Down-regulation of adhesion molecules and other inflammatory biomarkers after moderate wine consumption in healthy women: a randomized trial[1–3]

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ABSTRACT

Background: Moderate alcohol consumption is cardioprotective. The mechanism for this beneficial effect might be reduced inflammatory responses, as suggested by prospective studies and small clinical trials in men. No studies have evaluated the antiinflammatory effects of wine in women.

Objective: We investigated whether low-dose intake of white and red wines has differential effects on inflammatory markers in women.

Design: In a crossover study, we randomly assigned 35 healthy women to two 4-wk periods of 20 g ethanol/d as white or red wine, preceded by two 4-wk washout periods. Before and after interventions, we measured serum lipids, circulating inflammatory biomarkers, cellular adhesion molecules (CAMs), and adhesion of monocytes to stimulated endothelial cells.

Results: HDL cholesterol increased, and the serum concentrations of high-sensitivity C-reactive protein, intercellular adhesion molecule-1, CD40L, and interleukin-6 decreased after either wine ($P < 0.01$, all). Vascular CAM-1 and E-selectin decreased ($P < 0.01$) only after red wine. CAM expression by mononuclear cells was blunted after either wine, with a greater suppressant effect of red wine. Enhanced adhesion of monocytes to stimulated endothelial cells was reduced by 51% (95% CI: −57%, −45%) after white wine and by 89% (95% CI: −96%, −82%) after red wine ($P = 0.01$ for between-wine differences).

Conclusions: Moderate wine consumption is associated with beneficial effects on various inflammatory pathways related to endothelial activation in women. Probably because of its higher polyphenol content, red wine shows superior antiinflammatory effects than does white wine. Reducing low-grade inflammation and endothelial activation may be another potential mechanism by which alcoholic beverages exert their cardioprotective effect. *Am J Clin Nutr* 2007;86:1463–9.

KEY WORDS Inflammatory biomarkers, endothelium, adhesion molecules, wine, polyphenols

INTRODUCTION

The protective effect of moderate alcohol consumption against ischemic heart disease (IHD) has been established in many epidemiologic studies (1–5). Because only one-half of this protective effect may be attributed to the increase in serum HDL cholesterol observed in moderate alcohol drinkers (3), mechanisms other than lipid effects may be involved in the association between moderate alcohol consumption and reduced IHD rates (2, 6, 7). The atherosclerotic process underlying IHD is currently considered an inflammatory disease (8). Findings from large cohort studies suggest that moderate alcohol consumption is associated with a reduction in serum inflammatory biomarkers (7, 9–12). In addition, clinical trials in men have shown a reduction of both circulating markers of inflammation and monocyte adhesion to endothelial cells after daily intake of 30 g alcohol as red wine (13, 14). It is unknown, however, whether in women doses of alcohol lower than those considered safe in men taken as wine are sufficient to elicit antiinflammatory effects similar to those observed in men. Therefore, we performed a randomized crossover study in women to evaluate the effects of moderate consumption (20 g alcohol/d) of 2 alcoholic beverages with high (red wine) or low (white wine) polyphenol content, respectively, on inflammatory markers associated with endothelial dysfunction and on monocyte adhesion to endothelial cells.

SUBJECTS AND METHODS

Subjects

Thirty-six healthy female employees of our institution who reported an average daily ethanol intake ranging from 10 to 20 g during the past 5 y were recruited into a protocol approved by the institutional review board and gave informed consent. Eligibility criteria were age of 20–50 y; absence of family history of premature IHD; no tobacco smoking, hypertension, or diabetes mellitus; LDL cholesterol <160 mg/dL; and HDL cholesterol >35

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TABLE 1
Characteristics of study group[1]

	Value
Age (y)	38.0 ± 8.5
Body weight (kg)	63.3 ± 9.9
BMI (kg/m^2)	23.9 ± 3.8
Systolic blood pressure (mm Hg)	104.2 ± 12.0
Diastolic blood pressure (mm Hg)	71.2 ± 5.2
Glucose (mg/dL)	85.1 ± 7.1
Lipids (mg/dL)	
Total cholesterol	211.3 ± 37.7
HDL cholesterol	52.7 ± 10
LDL cholesterol	128.5 ± 32.9
Triacylglycerols	74.8 ± 43.9
Serum biomarkers	
hsCRP (mg/L)	1.88 ± 1.43
Interleukin-6 (pg/mL)	3.85 ± 3.06
VCAM-1 (ng/mL)	1146 ± 288
ICAM-1 (ng/mL)	405 ± 271
E-selectin (ng/mL)	241 ± 214
P-selectin (ng/mL)	386 ± 138
CD40L (ng/mL)	46.5 ± 20.3

[1] All values are $\bar{x}$ ± SD. n = 35. hsCRP, high-sensitivity C-reactive protein; VCAM-1, soluble vascular cell adhesion molecule 1; ICAM-1, soluble intercellular adhesion molecule 1.

mg/dL. None of the women was taking oral contraceptives, medication of any sort, or vitamin supplements. The reported prior intake of alcohol was 7.8 ± 6.3 g/d during a period of 16 ± 12 y. Participants received free wine but no monetary compensation. The baseline characteristics of the participants are shown in **Table 1**.

