Alcohol consumption is directly associated with circulating oxidized low-density lipoprotein

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Abstract

Findings on the association of alcohol consumption and oxidation of low-density lipoprotein (LDL), which is thought to play a crucial role in the generation of atherosclerotic lesion, are inconsistent. The aim of the present study was to investigate the association of total alcohol consumption and type of alcoholic beverage with circulating plasma LDL oxidation. This cross-sectional study included data of circulating oxidized LDL (ox-LDL) from a subpopulation of 587 men and women enrolled in a population-based survey conducted in 2000 in Girona (Spain). Multivariate analysis was performed to describe the independent association of alcohol consumption and ox-LDL. Increasing alcohol consumption was associated with high in vivo ox-LDL levels in the present population. The consumption of 10 g of alcohol was associated with an increase of 2.40 U/L of ox-LDL (p = 0.002). Adjustment for dietary variables, leisure-time physical activity, educational level, smoking, LDL-cholesterol, high-density lipoprotein-cholesterol, glycemia, triglycerides, diabetes, body mass index, waist circumference, and systolic and diastolic blood pressures only slightly modified this association (p = 0.003). In this full adjusted model the consumption of 10 g of alcohol per day was associated with an increase of 2.11 U/L of ox-LDL. Consumption of wine (ml/day) was associated with increasing ox-LDL levels (p = 0.029), however, attenuated after controlling for alcohol. No significant relationship of ox-LDL with alcohol-independent consumption of wine, beer, and spirits was observed. Alcohol consumption was independently and directly associated with circulating ox-LDL in the present population.

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Introduction

Alcohol is a constituent of the diet, and the amount, type, and frequency of alcohol consumption can have divergent effects on the organism [1]. Protective effects of alcoholic beverage consumption against coronary heart disease, mainly mediated through the effects of alcohol on high-density lipoproteins (HDL), have been reported in several epidemiological studies [2]. On the other hand, there is evidence that alcohol acts as an oxidant through several metabolic pathways [3–5]. Alcohol-induced radical production may damage molecular structures such as DNA or lipoproteins. Most importantly, oxidation of low-density lipoprotein (LDL) is thought to play a crucial role in the generation of the atherosclerotic lesion [6–8]. Hence, identifying variables, particularly modifiable variables such as dietary components that are associated with higher levels of oxidized LDL (ox-LDL), is of importance. Little information on the association of alcohol consumption with in vivo LDL oxidation exists [9]. In the study of Di Bari and colleagues [9] ox-LDL levels were measured indirectly through antibodies against LDL titers. However, the clinical significance of these antibodies is unclear because results about the association of anti-ox-LDL titers and the atherosclerotic disease are inconsistent [10,11]. Hence, direct measurement of in vivo circulating ox-LDL might be more appropriate when analyzing the relationship of an exposure variable and ox-LDL. It has been shown that polyphenolic components, particularly of wine, exert antioxidant properties and protect against in vitro LDL...
oxidation [12]. However, it is uncertain whether wine as a whole, or only the dealcoholized fraction of wine, protects against in vivo lipid peroxidation [13].

The aim of the present study was to analyze the association of total alcohol and alcohol-independent consumption and types of alcoholic beverages with the oxidation of LDL. This was carried out by direct measurement of in vivo ox-LDL in a subpopulation of a population-based cross-sectional survey conducted in Spain.

**Material and methods**

**Subjects**

Free-living Spanish men and women from the province of Girona, between 25 and 74 years of age, participated in this study from 1999 to 2000. Six thousand subjects were randomly selected from the general population of Girona according to the 1996 census. After excluding census errors (owing to errors in census data, institutionalization, temporally absent, and died before the study was conducted) 4539 eligible subjects remained of whom 3179 agreed to participate. In vivo oxidized LDL was measured in a subpopulation of 587 men and women. The selected subpopulation was matched by age and sex with the entire population. All participants signed an informed consent form to allow their personal data to be stored in a computer database and the acquisition of biological samples for the necessary analyses. The protocol was approved by an Ethics Committee and the results of the examination were sent to all participants.

**Assessment of alcohol intake**

Participants were asked to report their alcohol consumption of the previous week in a structured open-ended questionnaire. This questionnaire asked about the consumption of several typical alcoholic beverages of the region. The consumption for average alcohol grade (%) of wine, beer, and spirits was 12.5, 5, and 40%, respectively. The alcohol intake (g/day) was calculated by multiplying the amount of the beverage (ml) with the respective grade (%) and the constant 0.80 to transform alcohol volumes into weight.

