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ARTICLE

Changes in Antioxidant Endogenous Enzymes (Activity and Gene Expression Levels) after Repeated Red Wine Intake

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The antioxidant properties of wine have been largely related to the reactive oxygen species (ROS) scavenging ability of phenolic compounds. Polyphenolic compounds are hardly absorbed and quickly transformed into metabolites. Their antioxidant activities just as radical scavenging properties are therefore limited, but it is worth looking to other mechanisms. This study intended to test whether wine consumption affects antioxidant enzyme activity and gene expression. For this purpose, eight subjects drank 300 mL of red wine every day for a week and ate a low phenolic diet (LPD + W) specifically designed to avoid interferences from other polyphenols in the diet. The control period was a week with this diet, and volunteers refrained from drinking wine (LPD). Blood samples were taken at 0, 1, and 7 days. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities were determined in erythrocytes (SOD), plasma (CAT and GR), and blood (GPx). Gene expression was determined in macrophages. Oxidative stress caused by LPD reduced SOD, CAT, and GR activities. After wine consumption, these activities significantly increased ($P < 0.05$), and this overcame the effect of oxidative stress on enzyme activity. The modulation of CAT activity may be independent of changes in their gene expression, which significantly increased after LPD. However, SOD gene expression increased only during the LPD + W week. Enzyme activities are not all regulated in the same way. The results show that subacute moderate wine ingestion modulated antioxidant enzyme expression and activity, which is important for the prevention of ROS-associated diseases.

KEYWORDS: Antioxidant enzymes; antioxidant activity; gene expression; red wine

INTRODUCTION

It is widely accepted that the consumption of fruits and vegetables prevents diseases related to oxidative processes (*1*). The main antioxidants in foods are vitamin C, carotenoids, selenium, and polyphenolic compounds. Red wine is an excellent source of such polyphenolic compounds as phenolic acids, flavonoids, stilbenes, and tannin compounds (*2*), and a considerable body of research has focused on determining the chemical composition of wine and assaying its in vitro antioxidant properties (*3*). Polyphenols' activity to scavenge radicals ranks according to their chemical structure and the radical involved in the antioxidant assay (*4*). The more phenoxyl groups there are in the structure, the higher will be the antioxidant value obtained with 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) and 2,2′-diazobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) tests (*5*).

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The mechanism underlying the protective effect of polyphenolic intake is not yet well understood. The hypothesis of direct radical scavenging action needs the presence of the antioxidant at the exact place radicals are formed. This is limited by little polyphenolic absorption and quick transformation into glucuronides and methylated or sulfonated derivatives (*6*). Indeed, the compounds responsible for antioxidant action may not be those present in the food itself. Another point to consider is that endogenous antioxidant enzymes are designed to destroy reactive oxygen species quickly and may react before a radical scavenger does. Thus, the hypothesis that polyphenolic consumption has an influence on antioxidant enzyme activity and expression merits attention. If the endogenous antioxidant defense system acts better, the organism may be better protected from oxidative damage. Superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx) are part of our antioxidant defense system. SOD catalyzes the dismutation of superoxide to hydrogen peroxide, CAT catalyzes the conversion of H_2O_2 to water (preventing the generation of hydroxyl radicals), and GPx

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reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (*7*).

Previously published results prove that polyphenolic compounds have an effect on antioxidant enzymes after the in vitro incubation of cultured cells. In vitro incubation of dietary flavonoids with Chang liver cells (previously submitted to a cytokine mixture to induce oxidative stress) resulted in a reduction of the expression of SOD isoforms (*8*). The preincubation of the human hepatoma cell line (HepG2) with grape seed procyanidin extract resulted in an increase of the mRNAs GPx and GR (*9*). SOD and CAT increased their activities after the aortic smooth cells had been incubated with resveratrol (*10*).

