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Randomized Control Trials

Consumption of aged white wine modulates cardiovascular risk factors via circulating endothelial progenitor cells and inflammatory biomarkers

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SUMMARY

Background & aims: There is compelling evidence showing that moderate alcohol consumption reduces cardiovascular risk factors related to atherosclerosis. The aim of this study was to evaluate the effects of aged white wine (AWW) and gin on circulating endothelial progenitor cells (EPC) and the expression of cell adhesion molecules, inflammatory cytokines and chemokines related to atherosclerosis in high cardiovascular risk subjects.

Methods: This was an open, randomized, controlled, crossover study in 38 high-risk male volunteers between 55 and 80 years of age randomized to receive 30 g of ethanol/day as AWW or gin during 3 weeks. We used the paired two-tailed t-test to compare differences in outcome variables in response to each intervention. Carryover effects for the two periods were evaluated comparing the outcome variables before the AWW and gin interventions.

Results: Compared to gin, AWW intake was associated with a significant 39.6% increase in EPCs. Expression of CD31 and CD40 in T-lymphocytes and of CCR2 and CD36 in monocytes also decreased significantly after AWW intake. In addition, compared to gin, AWW was associated with a significant decrease of plasma pro-inflammatory biomarkers interleukin-8 and interleukin-18 and vascular and intercellular adhesion molecules-1. Lfa-1, Mac-1, VLA4, CD40 and CD31 expression in monocytes and interferon gamma (IFN-γ) concentrations significantly decreased after intake of both alcoholic beverages.

Conclusions: AWW shows a greater ability to repair and maintain endothelial integrity compared to gin. This effect is probably due to grape-derived minor components in AWW, which are absent in gin.

1. Introduction

Atherosclerosis, the main cause of coronary artery disease (CAD), is considered to be a low-grade inflammatory disease in which circulating T-cells and monocytes are recruited and migrate to the sub-endothelial space following activation by adhesion molecules and chemokines secreted by the endothelium [1]. In this setting, cardiovascular risk factors such as smoking, high cholesterol, hypertension, and insulin resistance trigger endothelial cell (EC) dysfunction and the development of atherosclerosis [2]. In parallel, circulating endothelial progenitor cells (EPCs) act as a defense mechanism by attaching to the damaged vascular endothelium [3], thereby repairing its integrity and restoring its function [4].

Results from epidemiological studies have consistently shown that moderate alcohol consumption is associated with reduced cardiovascular risk and mortality [5]. The protective effects of moderate alcohol consumption have been attributed to an increase in plasma high-density lipoprotein cholesterol (HDL) and apolipoprotein A-1 (Apo-A1) concentrations and a decrease of platelet aggregation and fibrinogen concentrations. Reports from several studies also suggest that the prevalence of CAD is lower in subjects who consume moderate amounts of red wine [6], an effect that has been attributed to the high polyphenol content of this beverage. In

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fact, these bioactive compounds have antioxidant and anti-inflammatory properties that improve endothelial dysfunction and other vascular abnormalities in both healthy subjects and patients with CAD [7,8]. To date, few studies have evaluated the effects of alcoholic beverages rich in polyphenols such as wine or beer on circulating EPCs in healthy individuals [7,9] or in those at high cardiovascular risk [10].

Therefore, the aim of the current study was to compare the effects of moderate intake of an alcoholic beverage without polyphenols (gin) with aged white wine (AWW), which has a high polyphenol content. Table 1 details the phenol composition of different types of alcoholic beverages, including the sherry variety AWW. The expression of cellular adhesion molecules related to atheroma plaque formation and circulating EPCs, as well as plasma levels of immunomodulatory biomarkers and endothelial adhesion molecules were assessed after 3 weeks of each intervention in individuals at high cardiovascular risk.

2. Subjects and methods

2.1. Study design

This was an open, randomized, controlled, crossover study with two intervention periods. After signing the informed consent form, volunteers were randomized using a computer-generated random-number table into two interventions in a crossover design. One intervention included the administration of 92 mL of gin (30 g ethanol/day), provided by the Gin Xoriguer company, and the other intervention included the same amount of ethanol as AWW of the sherry variety (13% ethanol content) also called "*veil of flower* wine" (255 mL-total phenols: 927.79 Eq gallic acid/day (EGA)/day) provided by the Andalusian Government. A description of the biological aging process of sherry wines can be found elsewhere [11]. Each intervention was carried out over 21 days with a 2-week washout period between the two interventions. During the washout period the volunteers were asked to consume no alcoholic beverages. Participants fasted for 12 h prior to venipuncture for blood analyses.

Table 1
Phenolic compounds of different alcoholic beverages.

	RW	WW	SW	Beer
Total phenols (mEqGA/L)	2154.8	321	927.8	278.3
Gallic acid (mg/L)	35.9	2.2	6.0	0.7
Protocatechuic acid (mg/L)	3.3	0.5	5.6	0.5
4-Hydroxybenzoic acid (mg/L)	5.5	0.2	0.3	9.6
Vanillic acid (mg/L)	3.2	0.4	0.7	0.7
Syringic acid (mg/L)	2.7	5.4×10^{-2}	1.5	0.2
Caffeic (mg/L)	18.8	2.4	1.0	0.3
Caffeoyl tartaric acid (mg/L)	33.5	21.5	17.5	–
Ferulic acid (mg/L)	0.8	0.9	0.2	2.6
p-Coumaric acid (mg/L)	5.5	1.5	0.2	1.0
p-Coumaroyl tartaric acid (mg/L)	11.8	21.5	24.2	–
Flavanols				
Catechin (mg/L)	68.1	10.8	23.7	1.1
Epicatechin (mg/L)	37.8	9.5	12.5	0.6
Procyanidin dimer B1 (mg/L)	41.7	8.4×10^{-2}	20.0	–
Procyanidin dimer B2 (mg/L)	49.7	4.7×10^{-2}	7.2	–
Procyanidin dimer B3 (mg/L)	94.7	2.9×10^{-2}	14.7	1.8
Procyanidin dimer B4 (mg/L)	72.9	7.1×10^{-2}	3.5	
Flavonols				
Quercetin (mg/L)	8.3	0.4	0.1	6.7×10^{-2}
Quercetin 3-O-arabinoside (mg/L)	4.9	2.2	–	5.8×10^{-3}
Quercetin 3-O-rutinoside (mg/L)	8.1	1.9	–	0.9
Other polyphenols				
Tyrosol (mg/L)	31.2	2.1	57.3	3.2
Vanillin (mg/L)	–	–	2.2	0.2
Resveratrol (mg/L)	2.7	0.4	–	–
trans-Resveratrol (mg/L)	1.8	0.3	–	–
cis-Resveratrol (mg/L)	1.3	0.2	–	–

RW: Red wine; WW: White Wine; SW: Sherry Wine.
Gin was not determined because of its phenol content is close to zero.

