Red Wine Mitigates the Postprandial Increase of LDL Susceptibility to Oxidation

Fausta Natella,* Andrea Ghiselli,∗ Alessia Guidi,∗ Fulvio Ursini,† and Cristina Scaccini*

*Free Radical Research Group, National Research Institute for Food and Nutrition, Roma, Italy; and †Department of Biological Chemistry, University of Padova, Padova, Italy

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Abstract—The aim of the present study was to verify the extent of oxidative stress induced by a meal at plasma and LDL level, and to investigate the capacity of red wine to counteract this action. In two different sessions, six healthy men ate the same test meal consisting of “Milanese” meat and fried potatoes. The meal was taken either with 400 ml red wine or with an isocaloric hydroalcoholic solution. Oxidative stress at plasma level was estimated through the measure of ascorbic acid, α-tocopherol, protein SH groups, uric acid, and antioxidant capacity, measured before and 1 and 3 h after the meal. The change in the resistance of LDL to oxidative modification was taken as an index of exposure to pro-oxidants. The susceptibility to Cu(II)-catalyzed oxidation of baseline and postprandial LDL was measured as conjugated dienes formation, tryptophan residues, and relative electrophoretic mobility. The experimental meal taken with wine provoked a significant increase in the total plasma antioxidant capacity and in the plasma concentration of α-tocopherol and SH groups. Postprandial LDL was more susceptible to metal-catalyzed oxidation than the homologous baseline LDL after the ethanol meal. On the contrary, postprandial LDL obtained after the wine meal was as resistant or more resistant to lipid peroxidation than fasting LDL. © 2001 Elsevier Science Inc.

Keywords—Wine, Polyphenols, Postprandial oxidative stress, Lipid hydroperoxides, LDL oxidation, Human, Free radicals

INTRODUCTION

Several observations corroborate the existence of a link between postprandial state and risk of cardiovascular disease. Postprandial concentration of triacylglycerols and triacylglycerol-rich lipoproteins better correlate with the risk for coronary heart disease than the concentrations of triacylglycerols in the postabsorptive state [1–3], and postprandial concentrations of chylomicron remnants are related to the rate of progression of coronary lesions in patients with premature coronary artery disease [4]. Postprandial lipemia induces alterations in metabolism and composition of all major lipoproteins [5–7] and LDL isolated during postprandial lipemia is more susceptible to oxidation in vitro than fasting isolated LDL [8]. Finally, postprandial LDL induces a higher cholesterol accumulation into cultured macrophages than fasting LDL [8].

The exact process of biochemical reactions that regulate the relationship between postprandial lipemia and CHD remains unclear, but the balance between antioxidant/pro-oxidant species in food may represent the key factor.

Epidemiological studies indicate that a moderate intake of wine reduces the risk of CHD [9–11]. Both the ethanol and the nonethanol component of wine can be responsible for this protective action with different mechanisms. In fact, a moderate intake of ethanol determines increase of HDL cholesterol [12,13], prevention of platelet aggregation, and increase of fibrinolysis [14]. Some chemical reactions and biological effects of polyphenols, the principal nonethanol component of wine, fit the observed protection against cardiovascular disease. Polyphenols are efficient antioxidants [15–18] and are endowed with anti-platelet [19,20] and vasorelaxing properties [21,22]. Recently, red wine antioxidants were proved to prevent the
activation of NF-κB in peripheral blood monocytes during postprandial lipemia [23].

Red wine and red wine phenolics increase in vitro the resistance of human LDL against oxidative modification [24–27]. Moreover, long-term consumption of red wine (either alcohol containing or alcohol free) has been associated with an increase of plasma antioxidant capacity [28–30]. Several studies report an increased resistance to oxidative modification of human LDL after long-term consumption of red wine and red wine polyphenols [25, 31–33]. In contrast, other studies report that red wine [34] and either grape juice [25] and a phenolic extract from red wine [35] do not affect LDL oxidizability. A recent study reports that the acute ingestion of either red wine or dealcoholized red wine fails to influence LDL oxidation [36].

The present study was designed to investigate the effect of red wine consumption on a meal-induced oxidative stress in plasma and on the susceptibility of postprandial LDL to lipid peroxidation. To discriminate the effect of ethanol from that of the nonalcohol component of red wine, the study was carried out by comparing the effect of the same meal when consumed with an isoalcoholic water solution.

