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Randomized Control Trials

Acute consumption of Andalusian aged wine and gin decreases the expression of genes related to atherosclerosis in men with high cardiovascular risk: Randomized intervention trial

Irene Roth [a], Rosa Casas [a, b], Margarita Ribó-Coll [a], Mónica Doménech [a], Rosa M. Lamuela-Raventós [b, c], Ramón Estruch [a, b, *]

[a] Department of Internal Medicine, Hospital Clínic, Institut d'Investigació Biomèdica August Pi i Sunyer, Universitat de Barcelona, Spain
[b] CIBER CB06/03 Fisiopatología de la Obesidad y la Nutrición, (CIBERobn), Instituto de Salud Carlos III (ISCIII), Spain
[c] Nutrition and Food Science Department-XaRTA, INSA, Pharmacy School, University of Barcelona, Spain

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SUMMARY

Background: Atherosclerosis is an inflammatory disease. Previous studies have suggested the beneficial effects of moderate consumption of alcoholic beverages on reducing cardiovascular risk (CVR). The aim of this study was to evaluate the effects of acute consumption of Andalusian aged wine (AAW) and gin by analyzing the expression of genes related to the appearance and progression of atherosclerosis in men with high CVR.

Methods: We performed an open, randomized, controlled, crossover trial including 41 men with high CVR between 55 and 80 years age, who received a single dose of AAW or gin (0.5 g ethanol/kg). The expression of 10 genes related to atherosclerosis was determined by RT-PCR at baseline and 4 h after the intervention.

Results: Gene expression analysis 4 h after consumption of each alcoholic beverage showed a significant decrease in Toll-like receptors 4 and 6 (TLR4, TLR6) and Caspase-1 ($p < 0.05$ all). Additionally, TLR2, Interleukin-1 receptor, chemokine receptor 3 and inflammasome expression decreased after AAW intake ($p < 0.05$, all) while only chemokine receptor 5 decreased after gin consumption ($p = 0.039$).

Conclusion: The decrease in the expression of several genes related to the appearance and progression of atherosclerosis was greater after AAW than gin intake, suggesting that the phenolic content of AAW may play a protective role against atherosclerosis.

1. Introduction

Chronic inflammatory status and oxidative stress leads to the development of atherosclerosis and, consequently, to cardiovascular disease (CVD) [1,2] (Ross R, 1999; Libby P, 2001). Inflammation contributes to all phases of atherogenesis: recruitment of monocytes and lymphocytes, followed by progression and propagation of arterial lesions until the development and rupture of atherosclerotic plaques, and finally, thrombosis of the vessel, which can lead to myocardial infarction or stroke [3,4]. From the onset, this inflammatory process is also related to the oxidation of lipids in low-density lipoprotein particles (LDL). In fact, oxidized LDL (oxLDL) and its components can activate innate immunity by binding to toll-like receptors (TLRs), leading to the recruitment of leukocytes and a high expression of adhesion molecules such as E-selectin and VCAM-1 on the endothelial surface of the artery [5]. In this process, two chemokine receptors (CCR5 and CXCR3) widely expressed on the surface of leukocyte populations and vascular cells [6] have been identified as possible modifiers of atherosclerotic disease [7].

Another main inflammatory mediator is interleukin-1-beta (IL-1β) which has an important role in the development of lipid plaques and the vulnerability of atherosclerotic plaques. The activation of cysteine protease caspase-1 present in macrophages leads to an autocatalytic activation of pro-IL-1β and pro-IL-18 [8–10], that is modulated by the activation of TLRs such as TLR2 and TLR4 [3,8,11].

Abbreviations: AAW, Andalusian aged wine; AM, dhesion molecules; CVR, Cardiovascular risk; EGA/day, Eq gallic acid/day; PBMC, Peripheral blood mononuclear cells; PBS, Phosphate buffer saline; MFI, Median fluorescent intensity.

* Corresponding author. Department of Internal Medicine, Hospital Clinic, Villarroel, 170, 08036 Barcelona, Spain. Fax: + 34 93 2275758.
E-mail address: restruch@clinic.ub.es (R. Estruch).

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Inflammasonme NLRP3, an intracellular signaling complex, also participates in the activation and regulation of inflammatory response [11].

