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ORIGINAL CONTRIBUTION

Effects of de-alcoholised wines with different polyphenol content on DNA oxidative damage, gene expression of peripheral lymphocytes, and haemorheology: an intervention study in post-menopausal women

Lisa Giovannelli · Vanessa Pitozzi · Cristina Luceri · Lucia Giannini · Simona Toti · Simonetta Salvini · Francesco Sera · Jean-Marc Souquet · Veronique Cheynier · Francesco Sofi · Lucia Mannini · Anna Maria Gori · Rosanna Abbate · Domenico Palli · Piero Dolara

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Abstract
Purpose Epidemiological studies suggest that a moderate consumption of wine is associated with a reduced risk of cardiovascular diseases and with a reduced mortality for all causes, possibly due to increased antioxidant defences. The present intervention study was undertaken to evaluate the in vivo effects of wine polyphenols on gene expression in humans, along with their supposed antioxidant activity.

Methods Blood haemorheology and platelet function were also evaluated. In order to avoid interferences from alcohol, we used de-alcoholised wine (DAW) with different polyphenol content. A randomised cross-over trial of high-proanthocyanidin (PA) red DAW (500 mL/die, PA dose = 7 mg/kg b.w.) vs. low-PA rosé DAW (500 mL/die, PA dose = 0.45 mg/kg) was conducted in 21 post-menopausal women in Florence, Italy. Oxidative DNA damage by the comet assay and gene expression by microarray was measured in peripheral blood lymphocytes, collected during the study period. Blood samples were also collected for the evaluation of haematological, haemostatic, haemorheological, and inflammatory parameters.
Results The results of the present study provide evidence that consumption of substantial amounts of de-alcoholised wine for 1 month does not exert a protective activity towards oxidative DNA damage, nor modifies significantly the gene expression profile of peripheral lymphocytes, whereas it shows blood-fluidifying actions, expressed as a significant decrease in blood viscosity. However, this effect does not correlate with the dosage of polyphenols of the de-alcoholised wine.
Conclusions More intervention studies are needed to provide further evidence of the health-protective effects of wine proanthocyanidins.

Keywords Proanthocyanidins · Wine · DNA damage · Comet assay · Microarray · Transcriptomics

L. Giovannelli (✉) · V. Pitozzi · C. Luceri · L. Giannini · S. Toti · P. Dolara
Department of Preclinical and Clinical Pharmacology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy
e-mail: lisa.giovannelli@unifi.it

S. Salvini · F. Sera · D. Palli
Molecular and Nutritional Epidemiology Unit, ISPO Cancer Prevention and Research Institute, Florence, Italy

J.-M. Souquet · V. Cheynier
INRA, UMR1083 Sciences Pour l'Oenologie, 34060 Montpellier, France

F. Sofi
Regional Agency of Nutrition, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

F. Sofi · R. Abbate
Centro Interdipartimentale per la Ricerca e la Valorizzazione degli Alimenti, University of Florence, Florence, Italy

L. Mannini · A. M. Gori · R. Abbate
Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Florence, Italy

A. M. Gori
Centro S. Maria agli Ulivi, Fondazione Don Carlo Gnocchi IRCCS, Impruneta, 50134 Florence, Italy

Introduction

There is consistent evidence that a diet rich in fruits and vegetables is associated with lower incidence of cardiovascular diseases and some types of cancer [1, 2]. It has

been hypothesised that this might be due to the phenolic content of these foods. Natural polyphenols have been extensively studied in experimental models for their antioxidant, antiinflammatory, anticancer, and cardiovascular-protective actions [3–5].

Wine is one of the main dietary sources of polyphenols in some western countries [6]. Epidemiological studies suggest that moderate consumption of wine is associated with a reduced risk of cardiovascular disease [7] and with reduced mortality for all causes [8]. These effects might derive at least in part from increased antioxidant defences [9]. In fact, intervention studies have shown that wine and wine extracts improve plasma antioxidant status [10, 11], increase protection from oxidation of LDL and DNA [10, 12], and reduce F_2 isoprostane production [13]. However, the antioxidant activity of wine and wine extracts has not been confirmed in other intervention studies with red wine [14, 15], and even a pro-oxidant action of red wine has been described [15], which, however, has been associated with the alcoholic component [16].

The different biological activities of polyphenols rely not only on their free-radical scavenging action but also on the ability of modulating protein activity and expression. An inhibitory effect of wine polyphenols on the expression of genes of inflammatory pathways has been described in vivo in the rat colon mucosa of naïve and carcinogen-treated animals [17, 18]. Grape seed procyanidins vary the expression of genes related to cholesterol metabolism in rat liver, and that could partly explain the beneficial effects of wine on the lipidemic profile [19]. In vitro, wine polyphenols have been shown to reduce smooth muscle cell proliferation in rat aorta through the inhibition of the expression of transcription factors and cyclin A [20] and to influence the expression of specific genes involved in cell adhesion and fibrinolysis in cultured primary human endothelial cells [21]. Pathways related to cholesterol and lipoprotein metabolism have also been shown to be modulated by wine polyphenols in cultured hepatocytes [22]. No gene expression data have been produced in humans up to date.

Blood haemorheology and platelet function are related to the pathogenesis of cardiovascular diseases [23, 24]. Recently, some studies showed an influence of dietary habits on lipid, inflammatory and haemorheological variables [25], but no data on the effect of polyphenols and such parameters have been published.

With these premises, the present intervention study was undertaken to evaluate the in vivo effects of wine polyphenols on gene expression and on haematological, haemorheological and inflammatory parameters in humans, along with their supposed antioxidant activity. We chose to use de-alcoholised wine to be able to significantly increase the daily intake of the polyphenols in the study, without interferences from alcohol.

Materials and methods

Recruitment of volunteers

This study was approved by the Azienda Universitaria Ospedaliera Careggi, Florence, Italy, Health Authority's Ethics Committee. All the employed procedures were in conformity with the Declaration of Helsinki for medical research involving human subjects. Flyers advertising for the trial were posted in clinics and laboratories at the University of Florence, at the blood donor clinics, among women's health initiative groups and in other out-patient clinics in Florence, Italy. The flyer briefly explained the study, the eligibility criteria, and invited interested women to telephone to our office for enrolment. Selection criteria included being a healthy post-menopausal woman (no menstrual periods in the last 12 months), non smoker or ex-smoker, with no history of treated hypercholesterolaemia and/or hypertension, no history of cancer, cardiovascular disease, diabetes or other important chronic conditions, no current use of aspirin, other medications, and antioxidant vitamins. Eligibility criteria included the willingness to adhere to the study protocol, involving consumption of de-alcoholised wine with high or low proanthocyanidin (PA) content and some dietary restrictions, as indicated in detail in the following text. Volunteers were not paid to participate. They all signed a written consent form.