Some experiments included feeding animals with a rich phenolic extract or food and measuring antioxidant enzyme activities and expression afterward (*11*). Others tested the effects of wine intake on antioxidant enzymes in renal cortex and renal papilla tissues in rats (*12*). The consumption of red wine, alcohol, or alcohol-free red wine for 10 weeks resulted in no statistical difference in SOD activity and a significant increase in CAT and GPx activities in groups consuming the ethanol or wine groups. To test the protective effect against oxidative stress conditions, rats were treated with wine or water and then submitted to myoglobinuric acute renal failure to induce oxidative stress. Rodrigo et al. showed that the SOD, GPx, and CAT activities were higher in the wine group than in the control group, thus demonstrating the attenuation of oxidative stress by wine consumption (*13*).

Therefore, the aim of this work is to test whether or not the activity and expression of antioxidant enzymes are modified by wine consumption in human subjects.

MATERIALS AND METHODS

Intervention Study. Eight volunteers aged 23−37 (28.75 ± 3.33) years, four men and four women, participated in the study. Biochemical laboratory determinations of hemoglobin, glucose, proteins, creatinine, glutamic oxalacetic transaminase (GOT), glutamate pyruvate transaminase (GPT), cholesterol, triglycerides, HDL, LDL, VLDL, urea, albumin, bilirubin, reactive "C" protein, and uric acid were in normal ranges for all volunteers. The subjects reported no previous cardiovascular, hepatic, gastrointestinal, or renal diseases. They did not smoke or drink. In the 4 week period before the start of the study, they had not taken vitamin or mineral supplements or consumed drugs or antibiotics.

Ethical approval for the study was obtained from the Ethical Research Committee of the University of Sevilla. Participants attended a training course during which they were informed about the intervention study before giving their written consent.

Special attention was taken to avoid the possible effect of other phenolics in the diet. As phenolic compounds are present in many foods and beverages, volunteers followed a low phenolic diet especially designed for the study. The organizer of the trial held a meeting with all participants in the study to explain all of the dietetic guidelines. They were also provided with a brochure with an extensive list of foods to be consumed or avoided so that this information was available to them in written forms at all times. The foods allowed or withdrawn from the diet are listed in **Tables 1** and **2**, respectively. Diet was devoid of almost all fruits, a large number of vegetables, virgin olive oil, tea, and chocolate. Food composition is taken from DIAL software (*14*). They were asked to avoid all alcoholic drinks, except wine, during the intervention period. Volunteers completed a 24 h dietetic questionnaire on every day of the intervention to make sure they had followed the recommendations appropriately. These were kept by the organizer. A copy of this questionnaire was returned to each volunteer, and they were asked to take the same food as in the previous intervention period. All volunteers drank the same red wine, which was chosen from a varied set of samples that had been analyzed in our laboratory. It was selected for its high values of in vitro antioxidant activity and total phenolic index. It was of the Monastrell variety, vintage 2001, aged for 6 months in oak wood and subsequently aged in the bottle for 3 years. The polyphenolic polymeric fraction of this wine was analyzed by CCC and HPLC-MS (*15*). It was provided by the Regulating Council of the Alicante Designation of Origin. The alcoholic content was 13.5% v/v. The phenolic composition of the wine selected was determined by the HPLC method (*16*), and the antioxidant activity was measured by ORAC (*17*, *18*) and FRAP (*19*) assays. Analyses were done in duplicate (see **Table 3**).

Table 1. Foods Allowed in the Diet during the Intervention Period and Their Antioxidant Compositions (Contents of Ascorbic Acid, Carotenoids, Tocopherols, and Polyphenols)

allowed food	ascorbic acid (mg/100 g of ep[a])	carotenoids (μg/100 g of ep)	tocopherols (μg/100 g of ep)	polyphenols (mg/kg of fresh wt[b] or mg/L)
potato	17.0	5.20	0.05	100
carrot	6.50	8731	0.55	−
cucumber	7.00	169	0.39	−
mushroom	4.00	0.00	0.13	−
pea	12.0	405	0.12	−
banana	11.5	228	0.23	−
pineapple	11.0	12.0	0.05	−
pear	5.20	16.2	0.89	−
marrow	13.8	287	0.06	−
rice	−[c]	−	0.076	70.0−90.0
pasta	−	−	0.13	−
milk	−	28.0	0.10	−
butter	−	200	3.60	−
cream	1.00	200	0.25	−
yogurt	0.70	4.10	0.04	−
egg	0.00	10.0	1.90	−
salmon	0.00	0.00	2.00	−

[a]ep, edible portion. [b]wt, weight. [c]−, not containing.

