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Effect of a Polyphenols-Enriched Chardonnay White Wine in Diabetic Rats

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A Chardonnay white wine enriched in polyphenols was obtained by modification of winemaking and characterized by its enrichment in total polyphenolic content (1346 mg/L as compared to 316 mg/L for traditional Chardonnay) and in various individual polyphenols (catechin, epicatechin, procyanidins dimers B1−B4, gallic acid, cafeic acid, and caftaric acid), as determined from HPLC coupled to a diode array detector. The polyphenols-enriched white wine (W) or its ethanol-free derivative (EFW) was then administered by gavage (10 mL/kg, twice a day) for 6 weeks to rats that have been rendered diabetic by a single iv injection of streptozotocin (55 mg/kg). Treatments had no effect on the symptoms associated with hyperglycemia. However, while a reduction in plasma antioxidant capacity was associated with the diabetic state, administration of W or EFW restored plasma antioxidant capacities to a level not significantly different from that of nondiabetic control animals. In addition, the effect of both treatments was manifested by the enlargement of mesenteric arteries, as determined by quantitative histomorphometry. In summary, our study indicates that white wine, when enriched in polyphenols, is able to induce ethanol-independent in vivo effects in a model of insulin-deficient diabetes characterized by a major oxidative stress.

KEYWORDS: Diabetes; antioxidant capacity; white wine; catechins; tanins; levels; phenolics; oxidative stress

INTRODUCTION

Considerable evidence accumulates now indicating that moderate prolonged red wine consumption is beneficial for health (*1*−*3*). This could be explained by significant amounts of natural antioxidant phenolics intake present in red wine (*4*, *5*). Indeed, epidemiological studies show that intake of these compounds is correlated with reduced incidence of coronary heart disease (CHD) (*6*).

Type 1 diabetes or insulin-dependent diabetes mellitus (IDDM, 10% of diabetic patients) generally occurs early in life (teen-agers), originates from an immunological destruction of the insulin-secreting cells of the islets of Langerhans, and requires a lifetime administration of insulin. Specific long-term complications of hyperglycemia include retina, kidney, and peripheral nervous system alterations which are the consequences of structural and hemodynamic changes occurring at the level of small blood vessels (microangiopathy) (*7*−*9*).

The streptozotocin (STZ-) -induced diabetic rat represents a model of insulin-deficient diabetes (type 1 diabetes) provoked by the specific destruction of insulin secreting β-cells. When STZ is injected (iv) at a dose of 60 mg/kg, 90% of insulin-secreting β cells are destroyed, but the remaining cells allow for the long-term survival of the animals with major hyperglycemia and development of diabetic complications. Insulin resistance in this model is secondary to hyperglycemia and prevented by the administration of insulin. A major oxidative stress is associated with this model and constitutes a pathophysiological factor of both insulin resistance and diabetic complications including cardiovascular disease (*10*−*13*).

Elevated indices of free radical mediated damage to lipids, proteins, and DNA in plasma and urine are indicative of enhanced oxidative stress and are related to a number of pathophysiological conditions including aging, obesity, atherosclerosis, and diabetes (*14*). In healthy subjects, hyperglycemia, elevated fatty acids, and hyperinsulinemia all induce a rise in such indices of oxidative damage in plasma. Once elevated oxidative stress occurs, accelerated disease pathogenesis may result. Antioxidants, which include many phenolic and polyphenolic compounds, scavenge free radicals, and the incorporation of high antioxidant produce into the diet may therefore be

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useful in preventing or delaying the development of atherosclerosis, diabetes, or obesity (*15*).

Oxidative stress is believed to be a pathogenetic factor in the development of diabetic complications.

Antioxidants are currently being tested for their potential to prevent or correct diabetes-related diseases. Some results suggest that diabetic pregnant rats and their neonates exposed to oxidative stress but with a vitamin E supplementation could in part reduce the imbalance between uncontrolled reactive oxygen species generation and scavenging enzyme activity and may potentially serve as a useful prophylactic factor against oxidative stress development (*16*). Recently, the effect of nitecapone, an inhibitor of the dopamine-metabolizing enzyme catechol-*O*-methyl transferase (COMT) and a potent antioxidant, on functional and cellular determinants of renal function in rats with streptozotocin-induced diabetes was found to provide a protective therapy against the development of diabetic nephropathy. Nitecapone-treated diabetic rats were protected from intracellular modifications and oxidative stress (*17*). The effects of an ethanolic extract of the *Andrographis paniculata* aerial parts were also investigated for antihyperglycemic and antioxidant effects in normal and streptozotocin-induced type I diabetic rats. The results show that oxidative stress is evident in streptozotocin-diabetic rats and indicate that the ethanolic extract of *A. paniculata* not only possesses an antihyperglycaemic property but may also reduce oxidative stress in diabetic rats (*18*).