One kick-off meeting was organised to describe the details of the study, to obtain informed consent, and to start distributing study material (questionnaires, measuring container for the de-alcoholised wine, vial for urine collection).

Eighty potentially eligible women were contacted to obtain the final sample of 21 volunteers. The characteristics of the subjects are reported in Table 2. One of the women withdrew from the study at the end of the second study period, because of temporary illness, leaving 20 subjects for data analyses.

Intervention design

The intervention was a crossover design in which the effect of a de-alcoholised red wine rich in PA and in other phenolic compounds (red DAW) was compared with that of a de-alcoholised rosé wine (rosé DAW), with a lower PA and polyphenol concentration (Fig. 1). Volunteers were randomly assigned to either treatment, and in the following 4 weeks (first period), they consumed 500 mL per day of DAW, divided in two portions of 250 mL during the two main meals of the day (lunch and dinner). After a 3-week wash-out period, the treatments were crossed over (subjects on red DAW in the first period were shifted to rosé DAW in the second and vice versa) for further 4 weeks (second period). Although the two de-alcoholised wines were visibly

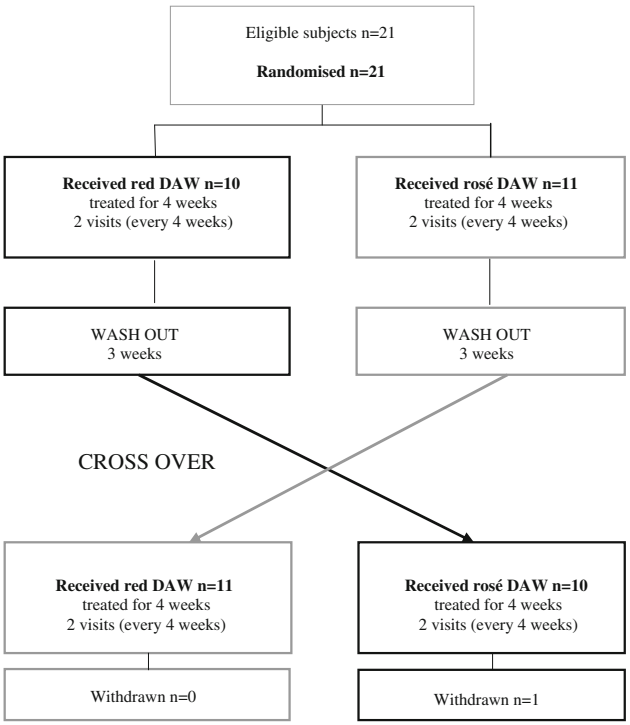

Fig. 1 Trial profile, summarising the flow of the study subjects. Red DAW = de-alcoholised wine, high in proanthocyanidines; rosé DAW = de-alcoholised wine, low in proanthocyanidines. For details of subjects and procedures, see Methods and Table 3

different (red vs. rosé), subjects were unaware of their PA concentration. In order to avoid interference with other dietary sources of PA, the subjects were asked to adhere to a low-proanthocyanidin (LPA) diet starting 2 weeks before the beginning of the trial and throughout the study, including the wash-out period between first and second study periods. The foods not allowed in the LPA diet were wine, beer and other alcoholic beverages, apples, plums and dried prunes, grapes, and raisins (other fruit permitted, if peeled), strawberries, blueberries, raspberries, blackberries, etc., fruit juices (except citrus), nuts, hazelnuts, almonds, pistachios, chocolate and cocoa, green tea, teas with berries, karkadé, rose hip tea (black tea maximum one cup/day), legumes with coloured skin (white beans and chickpeas permitted).

Volunteers were seen at the CSPO clinic at baseline and at the end of each treatment period for body measurements (height only at the first visit, weight without cloths, waist, and hip circumferences), blood pressure and heart rate measurements, blood drawing, urine collection and a detailed 24-hour diet recall interview. At the baseline visit, subjects were also asked to hand-in the EPIC self-administered dietary and lifestyle questionnaires [26] that they had received at the kick-off meeting and completed at home [27, 28]. After 2 weeks from enrolment, a phone interview was conducted to obtain a qualitative 24-hour diet recall.

Source and preparation of the wines used in the study

The DAW (360 L each) have been produced at the INRA experimental winery (Gruissan, France), from Syrah grapes harvested in September 2005 from the experimental domain. The low-PA DAW (rosé DAW) was made by destemming, crushing and immediately pressing the grapes, followed by fermentation of the liquid phase; the high-PA DAW (red DAW) was obtained by flash release (a process known to increase PA content by about 20–30%) [29] and fermentation on skins. After alcoholic and malolactic fermentation, de-alcoholisation was performed by evaporation under reduced pressure in 45 L batches at a temperature below 40 °C. The DAW batches were pooled and stored at 4 °C under nitrogen atmosphere and then formulated (with glycerol, sugar, and aromas), filtered, bottled, and pasteurised.

Composition of the de-alcoholised wines

Traditional oenological analyses (concentration of ethanol, glycerol, sugar, total and volatile acidities, pH) were performed according to the official methods of the European Union [30].

Anthocyanins, hydroxycinnamic acids, flavonol monomers, flavonols, and stilbenes were analysed by reversed phase high-performance liquid chromatography with diode array detection (HPLC–DAD) as described earlier [29]. Calibration curves were established using t-caftaric acid isolated in our laboratory to quantify hydroxycinnamic acids at 320 nm, malvidin 3-O-glucoside and quercetin 3-glucoside from Extrasynthese (Genay, France) to quantify, respectively, anthocyanin pigments at 520 nm and flavonols at 360 nm, trans resveratrol to quantify stilbenes at 305 nm, and (+) catechin and (−) epicatechin to quantify each of the flavanol monomers at 280 nm from Sigma (St Louis, MO,USA).

Proanthocyanidin composition was determined by phloroglucinolysis followed by HPLC analysis The concentrations of (+)-catechin, (−)-epicatechin, phloroglucinol derivatives of (−)-epigallocatechin, (+)-catechin, (−)-epicatechin, (−)-epicatechin 3-gallate and the total quantity of tannins in mg/L were determined from peak areas at 280 nm using calibration curves established using external standards, either commercial ((+)-catechin and (−)-epicatechin) or isolated, and purified in our laboratory. The mean degree of polymerisation (mDP) was calculated as the ratio between the summed molar concentrations of all released constitutive units and the summed molar concentrations of lower constitutive units.

