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Antioxidant Capacity of Plasma after Red Wine Intake in Human Volunteers

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Plasma antioxidant capacity (AC) has been assessed in eight healthy human volunteers after wine intake. Analytical methods used for evaluating AC included the ferric reducing ability of plasma (FRAP) and oxygen radical absorbance capacity using two different fluorescent probes, β-phycoerythrin (ORAC-PE) and fluorescein (ORAC-FL). In addition, the concentrations of endogenous antioxidants such as uric acid, albumin, and bilirubin were determined. The suitability of analytical methods was evaluated with two different biological matrixes: plasma and serum. Plasma AC was determined before ingestion of 300 mL of red wine (baseline value) and 30, 55, and 120 min afterward. Maximum average increase in AC values was reached at 55 min. Plasma AC determined by ORAC-PE at time zero was significantly correlated with albumin concentration. Plasma AC determined with FRAP at time zero is well correlated with uric acid. Moreover, a good linear correlation was found between uric acid concentration and AC determined by FRAP in each volunteer. The maximum concentration of uric acid occurred after 55 min. Uric acid increase accounts for a nonnegligible part in FRAP values and must be evaluated when using this method for assessing AC in plasma.

KEYWORDS: Antioxidant capacity; wine; plasma; serum; phenolic compounds; ORAC; phycoerythrin; fluorescein; FRAP; uric acid; human volunteers

INTRODUCTION

Epidemiological studies evidence that moderate wine consumption protects against cardiovascular disease due to its alcoholic content and phenolic compounds (*1*). Red wine is known to be a source of polyphenolic compounds with antioxidant properties (*2*, *3*). In vitro antioxidant activity of wine has been assessed thoroughly employing different methodologies (*4*, *5*). However, the need to evaluate its antioxidant activity in vivo has been stated several times (*6*, *7*). To date, little is known about the utility of phenolic compounds in humans, the extent to which they are absorbed, or their metabolism and biological actions.

The complete evaluation of the antioxidant activity of a given food or beverage needs to consider its influence on certain biomarkers before and after its intake. Plasma AC has been proposed as a good measure of antioxidant status (*8*). Plasma antioxidant capacity (AC) encompasses the action of antioxidant compounds from diet and other endogenous compounds that scavenge radicals in living systems. It is also modulated by radical oxygen species production. Endogenous nonenzymatic antioxidants such as albumin, bilirubin, and uric acid constitute a relevant contribution to antioxidant defenses. The use of different methods has been suggested as necessary to assess AC changes and to interpret results adequately (*9*). Besides, some methodological problems have been raised. For instance, there is not a consensus on whether measures must be performed in plasma (*8*, *10*, *11*) or serum (*12*, *13*), and a lack of repeatability has been reported for some assays (*14*). Among the most used methods to determine plasma AC, we discuss here FRAP and ORAC. The latter originally used β-phycoerythrin (*9*, *15*), but recently the use of fluorescein as a fluorescent probe has been proposed (*14*). Difficulties in interpreting results have been an object of scientific debate related to the role of endogenous antioxidants (*16*). This work has various purposes: first, to evaluate some methodological concerns for assessing AC such as the type of biological fluid involved in the assay (plasma or serum), the repeatability of selected methods, linearity, and sample dilution; second, to elucidate whether red wine intake modifies human plasma AC and to compare the results obtained by different methods; and third, to evaluate the contribution of endogenous antioxidants to obtain values with these analytical methods.

MATERIALS AND METHODS

Chemicals, Reagents, and Equipment. β-Phycoerythrin (β-PE), 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 H_3PO_4 85% were purchased from Sigma. HCl 32%, ascorbic acid, and acetonitrile (HPLC grade) were provided from MERCK. Fluorescein sodium salt and acetic acid glacial (HPLC grade) were supplied by Fluka. AAPH (2,2′-diazo bis amidine propane dihydrochloride) was from Wako Chemicals. K_2HPO_4 was purchased from Probus. Vacutainers with heparin were provided by Becton Dickinson (UK). Kits of albumin, bilirubin, and uric acid were puchased from reagents SPINREACT.

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A fluorescence spectrophotometer, UV−vis spectrophotometer, and liquid chromatographic equipment (Waters) connected in series to a Waters photodiode array detector and a Waters fluorescence detector were used.

The data were processed with Statistica StatSoft single user version and Excel softwares.