**MATERIALS AND METHODS**

**Subjects and test meal**

Six healthy men (25–40 years old) selected from the laboratory staff were studied after informed consent was obtained. The subjects were asked to keep their diet as constant as possible during the study period and none of them was taking any drug or vitamin supplement. The local ethics committee approved the study protocol.

The six subjects ate the same test meal in two different sessions (2 weeks apart) after a 16 h fast. The test meal consisted of “Milanese” meat (beef, egg, and bread crumbs, fried in maize oil) and fried potatoes, and was eaten either with 400 ml of red wine (Rubesco D.O.C., 1995, 12% vol.; total polyphenols: 3.2g/l by Folin Cha colteau method [37]), “wine-meal,” or with an isocaloric aqueous ethanol solution, “ethanol-meal.” The energy content of the meal was 40% of the estimated daily energy need, calculated as 1.5 × basal metabolic rate. The percentages of total energy intake derived from protein, fat, carbohydrate, and alcohol were 14, 24, 38, and 24%, respectively. Saturated, monounsaturated, polyunsaturated fatty acids represented 19, 33, and 48% of total fat, respectively.

**Plasma treatment and analyses**

Blood was collected in EDTA-K3 (1 mg/ml) before, and 1 and 3 h after the test meal. Plasma total antioxidant capacity and ascorbic acid were analyzed immediately after blood centrifugation. Plasma samples for metabolic and antioxidant control (glycemia, total cholesterol, triacylglycerols, SH groups, and uric acid) were stored at −80°C until the analysis.

Plasma glucose, total cholesterol, triacylglycerols, and urate were measured by commercial kits purchased from Sigma Chemical Co. (St. Louis, MO, USA). SH groups were measured according to Ellmann [38]. Ascorbic acid was measured by EC-HPLC, according to Kutnik [39]; dehydroascorbic acid was indirectly estimated by converting it to ascorbic acid after reduction with DL-homocysteine [40].

The total antioxidant capacity of plasma (TRAP) was measured as described previously by Ghiselli et al. [41]. TRAP expresses the mmoles of peroxy radicals trapped by 1 l of plasma.

**LDL preparation**

LDL (d 1.019–1.063 g/ml) was isolated from plasma (at time 0 and time 3) by sequential ultracentrifugation in salt solutions, according to Havel et al. [42], using a Beckman T-100 bench-top ultracentrifuge (T-100.3 rotor). LDL solution was flushed with N2, stored at −80°C and used within 1 week of the preparation. Protein was measured by the method of Lowry et al. [43], using bovine serum albumin as standard. α-tocopherol was measured, after extraction, by reversed phase HPLC with fluorescence detection [44].

For oxidation experiments, LDL was dialyzed in the dark for 18 h at 4°C against two changes of 2 l each (2000-fold volume) of 0.01 M phosphate buffered saline (PBS) 0.15 M NaCl, pH 7.4.

**LDL resistance to oxidative modification**

Dialyzed LDL (200 μg protein/ml) was oxidized in PBS at 37°C for 4 h in the presence of 5 μM CuCl2. Oxidation was stopped by refrigeration and addition of 3 mM EDTA and 100 μM BHT. For conjugated dienes formation, LDL (50 μg/ml) was incubated with 5 μM CuCl2 and the kinetic was followed by continuously monitoring the change in the 234 nm absorbance using a Beckman DU 70 spectrophotometer thermostated at 37°C. LDL electrophoresis was performed at pH 8.6 in 0.05 M barbital buffer on 0.5% agarose gel. The gels were stained with Sudan B black. The increased electrophoretic mobility of LDL was expressed relative to the mobility (REM) of native LDL. Tryptophan residues were determined by the measurement of intrinsic fluorescence in cetyltrimethyl ammonium bromide [45].
Table 1. Plasma Concentrations of Some Metabolic Parameters After Administration of a High-fat Fried Meal with 500 ml of Red Wine or a 12% Solution of Ethanol in Tap Water

<table>
<thead>
<tr>
<th></th>
<th>Red wine</th>
<th>Ethanol solution</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 h</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>162 ± 8</td>
<td>166 ± 7</td>
</tr>
<tr>
<td>Triacylglycerol, mg/dl</td>
<td>101 ± 19</td>
<td>143 ± 26*</td>
</tr>
<tr>
<td>Glycemia, mg/dl</td>
<td>93 ± 4</td>
<td>112 ± 6*</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE of six subjects. Means were compared by using paired t test.

