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Repeated Red Wine Consumption and Changes on Plasma Antioxidant Capacity and Endogenous Antioxidants (Uric Acid and Protein Thiol Groups)

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This study aims to ascertain the in vivo antioxidant properties of red wine by determining how it affects antioxidant biomarkers (plasma antioxidant capacity (PAC) and endogenous antioxidants such as uric acid or protein thiol groups). Antioxidant biomarkers have been assessed in eight healthy human volunteers after repeated intakes of 300 mL of red wine every day for a week. During this intervention period, volunteers followed a low phenolic diet designed to prevent the phenolic compounds in wine from interfering with the phenolics from other foods or beverages. This diet was followed throughout the week that the volunteers drank wine and for another control week when they drank water. Biomarkers were determined before the subjects taking part in the study started the intervention period with red wine (Monastrell variety) and 1, 3, and 7 days after. PAC was evaluated by the Ferric Reducing Ability of Plasma assay (FRAP), and the Oxygen Radical Absorbance Capacity assay using fluorescein (ORAC-FL). In addition, the concentrations of endogenous antioxidants such as uric acid, albumin, bilirubin, and protein thiol groups were analyzed. The FRAP method shows that PAC increased after the week of wine consumption but decreased after the week without wine consumption. The uric acid concentration did not show any changes that were significantly different from our results in acute wine intake studies. Protein thiol groups decreased significantly ($p < 0.05$) with the low phenolic diet, but this decrease was not statistically significant if the diet was taken with red wine ($p < 0.05$).

KEYWORDS: Antioxidant; wine; plasma; phenolic; ORAC; FRAP; protein thiol groups; humans volunteers; uric acid; in vivo

INTRODUCTION

Epidemiological studies demonstrate that moderate wine consumption protects against cardiovascular disease largely because of the alcohol and phenolic compounds that wine contains (*1*). Red wine is known to be a source of polyphenolic compounds with antioxidant properties (*2, 3*). Phenolic compounds are in vitro antioxidants, and their values have been reported with a variety of methods (*4*). Their concentrations affect wine's antioxidant activity (*5–7*).

However, what still remains to be proved is whether they exert an antioxidant activity once they have been ingested. The need to evaluate their antioxidant activity in vivo has been stated several times (*8, 9*). Nowadays, intense research is being conducted into the bioavailability of phenolic compounds in humans, the extent to which they are absorbed, their metabolism, and their biological actions. Another strategy is to determine an antioxidant biomarker before and after the intake of food or beverages.

PAC is one of the most widely used biomarkers as it is related to the antioxidant network in plasma (*10, 11*). It consists of a pool of compounds with antioxidant activities that are likely to be present in plasma (ascorbic acid, carotenoids, α-tocopherol, and other bioactives). In addition, endogenous nonenzymatic antioxidants such as albumin, bilirubin, and uric acid make an important contribution to antioxidant defenses. It has been suggested that a variety of methods need to be used if changes in PAC are to be assessed and the results interpreted appropriately (*12*). Among the most widely used methods for determining PAC are FRAP and ORAC. Most of the papers in the field assess PAC shortly after foods or beverages have been consumed (minutes or hours). Indeed, PAC increases after the acute intake of foods containing phenolic compounds such as wine (*10*), chocolate (*13*), pomegranate (*14*) juice, and cranberry juice (*15*) regardless of the antioxidant assay used. Therefore, there is a need to understand the effect that a dietetic component

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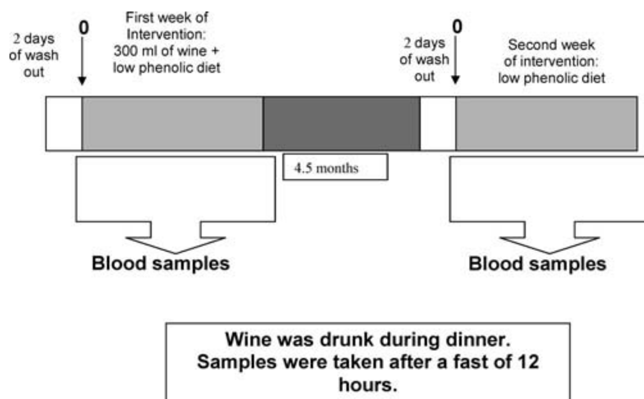