**Laboratory measurements**

Venous blood was obtained in the morning after an overnight fast for at least 12 h. Serum for lipid determination was separated and kept frozen at −40°C until assayed. For the measurements of ox-LDL concentrations, blood was drawn into tubes containing EDTA-2Na (1 mg/ml) and chilled on ice, and plasma was separated by centrifugation at 4°C.

The concentration of ox-LDL in plasma was measured by a sandwich enzyme-linked immunosorbent assay procedure using the murine monoclonal antibody mAb-4E6 as capture antibody bound to microtitration wells and a peroxidase-conjugated anti-apolipoprotein B antibody recognizing ox-LDL bound to the solid phase (ox-LDL; Mercodia AB, Uppsala, Sweden). Intra- and interassay CVs were 2.8 and 7.3%, respectively. Serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), and triglyceride (TG) levels were determined using enzymatic kits (Roche Diagnostic, Basel, Switzerland) adapted to a Cobas Mira Plus autoanalyzer (Hoffmann-La Roche, Basel, Switzerland). Low-density lipoprotein cholesterol (LDLC) was calculated by means of the Friedewald formula. Laboratory determinations for an individual were performed in duplicate. Analytical intraassay imprecision of the methods was assessed from 20 pairs of duplicate samples in the same run. Interassay imprecision was assessed from 20 day-to-day measurements of control samples. Both precision measurements were expressed as coefficient of variation (CV%). Interassay CVs were 2.8, 2.6, 4.6, and 2.9%, for glucose, total cholesterol, HDL cholesterol, and triglycerides, respectively.

**Dietary assessment**

Food consumption and nutrient intake were measured by a validated food frequency questionnaire (FFQ) [14] administered by a trained interviewer. In brief, the validity of a questionnaire is primarily defined as its ability to classify study subjects according to a rank of nutrient intake. The ability to rank subjects adequately according to their dietary intake was reflected by (a) Pearson’s correlation coefficients (average of 0.37), (b) intraclass correlation coefficients (average of 0.43), and (c) proportion of correct classification (average of 33.0%) into the same and extreme quartile and that of misclassification (average of 6.8%). These data indicated acceptable relative validity of the FFQ. Furthermore, the range of correlation coefficients between dietary intakes of protein (r = 0.26), vitamin C (r = 0.54), β-carotene (r = 0.17), and selenium (r = 0.26) and their corresponding biomarkers in urine and plasma were comparable with those found by other dietary assessment methods. The optically readable FFQ asked for the usual food intake over the previous year. The food list comprised 165 items. For each food item, participants were asked to indicate their usual consumption from the nine frequency categories, ranging from never or less than once a month to six or more times a day. The FFQ did not include standard questions on portion size but rather indicated specific medium servings defined by natural (e.g., one orange, one slice of bread) or household (e.g., one spoon, one cup, one glass) units. Energy consumption and nutrient intake were calculated from the FFQ using the Medisystem 2000 software (Conaycito, Madrid).

**Leisure-time physical activity**

Leisure-time physical activity was measured by the Minnesota leisure-time physical activity questionnaire that had been previously validated for Spanish men and women [15,16]. The questionnaire was administered by a trained interviewer. The participants were provided with detailed instructions and a list of physical activities. They were asked to mark those activities that they had undertaken during the previous year and the
Anthropometric measurements

A precision scale of easy calibration was used for weight measurement. Readings were rounded up to 200 g. Individuals wore only their underwear. Height was measured in the standing position and measurements were rounded up to 0.5 cm. Body mass index (BMI) was determined as weight divided by squared height (kg/m²). Waist and hip circumferences were obtained taking the maximum circumferential length of the buttocks in centimeters, with the subject standing, rounded to 0.5 cm.

Smoking

Information on smoking habits of the participants was obtained by a structured interview. Participants were categorized as people who had never smoked, former smokers (<1 year), and current smokers (at least 1 cigarette/day on average during the previous year). The latter were asked for the average daily amount of cigarettes smoked.

Educational status

Information on educational status was obtained through structured standard questionnaires, administered by trained personnel. Maximum level of education attained was elicited and for analysis purposes was recorded as basic education, secondary school, and university.

Blood pressure

Two blood pressure determinations were taken by trained personnel using a periodically calibrated mercury sphygmomanometer with strict standard procedures.