The results of the composition analyses of the de-alcoholised wines are provided in Table 1. The red DAW contained much higher levels of PA (14 fold) but also

Table 1 Composition of the de-alcoholised wines (DAW) used in this study

	DA Wine	
	Red	Rosé
Total proanthocyanidins (mg/L)	868.2	61.4
Flavanol monomers (mg/L)	14.0	–
Total anthocyanins (mg/L eq. malvidin 3-glucoside)	219.6	37.4
Total hydroxycinnamic acids (mg/L eq. caftaric acid)	145.1	75.5
Total flavonols (mg/L eq. quercetin 3 glucoside)	16.0	1.2
Total stilbenes (mg/L eq. *trans* resveratrol)	7.2	0.2
Alcohol (% at 20 °C)	0.30	0.56
pH	3.87	3.88
Total acidity (g/L H_2SO4)	3.35	3.2
Free sulphur anhydride (mg/L)	17	7
Total sulphur anhydride (mg/L)	77	65
Glycerol (g/L)	16.3	16.3
Glucose + fructose (g/L)	17.7	19.9
280 nm absorbance (10 mm)	72.7	17

higher levels of other phenolic compounds than the rosé DAW, while values for all other measured parameters were similar in both DAWs. Glycerol, glucose, and fructose have been added to improve the palatability of the wines.

The administered PA doses were 434 mg/die (i.e. about 7 mg/kg b.w./die) in the red DAW group, and 30 mg/die (i.e. about 0.45 mg/kg b.w./die) in the rosé DAW group.

Dietary intake measurements

At baseline, dietary habits were measured by the EPIC food frequency questionnaire. Frequencies of consumption of standard or specifically reported portions were transformed into grams of daily consumed food and beverages and daily nutrient intakes [28]. In addition, at each visit, subjects were interviewed by the study dietician about food and beverages consumed in the previous 24 h. Food consumption data were transformed into average daily consumption of food, beverages, and nutrients, by means of the nutritional software Microdiet, Downlee Systems Ltd, UK. The software includes, in addition to UK food composition data, also the same Italian food tables applied to the EPIC questionnaire [31].

Haematological, haemostatic and haemorheological parameters

Thirty mL of peripheral blood was collected from an antecubital vein at each visit. Blood was drawn in different vacutainers depending on the analyses to be performed. Samples for comet assay and gene expression analysis were collected in EDTA-treated tubes. Haematological determinations were performed using an automated assay with ADVIA 2400 and Centaur XP instruments (Siemens Medical Solutions Diagnostics S.r.l., Milano, Italy). Whole-blood viscosity (WBV, shear rates 0.512–94.5 s–1) and plasma viscosity (PLV) were measured in EDTA-anticoagulated samples using a Rotational Viscosimeter Low Shear 30 (Contraves, Zurich, Switzerland). Platelet aggregation was measured in citrate-anticoagulated blood using a PFA-100® (Platelet Function Analyzer–100) with Epinephrine-collagen and ADP-collagene cartridges. All analyses were carried out blindly by laboratory personnel.

Interleukin-1β (IL-1β), interleukin 1 receptor antagonist (IL-1ra), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), interferon-inducible protein (IP-10), interferon-γ (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1-α (MIP-1α), macrophage inflammatory protein 1-β (MIP-1β), tumour necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF) levels were determined by using the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA) according to manufacturer's instructions.

Lymphocyte isolation

One EDTA tube was brought within 3 h from collection to the laboratory for analyses of DNA damage. Lymphocytes were isolated utilising Lymphoprep separation medium (Gibco, UK). About 200 µL of whole blood was diluted 1:4 with cold RPMI 1640 medium (Sigma). Then, 200 µL of Lymphoprep medium was carefully layered to the bottom of the centrifuge tubes. After centrifugation at 1,000×g for 5 min, at room temperature, gradient-separated lymphocytes were recovered, diluted to 1.5 mL with RPMI 1640 medium and centrifuged again at 1,000×g for 5 min, at 4 °C. The resulting cell pellets were resuspended in PBS and counted in a Neubauer chamber. Membrane integrity was assessed by the Trypan Blue exclusion method.

Lymphocyte DNA damage and response to in vitro oxidative stress

The comet assay was used to measure both basal and induced DNA oxidation. Aliquots of the fresh lymphocyte suspension containing about 200,000 cells were further centrifuged at 250 ×g for 10 min, and the resulting pellets were re-suspended in LMA (low melting point agarose), layered on microscopic slides and run through the comet assay as previously described [32]. Detection of oxidative DNA damage was carried out by means of the Fpg enzyme

(formamidopyrimidine DNA glycosylase), which introduces breaks at sites of oxidised purines such as 8-oxo-2′-deoxyguanosine. The value of DNA damage obtained in slides without enzyme incubation estimated the basal number of DNA strand breaks, whereas specific DNA oxidative damage on purines was assessed for each subject by subtracting the basal number of breaks (buffer-incubated slides) from the number of breaks obtained incubating the slides with Fpg. The enzyme dilution was chosen based on a calibration curve of enzyme activity, performed as described in Pitozzi et al. [33]. Microscopic analysis was carried out by means of a Labophot-2 microscope (Nikon, Tokyo, Japan) provided with epifluorescence and equipped with a rhodamine filter (excitation wavelength 546 nm; barrier 580 nm). The images of 100 randomly chosen nuclei per slide were captured and analysed using a custom-made imaging software coupled with a CCD camera (model C5985, Hamamatsu, Sunayama-Cho, Japan). Each point was run in duplicate.

In vitro *exposure to H_2O_2*. In order to evaluate the response of lymphocytes to oxidative stress, immediately after the inclusion of the cells in agarose and the solidification of the gel, one slide from each volunteer was immersed for 5 min in a solution of 500 μM H_2O_2 in PBS [32]. The incubation was conducted at 4 °C to inhibit DNA repair. After completing the incubation with H_2O_2, the slides were transferred to the lysis solution and run through the rest of the procedure as described. The damage induced by H_2O_2 was measured as induced DNA strand breaks, without employing the Fpg enzyme.

Lymphocyte gene expression analysis

Ten mL of peripheral blood was collected in EDTA and processed within 3 h after blood drawn for gene expression analysis. Peripheral blood lymphocytes were isolated via density gradient centrifugation using Lymphoprep™ (Axis-Shield, Oslo, Norway) as described. Lymphocyte pellets were stored at −80 °C in RNAlater (Qiagen, Milan, Italy) until RNA extraction.

After thawing, the RNA was extracted using the RNeasy Midi kit (Qiagen, Milan, Italy) according to the manufacture's instruction; the RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Samples whose RNA integrity number was greater than 8.0 were used for mRNA profiling by microarray analysis. We performed 40 microarray analyses, corresponding to 20 paired samples (before and after treatment) and to ten subjects (after period I and after period II).