2.2. Study subjects

A total of 47 consecutive potential participants were screened from September 2012 to March 2014 in a primary care center associated with Hospital Clínic of Barcelona, Spain (Fig. 1). Of them, one did not fulfill inclusion criteria, four declined to participate and another was excluded because of excessive alcohol consumption. Of the remaining 41 participants selected and randomized, three dropped out during the study. Thus, a total of 38 individuals completed the study. Participants' age was between 55 and 80 years and all were moderate alcohol drinkers (around 30 g ethanol/day) and had 3 or more of the following cardiovascular risk factors: active smoking, hypertension, high low-density lipoprotein (LDL)-cholesterol concentrations (>160 mg/dL), low high-density lipoprotein (HDL)-cholesterol (<35 mg/dL) concentrations, overweight or obesity (body mass index (BMI) > 25 kg/m^2), and family history of premature cardiovascular disease (CVD). Exclusion criteria included documented CHD, stroke or peripheral vascular disease; human immunodeficiency virus infection; alcoholic liver disease, malnutrition, and neoplastic or acute infectious diseases. No participant consumed vitamin supplements or anti-inflammatory drugs (steroids, non-steroidal anti-inflammatory agents or aspirin) during the study period.

2.3. Diet and exercise monitoring

All participants were asked to abstain from alcohol during the 2 weeks prior to starting the first intervention (run-in period) and to maintain dietary habits and physical activity throughout the study. Fruits, vegetables and other foods rich in antioxidants were monitored to ensure a similar content of antioxidants in the individual diets throughout the study.

Exercise was also monitored before and after each intervention by using the Minnesota Leisure Time Physical Activity questionnaire [7]. The dietitian used a 3-day (2 week-days and 1 weekend day) food record for diet monitoring. After each intervention, the Food Processor Nutrition and Fitness Software (ESHA Research, Salem OR, 2012 10.10.0) was used to convert the dietary information into nutrient data. In addition, the dietitian made weekly phone calls to the participants to ensure adherence to the protocol. Intervention compliance was assessed by recount of empty bottles and measuring tartaric acid concentrations in urine. At the end of each intervention, a clinician assessed possible adverse effects using a symptom checklist.

2.4. Ethics statement

The Institutional Review Board of Hospital Clinic approved the study protocol, and all participants signed the informed consent form. This study was registered at controlled-trials.com (ISRCTN01319643).

2.5. Clinical and laboratory measurements

Main outcome measures were changes in classical cardiovascular risk factors, cellular expression of circulating adhesion molecules, and EPCs and plasma biomarkers related to atherosclerosis after 3 weeks of intervention.

A registered nurse measured blood pressure (BP) using an oscillometer (Omron 705 CP; Omrom Matsuasaka Co Ltd, Matsuasaka City, Japan) before and after each intervention (AWW or gin). BP and heart rate were measured 3 times at 5 min intervals in the

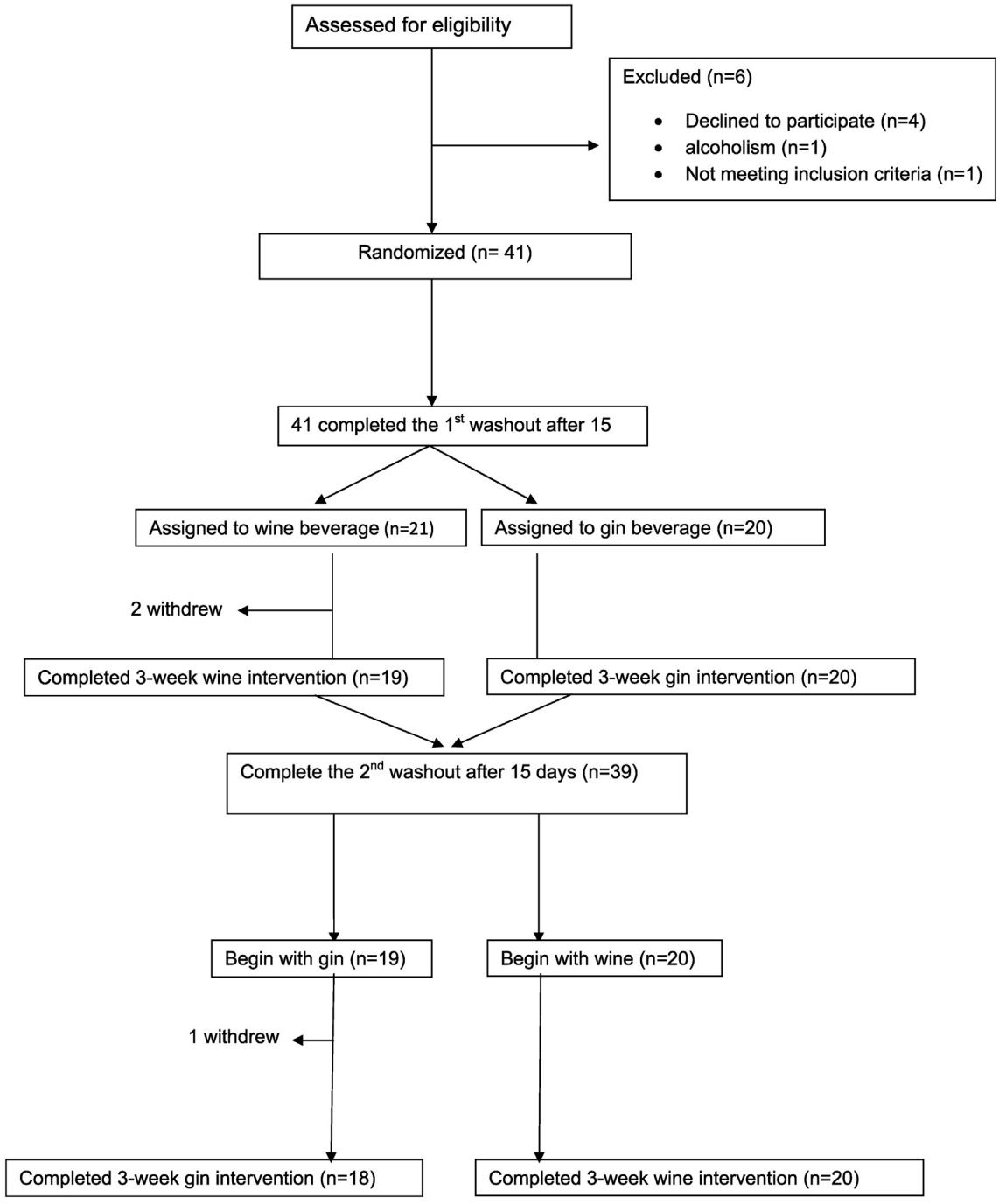

Fig. 1. Flow-chart.

non-dominant arm after 15 min resting in a seated position. The mean of the second and third measurements were taken as clinic BP. In addition, anthropometric measurements including height, body weight and waist and hip circumferences were performed using standardized methods [1,10].