Study design

The study was an open, randomized crossover trial. To equilibrate hormonal influences on adhesion molecules, all biological measurements were performed on the first day of the menstrual cycle. The study design included a 4-wk run-in period (coinciding with the menstrual cycle) during which all subjects abstained from alcohol and consumed a prescribed Mediterranean-type diet with average energy intake as 20% protein, 47% carbohydrate, and 33% fat (8% saturated fatty acids, 20% monounsaturated fatty acids, and 5% polyunsaturated fatty acids). After this period, participants were individually randomly assigned in a crossover design between 2 isocaloric diet sequences for 4-wk periods, containing either red wine or white wine. Between the first and second wine sequence, there was a 4-wk washout period with alcohol abstention. All subjects received daily doses of 20 g ethanol as red wine or white wine (1 glass of 100 mL at lunch and at dinner). Dietary habits and physical activity were monitored before and at the end of each intervention treatment, when body weight and blood pressure were measured and blood and urine samples were collected.

Wine polyphenol content

Red and white wines were obtained from Tempranillo and Xarello grapes, with an alcoholic strength of 13.5% and 13%, respectively. The selection of wines was based on the wine's polyphenol content, which was determined by HPLC as described (15). The total polyphenol, resveratrol, and anthocyanin contents of red wine were 1945 mg/L, 12.8 mg/L and 164.85 mg/L, respectively. White wine provided 308 mg/L polyphenols and 1.3 mg/L resveratrol, and anthocyanin was below detection limits.

Diet and exercise monitoring

The background diets were designed according to the subject's personal preferences. Consumption of dispensable foods rich in polyphenols or other potent antioxidants, such as onions, virgin olive oil, and green and black tea, was discouraged. Other foods with a high content in polyphenols, ascorbic acid, α-tocopherol, or β-carotene, such as cocoa, chocolate, orange and tomato juices, nuts, some fruit (oranges, lemons, strawberries, grapes, melon, apples, and apricots), some vegetables (spinach, turnips, carrots, parsley, peppers, garlic, and tomatoes), and soybean products were restricted. Natural foods rich in antioxidants, especially fruit and vegetables, were controlled so that individual diets had similar antioxidant content throughout the study.

Diet compliance was assessed from 3-d diet records administered by the same trained physician before and after each intervention (2 washout periods and 2 intervention treatments). These questionnaires were previously validated in our population (16). Foods were converted to nutrients by using the PROFESSIONAL DIET BALANCER software (Cardinal Health Systems Inc, Edina, MN). Physical activity was monitored with the Minnesota Leisure Time Physical Activity Questionnaire, which has also been validated in Spain (17). At the end of the study, a clinician assessed any adverse effects from the interventions by administering a checklist of symptoms, including bloating, fullness, or indigestion; altered bowel habit; dizziness; and other symptoms possibly associated with wine intake.

Laboratory measurements

Fasting blood samples and a spot urine specimen were obtained at the end of each 4-wk period (run-in, first wine, washout, and second wine). Blood lipid measurements and immunophenotyping of peripheral blood mononuclear cells (PBMCs) were performed immediately. Serum and EDTA-plasma samples were stored at −80 °C for analysis of inflammatory and cell adhesion molecules at the end of the study. Cholesterol and triacylglycerols were measured with the use of enzymatic procedures. HDL cholesterol was quantified after precipitation with phosphotungstic acid and magnesium chloride. Analyses determined by subject in frozen samples of whole serum or plasma as appropriate were homocysteine by fluorescence polarization immunoassay; high-sensitivity C-reactive protein (hsCRP) by particle-enhanced immunonephelometry; and interleukin-6, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, and CD40L by standard enzyme-linked immunoabsorbent assay (Bender MedSystems, Vienna, Austria). Intraassay and interassay CVs for hsCRP, interleukin-6, ICAM-1, VCAM-1, E-selectin, P-selectin, and CD40L ranged from 1.8% to 5.4% and 0.9% to 9.9%, respectively.

All analyses were done in duplicate. As a measure of intervention compliance, urinary resveratrol metabolites and anthocyanins were measured by HPLC before and after each intervention, as previously reported (18, 19).

