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Polyphenols-Enriched Chardonnay White Wine and Sparkling Pinot Noir Red Wine Identically Prevent Early Atherosclerosis in Hamsters

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The effects of a white wine enriched with polyphenols (PEWW) from Chardonnay grapes and of a sparkling red wine (SRW) from Pinot Noir and Chardonnay grapes were studied for the first time on early atherosclerosis in hamsters. Animals were fed an atherogenic diet for 12 weeks. They received by force-feeding PEWW, SRW, ethanol 12% (ETH), or water as control (mimicking a moderate consumption of ∼2 red wine glasses per meal for a 70 kg human). Plasma cholesterol concentrations were lower in groups that consumed PEWW and SRW accompanied by an increase in the ratio apo A-1/apo B. Liver-specific activities of superoxide dismutase and catalase were significantly increased by PEWW (38 and 16%, respectively) and by SRW (48 and 15%, respectively). PEWW and ETH significantly increased plasma antioxidant capacity and vitamin A concentrations. Aortic fatty streak area (AFSA) was significantly strongly reduced in the groups receiving PEWW (85%) and SRW (89%) in comparison with the control. AFSA was reduced by ethanol to a lesser extent (58%). These data suggest that tannins from the phenolics-enriched white wine induce a protective effect against early atherosclerosis comparable to that produced by sparkling red wine containing tanins and anthocyanins and dissociated from the antioxidant action of these compounds.

KEYWORDS: Atherosclerosis; phenolics-enriched white wine; sparkling red wine; phenolic compounds; tannins; ethanol; hamsters

INTRODUCTION

Over the past 15 years various epidemiological studies have strongly demonstrated that moderate consumption of alcoholic beverages is associated with reduced mortality and risk of cardiovascular disease (CVD) (*1*−*3*). It has also been reported that the consumption of red wine, which contains phenolic compounds that have a number of antioxidant properties (*4*), imparts greater benefits in the reduced risk of CVD in wine drinkers. The greatest degree of cardioprotection is related to the ingestion of red wine rather than white wine, beer, or spirits (*5*−*9*). It was proposed by Renaud and de Lorgeril (*10*) that the "French paradox," that is, a low mortality rate in France from CVD despite a high consumption of saturated fat and smoking habits similar to those of other countries, can be explained by the population's moderate and regular consumption of red wine. The unique atherogenic effect of red wine resides in the action of polyphenols (*11*). Ethanol alone does not account for the healthful benefits of wine. Nevertheless, recent authoritative reviews do not support this (*12*, *13*), and results from observational studies, where individual consumption can be assessed in detail and linked directly to coronary heart disease, provide strong evidence that a substantial proportion of the benefits of wine, beer, or spirits is attributable primarily to the alcohol content rather than to other components of each drink. Wine is rich in nonalcoholic ingredients such as flavonoids and other polyphenolic antioxidants that could prevent atherosclerosis by inhibiting the oxidation of low-density lipoprotein (LDL) (*14*) and can reduce the risk of heart disease. Nonalcoholic red wines have been shown to inhibit LDL oxidation by

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46−100%, whereas white varieties achieved only 3−6% inhibition (*15*−*17*). The antioxidant activity of wines positively correlated with their total phenol content, as well as with the concentration of individual phenolics.

