Differential effects of polyphenols and alcohol of red wine on the expression of adhesion molecules and inflammatory cytokines related to atherosclerosis: a randomized clinical trial[1–3]

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ABSTRACT

Background: Few clinical studies have focused on the alcohol-independent cardiovascular effects of the phenolic compounds of red wine (RW).

Objective: We aimed to evaluate the effects of ethanol and phenolic compounds of RW on the expression of inflammatory biomarkers related to atherosclerosis in subjects at high risk of cardiovascular disease.

Design: Sixty-seven high-risk, male volunteers were included in a randomized, crossover consumption trial. After a washout period, all subjects received RW (30 g alcohol/d), the equivalent amount of dealcoholized red wine (DRW), or gin (30 g alcohol/d) for 4 wk. Before and after each intervention period, 7 cellular and 18 serum inflammatory biomarkers were evaluated.

Results: Alcohol increased IL-10 and decreased macrophage-derived chemokine concentrations, whereas the phenolic compounds of RW decreased serum concentrations of intercellular adhesion molecule-1, E-selectin, and IL-6 and inhibited the expression of lymphocyte function-associated antigen 1 in T lymphocytes and macrophage-1 receptor, Sialil-Lewis X, and C-C chemokine receptor type 2 expression in monocytes. Both ethanol and phenolic compounds of RW downregulated serum concentrations of CD40 antigen, CD40 ligand, IL-16, monocyte chemotactic protein-1, and vascular cell adhesion molecule-1.

Conclusion: The results suggest that the phenolic content of RW may modulate leukocyte adhesion molecules, whereas both ethanol and polyphenols of RW may modulate soluble inflammatory mediators in high-risk patients. The trial was registered in the International Standard Randomized Controlled Trial Number Register at http://www.isrctn.org/ as ISRCTN88720134. *Am J Clin Nutr* 2012;95:326–34.

INTRODUCTION

CAD[4] is generally due to atherosclerosis and is the leading cause of morbimorbidity in developed countries. Atherogenesis is a multifactorial process that involves a combination of environmental, genetic, and metabolic components that act synergistically to induce oxidative stress and a chronic inflammatory state. Thus, atherosclerosis is considered a low-grade inflammatory disease in which the cell and endothelial expression of adhesion molecules and chemokines participate in the recruitment of circulating leukocytes to the vascular endothelium and further migration into subendothelial spaces (1), which leads to the formation of atherosclerotic lesions (2). In this process, chemokines, the secreted proteins that recruit specific cell types to inflammatory sites, have emerged as major contributors to vascular inflammation. Therefore, the study of cir-

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[4] Abbreviations used: CAD, coronary artery disease; CCR2, C-C chemokine receptor type 2; CD40a, CD40 antigen; CD40L, CD40 ligand; CRP, C-reactive protein; DRW, dealcoholized red wine; ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; Mac-1, macrophage-1 receptor; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIP-1α, macrophage inflammatory protein 1α; PBMC, peripheral blood mononuclear cell; RW, red wine; SLe^x, Sialil-Lewis X; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late activation antigen 4.

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culating chemokines may provide a greater understanding of the underlying pathophysiology of this disease (3).

Several epidemiologic studies have reported beneficial effects of moderate alcohol consumption on cardiovascular disease (4, 5). Other studies that differentiated the actions of different alcoholic beverages have observed that moderate consumption of RW has greater effects on lowering risk of CAD than do other beverages (6–9). RW contains alcohol and nonalcoholic compounds, mainly polyphenols such as anthocyanosides, catechins, proanthocyanidins, stilbenes, and other phenolic compounds (6). The cardiovascular effects of RW phenolic compounds have been well studied in vitro (10–12) and in animal models (13–16), but few human clinical trials have focused on the alcohol-independent effects of phenolic compounds of RW (17–20). Therefore, the differential cardiovascular effects of both components in RW are not well known.

In contrast, previous clinical trials in healthy men (21, 22) and women (23) have shown a reduction in circulating markers of inflammation and monocyte adhesion to endothelial cells after the daily intake of 20–30 g alcohol as RW, which potentially modulates atherosclerosis development. However, it is unknown whether these effects can be extrapolated to high–cardiovascular risk populations, and whether these effects are attributed to alcohol, polyphenols of RW, or the synergistic effect of both.

Therefore, we embarked on a randomized, crossover, controlled clinical trial to evaluate and compare the effects of moderate consumption of 30 g alcohol/d of gin (a nonpolyphenolic alcoholic beverage), RW (a high-polyphenolic alcoholic beverage), and the same amount of DRW (a high-polyphenolic nonalcoholic beverage) on the expression of soluble and leukocyte adhesion molecules as well as proinflammatory cytokines related to the early stages of atherosclerosis in subjects at high risk of CAD in whom diet and exercise were carefully monitored.