In the Zutphen elderly study (*19*), after adjustment for age, education, and smoking status, men with cardiovascular diseases (CVD)/diabetes and low-to-moderate alcohol intake had a significantly lower risk for poor cognitive functions (MMSE (Mini-Mental State Examination): <25) than abstainers (odds ratios of 0.3 for less than 1 drink and 0.2 for 1−2 drinks/day). Alcohol intake was not associated with cognitive decline. This study concluded that alcohol may result in an acute beneficial effect on cognitive function among those with CVD/ diabetes.

More recently, in a prospective study with 8663 men (aged 30−79 years old), fasting plasma glucose measurements and alcohol intake were classified into five groups (nondrinkers and four quartiles) according to the alcohol intake levels (*20*). Type 2 diabetes was diagnosed according to the 1997 American Diabetes Association criteria (fasting glycemia >1.26 g/L). There were 149 incident cases of type 2 diabetes during the 6 years of follow-up. A U-shaped association between alcohol intake and diabetes was observed with the lowest incidence of diabetes at 61.9−122.7 g/week (corresponding to the moderate consumption of 1−2 glasses/day) being obtained.

Nondrinkers and heavy drinkers had higher risk of developing diabetes (2.3- and 2-fold respectively) when compared with moderate drinkers (1 or 2 glasses of alcoholic beverages/day). These associations persisted after adjustment for age, fasting plasma glucose, smoking, BMI, blood pressure, serum triglyceride concentration, cardiorespiratory fitness, HDL-cholesterol, waist circumference, and parental diabetes. It was calculated that if nondrinkers become moderate drinkers, there would be a 10% reduction in diabetes in their population, and if heavy drinkers become moderate drinkers, a 24% incident reduction in diabetes would occur. The authors concluded that a light or moderate consumption of alcoholic beverages is associated with an improvement of insulin sensitivity. However, this study provided no insights into whether the beneficial effects of consuming moderate amounts of alcoholic beverages are due to alcohol and/or the presence of phenolic compounds which are present in especially high concentrations in red wine.

Furthermore, red wine and its antioxidant phenolic components increase serum antioxidant capacity in vivo (*21*, *22*), inhibit low-density lipoprotein (LDL) oxidation in vitro (*6*, *23*, *24*, *25*) and ex vivo (*22*, *26*), inhibit platelet aggregation in vitro (*6*), and demonstrate scavenging capacity against free radicals in vitro (*6*). Taken together, these observations indicate that red wine phenolic compounds provide protection against oxidation although the mechanism of this effect is not totally understood (*7*). Oppositely, it is interesting to note that white wine does not seem to have any of these protective effects (*22*, *27*, *28*). We therefore developed, for the first time, a white wine (Chardonnay) enriched in phenolics through a specific wine-making process (*29*). The intrinsic antioxidant capacities and phenolic levels of the resulting wine were assessed and compared to different varieties and vintages of red and white wines. Total phenolic compounds content and antioxidant capacity were significantly increased in our enriched white wine as compared with regular white wines of reference, getting therefore closer to red wines values (*30*).

In this study, we investigated the in vivo effect of this polyphenol-enriched white wine on plasma antioxidant capacity of streptozotocin (STZ-) -treated diabetic rats, an experimental model of diabetes characterized by a major oxidative injury (*10*). General physical and biochemical parameters were also determined. Because polyphenols have been described as vasoactive agents (*37*, *50*), we also studied the influence of polyphenol-enriched white wine (chardonnay) on the morphological changes of mesenteric arteries previously described in the same model of diabetes. For comparison purpose, we also determined the effect of the same polyphenol-enriched white wine from which ethanol had been removed.

MATERIALS AND METHODS

Phenolics-Enriched White Wine Production. Phenolics-enriched white wine (W) from the Chardonnay variety (alcohol: 13.5% vol) was produced as previously described (*29*). Briefly, viticultural areas of south of France were chosen for their phenolic potential. Grapes were fermented and treated by following a process close to red wine production with temperature variation control and maceration. Wine chemical analyses have already been reported (*30*). An alcohol-free enriched phenolic white wine (EFW) was obtained by removing alcohol (pervaporation under vacuum) from enriched phenolic white wine.

Phenolics Standard and Solvents. (+)-catechin, (−)-epicatechin, gallic acid, and cafeic acid were obtained from Aldrich (L'isle d'Abeau, St. Quentin Fallavier, France), and malvidine-3-glucoside, peonidine-3-glucoside, and cyanidine-3-glucoside, from Extrasynthése (ZI Lyon Nord, Genay, France). Caftaric acid was provided by Dr. Ursa Vorshek. Procyanidins dimers B1−B4 were obtained from grape seeds as described by Teissedre et al. (*31*). Methanol and acetonitrile were both HPLC grade and were obtained from Carlo Erba (Val de Reuil, France) and Merck (Nogent sur Marne, France), respectively. Orthophosphoric acid (85%) and ammonia were purchased from Panreac (Barcelona, Spain), and dihydrogen ammonium phosphate was obtained from Acros (Noisy le Grand, France).

