JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 2015−2021 **2015**

Dietary Wine Phenolics Catechin, Quercetin, and Resveratrol Efficiently Protect Hypercholesterolemic Hamsters against Aortic Fatty Streak Accumulation

CYRIL AUGER,[†,‡] PIERRE-LOUIS TEISSEDRE,[‡] PEGGY GÉRAIN,[†] NADINE LEQUEUX,[§] AURÉLIE BORNET,[‡] SAMUEL SERISIER,[†] PIERRE BESANÇON,[†] BERTRAND CAPORICCIO,[†] JEAN-PAUL CRISTOL,[§] AND JEAN-MAX ROUANET*[,†]

Equipe d'Accueil 3762, Nutrition & Aliment, Université Montpellier 2, 34095 Montpellier, France; Equipe d'Accueil 2993, Nutrition Humaine & Athérogénèse, Université Montpellier 1, CHU La Peyronie, 34925 Montpellier, France; and UMR 1083 INRA Sciences pour l'Œnologie, Centre d'Œnologie, Faculté de Pharmacie, Université Montpellier 1, 34093 Montpellier, France

The effects of the phenolic compounds catechin (Cat), quercetin (Qer), and resveratrol (Res) present in red wine on early atherosclerosis were studied in hamsters. Hamsters ($n = 32$) were divided into 4 groups of 8 and fed an atherogenic diet for 12 weeks. They received by force-feeding 7.14 mL/(kg of body wt·day) Cat, Qer, or Res in water [2.856 mg/(kg of body wt·day) for Cat and 0.1428 mg/(kg of body wt·dday) for Qer and Res], mimicking a moderate consumption of alcohol-free red wine (equivalent to that supplied by the consumption of about two glasses of red wine per meal for a 70 kg human), or water as control. Plasma cholesterol concentration was lower in groups that consumed phenolics than in controls. The increase in plasma apolipoprotein (Apo) A1 concentration was mainly due to Cat (26%) and Qer (22%) and to a lesser extent, but nonsignificantly, Res (19%). Apo-B was not affected. Plasma antioxidant capacity was not improved, and there was no sparing effect on plasma vitamins A and E. Plasma iron and copper concentrations were not modified nor were liver super oxide dismutase and catalase activities. A sparing effect of Qer on liver glutathione peroxidase activity appeared, whereas Cat and Res exhibited a smaller effect. Aortic fatty streak area was significantly reduced in the groups receiving Cat (84%) or Qer (80%) or Res (76%) in comparison with the controls. These findings demonstrate that catechin, quercetin, and resveratrol at nutritional doses prevent the development of atherosclerosis through several indirect mechanisms.

KEYWORDS: Red wine; catechin; quercetin; resveratrol; atherosclerosis; hamsters

INTRODUCTION

Emerging evidence from epidemiological studies suggests that moderate consumption of alcohol, particularly red wine, lowers mortality rates from coronary heart disease (*1*, *2*). Because red wines are rich in flavonoids and other phenolic constituents, various red wine components have been studied for antiatherogenic effects (*3*). Resveratrol (*trans*-3,4′,5-trihydroxystilbene), a phenolic compound present in red wine, has been thought to be the active ingredient responsible for cardiovascular benefits associated with wine consumption and used for numerous therapeutic purposes, including heart diseases (*4*, *5*), in the Oriental folk medicine kojo-kon. It has been shown to protect human low-density lipoprotein (LDL) against oxidation in vitro, not in vivo (*6*), and inhibit platelet aggregation (*7*) in vitro and in vivo. Recently, some authors proposed that cardioprotective effects of resveratrol are at least partly due to its negative regulatory effects on vascular smooth muscle cells (VSMCs) proliferation and survival (*8*) and reported that resveratrol may be capable of selectively eliminating abnormally proliferating VSMCs of the arterial walls in vivo. Nevertheless, the atheroprotective effect of resveratrol in vivo is yet to be demonstrated convincingly. Red wine contains other phenolic compounds, particularly the flavonoids, in much greater abundance than resveratrol (e.g., catechin up to 400 mg/mL and quercetin up to 20 mg/mL vs resveratrol, up to 10 mg/mL) (*9*). Moreover, these compounds were also shown to exhibit atheroprotective effects in vitro in various degrees (*3*). A vast amount of literature has been published that provides evidence that flavonoids possess antioxidant properties and free radical scavenging and chelation abilities (*10*, *11*−*15*). An increased consumption of phenolics has been correlated with a reduced risk of cardiovascular diseases and certain types of cancers (*16*). This was

* Address correspondence to this author at Equipe d'Accueil 3762, Nutrition et Aliment, CC 023, Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France [telephone (33) 04 67 14 35 21; fax (33) 04 67 63 36 49; e-mail jm.rouanet@univ-montp2.fr].

† Equipe d'Accueil 3762, Université Montpellier 2.

‡ UMR 1083, Université Montpellier 1.

§ Equipe d'Accueil 2993, Université Montpellier 1.

