

# Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation<sup>1,2</sup>

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**ABSTRACT** The effect of consumption of red or white wine (11% alcohol) with meals on the propensity of plasma and low-density lipoprotein (LDL) to undergo lipid peroxidation was studied in 17 healthy men who were divided into two groups: 8 received 400 mL red wine/d for 2 wk, and 9 received a similar amount of white wine. Red wine consumption for 2 wk resulted in a 20% reduction in the propensity of plasma to undergo lipid peroxidation (in the presence of a free-radical-generating system) as determined by the thiobarbituric acid reactive substances (TBARS) assay. In parallel, red wine consumption reduced the propensity of the volunteers' LDL to undergo lipid peroxidation (in response to copper ions) as determined by a 46%, 72%, and 54% decrease in the content of TBARS, lipid peroxides, and conjugated dienes in LDL, respectively, as well as by a substantial prolongation of the lag phase required for the initiation of LDL oxidation. On the contrary, dietary consumption of white wine for 2 wk resulted in a 34% increase in plasma's propensity to undergo lipid peroxidation and also in a 41% increased propensity of the LDL to undergo lipid peroxidation. The antioxidant effect of dietary red wine on plasma lipid peroxidation was not secondary to changes in the plasma vitamin E or  $\beta$ -carotene content but could be related to the elevation of polyphenol concentration in plasma and LDL. Thus, some phenolic substances that exist in red wine, but not in white wine, are absorbed, bind to plasma LDL, and may be responsible for the antioxidant properties of red wine. *Am J Clin Nutr* 1995;61:549–54

**KEY WORDS** Lipid peroxidation, red wine, white wine, low-density lipoprotein

## Introduction

An early atherosclerotic lesion consists of macrophages that are filled with cholesterol, which is mainly derived from the uptake of modified forms of low-density lipoproteins (LDL) including oxidized LDL (1–10). The susceptibility of LDL to lipid peroxidation positively correlates with the lipoprotein content of polyunsaturated fatty acids and inversely correlates with the concentration of monounsaturated fatty acids in LDL (11, 12). The peroxidation of LDL lipids is preceded by initial oxidation of the lipoprotein associated vitamin E and carotenoids (13–15). In addition, extrinsic factors such as transition

metal ions (16, 17), cellular oxygenases (18, 19), matrix components (20), and high-density lipoprotein (HDL) (21) have also been shown to affect the propensity of LDL to undergo lipid peroxidation. Flavonoids are components of a wide variety of edible plants, fruits, and vegetables, and of beverages such as tea, coffee, beer, and wine (22). This group of compounds appears to have antioxidative properties toward LDL lipid peroxidation (23–25), and they have been reported to exert free radical-scavenging capabilities (26–28). The flavonoids are soluble chain-breaking agents of the peroxy and alkoxy radicals (23), and they also possess high affinity for iron ions (26). Flavonoids were shown to possess an inhibitory effect on 5-lipoxygenase of rat peritoneal macrophages (29) and of human neutrophils (30), and on both the 12-lipoxygenase of bovine platelets and on 15-lipoxygenase of soybean (31). An epidemiological study of 800 Dutch subjects revealed that flavonoid intake was inversely associated with morbidity and mortality from coronary heart disease (32). Recently, *in vitro* inhibition of LDL oxidation by phenolic flavonoids derived from red wine has been demonstrated (33), and resveratrol has been suggested as one of the active ingredients of these polyphenols (34). Little is known, however, about the absorption status or about the metabolism of wine polyphenols, and hence their *in vivo* antioxidative effects have not yet been estimated.

The present study was undertaken to analyze the *in vivo* effect of red wine consumption (in comparison with white wine) on the susceptibility of plasma and LDL to lipid peroxidation.

## Subjects and methods

### Subjects

Seventeen healthy men, aged 25–45 y, who were nonsmokers, taking no medication, and consuming a standard Israeli diet (53% fat, 26% carbohydrate, and 21% protein) participated in this study. Before study entry the volunteers' alcohol consumption was only occasional, and not >1 L wine/wk. All of the

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volunteers were asked to maintain their habitual diet and lifestyle 1 wk before the beginning of the study and during the 2 wk of the study. The volunteers were divided into two groups, and each group consumed 400 mL (two glasses/d) of white or red wine (11% alcohol). One glass of wine was consumed at lunch and the other during the supper meal. The wine was a generous gift from Barkan Ltd (Barkan, Israel; red wine, Cabernet Sauvignon and white wine, Emerald Riesling). The wine provided during the study was the only alcohol allowed during the study period. Blood samples were drawn 12 h after the supper meal. Blood was taken before wine consumption and after 1 and 2 wk of wine consumption.