Amplification and labelling of RNA samples were performed according to the manufacturer's specifications, using the GeneChip Expression 3' Amplification OneCycle Target labeling and Control reagents (Affymetrix Cat#900493). After completion of the labelling protocol, the concentration of the cRNA was measured using the NanoDrop; the cRNA profiles were analysed using the Bioanalyzer to check that the overall length of cRNA was up to 1,000 bp. Hybridisation of labelled RNA samples to the GeneChip array was performed according to manufacturer's specifications. The arrays were scanned using the Affymetrix GeneChip Scanner 3000 7G. All these procedures were performed at the ServiceXS (Leiden, The Netherlands), using the "NuGO array", a GeneChip array, designed by the Network of Excellence NuGO specifically for Nutrigenomic studies; this array contains in part common probesets that are also present on the Affymetrix Human Genome U133 Plus 2.0 Array, and in part newly designed probesets (http://blog.bigcat.unimaas.nl/~martijn/NuGO/annotations/).

Affymetrix data in the form of CEL files were checked for quality control and normalised by the RMA method using the web-based Bioconductor software. A two-class, paired t-moderated analysis was performed by SAM [34]; each treatment (T1 = rosé wine; T2 = red wine) was first compared to the relative baseline, T1 vs B1, T2 vs B2; after that, for each subject, the log ratio of treatment 1 (T1/B1) was compared to the log ratio of treatment 2 (T2/B2).

Microarray data were also analysed via Gene Set Enrichment Analysis (GSEA) (http://www.broad.mit.edu/gsea/) a microarray data-mining method used to identify significant enrichment of pre-defined groups of gene sets, including metabolic or signalling pathways, groups of genes previously found to be up- or down-regulated by a particular stimulus, genes located in the same chromosomal location, etc.

Briefly, data were mapped on 1230 gene sets (from c2.symbols.gmt) downloaded with the GSEA package and measured for the enrichment of genes at the top or bottom of the gene list to determine their correlation with the gene set's phenotype. We used the GSEA pre-ranking tool applied to the SAM statistics calculated on log ratios and the following parameters: enrichment statistic, weighted; permutation type, gene_set; permutation number, 1000; and gene set size restrictions, 15 minimum, 500 maximum. Gene sets significantly modulated by the wine treatments were identified using a nominal p-value and a multiple hypothesis testing FDR < 0.05.

Statistical methods

Descriptive statistics were performed to provide general information on the sample of volunteers. The analyses on DNA damage, haemostatic, and haemorheological parameters were carried out with STATA 9.2 (Stata Statistical Software: Release 7. College Station, TX: StataCorp LP).

To evaluate the effect of treatment and period and the presence of carry-over, responses obtained during the first period were compared with those obtained in the second period, considering the sequence of the treatment, i.e. "Low–High" corresponding to rosé DAW in the first period and red DAW in the second period and "High-Low" corresponding to red DAW in the first period and rosé DAW in the second period.

Indicated with y_{ijk}, the logarithm of the response observed on k-th subject in the period $j = 1, 2$ of group i = Low, High, we use the usual parameterisation, based on [35], $y_{ijk} = \mu + s_{ik} + \pi_j + \tau_i + \lambda_{i,j-1} + \varepsilon_{ijk}$, where π_j ($j = 1, 2$) is the period effect; τ_i ($i =$ Low, High) is the direct treatment effect, $\lambda_{L,1}$ $\lambda_{H,1}$ are the carry-over effect parameters; s_{ik} is the random subject effect (i.i.d.) with mean 0 and variance σ_s and ε_{ijk} are white-noise random error.

Standard tests for two period cross-over trials were applied [35]. Two-sample t-tests were applied to assess, for each outcome, hypothesis of equality of carry-over effect $H_0 : \lambda_1 = \lambda_2$, treatment effect $H_0 : \tau_L = \tau_H/\lambda_1 = \lambda_2$, and period effect $H_0 : \pi_1 = \pi_2/\lambda_1 = \lambda_2$, and to derive point estimates with 95% confidence interval for the differences on treatment. The term exp $(\tau_H - \tau_L)$ gives the geometric mean ratio by treatment group, and the term exp $(\pi_1 - \pi_2)$ gives the geometric mean ratio by period.

To evaluate the differences of the response observed upon treatment compared to baseline values, the Mann–Whitney test was used unless otherwise indicated. Treatment assignment, period and their interaction, and clinical characteristics (age, sex, cardiovascular risk factors) were taken into account using a random effect general linear model.

Results

The study sample is described in Table 2. In the course of the study, the volunteers did not show any significant change in anthropometric parameters or in blood pressure. No change was found during the treatment in blood cell counts, haemoglobin content, glucose and urea, platelet function. At the end of period I, an increase in triglycerides (from 82.60 to 100.90 mg/dL, $p < 0.01$ paired Student's t test) and total cholesterol (from 221.60 to 241.00 mg/dL, $p < 0.05$ paired Student's t test) was found in the high-PA group in comparison to baseline, whereas no difference between the groups was detected in period II. Among the measured rheological parameters (plasma viscosity and blood viscosity measured at different flow rates), none was differentially modified by either treatment. As summarised in Tables 3 and 4, there was no significant difference between the two treatments (red vs. rosé) on any of the analysed biomarkers, including lymphocyte oxidative DNA damage. A significant period effect was found for DNA damage and some rheological and platelet aggregation parameters (Table 4).

A different statistical analysis was then performed to analyse the effect of the combined treatments compared to baseline, adjusting for the type of treatment and period. No significant effect on either basal or induced lymphocyte DNA oxidative damage was found at the end of the treatment with the two DAW (Table 3). A significant decrease in blood viscosity was detected at rate 0.512 and 2.370,

Table 2 Characteristics of the study subjects

	Mean	SD*	Min	Max
Subjects characteristics (unit)				
Age (years)	55.3	3.9	45.9	63.8
Weight (kg)	63.9	11.1	44.0	9.3
Height (cm)	158.9	7.4	142.0	173.0
BMI (kg/m^2)	25.3	4.0	18.3	34.0
Waist (cm)	79.8	8.0	68.0	96.0
Hip (cm)	100.8	7.7	85.0	117.0
Waist-to-hip ratio	0.79	0.05	0.77	0.88
Diastolic blood pressure (mmHg)	80.5	6.9	70.0	90.0
Systolic blood pressure (mmHg)	126.8	10.0	110.0	150.0
Concentration (unit)				
Glucose (mg/dL)	0.89	0.07	0.78	1.02
Triglycerides (mg/dL)	0.4	26.8	31.0	152.0
Total cholesterol (mg/dL)	220.3	27.5	168.0	278.0
HDL cholesterol (mg/dL)	65.0	12.0	51.0	91.0
LDL cholesterol (mg/dL)	140.5	25.2	99.0	202.0
Daily nutrient intake form FFQ ** (unit)				
Energy (kcal)	1,991	474	1,106	2,775
Energy (kJ)	8,331	1,981	4,628	11,607
Total protein (g)	76.2	18.2	38.3	109.2
Total fat (g)	75.8	20.8	43.8	112.3
Saturated fatty acids (g)	0.36	7.50	13.24	43.13
Monounsaturated fatty acids (g)	38.0	12.0	19.7	60.7
Polyunsaturated fatty acids (g)	8.3	2.1	5.5	12.1
Cholesterol (mg)	320.4	89.2	143.9	509.4
Carbohydrates (mg)	0.10	67.44	140.0	370.1
Dietary fibre (g)	21.7	5.0	14.4	31.5
Alcohol (g)	8.6	9.3	0	30.8
Vitamin C (mg)	147.2	57.6	47.2	250.9
Folic acid (μg)	280.7	68.2	174.8	443.7
Retinol equivalents (μg)	981	367	359	1,610
Retinol (mg)	377	189	128	769
Beta carotene equivalents (μg)	3,620	1,778	939	7,494
Vitamin E (mg)	7.8	2.1	3.5	11.0
Vitamin D (μg)	2.57	1.01	0.87	5.69