SUBJECTS AND METHODS

Subjects

A total of 73 high-risk subjects aged between 55 and 75 y were recruited for the study in the outpatient clinic of the Internal Medicine Department at Hospital Clínic of Barcelona. Subjects included in the trial were moderate alcohol consumers (1–3 drinks/d) and had diabetes or $\geq$3 of the following cardiovascular disease risk factors: tobacco smoking, hypertension, plasma LDL cholesterol concentrations $\geq$160 mg/dL, plasma HDL cholesterol concentrations $\leq$35 mg/dL, overweight or obesity [BMI (in kg/m^2) $\geq$25], and/or family history of premature CAD. Exclusion criteria included documented CAD, stroke, or peripheral vascular disease, HIV infection, alcoholic liver disease, malnutrition, and neoplastic or acute infectious diseases. The Institutional Review Board of the hospital approved the study protocol, and all participants gave written consent before participation in the study.

Study design

The study was an open, randomized, crossover, and controlled clinical trial, which included 3 4-wk periods. After a run-in period of 2 wk in which subjects were asked not to consume any alcoholic beverage, they received gin (100 mL; 30 g ethanol/d), RW (272 mL; 30 g ethanol/d), and the same amount of polyphenols as RW in the form of DRW (272 mL) according to a computer-generated random-number table. None of the subjects consumed multivitamin or vitamin E supplements or antiinflammatory drugs (steroids, nonsteroidal antiinflammatory drugs, or aspirin >100 mg/d). The RW and DRW were from the Penedès appellation and elaborated with the Merlot grape variety. The phenolic composition of the 3 beverages used in the study is detailed in **Table 1**. The total phenolic content of the 3 beverages was determined by using the Folin-Ciocalteu method (24), the phenolic profile of RW and DRW was determined by using HPLC–diode-array detection as described previously (25), and resveratrol and piceid contents were determined by using HPLC–diode-array detection as described by Romero-Pérez et al (26). No significant differences were observed between phenolic compositions of RW and DRW (Table 1).

Diet and exercise monitoring

Subjects were asked to exclude alcoholic beverages 15-d before the first intervention (run-in period) and during the study. Subjects were also asked not to change their dietary pattern during the study. Natural foods rich in antioxidants, especially fruit and vegetables, were especially monitored so that individual diets had similar antioxidant contents throughout the study. Participants were not blinded to the type of drink they ingested. At the beginning of the study and after each intervention period, a medical record and Minnesota Leisure Time Physical Activity Questionnaire validated in Spain (27) were performed, and a 7-d food record questionnaire (5 weekdays and 2 weekend days), which was also validated in our population (28), was used to assess nutrient intake and to monitor adherence to the study protocol. This information was converted into dietary data by using the Food Processor Nutrition and Fitness Software (*esha* Research). Nutritional variables analyzed included total energy, total protein, carbohydrates, dietary fiber, sugars, total lipids, saturated fatty acids, MUFAs, PUFAs, cholesterol, vitamins C, A, and E, folic acid, and total polyphenols. Subjects were asked to maintain their lifestyle habits and to report any illness or abnormality presented during the study period. At the end of each study period, a clinician assessed any adverse effects from the interventions by administering a checklist of symptoms, including bloating, fullness, or indigestion, altered bowel habits, dizziness, and other symptoms that were possibly associated with the interventions.

Methods

Clinical and laboratory measures

Anthropometric measures were performed with standardized methods (29). Fasting blood and 24-h urine samples were collected at baseline (the last day of the washout period) and the day after the last day of each intervention (RW, DRW, and gin). Serum, EDTA plasma, and urine samples were stored at −80°C until assayed. Clinical investigators and laboratory technicians were blinded to the interventions. To ensure that ethanol consumption did not cause side effects, plasma aminotransferases (aspartate aminotransferase and alanine aminotransferase), γ-glutamyl transpeptidase, albumin, vitamin B-12, and serum and intraerythrocytary folic acid concentrations were measured.

TABLE 1
Phenolic composition of beverages used in the study: RW, DRW, and gin[1]