The study protocol was approved by the Rambam Helsinki Committee. All subjects signed informed consent documents.

#### Plasma lipid peroxidation

Blood was drawn into Na<sub>2</sub>EDTA (1 mmol/L), and centrifuged at 200 × *g* for 10 min at room temperature. White and red wine samples were always run simultaneously. Four milliliters of plasma was dialyzed against phosphate-buffered saline overnight at 4 °C. Then 2 mL plasma was incubated for 2 h at 37 °C without (control) or with (100 mmol/L) 2,2'-azobis,2-amidinopropane hydrochloride (AAPH), (Polysciences, Warrington, PA). AAPH is a water-soluble azo compound that thermally decomposes and thus generates water-soluble peroxy radicals at a constant rate (35). Under these conditions, plasma lipid peroxidation was maximal. The samples were then kept at 4 °C and analyzed for their oxidation state within 2 h by using the thiobarbituric acid reactive substances (TBARS) assay, which measures malondialdehyde (MDA) equivalents (36).

#### LDL oxidation

LDL was separated from the plasma by discontinuous density-gradient ultracentrifugation (37) and dialyzed against saline with EDTA (1 mmol/L). LDL was diluted in phosphate-buffered saline to 200 mg protein/L and dialyzed overnight against phosphate-buffered saline at 4 °C to remove the EDTA.

Dialysis of LDL before the oxidation experiments did not affect the content of phenolic substances. Similar amounts of total polyphenols were found in freshly prepared LDL as in the same LDL sample after a night of dialysis (25.5 and 24.7 mg polyphenols/g LDL protein before and after overnight dialysis, respectively).

The lipoprotein was then incubated in the presence of (10 μmol/L) CuSO<sub>4</sub> at 37 °C for 5 h. Oxidation was terminated by refrigeration and the addition of EDTA (0.1 mmol/L). LDL oxidation was determined by 1) MDA analysis (36), 2) lipid peroxide determination with a commercially available kit [cholesterol color reagent, CHOD iodide method; Diagnostica MERK, Darmstadt, Germany (38)] and 3) conjugated diene formation (17). 4) LDL oxidation kinetics were continuously monitored by measuring the increase in absorbance at 234 nm (14).

#### Other assays

Plasma concentrations of vitamin E (α-tocopherol) and β-carotene were determined by HPLC analysis (14). Plasma concentration of polyphenols was determined spectrophotometrically (39) with phosphomolybdic phosphotungstic acid

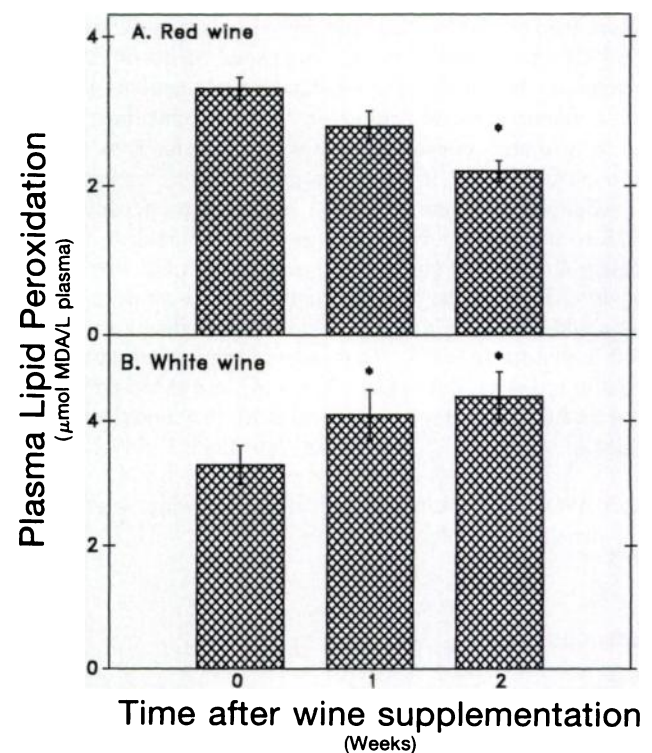
reagents. LDL protein concentration was determined with the Folin phenol reagent (40).