Figure 1. Scheme of the intervention.

exerts when the intervention period is longer. One of the drawbacks is that it is difficult to recruit volunteers who follow dietetic guidelines closely. Experimental designs to prove the effect of wine intake on antioxidant biomarkers are compared with the results with abstinence from alcoholic beverages (*16*), red and white wine (*17*), or drinking red wine and dealcoholized wine. Phenolic compounds are ubiquitous plant-derived metabolites; subsequently, they are natural in many food and beverage compositions (*18*). Many authors control the alcoholic beverages intake to prevent its interference. In our opinion the intake of polyphenols from another food source can be relevant enough to interfere with our results. Consequently, in our experimental design we controlled both alcohol and polyphenol intake.

Therefore, studies of this type are scarce. This study, then, aims to elucidate whether repeated red wine intake modifies human PAC or endogenous antioxidants.

MATERIALS AND METHODS

Chemicals, Reagents, and Equipment. TROLOX (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), $NaH_2PO_4 \cdot H_2O$, TPTZ (2,4,6-tripyridyl-*s*-triazine), $FeCl_3 \cdot 6H_2O$, $C_2H_3O_2Na \cdot 3H_2O$, $FeSO_4 \cdot 7H_2O$, NaF, and 85% H_3PO_4 were purchased from Sigma. 32% HCl, ascorbic acid, and acetonitrile (HPLC grade) were provided by MERCK. Fluorescein sodium salt and acetic acid glacial (HPLC grade) were supplied by Fluka. AAPH (2,2′-diazobis(2-amidinepropane)dihydrochloride) was from Wako Chemicals. K_2HPO_4 was purchased from Probus. Vacutainers with sodium citrate were provided by Eulabor. Albumin, bilirubin, and uric acid kits were purchased from SPINREACT reagents.

The equipment used was a UV-2800 Hitachi fluorescence spectrophotometer with a Peltier system at 25 °C, a UV–vis F-2500 Hitachi spectrophotometer connected to a bath to keep the temperature at 37 °C, and a liquid chromatograph (Agilent 1100) equipped with a DAD detector.

The data were processed with Statistica StatSoft software. Nonparametric statistics test included the Kolmogorov–Smirnov two sample test, the Mann–Withney U test, and the Kruskal–Wallis test. Linear regression analysis was also performed.

Collection of Samples. Eight healthy volunteers aged 23–37 (28.75 ± 3.33) were recruited for the study.

The subjects led a healthy life: they did not drink alcoholic beverages or smoke. In the four-week period before the start of the study, they had not taken vitamin or mineral supplements and had not consumed drugs or antibiotics that are known to interfere with intestinal absorption and/or the P450 enzymatic system.

Clinical history questionnaires reported no previous cardiovascular, hepatic, gastrointestinal, or renal diseases. The results of clinical laboratory tests for all volunteers (hemoglobin, glucose, proteins, creatinine, GOT, GPT, cholesterol, triglycerides, HDL, LDL, VLDL, urea, albumin, bilirubin, reactive “C” protein, and uric acid) were in normal ranges.

Table 1. Phenolic Compounds (Mean and Standard Deviation) and Antioxidant Activity of the Wine Used in the Study[a]

gallic acid	38.0 ± 0.09 mg/L	(+)-catechin	2.8 ± 0.10 mg/L
vanillic acid	2.9 ± 0.13 mg/L	(−)-epicatechin	1.3 ± 0.20 mg/L
syringic acid	8.5 ± 0.09 mg/L	resveratrol glucoside	9.9 ± 0.06 mg/L
caffeoyl tartaric acid	80.0 ± 0.3 mg/L	3-quercetin glucoside	2.5 ± 0.03 mg/L
caffeic acid	4.2 ± 0.00 mg/L	tyrosol	55.4 ± 0.2 mg/L
total phenolic index	2960 mg/L	ORAC (TROLOX equivalent)	11 359.79 μM
		FRAP (μM Fe^{2+} equivalent)	0.211 μM

[a] Total phenolic index expressed as gallic acid equivalent, ORAC expressed in TROLOX equivalent, and FRAP expressed as μM Fe^{2+} equivalent.