Wine Phenolics HPLC Analysis. A Hewlett-Packard model 1090 with three low-pressure pumps and a diode array UV detector coupled to an Hewlett-Packard Chemstation was used for solvent delivery system and detection. A Hewlett-Packard column Nucleosil 100 C18, 250 × 4 mm and 5 μm particle size, was used for the stationary phase with a flow of 0.7 mL/ min. The solvents used for separation were as follows: solvent A, 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B, 20% A with 80% acetonitrile; solvent C, 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5 and solvent gradient conditions as described by Lamuela-Raventos (*32*). Temperature was thermostated at 25 °C.

Treatment of STZ Diabetic Rats with Phenolics-Enriched and Alcohol-Free Phenolics-Enriched Chardonnay White Wines. Male

Table 1. Composition of Diet "A 04"

diet ingredients	std diet	diet ingredients	std diet
Constituents			
protein[a] (g/kg)	165	mineral ashes mix[b] (g/kg)	62
fat material (g/kg)	30	cellulose (g/kg)	40
Added Compounds			
vitamin A (UI)	6500	vitamin E (mg/kg)	30
vitamin D3 (UI)	800	copper (mg/kg)	25

[a] Protein mixture: wheat, corn, soya, fish protein. [b] Mineral mixture contained principally Ca_2PO_4 and $CaCO_3$.

Wistar rats (160−180 g) were obtained from Iffa-Credo (Arbresle, France) and maintained on a 12 h light/dark cycle (6:00 am−6:00 pm). Rats were fed the A04 standard chow commercialized by UAR (Villemoisson-sur-Orge, France) with a composition described in detail in **Table 1**. Animals were randomly assigned to 4 groups of 8 animals. One group of rats was used as untreated nondiabetic control (C), while the other animals were injected intravenously with streptozotocin (55 mg/kg). Streptozotocin was obtained from Sigma (L'isle d'Abeau, St Quentin Fallavier, France). The diabetic state (glycemia ≥ 16 mM) was checked 3 days later by measuring blood glucose from a cut to the tail using a Glucometer apparatus. Diabetic animals (≥95% of injected animals) were randomized according to glycemia and weight to the following groups: untreated diabetic (D); diabetic treated with phenolics-enriched white wine (D + W); diabetic treated with ethanol-free phenolics-enriched white wine (D + EFW). Treatments (10 mL/kg, twice daily) using W or EFW were administered for 6 weeks. Untreated animals received water only. Weight and water intake were recorded three times a week while food intake was recorded daily. At the end of the experiment, animals were killed by decapitation and blood was collected on a heparinized tube. Plasma was stored at −80 °C until further use.

Plasma (+)-Catechin Analysis. Plasma (+)-catechin was searched for according to a procedure already described (*33*). Plasma samples were collected at the end of treatment (1 mL for each animal) and pooled by animal groups to obtain 4 aliquotes of 8 mL of plasma placed in an ice bath. Acetonitrile (10 mL) was added to effect protein precipitation. The mixture was then centrifuged at 2000*g* and +4 °C for 4 min. A solvent evaporation was realized under vacuum with a rotovapor to obtain a dry residue to determine catechin concentration. A 300 μL volume of methanol was then added to the dry residue, and the solution was shaken for dissolution and filtered through a 0.5 μm pore size membrane (Millex-FH13 Millipore, St. Quentin Yvelines, France). A 25 μL volume of the filtered solution was directly injected into the HPLC system. A stock solution of (+)-catechin was prepared by dissolving 20 mg of the compound in 10 mL of methanol. It was stored at +4 °C and diluted with methanol before use. The stock solution was stable for at least 1 month at + 4 °C. Calibration samples were prepared before each assay by mixing the appropriate volume of (+)-catechin in 0.5 mL of water. Theses samples were analyzed according to the procedure described for sample preparation. Calibration curves were constructed by linear regression of the peak-area ratio vs concentration. A Hewlett-Packard model 1090 HPLC system with three low-pressure pumps coupled to an Hewlett-Packard Chem station was used for solvent delivery. A Shimadzu RF 530 fluorescence detector coupled with a Sefram recorder was also used for detection. The HPLC conditions (column, temperature, flow, solvent composition, and gradient) were the same as for the wine phenolics analysis (*32*) except for detection: fluorescence detection was monitored at an excitation wavelength of 280 nm and an emission wavelength of 310 nm.

Measurement of Plasma Antioxidant Capacity. At the end of treatment, blood samples were collected on heparinized tubes, centrifuged for 20 min (2000*g*), and plasma stored at −80°C until processed.

Plasma antioxidant capacity was determined by the total antioxidant status method of Randox. A Kit Randox catalog No. NX2332 (Randox Laboratories Ltd., Crumlin, U.K.) was used. This assay is based on 2,2′-azinobis(3-ethylbenzothiazoline sulfonate) (ATBS) incubated with a peroxidase (metmyoglobin) and hydrogen peroxide (H_2O_2) to produce the radical cation $ATBS^{\cdot+}$. This has a relatively stable blue-green color, which is measured at 600 nm.