#### Statistics

Student's paired *t* test was performed for all statistical analyses and results are given as  $\bar{x} \pm \text{SEM}$ . The computer software program *STATEASE* (version 1.00; DataPlus Systems Inc, New York) was used for computations.

#### Results

Dietary consumption of 400 mL red wine/d by eight men resulted in a gradual reduction in the susceptibility of their plasma to lipid peroxidation in the presence of the free radical-generating substance AAPH by 12% and 20% after 1 and 2 wk of consumption, respectively (Figure 1A). In contrast, plasma that was sampled after 1 and 2 wk of white wine consumption demonstrated a 24% and 33% greater propensity to undergo lipid peroxidation (Figure 1B). After 1 wk of red wine consumption no significant changes were found in the propensity of LDL to undergo lipid peroxidation in the presence of copper ions. However, 2 wk of red wine consumption resulted in a substantial reduction in the propensity of LDL to oxidize with a 44%, 40%, and 44% decrement in the formation of TBARS, lipid peroxides, and conjugated dienes, respectively (Figure 2

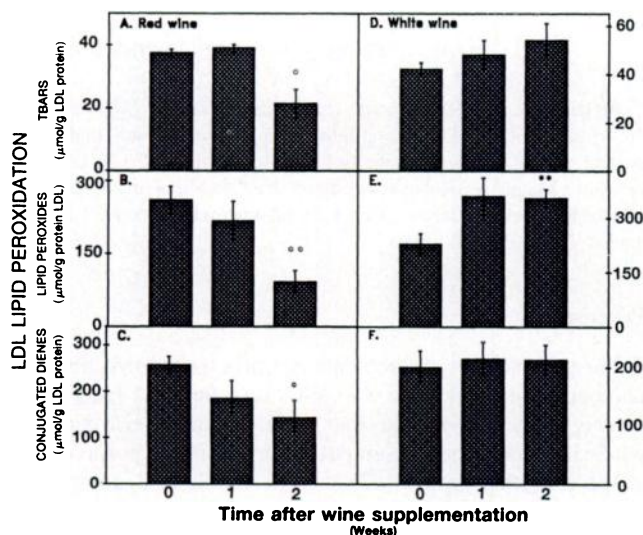


**FIGURE 1.** The effect of red ( $n=8$ ) or white ( $n=9$ ) wine consumption on plasma's propensity to undergo lipid peroxidation. Plasma that was obtained before (0 time) or after 1 and 2 wk of red or white wine consumption was incubated for 2 h at 37 °C without (control) or with 2,2'-azobis,2-amidinopropane hydrochloride (AAPH) (100 mmol/L). At the end of the incubation plasma lipid peroxidation was determined by the thiobarbituric acid reactive substances assay. Results were obtained by subtracting control values from AAPH-induced lipid peroxidation.  $\bar{x} \pm \text{SEM}$ . \*  $P < 0.01$  (vs 0 time). MDA, malondialdehyde equivalents.

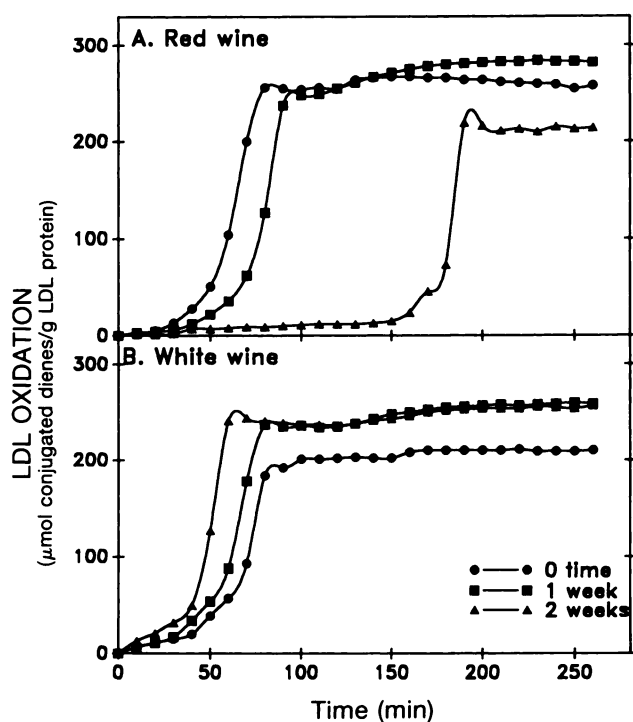


A–C). The propensity of LDL derived from subjects that consumed white wine to undergo lipid peroxidation, however, did not significantly change (Figure 2 D, and F), except for a 41% increment in LDL lipid peroxide formation after 2 wk of white wine consumption (Figure 2E).