10.1021/jf048177q CCC: $30.25
Published on Web 02/17/2005

recently reported by Auger et al. (*17*). Hence, it is expected that chronic diseases, particularly atherosclerosis, may be prevented by flavonoids. It has also been shown that wine phenolic compounds such as resveratrol (*18*), quercetin (*18*, *20*), and catechins (*21*) exerted considerable antioxidant potency toward LDL oxidation in vitro. Elsewhere, Revell et al. (*22*) reported that resveratrol, catechin, epicatechin, and quercetin protect against LDL oxidation in vitro but not ex vivo. By acting as free radical scavengers, flavonoids inhibit lipid peroxidation that can initiate LDL oxidation, a contributing factor to the development of atherosclerosis (*20*). They also can act as transient metal ion chelators (*21*) and control protein oxidation and advanced glycation end products (AGEs) formation (*23*, *24*). Indeed, apart from ethanol, the phenolic compounds are involved in the cardioprotective capacity of red wine (*23*, *24*), and one possible explanation is that they slow the progression of atherosclerosis by acting as antioxidants toward LDL (*27*). Atherosclerosis progresses when macrophages in the subendothelial space of an artery take up oxidized LDL through a nonregulated scavenger receptor and are converted to foam cells that contain excessive lipid, especially cholesterol ester. The continuing aggregation of foam cells and cholesterol esters in the subendothelial space leads to the formation of fatty streaks, which are the earliest identifiable lesions of atherosclerosis and can be referred to as early aortic atherosclerosis. We have reported that the main mechanism involved in the prevention of the development of aortic disease was not simply a direct antioxidant effect (*28*, *29*).

Because catechin, quercetin, and resveratrol (henceforth abbreviated Cat, Qer, and Res, respectively) have the potential to inhibit these atherosclerosis-promoting mechanisms, it was hypothesized that orally administered they would inhibit atherosclerotic development in hypercholesterolemic hamsters. The present study represents a part of an investigation aiming at determining what wine phenolic compounds are efficient in the prevention of early atherosclerosis in hamster. We have recently shown that wine hydroxycinnamic acids (caffeic and sinapic acids) are not the phenolic compounds involved in such a beneficial effect (*28*). There are no reports comparing the individual implication of some important dietary families of wine phenolics in this pathology, excepted the work reported by Xu et al. (*30*), which showed that vitamin E plus catechin inhibited the development of aortic lesions in hypercholesterolemic hamsters, using diet and experimental conditions different from those described in this work.

We therefore used the hamster model of atherosclerosis (*28*, *29*, *31*, *32*) to compare catechin, quercetin, and resveratrol given at a nutritional dose, that is, equivalent to that supplied by the consumption of about two glasses of red wine per meal for a 70 kg human.

MATERIALS AND METHODS

Phenolics. (+)-Catechin, quercetin, and *trans*-resveratrol were obtained from Aldrich, Saint Quentin Fallavier, France.

Animals. Weanling male Syrian golden hamsters were received from Elevage Janvier (Le Genest-St-Isle, France) weighing 60−80 g and were randomly separated into four groups ($n = 8$/group) of statistically equal weight. They were maintained in plastic cages in a temperature-controlled environment (23 ± 1 °C) subjected to a 12-h light/dark cycle (lights on at 7:00 a.m.) and allowed free access to both food and water. Hamsters were handled according to the guidelines of the Committee on Animal Care at the University of Montpellier and NIH guidelines (*33*).

Diets and Feeding Procedures. Hamsters were fed a semipurified atherogenic diet (**Table 1**) in which the cholesterol content had been set at 0.5% and which was supplemented with 15% lard at the expense of starch and sucrose; no selenium, vitamin C, or vitamin E was added to this diet. Animals were given food daily for 12 weeks, and uneaten food was weighed daily. The hamsters of each group were additionally force fed once a day either tap water (control) or a solution of catechin, quercetin, or resveratrol in water. The volumes of solutions force-fed were adjusted daily to the weight of hamsters: it was established by extrapolating 500 mL/day average wine consumption, that is, about two glasses per meal (wine containing 2 g/L total phenolic compounds) for a 70 kg human to the equivalent for the daily weight of hamsters. This represents a volume of 7.14 mL/(kg of body wt·day). Hamsters received either 2.856 mg of (+)-catechin/(kg of body wt·day), 0.1428 mg of quercetin/(kg of body wt·day), or 0.1428 mg of resveratrol/(kg of body wt·day) dissolved in the above-mentioned volume of water.

Table 1. Composition of the Diets (Grams per Kilogram)

diet ingredient	exptl diet	diet ingredient	exptl diet
casein	200	mineral mix[a]	35
DL-methionine	3	vitamin mix[b]	10
cornstarch	393	lard	150
sucrose	154	cholesterol	5
cellulose	50		

[a] Mineral mixture contained (mg/kg of diet): $CaHPO_4$, 17200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4 \cdot 7H_2O$, 200; trace elements, 400 [$MnSO_4 \cdot H_2O$, 98; $CuSO_4 \cdot 5H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 80; $CoSO_4 \cdot 7H_2O$, 0.16; KI, 0.32; sufficient starch to bring to 40 g (per kg of diet)]. [b] Vitamin mixture contained (mg/kg of diet): retinol, 12; cholecalciferol, 0.125; thiamin, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; *p*-aminobenzoic acid, 100; biotin, 0.6; sufficient starch to bring to 20 g (per kg of diet).

Analytical Procedures. At the end of the 12-week experimental period, hamsters were deprived of food for 18 h and were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/mL at a dosage of 60 mg/kg of body wt). Blood was drawn by cardiac puncture with heparin-moistened syringes, and plasma was prepared by centrifugation at 2000*g* for 10 min at 4 °C and then stored at −80 °C until analysis. Plasma total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were determined by commercially available enzymatic methods (respectively no. 401 and 352-4, Sigma Chemicals, Saint Quentin Fallavier, France). Plasma very low- and low-density lipoprotein cholesterol (nonHDL-C) were precipitated with phosphotungstate reagent (*34*), and HDL-C was measured in the supernatant. Plasma apolipoprotein A-1 (Apo-A1) and apolipoprotein B (Apo-B) concentrations were determined using Sigma turbidimetric immunoassay kits (no. 356 and 357, respectively) as previously described (*35*). Plasma iron and copper were assayed on a flame atomic absorption spectrophotometer (Spectra AA10/20, Varian Instruments, Les Ulis, France). Plasma samples (1 mL) were treated with 2 mL of pure nitric acid at room temperature during 24 h until digested, diluted to 10 mL if necessary, and filtered through ash-free filter paper under pressure before analysis as described earlier (*36*). Standards solutions were obtained from iron and copper standard solutions (Sigma, Steinheim, Germany) by dilution with deionized water twice distilled.