Table 2. Mean Values as Average and Standard Deviation of Volunteers ($n = 8$/Day) of PAC determined by FRAP and ORAC-FL assay, ORAC Expressed as TROLOX Equivalent (μmol/L), and FRAP Expressed as μM Fe^{2+} Equivalent[a]

	day	SH (μM) mean ± SD ($n = 8$)	ORAC-FL (μM) (TROLOX equivalent) man ± SD ($n = 8$)	FRAP (μM) (Fe^{2+} equivalent) man ± SD ($n = 8$)
wine period	day 0	0.185 ± 0.07	13433 ± 4141	324 ± 125
	day 1	0.176 ± 0.05	11003 ± 2987	284 ± 105
	day 3	0.148 ± 0.06	13596 ± 8026	382 ± 340
	day 7	0.150 ± 0.03	11965 ± 3461	510 ± 391
control period	day 0	0.195 ± 0.03	12911 ± 3513	345 ± 118
	day 1	0.175 ± 0.02	12178 ± 3314	330 ± 118
	day 3	0.164 ± 0.17	10602 ± 3250	273 ± 99.1
	day 7	0.160 ± 0.03	10032 ± 4188	237 ± 82.2

[a] Protein thiol groups (mM) during the period in which volunteers drank wine (0, 1, 3, and 7 days) and the control period (0, 1, 3, and 7 days). The average of 284, corresponding to “day 1”, was obtained as the mean of seven data points instead of eight because of the presence of an outlier.

Table 3. FRAP Difference along Time (Wine Group – Control Group)

	volunteers							
days	1	2	3	4	5	6	7	8
0	−149	105	6	−313	−107	160	13	2
1	−100	−282	−96	−234	−151	186	45	32
3	176	−30	40	44	−66	79	79	31
7	277	161	66	8	21	113	227	163

Ethical approval for the study was obtained from the Ethical Research Committee of the University of Seville. All subjects were informed and gave their written consent before taking part in the study.

Before starting the experiment, volunteers were given a two-hour training course. To avoid the possible effect of other phenolics in the diet, a low phenolic diet was designed. Dietetic guidelines were explained orally, and a brochure with an extensive list of foods to be avoided or consumed was given to all subjects so that they had information at all times. The foods withdrawn from the diet were as follows: fruit, vegetables, virgin olive oil, tea, and chocolate. They were asked to avoid all alcoholic drinks, except wine, during the intervention period.

Volunteers filled in a 24 h dietetic questionnaire on every day of the intervention to make sure they had followed the recommendations appropriately. The scheme of the intervention is shown in **Figure 1**. It started with a two day period of wash out. During the two day wash out period volunteers did not drink wine nor alcoholic beverages, and

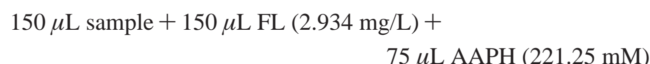
they followed the above described low phenolic diet. During the first week, the volunteers followed the low phenolic diet and drank 300 mL of red wine every day during dinner for a period of 7 days. During the second period they just followed the low phenolic diet. This period was also preceded by a two day wash out as previously described. 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 questionnaire they filled in the first time.

Each subject fasted 12–14 h before the blood extraction. Venous blood samples were obtained by antecubital venipuncture into a sodium citrate vacutainer (BD Vacutainer CPT, REF 362781). Blood was extracted on day 0, before wine ingestion (baseline value), and 1, 3, and 7 days after. Blood samples were immediately centrifuged at 12 000*g* for 3 min, and unnecessary exposure to light was avoided. The plasma obtained was separated into six different aliquots for the subsequent assays.

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. Red wine used in this study was from Monastrell variety, vintage 2001, aged six months in oak wood and subsequently aged in the bottle for three years. The Regulating Council of the Alicante Designation of Origin provided the wine. The antioxidant activities of the wine selected were determined by a variety of assays and are as follows: total polyphenolic index = 2960 mg/L, ORAC-FL = 11 359.79 μM, and FRAP = 0.211 μM. The alcoholic content was 13.5% (v/v). We also chose a variety of grape (Monastrell) with a considerable resveratrol content, whose healthy effects are widely recognized (*19, 20*).