Collection of Samples. Eight healthy female volunteers aged 25−35 (28.25 ± 3.33) were recruited for the study. The average body mass index (BMI) was 20.97 ± 2.73 kg/m^2 (range 17.30−25.59), which was within the normal range according to the WHO.

The subjects had healthy life habits; they were nonsmokers and nonalcoholic drinkers. They had not taken vitamin or mineral supplements and had not consumed drugs or antibiotics known to interfere with intestinal absorption and/or the P450 enzymatic system for 4 weeks before the start of the study.

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

Ethical approval for the study was obtained from the Ethical Research Committee of the University Hospital Virgen Macarena, Seville. All subjects were informed and signed a written consent prior taking part in the study.

Subjects were instructed to avoid consumption of dietary antioxidants the day before the study. They did not take vitamin supplements or any of the following foods: fruits, vegetables, virgin olive oil, tea, chocolate, and alcoholic drinks, which can interfere in the evaluation of plasma antioxidant capacity. Volunteers fulfilled a 24 h dietetic questionnaire to make sure recommendations were followed adequately. Each subject fasted for 12−14 h before the experience started. Volunteers were asked to consume 300 mL of red wine in 5 min. Venous blood sample was obtained by antecubital venipuncture, with a heparin vacutainer. Blood extraction was performed before wine ingestion (baseline value) and 30, 55, and 120 min after wine intake. Blood samples were immediately centrifuged at 12 000*g* for 3 min, avoiding unnecessary exposure to light. The plasma obtained was separated in four different aliquots for the subsequent assays. One of these aliquots was immediately stabilized with ascorbic acid (1%) and orthophosphoric acid (10 μL/mL) in order to analyze phenolic compounds by HPLC (*17*). Plasma samples were stored at −80 °C until analysis. In addition, the repeatability of the antioxidant methods was studied: blood was obtained by venipuncture and heparin vacutainer from a single volunteer donor. Two extractions of 15 mL of blood were performed, one to obtain plasma and the second one to obtain serum.

All volunteers drank the same red wine. Red wine was chosen between a varied and large set of red wine samples analyzed in our laboratory previously. It was selected according to its highest values for in vitro antioxidant activity and total phenolic index (*5*). The values of antioxidant activity of the selected wine determined with different assays were as follows: total polyphenolic index = 2498 mg/L, ORAC-PE = 10 614 ± 157 μM, ORAC-FL = 38 740 ± 533 μM and FRAP = 7021 ± 140 μM. The alcoholic content of wine was 12% v/v.

Oxygen Radical Absorbance Capacity with β-Phycoerytrin as Fluorescent Probe (ORAC-PE). The method was based on the detection of oxidative damage to a protein, (*R*)- or β-phycoerythrin (PE) of *Porphiridium cruentum*, through the decrease in its fluorescence emission after suffering oxidative attack (*9*). AAPH was used as a peroxyl radical generator and TROLOX as an antioxidant standard.

The reagent concentrations (*15*) were as follows: phosphate buffer (75 mM, pH 7), PE (68 mg/L), AAPH (160 mM), and TROLOX (20 μM). The plasma was diluted 150-fold with phosphate buffer. The final reaction mixture for the assay is described below:

150 μL sample + 150 μL PE (68 mg/L) + 75 μL AAPH (160 mM)

The assays were carried out at 37 °C in fluorimeter cuvettes in triplicate.

The fluorescence was recorded immediately (excitation wavelength, 540 nm; emission wavelength, 565 nm) every 5 min for a period of 60 min, until the final value was less than 5% of the initial one.

Antioxidant capacity was then related to the inhibition of the action of the reactive species and compared to TROLOX. This assay uses an area-under-curve (AUC) technique for quantification, and thus combines both the inhibition time and inhibition degree of the free radical action by antioxidants into a single quantity (*7*). Results are expressed as ORAC values or TROLOX equivalents (μmol/L): μmol of Trolox per liter of plasma sample (*15*).

ORAC Assay with Fluorescein as Fluorescent Probe (ORAC-FL). The reagent concentrations were as follows: phosphate buffer (75 mM, pH 7.4), fluorescein (FL) (2934 mg/L), AAPH (221,25 mM), and TROLOX (20 μM). The plasma was diluted 750-fold with phosphate buffer. The assay was carried out at 37 °C in fluorimeter cuvettes in triplicate. The final reaction mixture was composed of

150 μL sample + 150 μL FL (2934 mg/L) + 75 μL AAPH (221.25 mM)

The fluorescence was recorded (excitation wavelength, 490 nm; emission wavelength, 515 nm) every 5 min for a period of 60 min, until the final value was less than 5% of the initial one. Results were calculated as previously described for ORAC-PE assay (*14*).