A potentially important clinical corollary of the atherosclerosis oxidation theory is that inhibition of LDL oxidation may also inhibit atherosclerosis independent of lowering plasma cholesterol concentrations. Red wine has been seen to increase antioxidant capacity in some animal and in vivo/ex vivo studies. It was found that moderate consumption of red wine protects rats from oxidation in vivo (*18*). Red wine has been shown to increase serum antioxidant capacity in humans (*19*, *20*) and to reduce the susceptibility of human plasma lipid peroxidation (*21*, *22*). There is evidence that oxidatively modified LDL plays a crucial role in atherogenesis (*23*, *24*). Lipid peroxidation is indeed an initial step in the atherosclerosis pathology, and evidence is increasing that oxidative modification of LDL is involved (*23*). In humans, fatty streak lesions can usually be found in the aorta, coronary, and cerebral arteries (*25*). It has been shown that Golden Syrian hamsters fed a fat-rich diet develop dyslipidemia and atherosclerotic plaques, similar in many respects to human atheroma (*26*, *27*). Hamsters were selected for this study because decreased plasma cholesterol and aortic fatty streak area (AFSA) have been reported in hamsters fed hypercholesterolemic diets following consumption of phenolics (*28*−*30*), a red wine phenolic extract (*31*), or red wine or dealcoholized red wine (*32*).

White wines exhibit low polyphenol content and a low antioxidant potential in comparison to red wine (*33*). On the other hand, we previously produced a white wine called "Paradoxe blanc" enriched with polyphenols and in particular tanins (*34*) that exhibited beneficial effects on diabetes (*35*). This is the main reason for determining in the present study whether this polyphenols-enriched Chardonnay white wine (PEWW) administered to hamsters also affected the development of atherosclerosis. A sparkling Pinot Noir−Chardonnay red wine (SRW) was also tested; this wine contains red phenolic polymers (anthocyanins) and a lower level of tanins in comparison with PEWW. Comparison was made with 12% ethanol. To do so, an aortic wall response to a high-cholesterol/low-antioxidant diet was triggered in Syrian hamsters to induce fatty streak formation and atherosclerosis emergence; we then evaluated the possible preventive effect of the administration of the wines.

MATERIALS AND METHODS

Wines. PEWW from the Chardonnay variety (alcohol level of 13.5% vol) was produced as previously described (*34*) by Virginie-Castel Winery at Béziers (France); this is a commercial wine called "Paradoxe Blanc vintage 1999". Briefly, viticultural areas of southern France were chosen for their phenolic potential. Grapes were fermented and treated following a process close to red wine production with temperature variation control and maceration. Wine chemical analyses have already been reported (*36*). SRW was obtained from Pinot and Chardonnay grapes by Chandon do Brazil. This wine contains an alcohol level of 11.4% vol and was produced according to the closed tank method; this is a commercial wine called "Chandon, vintage 2002".

Standards and HPLC Analysis. (+)-Catechin, (−)-epicatechin, protocatechuic acid, caffeic acid, gallic acid, *p*-coumaric acid, and ellagic acid were obtained from Aldrich (St. Quentin Fallavier, France). Cyanidin-3-glucoside, peonidin-3-glucoside, delphinidin, peonidin, and myristin were obtained from Extra Synthèse (St. Quentin Fallavier, France). Caftaric acid was provided by Dr. Ursa Vorshek. Procyanidin dimers B1, B2, B3, and B4 were obtained from grape seeds as previously reported (*37*). HPLC analysis with UV detection was performed using a Hewlett-Packard model 1090 with three low-pressure pumps, and a diode array detector coupled to a Hewlett-Packard Chem Station was used for solvent delivery and detection. A Hewlett-Packard column packed with Nucleosil 100 C18 (250 × 4 mm, 5 μm particle size) was used for the stationary phase with a flow of 0.7 mL/min. The solvents used for separation (*38*) were as follows: solvent A, 50 mmol/L dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B, 20% A with 80% acetonitrile; solvent C, 200 mmol/L orthophosphoric acid adjusted with ammonia to pH 1.5. Elution was performed with a gradient previously described (*37*). Detection was carried out at 280, 313, 365, and 520 nm. Wine phenolics levels are shown in **Table 1**.

