels did not show any change in overweight subjects with wine consumption.

When the data were evaluated regarding to the group (healthy and overweight) (Figure 1); basal and copper stimulated diene levels on the 30th day of wine consumption were found as lower than the baseline levels in controls ($p = 0.035$

and $p = 0.011$, respectively). As it can be seen from Figure 1, basal diene levels significantly and stimulated diene levels insignificantly decreased on the 30th day of wine consumption regarding to basal levels in overweight subjects ($p = 0.047$, $p = 0.074$, respectively).

CRP values of overweight subjects were higher than these of the healthy subjects (Table 2) but wine had no effect on CRP values both in healthy and overweight subjects. There was no change in TNF- α , IL-6 and IL-10 levels following wine consumption in both groups. Plasma levels of adiponectin, as an insulin-sensitizer was lower, but not significantly, in overweight patients compared to controls and wine consumption led to an increase in adiponectin levels of overweight subjects.

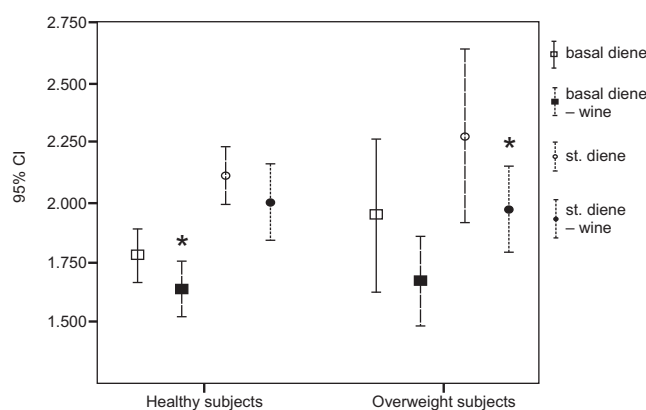


FIGURE 1. Effect of wine administration on basal and copper-stimulated LDL diene levels in healthy and overweight subjects. * $p < 0.05$ – comparisons were made to their own baseline levels.

TABLE 2. The baseline characteristics and serum inflammatory markers of subjects at baseline and after wine consumption. Data were given as mean±S.D.

	Healthy baseline	Healthy after wine consumption	Overweight baseline	Overweight after wine consumption
Hs-CRP (mg/L)	0.10±0.06	0.11±0.05	0.19±0.12*	0.16±0.08
TNF-alpha (ng/L)	17.3±17.3	13.3±11.8	13.8±11.4	19.1±20.5
IL-6 (ng/L)	5.2±4.1	6.9±12.6	5.5±6.2	6.6±9.8
IL-10 (ng/L)	2.7±0.3	2.6±0.2	2.7±0.4	2.7±0.3
Adiponectin	9.46±3.65	9.35±3.89	7.92±3.36	8.57±3.51

* p<0.01 (Student's t Test) versus to healthy controls.

DISCUSSION

Current evidence from epidemiologic and experimental studies has suggested a protective effect against the development of cardiovascular disease with moderate consumption of red wine [Wollin & Jones, 2001, Aviram & Fuhrman, 2002]. Phenolic compounds present in red wine cause an increase in serum total antioxidant capacity [Whitehead *et al.*, 1995], and thereby inhibit low-density lipoprotein (LDL) oxidation [Frankel *et al.*, 1993]. We investigated possible antioxidant and anti-inflammatory effect of red wine consumption in overweight subjects under the risk of atherosclerosis and metabolic syndrome due to insulin resistance and adipocyte mass.

Our data showed that moderate red wine consumption prevented LDL oxidation in both healthy and overweight subjects. The basal and copper-stimulated LDL diene levels of overweight subjects were not significantly higher than those of the healthy subjects, and wine consumption significantly decreased the basal and stimulated diene levels in healthy and overweight subjects (Figure 1). Several possible contributors to oxidative stress in obesity were reviewed by Vincent & Taylor [2006]. These factors comprise the hyperglycemia, lower intake of antioxidants, increased muscle activity to carry excessive weight, increased dietary fat intake, increased fat storage and excessive intracellular triglycerides and dyslipidemia, and chronic inflammation [Vincent & Taylor, 2006]. Our study group (overweight but not obese) was subjected to oxidative stress due to high glucose, triglyceride and LDL cholesterol levels. Although there are a number of studies suggesting the antioxidant effects of red wine on LDL oxidation, the data obtained from *in vivo* studies were conflicting [Frankel *et al.*, 1993; DeRijke *et al.*, 1996; Nigdikar *et al.*, 1998; Sarandol *et al.*, 2003; Egert *et al.*, 2009]. Previously it has been shown that both organic and inorganic wines consumed in the short term had no effect on basal and Cu-stimulated LDL oxidation [Akçay *et al.*, 2004; DeRijke *et al.*, 1996]. On the other hand, Sarandol *et al.* [2003] 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) TBARS levels before and after red wine consumption. In accordance with current data, Fuhrman & Aviram [2002] showed that LDL of volunteers had a reduced propensity for copper ion-induced lipid peroxidation in comparison to LDL obtained at the baseline.