	RW	DRW	Gin	P^2
Alcohol (%)	14.2	0.42	38	
Phenolic compounds[3]				
Total phenols (mEqGA/L)	2933.35 ± 377.31	2694.92 ± 86.79	ND	0.426
Gallic acid (mg/L)	68.48 ± 6.40	73.17 ± 7.01	ND	0.306
Protocatechuic acid (mg/L)	5.22 ± 0.62	5.85 ± 0.51	ND	0.246
Tyrosol (mg/L)	43.59 ± 4.73	47.81 ± 3.90	ND	0.298
Catechin (mg/L)	123.51 ± 11.30	126.45 ± 13.35	ND	0.786
Epicatechin (mg/L)	67.86 ± 7.74	70.57 ± 8.22	ND	0.699
*t*rans-Caftaric (mg/L)	18.62 ± 1.45	19.21 ± 1.62	ND	0.595
trans-Caffeic (mg/L)	11.50 ± 0.79	12.18 ± 0.92	ND	0.246
trans-Coutaric (mg/L)	5.21 ± 0.45	5.62 ± 0.52	ND	0.182
2-S-glutationylcaftaric (mg/L)	10.30 ± 1.00	10.76 ± 1.26	ND	0.956
Quercetin-3-glucuronide (mg/L)	11.88 ± 1.38	11.25 ± 1.42	ND	0.770
Quercetin (mg/L)	26.66 ± 0.78	23.82 ± 2.37	ND	0.161
Isorhamnetin (mg/L)	3.34 ± 0.27	2.96 ± 0.14	ND	0.114
Delphinidin-3-glucoside (mg/L)	15.25 ± 0.89	14.71 ± 1.62	ND	0.589
Petunidin-3-glucoside (mg/L)	12.29 ± 1.06	12.04 ± 1.15	ND	0.755
Peonidin-3-glucoside (mg/L)	6.78 ± 0.62	6.68 ± 0.57	ND	0.797
Malvidin-3-glucoside (mg/L)	48.83 ± 4.45	49.86 ± 4.27	ND	0.787
Malvidin-(6-acetyl)-3-glucoside (mg/L)	10.97 ± 0.96	10.41 ± 1.20	ND	0.563
Malvidin-(6-coumaroyl)-3-glucoside (mg/L)	4.15 ± 0.27	3.54 ± 0.33	ND	0.066
Total resveratrol (*trans* and *cis*) (mg/L)	5.26 ± 0.83	5.01 ± 0.86	ND	0.566
trans-Resveratrol (mg/L)	2.92 ± 0.36	2.73 ± 0.23	ND	0.352
cis-Resveratrol (mg/L)	2.79 ± 0.15	2.75 ± 0.15	ND	0.761
Total Piceid (*trans* and *cis*) (mg/L)	18.43 ± 1.57	17.98 ± 3.21	ND	0.785
trans-Piceid (mg/L)	9.41 ± 1.12	10.53 ± 0.96	ND	0.160
cis-Piceid (mg/L)	7.71 ± 0.34	7.08 ± 0.87	ND	0.226
Total stilbenes (mg/L)	23.61 ± 1.61	24.53 ± 1.49	ND	0.408

[1] DRW, dealcoholized red wine; mEqGA/L, mEq Gallic acid/L; ND, not detected; RW, red wine.
[2] P values are for comparison between RW and DRW polyphenols (Student's t test for independent samples).
[3] Mean ± SD (n = 2) (all such values).

Resveratrol conjugates derived from phase II and microbial metabolism were measured in 24-h urine samples by using the validated methodology described by Urpi-Sarda et al (30), which was adapted to this study, as a biochemical marker of RW- and DRW-intervention compliance. Ethylglucuronide was measured in 24-h urine samples by using liquid chromatography as a biomarker of alcohol intake. High-performance liquid chromatography was performed on an LC Agilent series 1200 (Agilent Technologies) coupled with a hybrid quadrupole time-of-flight mass spectrometer QSTAR Elite (Applied Biosystems/MDS Sciex).

The following serum-soluble adhesion molecules and cytokines and other regulator molecules of adhesion and inflammation processes were quantified by customized human multianalyte profiling (Human MAP; Rules Based Medicine Inc): CD40a, CD40L, CRP, E-selectin, ICAM-1, IL-1α, -10, -16, -18, and -6, MCP-1, -2, and -3, MDC, MIP-1α, myeloid progenitor inhibitory factor 1, tumor necrosis factor-α, and VCAM-1.

PBMC immunophenotyping

PBMCs were isolated from whole blood by Ficoll-Hypaque (Pharmacia) density gradient. The expression of adhesion molecules on the surface of PBMCs was analyzed via double direct immunofluorescence with the use of commercial monoclonal antibodies by following the manufacturer's instructions. The adhesion molecules analyzed were as follows: VLA-4 (CD49-d; Cytogmos), LFA-1 (CD11a; (Bender MedSystems), Mac-1 (CD11b/CD18; Bender MedSystems), SLe^x (CD15s; Beckman Coulter), CD40 (Caltag Laboratories), CD36 (Beckman Coulter), and CCR2 (R&D Systems). Monocytes were identified and selected with the CD14 monoclonal antibody (Caltag Laboratories), and T lymphocytes were identified and selected with the CD2 monoclonal antibody (Caltag Laboratories). Cell counting (5000 events for T lymphocytes and 2000 events for monocytes) and fluorescence analysis were performed in a FACSCalibur Flow Cytometer (Becton Dickinson) with the use of CellQuest software (version 3.3; BD Biosciences).

Statistical analysis

Statistical analysis was performed with SAS Statistical Analysis Systems software (version 9.2; SAS Institute Inc). Descriptive statistics (means ± SDs) were used for baseline characteristics of participants. Values with a skewed distribution (ICAM-1 and MCP-1) were transformed to their natural logarithm for analyses. ANCOVA with baseline value as the covariate was used to compare changes in outcome variables in response to intervention treatments. One-factor ANOVA for repeated measures was used to compare changes in outcome variables in response between intervention treatments and baseline. To exclude the presence of a carryover effect for the 3 periods, the interaction between treatment (RW, DRW, and gin) and sequence of treatment was analyzed in the repeated-measures ANCOVA analyses. Within- and between-group differences are expressed as means and 95% CIs. $P < 0.05$was considered to be significant.