Statistical analysis

The general linear modeling procedures (PROC.GLM; SAS Institute Inc., Cary, NC; version 8.0) were used to estimate the age- and sex-adjusted relationship of ox-LDL (tertiles) with nutrient intake, alcohol consumption, leisure-time physical activity, HDL-cholesterol, LDL-cholesterol, glyceremia, triglyceride, body mass index, and systolic and diastolic blood pressures. Age- and sex-adjusted logistic regression analysis with the use of the PROLOGISTIC procedure of SAS (SAS Institute Inc.; version 8.0) was used to determine p for linear trend of categorical variables. The association of alcohol consumption and ox-LDL was evaluated using linear regression analysis (PROREG, SAS Institute Inc.; version 8.0). Multiple linear regression models were fitted to determine first the age-controlled relationship, second the age-, lifestyle-(smoking, leisure-time physical activity, and dietary intake of vitamin C, vitamin E, beta-carotene, and polyunsaturated fat), and sociodemographic-variable- (educational level) controlled relationships, and third the age-, lifestyle-, and cardiovascular-risk-factor- (LDL-cholesterol, HDL-cholesterol, triglycerides, BMI, waist circumference, diabetes, and systolic and diastolic blood pressures) controlled relationships between alcohol consumption and ox-LDL. The data of these models followed a normal distribution.

To address the question of whether components of the nonalcoholic parts of wine, beer, and spirits were associated with ox-LDL we adjusted the consumption of these beverages for total alcohol ingestion in a linear regression analysis. Differences were considered significant if p < 0.05.

Results

The main characteristics of the study population are shown in Table 1. The median alcohol consumption was 5.7 g per day. This can be considered a moderate consumption.

Levels of circulating ox-LDL increased with total alcohol intake and age (Table 2). Sex- and age-adjusted prevalence of smoking and heavy smoking increased with higher levels of ox-LDL (Table 2). No differences of antioxidant vitamin (ascorbic acid, α-tocopherol, and β-carotene) and polyunsaturated fat intake according to tertile distribution of oxidized LDL among groups were observed (Table 3). Plasma concentrations of LDL-cholesterol and triglycerides increased across tertiles of ox-LDL, whereas an inverse association was observed for HDL-cholesterol. Men with higher concentrations of ox-LDL had a higher body mass index and higher waist circumference.
Higher systolic and diastolic blood pressures were found in participants with high ox-LDL levels (Table 4).

Because age was significantly associated with ox-LDL we adjusted the first model of linear regression analysis for this variable. Alcohol intake was directly associated with ox-LDL (Table 5). The consumption of 10 g alcohol per day (equivalent to the amount of alcohol in a 100-ml glass of wine) was associated with an increase of 2.40 U/L of ox-LDL. This association held after adjusting for several lifestyle variables (Model 2; Table 5). Further controlling for total alcohol consumption and circulating ox-LDL, a direct relationship of HDL (dependent variable) with wine, beer, and spirit consumption (mutually adjusted) revealed a significant association for wine (ml/day) [0.015 (CI 0.004–0.025)] but not for beer and spirit.

### Discussion

In the present study we analyzed the relationship between total alcohol consumption and circulating ox-LDL, a direct measurement of in vivo LDL oxidation. Alcohol consumption was directly associated with ox-LDL. This association held after controlling for lifestyle and cardiovascular risk factors.

Increased oxidative stress has been associated with several diseases [6,18–21]. Oxidized LDL is directly involved in...

### Table 3

Selected nutrients according to tertile distribution of oxidized LDL

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>First tertile (n = 195)</th>
<th>Second tertile (n = 196)</th>
<th>Third tertile (n = 196)</th>
<th>p for linear trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range oxidized LDL (U/L)</td>
<td>3.3–50.8</td>
<td>50.9–72.4</td>
<td>72.5–163.4</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (mg/day)</td>
<td>302</td>
<td>281</td>
<td>281</td>
<td>0.281</td>
</tr>
<tr>
<td>α-Tocopherol (mg/day)</td>
<td>16.3</td>
<td>16.4</td>
<td>15.6</td>
<td>0.368</td>
</tr>
<tr>
<td>β-Carotene (mg/day)</td>
<td>8.5</td>
<td>8.0</td>
<td>8.3</td>
<td>0.850</td>
</tr>
<tr>
<td>Polyunsaturated fat (mg/day)</td>
<td>16.9</td>
<td>18.1</td>
<td>16.9</td>
<td>0.963</td>
</tr>
</tbody>
</table>