Ferric Reducing Ability of Plasma (FRAP). This method is based on the measure of the reducing power of plasma. Antioxidants compounds can donate electrons, reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) at low pH. TPTZ in the reaction medium can bind the ferrous ion at low pH. An intense blue color is developed with an absorption maximum at 593 nm: $Fe(TPTZ)^{3+} \rightarrow Fe(TPTZ)^{2+}$. The color intensity is a measurement of the ferric reducing ability of plasma and, therefore, of its antioxidant capacity.

The reagent concentrations (*18*) were as follows: acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in HCl 40 mM), $FeCl_3 \cdot 6H_2O$ (20 mM). FRAP reagent was prepared by mixing: 10 volumes of acetate buffer, 1 volume of TPTZ, and 1 volume of $FeCl_3 \cdot 6H_2O$. 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 shaken and the absorbance recorded for 8 min at 593 nm. The measurements of absorbance were performed in triplicate.

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

Quantitative Determination of Albumin. Albumin in the presence of bromocresol green at a slightly acidic pH produces a color change from yellow-green to green-blue. The intensity of the color formed (λ = 630 nm) is proportional to the albumin concentration in the sample (*19*).

Quantitative Determination of Bilirubin. Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions present in serum, bilirubin−glucuronide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin, the direct is also determined; the results correspond to total bilirubin. The intensity of the color formed (λ = 555 nm) is proportional to the bilirubin concentration in the sample (*19*).

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

$$\text{uric acid} + 2H_2O + O_2 - \text{uricase} \rightarrow \text{allantoine} + CO_2 + 2H_2O_2$$

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

The intensity of the red color formed (λ = 520 nm) is proportional to the uric acid concentration in the sample (*19*).

Analysis of Wine Phenolic Compounds in Human Plasma by HPLC (High-Performance Liquid Chromatography). A plasma sample (1 mL) was treated with (1%, w/v) NaF and 4 mL of acidified

Table 1. Analysis of Repeatability[a]

ORAC-PE[b]		
dilution	plasma	serum
1:100	1744 ± 144	1947 ± 117
	cv 8.24	cv 6.02
1:200	2007 ± 215	2399 ± 229
	cv 10.71	cv 9.55
1:150	2288 ± 215	1965 ± 73.1
	cv 9.39	cv 3.72
ORAC-FL[b]		
dilution	plasma	serum
1:1000	19732 ± 2465	18824 ± 2221
	cv 12.49	cv 11.80
1:750	18873 ± 681	18603 ± 2242
	cv 3.61	cv 12.05
1:500	19091 ± 1769	14135 ± 702
	cv 9.27	cv 4.97
FRAP[c]		
dilution	plasma	serum
no dilution	395 ± 10.94	367 ± 16.29
	cv 2.77	cv 4.43
1:2	288 ± 2.19	188 ± 7.14
	cv 0.76	cv 3.79
1:3	217 ± 9.63	145 ± 8.32
	cv 4.44	cv 5.75

[a] Values represent mean ± SD calculated with each method (ORAC-PE, ORAC-FL, FRAP), in two different matrixes (plasma or serum) to 3 dilutions. Variation coefficient (cv) ($n = 6$) is shown. ORAC-PE, oxygen radical absorbance capacity-phycoerythrin; ORAC-FL, oxygen radical absorbance capacity-fluorescein; FRAP, ferric reducing ability of plasma. [b] Results expressed as μmol of Trolox per L sample. [c] Results expressed as μmol of Fe^{2+} per L sample.

ethanol (50 mM) to inhibit enzymatic activity and to perform the deproteinization, respectively. These treatments have been previously reported to have good recovery in quantification of phenolic compounds in biological fluids (*17*).

Chromatographic conditions were as follows: The column was a Merck LiChroCART 250-4 Superspher 100 RP-18 1.16056.0001, protected by a LiChroCART guard cartridge 1.50957.0001. The syringe filters were Anotop (Anopore) 0.2 μm Whatman 6809 1022. The elution gradient has been previously reported (*20*). Analyses were carried out in duplicate.

Fluorescence detection recorded the following λ_{ex} and λ_{em}: time interval 0−12 min, $\lambda_{ex} = 345$ nm and $\lambda_{em} = 380$ nm; time interval 12−27 min, $\lambda_{ex} = 290$ nm and $\lambda_{em} = 310$ nm; time interval 27−35 min, $\lambda_{ex} = 360$ nm and $\lambda_{em} = 435$ nm; time interval 35−60 min, $\lambda_{ex} = 350$ nm and $\lambda_{em} = 385$ nm.