Table 1. Phenolics Levels in Wines

	mg/L	
phenolic	PEWW[a]	SRW[b]
total phenols content[c]	1425	570
total catechins[d]	371.90	53.82
red polymers[c]	NF	272.10
catechin	98.00	7.31
dimer B1	27.20	17.95
dimer B2	58.20	3.49
dimer B3	59,20	NF
dimer B4	29.30	NF
protocatechuic acid	NF	NF
caftaric acid	83.40	3.69
cafeic acid	3.20	7.89
gallic acid	25.30	10.02
delphinidin	NF	1.27
cyanidin-3-glucoside	NF	0.21
peonidin-3-glucoside	NF	1.97
peonidin	NF	1.25
myristin	NF	1.23
p-coumaric acid	NF	3.09
ellagic acid	NF	1.69

[a] Phenolic-enriched white wine. [b] Sparkling red wine. [c] Expressed as gallic acid equivalent. [d] Procyanidin dimers B1, B2, B3, B4 + catechin + epicatechin.

Table 2. Composition of the Diet (Grams per Kilogram)

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

[a] Mineral mixture contained the following (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 the following (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).

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.

Diets and Feeding Procedures. Hamsters were fed for 12 weeks a semipurified atherogenic diet (**Table 2**) 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, and vitamin E were 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 daily either tap water (control), 12% ethanol (group ETH), or phenolics-enriched white wine (group PEWW) or sparkling red wine (SRW). The volume of solutions force-fed was

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 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).

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 weight). 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), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were determined according to commercially available enzymatic methods (respectively, no. 401, 352-4, and 343, Sigma Chemicals, Saint Quentin Fallavier, France). Plasma very low-density and low-density lipoprotein cholesterol (nonHDL-C) was precipitated with phosphotungstate reagent (*39*), 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 356 and 357, respectively, as previously described (*40*). 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 (*41*). Standards solutions were obtained from iron and copper standard solutions (Sigma, Steinheim, Germany) by dilution with deionized water twice distilled.

Plasma malondialdehyde (MDA) was measured using a fluorometric assay (*42*) initially performed by Yaggi et al. (*43*). After isolation of lipids by precipitating them with serum protein using phosphotungstic acid, the pellet obtained is incubated at 95 °C in the presence of thiobarbituric acid (TBA). By this procedure, TBA-reacting substances in blood serum are easily removed, and the reaction products are measured by fluorometric assay (excitation at 515 nm and emission at 535 nm).

Measurement of plasma advanced oxidation protein products (AOPP) level was performed using technical recommendations described by Witko-Sarsat et al. (*44*) and modified by Wratten et al. (*45*). 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 (Sigma, St. Louis, MO) was added to 1 mL of chloramine-T solution (0, 10, 30, 50, 70, or 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) (*42*) using the material from Waters chromatography (Millipore Waters, Les Ulis, France). 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 advanced glycation end products (AGEs) concentration was determined by fluorometric assay derived from Munch et al. (*46*). 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 fluorescence of 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 (*47*, *48*), 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′-azinobis-(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, and β-carotene) 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. 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. SOD was assayed according to the method of Paoletti and Mocali (*49*); 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 (*50*) 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 (*51*), 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. (*52*) 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, 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 corked, 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, rinsed in distilled water, and then quickly rinsed in 70% isopropyl alcohol; finally, they were stained in Oil Red O for 30 min according to the method of Nunnari et al. (*53*) and rinsed in 70% isopropyl alcohol and then in 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.

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 analysis of the data was carried out using the 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 AND DISCUSSION

Phenolics levels in wines (PEWW and SRW) are given in **Table 1**. The level of total phenols shows that PEWW is 2.5 richer in total phenols and 6.9 times richer in catechins (sum of procyanidin dimers B1, B2, B3, and B4 and monomeric catechins) in comparison with SRW. Red polymers (anthocyanins) are found in only SRW and represent 47.8% of SRW total