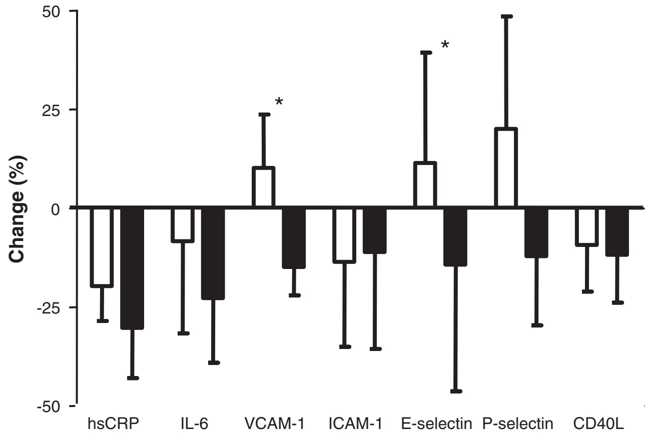

FIGURE 1. Changes from baseline in circulating adhesion and inflammatory molecules after intake of white wine (□) and red wine (■) in healthy women ($n = 35$). Error bars are 95% CIs. hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1. *Significant differences ($P < 0.007$) between effects of white wine and red wine by repeated-measures ANOVA.

PBMC immunophenotyping

PBMCs were obtained from whole blood by the Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) method (20). To measure PBMC expression of cell adhesion molecules (CAMs), a double direct immunofluorescence test was performed with commercial fluorochrome-conjugated monoclonal antibodies. Data analyses were performed with a FACScan Clinical Cytometer (Becton Dickinson, San Jose, CA) with the use of CELLQUEST software (version 7.5.3; Becton Dickinson, Aoust, Belgium). The following CAMs were measured: very late activation antigen-4 (VLA-4; anti-CD49d; Clone 44H6; Cytogmos, Barcelona, Spain), lymphocyte function-associated antigen-1 (anti-CD11a; (Clone R7.1; Bender MedSystems), $\alpha M\beta 2$ (Mac-1; anti-CD11b; Clone LM2/1; Bender MedSystems), and Syalil-Lewis X (anti-CD15s, Clone CSLEx1; BD Biosciences, San Jose, CA).

We also measured the expression of monocyte chemoattractant protein-1 (Pharmingen, San Diego, CA,) and CD40 on the cell surface. Monocytes and T lymphocytes were identified with the use of anti-CD14 and anti-CD2 monoclonal antibodies (Caltag Laboratories, Burlingame, CA), respectively.

Monocyte-endothelium adhesion assay

After obtaining a suspension of PBMCs with the Ficoll-Hypaque method, cells were labeled with microbeads (monoclonal antibodies bound to magnetic particles) and were submitted to a magnetic field, which resulted in the isolation of an enriched monocyte population (>95% $CD14^+$ cells, as assessed by flow cytometry). Cell viability was determined by the Trypan blue dye exclusion test (Sigma-Aldrich, Irvine, CA). The endothelial cell line used was Ea.hy926, which is a fusion product between the human umbilical vein endothelial cell line and the epithelial cell line A549, and was processed as previously reported (14). Endothelial Ea.hy926 cell monolayers were grown to confluence in 96-well tissue culture plates (Nunc, Roskilde, Denmark). The endothelium adhesion assay was performed under nonstimulated and stimulated conditions [human recombinant tumor necrosis factor-α (TNF-α) 10 ng/mL]. We added 1.5×10^5 human monocytes/well (30 min at 37 °C) to allow the adhesion. After that, nonadherent cells were removed by aspiration, and the wells were washed. Adherent cells were fixed and stained with 0.2% crystal violet in 20% methanol in phosphate-buffered saline for 20 min and were washed repeatedly with distilled water. After solubilization with 1% sodium dodecyl sulfate, adhesion was measured in units of absorbance with a spectrophotometer at a wavelength of 600 nm (Multiskan RC ThermoLabsystems, Helsinki, Finland). The adhesion assay, for each subject and condition, was performed in quadruplicate.

Statistical analyses

For a crossover design, statistical power calculations indicated that to detect mean differences of 10 mean fluorescence intensity in VLA-4 in monocytes with a conservative SD of 10 mean fluorescence intensity (13), 32 subjects per group would need to complete the study (α risk $= 0.05$; power $= 0.8$). Although the VLA-4 adhesion molecule measurement was used to set sample size, changes in all endpoints were of equal interest in this study. Descriptive statistics with means and SDs were used for the baseline characteristics of the participants. Values with a skewed distribution (hsCRP, VCAM-1, ICAM-1, and interleukin-6) were transformed to their ln for analyses. Changes in clinical outcomes were assessed with repeated-measures analysis of variance with 3 factors: wine (red wine compared with white wine), time (before compared with after intervention), and wine sequence. Treatment (wine) and time were factors with repeated measures. No carryover effect between wine treatments (period sequence) was found for any variable. Therefore, final analyses were performed with repeated-measures analysis of variance for the 2 factors wine and time and their interactions. Within- and between-group differences are expressed as means and 95% CIs. All statistical tests were 2-tailed, and the significance level was 0.05. Analyses were performed with the use of SPSS, version 11.0 (SPSS Inc, Chicago, IL).