RESULTS

Baseline characteristics, intervention compliance, diet, exercise monitoring, and side effects

Of the 73 subjects included, 6 subjects withdrew before completing the 3 phases of the study because of physical illness ($n = 2$), journeys ($n = 2$), or not being able to drink DRW ($n = 2$). Therefore, 67 subjects were included in the study. Baseline characteristics of the 67 participants are shown in **Table 2**. Most participants were overweight or obese (~91%), more than one-half of the population had hypertension (~57%), more than three-quarters of subjects had a family history of cardiovascular disease (~78%), and more than one-fifth of subjects had dyslipemia (~24%), had type-2 diabetes (~22%), or were smokers (~24%). Biochemical safety analytes (serum and intraerythrocytary folic acid, vitamin B-12, albumin, aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase) remained within the normal range throughout the study. None of the subjects reported adverse effects related to the interventions.

Protocol adherence was optimum in all subjects, and complete agreement was observed between the reports of participants and the number of empty bottles returned. All the subjects complained about the taste and texture of the DRW. As a measure of intervention compliance, a sum of total resveratrol metabolites, which is a marker of RW and DRW consumption (31), was determined in 24-h urine samples collected the last day of the run-in period and the last day of each intervention. After consumption of RW and DRW, 24-h urinary excretion of total resveratrol metabolites increased in relation to baseline amounts from 1.24 μmol (95% CI: 0.91, 1.65 μmol) to 4.69 μmol (95% CI: 3.86, 5.53 μmol) and 8.33 μmol (95% CI: 6.86, 10.19 μmol), respectively ($P < 0.001$, both). Resveratrol metabolites concentrations were statistically higher after the DRW compared with RW intervention ($P = 0.002$) and were also statistically higher after RW and DRW interventions than those obtained after gin consumption [0.76 μmol (95% CI: 0.48, 1.11 μmol); $P < 0.0001$]. After the gin intervention, the concentration of resveratrol metabolites did not change significantly compared with that at baseline ($P = 0.832$). Ethylglucuronide was used as a biomarker of alcohol consumption. Urinary ethylglucuronide concentrations increased significantly after the RW and gin periods compared with those observed at baseline, with increases of 342% (95% CI: 245%, 773%) and 256% (95% CI: 179%, 599%), respectively ($P < 0.001$, both). Moreover, concentrations after the RW and gin interventions were also higher than those obtained after the DRW intervention: 634% (95% CI: 468%, 1424%) and 491% (95% CI: 359%, 1121%), respectively ($P < 0.001$, both). No significant differences were observed between DRW and baseline periods [66% (95% CI: 64%, 75%); $P = 1.000$] and between RW and gin interventions [24% (95% CI: 24–25%); $P = 1.000$]. According to these results, compliance with the 3 interventions was excellent.

Dietary intake data for the 3 intervention periods are shown in **Table 3**. No significant differences were observed in nutrient intake at the beginning of the study and after each intervention. No significant differences were observed in the daily intake of antioxidants or fat before and after each intervention period or in the daily average energy expended in physical activity during the period of intake of RW, DRW, and gin. In addition, no changes were reported in drug intake (Table 2) in any subjects throughout the study. No effect of the treatment from the previous time period on the response in the current time period was observed ($P > 0.05$, all), and thus, no carryover effect was observed for any variables.

TABLE 2
Baseline characteristics of 67 subjects[1]

	Values
Age (y)	60 ± 8[2]
Current smokers [n (%)]	16 (23.9)
Sedentarism [n (%)]	40 (59.7)
Family history of premature CAD [n (%)]	52 (77.6)
BMI (kg/m^2)	29.6 ± 3.9
BMI ≥25 kg/m^2 [n (%)]	61 (91.0)
WHR	0.975 ± 0.045
Type 2 diabetes [n (%)]	15 (22.4)
Hypertension [n (%)]	38 (56.7)
Dyslipemia [n (%)]	16 (23.9)
Medications [n (%)]	
ACE inhibitors	28 (41.8)
Diuretics	5 (7.5)
Statins	22 (32.8)
Oral hypoglycemic drugs	14 (20.9)
Aspirin or antiplatelet drugs	15 (22.4)
Systolic blood pressure (mm Hg)	142 ± 18
Diastolic blood pressure (mm Hg)	81 ± 8
Heart rate (beats/min)	69 ± 10
Glucose (mg/dL)	111 ± 34
Triglycerides (mg/dL)	128 ± 60
Total cholesterol (mg/dL)	204 ± 33
LDL cholesterol (mg/dL)	133 ± 32
HDL cholesterol (mg/dL)	43 ± 7
LDL cholesterol:HDL cholesterol ratio	3.08 ± 0.10
Folic acid (serum) (ng/mL)	9.8 ± 4.0
Intraerythrocytary folic acid (ng/mL)	386 ± 98
Vitamin B-12 (pg/mL)	406 ± 163
Albumin (mg/mL)	45.4 ± 2.8
ASAT (UI/L)	25.8 ± 9.5
ALAT (UI/L)	29.2 ± 11.7
GGT (UI/L)	30.7 ± 12.6

[1] ACE, angiotensin-converting enzyme; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CAD, coronary artery disease; GGT, γ-glutamyl transpeptidase; WHR, waist-to-hip ratio.