Before and after each intervention, fasting blood, serum, ethylenediaminetetraacetic acid (EDTA) plasma and urine samples were collected and immediately centrifuged and stored at −80 °C until assayed. To determine the possible adverse effects of ethanol intake, plasma aspartate aminotransferases (AST), alanine transaminase (ALT), gamma glutamyltranspeptidase (GGT), albumin, cholesterol, prothrombin time, folic acid and vitamin B_{12} concentrations were also analyzed [1,10].

2.5.1. Endothelial progenitor cell determination

A 10 mL aliquot of whole blood was used for EPC quantification. Blood samples were processed within 1 h after collection, and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density-gradient centrifugation. Recovered cells were washed twice with phosphate buffer saline (PBS) and the pellet was resuspended at a concentration of 10^7 cells/mL of 2% fetal bovine serum (FBS). After counting in a Neubauer chamber, a volume of 100 μl of the suspension (10^6 cells) was incubated for 1 h in the dark at 4 °C with 10 μl of monoclonal antibodies against phycoerythrin (PE)-labeled human vascular endothelial growth factor receptor 2 (VEGFR-2), CD34-FITC and CD133-APC (MAC's Miltenyi Biotech, all), which are known to be expressed in EPC. At the end of the

incubation, 1 μl of 7-aminoactinomycin D _8722D) (Sigma–Aldrich) was added, and the cells were washed with PBS/2% FBS and fixed in PBS 1% paraformaldehyde before analysis. Each analysis was carried out at a maximum of 1 h after fixation and included 500,000 PBMCs. Fluorescence analyses were performed in a FACSCalibur Flow Cytometer (Becton–Dickinson, San Jose, CA) using CellQuest Pro software [10].

2.5.2. Peripheral blood mononuclear cell immunophenotyping

PBMCs were isolated from whole blood by Ficoll-Hypaque (Lymphoprep™, Axis-Shield PoC AC) density-gradient. Using double direct immunofluorescence, a FACSCalibur Flow Cytometer™ (BectoneDickinson, San Jose, CA, USA) analyzed the expression of adhesion molecules on the surface of PBMCs using commercial monoclonal antibodies according to the manufacturer's instructions [1]. The adhesion molecules analyzed were: very late activation antigen-4 (VLA4), CD49-d) (Cytogmos, Barcelona, Spain), lymphocyte function-associated antigen-1 (Lfa-1, CD11a) (Bender MedSystems, Vienna, Austria), macrophage-1 antigen (Mac-1) (CD11b/CD18) (Bender MedSystems), SLex (Sialil-Lewis X, CD15s) (Beckman Coulter, Fullerton, CA), CD40 (Caltag Laboratories, Burlingame, CA), CD36 (Beckman Coulter), and chemokine receptor type 2 (CCR2) (R&D Systems, Minneapolis, USA).

Sphero Rainbow calibration particles (6 peaks) of 6.0–6.4 μm (BD Biosciences, San Jose, CA) were used to monitor fluorescence. Anti-CD14 and anti-CD2 monoclonal antibodies (Caltag Laboratories, both) were used as markers of monocytes and T-lymphocytes. Cell counting was 5000 events for T-lymphocytes and 3500 for monocytes, and the fluorescence analysis was performed using the CellQuest Pro software. The results are expressed as mean fluorescence intensity (MFI) in arbitrary units [1].

2.5.3. Plasma inflammatory biomarkers

Plasma was obtained after centrifugation of blood at 1500 rpm for 15 min at room temperature and stored at −80 °C until analysis. The plasma concentrations of 12 inflammatory biomarkers related to different stages of the atherosclerotic process were measured. Interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12, IL-13, and interferon gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), IL-18, ICAM-1 and VCAM-1 were determined using the Bio-PlexPro™ cytokine, adhesion molecule and chemokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA). These kits are based on magnetic bead-based multiplex assays designed to measure multiple cytokines, adhesion molecules and chemokines. Plasma samples were diluted 1:4 with the diluents provided for each assay following the manufacturer's instructions. Data were acquired using the Luminex® 100™ System (Luminex, Austin, TX) and Bio-PLEX Manager 6.1 Software (Bio-Rad, Hercules, CA). Concentrations were obtained by standard calibration curves. The results are expressed in pg/mL.

2.6. Statistical analyses

The ENE 3.0 statistical program (GlaxoSmithKline, Brentford, United Kingdom) was used to determine sample size. Assuming a maximum loss of 10% of participants and to detect a mean difference of 10 MFI units in the expression of monocyte VLA4 with a conservative standard deviation (SD) of 10, 20 subjects would be needed for the study (α risk = 0.05, power = 0.9). The monocyte expression of VLA4 was considered the primary outcome, but changes in all endpoints were of equal interest in this study [12,13].

To describe the baseline characteristics of the participants and the outcome variables we used descriptive statistics [mean ± SD or n (%)]. To compare changes in outcome variables in response to each intervention period and carryover effects for the outcome variables observed before the AWW and gin interventions, we used the paired two-tailed t-test. This test was also used to compare differences in the effects of each intervention. Within- and between-group differences were expressed as estimated means and 95% confidence interval (CI). The level of significance was set at $p < 0.05$. The statistical analyses were carried out using the SPPS statistical Analysis System v. 20.0 (SPSS, Chicago, IL, USA).

2.7. Results

Of the 41 subjects included, 38 completed the study (Fig. 1). Table 2 shows the baseline characteristics of study participants. Most were overweight or obese (92%), and had dyslipidemia (53%), hypertension (71%), or type-2 diabetes (23%), while only 13% were smokers. Biochemical analyses showed that serum folic acid, vitamin B_{12}, albumin, AST, ALT and GGT values remained within the normal range throughout the study.

There were no individual deviations from the interventions in physical activity or food intake according to the participants' dietary reports (Table 3). Moreover, no significant changes were observed in nutrients and antioxidants during the two interventions. Protocol adherence was defined as optimum in all subjects, and complete agreement regarding intake of test beverages was observed between the reports of the participants and the number of empty bottles returned. As a measure of intervention

Table 2
Baseline characteristics of the 38 study subjects.

	Mean ± SD or %
Age (years)	68.7 ± 5.4[a]
Height (cm)	168.9 ± 6.2
Body weight (kg)	82 ± 11.1
BMI (kg/m^2)	28.7 ± 3.2
BMI ≥ 25 kg/m^2 [n (%)]	35 (92)[a]
Waist circumference (cm)	105 ± 7
Hypertension [n (%)]	27 (71)
Type 2 diabetes mellitus [n (%)]	8 (23)
Dyslipidemia [n (%)]	20 (53)
Current smokers [n (%)]	5 (13)
Sedentariness [n (%)]	4 (11)
Family history of premature CAD [n (%)]	4 (11)
Systolic blood pressure (mmHg)	144.2 ± 16.2
Diastolic blood pressure (mmHg)	76.9 ± 9.7
Heart rate (beats/min)	65.3 ± 9.2
Diuretics [n (%)]	10 (26)
Statins [n (%)]	15 (39)
Fibrates [n (%)]	13 (34)
ACE inhibitors [n (%)]	19 (50)
Oral hypoglycemic drugs [n (%)]	14 (37)
Aspirin or antiplatelet drugs [n (%)]	9 (24)
Others [n (%)]	17 (45)
Glucose (mg/dL)	111.3 ± 23.3
Total cholesterol (mg/dL)	170.8 ± 38.5
LDL-cholesterol (mg/dL)	103.9 ± 36.1
HDL-cholesterol (mg/dL)	44.8 ± 14.4
Triglycerides (mg/dL)	105.5 ± 54.5
ALT (IU/L)	25.5 ± 11.6
AST (IU/L)	25.3 ± 11.7
GGT (IU/L)	21.4 ± 13.8
Folic acid (serum) (ng/ml)	8.9 ± 2.9
Vitamin B12 (pg/ml)	399.5 ± 175.4
Apo A1 (mg/dL)	113.7 ± 18.9
Apo B (mg/dL)	79.3 ± 19.2
Lipoprotein (a) (mg/dL)	37.1 ± 54.1