Antioxidants in the added sample cause suppression of this color production to a degree proportional to their concentration. This analytical procedure has been applied to physiological antioxidant compounds and radical-scavenging drugs, and an antioxidant ranking based on their reactivity relative to a 1.0 mmol/L Trolox standard has been established. The Trolox equivalent antioxidant capacity of plasma from an adult reference population has been measured, and the method, optimized and validated (*34*). We used this automated method to investigate the total plasma antioxidant capacity of plasma rats and total plasma antioxidant capacity of wines (W and EFW).

Biochemical Parameters. Assessment of glycemia, total cholesterol, triglycerides, HDL (high-density lipoprotein), ALP (alkaline phosphatase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), and urea were performed on final blood sampling with an Hitachi 704 apparatus.

Triglycerides analyses were realized with a Kit No. 1488872 Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. This enzymatic triglycerides assay required a liporotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Triender end point reaction).

Glycemia. Analyses were realized with a Kit No. 1448668 Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. The procedure involves the sampling and addition of R1 (buffer/enzymes/4-aminophenazone/phenol) and start of a reaction where glucose is oxidized by glucose oxidase (GOD) to gluconolactone in the presence of atmospheric oxygen; H_2O_2 is also created. This resultant hydrogen peroxide oxidizes 4-(*p*-benzoquinonemonoimino)phenazone in the presence of peroxidase (POD). The color intensity of the red dye is directly proportional to the glucose concentration and can be measured photometrically.

Total Cholesterol. Analyses were realized with a Kit No. 1489232 Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. Sampling and addition of R1 (cholesterol reagent) are conducted to start the reactions. Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. Hydrogen peroxide created forms a red dyestuff 4-(*p*-benzoquinonemonoimino)phenazone by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

HDL. Analyses were realized with a Kit No. 1930672 Roche, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. The principle is based on a homogeneous enzymatic colorimetric test. Sample and addition of R1 (sulfated α-cyclodextrin/buffer) are realized. In the presence of slightly alkaline buffer and magnesium sulfate, sulfated α-cyclodextrin and dextran sulfate selectively form water-soluble complexes with LDL, VLDL, and chylomicrons which are resistant to PEG-modified enzymes (PEG = poly(ethylene glycol)). Addition of R2 (PEG-modified enzymes/4-aminophenazone/buffer) is followed by the start of reaction, where the cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminophenazone and HSDA (HDSA = *N*-2-hydroxy-3-sulfopropyl-3,5-dimethoxyaniline) to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Urea. Analyses were realized with a Kit No. 1489364 Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. The principle

Table 2. Levels of Phenolics (mg/L) and Antioxidant Capacity Comparison for Chardonnay Wines (Traditional or Phenolic Enriched) and a Traditional Red Wine[a]

wines	AC in mmol/L	CPT in mg/L	catechin	epicatechin	B1	B2	B3	B4	gallic acid	cafeic acid	caftaric acid	malvidine-3-glucoside	peonidine-3-glucoside	cyanidine-3-glucoside	catechins sum[b]
white traditional Chardonnay	2.31	379	7.5	7	0.8	4.7	7.6	1.1	2.6	4.9	24. 7	NF	NF	NF	27.9
white Chardonnay[c]	13.8	1425	98	100	27.2	58.2	59.2	29. 3	25. 3	3.2	83. 4	NF	NF	NF	371.9
traditional red	18.9	2155	41.3	29.4	15.2	28.6	58.7	5.2 1	30	11	51. 2	20	1.8	0.3	177.7

[a] Abbreviations: NF, not found; CPT, total phenol content; AC, antioxidant capacity. [b] Sum of catechins: monomers (catechin + epicatechin) and dimers (B1–B4). [c] Natural phenolic enriched by special winemaking.

is based on a kinetic UV assay with addition to the sample of R1 (buffer/NADH) and addition of R2 (buffer/enzymes/substrate) and then the start of reaction, where urea is hydrolyzed by urease to form CO_2 and ammonia. The ammonia formed then reacts with α-ketoglutarate and NADH in the presence of GLDH (glutamate dehydrogenase) to yield glutamate and NAD^+. The decrease in absorbance due to consumption of NADH is measured kinetically.

AST. Analyses were realized with a Kit No. 851124 Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. The principle is based on a UV test according to a standardized method with addition to the sample of R1 (buffer/enzyme/coenzyme) and addition of R2 (α-ketoglutarate) and then the start of reaction, where α-ketoglutarate and L-aspartate can give L-glutamate and oxaloacetate. AST is the enzyme that catalyzes this equilibrium reaction. The oxaloacetate increase is measured in a subsequent indicator reaction which is catalyzed by malate dehydrogenase. Oxaloacetate and NADH in H^+ presence give L-malate and NAD^+. In this second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (measured photometrically) is directly proportional to the rate of formation of oxaloacetate and, thus, the AST activity.