Classically, it has been considered that the protective effect of alcoholic beverages on arteriosclerosis is mediated by the effect of ethanol increasing HDL-cholesterol [12], inhibiting the oxidation of LDL-cholesterol, increasing sensitivity to insulin and decreasing fibrinogen [13,14]. However, other studies have suggested that other components present in alcoholic beverages, mainly polyphenols, may play a role in the protection against cardiovascular disease [15–19].

In this respect, there are notable discrepancies regarding the specific effects of different types of alcoholic beverages (wine, beer and liquors) on the cardiovascular system, the possible protective mechanisms of alcoholic beverages and also whether these possible mechanisms are due to their alcoholic component (ethanol), to the non-alcoholic products they contain, mainly polyphenols, or both. Thus, new studies are needed with different types of wine, such as, for example, Andalusian aged wine (AWW) (13% ethanol content) also called "*veil of flower* wine", in order to know whether peculiar chemical composition of this type of wine has a greater protective effect on the cardiovascular system.

Taking into account the *in vivo* evidence about how diet and some of its components can directly modulate the regulation of genes related to inflammation, the aim of the present study was to investigate the effect of acute intake of AAW on leukocyte expression and changes in the expression of 10 genes related to the atherosclerosis process in a free living population of men with high CVD risk.

2. Subjects and methods

2.1. Subjects

A total of 47 potential participants with high CVD risk between 55 and 80 years of age were recruited for the study in the outpatient clinic of the Medicine Department of the Hospital Clinic of Barcelona from September 2012 to March 2014. However, one did not fulfill the inclusion criteria, four declined to participate and another was excluded because of excessive alcohol consumption (AC). Thus, a total of 39 subjects completed the study (Fig. 1). All subjects included were men, who were moderate alcohol consumers (0.5 g/alcohol/day/Kg body) and had three or more of the following CVD risk factors: diabetes mellitus, hypertension, smoking, plasma concentrations of LDL cholesterol $\geq$ 160 mg/dL and HDL cholesterol < 35 mg/dL, overweight or obesity [body mass index (BMI) $\geq$ 25 kg/m^2] and/or a family history of premature coronary heart disease. The exclusion criteria included: documented coronary heart disease (CHD), stroke or peripheral vascular disease, human immunodeficiency virus infection, alcoholic liver disease, malnutrition and neoplastic or acute infectious diseases. None of the study subjects were consumers of vitamin supplements or anti-inflammatory drugs (steroids, non-steroidal anti-inflammatory agents or aspirin). The Institutional Review Board of the Hospital approved the study protocol, and all participants gave written consent before participation in the study.

2.2. Study design

The study was an open, randomized, controlled, crossover trial with four intervention periods. After signing the informed consent form, the volunteers were randomized using a computer-generated random-number table into two treatments in a crossover design to receive gin (0.5 g ethanol/kg) or AAW (0.5 g ethanol/kg and phenol of 927.79 Eq gallic acid/day-EGA/day), containing 13% of ethanol. A two-week washout period was established between the two interventions. During washout the volunteers were asked not to consume any alcoholic beverage. On the day of the experiment, each subject had fasted for at least 12 h before drinking the alcoholic beverage in 10 min.

Blood samples were collected at baseline and at 4 h after initiating the study to determine the expression of 10 genes and cellular adhesion molecules (AM) of inflammatory parameters related to atherosclerosis.

2.3. Diet and exercise monitoring

Subjects were asked not to drink any alcoholic beverage during the two weeks before the first intervention (run-in period). In addition, they were also asked not to change their dietary habits or physical activity during the study. Natural foods rich in antioxidants, especially fruits and vegetables, were especially monitored so that the diets of the individuals studied had similar antioxidant content along the study period. The participants were not blinded to the type of drink they ingested.

Diet and exercise were monitored before each intervention. Diet monitoring was performed using a validated 3-day (2 weekdays and 1 weekend day) recall questionnaire of food consumption [20]. The dietary information was registered by a dietitian and converted into nutrient data using the Food Processor Nutrition and Fitness Software (ESHA Research, Salem OR, 2012 10.10.0). Exercise monitoring was performed using the Minnesota Leisure Time Physical Activity questionnaire.

2.4. Ethics statement

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

2.5. Selection of beverages

In order to select the AAW to be used in the study, we determined the total polyphenol concentration by the Folin-Ciocalteu method in 11 samples of Fino and Sherry wines [21–25]. Three wines came from the *Fundación Condado de Huelva*, four more from Montilla-Moriles and, the last were wines from Jerez, that were coded with random numbers. According to the results showed in Table 1, we selected the wine that showed the greatest total polyphenol concentration.

