Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivolipoprotein oxidizability1–3

Rima Abu-Amsha Caccetta, Kevin D Croft, Lawrence J Beilin, and Ian B Puddey

ABSTRACT
Background: Reduced lipoprotein oxidizability by red wine phenols has been proposed as the basis for a relatively lower incidence of coronary heart disease in red wine drinkers. We showed previously that caffeic and protocatechuic acids isolated from red wine exhibit antioxidant activity in vitro. However, there is no information in the literature on the absorption of these compounds after red wine ingestion.

Objectives: We sought to determine whether certain phenolic acids can be detected in the circulation after red wine consumption and if their presence has an acute effect on serum and LDL oxidation ex vivo.

Design: Twelve healthy male nonsmokers consumed red wine, phenol-stripped red wine, dealcoholized red wine, or water, each at a separate visit, in random order and 1 wk apart. Beverages were consumed over 30 min and blood was sampled just before beverage consumption and 1, 2, and 4 h after consumption. Plasma caffeic, protocatechuic, and 4-O-methylgallic acids were measured by gas chromatography–mass spectrometry. We also measured copper-induced serum and LDL oxidizability ex vivo. Plasma caffeic, protocatechuic, and 4-O-methylgallic acids were measured by gas chromatography–mass spectrometry.

Results: Caffeic acid and 4-O-methylgallic acid concentrations increased significantly (P < 0.025) after consumption of red wine and dealcoholized red wine compared with water or phenol-stripped red wine. Uric acid increased significantly (P < 0.001) after ingestion of red wine, phenol-stripped red wine, and dealcoholized red wine. There was no effect on ex vivo serum or LDL oxidation after any of the beverages.

Conclusion: Although red wine and dealcoholized red wine consumption acutely increase plasma phenolic acid and serum uric acid concentrations, the increase is insufficient to influence ex vivo lipoprotein oxidizability.

KEY WORDS Wine, phenolic acids, caffeic acid, 4-O-methylgallic acid, protocatechuic acid, bioavailability, uric acid, LDL oxidation, men

INTRODUCTION
Epidemiologic studies suggest that alcohol consumption, irrespective of beverage type, can reduce the risk of atherosclerotic cardiovascular disease (CVD) (1, 2). However, red wine has received special attention in this regard because it contains phenolic compounds, which strongly inhibit LDL and serum oxidation in vitro (3–5). Because lipoprotein oxidation is believed to play a causative role in the development of CVD (6), the consumption of antioxidant compounds as beverage constituents has become an attractive approach in the prevention of this disease. However, relatively little information is available on the in vivo absorption of specific compounds contained in beverages such as red wine, and intervention studies measuring lipoprotein oxidation after red wine ingestion have shown conflicting outcomes.

Fuhrman et al (7) reported that daily consumption of 400 mL red wine for 2 wk reduced the susceptibility of plasma and LDL to oxidation, whereas a similar quantity of white wine, with approximately one-fifth the total polyphenol content, increased the susceptibility of plasma and LDL to oxidation. In contrast, de Rijke et al (8) found that consumption of 550 mL red wine or the same amount of white wine for 4 wk had no effect on LDL oxidation. In an uncontrolled study by Carbonneau et al (9), dietary supplementation with an extract of phenolic compounds from red wine to subjects for 2 wk increased the antioxidant capacity of plasma but had no effect on isolated LDL oxidizability. In another uncontrolled study, ingestion of red grape juice was shown to increase serum total antioxidant capacity and reduce the susceptibility of LDL to oxidation (10). Miyagi et al (11) found that red wine, acutely over 2 h, significantly inhibited copper-induced oxidation of LDL whereas grape juice did not. Recently, Nigdikar et al (12) observed a prolonged lag time to copper-catalyzed oxidation of LDL after subjects consumed red wine polyphenols for 2 wk. These authors suggested that the difference in the outcome of red wine’s effects on LDL oxidation may have been due to important differences in methodology.

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such as the concentration of EDTA in the LDL and methods used to remove it before oxidation.

There have been few reports on the absorption and bioavailability of specific phenolic compounds after consumption of red wine or grape juice by humans (13). In the studies by Fuhrman et al (7) and Nigdikar et al (12), inhibitory effects on LDL oxidation were related to phenolic compounds in LDL measured by using the Folin-Denis method, which is a nonspecific assay. In 1983, Jacobson et al (14) reported the detection of some phenolic acids in urine after ingestion of 1 g caffeic or ferulic acid, whereas, more recently, Bourne and Rice-Evans (15) reported the presence of some hydroxycinnamic acids in urine after ingestion of some high phenolic acid–containing fruit.