[2] Mean ± SD (all such values).

Changes in circulating inflammatory markers

Changes in circulating inflammatory markers are shown in **Table 4.** Serum E-selectin concentrations were lower after the DRW intervention than after the RW and gin interventions [$P = 0.009$ and 0.016, respectively (Bonferroni post hoc test)]. Serum ICAM-1 and IL-6 concentrations were significantly lower after the RW and DRW interventions than after the gin intervention ($P = 0.014$, 0.016, 0.026, and 0.023 for ICAM-1 and IL-6, respectively). The IL-10 concentration was shown to be significantly higher and MDC was shown to be significantly lower after the RW and gin interventions than after the DRW intervention ($P = 0.046$, 0.023, 0.003, and 0.007, for IL-10 and MDC, respectively). Serum concentrations of CD40a and CD40L decreased significantly after the 3 interventions (RW, DRW, and gin) compared with the baseline situation ($P = 0.008$, 0.043, 0.001, 0.001, 0.019, and 0.015 for CD40a and CD40L, respectively) as

TABLE 3
Daily energy and dietary intakes in 67 subjects at baseline and after 3 interventions[1]

	Baseline	RW intervention	DRW intervention	Gin intervention	*P*
Energy (kcal/d)	1816 ± 457	1782 ± 325	1862 ± 320	1887 ± 336	0.109
Total protein (g/d)	90.39 ± 20.60	89.06 ± 18.35	94.29 ± 17.56	95.04 ± 18.73	0.133
Carbohydrates (g/d)	193 ± 51	201 ± 44	193 ± 38	206 ± 40	0.111
Dietary fiber (g/d)	19.67 ± 9.83	20.63 ± 8.08	19.88 ± 6.82	22.05 ± 10.01	0.254
Sugars (g/d)	62.89 ± 23.55	66.74 ± 25.59	67.33 ± 20.08	70.11 ± 20.84	0.489
Total lipids (g/d)	74.87 ± 24.91	72.72 ± 16.27	78.55 ± 20.41	79.86 ± 21.89	0.194
SFA (g/d)	18.65 ± 8.66	18.40 ± 7.06	18.90 ± 6.15	19.01 ± 5.48	0.804
MUFA (g/d)	36.85 ± 12.06	35.84 ± 8.23	37.97 ± 10.18	38.31 ± 9.60	0.522
PUFA (g/d)	10.31 ± 3.89	10.65 ± 4.21	11.94 ± 4.42	10.71 ± 3.29	0.644
Cholesterol (mg/d)	346 ± 142	355 ± 125	342 ± 103	360 ± 162	0.710
Vitamin C (mg/d)	112 ± 75	125 ± 83	121 ± 68	133 ± 91	0.534
Vitamin A (μg retinol equivalent/d)	640 ± 360	688 ± 340	729 ± 343	791 ± 481	0.345
Vitamin E (mg/d)	9.49 ± 3.88	9.65 ± 3.22	9.47 ± 3.34	10.29 ± 4.65	0.153
Folic acid (μg/d)	426 ± 198	454 ± 155	443 ± 143	498 ± 226	0.181
Total polyphenols (mg/d)	340 ± 185	318 ± 142	311 ± 146	327 ± 170	0.555

[1] All values are means ± SDs. Energy, nutrient, and total polyphenol contributions from interventions were excluded. Changes in outcome variables were determined by using repeated-measures ANCOVA with the baseline value as the covariate. No changes were observed between baseline and interventions determined by repeated-measures ANOVA ($P > 0.05$, all). DRW, dealcoholized red wine; RW, red wine.

did IL-16 (P = 0.013, 0.005, and 0.008 for RW, DRW, and gin, respectively), MCP-1 (P = 0.016, 0.009, and 0.007 for RW, DRW, and gin, respectively) and VCAM-1 (P = 0.002, 0.003, and 0.027 for RW, DRW, and gin, respectively) concentrations. Serum ICAM-1 and IL-6 concentrations decreased significantly after the RW and DRW interventions compared with those at baseline (P = 0.005, 0.003, 0.019, and 0.009 for ICAM-1 and IL-6, respectively), and IL-10 concentrations increased and MDC concentrations decreased after the RW and gin interventions compared with those at baseline (P = 0.013, 0.023, 0.008, and 0.013 for IL-10 and MDC, respectively). Otherwise, serum concentrations of the other molecules evaluated were shown to remain practically unaltered between baseline and after the 3 interventions.