The susceptibility of LDL to lipid peroxidation in the presence of  $\text{CuSO}_4$  ( $10 \mu\text{mol/L}$ ) was continuously monitored by measuring the increase in the absorbance at 234 nm. This absorption develops in LDL during its oxidation through the formation of conjugated dienes, and it represents the initial step in LDL oxidation. The lag phase, which represents the resistance of LDL to undergo lipid peroxidation, was prolonged from  $45 \pm 3.8$  min to  $62 \pm 5.3$  and  $175 \pm 14.9$  min, after 1 and 2 wk of red wine consumption, respectively (Figure 3A). In addition, a 17% decrement in the maximal formation of conjugated dienes after 2 wk of red wine consumption was observed (Figure 3A). One and 2 wk of white wine consumption reduced the lag phase time from  $60 \pm 5.8$  min to  $50 \pm 4.85$  and  $40 \pm 3.9$  min, respectively (Figure 3B). This pattern was accompanied by an 18% increment in the maximal formation of conjugated dienes after 2 wk of white wine consumption (Figure 3B). Thus, red wine consumption retards the susceptibility of LDL to oxidation whereas white wine renders the LDL more susceptible to oxidation. Wine consumption may affect the propensity of plasma and LDL to undergo lipid peroxidation via changes in plasma antioxidants, as well as from a direct contribution of some wine constituents. No significant difference between red or white wine consumers was found for baseline plasma concentrations of carotenoids, vitamin A, and vitamin E. In addition, no significant changes were found in plasma concentrations of carotenoids, vitamin E, or vitamin A after red or white wine consumption (Table 1). Plasma cho-



**FIGURE 2.** The effect of red ( $n=8$ ) or white ( $n=9$ ) wine consumption on the susceptibility of LDL to lipid peroxidation. LDL ( $200 \text{ mg protein/L}$ ) collected before or 1 and 2 wk after red or white wine consumption was incubated for 4 h at  $37^\circ\text{C}$  without (control) or with  $\text{CuSO}_4$  ( $10 \mu\text{mol/L}$ ). Oxidation was measured by analyses of thiobarbituric acid reactive substances (A,D), lipid peroxidation (B,E), and conjugated dienes (C,F), and was calculated by subtracting the values obtained for the control, from the values obtained in the presence of  $\text{CuSO}_4$ .  $\bar{x} \pm \text{SEM}$ . \*  $P < 0.05$  (vs 0 time), \*\*  $P < 0.01$  (vs 0 time).



**FIGURE 3.** Kinetic analysis of copper-induced LDL oxidation using lipoprotein that was collected before as well as after 1 and 2 wk of red or white wine consumption. LDL ( $200 \text{ mg protein/L}$ ) was incubated with  $\text{CuSO}_4$  ( $10 \mu\text{mol/L}$ ) and the change in the absorbance at 234 nm was continuously monitored and recorded over a 3-h period. The length of the lag phase was determined as the intercept of the tangents drawn to the segments of the absorbance curve corresponding to the lag phase. The variability in the length of the lag phase was 8.5% between individuals in the red wine group, and 9.7% in the white wine group.

lesterol determination revealed no significant changes in total cholesterol but the HDL cholesterol fraction increased from  $1.25 \pm 0.31$  to  $1.59 \pm 0.29 \text{ mmol/L}$  after 2 wk of red wine consumption ( $P < 0.01$ ). No significant changes in HDL concentration were found after 2 wk of white wine consumption ( $1.22 \pm 0.16$  vs  $1.3 \pm 0.2 \text{ mmol/L}$  before and after white wine consumption, respectively). In addition, no significant changes were found in the cholesterol-protein ratio of LDL from either group after 2 wk of wine consumption ( $1.55 \pm 0.09$  and  $1.42 \pm 0.1$  before and after red wine consumption, and  $1.39 \pm 0.09$  and  $1.42 \pm 0.12$  before and after white wine consumption, respectively), suggesting that LDL composition was not significantly altered after wine intake.