SD: standard deviation; BMI: body mass index; CAD: coronary artery disease; ACE: angiotensin-converting enzyme; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transpeptidase; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B.

[a] Values are mean ± SD or n (%).

Table 3
Values of the mean nutrients and physical activity before and after the 3-week aged white wine (AWW) and gin interventions.

	Before AWW mean ± SD⁺	After AWW mean ± SD⁺	Mean differences (95% CI)‡	Before GIN mean ± SD⁺	After GIN mean ± SD⁺	Mean differences (95% CI)‡	*p* value
Physical activity (METS/week)	6560 ± 6333	6686 ± 6531	125.94 (−733.80 to 985.70)	5783 ± 4624	6477 ± 6379	694 (−1294 to 2682)	0.481
Energy (Kcal)	1753 ± 264	1965 ± 353	212.03 (83.95–340.10)*	1730 ± 301	1972 ± 397	242.02 (−90.50 to 393.54)*	0.003
Protein (g)	82.35 ± 15,63	86.07 ± 14.61	3.71 (−2.75 to 10.18)	84.15 ± 16.53	83.61 ± 18.01	−0.53 (−8.94 to 7.86)	0.896
Carbohydrates (g)	187.72 ± 39.05	196.64 ± 50.72	8.92 (−6.63 to 24.48)	187.81 ± 47.92	196.53 ± 54.37	8.71 (−14.96 to 32.39)	0.456
Dietary fiber (g)	17.19 ± 5.10	17.67 ± 6.96	0.48 (−1.81 to 2.78)	17.28 ± 6.25	16.90 ± 7.61	−0.38 (−3.24 to 2.47)	0.785
Total fat (g)	78.41 ± 17.82	73.01 ± 16.17	−5.41 (12.54 to 1.72)	76.55 ± 18.28	73.30 ± 18.22	−3.25 (−11.71 to 5.20)	0.436
Cholesterol (mg)	278.42 ± 63.70	281.69 ± 106.31	3.26 (−44.32 to 50.86)	281.37 ± 72.40	272.48 ± 85.56	−8.89 (−54.70 to 36.92)	0.693
Polyphenols (mg)	1192 ± 374	1428.41 ± 641.62	236.30 (28.80–443.80)*	1281 ± 588	1294 ± 608	12.99 −(−282.69 to 308.68)	0.929
Alcohol (g)	0.00 ± 0.00	26.17 ± 0.32	26.17 (26.04–26.30)*	0.00 ± 0.00	29.26 ± 1.88	29.26 (28.49–30.02)*	<0.001
Vitamins B (ug)	2896.29 ± 699.72	12636.21 ± 9277.65	9739.92 (−9365.64 to 28845.48)	2914.69 ± 717.62	3140.51 ± 951.04	225.81 (−185.51 to 637.15)	0.320
Vitamin A (ug)	4006.48 ± 3883.79	4255.52 ± 3605.65	249.04 (−1493.58 to 1991.67)	3235.45 ± 2823.49	3225.77 ± 2585.24	−9.68 (−1414.50 to 1395.14)	0.790
Vitamin C (ug)	13926.00 ± 4888.63	16343.12 ± 8128.64	2417.12 (−239.98 to 5073.22)	13926.00 ± 4888.63	16343.12 ± 8128.64	2417.12 (−238.98 to 5073.22)	0.708
Vitamin D (ug)	4.83 ± 2.50	4.84 ± 2.45	0.00 (−0.96 to 0.97)	4.91 ± 2.44	4.20 ± 2.29	−0.71 (−1.63 to 0.20)	0.072
Vitamin E (ug)	970.92 ± 336.24	902.51 ± 331.48	−68.40 (−214.57 to 77.76	969.85 ± 403.49	830.29 ± 244.92	−139.55 (−305.04 to 25.93)	0.934
Vitamin K (ug)	277.69 ± 312.48	336.71 ± 329.90	59.02 (−99.56 to 217.60)	261.24 ± 339.29	226.65 ± 310.02	−34.59 (−205.93 to 136.75)	0.781
Fe (mg)	12.68 ± 2.26	13.37 ± 3.99	0.69 (−0.69 to 2.08)	12.83 ± 4.58	12.92 ± 4.09	0.09 (−2.51 to 2.70)	0.638
Cu (mg)	1.30 ± 0.93	1.45 ± 1.27	0.15 (−0.00 to 0.18)	1.18 ± 0.49	1.49 ± 1.44	0.30 (−0.32 to 0.93)	0.505
Se (mg)	1.10 ± 0.25	1.19 ± 0.27	0.09 (0.37–0.24)	1.13 ± 0.30	1.14 ± 0.31	0.00 (−0.12 to 0.13)	0.247
Zn (mg)	7.88 ± 1.57	8.85 ± 2.09	0.97 (−0.95 to 1.95)	8.22 ± 2.51	8.38 ± 2.57	0.15 (−1.15 to 1.46)	0.467
Mn (mg)	2.41 ± 0.82	2.73 ± 1.06	0.31 (−0.20 to 0.83)	2.36 ± 1.05	2.31 ± 1.05	−0.47 (−0.58 to 0.49)	0.466

METS: metabolic equivalent; Results are expressed as ⁺mean ± standard deviation (SD) (n = 38) and ‡mean differences (95% confidence interval [CI]) between before and after each intervention. Before each intervention is the value of the previous intervention or the baseline in the first intervention. *p: Significant differences ($p < 0.05$) between before and after the intervention. p value: Significant differences ($p < 0.05$) between-group changes.

compliance, a biomarker of wine consumption (tartaric acid) [17] was determined in 24 h urine samples collected in the last day of the run-in period and after each intervention. We found that after AWW consumption the 24 h excretion of tartaric acid increased from 14.09 ± 31.80 to 63.79 ± 39.73 μg/mL ($p < 0.001$).

No changes in drug intake were reported, and no adverse effects were observed along the different interventions ($p > 0.05$, all).