RESULTS

Patient characteristics and diets

Of the 36 participants randomly assigned to intervention, 35 completed all study periods. One woman withdrew before completing the 2 phases of the study. Her baseline characteristics were similar to those of the overall group. The women who finished the trial had a mean age of 38 y (range: 23–50 y). Seventeen participants consumed first red wine for 4 wk and after the washout period switched to white wine for the ensuing 4 wk, whereas 18 subjects followed the same interventions in reverse order.

According to participants' reports and recounts of empty bottles, compliance with intake of both red and white wines was 100%. As an objective measure of intervention compliance, resveratrol metabolites were measured in urine. As expected, urine concentration of total resveratrol metabolites (*cis*- and *trans*-resveratrol glucoronides) increased from 51.2 ± 30.3 to 262.7 ± 76.4 nmol/g (mean change: 211.0 nmol/g; 95% CI: 168.1, 253.9; $P = 0.001$) at the end of the white wine period compared with its correspondent washout period. In addition, a significant increase from 49.5 ± 35.0 to 604.0 ± 425.6 nmol/g (mean change: 554.5 nmol/g; 95% CI: 229.1, 879.9; $P = 0.005$) was also observed in

TABLE 2
Mean fluorescence intensity of adhesion molecule and chemokine expression on T lymphocytes and monocytes before and after each intervention[1]

	Before intervention (n = 35)	After intervention (n = 35)	Differences[2]	P for treatment[3]	P for time[4]	P for interaction[5]
Lymphocytes						
LFA-1						
White wine	126.4 ± 35.6[6]	122.9 ± 31.2	−3.4 (−14.9, 8.1)[7]	0.514	0.457	0.900
Red wine	131.2 ± 33.6	129.8 ± 37.5	−1.4 (−11.2, 8.4)			
VLA-4						
White wine	28.2 ± 5.7	26.8 ± 4.6	−1.3 (−3.1, 0.48)	0.958	0.040	0.726
Red wine	28.4 ± 4.0	26.8 ± 4.3	−1.6 (−3.2, −0.10)			
CD40						
White wine	21.2 ± 9.2	19.1 ± 6.5	−2.1 (−5.4, 1.2)	0.720	0.484	0.716
Red wine	20.8 ± 7.9	20.5 ± 8.6	−0.4 (−3.8, 3.0)			
Syalil-Lewis						
White wine	16.8 ± 7.2	15.1 ± 5.2	−1.8 (−4.0, 0.46)	0.900	0.010	0.369
Red wine	17.7 ± 7.9	14.0 ± 4.9	−3.7 (−6.4, −1.0)			
Monocytes						
LFA-1						
White wine	131.6 ± 29.1	127.1 ± 33.6	−4.4 (−15.5, 6.6)	0.345	0.001	0.002
Red wine	139.9 ± 36.4	112.4 ± 28.0	−27.5 (−39.3, −15.7)			
Mac-1						
White wine	69.9 ± 22.6	60.6 ± 18.2	−9.3 (−16.7, −1.8)	0.116	0.002	0.998
Red wine	66.0 ± 20.1	55.7 ± 18.3	−10.3 (−18.3, −2.3)			
VLA-4						
White wine	42.0 ± 16.2	35.5 ± 12.1	−6.6 (−12.6, −0.46)	0.059	0.004	0.814
Red wine	37.4 ± 16.7	31.3 ± 6.30	−6.1 (−11.7, −0.38)			
CD40						
White wine	43.4 ± 21.1	35.2 ± 17.7	−8.2 (−17.1, 0.62)	0.211	0.017	0.409
Red wine	42.4 ± 20.7	30.6 ± 15.4	−11.8 (−20.9, −2.8)			
Syalil-Lewis						
White wine	59.6 ± 23.3	53.6 ± 24.2	−6.0 (−17.9, 5.9)	0.007	0.007	0.020
Red wine	61.1 ± 28.3	40.7 ± 15.8	−20.6 (−31.0, −10.3)			
MCP-1						
White wine	99.8 ± 51.3	81.9 ± 45.6	−17.9 (−39.8, 4.0)	0.831	0.020	0.701
Red wine	99.6 ± 49.7	76.7 ± 48.1	−22.9 (−44.1, −1.6)			

[1] LFA-1, lymphocyte function-associated antigen 1; VLA-4, very late activation antigen 4; Mac-1, $\alpha M\beta 2$; MCP-1, monocyte chemoattractant protein 1. There were no significant differences between the 2 groups before the intervention.
[2] Before and after intervention.
[3] Comparison between white and red wines (repeated-measures ANOVA).
[4] Comparison between before and after intervention (ANOVA).
[5] Comparison between measures obtained before and after intervention with white and red wines (ANOVA).
[6] $\bar{x}$ ± SD (all such values).
[7] $\bar{x}$; 95% CI in parentheses (all such values).

these metabolites after the red wine period in comparison to its correspondent washout period. The differences between the changes observed in urinary resveratrol concentrations after intakes of white and red wines significantly favored red wine (P = 0.005). In addition, urinary anthocyanin concentrations were measured, as a measure of red wine intake, which increased a mean of 0.077 ng malvidin glycoside/mg creatinine (95% CI: 0.015, 0.140 ng malvidin glycoside/mg creatinine) during red wine intake (P = 0.02), whereas it remained practically unchanged after white wine intake, with a mean change of 0.0002 ng malvidin glycoside/mg creatinine (95% CI: −0.0003, 0.0008 ng malvidin glycoside/mg creatinine).