ORAC Assay. In this method, fluorescein (FL) was used as fluorescent probe (*21, 22*). AAPH was used as a peroxyl radical generator and TROLOX as an antioxidant standard.

The reagent concentrations were phosphate buffer (75 mM, pH 7,4), FL (2.934 mg/L), AAPH (221.25 mM), and TROLOX (20 μM). The plasma was diluted 750 times with phosphate buffer. The assay was carried out at 37 °C in fluorimeter cuvettes in triplicate. The final reaction mixture was composed of



$$150\ \mu\text{L sample} + 150\ \mu\text{L FL (2.934 mg/L)} + 75\ \mu\text{L AAPH (221.25 mM)}$$

The fluorescence was recorded (excitation wavelength, 490 nm; emission wavelength, 515 nm) every 5 min for 60 min, until the final value was less than 5% of the initial one. The results are expressed as ORAC values or TROLOX equivalents (μmol/L): μmol TROLOX equivalents per liter plasma sample. ORAC results are expressed as TROLOX equivalents obtained integrating the area under the curve (AUC) described by the fluorescence signal along time (60 min)

$$\text{ORAC}\ (\mu\text{M}) = 20K(S_{\text{sample}} - S_{\text{blank}})/(S_{\text{TROLOX}} - S_{\text{blank}})$$

where 20 = TROLOX concentation (μM), K = sample dilution factor, S = AUC for sample, TROLOX, or blank as calculated. Results are expressed as TROLOX equivalents (μmol/L): micromoles TROLOX equivalent per plasma sample liter. (*23*).

Ferric Reducing Ability of Plasma (FRAP). This method measures the reducing power of plasma.

The reagent concentrations (*24*) were as follows: acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in HCl 40 mM), and $FeCl_3 \cdot 6H_2O$ (20 mM). The FRAP reagent was prepared by mixing 10 vol of acetate buffer, 1 vol of TPTZ, and 1 vol of $FeCl_3 \cdot 6H_2O$. The plasma samples were not diluted.

In the reaction, 3 mL of FRAP reagent, 100 μL of sample, and 300 μL of deionized water were mixed in a cuvette. This cuvette was then shaken, and the absorbance was recorded for 8 min at 593 nm. Samples were measured in triplicate.

A calibration curve was constructed with aqueous solutions of known Fe^{2+} concentrations (0, 100, 300, 500, 750, and 1000 μmol/L $FeSO_4 \cdot 7H_2O$). The results are expressed as micromoles per liter of antioxidant power.

Measurement of Protein Thiol Groups. Most of the plasma thiol groups (SH) were associated with proteins. Albumin is a relevant protein in plasma; its extracellular antioxidant capacity has been previously described (*25, 26*).

The assay for determining protein thiol groups used spectrophotometric methods. It used DTNB (10 mM in absolute methanol) and Tris base (0.25M)–ethylenediaminetetraacetic acid (EDTA; 20 mM) buffer, pH 8.2.

The procedure was the following. An aliquot of plasma (0.20 mL) was mixed in a 10 mL test tube with 0.6 mL of the Tris-EDTA buffer, and then 40 μL of 10 mM DTNB and 3.16 mL of absolute methanol were added. The test tube was capped, and the color was allowed to develop for 15–20 min. The mixture was then centrifuged at 3000*g* for 15 min at ambient temperature. The absorbance of the supernadant was measured at 412 nm (A) and subtracted from this were a DTNB blank (B) and a blank containing the sample without DTNB (C) (*27*). Total SH groups were calculated using an molar extintion coefficient of 13 600 cm^{-1} M^{-1} as follows:

$$(A - B - C) \times (4.0/0.2)/13.6 = (A - B - C) \times 1.47\ \text{mM}$$

where 4.0 is the final mixture volume and 0.2 the plasma aliquot volume.

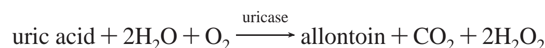
Quantitative Determination of Albumin. In the presence of bromcresol green at a slightly acidic pH, albumin produces a color change from yellow-green to green-blue. The intensity of the color (λ = 630 nm) is proportional to the albumin concentration in the sample (*28*).