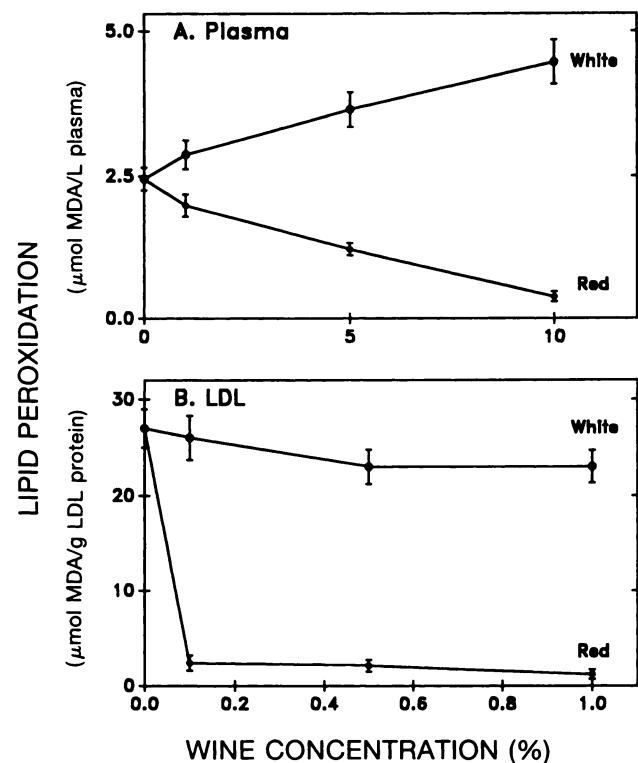
We next studied the direct in vitro effect of red and white wine on the lipid peroxidation of plasma and LDL. In vitro incubation of plasma with increased concentrations of red wine for 2 h at  $37^\circ\text{C}$  in the presence of AAPH ( $100 \text{ mmol/L}$ ) resulted in an up to 84% reduction in TBARS formation, and this was a wine concentration-dependent process (Figure 4A). Similar incubation with white wine resulted in increased TBARS formation by up to 82% (Figure 4A). In vitro incubation with LDL (instead of plasma) revealed that red wine, even at a concentration of 0.1%, completely abolished copper-mediated LDL oxidation (Figure 4B), whereas white wine did not significantly affect LDL oxidation at concentrations as high as 1% (Figure 4B).

**TABLE 1**The effect of red and white wine supplementation on plasma concentrations of carotenoids, vitamin A, and vitamin E<sup>1</sup>

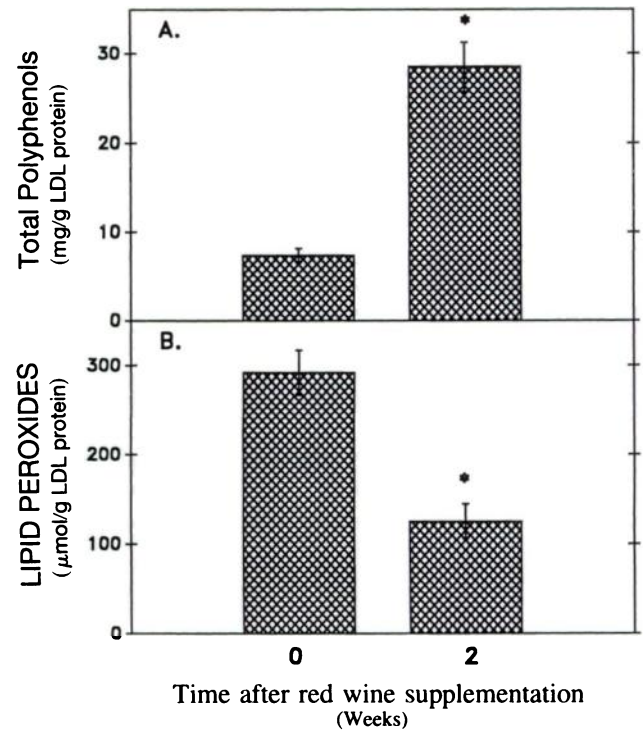
	Red wine (n=8)			White wine (n=9)		
	0 wk	1 wk	2 wk	0 wk	1 wk	2 wk
Carotenoids ( $\mu\text{mol/L}$ )	1.68 $\pm$ 0.20	1.94 $\pm$ 0.22	1.98 $\pm$ 0.24	2.28 $\pm$ 0.38	2.60 $\pm$ 0.46	2.70 $\pm$ 0.50
Vitamin A ( $\mu\text{mol/L}$ )	2.72 $\pm$ 0.24	3.08 $\pm$ 0.24	3.00 $\pm$ 0.12	3.28 $\pm$ 0.32	3.16 $\pm$ 0.24	2.96 $\pm$ 0.24
Vitamin E ( $\mu\text{mol/L}$ )	106.97 $\pm$ 9.30	120.93 $\pm$ 9.30	111.63 $\pm$ 9.30	132.56 $\pm$ 9.30	123.25 $\pm$ 6.97	118.60 $\pm$ 6.97