2.8. Chemokine and cytokine changes

We observed changes in plasma cytokine and chemokine concentrations after the 3-week AWW and gin interventions. Following the AWW intervention, significant decreases in VCAM-1 (−17%; $p = 0.012$), ICAM-1 (−11%; $p = 0.020$), IL-8 (−30%; $p = 0.048$), and IL-18 (−40%; $p = 0.048$) concentrations were observed, while there were no changes in IL-1β, IL-6, and TNF-α concentrations. In addition, IFN- γ concentrations significantly decreased after both AWW (−33%) and gin intake (−14%) ($p \leq 0.046$; both).

Differences between groups showed a significant reduction of IFN-γ (−5.73 vs. −1.93 pg/mL; $p = 0.027$), MCP-1 (−2.21 vs. 0.94 pg/mL; $p = 0.042$), IL-18 (−18.90 vs. −0.84 pg/mL; $p = 0.037$), and VCAM-1 (−5.44 vs. 2.32 pg/mL; $p = 0.035$) after AWW compared to gin (Table 4). No carryover effect was observed for any of the variables.

2.9. Changes in the expression of T-lymphocytes and monocyte cell adhesion molecules and in circulating EPCs

As shown in Table 5, CD40 and CD31 T-lymphocyte expression significantly decreased ($p \leq 0.045$, both) by 16 and 44%, respectively, after AWW intake. Only CD40 showed a significant 41% reduction ($p = 0.013$) after gin intake. With regard to monocyte expression after the AWW and gin interventions, we observed a

Table 4
Chemokines and cytokine changes at baseline and after the aged white wine (AWW) and gin interventions.

	Before AWW mean ± SD⁺	After AWW mean ± SD⁺	Mean differences (95% CI)‡	Before GIN mean ± SD⁺	After GIN mean ± SD⁺	Mean differences (95% CI)‡	*p* value
IL-β (pg/mL)	2.48 ± 6.07	1.88 ± 4.25	−0.60 (−1.32 to 0.12)	2.26 ± 2.33	2.09 ± 1.78	−0.16 (−0.42 to 0.09)	0.219
IL-6 (pg/mL)	241.91 ± 204.19	168.67 ± 151.30	−73.24 (−213.13 to 66.65)	233.47 ± 301.68	322.48 ± 465.1	89.01 (−1379.31 to 1557.33)	0.420
IL-8 (pg/mL)	34.18 ± 35.67	23.83 ± 26.37	−10.35 (−20.57 to −0.13)*	34.30 ± 14.33	28.10 ± 11.15	−6.19 (−13.51 to 1.12)	0.444
IL-10 (pg/mL)	26.72 ± 110.32	36.28 ± 144.91	9.55 (−36.05 to 55.17)	27.69 ± 26.36	36.29 ± 56.10	8.60 (−7.47 to 24.68)	0.184
IL-12 (pg/mL)	45.23 ± 116.82	45.99 ± 120.95	0.76 (−61.51 to 63.04)	48.56 ± 89.84	74.95 ± 122.28	26.39 (−15.05 to 67.83)	0.211
IL-13 (pg/mL)	7.9 ± 23.09	11.61 ± 36.67	3.70 (−5.24 to 12.66)	5.94 ± 2.87	10.84 ± 19.24	2.90 (−3.65 to 9.45)	0.179
IFN-γ (pg/mL)	46.78 ± 95.11	31.44 ± 54.89	−15.33 (−29.97 to −0.69)*	46.50 ± 31.51	39.98 ± 22.40	−6.51 (−12.89 to −0.13)*	0.027
MCP-1 (pg/mL)	90.58 ± 74.43	85.30 ± 58.61	−5.28 (−12.96 to 2.40)	99.41 ± 459.07	12.97 ± 12.89	−86.44 (−264.18 to 91.29)	0.042
TNF-α (pg/mL)	38.81 ± 123.52	32.93 ± 96.62	−5.87 (−19.63 to 7.87)	34.23 ± 44.72	31.62 ± 33.93	−2.60 (−6.74 to 1.53)	0.635
IL-18 (pg/mL)	43.06 ± 50.46	26.03 ± 14.61	−17.03 (−33.93 to −0.13)*	38.01 ± 17.13	34.52 ± 10.57	−3.48 (−8.12 to 1.15)	0.037
ICAM-1 (pg/mL)	52.31 ± 54.39	46.41 ± 47.38	−5.89 (−10.82 to −0.97)*	54.61 ± 53.39	53.70 ± 44.42	−4.91 (−11.31 to 1.48)	0.616
VCAM-1 (pg/mL)	62.88 ± 56.37	52.05 ± 42.62	−10.82 (−19.14 to −2.50)*	63.40 ± 58.41	58.68 ± 49.37	−4.71 (−11.62 to 2.20)	0.035

Results are expressed as ⁺mean ± SD (n = 38) and ‡mean differences (95% confidence interval [CI]) between before and after each intervention. Before each intervention is the value of the previous intervention or the baseline in the first intervention. *p: Significant differences ($p < 0.05$) between before and after the intervention. p value: Significant differences ($p < 0.05$) between-group changes.

Table 5
Mean T-lymphocyte and monocyte values before and after 3 weeks of the aged white wine (AWW) and gin interventions.

	Before AWW mean ± SD⁺	After AWW mean ± SD⁺	Mean differences (95% CI)‡	Before GIN mean ± SD⁺	After GIN mean ± SD⁺	Mean differences (95% CI)‡	*p* value
Lymphocytes (MFI)							
Lfa-1	124.52 ± 63.59	112.70 ± 45.42	−11.82 (−30.75 to 7.11)	128.54 ± 55.23	115.97 ± 45.83	−12.56 (−32.42 to 7.30)	0.514
Mac-1	86.10 ± 50.11	83.09 ± 47.71	−3.00 (−16.03 to 10.01)	82.74 ± 57.69	81.92 ± 47.49	−0.81 (−20.58 to 18.96)	0.350
VLA4	38.74 ± 15.01	38.18 ± 6.53	−0.55 (−5.36 to 4.25)	35.75 ± 12.68	35.37 ± 8.44	−0.38 (−6.08 to 5.31)	0.043
CD15	42.56 ± 18.33	39.77 ± 11.71	−2.78 (−8.89 to 3.31)	46.67 ± 36.44	36.48 ± 12.20	−10.19 (−24.53 to 4.15)	0.210
CD40	56.39 ± 21.47	47.43 ± 18.70	−8.95 (−17.71 to −0.20)*	56.43 ± 49.35	33.46 ± 19.25	−22.96 (−40.63 to −5.29)*	0.141
CD31	396.62 ± 381.61	223.38 ± 162.02	−173.23 (−280.23 to −66.24)*	388.09 ± 707.35	175.13 ± 269.17	−197.41 (−461.28 to 66.45)	0.044
Monocytes (MFI)							
Lfa-1	76.49 ± 18.17	67.56 ± 19.12	−8.93 (−15.79 to −2.07)*	80.62 ± 37.22	63.40 ± 18.60	−17.22 (−29.57 to −4.87)*	0.428
Mac-1	51.90 ± 14.39	45.13 ± 15.58	−6.76 (−12.61 to −0.92)*	56.55 ± 19.07	44.42 ± 16.59	−12.13 (−21.00 to −3.25)*	0.118
VLA4	44.74 ± 19.05	34.76 ± 12.58	−9.97 (−16.93 to −3.02)*	45.24 ± 15.63	36.73 ± 16.47	−8.51 (−14.68 to −2.33)*	0.144
CD15	29.04 ± 6.86	28.84 ± 9.61	−0.20 (−4.11 to 3.70)	33.12 ± 15.27	29.82 ± 8.41	3.3 (−9.87 to 3.27)	0.266
CD40	31.19 ± 11.22	26.70 ± 6.31	−4.49 (−8.76 to −0.22)*	35.95 ± 17.33	27.15 ± 6.90	−8.80 (−15.52 to −2.08)*	0.047
CD31	631.03 ± 372.24	330.44 ± 225.45	−300.59 (−433.06 to −168.11)*	591.29 ± 479.45	358.42 ± 281.86	−232.87 (−398.42 to −67.31)*	0.038
CD36	711.75 ± 627.10	495.70 ± 400.81	−216.04 (−421.14 to −10.93)*	733.14 ± 598.38	611.37 ± 523.34	−121.77 (−340.16 to 96.61)	0.042
CCR2	123.23 ± 107.87	54.66 ± 19.08	−68.57 (−107.51 to −29.63)*	110.19 ± 172.77	65.44 ± 42.32	−44.75 (−111.71 to 22.21)	0.038
EPC	48.92 ± 43.13	68.31 ± 52.39	19.38 (4.40–34.37)*	48.33 ± 68.81	60.42 ± 72.98	12.09 (−5.77 to 29.95)	0.044