On the other hand, the alcoholic beverage chosen as a beverage with an undetectable content of polyphenols was a gin provided by the "Gin Xoriguer Company".

2.6. Clinical and laboratory measurements

Blood pressure (BP) was measured in the left brachial artery by a nurse using with an oscillometer (Omron 705 CP; Omrom Matsuasaka Co Ltd, Matsuasaka City, Japan) at baseline and 4 h after the intervention with AAW or gin. BP and heart rate were measured 3 times in the nondominant arm at 5-min intervals after 15 min resting in a seated position. The mean of the second and the third measures was considered for statistical analysis. In addition, anthropometric measurements were performed according to standardized methods (BMI and waist-to-hip ratio).

Before and 4 h after each intervention period, fasting blood, serum and EDTA plasma samples were collected and immediately centrifuged and stored at −80 °C until assayed. The urine samples were collected at 0–3 h and 3–6 h after the intervention.

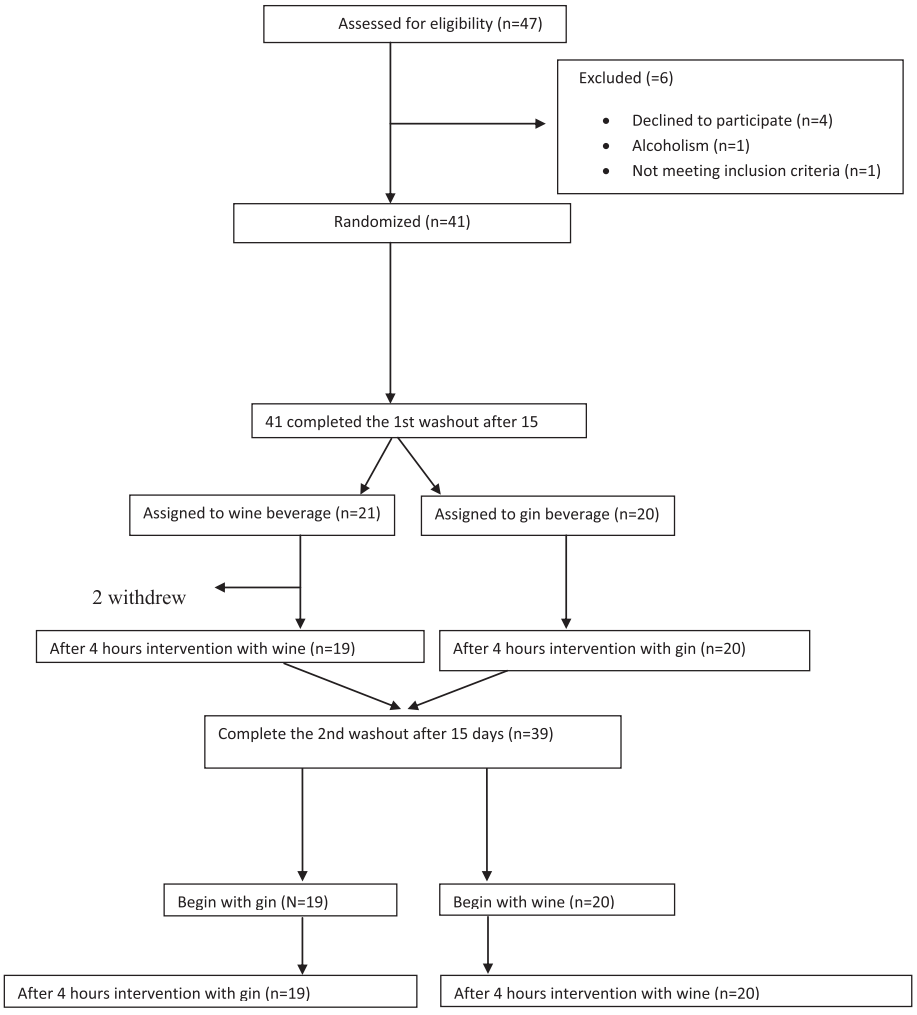

Fig. 1. Flow-chart of the subjects included in the trial.

Table 1
Polyphenol content of the different Andalusian age wine analyzed.