In our previous studies we showed that phenolic acids, such as caffeic and protocatechuic acids, were among the most potent inhibitors of in vitro LDL oxidation isolated from antioxidant fractions of red wine (3). In this study we wished to determine whether phenolic acids are absorbed into the bloodstream after acute ingestion of red wine and whether they have ex vivo antioxidant activity against LDL and serum oxidation. In addition, we identified a further phenolic acid metabolite that may serve as a useful marker of ingestion of phenolic acid–containing beverages such as red wine.

SUBJECTS AND METHODS

Study design

Twelve healthy male volunteers aged 40–63 y [mean BMI (in kg/m²): 25.7; range: 18.8–35.3] who were normolipidemic, non-smokers, light-to-moderate drinkers (<4 standard alcoholic drinks/d), and taking no medications were recruited. Subjects were their own controls and were asked to avoid taking any antioxidant supplements for 2 wk before their first visit and throughout the ensuing study periods. They were also asked to avoid grape products and any other high phenol-containing foods or drinks throughout the study. Subjects were required to abstain from drinking any alcoholic beverages for 2 d before each visit. Subjects ceased drinking coffee and tea 24 h before each visit and fasted for ≥12 h before each visit. The study was approved by the Human Rights Committee of the University of Western Australia and was carried out under the guidelines of the National Heart and Medical Research Council of Australia.

Four beverages were tested, one beverage per week in random order: 1) red wine (Cabernet Shiraz 1994, 13% alcohol by vol; Hardys Nottage Hill, McLaren, South Australia), 2) the same red wine but deacelholized, 3) the same red wine but with the phenols removed (phenol stripped), and 4) water. Nine volunteers tested all 4 beverages and 3 volunteers tested each beverage except water. Volunteers were seated for 10 min before blood samples were taken at 0, 1, 2, and 4 h. After their first fasting blood sample was taken, each volunteer was given 2 plain, low-fat bagels and a beverage (5 mL red wine equivalents/kg body wt) to consume within 30 min.

Preparation of red wines

Red wine was deacelholized by vacuum distillation with gentle heating to 50°C. Phenol-stripped red wine was prepared by passage through a column of polyvinylpolypyrrolidone (PVPP; Sigma Chemical Co, St Louis) made up in double-distilled water containing 12% redistilled ethanol and prewashed 3 times with 12% ethanol in water solution; 145 g PVPP was used to remove the phenols from 1.7 L red wine. The total polyphenol content of the red wine was determined before and after the phenolic compounds were stripped from it; >95% of phenols were removed by this procedure. Measurement of phenolic acids by gas chromatography–mass spectrometry (GC-MS) before and after stripping revealed that 99% of caffeic acid, 97% of protocatechuic acid, and 99.9% of gallic acid were removed by this method (Table 1).

TABLE 1

<table>
<thead>
<tr>
<th>Phenolic acid</th>
<th>Red wine</th>
<th>Phenol-stripped red wine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols (mg/L)</td>
<td>2014</td>
<td>87</td>
</tr>
<tr>
<td>Caffeic acid (mg/L)</td>
<td>11.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Protocatechuic acid (mg/L)</td>
<td>1.58</td>
<td>0.04</td>
</tr>
<tr>
<td>Gallic acid (mg/L)</td>
<td>9.5</td>
<td>0.01</td>
</tr>
<tr>
<td>4-O-Methylgallic acid (mg/L)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values of specific phenolic acids are the mean of duplicate determinations that were <5% different from each other.

LDL purification

Blood was collected by venipuncture into EDTA (1 g/L) and centrifuged immediately at 1000 × g for 10 min at 4°C. Plasma was stored at 4°C and LDL was isolated from all 4 samples (0, 1, 2, and 4 h) at the end of each day by density-gradient ultracentrifugation as described previously (16). Briefly, plasma density was increased to 1.07 by adding sodium chloride. A 4-step gradient was then constructed over the plasma by using the following densities (kg/L): 0.5 mL of 1.063 NaCl, 0.5 mL of 1.04 NaCl, 0.5 mL of 1.02 NaCl, and 0.9 mL of double-distilled water. To protect the LDL against oxidative modification during ultracentrifugation, each density solution contained 100 mg NaEDTA/L. Samples were ultracentrifuged at 205000 × g (average) for 20 h at 4°C with a Centrikon T-1190 ultracentrifuge (Kontron Instruments, Milano, Italy). The LDL band was collected by aspiration and passed through a PD10 Sephadex column (Amersham Pharmacia Biotech, Upssala, Sweden) to remove the excess salt and most of the EDTA.