MFI, Mean fluorescence intensity.
Results are expressed as ⁺mean ± SD (n = 38) and ‡mean differences (95%CI) between before and after each intervention. Before each intervention is the value of the previous intervention or the baseline in the first intervention. *p: Significant differences ($p < 0.05$) between before and after the intervention. p value: Significant differences ($p < 0.05$) between-group changes.

significant decrease of Lfa-1 ($p \leq 0.012$; both), Mac-1 ($p \leq 0.024$; both), VLA4 ($p \leq 0.009$; both), CD40 ($p \leq 0.040$; both), and CD31 ($p \leq 0.007$; both). However, only after AWW intake the expression of CD36 and CCR2 significantly decreased ($p \leq 0.040$; both) by 3% and 56%, respectively.

There was also a significant 40% increase in EPC expression following AWW intake ($p = 0.013$), while gin had no effect. Comparing AWW and gin intake we observed that AWW intake was associated with a significant decrease in CD31and CCR2 (−229.77 and −45.36 MFI, respectively; $p = 0.038$; both), CD40 (−9.48 MFI; $p = 0.047$), and CD36 (−295.07 MFI; $p = 0.042$), and lastly, a significant increase in EPC expression (23.96 MFI; $p = 0.044$).

2.10. Changes in cardiovascular risk factors

No changes in body weight, BMI or waist circumference were observed during the study, even though total energy consumption increased slightly in both groups. During the 3-week interventions systolic and diastolic BP values of subjects drinking AWW decreased by 3% and 4%, respectively ($p = 0.033$; both), while HDL-cholesterol concentrations increased by 9% ($p = 0.001$). There were no changes of these parameters after the gin intervention. On the other hand, gin intake was associated with increases of 5% ($p = 0.025$) in plasma glucose concentration, 0.7% ($p = 0.001$) in total cholesterol, 10% ($p = 0.005$) in LDL-cholesterol, and 9% ($p = 0.001$) in apolipoprotein B. Apolipoprotein AI concentration and prothrombin time increased after both interventions ($p \leq 0.022$; all). No significant changes were observed in CRP, creatinine, ASAT, ALAT, GGT, lipoprotein(a), thromboplastin time, fibrinogen, folic acid, vitamin B12, homocysteine, leukocytes, platelets, lymphocytes, monocytes, or hemoglobin A1c. Between-group differences revealed only reductions in systolic and diastolic BP ($p = 0.039$; both) favoring AWW and increases in GGT concentrations ($p = 0.019$) and prothrombin time ($p = 0.038$) favoring gin consumption (Table 6).

3. Discussion

Our results show that moderate consumption of AWW down-regulates various biological biomarkers related to the development and progression of atheroma plaque, including the expression of inflammatory adhesion molecules on circulating T-lymphocyte and monocyte surfaces, EPC expression and the concentrations of plasma pro-inflammatory molecules. In addition, moderate AWW intake reduced systolic and diastolic BP and increased HDL-cholesterol, apolipoprotein A1, folic acid and iron concentrations. Since there were no changes in dietary intake of other antioxidants or after gin intervention, the main anti-inflammatory effects observed in this study can be attributed mainly to polyphenols in AWW.

With regard to biomarkers of cardiovascular risk, a large body of scientific evidence supports the cardioprotective effects of ethanol, mediated in part by dose-dependent increases in HDL-cholesterol [14,15] and moderate reduction of LDL-cholesterol [16,17]. However, there are conflicting data on the effects of wine consumption on the lipid profile beyond its alcohol content. Nicod et al. [18] investigated the possible mechanisms of polyphenols in increasing HDL-cholesterol in a Caco-2 Transwell cell model and these authors found no association between polyphenols from red wine and increased HDL-cholesterol secretion by intestinal cells or enhancement of HDL functionality. In addition, in a long-term (12-months), prospective, multi-center, randomized trial performed in 157 healthy subjects, Taborsky et al. [19] found no significant differences in the lipid profile, CRP, fasting blood glucose, or other markers of atherosclerosis after consumption of red and white wine. On the other hand, in clinical studies in high cardiovascular risk subjects, Estruch et al. [4] and Chiva-Blanch et al. [20] observed that red wine consumption, but not gin, increased HDL-cholesterol and reduced lipoprotein(a) concentrations. In a recent study [21], moderate alcohol consumers showed a significant increase in HDL-cholesterol and apolipoprotein A1 after one month of white wine intake (375 mL daily).

Concerning BP, results of clinical studies and meta-analyses thereof [22] have shown that moderate alcohol consumption does not affect BP. Chiva-Blanch et al. [23] observed that moderate consumption of alcoholic beverages such as gin or red wine did not modify BP, but dealcoholized red wine significantly decreased BP. Concurring with these results, Cheng et al. [24] reported a reduction in BP after the consumption of resveratrol in fructose-fed rats. Resveratrol may decrease BP through the phosphorylation of activated protein kinase (AMPK), which reduces the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and activates the Akt-neuronal NOS (nNOS) signaling pathway in the brain, leading to a decrease in the generation of reactive oxygen

Table 6
Mean biomarker values before and after the 3-week aged white wine (AWW) and gin interventions.