Measurement of plasma advanced oxidation protein products (AOPP) level was performed using technical recommendations described by Witko-Sarsat et al. (*37*) and modified by Wratten et al. (*38*). Briefly, AOPP were measured by spectrophotometry using a microplate reader (Spectra Kontron, Tecan-France, Trapes, France) and were calibrated with chloramine-T (Sigma, St. Louis, MO) solutions, which absorb in the presence of potassium iodide at 340 nm. In test wells, 200 μL of plasma was diluted 1/5 in phosphate-buffered saline (PBS). In standard wells, 50 μL of 1.16 M KI was added to 1 mL of chloramine-T solution (0, 10, 30, 50, 70, 100 μM). Then, 100 μL of acetic acid was added in both test and standard wells. Finally, after a 2-min gentle agitation, absorbance of the reaction mixture was read at 340 nm. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents.

Plasma vitamin A and E concentrations were measured simultaneously by high-performance liquid chromatography (HPLC) (Waters

Chromatography, Millipore Waters, Les Ulis, France) as previously reported (*39*). Vitamins A and E were extracted from plasma in the presence of tocopherol acetate as an internal standard. After centrifugation, the supernatant was separated and extracted with hexane and evaporated until dryness; the dry residue was redissolved in methanol and used for chromatography separation. The HPLC apparatus was equipped with a Novopack 150 × 3.9 mm column (4-μm particle size, reverse phase) and a spectrophotometric detector (Millipore-Waters Lambda max 480) with ultraviolet detection at 292 nm.

Plasma AGEs concentration was determined by fluorometric assay derived from Munch et al. (*40*). Briefly, plasma was diluted 50-fold with H_2O. Fluorescence was then recorded in duplicate on a Wallac Victor fluorometer (excitation at 355 nm and emission at 460 nm) and expressed in arbitrary units (AU) after subtraction of the fluorescence of the blank (H_2O).

The antioxidant capacity of plasma was measured as Trolox equivalents, that is, a quantitative value for general antioxidant levels in biological samples (*41, 42*), which was assayed in plasma with a quantitative colorimetric technique according to the kit supplier's instructions (Kit NX2332; Randox, Mauguio, France). The assay is based on the incubation of a peroxidase and H_2O_2 with 2,2′-azinodi-(3-ethylbenzthiazoline sulfonate) (ABTS) to produce the radical cation $ABTS^{\bullet+}$. This has a relatively stable blue-green color, which is measured at 600 nm. Antioxidants (albumin, uric acid, ascorbic acid, α-tocopherol, glutathione, β-carotene, etc.) in the sample suppressed $ABTS^{\bullet+}$ color production to a degree proportional to their concentration.

The liver was perfused with 0.15 mol/L KCl to remove residual blood, rapidly excised, rinsed in ice-cold saline, blotted dry, weighed, sectioned for analyses, and stored in liquid nitrogen. The liver was homogenized in 4 volumes of ice-cold 0.1 mol/L potassium phosphate buffer (pH 7.4), and the homogenate was spun at 13000*g* for 15 min at 4 °C. The supernatant was then centrifuged at 105000*g* for 60 min at 4 °C, and cytosols were stored at 80 °C for subsequent assay of superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT) activities. Superoxide dismutase was assayed according to the method of Paoletti and Mocali (*43*); 1 unit of SOD activity was defined as the amount of protein that produced 50% inhibition of the rate of NADH oxidation observed in a control without sample and in a medium containing triethanolamine−diethanolamine−HCl buffer (100 mM), NADH (7.5 mM), EDTA (100 mM), $MnCl_2$ (50 mM), and mercaptoethanol (10 mM). This activity of Se-GSHPx was measured according to the method of Wendel (*44*) using 0.2 mmol/L hydrogen peroxide as the substrate and including 1.0 mmol/L sodium azide to inhibit catalase, so that only GSHPx activity was measured. Catalase was assayed according to the method of Aebi (*45*), which measures the degradation of H_2O_2 at 240 nm in potassium phosphate buffer (50 mM) at pH 7.0. The cytosolic protein content was determined by using a commercial protein assay (Sigma, Saint Quentin Fallavier, France) according to the method of Smith et al. (*46*) and using bovine serum albumin as standard.

Aortic Tissue Processing. Following blood collection and liver removal, the intact aorta was first perfused with phosphate-buffered saline containing 1 mmol/L $CaCl_2$ and 15 mmol/L glucose for 5 min and then with 0.1 mmol/L sodium cacodylate buffer (pH 7.4) containing 2.5 mmol/L $CaCl_2$, 2.5% paraformaldehyde, and 1.5% glutaraldehyde for the fixation of the vasculature. The aorta was carefully dissected between sigmoid valves and 3−4 cm after the aortic arch and thoroughly cleaned of loose adventitial tissue; the aortic arch was cut free, opened longitudinally along the outside of the arch, pin cork, immersed in fresh fixative solution, and stored at 4 °C until staining. The aortic arches were then first rinsed for 48 h in 0.1 mol/L sodium cacodylate buffer (pH 7.4) containing 30 mmol/L $CaCl_2$ and 250 mmol/L sucrose. The arches were then rinsed in distilled water, stained for 40 s in Harris hematoxylin, and then rinsed in distilled water and quickly in 70% isopropyl alcohol; finally, they were stained in Oil Red O for 30 min according to the method of Nunnari et al. (*47*), rinsed in 70% isopropyl alcohol, and back to distilled water. Each aortic arch was then directly displayed on a glass slide, endothelium side up, covered with Aquamount mounting medium and cover slips and observed *en face* by light microscopy. All segments were photographed using a video digitizer. The area covered by foam cells (aortic fatty streak lesion) was analyzed quantitatively using a computer-assisted morphometry system and expressed as a percentage of the total area surveyed.