LDL oxidation ex vivo

The ex vivo LDL oxidation procedure was similar to that described by us previously (3). Briefly, freshly isolated LDL was passed through a second PD10 Sephadex column to remove any remaining EDTA. The cholesterol concentration of the LDL was measured by using a standard enzymatic method (Monotest; Boehringer Mannheim, Mannheim, Germany) and the LDLs were diluted with phosphate buffered saline pretreated with Chelex chelating resin (Sigma Chemical Co) to a standard concentration of 0.3 mmol cholesterol/L (≈0.1 g protein/L). Oxidation reactions were initiated by adding freshly prepared CuCl₂ (final concentration 5 µmol/L). Oxidation kinetics were determined by monitoring the change in absorbance at 234 nm by using a DU650 UV-Vis spectrophotometer (Beckman Instruments Inc, Fullerton, CA) with absorbance readings made every 5 min at 37°C until there was no further increase in the formation of conjugated dienes. The plot of absorbance against time was divided into a lag phase and a propagation phase. The lag time was defined as the intercept between the baseline and the tangent of the absorbance curve during the propagation phase.
and was expressed in minutes. All 4 samples collected at each visit (at 0, 1, 2, and 4 h) from each volunteer were assayed together in the same experiment, under the same conditions.

**Serum oxidation ex vivo**

Serum was prepared from blood taken and left to clot in the dark at room temperature for 30 min, then centrifuged immediately at 1000 × g for 10 min at 4°C. Serum samples for all time points were stored overnight at −80°C and assayed together in the same experiment, the following day. Cu²⁺-induced serum oxidation (0.67% serum diluted in phosphate-buffered saline) was carried out as described by Regnström et al (17) with 12.5 μmol Cu²⁺/L and as previously done so in our laboratory (3, 18). Oxidation kinetics were determined by measuring the absorbance at 234 nm every 5 min at 37°C until there was no further increase in the formation of conjugated dienes. The serum oxidation lag phase was defined as for the LDL-oxidation curves.

**Serum and LDL oxidation in vitro**

Isolated LDL and serum were oxidized separately by using copper, as described above, in the presence and absence of caffeic, protocatechuic, gallic, and 4-O-methylgallic acids. The phenolic acids were tested over a concentration range of 0.1–10 μmol/L in the LDL oxidation system and 0.01–1.0 μmol/L in the serum oxidation system. 4-O-Methylgallic acid was also tested at 10 μmol/L in the serum oxidation system. The percentage inhibition of lipid oxidation was calculated as the change in lag time compared with control oxidation.

**Measurement of total polyphenols**

The total polyphenol content in beverages and in EDTA-treated plasma, stored at 4°C until the end of each week, was quantified with a colorimetric assay with Folin-Denis reagent according to the method published by the Association of Official Analytical Chemists (19). This method estimates polyphenols at 760 nm in relation to a standard curve for tannic acid.

**Extraction and derivatization of phenolic acids in plasma and red wine**

Plasma samples collected at all time points were stored at −80°C until the end of the study. All samples from each volunteer collected from all visits were extracted and assayed together on the same day. Glycosides and glucuronides were hydrolyzed by using a modified version of the technique reported by Schultz et al (20). A 2-mL aliquot of EDTA-treated plasma, diluted (1/100) red wine, or undiluted phenol-stripped red wine was lyophilized. The internal standard, 100 ng 1-hydroxy-2-naphthoic acid (Sigma Chemical Co.), was added to each lyophilized sample. The dry pellet was then resuspended in 2 mL of 100 mmol acetic acid buffer/L (pH 4.5) containing 4000 U β-glucuronidase (EC 3.2.1.31) plus 200 U sulfatase (type HP-2; Sigma Chemical Co), and 15.4 U β-glucosidase (EC 3.2.1.21; Sigma Chemical Co). This solution was then incubated for 4 h at 37°C. The mixture was then further acidified with 6 mol HCl/L to a pH of <3 and then extracted with ethyl acetate. The ethyl acetate layer was extracted with 5% NaHCO₃, which was then removed, immediately acidified with 6 mol HCl/L, and reextracted with ethyl acetate. The ethyl acetate extract was then dried under nitrogen. The dried extracts were derivatized with 30 μL bis(trimethylsilyl)-trifluoroacetamide and 30 μL dry pyridine heated at 50°C for 20 min. Samples were diluted 2-fold in redistilled isooctane before GC-MS analysis.