<sup>1</sup>  $\bar{x} \pm \text{SEM}$ .

Red wine contains a substantial amount of polyphenols in comparison with white wine (1.4 g quercetin equivalents/L in red wine compared with 0.25 g/L in white wine). Because it was shown previously that phenolic substances in red wine possess antioxidative characteristics against LDL lipid peroxidation in vitro (33), we questioned whether phenolic substances are absorbed after red wine ingestion and whether they are associated with the LDL fraction. The total polyphenol concentration in the LDL fraction was elevated fourfold after 2 wk of red wine consumption (Figure 5A). This elevation was associated with a significant ( $P < 0.01$ ) decrease (by 2.3-fold) in LDL susceptibility to copper-induced lipid peroxidation (Figure 5B).



**FIGURE 4.** In vitro effect of red and white wine on plasma (A) and on LDL (B) susceptibility to lipid peroxidation. Plasma (2 mL) or LDL (200 mg protein/L) was incubated with 2,2'-azobis,2-amidinopropane hydrochloride (100 mmol/L) or  $\text{CuSO}_4$  (10  $\mu\text{mol/L}$ ), respectively, for 4 h at 37 °C, in the presence of the indicated concentrations of red or white wine. Lipid peroxidation was measured by the thiobarbituric acid reactive substances assay.  $\bar{x} \pm \text{SEM}$ ;  $n=3$ . \*  $P < 0.01$  at each concentration examined (vs 0 concentration).



**FIGURE 5.** The effect of red wine consumption on LDL-associated polyphenols and on LDL susceptibility to lipid peroxidation. Total phenol content of LDL (A) was determined as described in Methods. LDL susceptibility to lipid peroxidation was determined after lipoprotein incubation with copper ions by analysis of the LDL lipid peroxide content.  $\bar{x} \pm \text{SEM}$ ;  $n=8$ . \*  $P < 0.01$  (vs 0 time).

## Discussion

The present in vivo study demonstrates for the first time that daily consumption of red wine (400 mL) for 2 wk reduced the susceptibility of plasma and LDL to lipid peroxidation, whereas white wine consumption showed the opposite effect.

We and other groups (33) have been able to demonstrate a similar in vitro inhibitory effect of red wine on plasma and on LDL propensity to oxidize, and this effect may be attributed to the phenolic substances that are present in red wine. As was already demonstrated (33, 34), red wine contains phenolic substances with antioxidative properties toward LDL oxidation in vitro. Our study expanded these findings to an in vivo situation, demonstrating that total polyphenol content in LDL indeed increased after dietary consumption of red wine. These results are consistent with the in vitro effect of polyphenols, which have been shown to increase the resistance of LDL