Table 2. Effects of Daily Force Feeding of Water (Control), Catechin, Quercetin, or Resveratrol in Water on Body Weight and Food Intake of Hamsters Fed an Atherogenic Diet for 12 Weeks[a]

exptl group	controls	catechin	quercetin	resveratrol
initial body wt, g	70.8 ± 1.7	69.0 ± 1.5	70.6 ± 1.5	69.1 ± 0.9
final body wt, g	107.9 ± 4.0	106.1 ± 1.9	107.7 ± 3.2	108.1 ± 3.4
food intake, g/day	5.47 ± 1.16	5.17 ± 1.22	5.42 ± 1.44	5.26 ± 0.60

[a] Values are means ± SEM, $n = 8$. Data were analyzed by one-way ANOVA followed by the least significant difference test. For each dietary treatment, means in a column with different letters differ, $P < 0.05$.

Table 3. Effects of Daily Force Feeding of Water (Control), Catechin, Quercetin, or Resveratrol in Water on Plasma Lipid and Apolipoprotein Concentrations in Hamsters Fed an Atherogenic Diet for 12 Weeks[a]

exptl group	controls	catechin	quercetin	resveratrol
TC,[b] mmol/L	10.03 ± 0.25a	9.10 ± 0.31b	9.00 ± 0.23b	9.25 ± 0.12b
HDLC,[c] mmol/L	3.61 ± 0.36	3.62 ± 0.15	3.68 ± 0.17	3.83 ± 0.24
TG,[d] mmol/L	1.37 ± 0.19a	1.20 ± 0.05a	1.11 ± 0.05b	1.45 ± 0.08a
Apo-A1, g/L	1.70 ± 0.21a	2.14 ± 0.13b	2.07 ± 0.13ab	2.02 ± 0.09a
Apo-B, g/L	0.36 ± 0.03	0.33 ± 0.02	0.32 ± 0.02	0.35 ± 0.03
Apo-A1/Apo-B	4.72 ± 0.59a	6.48 ± 0.35b	6.45 ± 0.54bc	5.80 ± 0.42ac

[a] Values are means ± SEM, $n = 8$. Data were analyzed by one-way ANOVA followed by the least significant difference test. For each dietary treatment, means in a column with different letters differ, $P < 0.05$. [b] Total cholesterol. [c] High-density lipoprotein cholesterol. [d] Triglycerides.

Statistical Analyses. Data are shown as the means ± SEM, $n = 8$ measurements/group. Data were subjected to logarithmic transformation when necessary to achieve homogeneity of variances. Statistical analyses of the data were carried out using Stat View IV software (Abacus Concepts, Berkeley, CA) by one-way ANOVA followed by Fisher's protected least significant difference test. Differences were considered to be significant at $P < 0.05$.

RESULTS

There was no difference in the final body weight or in the food consumption among the four groups (**Table 2**). The plasma lipid and apolipoprotein profile is shown in **Table 3**: plasma TC was 10% lower in hamsters that received Cat, Qer, or Res than in controls; whatever the experimental group, treatment was without effect on plasma HDL-C, and TG was reduced by only Qer (18%) compared with controls. Catechin triggered an increased Apo-A1 concentration (26%), and treatments did not affect Apo-B concentration. The Apo-A1/Apo-B ratio was equally increased by feeding Cat or Qer (37%) but not Res compared with controls. Average aortic fatty streak accumulation (AFSA), measured as the percentage of Oil Red O staining relative to the total area surveyed (**Figure 1**), was significantly decreased in hamsters receiving catechin (84%), quercetin (80%), or resveratrol (76%) in comparison with controls. Plasma AGEs concentration was only significantly reduced in hamsters receiving quercetin in comparison with those fed catechin (∼9%) (**Table 4**); the group receiving quercetin exhibited a reduced plasma AOPP concentration (41%) compared with controls, whereas the other groups did not. PAC values are also displayed in **Table 4**; they were not affected by the phenolic compounds tested. In all flavonoid groups, plasma vitamin A and vitamin E concentrations as well as those of iron and copper were not modified when compared with controls (**Table 4**). Catechin, quercetin, and resveratrol had no effect on liver SOD activity (**Table 5**); feeding quercetin significantly reduced GSHPx

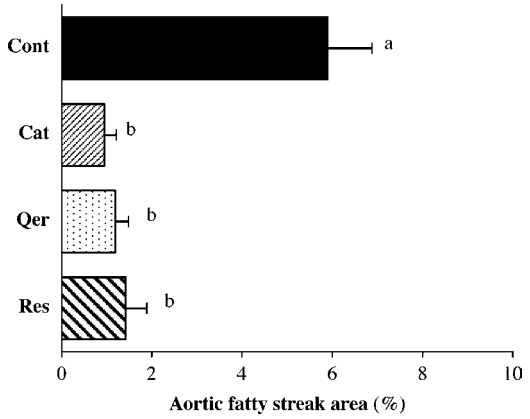

Figure 1. Effects of daily force feeding of water (control, Cont), catechin (Cat), quercetin (Qer), or resveratrol (Res) on aortic fatty streak area (AFSA) in hamsters fed an atherogenic diet for 12 weeks. AFSA is expressed as a percentage of the total aortic area surveyed. Bars with different letters differ, $P < 0.05$.

activity by 31%, whereas hamsters receiving Cat or Res exhibited nonsignificantly reduced GSHPx activities (19 and 21%, respectively). This was due to high SEM values coming from two animals in the control and Res groups. Catalase activity was not modified by the ingestion of the phenolic compounds except by Cat.