The energy, nutrient, and antioxidant vitamin contents of the self-reported diets were close to that of the planned diets. No consumption of discouraged polyphenol-rich foods was reported. Nutrient intake was similar in all study phases (data not shown). Participants reported no adverse effects during the wine consumption periods. The average daily energy expended in physical activity was similar throughout the study. No weight or blood pressure changes were observed (data not shown).

Changes in lipids, homocysteine, and soluble inflammatory markers

In comparison with baseline, red wine and white wine intakes produced a significant increase in serum HDL cholesterol with a mean change of 6.7 mg/dL (95% CI: 0.5, 12.8 mg/dL) and 2.8 mg/dL (95% CI: 0.2, 5.3 mg/dL), respectively (P = 0.034; both). However, no significant differences were observed between the effects of both interventions on mean serum HDL cholesterol (3.9 mg/dL; 95% CI: 2.2, 10.0 mg/dL; P = 0.202). No changes were observed in other lipid values. However, homocysteine serum concentration showed an imperceptible mean increase of 0.008 μmol/L (95% CI: −0.50, 0.52 μmol/L; P = 0.97) after the white wine intake and a not significant decrease of 0.22 μmol/L (95% CI: −0.85, 0.41 μmol/L; P = 0.47) after the red wine period.

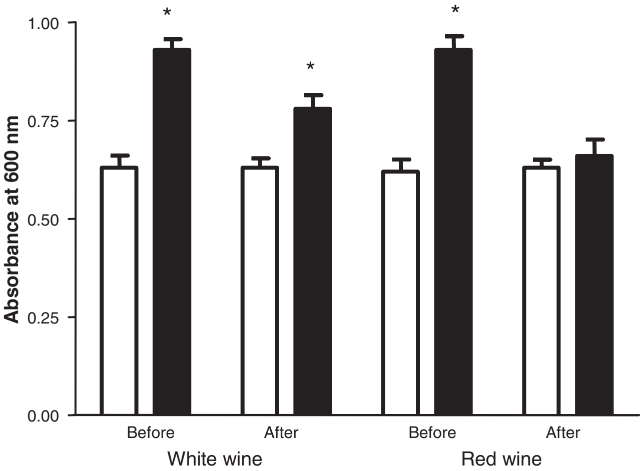

FIGURE 2. Changes in monocyte adhesion to unstimulated (□) and tumor necrosis factor-α (TNF-α)–stimulated (■) endothelial cells (Ea.hy926 line) before and after white wine and red wine in healthy women ($n = 35$). Noticeably, unlike the situation with white wine, adhesion of monocytes to stimulated endothelial cells was similar to that observed in unstimulated conditions after red wine (ie, monocytes were little activated only after red wine). Error bars are 95% CIs. *Significant differences ($P < 0.001$) between unstimulated and TNF-α–stimulated endothelial cells by paired Student's t test.

Changes in circulating concentrations of inflammatory markers are shown in **Figure 1**. hsCRP, interleukin-6, ICAM-1, and CD40L decreased significantly ($P < 0.05$) from 12% to 29% after the white wine intake, whereas hsCRP, interleukin-6, VCAM-1, ICAM-1, E-selectin, P-selectin, and CD40L were significantly ($P < 0.05$) reduced from 17% to 39% after the red wine intake. Significant differences were observed in the effects of the 2 interventions on VCAM-1 and E-selectin, with mean changes after the red wine above those observed after the white wine of −25% (95% CI: −42%, −10%; $P = 0.007$) and −39% (95% CI: −72%, −8%; $P = 0.005$), respectively.

Adhesion molecule expression by PBMCs

The changes in PBMC expression of CAMs and related proteins are shown in **Table 2**. After the red and white wines, the mean expression of VLA-4 and Syalil-Lewis on lymphocyte surface membranes decreased by 10% and 18% ($P < 0.05$, both) and 5% and 6% (NS), respectively. On monocyte membranes, the mean expression of Mac-1, VLA-4, monocyte chemoattractant protein-1, and CD40 decreased between 13% and 28% after both interventions ($P < 0.02$, all). Lymphocyte function-associated antigen-1 and Syalil-Lewis expression, however, decreased only after the red wine intake (20% and 33%, respectively; $P < 0.001$, both).