Table 2. Foods Withdrawn from the Diet during the Intervention Period and Their Antioxidant Compositions (Contents of Ascorbic Acid, Carotenoids, Tocopherols, and Polyphenols)

withdrawn food	ascorbic acid (mg/100 g of ep[a])	carotenoids (μg/100 g of ep)	tocopherols μg/100 g of ep)	polyphenols (mg/kg of fresh wt or mg/L)
aubergine	5.90	50.6	0.03	7500
onion	6.90	8.40	0.45	350−1200
broccoli	100	863	0.62	40.0−100
strawberry	54.9	18.0	0.53	150−750
kiwi	43.1	37.2	0.93	600−1000
cherry	15.0	34.8	0.13	180−1150
apple	12.4	18.0	0.36	50.0−600
orange	50.6	201	0.81	215−685
lemon	51.0	3.40	0.80	50.0−350
green tea	−[c]	−	−	50.0−450
chocolate	−	13.0	0.70	920−1220

[a]ep, edible portion. wt, weight. [c]−, not containing.

Figure 1 shows the intervention design. The preliminary wash-out period lasted for 2 days. During this time volunteers did not drink wine or alcoholic beverages, and they followed the low phenolic diet described above. Then, during the first week, volunteers followed the low phenolic diet and drank 300 mL of red wine every day for dinner for a period of 7 days. During the second period, they just followed the low phenolic diet. This period was also preceded by a 2 day wash-out as described previously. To minimize dietetic interferences, we asked the subjects to consume the same foods as they did in the first period. To this end, we provided them with a copy of the questionnaires they had filled in every day during the first week of intervention (*20*).

Sampling. Blood was extracted on day 0, before wine ingestion (baseline value), day 1, and day 7 after wine had been consumed every day. Each subject fasted for 12−14 h before blood extraction. Venous blood samples were obtained by antecubital venipuncture into a sodium citrate vacutainer (BD Vacutainer CPT, REF 362781) so that macrophages could be separated. An aliquot of blood was separated to determine GPx activity. Blood samples were immediately centrifuged at

Table 3. Phenolic Content and Antioxidant Activity of the Wine Used in the Study

phenolic compound	mean (mg/L)	SD
gallic acid	38.0	0.09
vanillic acid	2.90	0.13
syringic acid	8.50	0.09
caffeoyltartaric acid	80.0	0.30
caffeic acid	4.20	0.00
(+)-catechin	2.80	0.10
(−)-epicatechin	1.30	0.20
resveratrol glucoside	9.90	0.06
tyrosol	55.4	0.20
3-quercetin glucoside	2.50	0.03
total phenolic index (mg/L)[a]	2960	
antioxidant activity		
ORAC[b] (μM TE)	11360	
FRAP[c] (μM Fe^{2+} E)	0.21	

[a]Total phenolic index expressed as gallic acid equivalent. [b]ORAC, oxygen radical absorbance capacity, expressed in Trolox equivalents (TE). [c]FRAP, ferric reducing ability of plasma, expressed as μM Fe^{2+} equivalents (E).