*p < .05 from time 0; **p < .01 from time 0.

Statistical analysis

Data represent mean and standard error of the six subjects. The comparison between wine and ethanol-meal was performed by paired Student’s t-test.

RESULTS

Red wine minimizes the consequences of a high-fat fried meal on plasma antioxidants

Plasma concentrations of total cholesterol, triacylglycerols, and glucose, before and after either wine- or ethanol-meal, are shown in Table 1. As expected, there was only an increase in plasma triacylglycerols and glucose after both meals, while cholesterol concentration did not change significantly in the postprandial period.

Table 2 reports the effect of meals consumed with or without wine on plasma antioxidants. Ascorbic acid concentration decreased significantly after both meals (p < .01, 3 h after the test meal), although the reduction was lower after the wine-meal. The reduced form of ascorbic acid (expressed as % of total ascorbic acid) also tended to decrease, although the difference from baseline was not statistically significant. When the meal was supplemented with wine, α-tocopherol increased significantly 3 h after the test meal (p > .01 from baseline), while the increase was not statistically significant following the ethanol-meal. A similar increase in the concentration of titrable SH groups was observed 1 h after the wine-meal (p < .05 from baseline). Urate concentration increased significantly after both meals, the increase being more pronounced after the wine-meal than after the ethanol-meal (p < .01 at 1 and 3 h; p < .05 at 1 h and p < .01 at 3 h, respectively).

The antioxidants above mentioned should account for the largest part of the plasma antioxidant capacity, as measured by the TRAP test. However, the TRAP value increases significantly 3 h after the wine-meal (p = 0.003 from baseline), but not after the ethanol-meal (Fig. 1).

From our results, we cannot report an impressive postprandial oxidative stress in plasma after both meals (with and without red wine), except for a significant decrease of the concentration of total ascorbic acid. Yet, red wine seems to confer a further protection.

Decrease of α-tocopherol in postprandial low density lipoprotein is prevented by red wine

The concentration of α-tocopherol was measured at baseline and 3 h after the meal (Fig. 2). When meal was supplemented with ethanol, α-tocopherol concentration in postprandial LDL was significantly lower (p = .05) than in baseline LDL (from 5.4 ± 1 μg/mg protein to 4.6 ± 1 μg/mg protein at 3 h). When wine was assumed with the test meal, α-tocopherol concentration did not change significantly (5.8 ± 2 μg/mg protein at baseline, to 5.5 ± 1 μg/mg protein at 3 h).

Table 2. Plasma Ascorbic Acid (Total and % of the Reduced Form), α-Tocopherol, SH Groups, and Uric Acid

<table>
<thead>
<tr>
<th></th>
<th>Red wine</th>
<th>Ethanol solution</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 h</td>
</tr>
<tr>
<td>Total ascorbic acid, μM</td>
<td>62.9 ± 7.4</td>
<td>63.9 ± 7.7</td>
</tr>
<tr>
<td>Dehydroascorbic acid, μM</td>
<td>61.7 ± 7.1</td>
<td>61.0 ± 6.9</td>
</tr>
<tr>
<td>% of total ascorbic acid</td>
<td>98.3 ± 0.8</td>
<td>96.2 ± 1.5</td>
</tr>
<tr>
<td>α-tocopherol, μM</td>
<td>21.1 ± 1.6</td>
<td>22.5 ± 1.2</td>
</tr>
<tr>
<td>SH group, μM</td>
<td>279 ± 7</td>
<td>304 ± 10**</td>
</tr>
<tr>
<td>Uric acid, μM</td>
<td>291 ± 12</td>
<td>363 ± 18*</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE of six subjects. Means were compared by using paired t-test.