For this determination we used the enzymatic kit Spinreact 1001020.

Quantitative Determination of Bilirubin. Bilirubin was converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions present in serum, bilirubin–glucuromide and free bilirubin loosely bound to albumin, only the former reacted directly in aqueous solution (bilirubin direct), while free bilirubin required solubilization with dimethylsulfoxide (DMSO) to react (indirect bilirubin). When determining indirect bilirubin, direct bilirubin was also determined, so the results are for total bilirubin. The intensity of the color formed (λ = 555 nm) was proportional to the bilirubin concentration in the sample (*28*).

The enzymatic kit used for this determination was SPINREACT 1001042.

Quantitative Determination of Uric Acid. Uric acid was oxidized by uricase to allantoine and hydrogen peroxide ($2H_2O_2$), which under the influence of peroxidase (POD), 4-aminophenazone (4-AP), and 2,4-dichlorophenol sulfonate (DCPS) formed a red quinoneimine compound:



$$\text{uric acid} + 2H_2O + O_2 \xrightarrow{\text{uricase}} \text{allontoin} + CO_2 + 2H_2O_2$$

$$2H_2O_2 + \text{4-AF} + \text{DCPS} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

The intensity of the red color formed (λ = 520 nm) was proportional to the uric acid concentration in the sample (*28*). The enzymatic kit used for this determination was SPINREACT 1001013.

Quantitative Determination of "C" Reactive Protein. The effects of "C" reactive protein (CRP) were monitored using a turbidimetric method (*29*). The latex particles covered with human anti CRP antibodies were gathered by the CRP present in the volunteer's sample. This effect led to a change in the absorptivity which was proportional to the CRP concentration of the sample.

Analysis of Wine Phenolic Compounds by HPLC. A total of 10 μL of wine sample was injected in a chromatograph Agilent 1100 with the following characteristics: the column was a Zorbax SB-C18, 4.6 × 12.5 mm, 5 μm by Agilent, PN 820950-920, protected by a Zorbax SB-C18 4.6 × 30 mm rapid resolution cartridge, PN 833975-902. The syringe filters were Millex LCR (Millipore) 0.45 μm.

The elution gradient has been reported previously (*30*). The solutions used here were Solvent A, water and trifluoroacetic acid (TFA; 99.8,

Table 4. Comparison between the FRAP Results for Control and Wine Subgroups the Seventh Day of Intervention, Difference of FRAP Results between "Day 0" and "Day 7" for the Control Period, and Difference of FRAP Results between the Control and Wine Periods at the Seventh Day

volunteer	comparison between the FRAP results for control and wine subgroups at the seventh day of intervention[a]		difference of FRAP results between day 0 and day 7 for control period[b]	difference at the seventh day between control and wine periods[c]
	control	wine		
1	229.4	506	−0.001	−276.6
2	358.3	519	−0.066	−160.7
3	222.7	289	−0.037	−66.3
4	276.8	285	−0.037	−8.2
5	98.3	321	−0.041	−222.7
6	285.7	399	−0.047	−113.3
7		464		
8	188.3	351	−0.027	−162.7

[a] Kolmogorov–Smirnov test: significant difference with $p < 0.005$. Mann–Withney test: significant difference with $p = 0.008$. [b] Kolmogorov–Smirnov test: significant difference with $p < 0.005$. Mann–Withney test: significant difference with $p = 0.0011$. [c] Kolmogorov–Smirnov test: significant difference with $p < 0.005$. Mann–Withney test: significant difference with $p = 0.0004$.