DISCUSSION

Epidemiological studies have shown that the lower incidence of cardiovascular disease (CVD) mortality in southern France than in other European countries, despite a high consumption of saturated fats (*48*), was correlated with an increased intake of red wine (*1*, *49*). It is not clear which phenolic compound of wine is responsible for this protective effect. Three in particular [(+)-catechin, quercetin, and resveratrol] are powerful antioxidants that can prevent in vitro and in vivo LDL free radical-mediated oxidation (*3*, *6*, *50*, *51*). These properties are associated with their ability to scavenge free radicals and chelate metals, which are a known feature of the atherosclerotic plaque where oxidized LDL contributes to the development of fatty lesions (*52*). To provide insight into the mechanism that mediates the protective effects of wine and also of fruits and vegetables, attention has been directed to the phenolic compounds, and this study represents a part of a work aimed at identifying the main class of active components that protect against atherosclerotic lesion formation. It resulted in several major observations: (1) Different phenolic compounds belonging to different families exhibited similar efficacies in reducing the amount of lipid deposits, for example, in preventing early atherosclerosis in hamsters. (2) Each of them equally reduced plasma TC. (3) The Apo-A1 to Apo-B ratio was improved by each phenolic compound, except for the Res group ratio, which is not statistically different from control albeit at the limit of the significance level ($p = 0.07$). (4) Each marker of plasma antioxidative status (PAC, vitamins, and transition metal ions) was not modified. (5) A sparing effect of hepatic antioxidant enzymes appeared for only GSHPx. The ability of polyphenols to chelate metal ions, such as iron and copper, may contribute to their antioxidant activity by preventing redox-active transition metals from catalyzing free radical formation (*20*, *53*). These metal-chelating properties likely explain the ability of polyphenols to inhibit copper-mediated LDL oxidation and other transition metal-catalyzed oxidations in vitro (*54*). However, it is not clear whether metal chelation is a physiologically relevant antioxidant activity, because most transition metal ions are bound to proteins in vivo where they cannot participate in metal-catalyzed free radical formation. This may have happened here. Moreover, *trans*-resveratrol could not chelate ferrous ions (*55*). Elsewhere, polyphenols can be good candidates for controlling protein oxidation and AGEs and AOPP formation, and may exert their protective effects against chronic diseases via controlling protein modification. In fact, it has been shown that wine phenolic compounds (*18*−*21*) exert considerable antioxidant potency toward LDL oxidation in vitro and also inhibit the oxidation of LDL and other tissue proteins in vitro and in vivo (*56*, *57*). Also, it has been found that flavonoids can inhibit the glycation and autoxidation of glucose, which may initiate and propagate protein modification (*56*, *58*). Apart from Qer (and

Table 4. Effects of Daily Force Feeding of Water (Control), Catechin, Quercetin, or Resveratrol in Water on Plasma AGEs,[a] AOPP,[b] Vitamins A and E, PAC,[c] and Iron and Copper Concentrations in Hamsters Fed an Atherogenic Diet for 12 Weeks[d]

exptl group	controls	catechin	quercetin	resveratrol
AGEs, arbitrary units	7.28 ± 0.30ab	7.60 ± 0.20a	6.89 ± 0.23b	7.56 ± 0.2ab
AOPP, μmol/L	74.16 ± 9.36a	78.65 ± 8.13a	43.27 ± 4.98b	62.66 ± 12.56a
PAC, mmol/L	0.98 ± 0.03	1.00 ± 0.03	1.04 ± 0.02	0.99 ± 0.03
vitamin A, mmol/L	1.25 ± 0.11	1.33 ± 0.04	1.39 ± 0.05	1.51 ± 0.17
vitamin E, mmol/L	4.03 ± 0.40	4.63 ± 0.55	4.90 ± 0.37	3.75 ± 0.42
iron, mg/L	1.50 ± 0.11	1.50 ± 0.09	1.71 ± 0.41	1.40 ± 0.32
copper, mg/L	0.66 ± 0.13	0.62 ± 0.05	0.59 ± 0.04	0.50 ± 0.04

[a] Advanced glycation end products. [b] Advanced oxidation protein products. [c] Plasma antioxidant capacity. [d] Values are means ± SEM, $n = 8$. Data were analyzed by one-way ANOVA followed by the least significant difference test. For each dietary treatment, means in a column with different letters differ, $P < 0.05$.

Table 5. Effects of Daily Force Feeding of Water (Control), Catechin, Quercetin, or Resveratrol in Water on Liver Superoxide Dismutase (SOD), Glutathione Peroxidase (GSHPx), and Catalase (CAT) in Hamsters Fed an Atherogenic Diet for 12 Weeks[a]

exptl group	controls	catechin	quercetin	resveratrol
SOD, units/mg of protein	7.28 ± 1.15	6.38 ± 0.36	7.50 ± 0.60	7.43 ± 1.00
GSHPx ($\times 10^{-2}$), units/mg of protein	11.47 ± 1.93a	9.30 ± 0.78ab	7.90 ± 0.79b	8.70 ± 1.10ab
CAT, units/mg of protein	4.51 ± 0.40a	6.03 ± 0.35b	4.69 ± 0.32a	4.03 ± 0.33a

[a] Values are means ± SEM, $n = 8$. Data were analyzed by one-way ANOVA followed by the least significant difference test. For each dietary treatment, means in a column with different letters differ, $P < 0.05$.