* Adjusted for sex and age.
Table 6
Regression coefficients for the association of oxidized LDL with the consumption of several types of alcohol beverages in men and women

<table>
<thead>
<tr>
<th>Model</th>
<th>Oxidized LDL (U/L)</th>
<th>( R^2 )</th>
<th>( \beta ) Coefficient</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine consumption (ml/day)</td>
<td>0.044</td>
<td>0.027</td>
<td>0.003; 0.051</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>Beer consumption (ml/day)</td>
<td>0.044</td>
<td>0.001</td>
<td>-0.016; 0.019</td>
<td>0.377</td>
<td></td>
</tr>
<tr>
<td>Spirit consumption (ml/day)</td>
<td>0.044</td>
<td>0.180</td>
<td>-0.070; 0.429</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine consumption (ml/day)</td>
<td>0.044</td>
<td>0.010</td>
<td>-0.034; 0.053</td>
<td>0.665</td>
<td></td>
</tr>
<tr>
<td>Beer consumption (ml/day)</td>
<td>0.044</td>
<td>-0.011</td>
<td>-0.032; 0.010</td>
<td>0.300</td>
<td></td>
</tr>
<tr>
<td>Spirit consumption (ml/day)</td>
<td>0.044</td>
<td>0.077</td>
<td>-0.213; 0.367</td>
<td>0.602</td>
<td></td>
</tr>
</tbody>
</table>

Model 1: adjusted for sex and age. Wine, beer, and spirit consumption was mutually adjusted. Model 2: adjusted for sex, age, and total alcohol consumption.

have been associated with ox-LDL plasma concentration [32,33]. Additionally, alcohol consumption is associated with these variables. Hence, controlling for confounding lifestyle and cardiovascular risk variables is important when analyzing the association of alcohol consumption and ox-LDL. In the present study, the significant association of alcohol consumption and ox-LDL held after adjusting for several lifestyle factors including antioxidant vitamins. Most importantly, we observed no attenuation of this association even after controlling for several cardiovascular risk factors. This results points to a strong independent association of alcohol consumption and ox-LDL in the present population.

The mechanism by which alcohol can exert its prooxidant properties differs from that of cigarette smoke. Cigarette smoke contains a great variety and large amounts of free radicals and prooxidants such as superoxide anion, reactive aldehyde species, nitric oxide, and peroxinitrite [34]. Higher plasma levels of lipid peroxidation in smokers than in nonsmokers have been reported [35,36]. In the present study, smoking, and particularly heavy smoking, was directly associated with higher levels of circulating oxidized LDL. Yamaguchi and colleagues [37,38] showed that cigarette smoking increased the level of oxidatively modified LDL. Of all the prooxidants present in tobacco smoke, peroxinitrite, which causes oxidative damage of a variety of molecular structures [38], is thought to be strongly involved in the oxidation of LDL in plasma [37,38]. Furthermore, Yamaguchi and colleagues [37] found higher levels of 3-nitrotyrosine, a biomarker for peroxynitration, in plasma and LDL of smokers than in nonsmokers. Based on these findings they suggest that the aqueous extract of the gas phase of cigarette smoke reaches the epithelial lining fluid and mucus. Then, peroxynitrite is released from the aqueous extract through the alveolar membrane, reacting with circulating LDL. The prooxidant properties of alcohol are mediated through intracellular metabolic processes [4]. It is generally recognized that NAD(P)H oxidase is the major source of reactive oxygen species in the artery wall. It has been shown that ethanol stimulates the release of superoxide anion to the extracellular space via activation of NAD(P)H oxidase in sinusoidal endothelial and Kupffer cells [39]. Formation of superoxide anion by vascular cells stimulates the conversion of LDL to oxidized LDL [40,41]. On the basis of these findings, one might speculate that alcohol stimulates vascular NAD(P)H oxidase leading to an increase of the release of superoxide anion into the artery wall and causing LDL oxidation. Another possible mechanism is the alcohol-induced increase in angiotensin II receptor expression [42]. Angiotensin is a major stimulus for activation of NAD(P)H oxidase. Moreover, alcohol detoxification by cytochrome P450 leads to the formation of reactive oxygen species [4].