RESULTS AND DISCUSSION

Plasma Antioxidant Capacity: Methodological Considerations. Different analytical methods have been applied to assess antioxidant capacity of biological fluids (*7*). In this work ORAC-PE , ORAC-FL, and FRAP have been selected, as they have been used in most studies. Some authors use plasma (*11*, *17*), while others, serum (*12*, *13*). To ensure an appropriate application of analytical assays, repeatability of the methods has been evaluated both in human plasma and human serum extracted from the same human volunteer. Results of the five determinations at three different sample dilution levels for plasma and serum for the three methods under study are shown in **Table 1**. As can be observed, the coefficients of variation (CV) are below the 15% accepted for biological samples according to the AOAC criteria (*21*). Therefore, these methods were sufficiently repeatable to allow assessment of antioxidant capacity both in serum and human plasma.

Table 2. Analysis of Linearity[a]

	r^2[b] (dilution-antioxidant capacity)		
	ORAC-PE	ORAC-FL	FRAP
plasma	0.91511	0.99873	0.99978
serum	0.97930	0.94436	0.99489

[a] Values represent correlation coefficients between antioxidant capacity measured with each method (ORAC-PE, ORAC-FL, FRAP) and sample dilution (plasma or serum). ORAC-PE, oxygen radical absorbance capacity-phycoerythrin; ORAC-FL, oxygen radical absorbance capacity-fluorescein; FRAP, ferric reducing ability of plasma. [b] Correlation coefficient.

Values obtained for antioxidant capacity of plasma were higher than those determined in serum. Indeed, analysis of variance showed statistical differences among them ($p < 0.5$). It has been suggested that plasma and not serum is to be preferred, as serum is obtained after clotting blood at room temperature. During aggregation, platelets release radical oxygen species (ROS) and this could be a possible explanation of the lower values of serum with regard to plasma (*22*). Moreover, in our study, endogenous antioxidants such as albumin, uric acid, and bilirubin have been determined. Plasma is usually employed for their assay in normal clinical practice, and antioxidant determinations should be done in the same biological fluid. For all the above-explained reasons, plasma was used in this study of human volunteers. Linearity of the different dilutions has been checked, and correlation coefficients are shown in **Table 2**.

This previous experience allowed us to choose an adequate dilution of plasma for the subsequent analysis. In the case of ORAC-PE, a dilution of 1:150 was selected. At this level, the final β-phycoerythrin fluorescence was 5% of the initial value in the time of analysis (1 h). Thus, it can be considered a normal decay, while higher concentration did not achieve this requirement. For FRAP assay, dilution of plasma is not recommended, as absorbance values obtained with 1:2 and 1:3 dilutions were too low and the error was higher. The determinations were performed with a dilution of 1:750, and the ORAC-FL method gave the lowest variation coefficients and was therefore chosen for subsequent analysis.

Plasma Antioxidant Capacity after Wine Intake. Table 3 shows plasma antioxidant capacities determined in the eight volunteers with the three assays under study. Plasma samples were collected at different times (time 0 or baseline value, and 30, 55, and 120 min after wine intake). **Table 3** includes mean value and standard deviation of the triplicate measurements for each sample. Mean values for the whole group taking part in the study were calculated. Analysis of variance (ANOVA) revealed that antioxidant values determined before wine intake were statistically different from those measured 30 min after wine ingestion ($p < 0.5$) for the whole group of subjects taking part in the study. Statistical differences for antioxidant value between baseline and 55 min after wine intake were also tested. These ANOVA results were identical with the three methods under study. Thus, conclusions extracted can be considered consistent.