Table 3. Effects of Force-Feeding Water (Control), 12% Ethanol, Polyphenols-Enriched Chardonnay White Wine (PEWW), or Sparkling Pinot Noir Red Wine (SRW) on Body Weight, Food Intake, and Plasma Lipid and Apolipoprotein Concentrations of Hamsters Fed an Atherogenic Diet for 12 Weeks[a]

	control	ethanol 12%	PEWW	SRW
initial body weight, g	70.8 ± 1.7a	71.3 ± 1.10ab	67.3 ± 1.7b	67.9 ± 1.6c
final body weight, g	107.9 ± 4.0b	115.6 ± 2.4ab	116.8 ± 2.3a	114.0 ± 1.9ab
food intake, g/day	5.47 ± 1.16a	5.46 ± 1.32a	5.29 ± 0.36a	5.20 ± 0.97a
TC,[b] mmol/L	10.39 ± 0.65a	9.65 ± 0.54ab	8.96 ± 0.20b	8.90 ± 0.28b
HDL-C,[c] mmol/L	3.74 ± 0.37	3.71 ± 0.15	3.61 ± 0.14	3.50 ± 0.17
TG,[d] mmol/L	1.37 ± 0.17	1.15 ± 0.10	1.19 ± 0.12	1.26 ± 0.07
apo A-1, g/L	1.70 ± 0.21a	2.20 ± 0.12b	1.98 ± 0.09ab	1.94 ± 0.15ab
apo B, g/L	0.36 ± 0.03	0.29 ± 0.02	0.32 ± 0.01	0.30 ± 0.02
apo A-1/apo B	4.64 ± 0.58a	7.38 ± 0.66b	6.07 ± 0.24b	6.44 ± 0.77b

[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.

Table 4. Effects of Daily Force-Feeding Water (Control), 12% Ethanol (ETH), Polyphenols-Enriched Chardonnay White Wine (PEWW), or Sparkling Pinot Noir Red Wine (SRW) on Plasma AGEs,[a] AOPP,[b] MDA,[c] Vitamins A and E, PAC,[d] and Iron and Copper Concentrations in Hamsters Fed an Atherogenic Diet for 12 Weeks[e]

	control	ETH	PEWW	SRW
AGEs, arbitrary units	7282 ± 301	6638 ± 279	6695 ± 220	7189 ± 220
AOPP, μmol/L	74.16 ± 9.36	46.95 ± 10.10	55.14 ± 10.09	77.54 ± 21.02
MDA, mmol/L	4.51 ± 0.40a	6.81 ± 0.44b	5.44 ± 0.29a	2.73 ± 0.45c
PAC, mmol/L	0.976 ± 0.034a	1.081 ± 0.050b	1.126 ± 0.024b	0.932 ± 0.103a
vitamin A, mmol/L	1.25 ± 0.11a	1.61 ± 0.05b	1.67 ± 0.09b	1.45 ± 0.04ab
vitamin E, mmol/L	4.03 ± 0.40	4.35 ± 0.26	3.75 ± 0.37	3.89 ± 0.24
iron, mg/L	1509 ± 118	1595 ± 55	1843 ± 900	1306 ± 132
copper, mg/L	661 ± 136	647 ± 60	696 ± 81	662 ± 130

[a] Advanced glycation end products. [b] Advanced oxidation protein products. [c] Malondialdehyde. [d] Plasma antioxidant capacity. [e] 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, $P < 0.05$.

phenols. Administration of PEWW significantly increased the final body weight of animals in comparison with the control group (**Table 3**), although no groups differed in food consumption. After 12 weeks, plasma lipids (**Table 3**) indicated a decreased TC level in animals receiving SRW or PEWW in comparison with control and ETH groups. A nonsignificant decrease in TC level appeared in the ETH group in comparison with controls. Triglycerides and HDL-C are found at equal levels for the different groups. Ethanol induced a greater increase in apo A-1 concentration (29.4%) than wines (15.3% on average) in comparison with controls, although the wine effect was nonsignificant. Apo B plasma levels decreased in PEWW, SRW, and ethanol groups in comparison with the control. Therefore, the ratio apo A-1/apo B is significantly higher for animals receiving ethanol, PEWW, and SRW in comparison with controls. Giving PEWW and SRW to hamsters induced decreased cholesterol levels, favoring a preventive effect against atherosclerosis because hypercholesterolemia is known as a major risk of cardiovascular diseases. Plasma TC decreases without modification of HDL-C concentrations can be related to an increase in the part of HDL induced by PEWW and SRW. This is in agreement with data obtained in humans after ingestion of small volumes of ethanol (*54*) or wine (*55*). The tendency of PEWW and SRW to decrease apo B and to increase apo A-1 levels suggested a possible modification of plasma lipoproteins metabolism through a hepatic elimination of cholesterol, resulting in an increased apo A-1/apo B ratio. These effects are close to those obtained with red wine, that is, a LDL decrease in rat (*56*) and an apo A-1 increase in humans (*55*).