Monocyte adhesion to endothelial cells

As expected, after the run-in and washout periods monocyte adhesion to the TNF-α–stimulated endothelial cells increased similarly by 46% (95% CI: 38%, 55%; $P < 0.001$) and 48% (95% CI; 39%, 51%; $P < 0.001$), respectively. The results were different after wine intake. Thus, with consumption of white wine, monocyte adhesion to TNF-α–stimulated endothelial cells increased only by 24% (95% CI: 17%, 28%; $P < 0.001$), whereas no significant changes occurred with red wine (5%; 95% CI: −4%, 13%); $P = 0.17$) (**Figure 2**). Compared with data obtained at baseline, white and red wine intakes decreased monocyte adhesion to TNF-α–stimulated endothelial cells by 51% (95% CI: −57%, −45%; $P < 0.001$) and 89% (95% CI: −96%, −82%; $P < 0.001$), respectively. Regarding effects of wine intake on monocyte adhesion to stimulated endothelial cells, a significantly ($P = 0.010$) greater decrease was observed after the red wine than after the white wine (**Table 3**).

DISCUSSION

In this clinical trial performed in 35 healthy women, consumption of 20 g alcohol/d as red wine or white wine was associated with increased HDL cholesterol and decreases in serum inflammatory biomarkers, CAM expression on monocyte surface membranes, and monocyte adhesion to endothelial cells. Red wine was usually more potent than white wine to elicit such changes.

Many epidemiologic studies have related moderate alcohol consumption to reduced rates of cardiovascular morbidity and mortality (1, 3, 5). Part of these beneficial effects are attributed to alcohol-associated increases in both HDL cholesterol and fibrinolytic activity, as well as decreased platelet aggregation (2, 3), although other mechanisms may be involved (2). Thus, the results of prospective studies and clinical trials show that, compared with abstainers or heavy drinkers, moderate drinkers have lower serum concentrations of inflammatory markers, such as hsCRP and interleukin-6 (7, 9, 10, 12, 21). In small clinical trials, wine (13, 14) and beer (21) reduced circulating and cellular inflammatory molecules related to early stages of atheroma plaque formation. Suppression of the postprandial activation of transcription factor nuclear factor-κB in circulating mononuclear cells by a single red wine drink was suggested to play a key

TABLE 3

Changes in monocyte adhesion to nonstimulated and tumor necrosis factor-α–stimulated endothelial cells (line Ea.hy926) associated with consumption of white wine and red wine in healthy women ($n = 35$)

	Before intervention ($n = 35$)	After intervention ($n = 35$)	Differences[1]	P for Treatment[2]	P for Time[3]	P for Interaction[4]
White wine	0.31 (0.27, 0.34)[5]	0.15 (0.12, 0.19)	−0.16 (−0.21, −0.10)	0.005	<0.001	0.010
Red wine	0.29 (0.25, 0.34)	0.03 (−0.01, 0.06)	−0.26 (−0.31, −0.21)			

[1] Before and after intervention.
[2] Comparison between white and red wines (2-tailed paired Student's t test).
[3] Comparison between before and after intervention (ANOVA).
[4] Comparison between measures obtained before and after intervention with white and red wines (ANOVA).
[5] $\bar{x}$; 95% CI in parentheses (all such values).

role in this antiinflammatory effect (22). These beneficial effects on arterial wall inflammation-related processes add biological plausibility to the epidemiologic evidences supporting a cardioprotective effect of alcoholic beverages (23).

It is unclear, however, whether the beneficial effects of moderate alcohol consumption depend on sex, dose, or type of alcoholic beverage. Women appear to be more susceptible than men to alcoholic liver injury (24), brain disorders (25), and cardiomyopathy (26). Accordingly, a threshold of moderate ethanol consumption lower than that stipulated for men has been defined for women (27). In the present study, we observed that 2 daily glasses of 100 mL wine during 4 wk reduced cellular and serum inflammatory biomarkers in women, in addition to decreasing monocyte adhesion to endothelial cells, the postulated first step in the atherosclerotic process (8). With respect to the alcohol dose that may be safe in women, we found that the lower dose (20 g/d) taken by women was associated with beneficial effects on atherosclerotic markers similar to those observed in men consuming higher doses (30 g/d). In fact, an updated meta-analysis of 34 prospective studies has shown that up to 2 drinks/d (20 g/d) are inversely associated with total mortality in women (28).

Whether the beneficial effects of alcohol depend on the type of alcoholic beverage consumed has been a matter of debate. Although prospective studies found no differences among different alcoholic beverages in protection against IHD (1) or reduction in circulating inflammatory markers (7), small clinical trials showed that polyphenol-rich red wine had higher antiinflammatory effects than did gin, which is devoid of polyphenols (13, 14). Red wine and white wine have equivalent ethanol content but dissimilar quantities of polyphenols. In our study red wine was associated with a greater reduction in inflammatory biomarkers, CAM monocyte expression, and monocyte adhesion to endothelial cells than was white wine, suggesting that the polyphenols in even small amounts of red wine are responsible in part for these beneficial effects.