However, absolute values can vary from one subject to another, and maximum antioxidant values were reached at different times. This can be due to individual variability in absorption and metabolism processes, though the group was rather homogeneous concerning their physical characteristics and life habits. Despite ANOVA-proved statistical significant

Table 3. Plasma Antioxidant Capacity Measured in Plasma of Human Volunteers (V1–8) at Baseline, 30, 55, and 120 min after Consumption of 300 mL of Red Wine[a]

volunteer	time (min)	ORAC-PE[b]	ORAC-FL[b]	FRAP[c]
V1	0	1896 ± 4.55	21 668 ± 1013	293 ± 13.0
	30	1840 ± 123	28 155 ± 545	419 ± 12.7
	55	1947 ± 206	29 725 ± 4602	426 ± 11.7
	120	1758 ± 23.1	15 310 ± 1224	373 ± 18.2
V2	0	1527 ± 15.9	18 162 ± 653	401 ± 23.2
	30	1747 ± 262	17 979 ± 335	455 ± 3.08
	55	1574 ± 20.2	21 727 ± 209	476 ± 10.2
	120	1417 ± 82.1	16 753 ± 302	443 ± 18.0
V3	0	1330 ± 16.6	15 657 ± 33.5	251 ± 8.27
	30	1372 ± 132	15 752 ± 16.8	364 ± 4.06
	55	1531 ± 39.8	19 409 ± 1059	365 ± 2.41
	120	1534 ± 69.8	17 425 ± 1796	325 ± 2.61
V4	0	1702 ± 63.5	20 028 ± 50.3	440 ± 9.20
	30	1816 ± 67.2	22 274 ± 1322	457 ± 6.29
	55	2004 ± 8.35	23 291 ± 377	493 ± 3.75
	120	1877 ± 51.6	18 606 ± 1187	553 ± 3.95
V5	0	1933 ± 46.3	14 366 ± 50.3	398 ± 7.14
	30	1897 ± 0.38	14 224 ± 863	461 ± 9.20
	55	2081 ± 86.1	17 052 ± 431	525 ± 2.79
	120	1710 ± 86.9	13 342 ± 1181	534 ± 5.61
V6	0	1617 ± 84.6	19 222 ± 410.4	565 ± 3.10
	30	1981 ± 92.2	18 615 ± 1813	608 ± 5.42
	55	1922 ± 107	22 334 ± 1202	671 ± 3.82
	120	2121 ± 157	18 144 ± 1173	659 ± 6.58
V7	0	1691 ± 120	17 428 ± 879	357 ± 4.57
	30	2678 ± 107	22 216 ± 1269	428 ± 6.52
	55	2405 ± 143	21 419 ± 1164	478 ± 6.28
	120	1842 ± 155	16 984 ± 469	462 ± 7.40
V8	0	1454 ± 103	18 887 ± 763	479 ± 3.97
	30	1501 ± 97.1	19 062 ± 1332	522 ± 4.51
	55	1801 ± 63.7	24 141 ± 1102	589 ± 8.96
	120	1618 ± 109	17 416 ± 704	570 ± 1.81
mean value	0	1644 ± 207	18 177 ± 2349	398 ± 101
	30	1854 ± 390	19 785 ± 4384	464 ± 73.3
	55	1908 ± 281	22 387 ± 3712	503 ± 94.6
	120	1735 ± 219	16 748 ± 1692	490 ± 110

[a] Values presented are means and their standard deviations (SD). ORAC-PE, oxygen radical absorbance capacity-phycoerythrin; ORAC-FL, oxygen radical absorbance capacity-fluorescein; FRAP, ferric reducing ability of plasma. [b] Results expressed as μmol of Trolox equivalents per L sample. [c] Results expressed as μmol of Fe^{2+} per L sample.

differences for the whole group, data were examined in detail. Student's test (*t*-Student) was applied to verify whether the increases in antioxidant capacity of each volunteer determined at different times were significant. Therefore, antioxidant capacity values of each single volunteer at times zero and 30 min; zero and 55 min; 30 and 55 min; and zero and 120 min were compared. Results are shown in **Table 4** and depend on the method used. If ORAC-PE is considered, it can be observed that AC of plasma taken at the different times did not show significant differences in four volunteers of a total of eight at any of the times compared. Seven subjects have different AC values after wine intake (30 or 55 min) if the ORAC-FL method is used. In addition, FRAP method shows differences at every time considered. These results can be explained by the larger CV of ORAC-PE at the dilution of analysis (cv = 9.39) instead of the cv = 3.61 for the ORAC-FL. Therefore, it is advisable to use ORAC-FL to follow plasma AC changes.