Res, which is not a flavonoid), such an effect did not occur here. Moreover, no sparing effect of phenolics on vitamins A and E happened. In a previous study (*28*) we have demontrated that caffeic and sinapic acids, the major hydroxycinnamic acids present in wine, which are potent antioxidants (*58*, *59*), did not exhibit any effect on aortic fatty streak accumulation, and we had suggested that the main mechanism involved in the prevention of the development of the aortic disease was not simply a direct antioxidant effect and that indirect mechanisms may take place, such as sparing of antioxidant enzymes and vitamins, metal chelation, binding to transferrin (*60*) and/or inhibition of endothelin-I synthesis (*61*). A favorable influence on eicosanoid metabolism and platelet aggregation (*62*) and induction of endothelium-dependent relaxation via an enhancement of endothelial NO synthesis could also occur (*30*). Our results now suggest that sparing of antioxidant vitamins and metal chelation by phenolics are unlikely here. Sparing of GSHPx exists but does not seem to be essential. A recent work by Stocker and O'Hallaran (*63*) demonstrated that dealcoholized red wine decreases atherosclerosis in apolipoprotein E gene-deficient mice, independently of inhibition of lipid peroxidation in the artery wall, and corroborated our previous results suggesting that the main mechanism involved in the prevention of the development of the aortic disease was not simply a direct antioxidant effect (*28*, *30*). It must not be ruled out that the effect of wine on atherosclerosis may be a result of synergism between the phenolic compounds, as previously suggested by us from their vasorelaxant effects on rat aortic rings (*30*) and shown (*64*, *65*) for antioxidant and antiplatelet effects, or between phenolic compounds and vitamin E (*63*).

In conclusion, whatever are the involved mechanisms, we have demonstrated that catechin, quercetin, and resveratrol are among the phenolic compounds of red wine that contribute to its protective effect against early atherosclerosis; however, the contribution of other wine constituents cannot be excluded. The effects of these red wine constituents may be of potential therapeutic relevance, their concentrations used in our experiments being comparable to those found in humans following the ingestion of two glasses of red wine per meal, that is, nutritional and moderate doses.

LITERATURE CITED

(1) Rimm, E. B.; Klatsky, A.; Grobbe, D.; Stampfer, M. J. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. *Br. Med J*. **1996**, *312*, 731−736.

(2) Booyse, F. M.; Parks, D. A. Moderate wine and alcohol consumption: beneficial effects on cardiovascular disease. *Thromb. Haemost*. **2001**, *86*, 517−528.

(3) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as biological fluid: history, production, and role in disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313.

(4) Nonomura, S.; Kanagawa, H.; Makimoto, A. Chemical constituents of polygonaceous plants: I. Studies on the components of Ko-jo-kon. *Yakugaku Zasshi* **1963**, *83*, 988.

(5) Sieman, E. H.; Creasy, L. L. Concentration of the phytoalexin resveratrol in wine. *Am. J. Enol. Vitic.* **1992**, *43*, 49−52.

(6) Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993**, *341*, 1103−1104.

(7) Bertelli, A. A.; Giovannini, L.; Giannessi, D.; Migliori, M.; Bernini, W.; Fregoni, M.; Bertelli, A. Antiplatelet activity of synthetic and natural resveratrol in red wine. *Int. J. Tissue React.* **1995**, *17*, 1−3.

(8) Mnjoyan, Z. H.; Fujise, K. Profound negative regulatory effects by resveratrol on vascular smooth muscle cells: a role of p53-$p21^{WAF1/CIP1}$ pathway. *Biochem. Biophys. Res. Commun.* **2003**, *311*, 546−522.

(9) Cabanis, J. C.; Cabanis, M. T.; Cheynier, V.; Teissedre, P. L. Tables de composition. In *Œnologie, Fondements Scientifiques et Technologiques*; Flanzy, C., Coordonateur; Lavoisier Tec & Doc: Paris, France, 1999; pp 315−336.

(10) Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds—nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric*. **2000**, *80*, 1094−1117.

(11) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived phenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.

(12) Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *J. Agric. Food Chem*. **1995**, *43*, 2800−2802.

(13) Morel, I.; Lescoat, G.; Cillard, P.; Cillard, J. Role of flavonoids and iron chelation in antioxidant action. *Methods Enzymol.* **1994**, *234*, 437−443.

(14) Dell'Agli, M.; Busciala, A.; Bosicio, E. Vascular effects of wine polyphenols. *Cardiovasc. Res*. **2004**, *63*, 593−602.

(15) Stoclet, J. C.; Chataigneau, T.; Ndiaye, M.; Oak, M. H.; El Bedoui, J.; Chataigneau, M.; Schini-Kerth, V. B. Vascular protection by dietary polyphenols. *Eur. J. Pharm*. **2004**, *500*, 299−313.

(16) Duthie, G.; Crozier, A. Plant-derived phenolic antioxidants. *Curr. Opin. Lipidol.* **2000**, 11, 43−47.

(17) Auger, C.; Al-Awwadi, N.; Bornet, A.; Rouanet, J.-M.; Gasc, F.; Cros, G.; Teissedre, P.-L. Catechins and procyanidins in Mediterranean diets. *Food Res. Int*. **2004**, *37*, 233−245.