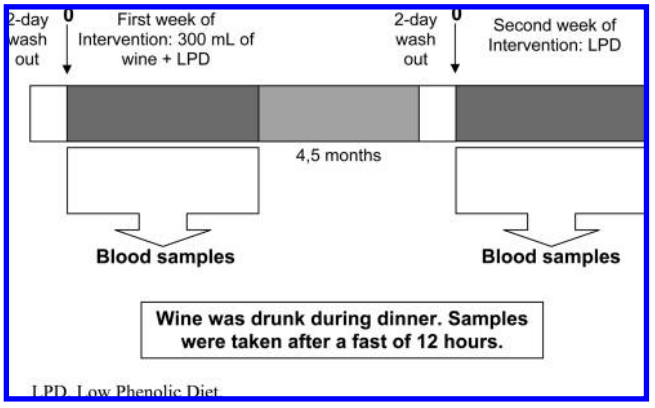

Figure 1. Scheme of the intervention.

12000*g* for 3 min, and unnecessary exposure to light was avoided. Plasma aliquots (for GR and CAT activity determinations), erythrocytes (for SOD activity determination), and macrophages (for CAT and SOD gene expression evaluation) were obtained and stored at −80 °C until analysis.

Antioxidant Enzyme Activity. SOD activity was spectrophotometrically determined in erythrocytes. The assay principle is based on the fact that SOD converts toxic superoxide radicals produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. The method uses xanthine and xanthine oxidase (XOD) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye, detectable at 560 nm. SOD activity is measured by the degree of inhibition of this reaction. Determinations were performed in duplicate.

GR activity was determined in plasma. The method reduced glutathione in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in absorbance at 340 nm is measured in a U-2800 Digilab Hitachi spectrophotometer. Determinations were performed in duplicate.

GPx activity was measured in whole blood. GPx catalyzes the oxidation of glutathione by cumen hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) was immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured. Drabkin's reagent (Randox catalog no. MS181) was used to dilute blood. Determinations were performed in duplicate.

The method for determining CAT activity was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced was measured spectrophotometrically

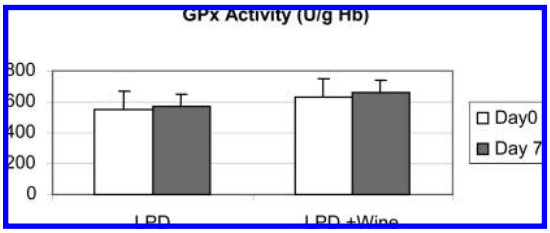

Figure 2. Activity values (U/g of Hb) for glutathione peroxidase (GPx) for the whole group of volunteers (n = 8) during both intervention periods: low phenolic diet (LPD) and low phenolic diet plus wine (LPD + W) at two sampling moments (days 0 and 7).

at 540 nm with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Determinations were performed in duplicate.

The activity of the antioxidant enzymes (SOD, GR, GPx, and CAT) was measured in a U-2800 Digilab Hitachi spectrophotometer and a microplate reader.

Enzymatic kits were purchased from Randox Laboratories Ltd. U.K. (superoxide dismutase SD125, glutathione reductase GR2368, and glutathione peroxidase RS504) and Cayman Chemical (catalase activity catalog no. 707002).

Real-Time Quantitative Polymerase Chain Reaction (PCR). Macrophage RNA was extracted with a specific RNA protocol. RNA was prepared using the Trizol reagent (Invitrogen) according to the instructions. The purity of the RNA extracted was measured in a spectrophotometer at 260 nm. Two micrograms of total RNA was reverse transcribed using TaqMan Universal PCR Master Mix (Applied Biosystems). The cDNA was amplified by quantitative real-time PCR using an ABI prism 7700 Sequence Detection System (Applied Biosystems). Quantitative real-time PCR reactions were performed in triplicate using 5 μL of cDNA, 10 μL of TaqMan Universal PCR Master Mix 2×, and 1 μL of 20× mix of probe and primers in a final volume of 20 μL. Primers and probes for genes were from Applied Biosystems Assay on-Demand Gene expression products: *catalase* (Hs 00156308_m1), *superoxide dismutase 2* (Hs 00167309_m1), and *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* (Hs 02758991_g1) as a housekeeping gene. The Applied Biosystems protocol was followed by using selecting primers with introns between two exons of each gene to avoid potential amplification of contaminating genomic DNA. Amplifications were normalized to GAPDH, and gene expression was quantified using the ΔΔCt calculation, where Ct was the threshold cycle. The amount of the target gene was normalized to GAPDH relative to the calibrator (blood from control group) using the value $2^{-\Delta\Delta Ct}$.