The effects of red wine consumption on LDL oxidation could be related to an elevation in the total polyphenols content in the plasma as well as in the LDL particle [Akçay *et al.*, 2004; Nigdikar *et al.*, 1998] and this current data also supported this notion. Since FRAP levels of controls and overweight subjects following wine consumption were observed to be higher than their baseline levels, we proposed that the protective effects of wine against oxidative stress directly depends on the antioxidative effect of its phenolic compounds (phenolic acids and flavonoids). The main polyphenolic groups (flavon and flavonols, catechins, proanthocyanidins, benzoic acids) that are found in wines are efficient scavengers of free radicals and breakers of lipid peroxidative chain reactions [Teissedre & Landrault, 2000]. They exert their antioxidant activity by chelating transition metals such as iron and copper ions, which are involved in free radical generation [Sagin & Sozmen, 2004].

It has been clearly shown that paraoxonase (PON1) prevents LDL from oxidation by removing oxidized phospholipids from LDL [Sozmen *et al.*, 2008]. Paraoxonase activity increased with wine consumption in control subjects and arylesterase activity in overweight subjects. Van der Gaag *et al.* [2009] observed that the alcohol-induced increase in serum paraoxonase activity after 3 weeks of red wine consumption correlated strongly with the elevation in HDL-C and apo A-I concentration. Our hypothesis is that the elevation in paraoxonase activity might be due to a decrease in lipid peroxidation *via* phenolic components in wine. Although it has been shown that inflammatory cytokines especially CRP produced by adipocytes in diabetic patients were closely related to depleted PON1 activity [Dullaart *et al.*, 2009], wine had no effect on the inflammatory cytokines in this study. Therefore, we supposed that this increase in PON1 activity is likely to be depleted in oxidative stress. The main substrate for PON1 is lipid hydroperoxide and PON1 is reduced through oxidative inactivation during detoxification of lipid hydroperoxides by its esterolytic activity [Rice-Evans *et al.*, 1996]. It has been suggested that other antioxidant enzymes might prevent this inhibition of PON1 activity. These enzymes play an important role in all stages of atherosclerosis and elevation in oxidative stress might inhibit them [Sozmen *et al.*, 2008]. The current *in vivo* study showed that the long term wine consumption had no effect on erythrocyte antioxidant enzymes, eSOD and catalase, both in healthy and overweight subjects.

Besides this well-known antioxidant activity, polyphenols also have anti-inflammatory effects. In this study, we inves-

tigated some inflammatory markers in overweight subjects compared to healthy controls and a possible effect of red wine on these parameters. We determined that overweight subjects had higher CRP values than controls, which was in accordance with a study by Warnberg *et al.* [2006] who showed that CRP concentrations were significantly associated with overweight and obesity in both females and males. They suggested that obesity and being overweight during adolescence were associated with a chronic low-grade inflammatory response.

The data related to the effects of red wine consumption on inflammatory markers were also conflicting. While some authors found no change in inflammatory markers (IL-6, CRP) following acute intake of red wine and beer [Tousoulis *et al.*, 2008], others reported depletion in serum IL-6 levels [Sacanella *et al.*, 2007] and an amplification of IL-10 and reduction of NF-kappaB [Natella *et al.*, 2001] following acute red wine intake. Avogaro *et al.* [2003] observed that moderate red wine intake improved post-glucose free fatty acid profiles but did not modify the plasma concentrations of both TNF- α and adiponectin concentrations. We observed no change in CRP, TNF- α , IL-6 and IL-10 levels following long-term wine consumption compared to their baseline levels in this study. These discrepancies between the different studies probably resulted from the dose and administration period of wine.

Previously Matsushita *et al.* [2006] found that the adiponectin level was a more significant predictor than TNF- α , IL-6 or CRP for prevalent metabolic syndrome. Our observation supported this data, and the adiponectin level was lower in overweight subjects at high risk of metabolic syndrome than those of healthy controls. Hung *et al.* [2008] showed that plasma adiponectin concentrations were inversely associated with insulin resistance scores independent of obesity and higher circulating adiponectin concentrations were associated with a reduced prevalence of metabolic syndrome and that this relationship was independent of the level of obesity and inflammatory markers. We determined that there was an increase in adiponectin levels following wine consumption in overweight subjects. Adiponectin is not only an insulin-sensitizer, but also represses the pro-inflammatory effects of TNF- α on the vascular system and probably prevents the progression of atherosclerosis [Ouchi *et al.*, 1999]. In accordance with our data, Imhof *et al.* [2009] showed that the adiponectin levels among women significantly increased after consuming red wine and increased among men after ethanol solution and consuming beer.

There was a significant correlation between the adiponectin level and PON1 arylesterase activity and a negative correlation between the adiponectin level and fasting glucose levels in this study. Our data is consistent with findings of other studies [Koncsos *et al.*, 2010; Seres *et al.*, 2010] that showed that adiponectin proved to be an independent factor of PON1 activity in both childhood and adult obesity.

CONCLUSIONS

In conclusion, chronic moderate wine consumption (in a dose of 200 mL/day for 4 weeks) decreased basal and stimulated serum oxidation, increased antioxidant activity (FRAP) and had no effect on inflammatory cytokines

(CRP, IL-6, IL-10 and TNF α) in both healthy and overweight subjects. One of the important data of this study is a decline in glucose levels and elevation in adiponectin levels in overweight subjects after wine consumption. We suggest that moderate wine consumption might delay the onset of atherosclerosis and metabolic syndrome by increasing paraoxonase/arylesterase activity and adiponectin levels, as well as preventing LDL oxidation.

Since we do not have measures of insulin resistance we cannot discuss about the possible relationship between oxidative stress, adiponectin and insulin resistance. The number of volunteers who accept to drink wine regularly for a month has limited to get significant results. With the same reason we could not conduct a statistical analysis between men and women.

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