Expression of cell adhesion molecules on leukocyte cell surfaces

Changes in the expression of cell adhesion molecules on leukocyte cell surfaces are shown in **Table 5**. LFA-1 expression on T-lymphocyte membrane was significantly higher after the gin intervention than after the RW and DRW interventions [P =

TABLE 4
Expression of soluble adhesion molecules and cytokines in 67 subjects at baseline and after 3 interventions[1]

	Baseline	RW intervention	DRW intervention	Gin intervention	*P*
CD40a (ng/mL)	0.865 ± 0.225	0.805 ± 0.195[2]	0.818 ± 0.201[2]	0.799 ± 0.202[2]	0.734
CD40L (ng/mL)	2.15 ± 1.16	1.62 ± 1.23[2]	1.81 ± 1.24[2]	1.79 ± 1.24[2]	0.477
CRP (μg/mL)	2.18 ± 0.31	2.17 ± 0.33	1.76 ± 0.20	2.15 ± 0.28	0.210
E-selectin (ng/mL)	9.38 ± 4.30	8.78 ± 3.25[a]	8.33 ± 3.17[b]	8.91 ± 3.93[a]	0.008
ICAM-1 (ng/mL)	91.41 ± 28.63	81.33 ± 25.98[a,2]	79.31 ± 21.46[a,2]	87.82 ± 20.39[b]	0.029
IL-1α (pg/mL)	4.46 ± 2.23	4.24 ± 2.23	4.41 ± 2.58	4.82 ± 2.77	0.188
IL-10 (pg/mL)	6.44 ± 2.63	7.68 ± 1.96[a,2]	6.69 ± 2.63[b]	7.89 ± 2.38[a,2]	0.043
IL-16 (pg/mL)	478 ± 142	450 ± 143[2]	428 ± 168[2]	436 ± 147[2]	0.582
IL-18 (pg/mL)	399 ± 123	384 ± 150	384 ± 129	383 ± 127	0.985
IL-6 (pg/mL)	3.67 ± 1.15	2.76 ± 1.06[a,2]	2.44 ± 1.29 [a,2]	3.70 ± 1.14[b]	0.039
MCP-1 (pg/mL)	488 ± 205	455 ± 201[2]	454 ± 196[2]	446 ± 219[2]	0.817
MCP-2 (pg/mL)	43.67 ± 12.57	43.67 ± 10.37	43.17 ± 12.19	43.02 ± 11.60	0.724
MCP-3 (pg/mL)	60.64 ± 20.03	60.72 ± 17.50	59.10 ± 17.69	59.10 ± 17.11	0.546
MDC (pg/mL)	450 ± 104	416 ± 115[a,2]	446 ± 120[b]	418 ± 104[a,2]	0.009
MIP-1α (pg/mL)	141 ± 30	138 ± 30	140 ± 31	142 ± 29	0.248
MPIF-1 (ng/mL)	1.41 ± 0.29	1.41 ± 0.27	1.43 ± 0.31	1.39 ± 0.31	0.274
TNF-α (pg/mL)	7.60 ± 3.79	8.09 ± 5.59	7.034 ± 3.96	7.64 ± 5.95	0.121
VCAM-1 (ng/mL)	608 ± 114	572 ± 112[2]	572 ± 113[2]	576 ± 112[2]	0.952

[1] All values are means ± SDs. Changes in outcome variables in response to the intervention treatment were determined by repeated-measures ANCOVA with the baseline value as the covariate. Values in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni post hoc test). CD40a, CD40 antigen; CD40L, CD40 ligand; CRP, C-reactive protein; DRW, dealcoholized red wine; ICAM-1, intercellular adhesion molecule 1; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIP-1α, macrophage inflammatory protein 1α; MPIF-1, myeloid progenitor inhibitory factor 1; RW, red wine; VCAM-1, vascular cell adhesion molecule 1.

[2] Significantly different from baseline, $P < 0.05$ (repeated-measures ANOVA and Bonferroni post hoc test).

TABLE 5
Expression of adhesion molecules on the surface of T lymphocytes and monocytes in 67 subjects at baseline and after 3 interventions[1]

	Baseline	RW intervention	DRW intervention	Gin intervention	*P*
T lymphocytes					
LFA-1 (MFI)	66.03 ± 15.72	65.95 ± 17.51[a]	66.98 ± 13.61[a]	75.92 ± 12.98[b]	0.003
Mac-1 (MFI)	43.44 ± 13.79	39.73 ± 10.90	37.80 ± 14.29	40.76 ± 15.79	0.271
VLA-4 (MFI)	36.67 ± 8.99	36.96 ± 8.34	37.27 ± 7.73	37.08 ± 7.97	0.775
SLe^x (MFI)	77.30 ± 14.06	68.46 ± 13.62[2]	68.94 ± 14.21[2]	73.28 ± 14.31	0.268
CD40 (MFI)	38.44 ± 12.73	37.36 ± 13.84	38.72 ± 11.39	38.37 ± 12.12	0.585
Monocytes					
LFA-1 (MFI)	30.59 ± 8.41	31.50 ± 9.29	31.01 ± 8.90	31.66 ± 9.19	0.781
Mac-1 (MFI)	30.05 ± 12.78	26.32 ± 9.60[a,b,2]	25.46 ± 6.41[a,2]	28.83 ± 11.48[b]	0.044
VLA-4 (MFI)	23.16 ± 8.07	22.74 ± 6.22	23.03 ± 9.57	22.69 ± 6.25	0.909
SLe^x (MFI)	40.12 ± 10.95	36.90 ± 10.40[2]	36.93 ± 11.05[2]	37.39 ± 10.53	0.861
CD40 (MFI)	23.83 ± 6.94	24.22 ± 5.23	23.67 ± 5.49	23.53 ± 5.21	0.266
CD36 (MFI)	19.89 ± 7.67	20.73 ± 7.90	20.50 ± 8.23	20.79 ± 7.74	0.279
CCR2 (MFI)	169.53 ± 36.78	116.94 ± 41.04[a,2]	109.52 ± 38.81[a,2]	168.83 ± 35.10[b]	0.009