*p < .01 from time 0; **p < .05 from time 0.
Red wine reduces susceptibility to oxidative modification of postprandial LDL

The resistance of baseline (fasting) and postprandial LDL to oxidative modification catalyzed by 5 μM Cu (II) was measured by recording the formation of conjugated dienes (Fig. 3) and the time course of some other indicators of oxidative damage, such as tryptophan residues and relative electrophoretic mobility (Table 3).

The kinetic of conjugated dienes formation indicates that, after the ethanol-meal, postprandial LDL was more susceptible (see propagation phase) to oxidation than baseline LDL. On the contrary, when meal was consumed with wine, postprandial LDL did not show any difference in the antioxidant resistance when compared to LDL isolated under fasting conditions (Fig. 3).

In agreement with diene conjugation data, LDL iso-
Fig. 3. Ex vivo lipoprotein oxidation: time course of conjugated diene formation. LDL (50 µg/ml) was oxidized in PBS at 37°C with 5 µM Cu(II) and absorbance was continuously monitored at 234 nm. Each point represents the mean ± SE of the values of the six subjects.

Table 3. Time Course of Cu(II)-catalyzed Oxidation In Vitro of Low-Density Lipoprotein (LDL)

<table>
<thead>
<tr>
<th>Time of oxidation (h)</th>
<th>Red wine</th>
<th>Ethanol solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline LDL</td>
<td>Postprandial LDL</td>
</tr>
<tr>
<td>Tryptophan residues %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>66 ± 5</td>
<td>61 ± 5</td>
</tr>
<tr>
<td>4</td>
<td>37 ± 5</td>
<td>41 ± 8</td>
</tr>
<tr>
<td>REM&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3.7 ± 0.3</td>
<td>2.9 ± 0.4</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE of six subjects. Means were compared by using paired t-test.

<sup>a</sup>p < .02 postprandial LDL after ethanol-meal vs. postprandial LDL after wine-meal.
<sup>b</sup>p < .01 from respective baseline LDL.
<sup>c</sup>REM = relative electrophoretic mobility.
lating after the wine-meal tended to be more resistant or, at least, as resistant to oxidative modification as LDL isolated in fasting conditions (Table 3). When the test meal was assumed with ethanol, the decrease of tryptophan residues in LDL following the meal was more pronounced than in baseline LDL (Table 3).

Comparing the two sets of data, it appears evident that postprandial LDL after the wine-meal was more resistant to oxidative modification than postprandial LDL after the ethanol-meal. In fact, tryptophan residues at 2 h, and REM at 4 h differed significantly ($p = .02$ and $p < .02$, respectively) in the postprandial LDL isolated after the two different meals.

**DISCUSSION**

According to the most widely accepted theory of atherogenesis, oxidatively modified LDL activates a series of cellular events in the arterial wall ultimately leading to plaque formation [46]. Yet, the mechanism of formation in vivo of modified LDL is still uncertain, even though the evidence that human plasma contains oxidatively modified lipoproteins has been achieved [47–49]. It has been proposed that diet-derived lipid hydroperoxides may be incorporated into lipoproteins and thus act as initiators for further lipoprotein oxidative modification, after escaping reduction or degradation while crossing the intestinal border [48–54]. Thus, a meal containing fat must be a relevant source, as various foods or food preparations contain considerable amounts of peroxides. The typical intake of lipid hydroperoxides in a western diet has been estimated as 1.5 mmol/d [54].

The present study reports the effect of a high-fat meal (likely to contain oxidized lipids) on plasma antioxidants and on oxidative stability of LDL. Red wine vs. an ethanol solution was administered with the meal to identify the possible mechanism of the largely suggested protective mechanism of red wine. Only the observed postprandial decrease of ascorbic acid, after both wine- and ethanol-meal, could be in agreement with a condition of oxidative stress. The increase of plasma $\alpha$-tocopherol is justified by the $\alpha$-tocopherol content in the meal (about 6 mg, as calculated using the Italian Tables of Food Composition [55]) but the rise was significant only when the test meal was supplemented with red wine. The increase in plasma concentration of uric acid was, as well, more pronounced after the wine-meal. This change can be easily due to both the content of purines in fermented beverages and by the interference of phenols with uric acid secretion and reabsorption [56,57].