	Before AWW mean ± SD[+]	After AWW mean ± SD[+]	Mean differences (95% CI)[‡]	Before GIN mean ± SD[+]	After GIN mean ± SD[+]	Mean differences (95% CI)[‡]	*p* value
Systolic BP (mmHg)	141.78 ± 14.94	136.87 ± 13.17	−4.91 (−9.41 to −0.418)*	137.43 ± 15.87	139.55 ± 17.51	2.12 (−2.31 to 6.56)	0.039
Diastolic BP (mmHg)	76.34 ± 9.29	73.44 ± 10.50	−2.90 (−5.50 to −0.295)*	72.83 ± 11.87	73.44 ± 910.87	0.61 (−1.64 to 2.86)	0.039
Heart rate (lpm)	65.15 ± 8.64	64.66 ± 9.69	−0.49 (−3.52 to 2.53)	65.76 ± 10.26	65.91 ± 9.45	0.15 (−3.26 to 3.57)	0.538
Weight (Kg)	81.77 ± 10.94	82.06 ± 10.64	0.28 (−0.21 to 0.78)	82.00 ± 10.86	82.29 ± 10.66	0.29 (−0.07 to 0.66)	0.496
Body mass index (Kg/m^2)	28.73 ± 3.12	28.84 ± 3.04	0.11 (−0.07 to 0.30)	27.91 ± 5.15	28.73 ± 3.02	0.81 (−0.84 to 2.48)	0.285
Waist circumference (cm)	103.37 ± 7.61	102.98 ± 8.30	−0.38 (−1.70 to 0.92)	102.79 ± 8.06	102.95 ± 7.39	0.16 (−0.56 to 0.90)	0.484
CRP (mg/dL)	0.44 ± 1.49	0.21 ± 0.24	−0.22 (−0.71 to 0.26)	0.25 ± 0.05	0.29 ± 0.48	0.04 −(−0.185 to 0.27)	0.174
Creatinine (mg/dL)	1491 ± 363	1570 ± 374	79.57 (−42.73 to 201.87)	1560 ± 404	1502 ± 416	−57.88 (−183.54 to 67.77)	0.265
Glucose (mg/dL)	105.08 ± 15.29	105.72 ± 13.06	0.64 (−3.19 to 4.47)	114.94 ± 30.10	120.60 ± 32.85	5.65 (0.75–10.55)*	0.791
Cholesterol (mg/dL)	221.18 ± 320.07	203.72 ± 140.99	−17.45 (−101.24 to 136.16)	169.23 ± 40.71	170.51 ± 41.42	19.02 (11.09–26.95)*	0.278
Triglycerides (mg/dL)	107.94 ± 57.28	111.71 ± 50.82	3.76 (−5.93 to 13.45)	107.52 ± 53.63	121.26 ± 63.45	13.73 (−1.73 to 29.20)	0.122
ASAT (U/L)	23.34 ± 6.31	23.31 ± 5.22	−0.02 (−1.63 to 1.57)	23.63 ± 12.09	22.97 ± 4.87	−0.65 (−4.19 to 2.87)	0.605
ALAT (U/L)	25.34 ± 11.53	25.18 ± 10.08	−0.15 (−2.16 to 1.85)	28.10 ± 31.33	24.55 ± 9.53	−3.55 (−12.84 to 5.73)	0.395
GGT (U/L)	19.96 ± 9.92	20.81 ± 7.99	0.84 (−0.70 to 2.40)	23.00 ± 18.10	26.92 ± 19.32	3.92 (−1.94 to 9.78)	0.019
LDL-cholesterol (mg/dL)	102.79 ± 41.21	106.89 ± 38.52	4.10 (−1.04 to 9.24)	106.63 ± 38.13	117.63 ± 40.03	11.00 (3.47–18.52)*	0.221
HDL-cholesterol (mg/dL)	41.89 ± 11.46	45.63 ± 12.12	3.73 (2.08–5.39)*	43.76 ± 14.86	46.36 ± 11.02	2.60 (−1.18 to 6.39)	0.541
Apolipoprotein A (mg/dL)	113.44 ± 18.88	121.92 ± 18.02	8.47 (4.33–12.60)*	116.71 ± 16.84	127.57 ± 16.34	10.86 (7.46–14.27)*	0.382
Apolipoprotein B (mg/dL)	77.52 ± 21.11	79.92 ± 19.82	2.40 (−0.94 to 5.74)	81.84 ± 20.37	88.94 ± 23.60	7.10 (2.92–11.28)*	0.121
Lipoprotein A (mg/dL)	28.80 ± 45.31	28.28 ± 47.98	−0.52 (−5.65 to 4.60)	29.89 ± 45.91	25.79 ± 44.78	−4.09 (−8.38 to 0.19)	0.361
Thromboplastin time (seg)	29.30 ± 1.93	28.74 ± 1.59	−0.56 (−1.11 to −0.01)*	29.08 ± 1.94	28.53 ± 1.91	−0.54 (−0.99 to −0.10)*	0.668
Fibrinogen (g/L)	3.42 ± 0.83	3.42 ± 0.64	−0.02 (−0.30 to 0.30)	3.48 ± 0.62	3.37 ± 0.64	−0.11 (−0.35 to 0.12)	0.663
Serum folic acid (ng/mL)	8.61 ± 2.51	8.05 ± 2.37	−0.56 (−1.34 to 0.22)	8.54 ± 3.24	9.97 ± 10.81	1.43 (−2.43 to 5.30)	0.264
Vitamin B_{12} (pg/mL)	380.78 ± 185.88	367.40 ± 212.19	−13.37 (−41.08 to 14.33)	417.72 ± 230.29	400.82 ± 213.30	−16.89 (−55.90 to 22.11)	0.193
Homocysteine (μmol/L)	12.88 ± 3.61	13.34 ± 3.33	0.46 (−0.91 to 1.85)	13.32 ± 3.25	19.49 ± 27.38	6.17 (−4.03 to 16.37)	0.234
Albumin	42.36 ± 2.35	43.09 ± 2.28	0.72 (−0.03 to 1.48)	42.12 ± 1.97	42.90 ± 2.19	0.77 (−0.06 to 1.60)	0.310
Leukocytes (10^9/L)	5.93 ± 1.48	5.85 ± 1.33	−0.08 (−0.36 to 0.19)	5.88 ± 1.41	6.03 ± 1.63	0.14 (−0.20 to 0.48)	0.542
Platelets (10^9/L)	221.00 ± 73.30	203.44 ± 44.06	−17.55 (−40.55 to 5.44)	208.43 ± 48.79	212.56 ± 49.55	4.13 (−5.56 to 13.84)	0.049
Lymphocytes (10^9/L)	1.66 ± 0.60	1.68 ± 0.55	0.02 (−0.14 to 0.18)	1.80 ± 0.60	1.76 ± 0.52	−0.03 (−0.14 to 0.07)	0.315
Monocytes (10^9/L)	0.37 ± 0.10	0.37 ± 0.10	−0.02 (−0.02 to 0.02)	0.38 ± 0.13	0.39 ± 0.13	−0.01 (−0.03 to 0.04)	0.543
Glycated hemoglobin (%)	5.84 ± 0.89	5.83 ± 0.93	−0.005 (−0.05 to 0.04)	7.29 ± 8.78	5.80 ± 0.87	−1.48 (−4.39 to 1.42)	0.293
Prothrombin time (%)	94.50 ± 10.79	96.41 ± 11.10	1.91 (0.29–3.54)*	95.58 ± 8.83	97.59 ± 3.90	2.00 (0.36–3.64)*	0.038

ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; BP: blood pressure; CRP: C-reactive protein; GG: gamma glutamyltranspeptidase; HDL: high density lipoprotein; LDL: low density lipoprotein.
Results are expressed as [+]mean ± SD (n = 38) and [‡]mean differences (95%CI) between before and after each intervention. Before each intervention is the value of the previous intervention or the baseline in the first intervention. *p: Significant differences ($p < 0.05$) between before and after the intervention. p value: Significant differences ($p < 0.05$) between-group changes.

species (ROS) and an increase in superoxide dismutase 2 (SOD2) levels. The BP-lowering effects also observed in the present study could be attributed to polyphenols (such as resveratrol or others) and/or other minor components of AWW and not to alcohol.

According to our results, AWW exerts significantly greater anti-inflammatory effects than gin. Pro-inflammatory cytokines and chemokines such as VCAM-1, ICAM-1, IL-18 and IL-8 decreased after AWW intake, and IFN-γ also decreased after both interventions. In addition, participants drinking AWW showed a significant decrease in the expression of CD31 cell adhesion molecules in T-lymphocytes and CD36 and CCR2 expression in monocytes. We also observed a decrease in T-lymphocytes CD40 and in monocyte expression of Lfa-1, Mac-1, VLA4, CD40, and CD31 after both interventions. The anti-inflammatory effects of red wine-derived drinks can be ascribed to polyphenols, with significant reductions in VLA4 in lymphocyte expression, and VLA4, Lfa-1, Mac-1 and CCR2 in monocyte expression, as well as significant reductions in the plasma concentrations of CD40 antigen, CD40 ligand, ICAM-1, VCAM-1, IL-6 and MCP-1 [25,27], compared to gin. In contrast with other studies in which no differences in the acute effects of red and white wine on IL-6, ICAM-1, or VCAM-1 levels were observed [28], others [26,29] have found a higher anti-inflammatory effect of red wine compared to white wine in trials that included healthy subjects or patients at high risk of CHD. *In vitro* studies [30] with human endothelial cells that were incubated with different concentrations of polyphenolic extracts from Italian red wines have also shown that these extracts inhibited monocyte adhesion and down-regulated ICAM-1, VCAM-1, E-Selectin, MCP-1 and macrophage colony-stimulating factor (M-CSF) expression at mRNA and protein levels, as well as NF-kβ and AP-1 activation. These findings together with our results strongly suggest that the polyphenol fraction or other minor components of wine may be responsible for down-regulating inflammation.

During the last two decades, several studies have focused on circulating EPCs as a novel biomarker of endothelial function and as a protective mechanism against CAD events [31–34]. EPCs play a prominent role in neovascularization since they converge into sites of endothelial damage, help to maintain endothelial integrity, and contribute to accelerating re-endothelialization and vascular homeostasis [35]. EPC levels are negatively correlated with cardiovascular risk factors [36], atherosclerotic disease and cardiovascular risk [35]. In addition, a lower risk of cardiovascular death seems to be associated with an increase in the amount of EPCs [31]. In our study, EPC expression significantly increased by 40% after the 3-week intervention with AWW. Recent clinical studies have also shown that a high number of circulating EPCs is associated with cardiovascular risk factors, drug treatments, chronic heart failure, and the age or gender of the patients, while low EPC levels are associated with an increased likelihood of abnormal left ventricular mass and worse cardiac remodeling [37,38]. There is also scientific evidence showing that red wine and/or its polyphenols (mainly resveratrol) increase EPC functional activity [7]. In a study of 15 healthy volunteers asked to drink 250 mL of red wine daily for 3 weeks, circulating $CD133^+$, $CD34^+/CD133^+$, $KDR^+/CD133^+$, and $CD34^+/KDR^+/CD133^+$ cells increased significantly from baseline, meaning improved endothelial function [7]. Huang et al. [9] also observed significant increases in EPCs after 3-week red wine intake compared to other beverages (water, beer or vodka). Finally, we have also shown that, compared to gin, daily intake of regular beer and non-alcoholic beer for 3 weeks was associated with a

significant increase in the number of circulating EPCs [10]. The lack of effect of gin on EPCs in the present study concurs with the results of Chiva-Blanch et al. [10] with gin and of Huang et al. [9] with vodka (both gin and vodka are distilled beverages lacking polyphenols) and suggests that ethanol plays no role in the effects of alcoholic beverages on EPC expression. The increase of circulating EPCs may be explained by an increase in nitric oxide availability, as shown by Huang et al. [9] after red wine consumption.

Our study has limitations. One limitation is the small sample size. However, the results of the study are in agreement with previous studies with similar methodologies and sample sizes [10]. Another limitation is that, based on the characteristics of the study design, the specific compounds responsible for the observed effects cannot be identified. Lastly, the results of this study cannot be extrapolated to other populations, and a 3-week intervention may not represent the potential effects of long-term consumption.

4. Conclusion

To best of our knowledge, this is the first study to investigate the effects of a 3-week intervention of AWW intake on leukocyte adhesion molecules, circulating EPCs and pro-inflammatory biomarkers related to atheroma plaque development in men at high cardiovascular risk. Our results suggest that the effect of alcohol alone is less than the sum of the effects of alcohol, polyphenols and other minor components of some alcoholic beverages, contributing to the down-regulation of pro-inflammatory biomarkers related to the atherosclerotic process. Increased EPC expression after intake of AWW, but not gin, might be explained by the non-alcoholic content of AWW.

Authors' contributions

The contributions of the different authors were as follows – RE, IR, RC: study conception and design; IR, RC: laboratory and clinical data; IR, RC and RE: analysis and interpretation of the data; IR, RC, AM, RML-R and RE: draft of the article; and IR, RC, AM, RML-R and RE: critical revision and final approval. IR, RC, AM, RML-R and RE wrote the paper. RE had primary responsibility for the final content. All the authors have read and approved the final manuscript. None of the authors declare any conflict of interest related to the study.

Conflict of interest and funding disclosure

RE reports serving on the board of and receiving lecture fees from the Research Foundation on Wine and Nutrition (FIVIN), serving on the boards of the Beer and Health Foundation and the European Foundation for Alcohol Research (ERAB), receiving lecture fees from Cerveceros de España and Sanofi-Aventis, and receiving grant support through his institution from Novartis. RML-R reports serving on the board of and receiving lecture fees from FIVIN, receiving lecture fees from Cerveceros de España and receiving lecture fees and travel support from PepsiCo. No other potential conflict of interest relevant to this article was reported.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2018.06.001.

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