ALT. Analyses were realized with a Kit No. 851132 Roche/Hitachi, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. The principle is based on a UV test according to a standardized method with addition to the sample of R1 (buffer/enzyme/coenzyme) and addition of R2 (α-ketoglutarate) and then the start of reaction, where α-ketoglutarate and L-alanine can give L-glutamate and pyruvate. ALT is the enzyme that catalyzes this equilibrium reaction. The pyruvate increase is measured in a subsequent indicator reaction which is catalyzed by lactate dehydrogenase. Pyruvate and NADH in H^+ presence give L-lactate and NAD^+. In this second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (measured photometrically) is directly proportional to the rate of formation of pyruvate and, thus, the ALT activity.

ALP. Analyses were realized with a Kit No. 816388 Roche, Roche Diagnostics, Gmbh-D-68298 Mannheim, Germany. The principle is based on a Colorimetric assay in accordance with a standardized method with addition of R1 (buffer: diethanolamine and magnesium chloride) to sample and addition of R2 (buffer, diethanolamine and magnesium chloride; substrate, *p*-nitrophenyl phosphate) and start of reaction. In the presence of magnesium and zinc ions, *p*-nitrophenyl phosphate is hydrolyzed by phosphatases (ALP) to form phosphate and *p*-nitrophenol. The *p*-nitrophenol released is proportional to the ALP activity and can be measured photometrically

Histomorphometric Study of Arterial Mesenteric System. Histomorphometric analysis of the mesenteric arterial system was performed as previously described (*35*). Briefly, the mesenteric vascular tree was collected by dissecting the superior mesenteric artery and its branches until their penetration into the jejunum, immerged for 6 h in Bouin solution, and kept in 70% ethanol until processed. Tissue samples were cut cross-way to the general direction of vessels at the distal portion of jejunal arteries resulting from the division of the cranial mesenteric artery and embedded in paraplast (Histomed Standard, Labo Moderne, France); 3 μm thick transverse sections, obtained at ≈5 mm from the distal end of the arteries, were stained with PAS for histomorphometric analysis to visualize elastic laminae.

Morphometric measurements were performed using the axioHOME system (Carl Zeiss, Oberkochen, Germany). Briefly, the system consists of an IBM-PC compatible computer using the 2.04 version of the Zeiss-Alcatel TITN Answare software (Meylan, France) and a light microscope in which a built-in high-resolution image is superimposed on the optical image of the specimen (*36*). A total of 5−13 arteries (vessels showing several elastic laminae)/animal were quantified, for a total of 66−75 vessels/group. For each artery examined, the following parameters were measured: diameter (measured for each vessel at its minimal value, *D*); total vessel (T), media + lumen (ML), and lumen (L) areas. Adventitia (A) and media (M) areas were calculated as T − L, T − ML, and ML − L, respectively, assuming that intima area was negligible in the conditions of quantification.

Statistical Analysis. Data are shown as the means ± SEM of 8 measurements/group. Data were subjected to logarithmic transformation where necessary to achieve homogeneity of variances. Separately for each dietary treatment, statistical analysis of data was performed by one-way ANOVA followed by Fisher's protected least significant difference post-hoc procedure using a Stat View 512+ microcomputer program (Brain Power, Calabasas, CA). Differences were considered significant when $P < 0.05$. Baseline values were not included in the statistical analysis and are presented for comparative purpose.

For morphometric data, areas were transformed into their logarithmic values, so that normal distribution and homogeneity of variance between groups were obtained for each measured or calculated parameter. Nested (hierarchical) analysis of variance was performed on the 285 vessels examined (66−75 vessels/group) for comparison of treatment groups (C, D, D + W, D + EFW), the "rat" factor being nested within the "group" factor. The ANOVA analysis for each parameter tested the significance of each of the two factors.

RESULTS AND DISCUSSION

The chardonnay white wine enriched in phenolics (W) contains 1425 mg/L of phenolics in the gallic acid equivalent. This wine was obtained by crushing the grapes with must, seeds, and skins fermentation: the winemaking was the same as for a red wine, including a maceration step (6 days) with an increase of temperature to 28 °C. Total antioxidant capacities were similar between W and EFW (13.8 mmol/L) and higher than in traditional Chardonnay wine (2.3 mmol/L). The antioxidant capacity levels of W and EFW 13.8 mmol/L are within the range of some red wines (9.6−29.9 mmol/L) (*30*).

The comparison of phenolics levels for chardonnay wines (traditional or phenolic enriched) is given in **Table 2**. For W and EFW the levels were the following: 98 mg/L for catechin; 100 mg/L for epicatechin; 173.9 mg/L for procyanidins dimers (B_1−B_4); 25.3 mg/L for gallic acid; 83.4 mg/L for caftaric acid (*29*). These levels are above those obtained for the chardonnay white wine obtained from the same grapes with traditionnal winemaking (respectively 12.8-, 22.6-, 7.7-, 34.5-, and 3.5-fold more). The catechins or tanins level was richest in W or EFW (13.3-fold more than the traditional chardonnay and 2.1-fold more than for the traditonal red wine). But in both white wines (W or EFW) anthocyanins were absent in comparison with the traditional red wine. Anthocyanins are responsible for the coloring of black grapes and red wines. They are lacking in white grapes due to a biosynthetic blockage. The amount and concentration of anthocyanins in red grapes will vary depending on the variety, maturity, climate, terroir, and the fruit yield. The