(18) Frémont, L.; Belguendouz, L.; Delpal, S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Sci*. **1999**, *64*, 2511−2521.

(19) Meyer, A. S.; Heinonen, M.; Frankel, E. N. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food Chem.* **1998**, *61*, 71−75.

(20) Yamamoto, N.; Moon, J.-H.; Tsuhida, T.; Nagao, A.; Terao, J. Inhibitory effect of quercetin metabolites and their relative derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein. *Arch. Biochem. Biophys*. **1999**, *372*, 347−354.

(21) Hanasaki, Y.; Ogawa, S.; Fukui, S. The correlation between active oxygens scavenging and antioxidative effects of flavonoid. *Free Radical Biol. Med.* **1994**, *16*, 845−850.

(22) Revell, A. F.; Legge, M.; Sutherland, W. H. F.; McCormick, S. P. A. The effect of resveratrol-rich wine on LDL oxidation. *Atheroscler. Suppl*. **2002**, *3*, 192.

(23) Exner, M.; Hermann, M.; Hofbauer, R.; Kapiotis, S.; Quehenberger, P.; Speiser, W.; Held, I.; Gmeiner, B. M. Genistein prevents the glucose autoxidation mediated atherogenic modification of low-density lipoprotein. *Free Radical Res.* **2001**, *34*, 101−112.

(24) Holvoet, P.; Collen, D. Oxidation of low-density lipoproteins in the pathogenesis of atherosclerosis. *Atheroscler. Suppl*. **1998**, *137*, 33−38.

(25) Das, D. K.; Sato, M.; Ray, P. S.; Maulik, G.; Engelman, R. M.; Bertelli, A. A.; Bertelli, A. Cardioprotection of red wine: role of phenolic antioxidants. *Drugs Exp. Clin. Res*. **1999**, *25*, 115−120.

(26) Agewall, S.; Wright, S.; Doughty, R. N.; Whalley, G. A.; Duxbury, M.; Sharpe, N. Does a glass of red wine improve endothelial function? *Eur. Heart J.* **2000**, *21*, 74−78.