* Standard deviation

** Food frequency questionnaire, administered at baseline

Table 3 Measurements of the outcome variables during the study

		Baseline 1	End of treatment 1	Baseline 2	End of treatment 2
DNA Fpg-sensitive sites	Low–high	15.36 ± 1.69	11.17 ± 1.24	11.84 ± 2.43	14.37 ± 1.10
	High-low	15.29 ± 1.60	12.24 ± 1.57	8.70 ± 1.23	14.55 ± 1.38*
Induced DNA oxidative damage	Low–high	47.18 ± 2.80	45.29 ± 2.39	42.65 ± 2.90	45.49 ± 2.91
	High–low	45.28 ± 2.60	47.00 ± 2.56	40.71 ± 2.35	48.59 ± 1.52*
Platelet aggregation time (epi)	Low–high	159.00 ± 13.91	144.09 ± 9.71	127.00 ± 9.72	125.44 ± 11.72
	High–low	132.50 ± 9.31	124.70 ± 8.83	128.18 ± 7.61	122.36 ± 4.95
Platelet aggregation time (ADP)	Low–high	103.64 ± 5.72	106.00 ± 5.52	87.60 ± 6.13	84.11 ± 6.17
	High–low	88.20 ± 5.11	99.50 ± 7.46	91.18 ± 5.32	91.00 ± 3.85
(PLV)	Low–high	1.28 ± 0.01	1.28 ± 0.01	1.27 ± 0.01	1.28 ± 0.01
	High–low	1.29 ± 0.01	1.25 ± 0.01	1.27 ± 0.01	1.30 ± 0.01*
Fibrinogen	Low–high	368.36 ± 14.77	365.18 ± 15.40	372.80 ± 17.87	365. 56 ± 12.43
	High–low	371.40 ± 17.96	378.20 ± 16.21	369.64 ± 10.34	363.00 ± 17.97
WBV (0.512)	Low–high	26.00 ± 0.95	23.89 ± 0.57**	22.64 ± 0.55	22.97 ± 0.61
	High–low	25.92 ± 1.08	23.92 ± 0.53**	23.14 ± 0.64	23.48 ± 0.65
WBV (2.370)	Low–high	12.15 ± 0.52	10.81 ± 0.25*	10.53 ± 0.24	10.66 ± 0.31
	High–low	11.97 ± 0.64	10.84 ± 0.20	10.73 ± 0.31	10.81 ± 0.25
WBV (1.040)	Low–high	7.81 ± 0.35	7.07 ± 0.13	7.10 ± 0.14	7.02 ± 0.11
	High–low	7.91 ± 0.43	7.14 ± 0.13	6.86 ± 0.06	7.01 ± 0.11
WBV (20.400)	Low–high	5.87 ± 0.15	5.61 ± 0.09*	5.73 ± 0.05	5.60 ± 0.04
	High–low	5.84 ± 0.11	5.84 ± 0.31	5.55 ± 0.09	5.57 ± 0.06
WBV (94.500)	Low–high	4.29 ± 0.02	4.26 ± 0.01*	4.26 ± 0.01	4.26 ± 0.01
	High–low	4.28 ± 0.02	4.26 ± 0.01	4.25 ± 0.01	4.27 ± 0.01
Total cholesterol	Low–high	223.09 ± 7.28	231.36 ± 14.39	244.20 ± 12.68	235.22 ± 12.76
	High–low	221.60 ± 10.75	241.00 ± 12.48	226.55 ± 6.18	231.82 ± 6.67

Values of the main outcome variables, measured at the beginning (baseline) and at the end of each period (mean ± S.E. of 9–10 subjects)

Fpg-sensitive sites and induced DNA oxidative damage measured with the comet assay were expressed as % DNA in tail. Platelet aggregation time (induced by epinephrine or ADP) was expressed in seconds (s), PLV= plasma viscosity in mPA*s and fibrinogen plasma levels in mg/dL. WBV=whole blood viscosity at different shear rates (from 0.512 to 94,500 s^{-1}) was expressed as mPA*s. Total plasma cholesterol was expressed as mg/dL. *$p < 0.05$, ** $p < 0.01$ statistically significant difference from baseline

Table 4 Comparison of treatment with red vs. rosé DAW

	Estimated treatment effect: red vs. rosé DAW	95% CI	Estimated period effect: II vs. I	95% CI
DNA Fpg-sensitive sites	1.01	0.64;1.58	1.75*	1.11;2.74
Induced DNA oxidative damage	1.11	0.87;1.41	1.06	0.83;1.35
Platelet aggregation time (epi)	0.88	0.71;1.10	0.85	0.68;1.05
Platelet aggregation time (ADP)	1.04	0.88;1.23	0.72**	0.61;0.85
Plasma viscosity (PLV)	0.99	0.97;1.02	1.04**	1.01;1.07
Fibrinogen	0.99	0.89;1.11	0.99	0.88;1.10
Blood viscosity (0.512)	0.82	0.92;1.05	0.94	0.88;1.00
Blood viscosity (2.370)	0.99	0.93;1.06	0.98	0.92;1.04
Blood viscosity (1.040)	1.00	0.94;1.07	0.98	0.92;1.05
Blood viscosity (20.400)	1.05	1.01;1.08	0.95**	0.92;0.98
Blood viscosity (94.500)	1.00	0.99;1.01	1.00	0.99;1.01
Total plasma cholesterol	1.03	0.90;1.17	1.01	0.89;1.15

The estimated treatment effect is expressed as the ratio of the geometric means of the repeated measures (red/rosé DAW, $n = 10$). The estimated period effect is expressed as the ratio of the geometric means of the repeated measures (study period II/I, $n = 10$). 95% CI = 95% confidence intervals of the ratios. * $p < 0.05$, ** $p < 0.01$ parametric tests; Jones & Kenward (1989). For details of subjects and procedures, see Methods and Table 2

which was independent of the type of treatment (Tables 3 and 4). No treatment effect was found on any of the measured inflammatory cytokines (data not shown).