Plasma antioxidant capacity (PAC) is significantly increased in PEWW and ETH groups in comparison with control and SRW (**Table 4**); this suggests a protective effect of the phenolics-enriched white wine comparable to the reported red wine effect in humans (*20*), although increased PAC in the ethanol-treated group is unclear and could be attributed to some high values coming from three animals in the ETH group. Nevertheless, it has been reported by Waddington et al. (*57*) that in apo E-deficient mice, red wine polyphenols had no effect on markers of lipid peroxidation. Because it has been shown that the phenolic fraction plays a role in the increase of PAC (*23*), the phenolic enrichment of the white wine (especially in tannins) is probably responsible for this comparative antioxidant effect obtained with red wines. In the case of SRW, plasma antioxidant capacity was not increased in comparison with control. Plasma vitamin A concentration was significantly increased by PEWW and ethanol treatments (**Table 4**) but not by SRW (nonsignificant increase). Any treatment was able to modify significantly plasma vitamin E levels (**Table 4**). No difference was observed in plasma iron and copper. Plasma malondialdehyde level (lipid peroxidation marker) was found to be significantly increased in the ETH group, but in SRW an important significant decrease in this marker was observed in comparison with control and PEWW groups (**Table 4**). SRW is able to reduce lipid peroxidation. An explanation could be in a protective effect of plasma and membrane poly-unsaturated fatty acids by red wine phenolics, as yet reported (*58*). Cellular peroxidases, responsible of lipoproteins oxidation (*59*), could be less inhibited by white wines, and this can explain the slight effect of PEWW on MDA level. In addition, 12% ethanol and PEWW reduced plasma AOPP and AGEs concentrations, which are two markers for protein peroxidation. Liver enzyme activities involved in body antioxidant system are given in **Table 5**. GSHPx activity did not change in the experimental groups; however, selenium deficiency in the experimental diet probably induced a low activity of this enzyme. PEWW and SRW significantly increased liver SOD and CAT activities in com-

Table 5. Effects of Force-Feeding Water (Control), 12% Ethanol (ETH), Polyphenols-Enriched Chardonnay White Wine (PEWW), or Sparkling Pinot Noir Red Wine (SRW) on Liver Superoxide Dismutase (SOD), Glutathione Peroxidase (GSHPx), and Catalase (CAT) in Hamsters Fed an Atherogenic Diet for 12 Weeks[a]

	units mg^{-1} of protein			
	control	ETH	PEWW	SRW
SOD	7.28 ± 1.15a	6.74 ± 0.97a	10.07 ± 1.51b	10.78 ± 1.82b
GSHPx ($\times 10^{-2}$)	11.47 ± 1.93	9.77 ± 1.77	10.93 ± 2.37	11.32 ± 2.95
CAT	108.27 ± 4.94a	100.32 ± 6.62a	125.20 ± 8.49b	125.04 ± 6.59b

[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, $P < 0.05$.