Average increases in AC expressed as a percentage of baseline level for the whole group are represented in **Figures 1**−**3** for all methods under study. As can be observed, the maximum value was reached at 55 min after wine intake with all methods, detailed as follows: 16.4% for ORAC-PE, 22.8% for ORAC-FL, and 28.6% for FRAP of baseline antioxidant capacity. ORAC-PE and FRAP have been used previously to determine plasma antioxidant values after food or wine intake. After the ingestion of wine or dealcoholized wine, 16.9% (*6*)

Table 4. Statistical Analysis of Data: *t*-Student Test for Comparison in Antioxidant Capacity for Each Single Volunteer (V1–8) at Different Time Intervals (0 and 30, 0 and 55, 0 and 120, and 30 and 55 min) after Consumption of 300 mL of Red Wine and Analysis of Variance (ANOVA) for the Whole Group (ΣV)[a]

		time intervals			
volunteer	method	0–30	0–55	0–120	30–55
V1	ORAC-PE	0.579673	0.761846	0.014154*	0.505032
	ORAC-FL	0.000415*	0.136802	0.009202*	0.483492
	FRAP	0.000009*	0.000005*	0.000360*	0.487333
V2	ORAC-PE	0.340979	0.069962	0.173080	0.317487
	ORAC-FL	0.757405	0.018014*	0.109332	0.005507*
	FRAP	0.003862*	0.001101*	0.029311*	0.007888*
V3	ORAC-PE	0.593955	0.003732*	0.013416*	0.244391
	ORAC-FL	0.070019	0.037673*	0.298675	0.039507*
	FRAP	0.000029*	0.000021*	0.000117*	0.776399
V4	ORAC-PE	0.149613	0.007923*	0.049235*	0.059371
	ORAC-FL	0.106993	0.006720*	0.206613	0.386249
	FRAP	0.022011*	0.000039*	0.000001*	0.000062*
V5	ORAC-PE	0.389491	0.165943	0.085576	0.094747
	ORAC-FL	0.837697	0.012820*	0.345012	0.053534
	FRAP	0.000037*	0.000020*	0.000000*	0.000759*
V6	ORAC-PE	0.054231	0.086650	0.057300	0.613750
	ORAC-FL	0.689612	0.074125	0.344654	0.136816
	FRAP	0.000280*	0.000003*	0.000024*	0.000082*
V7	ORAC-PE	0.013002*	0.032443*	0.389787	0.162678
	ORAC-FL	0.048248*	0.060775	0.592938	0.580173
	FRAP	0.000099*	0.000001*	0.000030*	0.000159*
V8	ORAC-PE	0.687141	0.055965	0.261150	0.067288
	ORAC-FL	0.858212	0.002456*	0.118860	0.018207*
	FRAP	0.000046*	0.000007*	0.000004*	0.000011*
ΣV	ORAC-PE	0.037933*	0.004195*	0.255161	0.717248
	ORAC-FL	0.049621*	0.000319*	0.045130*	0.247328
	FRAP	0.002024*	0.000058*	0.000775*	0.081531

[a] Statistically significant differences are marked with an asterisk* ($p < 0.05$). ORAC-PE, oxygen radical absorbance capacity-phycoerythrin; ORAC-FL, oxygen radical absorbance capacity-fluorescein; FRAP, ferric reducing ability of plasma.

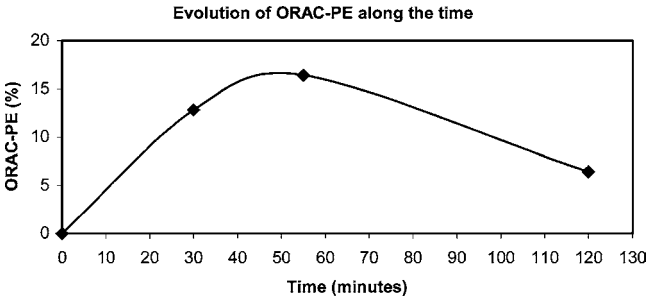

Figure 1. Changes (%) in ORAC-PE after consumption of 300 mL of red wine. Values are means for eight subjects. ORAC-PE, oxygen radical absorbance capacity-phycoerythrin.

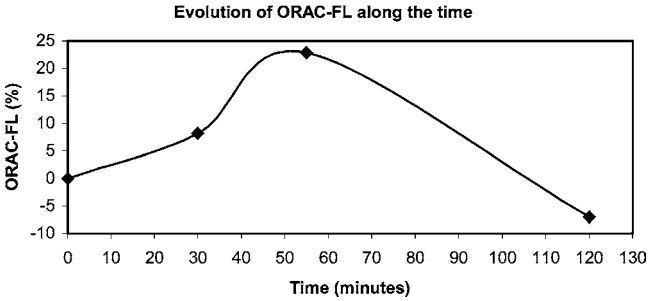

Figure 2. Changes (%) in ORAC-FL after consumption of 300 mL of red wine. Values are means for eight subjects. ORAC-FL, Oxygen radical absorbance capacity-fluorescein.