For gene expression analysis, 4 of 40 analysed arrays did not pass the quality control; these arrays plus their 4 paired arrays were consequently removed. After this step, it was possible to analyse data from 8 subjects after period I and after period II.

SAM analysis did not find differences comparing each treatment to its baseline. Comparing the log ratio of treatment 1 versus the log ratio of treatment 2, we did not find differences either. Therefore, we decided to perform a GSEA analysis which is a computational method that uses predefined gene sets and ranks of genes to identify significant biological changes in microarray data sets and it is especially useful when gene expression changes in a given microarray data set is minimal or moderate.

GSEA produced an enrichment score (ES) of pathways modulated by the treatments: 269 pathways were significantly modulated after red DAW treatment compared to rosé (nominal p value < 0.05), 12 of them had also a FDR q-value, which takes into account the multi-test, less then 0.05 (Table 5). The microarray data were submitted to ArrayExpress (http://www.ebi.ac.uk/microarray-as/ae/; Experiment name: Effects of de-alcoholised wines in post-menopausal women ArrayExpress accession code: E-MEXP-2616).

Discussion

The main positive effect observed in this study was a reduction in blood viscosity upon DAW consumption. This effect was not dependent on the amount of PA in the wine, as it was induced by both red and rosé.

Our data did not show any significant change in the basal levels of DNA oxidative damage in peripheral lymphocytes, measured as Fpg sites with the comet assay. Nor did we observe any protective effect of either treatment towards in vitro–induced oxidative stress in the same cells. A marked decrease in 8-hydroxydeoxyguanosine (8-oxodG) levels in blood leucocyte DNA upon wine consumption in humans has been reported by Leighton et al. [10]. However, antioxidant effects have not been consistently found in human studies with wine [14, 15, 36, 37].

Epidemiological studies showed that increased blood viscosity is associated with several cardiovascular risk factors as well as with both prevalent and incident cardiovascular diseases [24, 38]. Mechanisms by which elevations in rheological factors may promote cardiovascular events are different and include increases in blood pressure, shear stress, ischaemia, and blood-vessel wall interactions. Recently, an influence of diet on haemorheological and inflammatory parameters has been showed [26], indicating a possible beneficial role of nutrition in decreasing the thrombotic burden related to these alterations. The mechanism through which DAW induced a decrease in blood viscosity, not dose-related with polyphenol content, is not clear. One possibility is that this effect is simply related to higher water intake with DAW. The hyper-hydrating activity of glycerol [39], which is present in substantial amounts in DAW, might also contribute to such an effect.

Previous human studies with wine have shown a tendency towards amelioration of the lipidemic profile, particularly for cholesterol levels [11, 15, 36]. In the present study, we detected instead a tendency towards an increase in total cholesterol and triglyceride levels: however, such an effect was rather small and present only in the first period of treatment. It is possible that slight oscillations in the lipidemic profile were induced by the diet changes involuntarily associated with DAW consumption rather than by the DAW itself. Another possibility is that these changes were at least partly due to a higher glucose–fructose intake (9–10 g/die) associated with DAW. High fructose intake is increasingly recognised as a causative factor in the development of pre-diabetes and the metabolic syndrome [40].

The above-mentioned discrepancies with previous studies employing wine might be partly due to the alcohol component, which besides having its own biological effects might modify polyphenol bioavailability. This last point is still a matter of debate: polyphenol absorption in healthy human subjects has been reported to be similar in aqueous and alcoholic matrices [41], whereas alcohol did modify quercetin absorption and metabolism in the rat everted jejunal sac model [42]. Ethanol, fat, and emulsifiers can modify polyphenol solubility and as a consequence absorption [43]. Thus, it is conceivable that different matrices can give rise to differences in plasma levels and lifetime of the parent polyphenolic compounds and their metabolites. Finally, these discrepancies might also be due to the interference at the functional level from other DAW components such as glycerols and sugars, present in lower amounts in non-de-alcoholised wine.

Microarray data were analysed using two approaches: SAM, to identify statistically significant changes in gene expression, GSEA, to determine any coordinated differential expression (enrichment) in sets of functionally related genes. While SAM did not detect significant changes in gene expression, GSEA highlighted subtle but extensive differences between the treatments. SAM and GSEA results are not in contrast: if several genes of a given pathway are up- or down-regulated with a small variation of fold change, this may not be detected by traditional analyses, although the difference between treatments may

Table 5 List of pathways with a FDR q-value less than 0.05, up (positive ES value) or down-regulated (negative ES value) in red wine drinkers compared to the same subjects after rosé wine treatment

Name	Brief description	Size	ES	NOM *p*-val	FDR q-val	FWER *p*-val
CROONQUIST_IL6_RAS_DN	Genes down-regulated in multiple myeloma cells exposed to the pro-proliferative cytokine IL-6 versus those with N-ras-activating mutations	22	−0.75	0	0	0
CROONQUIST_IL6_STARVE_UP	Genes up-regulated in multiple myeloma cells exposed to the pro-proliferative cytokine IL-6 versus those that were IL-6-starved	32	−0.72	0	0	0
BENNETT_SLE_UP	Genes significantly up-regulated in SLE patient blood mononuclear cells	27	0.77	0	0.002	0.002
Breast_ductal_carcinoma_genes	Genes up-regulated in breast tumours that are identified as high tumour grade and that are progressing from preinvasive ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC)	18	−0.65	0	0.013	0.063
Goldrath_cellcycle	Cell cycle genes induced during antigen activation of CD8+ T cells	27	−0.66	0	0.014	0.042
Striated_muscle_contraction	–	28	−0.66	0	0.014	0.055
Greenbaum_E2A_up	Transcripts up-regulated threefold or greater in the E2A-deficient cell lines	32	−0.63	0	0.018	0.120
DOX_resist_gastric_up	Up-regulated in gastric cancer cell lines resistant to doxorubicin, compared to parent chemosensitive lines	40	−0.64	0	0.020	0.113
IFNA_HCMV_6 h_up	Up-regulated in fibroblasts at 6 h following treatment with interferon-alpha	49	0.69	0	0.023	0.046
CMV_HCMV_time course_12 h_up	NOT AVAILABLE	25	0.67	0	0.030	0.113
IFNA_UV-CMV_common_HCMV_6 h_up	Up-regulated in fibroblasts at 6 h following either infection with UV-inactivated CMV or interferon-alpha	25	0.67	0	0.031	0.090
NF90_UP	Up-regulated by ectopic expression of NF90 in GHOST(3)CXCR4 cells	23	0.65	0	0.042	0.220