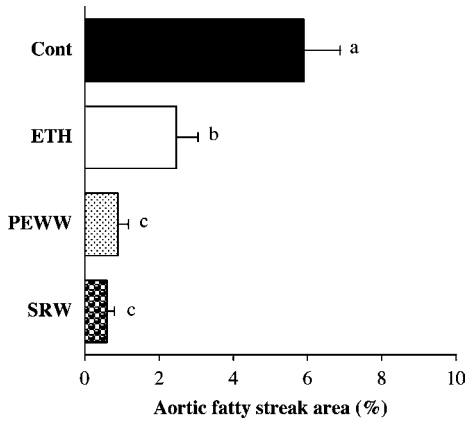

Figure 1. Effects of daily force-feeding water (control), 12% ethanol (ETH), phenolics-enriched white wine (PEWW), or sparkling red wine (SRW) on aortic fatty streak area (AFSA) in hamsters fed an atherogenic diet for 12 weeks. Values are means ± SEM, $n = 8$. Bars with different index letters differ, $P < 0.05$.

parison with control and ethanol groups. As SOD produces hydrogen peroxide from superoxide anion, the increased SOD specific activity could partly explain the increase in CAT activity, allowing hydrogen peroxide degradation.

Our most interesting finding is summarized in **Figure 1**, showing a significant reduction of hamster aortic fatty streak development by PEWW (85%), SRW (89%), and ethanol, to a lesser extent (58%) in comparison with controls. Phenolics enrichment of a white wine give a preventive effect on atherosclerosis comparable to that supplied by a sparkling red wine. For comparison, in our previous works on hypercholesterolemic hamsters, a red wine phenolic extract dissolved in ethanol reduced AFSA by only 62% (*31*), and pure phenolic molecules given at nutritional doses (*30*) induced significant AFSA reductions, that is, (+)-catechin (84%), quercetin (80%), and *trans*-resveratrol (76%). Our results show that white wine obtained following a process close to that of red wine production contained a remarkable concentration of polyphenols. On the contrary, the original white wine exhibited a low polyphenol content (*34*). Elsewhere, **Figure 1** demonstrates that PEWW, despite its 3-fold higher concentration of polyphenols, reduces AFSA similarly, or even higher than the SRW. The phenolics concentration of PEWW accounts for this beneficial effect because some authors (*32*, *33*, *60*) have previously demonstrated that white wine polyphenols are more potent antioxidants than red wine polyphenols when compared on similar concentration bases. Elsewhere, recent works by Stocker and O'Hallaran **(***61***)** and Waddington et al. (*57*), respectively, demonstrated that dealcoholized red wine and red wine polyphenols decrease 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 only a direct antioxidant effect (*28*). As suggested by others (*57*), it is possible that alternative actions for wine polyphenols took place, such as the inhibition of smooth muscle cell proliferation (*62*) or the regulation of adhesion molecules (*63*−*65*), which may influence atherosclerotic development. Also, 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 (*29*) and shown (*66*, *67*) for antioxidant and antiplatelet effects, or between phenolic compounds and vitamine E (*61*). These authors suggested that phenolic acids accounted for most of the radical-reducing activity of red wine; in this way, phenolic acids and total catechins content of PEWW, which were 5- and 7-fold higher, respectively, than those of SRW could be responsible for the beneficial effects of PEWW.

In conclusion, white wine with red wine properties not only was efficient in inducing plasma antioxidant capacity similar to that of a red wine (*33*) but also had beneficial effects on diabetes, a pathology associated with atherosclerosis as risk factor on cardiovascular diseases (*35*). Moreover, whatever are the involved mechanisms, we have shown here that tannins from the phenolics-enriched white wine induce a protective effect comparable to that obtained with sparkling red wine containing tannins and anthocyanins. Because wine phenolic compounds such as catechin, quercetin, and resveratrol given at nutritional doses mimicking a moderate consumption of two glasses of red wine per meal prevent the development of atherosclerosis through several indirect mechanisms independent of the inhibition of lipid peroxidation (*30*), it will be essential in the future to study the effects of the different individual tannin structures alone (monomers, oligomers, polymers) or in mixture and to explore their mechanisms of action involved in atherosclerosis prevention.

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