**Gas chromatography–mass spectrometry**

Samples were analyzed on an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) coupled with an HP 5970 series mass-selective detector and an HP ChemStation by using HP G1034C MS ChemStation software (Rockville, MD). Samples (1 μL) were injected onto an HP-1 cross-linked methyl silicone column (12 m × 0.20 mm, 0.33 μm film thickness; Hewlett-Packard) by using helium as carrier gas with an inlet pressure of 30 kPa. Injections were made in the splitless mode. The initial column temperature of 120°C was held for 0.5 min and then programmed to increase at 15°C/min to 280°C, at which it was held for 5 min. The mass spectrometer was operated in the electron-impact mode (70 eV). Mass chromatograms in the scan mode were recorded in the mass range of 50–550 atomic mass units. Selected ion monitoring was carried out to monitor the major characteristic ion for each compound; molecular ions of caffeic [mass-to-charge ratio (m/e) = 396], protocatechuic (m/e = 370), and gallic (m/e = 458) acids, molecular ion minus 30 for 4-O-methylgallic acid (m/e – 30 = 370), and the molecular ion minus a methyl group for identification of the internal standard (m/e – 15 = 317). The retention times used to further verify these compounds were as follows: 5.74 min for protocatechuic acid, 6.16 min for 4-O-methylgallic acid, 7.84 min for caffeic acid, and 6.79 min for 1-hydroxy-2-naphthoic acid (internal standard). Standards were run every day before and after running a batch of samples. The structures of these molecules are shown in Figure 1.

Peak identification was based on the relative retention time and mass spectra compared with authentic standards. The authentic standard of 4-O-methylgallic acid was prepared as described previously (21). Briefly, gallic acid (1 mol equivalent) was methylated with methyl iodide (2 mol equivalents) and potassium carbonate (2 mol equivalents) in acetone at room temperature for 12 h. The methyl ester was hydrolyzed with 1 mol KOH/L under nitrogen to give 4-O-methylgallic acid, which was purified by preparative thin-layer chromatography. For quantitation, calibration curves were established by measuring peak areas versus response in comparison with the internal standard over a range of analyte concentrations. The minimum level of detection (per mL plasma) for this assay was <0.2 ng for caffeic

![Chemical structures of caffeic acid (A), protocatechuic acid (B), gallic acid (C), 4-O-methylgallic acid (D), and 1-hydroxy-2-naphthoic acid (E).](https://academic.oup.com/ajcn/article-abstract/71/1/67/4729211/FIGURE1.png)
acid, <0.5 ng for protocatechuic acid, and <0.4 ng for 4-O-methylgallic acid. The intraassay variability was 5%.

Other measurements

Serum uric acid was determined in fresh samples by using a Boehringer Mannheim colorimetric enzymatic technique with uricase as the enzyme. The assay has a CV ≤ 1.4%. Serum ethanol was quantitated, from samples stored at −80°C, until the end of the study period by using the Emit Ethyl Alcohol Assay (designed by Behring Diagnostics, Inc, Summerville, NJ). This kit enables the enzymatic conversion of ethanol, in the presence of nicotinamide adenine dinucleotide (NAD) and alcohol dehydrogenase, to acetaldehyde. During this reaction, NAD is reduced to NADH and the increase in absorbance at 340 nm is proportional to the concentration of alcohol in the sample.

Statistics

Values are reported as means ± SEMs. The trapezoidal areas under the different curves were compared by analysis of variance in general linear modeling by using the Statistical Package for the Social Sciences (SPSS Inc, Chicago) with correction for baseline shift.

RESULTS

Passage of red wine through PVPP effectively removed >95% of the total phenolic compounds from the beverages (Table 1) and this was confirmed with the measurement of the specific phenolic acids by GC-MS. Plasma ethanol concentrations increased significantly in subjects consuming the 2 alcohol-containing beverages (Figure 2).