In the present population, higher LDL-cholesterol and triglycerides have been associated with ox-LDL plasma concentration [32,33]. Additionally, alcohol consumption is associated with these variables. Hence, controlling for confounding lifestyle and cardiovascular risk variables is important when analyzing the association of alcohol consumption and ox-LDL. In the present study, the significant association of alcohol consumption and ox-LDL held after adjusting for several lifestyle factors including antioxidant vitamins. Most importantly, we observed no attenuation of this association even after controlling for several cardiovascular risk factors. This results points to a strong independent association of alcohol consumption and ox-LDL in the present population.

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In the present population, higher LDL-cholesterol and triglycerides and lower HDL-cholesterol were associated with an increase in oxidized LDL. These data agree with previous published results of our group [25] in which significant positive correlations between ox-LDL and LDL-cholesterol and triglyceride values and a significant negative correlation between ox-LDL and HDL-cholesterol levels were obtained.
in a case–control study with stable CHD patients. In contrast, Di Bari and colleagues [9] did not find significant associations of antibodies against ox-LDL with these variables. This might be partially due to the different analytical approaches used in measuring the degree of LDL in the study of Di Bari et al. and ours.

Alcoholic consumption has been related to a number of effects on hemostatic factors, endothelial function, and lipoprotein metabolism [43,44]. The most important protective effect of alcohol is thought to be mediated by an increase of HDL lipoprotein [45,46]. Our findings that even light alcohol consumption was associated with an increment in ox-LDL seems surprising in this context. The subjects of the present population were predominantly wine consumers. Wine consumption provides not only alcohol but also polyphenols with antioxidant properties [47]. However, from our data the oxidative effect of the alcohol on the in vivo LDL oxidation seems to be displayed despite the concomitant ingestion of polyphenols from wine. The results of clinical intervention studies on the role of red wine polyphenols inhibiting the LDL oxidation are controversial, and no clear scientific evidence concerning this issue has emerged. Moreover, most studies used ex vivo methodologies to assess in vivo effects of wine ingestion on lipoprotein oxidation. Hence, comparison of these data with those of our present study is difficult. Currently, it is thought that the cardioprotective effect of grape polyphenols could be mediated both through an improvement of the plasma lipid profile and by reducing inflammation [48]. We found no significant association of ox-LDL with wine, beer, or spirit consumption after adjusting for total alcohol intake and age. A healthy lifestyle and higher socioeconomic status have been associated with wine consumption by several authors [49]. However, including these variables in the linear regression model did not modify the association between wine consumption and ox-LDL. In contrast, Di Bari and colleagues [9] observed an inverse association of wine consumption and antioxidized LDL antibodies in a predominantly wine-consuming population. As mentioned above, differences in the average age of the population and the methodological approach of measuring ox-LDL may account for these differences.

There are several limitations of the present study. Causality between alcohol consumption and degree of ox-LDL cannot be drawn because of the cross-sectional design of the present study. Furthermore, all dietary instruments measuring past food and alcohol intake are vulnerable to random and systematic measurement errors. A major problem with food frequency questionnaires is the difficulty to precisely determine the amount of food intake. In particular, this dietary measurement instrument tends to overestimate vegetable and fruit consumption, the main sources of antioxidant intake. However, the validation of the present food frequency questionnaire with biomarkers yielded acceptable correlation coefficients among variables of interest [14]. The validity of self-reported alcohol intake may be questioned. However, this is a general problem of recording alcohol consumption, particularly for high intakes, and is not specific for a 1-week recall of alcoholic beverages. However, self-reported alcohol consumption was directly associated with HDL in the present study. This association is concordant with other observational and experimental data and supports the validity of our measurement of alcohol consumption [50,51].

In conclusion, increasing alcohol consumption was significantly associated with higher levels of ox-LDL plasma concentrations. Controlling for age, leisure-time physical activity, smoking, educational level, and intakes of polyunsaturated fat, vitamin C, vitamin E, and β-carotene did not change the direction or magnitude of this association. Furthermore, additional adjustment for HDL-cholestrol, LDL-cholestrol, triglycerides, glycemia, body mass index, waist circumference, diabetes, and systolic and diastolic blood pressures did not attenuate the relationship between alcohol consumption and ox-LDL. Alcohol-adjusted consumption of wine, beer, or spirit was not significantly related to ox-LDL. These results suggest that alcohol is strongly and independently associated with increased levels of circulating oxidized LDL.

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