Table 3. Water and Food Consumption and Average Body Weight for Nondiabetic and Diabetic Rats with or without Enriched Phenolic White Wine (W) or Alcohol-Free Enriched Phenolic White Wine (EFW)[a]

	av water consumption (mL/day)	av food intake (g/day)	av body weight (g)
nondiabetic control (C)	27.3 ± 5^{a}	22.7 ± 3.3^{a}	326.1 ± 10.1^{a}
untreated diabetic (D)	157.6 ± 23.45^{b}	38.1 ± 6.4^{b}	252.4 ± 13.4^{b}
diabetic + W	157.7 ± 33.4^{b}	36.2 ± 3.9^{b}	247.8 ± 9.9^{b}
diabetic + EFW	158.7 ± 24^{b}	38.9 ± 4.3^{b}	264.6 ± 10.8^{b}

[a] Values are means ± SEM ($n = 8$). For each dietary treatment, means in a column with different superscripts differ, $P < 0.05$.

Table 4. Effects of Phenolic-Enriched White Wine (W) and Alcohol-Free Phenolic-Enriched White Wine (EFW) Administration on Biochemical Parameters in Diabetic Rats[a]

animals groups	TC (g/L)	HDL (g/L)	TG (g/L)	GLY (g/L)	urea (g/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
nondiabetic control (C)	0.52 ± 0.09^{a}	0.37 ± 0.05^{a}	1.12 ± 0.32	1.51 ± 0.09^{a}	0.33 ± 0.04^{a}	41.63 ± 6.13^{a}	103.63 ± 17.72	211.13 ± 52.16^{a}
untreated diabetic (D)	$0.67 \pm 0.05^{b,c}$	$0.46 \pm 0.07^{a,b}$	1.19 ± 0.49	6.95 ± 1.02^{b}	0.46 ± 0.07^{b}	59.57 ± 11.35^{b}	88.57 ± 12.78	577.86 ± 166.45^{b}
diabetic + W	0.76 ± 0.07^{c}	$0.48 \pm 0.07^{a,b}$	1.68 ± 0.54	6.11 ± 1.48^{b}	0.45 ± 0.06^{b}	69.75 ± 13.69^{b}	91.25 ± 13.06	552.75 ± 174.69^{b}
diabetic + EFW	0.78 ± 0.13^{c}	0.56 ± 0.08^{b}	1.06 ± 0.22	6.24 ± 1.37^{b}	0.48 ± 0.05^{b}	58.00 ± 9.43^{b}	100.57 ± 17.35	474.57 ± 159.96^{b}

[a] Values are means ± SEM ($n = 8$). For each dietary treatment, means in a column with different superscripts differ, $P < 0.05$.Abbreviations: TG, triglycerides; AST, aspartate aminotransferase; TC, total cholesterol; ALT, alanine aminotransferase; GLY, glycemia; ALP, alkaline phosphatase.

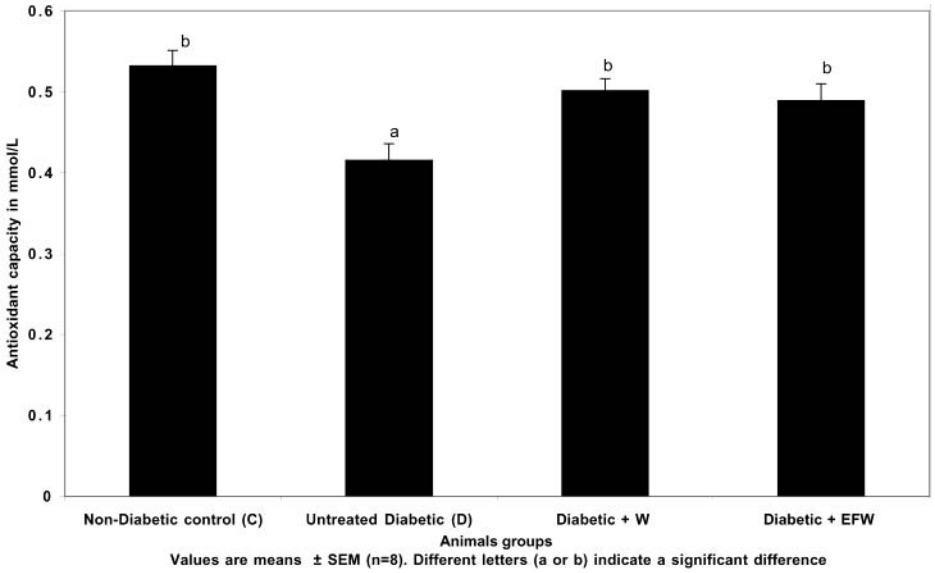

Figure 1. Effects of phenolics-enriched white wine (W) or alcohol-free phenolics-enriched white wine (EFW) administration on plasma antioxidant capacity of diabetic rats.

anthocyanins in grape skins are predominately the 3-*O*-glucosides of delphinidin, cyanidin, petunidin, peonidin, and the major compound malvidin, although coumaric, caffeic, and acetic acid esters have also been detected. Anthocyanins are readily extracted from grape skins and provide the vibrant redish-purple tones of young red wines. They are also known to contribute to the antioxidant and vasodilation activity of red wines (*37*).