oxidation. It is thus suggested that the phenolic substances in red wine with antioxidant properties toward LDL oxidation, are absorbed from the gut into the circulation, bind to LDL, and thus exert their antioxidant characteristics. Phenolic substances' antioxidative characteristics include reaction with superoxide anions (27), hydroxyl radicals (41), and lipid peroxyl radicals (23). The antioxidative effect of red wine *in vivo* was not shown to be related to the carotenoid, vitamin E, or vitamin A content of LDL. The reduced propensity of plasma (not LDL) that was derived from subjects who consumed red wine to undergo lipid peroxidation, may be attributed, at least in part, to the red wine-mediated elevation of the HDL fraction. In addition to the antiatherogenic properties of HDL because of its role in reverse cholesterol transport, HDL was also shown to inhibit LDL oxidation (21, 42). Alcohol intake increases plasma concentration of HDL (43, 44) and thus it is of interest that although both red and white wine contain similar concentrations of alcohol, only red wine (for only 2 wk) resulted in the elevation of plasma HDL. Previously, HDL elevation after alcohol intake was demonstrated only after >3 wk of consumption (44), but the present study was conducted for only 2 wk and thus it is possible that the lack of change in plasma HDL concentration after white wine supplementation is related to the short period of consumption.

The increased plasma HDL concentration after red wine consumption could be related to lipid transfer between HDL and LDL, which may change the composition of the lipids in LDL. However, our results demonstrated that the composition of the LDL particles in both groups was not significantly altered after 2 wk of wine consumption. Thus, under the experimental conditions used in this study, we tend to attribute the difference in LDL oxidizability of the red wine and the white wine drinkers to the antioxidant effect of the phenolic compounds that exist in red wine. It is also possible that some constituents other than phenolic substances, which are unique to red wine, are responsible for this phenomenon. The elevation in the HDL fraction after red wine consumption, however, cannot explain the reduced propensity to undergo lipid peroxidation of the isolated LDL. Furthermore, the direct *in vitro* antioxidative effect of red wine on plasma and LDL, which was similar to the *in vivo* effect, suggests that some red wine-derived polyphenols play a major role in the antioxidative properties of red wine consumption toward LDL oxidation.

It is also possible that the phenolic substances in red wine can act to inhibit the formation of lipid hydroperoxides *ex vivo*, and the delayed oxidation of plasma and LDL can then result from reduced *ex vivo* peroxide formation. No lipid peroxides however could be found *ex vivo*, in control plasma or in LDL (that were obtained at zero time, before wine consumption).

In contrast to red wine, white wine consumption resulted in an increased propensity of plasma and LDL to undergo lipid peroxidation. Because white wine contains only a minimal amount of polyphenol compared with red wine, this may underline the inability of white wine to act as an antioxidant. The prooxidant activity of white wine consumption toward plasma and LDL may thus be related to the prooxidative properties of alcohol consumption (45). Prooxidative characteristics of alcohol suggest that the antioxidant capabilities of some red wine-derived polyphenols are even greater than those shown in red wine (where polyphenols are associated with the alcohol) because both red and white wine contain similar alcohol content.

Red wine intake thus overcomes the prooxidant properties of alcohol, and in addition, causes a significant inhibition of LDL oxidation. The higher MDA production in plasma taken from the white wine group may reflect a higher proportion of polyunsaturated fatty acid in plasma or in LDL. However, it was demonstrated (46) that the postprandial plasma concentration of free fatty acids was similar after 2 wk of an isoenergetic diet with or without wine supplementation. Furthermore, in animal experiments it was demonstrated that alcohol intake resulted in a decrease in the relative concentration of plasma linoleic acid and in an increase in the saturated palmitic acid (47). In humans, higher alcohol intakes were also shown to be associated with lower proportions of linoleic acid and with higher proportions of palmitic acid (48). Thus, the increase in LDL and plasma oxidation rate that was associated with white wine consumption cannot be attributed to an increased LDL or plasma polyunsaturated fatty acid concentration.

Because LDL oxidation has an important role in atherogenesis (49) the inhibition of this process by moderate red wine supplementation may be beneficial in the attenuation of the atherosclerotic process. It is possible that the French paradox [a high-fat diet with a low incidence of coronary atherosclerosis (50)], can be explained by the relatively high consumption of red wine by French people. Phenolic substances in the red wine are absorbed and bind to LDL and thus offer protection to this lipoprotein against lipid peroxidation, which can further contribute to the lower incidence of cardiovascular disease in French people. The importance of the present study is in its *in vivo* demonstration for the first time that red wine consumption inhibits the propensity of LDL to undergo lipid peroxidation, and this may contribute to attenuation of atherosclerosis. The characterization of the specific red wine constituents responsible for the antioxidative effect of red wine may enable us to purify it and test it directly for an independent effect in reducing risk of atherosclerosis. ■

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