However, although both wines decreased serum ICAM-1 concentrations, only red wine diminished serum concentrations of VCAM-1 and E-selectin. In fact, these adhesion molecules differ in their origin and regulation of expression. Although regulated by inflammatory cytokines, ICAM-1 is constitutively expressed by endothelial cells, whereas VCAM-1 and E-selectin are only expressed on activated endothelium. In addition, serum ICAM-1 may be synthesized by leukocytes and endothelial cells, whereas serum VCAM-1 and E-selectin are mainly synthesized by endothelium (29, 30). These differences may explain, at least in part, why the effects of red wine were different from those of white wine on serum inflammatory biomarkers.

Limitations

One limitation is the inherent difficulty in ensuring compliance with dietary instructions, wine intake, and overall lifestyle in free-living persons. This is particularly important in a study such as ours, because diet and exercise may modify the concentrations of inflammatory markers (31, 32). Nonetheless, adherence to the recommended diets and wine intake was good, as judged by self-reports and objective measurements, and physical activity remained constant throughout the study. However, real-life conditions may be considered a study's strength. Finally, we studied healthy women; thus, it is not possible to determine whether these salutary effects of moderate wine consumption against arterial wall inflammation are also applicable to women at high cardiovascular risk, such as those who are postmenopausal.

From our data we cannot tell which alcoholic or nonalcoholic component of wine may be responsible for the antiinflammatory effects. In this sense, previous in vitro studies have shown that different red wine polyphenols induce down-regulation of ICAM-1 and VCAM-1, reduce adhesion of U937 monocytic cells to stimulated endothelium (33–35), prevent platelet-leukocyte interactions (36, 37), and inhibit the expression of matrix metalloproteinase-2, which is involved in atherosclerotic plaque growth and instability (38). In recent studies comparing the effects of red wine and gin (13, 14), we showed that both beverages (ie, ethanol itself) were associated with reduction of plasma fibrinogen, hsCRP, and interleukin-1α, but polyphenol-rich red wine had the additional effect of decreasing monocyte CAM expression. Thus, both ethanol and nonalcoholic compounds appear to contribute to the antiinflammatory effects of alcoholic beverages.

Conclusions

The mechanisms underlying the cardioprotective effect of moderate alcohol consumption are probably multifactorial (6). Our results indicate that the beneficial effects of moderate wine consumption on the vascular system may be mediated, at least in part, by a reduction in circulating inflammatory molecules, adhesion molecule expression by peripheral monocytes, and monocyte adhesion to endothelium. These salutary effects are observed in women after consumption of lower doses of alcohol as wine than those showing similar benefit in men. In probable relation with its higher polyphenol content, red wine intake is associated with superior antiinflammatory effects than is white wine in women. The results provide additional information on the beneficial role of alcoholic beverages in the prevention of low-grade inflammation and endothelial dysfunction in the arterial wall in women.

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REFERENCES

1. Gronbaek M, Becker U, Johansen D, et al. Type of alcohol consumed and mortality from all causes, coronary heart disease and cancer. Ann Intern Med 2000;133:411–9.
2. Estruch R. Wine and cardiovascular disease. Food Res Int 2000;33:219–26.
3. Gaziano JM, Buring JE, Breslow JL, et al. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. N Engl J Med 1993;329:1829–34.
4. Mukamal KJ, Jensen MK, Gronbaek M, et al. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation 2005;112:1406–13.
5. Tolstrup J, Jensen MK, Tjonneland A, Overvad K, Mukamal KJ, Gronbaek M. Prospective study of alcohol drinking patterns and coronary heart disease in women and men. BMJ 2006;332:1244–8.
6. Lucas DL, Brown RA, Wassef M, Giles TD. Alcohol and the cardiovascular system research challenges and opportunities. J Am Coll Cardiol 2005;45:1916–24.
7. Imhof A, Woodward M, Doering A, et al. Overall alcohol intake, beer,