Statistical Analysis. The ANOVA paired-test was performed with the Statistica software package to assess whether there were any differences between the measurements over time. The p value was set at ≤0.05 for significant differences.

RESULTS

Figures 2−5 show the activity values for the enzymes under study: GPx, GR, SOD, and CAT, respectively. The figures present the average values for the whole group of volunteers (n = 8) for both intervention periods at two sampling moments: low phenolic diet (LPD) and low phenolic diet plus wine (LPD + W) on days 0 and 7. SOD, CAT, and GR activity decreased after the volunteers followed the LPD for 7 days (data compared for LPD day 0 versus LPD day 7). However, this decrease is only significant in CAT ($p < 0.05$). On the other hand, enzyme activity increased significantly ($p < 0.05$) after the volunteers followed the LPD + W diet (data compared for LPD + W day 0 versus LPD + W day 7). Only for GPx was this increase not statistically significant ($p < 0.05$). The activities of the enzymes under study on day 0 did not present statistically significant differences between the groups (LPD and LPD + W). However, on the seventh day of intervention SOD and CAT activities were statistically higher ($p < 0.05$) in the period that volunteers drank wine.

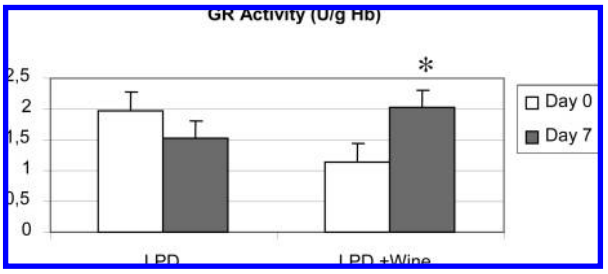

Figure 3. Activity values (U/g of Hb) for glutathione reductase (GR) for the whole group of volunteers (n = 8) during both intervention periods: low phenolic diet (LPD) and low phenolic diet plus wine (LPD + W) at two sampling moments (days 0 and 7). ∗, mean value significantly increased from day 0 to day 7 ($p < 0.05$) during LPD + wine week.

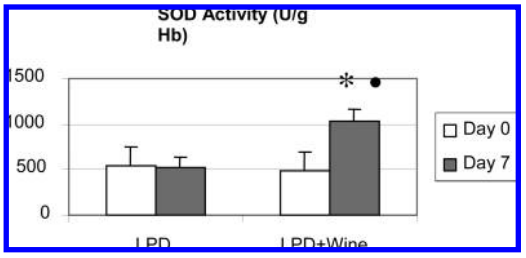

Figure 4. Activity values (U/g of Hb) for superoxide dismutase (SOD) for the whole group of volunteers (n = 8) during both intervention periods: low phenolic diet (LPD) and low phenolic diet plus wine (LPD + W) at two sampling moments (days 0 and 7). ∗, mean value increased significantly from day 0 to day 7 ($p < 0.05$) during LPD + wine week. ●, mean value at day 7 in LPD + wine week is significantly different from that in LPD week ($p < 0.05$).