(27) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.
(28) Auger, C.; Laurent, N.; Laurent, C.; Besançon, P.; Caporiccio, B.; Teissedre, P. L.; Rouanet, J.-M. Hydroxycinnamic acids do not prevent aortic atherosclerosis in hypercholesterolemic golden Syrian hamsters. *Life Sci.* **2004**, *74*, 2365−2377.
(29) Auger, C.; Gérain, P.; Laurent-Bichon, F.; Portet, K.; Bornet, A.; Caporiccio, B.; Cros, G.; Teissedre, P. L.; Rouanet, J. M. Phenolics from commercialized grape extracts prevent early atherosclerotic lesions in hamsters by mechanisms other than antioxidant effect. *J. Agric. Food Chem.* **2004**, *52*, 5297−5302.
(30) Xu, R.; Yokoyama, W. H.; Irving, D.; Rein, D.; Walzem, R. L.; German, J. B. Effect of dietary catechin and vitamin E on aortic fatty strea accumulation in hypercholesterolemic hamsters. *Atherosclerosis* **1998**, *13*, 29−36.
(31) Vinson, J. A.; Teufel, K.; Wu, N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis* **2001**, *156*, 67−72.
(32) Auger, C.; Caporiccio, B.; Landrault, N.; Teissedre, P. L.; Laurent, C.; Cros, G.; Besançon, P.; Rouanet, J.-M. Red wine phenolic compounds reduce plasma lipids and apolipoprotein B, and prevent early aortic atherosclerosis in hypercholesterolemic Golden Syrian hamsters (*Mesocricetus auratus*). *J. Nutr.* **2002**, *132*, 1207−1213.
(33) National Research Council. *Guide for the Care and the Use of Laboratory Animals*; National Institutes of Health Publication 85-23 (rev.).; U.S. Government Printing Office: Washington, DC, 1985.
(34) Weingand, K. W.; Daggy, B. P. Quantitation of high-density lipoprotein cholesterol in plasma from hamsters by differential precipitation. *Clin. Chem.* **1990**, *36*, 575−580.
(35) Rifai, N.; King, M. E. Immunoturbidimetric assays of apolipoprotreins A, AI, AII, and B in serum. *Clin. Chem.* **1996**, *32*, 957−961.
(36) Clegg, M. S.; Keen, C. L.; Lönnerdal, B.; Hurley, L. S. Influence of ashing techniques on the analysis of trace elements in animal tissue. I. Wet ashing. *Biol. Trace Elem. Res.* **1981**, *3*, 107−115.
(37) Witko-Sarsat, V.; Friedlander, M.; Capeillere-Blandin, C.; Nguyen-Khoa, T.; Nguyen, A. T.; Zingraff, J.; Jungers, P.; Descamps-Latscha, B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int.* **1996**, *49*, 1304−1313.
(38) Wratten, M. L.; Sereni, L.; Tropea, F.; Tetta, C. Hemolipodialysis removes hydrophobic toxins and reduces oxidative stress in an in vitro model system. *Nephrol., Dial., Transplant.* **1998**, *13*, A184.
(39) Cristol, J. P.; Bosc, J. Y.; Badiou, S.; Leblanc, M.; Lorrho, R.; Descomps, B.; Canaud, B. Erythropoietin and oxidative stress in haemodialysis: beneficial effects of vitamin E supplementation. *Nephrol., Dial., Transplant.* **1997**, *12*, 2312−2317.
(40) Münch, G.; Keis, R.; Wessels, A.; Riederer, P.; Bahner, U.; Heidland, A.; Niwa, T.; Lemke, H. D.; Schinzel, R. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. *Eur. J. Clin. Chem. Clin. Biochem.* **1997**, *35*, 669−677.
(41) Rice-Evans, C.; Miller, N. J. Total antioxidant status in plasma and body fluids. *Methods Enzymol.* **1994**, *234*, 279−293.
(42) Koechlin, C.; Couillard, A.; Cristol, J. P.; Chanez, P.; Hayot, M.; Le Gallais, D.; Prefaut, C. Does systemic inflammation trigger local exercise-induced oxidative stress in COPD? *Eur. Respir. J.* **2004**, *23*, 538−44.
(43) Paoletti, F.; Mocali, A. Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation. *Methods Enzymol.* **1990**, *186*, 209−220.
(44) Wendel, A. Glutathione peroxidase. *Methods Enzymol.* **1981**, *77*, 325−333.
(45) Aebi, H. Catalase. *Methods Enzymol.* **1984**, *105*, 121−126.
(46) Smith, S. K.; Krohn, R. I.; Mallia, A. K.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. K. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **1985**, *150*, 76−85.
(47) Nunnari, J. J.; Zand, T.; Joris, I.; Majno, G. Quantification of Oil red O staining of the aorta in hypercholesterolemic rats. *Exp. Mol. Pathol.* **1989**, *51*, 1−8.
(48) Tunstall-Pedoe, H.; Kuulasmaa, K.; Mahonen, N.; Tolonen, H.; Ruokokoski, E.; Amouyel, P. Contribution of trends in survival and coronary-event rates to changes in coranary heart disease mortality: 10-year results from 37 WHO MONICA project populations: monitoring trends and determinants in cardiovascular disease. *Lancet* **1999**, *353*, 1547−1557.
(49) Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1999**, *339*, 1523−1523.
(50) Frankel, E. N.; Waterhouse, A. L.; Teissedre, P.-L. Principal phenolic phytochemicals in selected Californian wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.
(51) Teissedre, P.-L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wine. *J. Sci. Food Agric.* **1996**, *70*, 55−61.
(52) Steinberg, D.; Parthasarathy, S.; Carew, T.; Khoo, J. C.; Witzum, J. L. Beyond cholesterol: modifications of low-density lipoprotein that increase atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915−924.
(53) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152−159.
(54) Brown, J. E.; Khodr, H.; Hider, R. C.; Rice-Evans, C. A. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. *Biochem. J.* **1998**, *330*, 1173−1178.
(55) Belguendouz, L.; Frémont, L.; Linard, A. Resveratrol inhibits metal ion-dependent and independent peroxidation of porcine low-density lipoproteins. *Biochem. Pharmacol.* **1997**, *53*, 1347−1355.
(56) de Whalley, C. V.; Rankin, S. M.; Hoult, J. R. S.; Jessup, W.; Leake, D. S. Flavonoids inhibit the oxidative modification of low-density lipoproteins by macrophages. *Biochem. Pharmacol.* **1990**, *39*, 1743−1750.
(57) Nagasawa, T.; Hayashi, H.; Fujimaki, N.; Nishizawa, N.; Kitts, D. D. Induction of oxidatively modified proteins in skeletal muscle by electrical stimulation and its suppression by dietary supplementation of (−)-epigallocatechin gallate. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 1004−1010.
(58) Andreasen, M. F.; Landbo, A.-K.; Christensen, L. P.; Hansen, A. A.; Meyer, A. S. Antioxidant effects of phenolic rye (*Secale cereale* L.) extracts, monomeric hydroxycinnamates, and ferulic acid dehydrodimers on human low-density lipoproteins. *J. Agric. Food Chem.* **2001**, *49*, 4090−4096.
(59) Abu-Amsha, R.; Croft, K. D.; Puddey, I. B.; Proudfoot, J. M.; Beilin, M. J. Phenolic contents of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation in vitro: identification and mechanism of action of some cinnamic acid derivatives from red wine. *Clin. Sci.* **1996**, *91*, 449−458.
(60) Brunet, M. J.; Bladé, C.; Salvado, M. J.; Arola, L. Human Apo A-I and rat transferrin are the principal plasma proteins that bind wine catechins. *J. Agric. Food Chem.* **2002**, *50*, 2708−2712.
(61) Khan, N. Q.; Lees, D. M.; Douthwaite, J. A.; Carrier, M. J.; Corder, R. Comparison of red wine extract and polyphenol constituents on endothelin-I synthesis by cultured endothelial cells. *Clin. Sci.* **2002**, *103* (Suppl. 48), 72S−75S.
(62) Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin. Chim. Acta* **1995**, *235*, 207−219.

(63) Stocker, R.; O'Hallaran, R. A. Dealcoholized red wine decreases atherosclerosis in apolipoprotein E gene-deficient mice independently of inhibition of lipid peroxidation in the artery wall. *Am. J. Clin. Nutr*. **2004**, *79*, 123−130.
(64) Saint-Cricq de Gaujelac, N.; Provost, C.; Vivas, N. Comparative study of polyphenols scavenging activities assessed by different methods. *J. Agric. Food Chem.* **1999**, *47*, 425−431.
(65) Shanmganayagam, D.; Beahm, M. R.; Osman, H. E.; Krueger, C. G.; Reed, J. D.; Folts, J. D. Grape seed and grape skin extract elicit a greater antiplatelet effect when used in combination than when used individually in dogs and humans. *J. Nutr*. **2002**, *132*, 3592−3598.

Received for review November 2, 2004. Revised manuscript received January 4, 2005. Accepted January 18, 2005. C.A. was supported by a grant from the French Research and Technology Ministry. This work was supported in part by a grant from the Languedoc-Roussillon Region.

JF048177Q