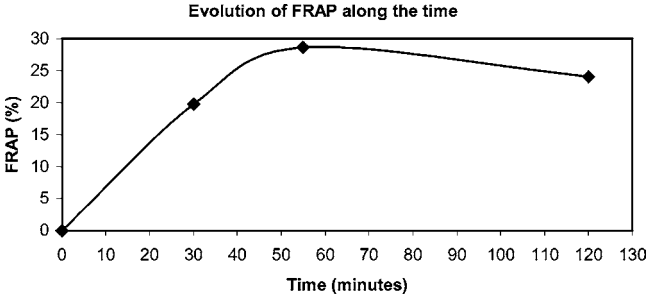

Figure 3. Changes (%) in FRAP after consumption of 300 mL of red wine. Values are means for eight subjects. FRAP, ferric reducing ability of plasma.

and 14.35% (*10*) increases in activity, respectively have been reported for ORAC-PE. These results are very similar to ours. Besides, wine intake produces a higher increase than the 9% AC increase determined after the intake of 294 g of spinach (*6*) and similar to the 14% reported after the intake of 240 g of strawberries (*6*) or the 15% obtained after the intake of 100 g of blueberries (*13*). On the other hand, only a 5.5% of increase was obtained using FRAP assay after the intake of the same volume of wine by elderly female volunteers (*6*). This value is considerably lower than that of our work. ORAC-PE and ORAC-FL values present a signficant linear correlation ($r = 0.81240$). However, the correlation decreased when the methods ORAC-PE and FRAP were compared ($r = 0.76991$). In addition, ORAC-FL and FRAP are not correlated.

Antioxidant Determination in Plasma: Phenolic and Endogenous Compounds. Phenolic compounds, including benzoic acids, cinnamic acids, flava-3-ols, and resveratrol, were considered. Plasma sample handling and treatments to obtain recoveries near 80% were described previously in an analytical in vitro study (*17*). Though these recommendations were followed exhaustively, phenolic compounds could not be detected in volunteer plasma after wine intake. If they are absorbed, quantities are below quantification limits described in the above-mentioned paper ((+)-catechin (0.6 mg/L); (−)-epicatechin (0.4 mg/L); caffeic acid (0.1 mg/L)). These data are in agreement with the poor absorption reported for flavonoids from different food sources (as after the ingestion of apples) (*23*).

On the other hand, concentrations of albumin, bilirubin, and uric acid determined in plasma are shown in **Table 5**. Linear regression analysis showed correlations between these compounds and antioxidant values before wine intake: Indeed, correlations between uric acid and AC determined with FRAP are significant ($r = 0.819$). Besides, ORAC-PE and albumin values are correlated ($r = 0.815$). Moreover, a linear correlation was found for the increases in FRAP values and uric acid along time in each volunteer (r values range between 0.943 and 0.995). Uric acid has been reported to contribute greatly to FRAP, accounting for 60% of the value (*24*). In our case, the average increase in uric acid concentration was 26%. We calculated 60% for FRAP, which is the expected contribution of uric acid according to the above published data. This theoretically calculated value increases up to 26%. Thus, the increase in AC determined with FRAP is partly explained by an increase in uric acid. The effect of alcohol intake in serum levels of uric acid has been a matter of discussion very recently (*25*), as the mechanisms involved are not completely known. Indeed, after ethanol intake (0.6 g/kg), uric acid did not increase (*26*), and neither did plasma xanthine oxidase activity after oral administration of ethanol (*27*). During short-term intravenous ethanol administration, serum urate levels, urate clearance, and urinary uric acid excretion were not substantially altered from the baseline period (*28*). Alcohol intake made no difference in uric acid concentration for no drinkers but increased by about 6.7 mg/L in regular drinkers (*29*) who drank half the dose of ethanol used in our study. However, contrary results have been reported, as uric acid increased after the administration of wine but not after dealcoholized wine intake. In this study, authors suggested that alcohol intake produces an increase in ATP consumption in the hepatocyte and thus an increase in uric acid formation by purin metabolism (*30*). In our opinion, contribution of uric acid to FRAP values has to be taken into account when assessing the effects of food intake in plasma AC in order to interpret data adequately. Our results show that uric acid cannot explain all the observed increase in antioxidant capacity, especially because the other methods used are not related to it. Indeed, the antioxidant activity of uric acid determined by ORAC-FL is very low (1.38 μM) and has a negligible contribution to plasma ORAC-FL values. It has been suggested that polyphenols might regenerate urate from its radical species (*23*). This explanation for the underlying antioxidant mechanism fits our results, as we have observed an increase in uric acid and the determination of phenolic compounds in plasma yielded poor results (all under detection limits).