Table 5. Mean Values and Standard Deviation ($n = 8$) of Uric Acid, Albumin, Reactive "C" Protein, and Bilirrubin for Control and Wine Periods

	group	0	1	3	7
albumin (g/dL)	control	4.0 ± 0.2	3.8 ± 0.2	3.7 ± 0.1	3.8 ± 0.1
	wine	4.3 ± 0.2	4.0 ± 0.2	3.9 ± 0.1	4.0 ± 0.1
uric acid (mg/dL)	control	4.0 ± 1.5	3.9 ± 1.2	4.3 ± 1.4	4.5 ± 1.1
	wine	4.4 ± 1.4	4.5 ± 1.4	4.4 ± 1.2	4.7 ± 1.3
bilirrubin (mg/dL)	control	0.71 ± 0.54	0.71 ± 0.44	0.76 ± 0.32	0.81 ± 0.24
	wine	0.9 ± 0.4	n.d.	0.9 ± 0.2	0.8 ± 0.2
triglycerides (mg/dL)	control	54.5 ± 19.3	60.7 ± 23.1	58.1 ± 20.1	60.7 ± 25.5
	wine	59.5 ± 20.5	57.8 ± 19.2	59.5 ± 19.4	59.5 ± 17.6
total cholesterol (mg/dL)	control	154.2 ± 24.1	155.0 ± 22.5	157.2 ± 23.1	156.7 ± 23.7
	wine	185.0 ± 25.0	155.7 ± 26.3	156.1 ± 25.2	157.0 ± 2.0
reactive "C" protein (mg/L)	control	1.6 ± 1.1	2.1 ± 1.2	2.4 ± 1.1	2.3 ± 1.4
	wine	2.2 ± 1.9	2.1 ± 1.6	1.8 ± 0.9	1.8 ± 0.3

0.2), and solvent B, acetonitrile and TFA (99.8, 0.2). Analyses were carried out in duplicate.

Phenolic compounds were quantified by external calibration with standards. Resveratrol glucoside was quantified on the basis of a molar absorptivity coefficient that was similar to that of its free counterpart.

RESULTS AND DISCUSSION

Table 1 shows the antioxidant activity and the concentration of phenolic compounds in the wine that was given to the volunteers to drink. **Table 2** presents protein thiol groups and PAC mean values as average of the group of volunteers ($n = 8$). Pellegrini et al. (*31*) reported baseline antioxidant activities determined by the TRAP method from 827 μmol/L to 1564 from one subject to another. The work by Heneman et al. (*32*) reports data with remarkable variation in antioxidant capacity in agreement with our results.

The data in our study have been assembled into two different subgroups: the control and the wine periods, to check for the effect of wine. Besides, another factor taken into account was the day when the blood was collected. At a glance, it can be seen that antioxidant values determined with the FRAP assay, after wine intake, increase with time in the wine group against the control one. In the control group, FRAP values range between 345 and 237 with time while in the wine group the gap is systematically higher: 324–510. Just if we consider the differences in FRAP between the wine and the control groups per day, we obtain the sequence −21, −46, +109, +154. The difference corresponding to the seventh day is noticeable. To assess suitably these observations, statistical analysis has been done. According to the sample size, normality cannot be reliably assessed, and the comparisons between the subgroups have been performed by using nonparametric statistics: The Kolmogorov–Smirnov two sample test, the Mann–Withney U test, and the Kruskal–Wallis test (a one-way nonparametric ANOVA with "repeated" measurements) (*33*). To determine the effect of the wine over time, we have considered the Kruskal–Wallis test for the FRAP difference wine – control group as indicated in **Table 3**. The null hypothesis is the equivalence of mean effects on volunteers over days. This test uses the ranks of the observations, by considering them as elements of the same sample. The Kruskal–Wallis statistics is $H(3, 32) = 9.33$ with a significance p-level = 0.025. This ensures that the null hypothesis is false, and accordingly, the FRAP difference between the wine and the control groups increases over days with statistical significance. Moreover, other paired nonparametric tests can be also applied to support this issue. The Kolmogorov–Smirnov two sample test and the Mann–Withney U test revealed that PACs determined with the FRAP assay (**Table 4**) were statistically larger after 7 days of wine intake ($p < 0.05$). Ethanol metabolism can produce free radicals (*34*) as well as reduce glutathioine levels (*35*). Despite the alcohol intake (13.5% v/v, 300 mL/d), FRAP values increases after seven days of wine consumption. However, during the control period, the low phenolic diet led to a significant decrease ($p < 0.05$) in the FRAP when comparing the mean difference between the results of "day 0" and "day 7" (**Table 4**). Indeed, the differences between the FRAP values of the wine and the control group were significatively different ($p < 0.05$) on the seventh day of the intervention (**Table 4**).