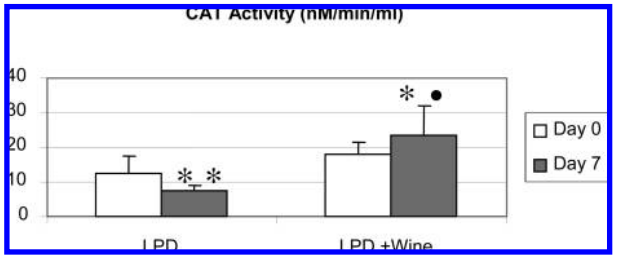

Figure 5. Activity values (nM/min/mL) for catalase (CAT) for the whole group of volunteers (n = 8) during both intervention periods: low phenolic diet (LPD) and low phenolic diet plus wine (LPD + W) at two sampling moments (days 0 and 7). ∗, mean value decreased significantly from day 0 ($p < 0.05$) to day 7 during LPD and increased significantly from day 0 ($p < 0.05$) to day 7 during LPD + wine week. ∗∗, mean value at day 7 is significantly different from that at day 0 ($p < 0.05$) during LPD week. ●, mean value at day 7 in LPD + wine week is significantly different from that at day 7 in LPD week ($p < 0.05$).

Figure 6 shows the relative expression of CAT in the LPD period and in the same volunteers after 1 and 7 days of ingesting 300 mL of wine ($n = 8$). As shown in **Figure 6A**, CAT expression significantly increased ($p < 0.05$) after the antioxidants in the diet had been withdrawn, reaching its maximum expression 7 days after withdrawal. **Figure 6B** indicates that the ingestion of 300 mL of wine annuls the significant increase observed in CAT expression ($n = 8$).

Figure 7 shows the relative expression of SOD under LPD and, in the same volunteers, after 1 and 7 days of ingesting 300 mL of wine (n = 8). SOD expression does not change after the LPD (**Figure 7A**), but it significantly increased ($p < 0.05$) after the ingestion of 300 mL of wine ($n = 8$) (**Figure 7B**).

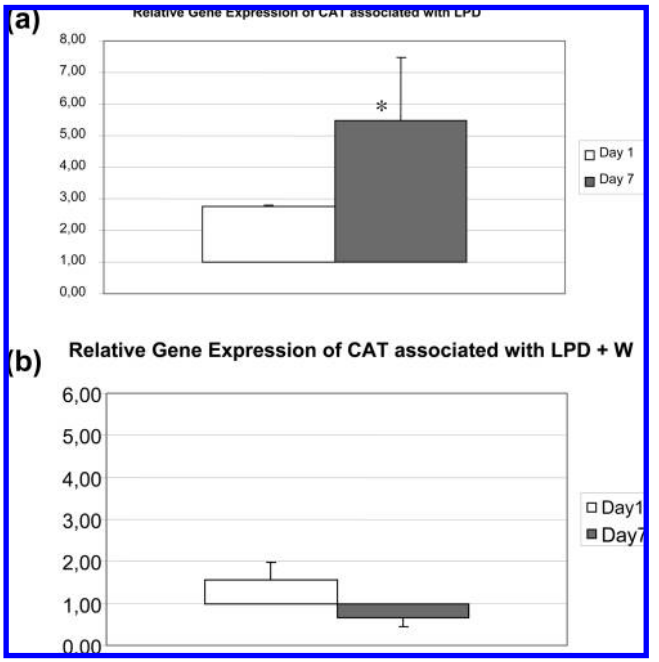

Figure 6. Relative gene expression of catalase (CAT) for the whole group of volunteers (n = 8) at three sampling moments (days 0, 1, and 7) during the intervention periods: (**a**) low phenolic diet (LPD) [∗, CAT expression at day 7 is significantly different from that at day 0 ($p < 0.05$) during LDP week]; (**b**) low phenolic diet plus wine (LPD + W).