In conclusion, the studied methods are sufficiently repeatable to allow assessment of antioxidant capacity both in serum and in human plasma. The antioxidant capacity maximum value is reached 55 min after red wine intake with all methods, and the increases were as follows: 16.4% for ORAC-PE, 22.8% for ORAC-FL, and 28.6% for FRAP of baseline value. The

Table 5. Concentrations of Albumin, Bilirubin, and Uric Acid in Plasma from Volunteers (V1–8) at Different Times (Baseline, 30, 55, and 120 min) Following Administration of 300 mL of Red Wine[a]

volunteer	time (min)	albumin (g/dL)	bilirubin (mg/dL)	uric acid (mg/dL)
V1	0	3.7 ± 0.05	0.8 ± 0.05	2.8 ± 0.05
	30	3.7 ± 0.05	0.6 ± 0.00	3.2 ± 0.10
	55	3.8 ± 0.03	0.7 ± 0.03	3.4 ± 0.03
	120	3.8 ± 0.06	0.6 ± 0.10	3.1 ± 0.05
V2	0	3.8 ± 0.04	1.5 ± 0.04	2.7 ± 0.10
	30	3.7 ± 0.03	1.5 ± 0.01	3.1 ± 0.00
	55	4.0 ± 0.01	1.7 ± 0.03	3.3 ± 0.06
	120	3.9 ± 0.02	1.5 ± 0.02	3.2 ± 0.03
V3	0	4.4 ± 0.03	0.6 ± 0.08	1.6 ± 0.06
	30	4.5 ± 0.05	0.7 ± 0.05	2.4 ± 0.05
	55	4.5 ± 0.03	0.7 ± 0.03	2.5 ± 0.02
	120	4.6 ± 0.02	0.7 ± 0.10	2.3 ± 0.10
V4	0	3.7 ± 0.00	0.3 ± 0.00	2.5 ± 0.08
	30	3.8 ± 0.04	0.4 ± 0.02	2.6 ± 0.03
	55	3.8 ± 0.01	0.4 ± 0.05	2.9 ± 0.04
	120	3.7 ± 0.05	0.4 ± 0.07	3.2 ± 0.04
V5	0	3.7 ± 0.02	0.7 ± 0.06	2.4 ± 0.00
	30	3.9 ± 0.04	0.8 ± 0.05	3.1 ± 0.02
	55	3.9 ± 0.01	1.0 ± 0.01	3.6 ± 0.00
	120	4.0 ± 0.05	1.1 ± 0.80	3.8 ± 0.05
V6	0	3.8 ± 0.01	0.4 ± 0.10	4.8 ± 0.06
	30	3.8 ± 0.03	0.4 ± 0.05	5.2 ± 0.03
	55	4.0 ± 0.03	0.5 ± 0.00	5.3 ± 0.02
	120	4.0 ± 0.01	0.5 ± 0.03	5.0 ± 0.07
V7	0	3.9 ± 0.04	0.6 ± 0.02	3.0 ± 0.05
	30	4.1 ± 0.02	0.7 ± 0.05	3.7 ± 0.05
	55	4.1 ± 0.06	0.7 ± 0.04	4.3 ± 0.02
	120	3.8 ± 0.00	0.7 ± 0.06	4.0 ± 0.06
V8	0	4.0 ± 0.03	1.0 ± 0.07	3.3 ± 0.00
	30	4.0 ± 0.04	1.6 ± 0.00	3.7 ± 0.05
	55	4.0 ± 0.01	1.6 ± 0.03	4.0 ± 0.00
	120	3.9 ± 0.02	1.5 ± 0.05	3.8 ± 0.05

[a] Values represent the means and their standard deviations (SD) in each subject.

contribution of uric acid to FRAP values should be taken into account in further studies of plasma antioxidant capacity.

ABBREVIATIONS USED

AC, antioxidant capacity; FRAP, ferric reducing ability of plasma; ORAC, oxygen radical absorbance capacity; FL, fluorescein; β-PE, β-phycoerythrin; 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; DMSO, dimethylsulfoxide; POD, peroxidase; 4-AP, 4-aminohenazone; DCPS, 2-4 dichlorophenol sulfonate.

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