As expected, diabetic animals displayed the classical symptoms of insulin deficiency previously described in this model (*35*). Indeed, untreated diabetic (D group) had a significantly lower body growth (+43 g) than nondiabetic control (C group, +123 g) animals. Food (+87%) and water (+51%) intakes were also higher in the D droup compared to that in the C group. Treatment of diabetic animals by W (D + W group) or EFW (D + EFW group) had no significant effect on the changes in body growth or in food or water intakes induced by diabetes (**Table 3**). Glycemia, total cholesterol, GPT, and ALT were significantly higher in diabetic (untreated or treated) than in nondiabetic animals, while no significant difference was found among the three diabetic groups, indicating that treatments by W or EFW had no effect on the correction of the diabetic state. For other biochemical parameters, no significant difference was found between treatment groups (**Table 4**). Untreated diabetic rats had a significantly lower plasma antioxidant capacity than the nondiabetic control group. Interestingly, treatment with either W or EFW restored the plasma antioxidant capacity of diabetic animals to a level not significantly different from that of nondiabetic animals (**Figure 1**). These results indicate that the administration of phenolics-enriched white wine or ethanol-free derivative restores plasma antioxidant capacity impaired by diabetes. Our results clearly indicate that phenolics from white wine (principally tannins) in the presence or absence of alcohol are actively playing a role as antioxidant in vivo. The increase due to the white wine phenolics was significantly different from that of the antioxidant capacity of the untreated diabetic rats. These results are in agreement with a recent study concerning the effects of lemon flavonoids, as crude flavonoids prepared from lemon juice, investigated in diabetic rats. The oxidative stress of eriocitrin (eriodictyol 7-*O*-β-rutinoside) and hesperidin (hesperetin 7-*O*-β-rutinoside) on streptozotocin-induced diabetic rats was suppressed with a diet which contained 0.2% crude flavonoids, 0.2% eriocitrin, and 0.2% hesperidin. After the 28-

Table 5. Histomorphometric Parameters of Mesenteric Vessels in Nondiabetic Control (C), Untreated Diabetic (D), and Diabetic Treated with Polyphenol-Enriched White Wine (D + W) or Ethanol-Free White Wine (D + EFW)[a]

groups	diameter (μm)	lumen [ln(μm^2)]	media [ln(μm^2)]	adventice [ln(μm^2)]
C	162.6 ± 2.6^a	8.26 ± 0.05^a	9.32 ± 0.03^a	9.22 ± 0.04^a
D	177.6 ± 3.1^b	8.53 ± 0.07^b	9.47 ± 0.04^b	9.58 ± 0.04^b
D + W	185.9 ± 3.0^c	8.64 ± 0.06^b	9.57 ± 0.04^c	9.63 ± 0.03^b
D + EFW	186.2 ± 3.4^c	8.60 ± 0.06^b	9.58 ± 0.04^c	9.60 ± 0.04^b

[a] Data are given as mean ± sem of a total of 66–75 arteries/group (5–13 arteries/animal, 8 animals/group). For each treatment group, means with different superscripts differ significantly ($P \leq 0.05$).

day feeding period, the concentration of the thiobarbituric acid-reactive substance in the serum, liver, and kidney of diabetic rats administered crude flavonoids, eriocitrin, and hesperidin significantly decreased as compared with that of the diabetic group. These results demonstrated that dietary flavonoids play a role as antioxidants in vivo (*38*).

As previously shown, diabetes was associated with major morphological changes of the mesenteric arterial system, with a significant increase in vessels diameter and lumen, media, and adventice areas. Treatment of diabetic animals by W or EFW was associated with further enlargement of vessels manifested by increases in diameter and media area (Table 5).