Size = number of genes in the gene set; *ES* enrichment score

Up-regulated

B4GALT5, BIRC4BP, BST2, C2, CASP1, CCL8, CKAP4, CLEC2B, CTSO, ECGF1, EMP1, GBP2, GCH1, IFI35, IFI44L, IFIT5, IFITM3, IL15, INDO, IRF7, ISG15, LY6E, MX1, MX2, NMI, OAS1, OAS2, OASL, PSMB9, RHOB, RSAD2, SERPING1, STAT1, TAP1, TDRD7, TNFAIP6, TRIM22, UBE2L6, WARS

Down-regulated

ANLN, AURKA, AURKB, BUB1, BUB1B, CA2, CCNA2, CCNB2, CDC2, CDC20, CDC25C, CDC45L, CDKN3, CHEK1, EXO1, FGFR2 GALE, GMNN, GSG2, H2AFX, KIF11, KIF22, KIF2C, KIF4A, MAD2L1, MCM3, MKI67, MYBL2, NCAPH, NEK2, PLK1, PLK4, POLD1, SLC12A2, SLC7A5, SPBC25, TACC3, TOP2A, TTK, UBE2C, WBP5

be biologically relevant. In our experiments, GSEA suggests a possible immunomodulatory activity of red wine; red DAW treatment, in fact, compared to the rosé, induced up-regulation of gene pathways related to the interferon response cascade, encoding proteins with an antiviral role and apoptosis regulators.

Beverages rich in antioxidants, such as green tea, exhibit immunomodulatory properties in vitro [44]. It has been reported that a moderate consumption of red wine did not affect immune responses in healthy men [45, 46]. However, a large cohort study, carried out to test whether alcohol intake has any effect on the risk of developing a common cold, demonstrated that wine consumption (especially red wine) was inversely associated with the risk of common cold [47].

The small effects on gene expression in vivo reported in the present study appear to be in contrast with those obtained in experimental animal models in vivo, where the modulation of pathways, such as the inflammatory ones, has been repeatedly shown [17, 18]: however, it is necessary to consider that in animals the attained doses are about 10 times higher than those used in human studies including the one reported here and that many observations were carried out on the gastrointestinal tract, where the attained concentrations of polyphenols or their metabolites are considerably higher.

Furthermore, plasma levels of a panel of inflammatory cytokines did not change significantly upon the employed treatments, although some polyphenol metabolites have been reported to have anti-inflammatory activity [48].

In conclusion, the results of the present human study provide evidence that consumption of substantial amounts of de-alcoholised wine for 1 month does not exert a protective activity towards oxidative DNA damage in peripheral lymphocytes, but shows blood-fluidifying actions. However, this effect does not depend on the amount of PA in the de-alcoholised wine, since it was observed both with high and low PA content wine.

More intervention studies are definitely needed to provide further evidence of the health-protective effects of wine PA. The use of de-alcoholised wine in these studies allows the separation of the effects of the alcoholic and non-alcoholic components, but introduces at the same time a series of variables, such as modifications in PA bioavailability and, above all, the intake of significant amounts of sweeteners, which can make the interpretation of the results more difficult. On the other hand, sweeteners are unavoidable components of DAW, which would otherwise be not palatable for consumers.

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References

1. WHO (2003) World Health Report
2. WCRF—World Cancer Prevention Fund and American Institute for Cancer Research (2007). Food, nutrition, physical activity and the prevention of cancer: a global perspective. http://www.dietandcancerreport.org
3. Biesalski HK (2007) Polyphenols and inflammation: basic interactions. Curr Opin Clin Nutr Metab Care 10(6):724–728
4. Thomasset SC, Berry DP, Garcea G, Marczylo T, Steward WP, Gescher AJ (2007) Dietary polyphenolic phytochemicals-promising cancer chemopreventive agents in humans? A review of their clinical properties. Int J Cancer 120(3):451–458
5. Zern TL, Fernandez ML (2005) Cardioprotective effects of dietary polyphenols. J Nutr 135(10):2291–2294
6. Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols. J Nutr 130(Suppl 8S):2073S–2085S
7. Szmitko PE, Verma S (2005) Antiatherogenic potential of red wine: clinician update. Am J Physiol Heart Circ Physiol 288(5):H2023–H2030
8. Ruf JC (2003) Overview of epidemiological studies on wine, health and mortality. Drugs Exp Clin Res 29(5–6):173–179
9. Covas MI, Gambert P, Fitó M, de la Torre R (2010) Wine and oxidative stress: up-to-date evidence of the effects of moderate wine consumption on oxidative damage in humans. Atherosclerosis 208(2):297–304
10. Leighton F, Cuevas A, Guasch V, Pérez DD, Strobel P, San Martín A, Urzua U, Díez MS, Foncea R, Castillo O, Mizón C, Espinoza MA, Urquiaga I, Rozowski J, Maiz A, Germain A (1999) Plasma polyphenols and antioxidants, oxidative DNA damage and endothelial function in a diet and wine intervention study in humans. Drugs Exp Clin Res 25(2–3):133–141
11. Tsang C, Higgins S, Duthie GG, Duthie SJ, Howie M, Mullen W, Lean ME, Crozier A (2005) The influence of moderate red wine consumption on antioxidant status and indices of oxidative stress associated with CHD in healthy volunteers. Br J Nutr 93(2):233–240
12. Chopra M, Fitzsimons PE, Strain JJ, Thurnham DI, Howard AN (2000) Nonalcoholic red wine extract and quercetin inhibit LDL oxidation without affecting plasma antioxidant vitamin and carotenoid concentrations. Clin Chem 46:1162–1170
13. Abu-Amsha Caccetta R, Burke V, Mori TA, Beilin LJ, Puddey IB, Croft KD (2001) Red wine polyphenols, in the absence of alcohol, reduce lipid peroxidative stress in smoking subjects. Free Radic Biol Med 30(6):636–642
14. de Rijke YB, Demacker PN, Assen NA, Sloots LM, Katan MB, Stalenhoef AF (1996) Red wine consumption does not affect oxidizability of low-density lipoproteins in volunteers. Am J Clin Nutr 63(3):329–334
15. Cartron E, Fouret G, Carbonneau MA, Lauret C, Michel F, Monnier L, Descomps B, Léger CL (2003) Red-wine beneficial long-term effect on lipids but not on antioxidant characteristics in plasma in a study comparing three types of wine-description of