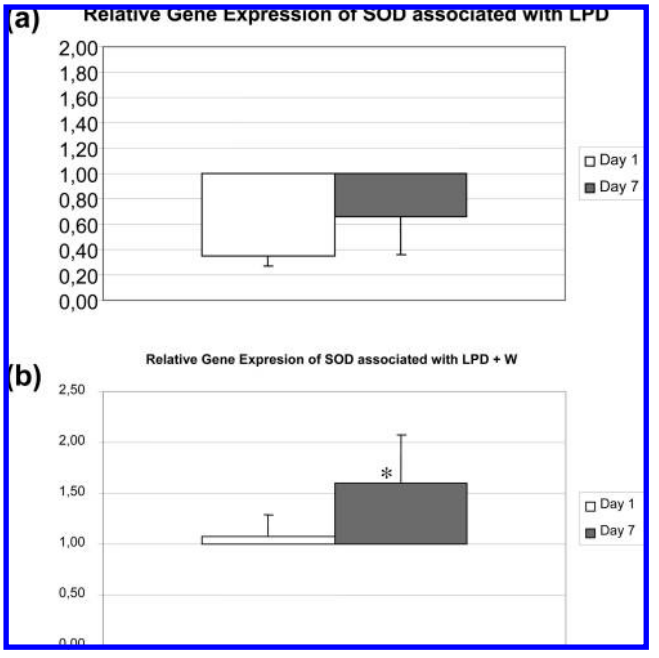

Figure 7. Relative gene expression of superoxide dismutase (SOD) for the whole group of volunteers (n = 8) at three sampling moments (days 0, 1, and 7) during the intervention periods: (**a**) low phenolic diet (LPD); (**b**) low phenolic diet plus wine (LPD + W) [∗, SOD expression at day 7 is significantly different from that at day 0 ($p < 0.05$) during LDP + wine week].

DISCUSSION

Certain difficulties arose when these experiments were designed. First, polyphenolic compounds are widespread. Therefore, if a group consuming a particular food or beverage is to be compared with a control group not consuming it, other foods that may provide the same polyphenolic compounds must be

controlled. For instance, wines contain catechins that are also present in tea or chocolate; caffeic acid is also in coffee, ferulic acid is found in aubergines, and anthocyanins are found in strawberries and blackberries (*21*). The consumption of these foods interferes with the experiment and can also mask the real effects the food or beverage under study may exert. In our experiment, then, dietetic guidelines were provided to ensure consistency of results. The resulting LPD left out many foods and beverages, and volunteers reported that it would be difficult to follow for a longer time. An additional value of these results is that the doses are in the dietetic range and the results can be extrapolated to daily life. Finally, it must be taken into account that the effect exerted by a food may be due to a mixture of different compounds and the synergic mechanism among them.

In our study, we have determined the influence of a LPD and a LPD + W on the antioxidant enzyme activities. SOD activity was measured in erythrocytes and enzyme expression in macrophagues. The average life of erythrocytes is 120 days, and the long time period between the two interventions allows their turnover. Thus, this time period is enough to guarantee that no effect of previous treatment is still present or interfering with the second intervention period.

Evidence showing that vascular oxidative stress is lower in women than in men has been provided in a recent study (*22*). Unfortunately, in the paper, the mechanism is not well established, and the role of gender on antioxidant enzymes is currently being explored. The excessive production of radicals by NADPH might be a cause of oxidative stress. Thus, the influence of gender on NADPH-oxidase activity could explain the differences observed on oxidative stress. For example, NADPH-oxidase activity and function are lower in cerebral arteries of female rats. However, SOD1, SOD2, and SOD3 expressions did not differ between genders (*23*). On the other hand, some years ago Guemori et al. measured the biological variability of antioxidant enzymes, such as SOD, GPx, and CAT, in plasma and erythrocytes of 1836 subjects. This study did not find any statistically significant variation between males and females (*24*). More recently, another study (*25*) showed the same results in whole blood GPx and SOD in 118 subjects. In our opinion, according to these authors who performed large population studies, we can assume than there do not exist differences by gender in SOD, CAT, and GPx enzymes. Due to the fact that our study includes a small number of volunteers (eight volunteers: four males, four females), we analyzed the data for the whole group to achieve statistical significance.