However, the results obtained with the ORAC-FL method for subgroup comparison were not significant during the intervention.

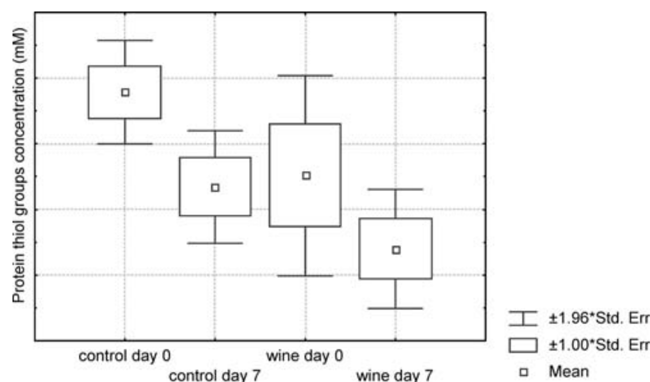


Figure 2. Protein thiol groups on days 0 and 7 with the control period and wine period.

Our data are different from those that have already been reported. After the intake of 200 mL of red wine or 250 mL of dealcoholized red wine, white wine, or champagne no changes in PAC were determined (*36*). On the other hand, PAC increased after acute but not repeated intake of grapeseed extract, fruit juice, fruit–vegetable concentrate, mushrooms, and red wine. The intake of orange juice (*37, 38*), tea (*39*), red wine, or dealcoholized red wine (*40, 41*) increased PAC after acute but not repeated consumption.

There are fundamental differences between our work and the work that has already been published (*36, 40, 41*). First, volunteers drank 300 mL of wine per day; second, we used the FRAP method; and third, and in our opinion the most important, the volunteers followed precise dietetic guidelines designed to test the effect of wine by avoiding the intereference of other dietetic phenolics.

The role of uric acid in PAC has already been pinpointed. After an hour of acute intake of 300 mL of red wine, the increase in the concentration of uric acid explains 60% of the PAC determined by the FRAP method (*41*). The increase in the uric acid concentration was associated with the increase in PAC determined after apples had been consumed. Plasma treatment with uricase diminished PAC (*42*).

Therefore, we included in our study the determination of endogenous antioxidants such as uric acid, albumin, bilirrubin, and protein thiol groups. The results for both intervention periods are summarized in **Table 5**. Linear correlation analysis showed a significant correlation $\beta = 0.53$ for FRAP and uric acid ($p < 0.05$) and also significant $\beta = 0.32$ for albumin concentration and ORAC ($p < 0.05$). The β coefficients represent the independent contributions of the variable to the prediction of the dependent variable. However, FRAP and ORAC values have no significant correlation. No significant differences were found for uric acid concentration in the control or wine groups. Therefore, the increase in FRAP values cannot be attributed to uric acid increase as it is in short-term studies (*41*).

From our results it can be deduced that a low phenolic diet causes a significant decrease in protein thiol groups ($p < 0.05$; see **Figure 2**). Plasma thiol groups are susceptible to oxidative damage, which explains why they are low in patients suffering from such diseases as rheumatoid arthritis or coronary artery disease (*43*). There are fewer protein thiol groups in patients suffering from essential hypertension than in normal subjects (*44*). These lower levels have been related to oxidative stress and proposed as a biomarker of aging-related diseases (*45*). Protein thiols have also been shown to have an emerging role in redox signaling as an adaptative stress response (*46*). Our results show that after a week the low phenolic diet causes a decrease in protein thiol concentration (**Figure 2**). But if this diet is accompanied with red wine the decrease is not significative ($p < 0.05$). In conclusion, the consumption of 300 mL of Monastrell red wine reduces the harmful effect of low phenolic diet on protein thiol groups and increases PAC. This is proof that red wine continues to exert antioxidant properties after having been consumed.

ABBREVIATIONS USED

PAC, plasma antioxidant capacity; FRAP, ferric reducing ability of plasma; ORAC, oxygen radical absorbance capacity; FL, fluorescein; TROLOX, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; TPTZ, 2,4,6-tripyridyl-*s*-triazine; AAPH, 2,2′-diazo bis amidine propane dihydrochloride.

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