two O-methylated derivatives of gallic acid in humans. Free Radic Res 37(9):1021–1035
16. van Golde PH, Sloots LM, Vermeulen WP, Wielders JP, Hart HC, Bouma BN, van de Wiel A (1999) The role of alcohol in the anti low density lipoprotein oxidation activity of red wine. Atherosclerosis 147(2):365–370
17. Luceri C, Caderni G, Sanna A, Dolara P (2002) Red wine and black tea polyphenols modulate the expression of cycloxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced F344 rat colon tumors. J Nutr 132(6):1376–1379
18. Dolara P, Luceri C, De Filippo C, Femia AP, Giovannelli L, Caderni G, Cecchini C, Silvi S, Orpianesi C, Cresci A (2005) Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutat Res 591(1–2):237–246
19. Del Bas JM, Fernández-Larrea J, Blay M, Ardèvol A, Salvadó MJ, Arola L, Bladé C (2005) Grape seed procyanidins improve atherosclerotic risk index and induce liver CYP7A1 and SHP expression in healthy rats. FASEB J 19(3):479–481
20. Iijima K, Yoshizumi M, Ouchi Y (2002) Effect of red wine polyphenols on vascular smooth muscle cell function–molecular mechanism of the 'French paradox'. Mech Ageing Dev 123(8): 1033–1039
21. Canali R, Ambra R, Stelitano C, Mattivi F, Scaccini C, Virgili F (2007) A novel model to study the biological effects of red wine at the molecular level. Br J Nutr 97(6):1053–1058
22. Pal S, Ho N, Santos C, Dubois P, Mamo J, Croft K, Allister E (2003) Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of ApoB100 from human HepG2 cells. J Nutr 133(3):700–706
23. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG (2007) Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh Artery Study. Circulation 115(16):2119–2127
24. Davì G, Patrono C (2007) Platelet activation and atherothrombosis. N Engl J Med 357(24):2482–2494
25. Sofi F, Buccioni A, Cesari F, Gori AM, Minieri S, Mannini L, Casini A, Gensini GF, Abbate R, Antongiovanni M (2010) Effects of a dairy product (pecorino cheese) naturally rich in cis-9, trans-11 conjugated linoleic acid on lipid, inflammatory and haemorheological variables: a dietary intervention study. Nutr Metab Cardiovasc Dis 20(2):117–124
26. Pisani P, Faggiano F, Krogh V, Palli D, Vineis P, Berrino F (1997) Relative validity and reproducibility of a food frequency dietary questionnaire for use in the Italian EPIC centres. Int J Epidemiol 26(Suppl 1):S152–S160
27. Slimani N, Deharveng G, Charrondière RU, van Kappel AL, Ocké MC, Welch A, Lagiou A, van Liere M, Agudo A, Pala V, Brandstetter B, Andren C, Stripp C, van Staveren WA, Riboli E (1999) Structure of the standardized computerized 24-h diet recall interview used as reference method in the 22 centers participating in the EPIC project. European Prospective Investigation into Cancer and Nutrition. Comput Methods Programs Biomed 58(3):251–266
28. Pala V, Sieri S, Palli D et al (2003) Diet in the Italian EPIC cohorts: presentation of data and methodological issues. Tumori 89:594–607
29. Morel-Salmi C, Souquet JM, Bes M, Cheynier V (2006) The effect of flash release treatment on phenolic extraction and wine composition. J Agric Food Chem 54:4270–4276
30. Analyse des moûts et des vins (1990) Réglement (CEE) n°2676/90 de la commision du 17 septembre 1990 déterminant des méthodes d'analyse communautaires applicables dans le secteur du vin. Journal Officiel des Communautés Européennes
31. Salvini S, Parpinel M, Gnagnarella P, Maisonneuve P, Turrini A (1998) Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia. Istituto Europeo di Oncologia, Milano
32. Giovannelli L, Pitozzi V, Riolo S, Dolara P (2003) Measurement of DNA breaks and oxidative damage in polymorphonuclear and mononuclear white blood cells: a novel approach using the comet assay. Mutat Res 538(1–2):71–80
33. Pitozzi V, Pallotta S, Balzi M, Bucciolini M, Becciolini A, Dolara P, Giovannelli L (2006) Calibration of the comet assay for the measurement of DNA damage in mammalian cells. Free Radic Res 40(11):1149–1154
34. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A 98(9):5116–5121
35. Jones B, Kenward MG (1987) Modelling binary data from a three-period cross-over trial. Stat Med 6(5):555–564
36. Sharpe PC, McGrath LT, McClean E, Young IS, Archbold GP (1995) Effect of red wine consumption on lipoprotein (a) and other risk factors for atherosclerosis. QJM 88(2):101–108
37. van der Gaag MS, Sierksma A, Schaafsma G, van Tol A, Geelhoed-Mieras T, Bakker M, Hendriks HF (2000) Moderate alcohol consumption and changes in postprandial lipoproteins of premenopausal and postmenopausal women: a diet-controlled, randomized intervention study. J Womens Health Gend Based Med 9(6):607–616
38. Velcheva I, Antonova N, Titianova E, Damianov P, Dimitrov N, Ivanov I (2006) Hemorheological parameters in correlation with the risk factors for carotid atherosclerosis. Clin Hemorheol Microcirc 35(1–2):195–198
39. Nelson JL, Robergs RA (2007) Exploring the potential ergogenic effects of glycerol hyperhydration Sports Med 37(11):981–1000
40. Miller A, Adeli K (2008) Dietary fructose and the metabolic syndrome. Curr Opin Gastroenterol 24(2):204–209
41. Goldberg DM, Yan J, Soleas GJ (2003) Absorption of three wine-related polyphenols in three different matrices by healthy subjects. Clin Biochem 36(1):79–87
42. Dragoni S, Gee J, Bennett R, Valoti M, Sgaragli G (2006) Red wine alcohol promotes quercetin absorption and directs its metabolism towards isorhamnetin and tamarixetin in rat intestine in vitro. Br J Pharmacol 147(7):765–771
43. Scholz S, Williamson G (2007) Interactions affecting the bioavailability of dietary polyphenols in vivo. Int J Vitam Nutr Res 77(3):224–235
44. Zvetkova E, Wirleitner B, Tram NT, Schennach H, Fuchs D (2001) Aqueous extracts of *Crinum latifolium* (L.) and Camellia sinensis show immunomodulatory properties in human peripheral blood mononuclear cells. Int Immunopharmacol 1:2143–2150
45. Watzl B, Bub A, Briviba K, Rechkemmer G (2002) Acute intake of moderate amounts of red wine or alcohol has no effect on the immune system of healthy men. Eur J Nutr 41(6):264–270
46. Watzl B, Bub A, Pretzer G, Roser S, Barth SW, Rechkemmer G (2004) Daily moderate amounts of red wine or alcohol have no effect on the immune system of healthy men. Eur J Clin Nutr 58(1):40–45
47. Takkouche B, Regueira-Méndez C, García-Closas R, Figueiras A, Gestal-Otero JJ, Hernán MA (2002) Intake of wine, beer, and spirits and the risk of clinical common cold. Am J Epidemiol 155(9):853–858
48. Larrosa M, Luceri C, Vivoli E, Pagliuca C, Lodovici M, Moneti G, Dolara P (2009) Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. Mol Nutr Food Res 53(8):1044–1054