For the purposes of this study, we developed a selective and sensitive method to measure specific phenolic acids in plasma and beverages. Typical GC-MS selected ion chromatograms of plasma extracts obtained from a volunteer before and 2 h after red wine consumption, respectively, are shown in Figure 3 (A and B). This trace shows a clear increase in 4-O-methylgallic acid at 6.16 min and in caffeic acid at 7.84 min. The unknown peak at 5.5 min did not show any significant increase after red wine consumption. Mean plasma concentrations of caffeic, protocatechuic, and 4-O-methylgallic acids are shown in Figure 4. Both caffeic and 4-O-methylgallic acid increased significantly within the first 4 h after consumption of a single dose of red wine (5 mL/kg body wt) and after the same amount of dealcoholized red wine as compared with water or phenol-stripped red wine. There was no significant increase in the plasma concentration of protocatechuic acid. Alcohol consumption did not significantly slow the absorption of phenolic acids, the presence of alcohol in the red wine having no significant effect on the absorption of caffeic acid or the production of 4-O-methylgallic acid over the first 4 h after consumption. The maximum concentrations of caffeic and 4-O-methylgallic acids were 84 ± 18 and 176 ± 19 mmol/L, respectively, after red wine consumption and 91 ± 22 and 184 ± 18 mmol/L, respectively, after dealcoholized red wine consumption (Figure 4). Plasma total phenols, measured by a colorimetric assay in relation to a tannic acid standard curve, were unaffected by beverage consumption (data not shown). Although the bagels may have contributed a small amount of phenolic acids, all volunteers ate 2 bagels at each visit during the first 30 min; therefore, this should not have contributed to the differences between beverages.

The consumption of either red wine or dealcoholized red wine had no significant effect on the lag time of ex vivo Cu²⁺-induced serum or LDL oxidation (Figure 5) measured over 4 h. Conjugated diene formation was also unaltered (data not shown). The results of in vitro experiments carried out to determine the dose of phenolic acids, which showed a significant inhibition of lipoprotein oxidation, are shown in Table 2. Caffeic acid was the most potent inhibitor, with an IC₅₀ value (the dose at which 50% inhibition of oxidation is observed) < 1 µmol/L for both serum and LDL oxidation. Methylation of gallic acid significantly reduced its effectiveness as an antioxidant in this assay system. LDL oxidation was not significantly inhibited in the presence of concentrations ≤ 10 µmol 4-O-methylgallic acid/L. However, serum oxidation showed ≈20% inhibition with 10 µmol 4-O-methylgallic acid/L (data not shown).

Serum uric acid concentrations increased significantly in response to red wine, phenol-stripped red wine, and dealcoholized red wine compared with water (Figure 6). To assess whether the increase in serum uric acid may have been a response to lactic acid in the beverages (22), we determined the lactate content of the beverages. Red wine contained 17.5 mmol lactate/L and phenol-stripped red wine contained 11.1 mmol lactate/L.

DISCUSSION

The present study provides novel evidence of the absorption of specific phenolic acids into the bloodstream after ingestion of red wine or dealcoholized red wine. However, the ex vivo oxidation of LDL and serum was not significantly influenced by acute consumption of these beverages.

In our previous research we showed that one of the most potent antioxidant fractions obtained from acid-hydrolyzed red wine contained phenolic acid compounds (3). In the present study, we developed a GC-MS assay to measure caffeic and protocatechuic acids in plasma to determine their absorption. Our results indicate that caffeic, but not protocatechuic acid, was absorbed after ingestion of red wine or dealcoholized red wine. However, the concentrations reached in plasma were not sufficient to produce an effect on ex vivo LDL or serum oxidation. The wine used contained ≈10 times more caffeic than protocatechuic acid (Table 1) and this may
explain the undetected absorption of protocatechuic acid. In addition, we also detected a significant increase in 4-O-methylgallic acid, a known major metabolite of gallic acid in humans (23), after consumption of red wine and dealcoholized red wine. Red wine contains a significant amount of tannins, which are partly made up of gallic acid esters. 4-O-Methylgallic acid has not been detected in the plasma of humans after consumption of red wine, but, given the clear increase in this metabolite, it may prove to be a useful marker of consumption of phenolic acid–containing beverages, such as red wine. The commonly used spectrophotometric assay for phenols is unreliable when applied to plasma and, as Waterhouse et al (24) commented in a recent editorial, gives total polyphenol values in plasma that are unrealistic. Some researchers have used the total radical-trapping antioxidant measure to show increases in polyphenol absorption after consumption of high phenolic acid–containing beverages (25), but this too is nonspecific.