[1] All values are means ± SDs. Changes in outcome variables in response to the intervention treatment were determined by repeated-measures ANCOVA with the baseline value as the covariate. Values in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni post hoc test). CCR2, C-C chemokine receptor type 2; DRW, dealcoholized red wine; LFA-1, lymphocyte function-associated antigen 1 (CD11a); Mac-1, macrophage-1 receptor; (CD11b/CD18); MFI: mean fluorescence intensity; RW, red wine; SLe^x, Sialil-Lewis X, CD15s; VLA-4, very late activation antigen 4 (CD49-d).

[2] Significantly different from baseline, $P < 0.05$ (repeated-measures ANOVA and Bonferroni post hoc test).

0.003 and 0.005, respectively (Bonferroni post hoc test)], Mac-1 monocyte expression was lower after the DRW intervention than after the gin period ($P = 0.014$), and CCR2 monocyte fluorescence intensity was also lower after the RW and DRW interventions than after the gin intervention ($P = 0.019$ and <0.0001, respectively). In addition, compared with the baseline situation, we observed that SLe^x lymphocyte and monocyte expression decreased significantly after the RW and DRW interventions (P = 0.006, 0.012, 0.027, and 0.037 for lymphocyte and monocyte SLe^x, respectively) as did the Mac-1 ($P = 0.018$ and 0.003, respectively) and CCR2 monocyte fluorescence intensity ($P = 0.024$ and 0.006, respectively). The expression of VLA-4, Mac-1, and CD40 on the lymphocyte surface remained practically unaltered. The expression of LFA-1, VLA-4, CD40, and CD36 on monocyte cell membrane were not altered.

DISCUSSION

Atherosclerosis is a low-grade inflammatory disease characterized by local inflammation in the vessel wall, as well as a systemic immune response (32), in which recruitment and migration of leukocytes through cell adhesion molecules into the arterial wall is a crucial step in early atherogenesis (33). We measured several chemokines and adhesion molecules implicated in the onset and progression of the atherosclerotic process after a 1-mo intervention with RW (constituted mainly by alcohol plus polyphenols), DRW (polyphenols), and gin (alcohol) in high-risk subjects. A downregulation of serum concentrations of CD40a, CD40L, IL-16, MCP-1, VCAM-1, and E-selectin was observed after the RW, DRW, and gin interventions. Therefore, these effects could be attributed to both ethanol and the phenolic compounds of RW but in a nonadditive manner. However, an additive effect of ethanol and RW polyphenols was suggested in the case of CD40L, albeit without achieving significance. RW polyphenols also exert a protective effect on atherosclerosis because the RW and DRW interventions decreased ICAM-1 and IL-6 serum concentrations and inhibited the expression of LFA-1 and SLe^x in the T-lymphocyte surface and Mac-1, SLe^x, and CCR2 expression in monocytes. Finally, ethanol by itself exerts a dual protective effect by increasing IL-10 and decreasing MDC concentrations.

To our knowledge, no previous consumption trials have compared the antiinflammatory effects of DRW with those of RW and gin in humans. However, our results agree with those obtained in experimental studies such as the study by Norata et al (34) in which MCP-1, MIP-1α and-1β, and IL-6 decreased in the arterial wall of apolipoprotein E$^{-/-}$ mice after oral administration of a mixture of polyphenols. Similarly, in the human umbilical vein endothelial cells cell line, Cullen et al (35) showed that ethanol inhibited the endothelial production of MCP-1, and Carluccio et al (36) observed that resveratrol inhibits the synthesis of VCAM. Lastly, the incubation platelets from healthy humans with extracts of grape seed and skin inhibited the release of soluble CD40L (37).

We observed increased antiinflammatory IL-10 concentrations only after the gin intervention. Similarly, Mandrekar et al (38) showed an increase of IL-10 in human monocytes after acute alcohol intake. We also showed a diminution of MDC after the RW and gin interventions. To our knowledge, this is the first time that MDC and IL-16 expression has been studied after moderate RW or ethanol consumption.