Previous studies showed that the ingestion of red wine or ethanol-free red wine causes a significant increase in fasting plasma polyphenol concentration, and plasma antioxidant capacity [26,28,58]. In our study, the changes in the plasma antioxidant capacity brought by the two test meals are reasonably due to the simultaneous absorption of a complex mixture of pro-oxidants and antioxidants with the diet. The resulting effect is the increase of plasma antioxidant capacity, as detected by the TRAP test, brought by wine but not by ethanol. Thus, our data support the hypothesis of a sparing effect of wine antioxidants on $\alpha$-tocopherol and protein thiols. Indirectly, the present study also demonstrates that intake of red wine with a single high-fat meal counteracts at least in part the pro-oxidative effect brought about by the meal itself (see Table 2).

Red wine phenolics are known to protect human LDL against oxidative modification in vitro [24,32,59,60] and in vivo, after long-term wine supplementation [25,31–33]. The novelty of our approach consists in studying the effect of red wine when consumed together with a single meal, looking at a direct acute effect rather than to a stabilized increase of antioxidant resistance following a long-lasting wine intake.

Our observation of a postprandial decrease of $\alpha$-tocopherol concentration in LDL (but not in plasma) is in agreement with the $\alpha$-tocopherol decrease observed 3 h after a fat-rich meal supplemented with fat-soluble vitamins ($\alpha$- and $\gamma$-tocopherol) [61]. This postprandial decrease is justified by a movement of $\alpha$-tocopherol from LDL to triglyceride-rich lipoproteins (chilomicrons and VLDL) determined by the relative amount of lipid present in the exchanging lipoproteins. However, in our study the postprandial decrease was not evident when subjects consumed the meal with wine, thus confirming the above-mentioned antioxidant sparing effect of wine.

Lechleitner et al. [8] showed that LDL isolated during postprandial lipemia is more susceptible to in vitro oxidation than fasting isolated LDL. We obtained a similar result but only after the ethanol-meal. In fact, postprandial LDL was as resistant or more resistant than baseline LDL when the test meal was consumed with red wine (Fig. 3 and Table 3).

In vitro susceptibility of LDL to oxidative modification depends on content of lipid hydroperoxides from which metal ions generate initiating free radicals, content of antioxidants, fatty acid composition, and, reasonably, some intrinsic structural factors not yet clearly defined. With the exception of the fatty acid composition, wine could affect almost the totality of these factors. In fact, dietary lipid hydroperoxides are secreted into chylomicrons and intestinal VLDL, and it is reasonable to assume that they can be transferred, at least in part, to the LDL by the action of plasma lipid transfer proteins. Antioxidants in red wine could minimize the postprandial increase of lipid hydroperoxides in plasma [30] and then in LDL by protecting against the buildup of new peroxides in the digestive tract and/or by contributing (if
bioavailable) to the plasma antioxidant capacity. In both cases they exert a sparing effect on endogenous antioxidants at LDL and plasma level, confirmed by the lack of decrease of LDL-α-tocopherol when the meal was consumed with red wine. Still, the possibility of the incorporation of a small amount of wine phenolics into LDL, as reported for soybean isoflavones [62], cannot be excluded. In this case, wine phenolics could bind reactive sites on apo B, influencing the susceptibility of LDL to oxidation reasonably through a structural modification.

In conclusion, our study gives further contribution to the novel, scarcely explored approach to the oxidative hypothesis of atherosclerosis, centered on the critical importance of the antioxidants/pro-oxidants balance in the postprandial phase. This hypothesis is supported by the observation that pretreatment with antioxidants blocks the impairment of endothelial functions caused by a single high-fat meal in human [63]. Following this hypothesis, minimally modified LDL (or LDL−[64]) can generate transiently after a lipid hydroperoxide-containing meal and dietary antioxidants can reduce this formation, as suggested in our study.

In this view, wine antioxidants may attenuate the risk of CHD, independently from ethanol, by reducing the potential pro-oxidant effect of fat (and hydroperoxide) rich food, thus bridging the gap with another set of epidemiological evidence. In this view, our data can contribute to better explain the controversial results obtained in different trials [9,11,65–68]. In fact, the modality of drinking (either during or out of meal) can represent the confusing factor in epidemiological studies on the association of wine consumption with CHD risk.

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