The decreased enzyme activity of SOD, CAT, and GR suggests that these enzymes might be affected by oxidative stress caused by LPD, although the decrease is significant for CAT. A high-fat refined carbohydrate diet designed to mimic the diet consumed in Western countries resulted in the up-regulation of NADPH oxidase (a ROS-generating enzyme) and the down-regulation of kidney Mn-SOD and kidney and aorta GPx. There was no clear effect on CAT (*26*). The LPD in our experiment has the same effect on SOD, CAT, and GR. Roberts et al. proposed that these events associated with antioxidant enzymes account for oxidative stress and endothelial dysfunction (*26*).

Wine consumption may overcome this deleterious role of diet. Enzyme activity increased significantly after the volunteers had followed the LPD + W (with the only exception of GPx). Other antioxidants induced a significant increase in CAT and SOD activities in agreement with our results: hydroxytyrosol and its triacetylated derivative increased the activities of SOD and CAT in the liver of rats fed a cholesterol-rich diet (*27*). Hepatic SOD and CAT activities in rabbits fed a high-cholesterol diet supplemented with naringin or probucol were significantly higher than in a group fed a high-cholesterol diet. Moreover, GPx activity did not differ significantly between the groups (*28*). However, if animals are fed with a balanced diet and wine (*12*), SOD activity in the renal cortex was not significantly different from that induced by a balanced diet and water even after long periods (10 weeks). Similarly, plasma SOD in elderly subjects did not change significantly over a 4 week intervention period. Volunteers drank either apple or pomegranate juices, but the rest of the diet was not controlled (*29*).

Therefore, those studies that used a controlled diet and restricted phenolic compounds from other food sources find a statistically significant effect. If diet is not controlled, other phenols are likely to be ingested, and wine will make no difference by itself.

The effects that polyphenols have on the gene expression of antioxidant enzymes have been determined by cell cultures or animal experiments (*11*, *28*). Results are also related to high doses of antioxidant extracts. Comparison is, therefore, limited.

Panaxadiol ginsenosides (100 μg/mL) extracted from *Panax ginseng* activated the transcription of SOD and CAT genes in HepG2 human cell culture (*30*). In H_2O_2-stressed K562 human cells pretreated with *Rhamnus alaternum* extracts, the level of transcripts related to SOD was higher that in H_2O_2-stressed cells. *R. alaternum* extracts were screened for the presence of flavonoids and tannins (*31*). SOD mRNA expression was significantly higher in rabbits fed a high-cholesterol diet supplemented with probucol than in the control group, but probucol did not induce changes in CAT expression (*28*). The total phenolic and flavonoid contents in Mauritian endemic plant extracts were proportionally associated with the transcriptional activity of Cu,Zn-SOD in HeLa human cell culture, and they were inversely correlated with CAT promoter activity (*32*). These results are in agreement with the changes in CAT and SOD gene expression after the chronic consumption of wine phenolics. The increase in CAT gene expression could be interpreted as the effort made by the body to overcome oxidative stress induced by a LPD. Consequently, CAT expression did not vary in the presence of phenolics. On the other hand, wine consumption increases SOD gene expression. Therefore, enzyme activities are not all regulated in the same manner.

Wine consumption increases both SOD activity and SOD gene expression. However, wine consumption maintains gene expression but does not significantly increase CAT activity. In this case, the modulation of CAT activity may be independent of changes in gene expression.

In conclusion, this study demonstrates that the intake of polyphenols through the diet affects both antioxidant enzymes and their expression and that this mechanism may explain how wine can prevent ROS-associated diseases.

ABBREVIATIONS USED

ROS, reactive oxygen species; LPD + W, low phenolic diet plus wine; LPD, low phenolic diet; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, gutathione reductase; DPPH, 2,2-diphenyl-1,1-picrylhydrazyl; ABTS, 2,2′-diazobis(3-ethylbenzothiazoline-6-sulfonic acid); GOT, glutamate-oxaloacetate transaminase; GPT, glutamic pyruvic transaminase; ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing ability of plasma; XOD, xanthine oxidase; GSSG, oxidized glutathione; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

ACKNOWLEDGMENT

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