There is some recent evidence suggesting that dietary phenols are absorbed in the glycosidic form (26). Absorption of some phenolic compounds may also be enhanced when conjugated with glucose (27). The mammalian gastrointestinal tract contains glycosidases capable of hydrolyzing α-1,4-glycosidic bonds (28) and, therefore, the phenolic moieties of the many glycosides from the diet would not be cleaved during absorption because the aglycones are bonded to sugar molecules by β-glycosidic bonds (29). However, gut microflora may hydrolyze some of these β-glycosidic bonds, which may explain the absorption of free phenolic molecules (aglycones). There is further evidence that in humans, phenolic compounds undergo glucuronidation, methylation, and sulfate conjugation (30). We therefore used β-glucosidase and β-glucuronidase, which also has some sulfatase activity, to hydrolyze such conjugates, releasing either the free phenolic compound or its methylated form.

Despite the demonstrated bioavailability of these phenolic acids and the possible presence of other phenolic compounds not
measured, there was no significant effect on serum or LDL oxidation ex vivo. The likely reason is that the concentrations reached in plasma were much less than those required to produce an effect on the ex vivo oxidation of serum or isolated LDL. For example, in vitro dose-response studies indicate that concentrations of 400 nmol caffeic acid/L, 2 μmol protocatechuic acid/L, or 2.6 μmol gallic acid/L are required to produce 50% inhibition of LDL oxidation and that concentrations up to 10 μmol 4-O-methylgallic acid/L did not affect in vitro LDL oxidation (Table 2). Even if it is assumed that all phenolic acids in the plasma were associated with LDL, the total increase in concentrations of the compounds measured in plasma would not exceed 250 nmol/L. This concentration is inadequate for an effect on serum or LDL oxidation in vitro. Although the phenolic acids measured represent only a fraction of the total phenols in red wine, there still was no observed effect of ingestion of red wine on lipoprotein oxidation ex vivo. In addition, gallic acid, which has potent antioxidant activity in vitro, may only be transiently bioavailable and appears to be rapidly converted to 4-O-methylgallic acid, which does not have the same antioxidant potential (Table 2).

Uric acid, a major determinant of the antioxidant capacity of serum, has been reported to increase after consumption of alcoholic beverages and offers another potential antioxidant mechanism. Day and Stansbie (31) reported an elevation of serum uric acid concentrations in response to port consumption, which was correlated with an increase in serum total antioxidant capacity. In the present study, serum uric acid concentrations increased in response to consumption of red wine, phenol-stripped red wine, and dealcoholized red wine. This indicates an effect unrelated to alcohol content and was likely due to the lactate content of these beverages. Burch and Kurke (22) reported a rise in serum uric acid of 0.03 mmol/L in 4 volunteers after intravenous infusion of 1 L of 167 μmol racemic sodium lactate/L, which was associated with a decrease in urinary uric acid concentrations. Therefore, they suggested that the renal excretion of uric acid is diminished because of a direct effect of lactate. Although the small increase in serum uric acid in our study was significant, it had no significant effect on ex vivo serum oxidation. In the ex vivo serum oxidation method, the uric acid concentration was ≈2.7 μmol/L in the final diluted mixture. Uric acid is proposed to inhibit lipoprotein oxidation by chelating metal ions and a ratio of uric acid to metal ions of ≥1 is required for such activity (18). In our

FIGURE 4. Mean (±SEM) plasma concentrations of caffeic, protocatechuic, and 4-O-methylgallic acids by gas chromatography–mass spectrometry in plasma after ingestion of red wine (○; n = 12), phenol-stripped red wine (●; n = 12), dealcoholized red wine (●; n = 12), and water (□; n = 9). Calibration curves were established by measuring peak areas versus response compared with the internal standard 1-hydroxy-2-naphthoic acid. Caffeic acid concentrations increased significantly after ingestion of red wine (F = 4.95, P < 0.025) and dealcoholized red wine (F = 14.4, P < 0.001) compared with water. Protocatechuic acid concentrations were not affected significantly by beverage intake. 4-O-Methylgallic acid concentrations increased significantly after ingestion of red wine (F = 96.3, P < 0.001) and dealcoholized red wine (F = 123.55, P < 0.001) compared with water.