More interestingly, the polyphenols of RW after the RW and DRW interventions decreased plasma concentrations of ICAM-1 and IL-6 and inhibited the expression of LFA-1 and SLe^x in the T-lymphocyte surface and that of Mac-1, SLe^x, and CCR2 on monocytes. These results agree with in vitro or animal models that analyzed the effect of polyphenols of RW, mainly resveratrol, in the regulation of these molecules. Resveratrol reduced ICAM-1 expression in human umbilical vein endothelial cells (39) and IL-6 in vascular smooth-muscle cells (40). The preincubation of polymorphonuclear leukocytes with *trans*-resveratrol resulted in a concentration-dependent inhibition of fMLP (formyl methionyl leucyl phenylalamine)-induced Mac-1 expression (41). Norata et al also showed a decrease in CCR2 expression in the arterial

wall of apolipoprotein $E^{-/-}$ mice after oral administration of resveratrol (34), and Cullen et al (42) showed that CCR2 expression was inhibited in a time- and dose-dependent manner by resveratrol in THP-1 monocytes.

Only a few clinical trials have analyzed the changes in the cellular expression of adhesion molecules (21, 23) or in the inflammatory biomarkers related to atherosclerosis (43) after 1-mo of moderate RW consumption in healthy humans. Therefore, we wondered whether the results observed may be extrapolated to a high–cardiovascular risk population. In a previous study in which 30 g ethanol/d in the form of gin and RW was administered to healthy men, significant reductions of VCAM-1 (16.5%), ICAM-1 (9.2%), and IL-1α (20.3%) concentrations were shown after RW consumption (21). In addition, a reduction of 13.9% in VLA-4 lymphocyte expression and LFA-1 (27.1%), Mac-1 (26.7%), VLA-4 (32.5%), and MCP-1 (45.7%) monocyte expression was also observed after RW consumption. However, no significant effects were shown after the gin intervention, except for a decrease in IL-1α concentrations (22.6%). No significant effects were detected in TNF-α, transforming growth factor β1, IL-6, ICAM-1, and VCAM-1 in another human trial performed by Djurovic et al (43) that included healthy subjects (men and women) who consumed 15 g alcohol/d of RW during 3 wk. Differences in the cardiovascular effects observed between the 2 studies (21, 43) may have been attributed to the different amount of alcohol administered (15 compared with 30 g ethanol/d). In the current study, in which 30 g ethanol/d was administered, reductions of ~6%, 11%, and ~12% of VCAM-1 and ICAM-1 concentrations and Mac-1 monocyte expression, respectively, were observed after RW consumption. Nevertheless, in difference from the aforementioned studies, we also observed a significant decrease of ~6% and ~7% in E-selectin and MCP-1 concentrations, respectively, as well as an increase of 15% in LFA-1 lymphocyte expression after the gin period. Thus, the effects of moderate RW consumption observed in healthy people could not be strictly extrapolated to the high–cardiovascular risk population. The fact that more than one-half of the high-risk subjects consumed drugs with known antiinflammatory effects (mainly statins and aspirin) (44) may explain, in part, the differences observed. However, ethanol (gin) exerted some antiinflammatory effect in high-risk subjects, which were actions that were not previously observed in healthy subjects.

One of the main limitations of our study is that there were no washout periods between interventions. Washout periods between interventions would have extended the study 6 wk more, which would have made it difficult to ensure compliance and subject withdrawal more likely. However, because previous studies have shown that changes in cellular and soluble adhesion molecules were already observed after 2 wk of intervention (21, 45), and no carryover effect was observed, the absence of a washout period would probably not have changed the results.

Although the study was far too short to deal with atherosclerosis itself and morbid mortality due to cardiovascular disease, RW polyphenols may provide additional benefits because of its antiinflammatory effects, namely the decreased expression of adhesion molecules related to early stages of atherosclerosis. Thus, another limitation of our study was that our results can only lead to limited conclusions that link the findings to a reduction in atherosclerosis because the biomarkers used in our study are not commonly examined in the clinical setting (46). Moderate alcohol consumption (independent of the type of beverage) is associated with lower risk of cardiovascular disease mortality and endpoints for CAD (47). Because most alcoholic beverages contain alcohol and polyphenols (except for gin and vodka among other alcoholic beverages), it is very difficult to exclude the effects of ethanol from other compounds of alcoholic beverages. Few studies have shown an inverse association between moderate RW consumption and risk of stroke in men (48). Therefore, we cannot conclude that the changes observed were of a potentially clinical relevant magnitude, and thus, additional studies are required regarding the clinical implication of the modulation of these biomarkers (and the role of each compound of alcoholic beverages) on the beneficial effects of alcoholic beverages on the cardiovascular system.

In conclusion, to our knowledge, this is the first randomized, controlled, clinical trial that studied the effects of RW and DRW separately on the expression of adhesion molecules and inflammatory cytokines related to early stages of atherosclerosis and provided information on the separate role of ethanol and phenolic compounds of RW in low-grade inflammation in the arterial wall and endothelial dysfunction. Our results suggest that both ethanol and nonalcoholic compounds contribute to antiinflammatory effects of RW. The phenolic content of RW may modulate leukocyte adhesion molecules, whereas both ethanol and polyphenols of RW may modulate soluble inflammatory mediators in patients at high risk of cardiovascular disease. These positive changes in the inflammatory profile in high–cardiovascular risk patients could contribute to the benefits of moderate wine consumption against early stages and the progression of atherosclerosis.

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