wine, and systemic markers of inflammation in Western Europe: results from three MONICA samples (Augsburg, Glasgow, Lille). Eur Heart J 2004;25:2092–100.
8. Hanson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685–95.
9. Sacanella E, Badia E, Nicolas JM, et al. Differential effects of moderate or heavy alcohol consumption on circulating adhesion molecule levels. Thromb Haemost 2002;88:52–5.
10. Imhof A, Froehlich M, Brenner H, Boeing H, Pepys MB, Koenig W. Effect of alcohol consumption on systemic markers of inflammation. Lancet 2001;357:763–7.
11. Pai JK, Hankinson SE, Thadhani R, Rifai N, Pischon T, Rimm EB. Moderate alcohol consumption and lower levels of inflammatory markers in US men and women. Atherosclerosis 2006;186:113–20.
12. Albert MA, Glynn RJ, Ridker PM. Alcohol consumption and plasma concentration of C-reactive protein. Circulation 2003;107:443–7.
13. Estruch R, Sacanella E, Badia E, et al. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial. Effects of wine on inflammatory markers. Atherosclerosis 2004;175:117–23.
14. Badia E, Sacanella E, Fernandez-Sola J, et al. Decreased tumor necrosis factor-induced adhesion of human monocytes to endothelial cell after moderate alcohol consumption. Am J Clin Nutr 2004;80:225–30.
15. Ibern-Gomez M, Andres-Lacueva C, Lamuela-Raventos RM, Waterhouse AL. Rapid HPLC analysis of phenolic compounds in red wines. Am J Enol Vitic 2002;53:218–21.
16. Schröder H, Covas MI, Marrugat J, et al. Use of a three-day estimated food record, a 72-hour recall and food frequency questionnaire for dietary assessment in a Mediterranean Spanish population. Clin Nutr 2001; 20:429–7.
17. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish Men. MARATHOM Investigators. Am J Epidemiol 1994;139:1197–209.
18. Andres-Lacueva C, Shukitt-Hale B, Galli RL, Jauregui O, Lamuela-Raventos RM, Joseph JA. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci 2005;8:111–20.
19. Zamora-Ros R, Urpi-Sala M, Lamuela-Raventós RM, et al. Diagnostic performance of urinary resveratrol metabolites as a biomarker of moderate wine consumption. Clin Chem 2006;52:1373–80.
20. Sacanella E, Estruch R, Gaya A, et al. Upregulated expression of VLA proteins and CD29 in peripheral blood lymphocytes of chronic alcoholics without ethanol-related diseases. Alcohol Clin Exp Res 1999;23: 371–5.
21. Sierksma A, van der Gaag MS, Kluft C, Hendriks HF. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur J Clin Nutr 2002; 56:1130–6.
22. Blanco-Colio LM, Valderrama M, Alvarez-Sala LA, et al. Red wine intake prevents nuclear factor-kappa B activation in peripheral blood mononuclear cells of healthy volunteers during postprandial lipemia. Circulation 2000;102:1020–6.
23. Lorgeril M, Salen P. Is alcohol anti-inflammatory in the context of coronary heart disease? Heart 2004;90:355–7.
24. Morgan MY, Sherlock S. Sex-related differences among 100 patients with alcoholic liver disease. BMJ 1977;1:939–41.
25. Mann K, Ackermann K, Croissant B, Mundle G, Nakovics H, Diehl A. Neuroimaging of gender differences in alcohol dependence: are women more vulnerable? Alcoholism Clin Exp Res 2005;29:896–901.
26. Fernandez-Sola J, Estruch R, Nicolas JM, et al. Comparison of alcoholic cardiomyopathy in women versus men. Am J Cardiol 1997;80:481–5.
27. Gunzerath L, Faden V, Zakhari S, Warren K. National Institute on Alcohol Abuse and Alcoholism Report on moderate drinking. Alcohol Clin Exp Res 2004;28:829–47.
28. Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L, de Gaetano G. Alcohol dosing and total mortality in men and women. Arch Intern Med 2006;166:2437–45.
29. Coll-Vinent B, Vilardell C, Font C, et al. Circulating soluble adhesion molecules in patients with giant cell arteritis. Correlation between soluble intercellular adhesion molecule-1 (sICAM-1) concentrations and disease activity. Ann Rheum Dis 1999;58:189–92.
30. Frenette PS, Wagner DD. Adhesion molecules, part II: blood vessels and blood cells. N Engl J Med 1996;335:43–5.
31. Katja B, Tiina L, Veikko S, Pekka J. Associations of leisure time physical activity, self-rated physical fitness, and estimated aerobic fitness with serum C-reactive protein among 3803 adults. Atherosclerosis 2006;185: 381–7.
32. Lopez-Garcia E, Schulze MB, Fung TT, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2004;80:1029–35.
33. Ferrero ME, Bertelli AE, Bertelli A. Activity in vitro of resveratrol on granulocyte and monocyte adhesion to endothelium. Am J Clin Nutr 1998;68:1208–14.
34. Koga T, Meydani M. Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. Am J Clin Nutr 2001;73:941–8.
35. Carluccio MA, Siculella L, Ancora MA, et al. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation. Arterioscler Thromb Vasc Biol 2003;23:622–9.
36. Rotondo S, Rajtar G, Manarini S, et al. Effect of trans-resveratrol, a natural polyphenolic compound, on human polymorphonuclear leukocyte function. Br J Pharmacol 1998;123:1691–9.
37. Appeldoorn CM, Bonnefoy A, Lutters BHC, et al. Gallic acid antagonizes P-selectin-mediated platelet-leukocyte interactions. Implications for the French paradox. Circulation 2005;111:106–12.
38. Oak MH, El Bedoui J, Anglard P, Schini-Kerth VB. Red wine polyphenolic compounds strongly inhibit pro-matrix metalloproteinase-2 expression and its activation in response to thrombin via direct inhibition of membrane type-1 matrix metalloproteinase in vascular smooth muscle cells. Circulation 2004;110:1861–7.