Our data also indicate that treatment of diabetic animals by polyphenols-enriched white wine amplified the effects of diabetes on mesenteric arteries. We have previously described the enlargement of mesenteric vessels in diabetic animals (*35*). In particular, the lumen enlargement was in accordance with the vasodilatation of the mesenteric arterial system previously shown in this model, while media (the smooth muscle layer of the arteries) was hypertrophic. The fact that media is further enlarged by treatment indicates that the effect of wine is not only expressed by a modification in circulating antioxidant activity but also by some significant histological changes. Whether or not those changes are beneficial in the diabetic state as well their mechanism of occurrence deserves to be further studied. Moreover, concentration of (+)-catechin in plasma was 1.63-fold more in the untreated nondiabetic group (24.54 μg/L) in comparison to untreated diabetic group (15.07 μg/L) and 1.54-fold more in the white wine treated diabetic group (23.31 μg/L) in comparison to alcohol-free white wine treated diabetic group (13.13 μg/L). In our animal model, possibly alcohol could help to extent biodisponibility of some phenolics as catechin. It was already demonstrated that for subjects consuming fruits, vegetables, and wine the highest level of (+)-catechin in plasma was observed when wine is added to a vegetables and fruits diet (*33*). Also, catechin was able to reduce the progression of atherosclerosis in apolipoprotein E-deficient mice (*39*). In addition it has been showed that dietary catechin and vitamin E inhibit the atherogenesis process via circulating plasma LDL cholesterol in hypercholesterolemic hamsters (*40*). Low intakes of alcohol have also been shown to stimulate the production of prostacyclin, a potent vasodilatator and platelet antiaggregator (*41*). Some studies with ethanol reported a significant beneficial effect of ethanol on atherosclerosis in mice (*42*, *43*). More recently, it has been shown (*44*) that ethanol by itself act as an antioxidant toward in vitro LDL peroxidation initiated by RO°_2/O_2°: using a low ethanol concentration added to LDL solutions (0.42×10^{-2} mol/L), all °OH was scavenged by ethanol to give RO°_2, which are much less efficient initiators of lipid peroxidation than °OH; these authors concluded that this only contributes to the beneficial effect of moderate wine drinking with regard to cardiovascular disease, apart from the specific antioxidant activity of polyphenols. In addition it has been speculated that the presence of alcohol in wine improves phenolics availability by increasing intestinal absorption and either by delaying excretion or perhaps by altering its course through xenobiotic excretion pathways (*45*). Others postulated that it may do this by preventing the precipitation of the polyphenolic tannins in the digestive tract (*46*). Although alcohol is not a treatment for atherosclerosis or diabetes, these reports on its effects in antioxidative processes are in agreement with and support our findings. Our results relative to the oxidative stress preventative effect of phenolics from white wine suggested that phenolics can induces a direct antioxidant effect but may also act by other indirect mechanisms: selenium enzymes and vitamins E and C economy; metal chelation (Cu^{2+} , Fe^{2+}). For example, it was demonstrated that the effect of oral administration of selenium plays a role in reducing the oxidative stress associated with diabetes: after 5 weeks of treatment, STZ-induced diabetic mice treatment as compared to normal control animals changed parameter activities such as lipid peroxidation, glutathione (GSH), glutathione peroxidase (GPx), and glutathione S-transferase (GST) to near control values in almost all cases (*47*). Recently, it was suggested that ethanol is not the major component in the benefits of red wine for the hamster and that chronic ingestion of RWPE (red wine phenolic extract) prevents the development of atherosclerosis through several mechanisms. At the time of a moderate consumption of red wine, ethanol can improve the effects of phenolic compounds. However, alcohol-free red wine appears as a very good alternative to red wine (*48*).

It was then demonstrated that red wine consumption was associated, in vivo, with higher protection against oxidation (*21*). Several red wine compounds were therefore proposed to explain the beneficial effects of this beverage on health and more particularly on the prevention of cardiovascular diseases (*25*, *49*, *50*). In the meantime, evidence from human studies was brought indicating that white wine does not provide protection against oxidation (*22*, *27*, *28*).

In this context we developped a new generation of "polyphenol-enriched" white wine Chardonnay (principally tanins and phenolic acids) through a specific production procedure (*29*) and tested antioxidative properties in vivo in an experimental animal model of diabetes (the streptozotocin-induced diabetic rat) characterized by a major oxidative stress. The main observation of our study was that treatment of diabetic rats with phenolics-enriched Chardonnay wine restored plasma antioxidant capacities to a similar level to that of nondiabetic animals. This observation is in accordance with previous reports from human studies indicating that antioxidant capacities can be transferred in vivo from wine to the organism (*37*, *51*). In our case, however, it is the first time that antioxidant properties are transferred *from a white wine*, as opposed to previous data obtained with red wine.

We show in this work that its natural enrichment in phenolics increased total wine antioxidant intrinsic capacity and restores the impaired plasma antioxidant capacities when wine is administered in vivo. This result confirms that polyphenols intake is correlated with an improved in vivo defense against free radicals. Our data also indicate that polyphenols present in white wine (phenolic acids, tanins) have in vivo properties similar to those of red wine (anthocyanins, tanins, ...) when similar concentrations are administered. At last, because the effects of wine were observed with both white wine and its ethanol-free counterpart, they can be attributed to the presence

of phenolic compounds and appear independent from the presence of ethanol, as previously shown for polyphenols from red wine (*51*). Interestingly, our preliminary data obtained on catechin levels from plasma pools collected from the four treatment groups indicate that levels of free catechin were higher in the control than in untreated diabetic animals and restored to the control level by polyphenols-enriched white wine but not by the ethanol-free wine treatment. Those data suggest that polyphenols absorption and/or metabolization are altered in diabetes and that wine is susceptible to restore their plasma levels, although the exact role of ethanol deserves further studies.

In conclusion, our study indicates that white wine, when enriched in polyphenols, induces ethanol-independent in vivo effects in a model of insulin-deficient diabetes characterized by a major oxidative stress. Further studies are required to determine the nature of the polyphenols involved as well as their mechanism of action.

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