FIGURE 5. Mean (±SEM) lag time of ex vivo Cu²⁺-induced serum (with 12.5 μmol Cu²⁺/L at 37 °C) and LDL (with 5 μmol Cu²⁺/L at 37 °C) oxidation after ingestion of red wine (○; n = 12), phenol-stripped red wine (●; n = 12), dealcoholized red wine (●; n = 12), and water (□; n = 9). There were no significant differences between any of the groups.
method, the ratio of uric acid to copper ion was 1:5; thus, the increase in uric acid concentrations in response to the consumption of the beverages was insufficient to produce an inhibitory effect on ex vivo copper-induced serum oxidation.

In a recent study, Nigdikar et al (12) showed that the presence of EDTA in the LDL oxidation matrix diminishes any effects of red wine on lipoprotein oxidation. They presented this as a plausible explanation of the difference in the results obtained by other groups in previous studies of the effects of red wine phenols on LDL oxidation (7, 8). However, such an explanation does not apply to our findings because our LDL samples were passed through 2 Sephadex PD-10 columns to remove the EDTA. It is possible that passage through PD10 columns may remove any associated phenolic compounds from the LDL, decreasing any possible effects on isolated LDL oxidation. However, the fact that there was no significant effect on ex vivo serum oxidation supports our overall observation. Generally, studies of the effects of red wine have used ex vivo methods to assess lipoprotein oxidation, such as the monitoring of conjugated dienes and malondialdehyde. Other methods include the estimation of the total antioxidant capacity of plasma. With the use of such methods, serum antioxidant activity has been shown to increase acutely after ingestion of red wine (32, 33). However, all of these methods are indirect and although they may indicate the resistance of lipoproteins to oxidative stress, they do not measure in vivo oxidative damage. The measurement of specific oxidation products such as F₂-isoprostanes in plasma and urine may provide better markers of in vivo oxidative damage (34). Because the goal of our study was to ascertain the acute antioxidant effects of red wine consumption, we chose not to measure isoprostanes because it is unlikely that plasma concentrations would be significantly altered in such a short time period (35).

There is some evidence that alcohol consumption can induce oxidative stress, either by free radical formation through alcohol metabolism, by interfering with antioxidant defense mechanisms, or by interfering with antioxidant enzymes (36–39). The overall effect of alcoholic beverages on lipoprotein oxidation may be a balance between prooxidant and antioxidant actions of the various components of the beverage. No differences were seen between the presence or absence of alcohol in our studies, possibly suggesting that the ex vivo methods used to assess lipoprotein oxidation are not sensitive enough to measure prooxidant effects that have been observed under in vivo conditions.

In conclusion, consumption of red wine or dealcoholized red wine significantly increased plasma phenolic acid concentrations. Red wine (whether dealcoholized, phenol stripped, or as is) also caused a significant elevation in serum uric acid. Despite these changes, there was no effect on ex vivo lipoprotein oxidation over a 4-h time period. Longer-term controlled studies, measuring appropriate markers of in vivo oxidative damage, are required to further clarify the possible antioxidative benefits of phenolic compounds in red wine and other beverages.

We thank Valerie Burke for her statistical advice, Jessie Prestage for nursing assistance, and the volunteers for their participation and tolerance.

REFERENCES


### TABLE 2

Concentrations of the phenolic acids of interest that produce a 50% increase in lag time during serum and LDL oxidation induced by 12.5 and 5 μmol Cu/L, respectively

<table>
<thead>
<tr>
<th>Phenolic Acid</th>
<th>Serum Oxidation (μmol/L)</th>
<th>LDL Oxidation (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>0.5 (0.4–0.6)</td>
<td>0.4 (0.3–0.5)</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>0.9 (0.7–1.0)</td>
<td>2.0 (1.8–2.2)</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.8 (1.6–2.0)</td>
<td>2.6 (2.5–2.7)</td>
</tr>
<tr>
<td>4-O-Methylgallic Acid</td>
<td>&gt; 10</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>

1 Mean of triplicate experiments; range in parentheses.

### FIGURE 6

Mean (±SEM) serum uric acid concentrations in vivo after ingestion of red wine (●, n = 12), phenol-stripped red wine (●, n = 12), dealcoholized red wine (●, n = 12), and water (●, n = 9). Serum uric acid increased significantly (F > 16, P < 0.001) after ingestion of all red wine–based beverages compared with water.