Codes	mg EAG/L sample (n = 6)	SD
1	245.34	11.73
2	334.11	21.78
3	206.20	10.71
4	302.59	15.42
5	467.25	46.58
6	304.86	15.48
7	289.37	12.89
8	**927.79**	**21.79**
9	166.75	18.02
10	220.49	14.50
11	245.79	17.23

Bold represents code 8.

2.7. Gene expression analysis

The expression of a total of 10 genes related to inflammatory stages of atherosclerosis and two endogenous controls (18S and nicotinamide adenine dinucleotide phosphate [NADPH]) were measured at baseline and 4 h after the intervention. The ISOLATE II RNA/DNA/Protein Kit (Bioline(Aust) Pty Ltd, Australia, NSW) was used for RNA extraction following the manufacturer's instructions. cDNA was synthesized using 1 g total RNA in a final volume of 50 μL using random hexamer priming. Samples were stored at −80 °C until use. Microfluidic cards (TaqMan® Arrays Cards, Applied Biosystems, Foster City, CA, USA) were used to analyze the following: TLR2, TLR4, TLR6, inflammasome (NLRP3), caspase-1, IL1R1, chemokine (C-C motif) receptor 2 and 5 (CCR2 and CCR5), chemokine (C-X-C motif) receptor 2 and 3 (CXCR2 and CXCR3). GE was analyzed with the ViiA 7 Real-Time PCR System (Applied Biosystems, Foster, City, CA, USA). The cycling parameters used were those indicated by the manufacturer. A comparative Ct method was used to assess the relative GE [26]. All samples were normalized to the expression of the endogenous control NADPH, and values were expressed as relative units. The inter-assay variation coefficient was less than 7.1%.

2.8. Peripheral blood mononuclear cell immunophenotyping

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by the Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density-gradient. The expression of AM on the surface of PBMC was analyzed via double direct immunofluorescence with the use of commercial monoclonal antibodies following the manufacturer's instructions. The AM analyzed were as follows: VLA-4 (very late activation antigen-4, CD49-d) (Cytogmos, Barcelona, Spain), LFA-1 (lymphocyte function-associated antigen-1, CD11a) (Bender MedSystems, Vienna, Austria), Mac-1 (macrophage-1 antigen, CD11b/CD18) (Bender MedSystems), SLex (Sialil-Lewis X, CD15s) (Beckman

Coulter, Fullerton, CA), CD40 (Caltag Laboratories, Burlingame, CA), CD36 (Beckman Coulter) and CCR2 (R&D Systems, Minneapolis, USA). Fluorescence was monitored with the Sphero Rainbow calibration particles (6 peaks) of 6.0–6.4 μm (BD Biosciences, San Jose, CA). Monocytes were identified and selected with the CD14 monoclonal antibody, and T-lymphocytes were identified and selected with the CD2 monoclonal antibody (Caltag Laboratories, both). Cell counting (5000 events for T-lymphocytes and 3500 for monocytes) and fluorescence analysis were performed in a FACS-Calibur Flow Cytometer (BectoneDickinson, San Jose, CA) using the CellQuest software.

2.9. Statistical analysis

For a parallel design, the sample size was determined with the ENE 3.0 statistical program (GlaxoSmithKline, Brentford, United Kingdom) assuming a maximum loss of 10% of participants. To detect a mean difference of 10 mean fluorescence intensity units in the expression of monocyte CD49d with a conservative standard deviation (SD) of 10, a total of 20 subjects would be needed (α risk = 0.05, power = 0.9). Monocyte expression of CD49d was considered the primary outcome and was used to determine the sample size, but changes in all endpoints were of equal interest in this study.

The statistical analyses were carried out using the SPPS statistical Analysis System v.20.0 (SPSS, Chicago, IL, USA). Descriptive statistics [mean ± SD or n (%)] were used to describe the baseline characteristics of the participants and the outcome variables. The paired two-tailed t-test was used to compare changes in outcome variables in response to each intervention period and carryover effects for the outcome variables observed before the AAW and gin periods. We also compared the differences in the parameters obtained between the group starting with AAW and that starting with gin. The paired two-tailed t-test was also used to compare differences in the effects of each intervention. The effects of each intervention as well as differences between the two interventions are expressed as mean change (95% confidence interval, CI).

3. Results

Of the 41 subjects initially included two withdrew before completing the study (Fig. 1). Therefore, 39 subjects finished the trial. The baseline characteristics (Table 2) showed that 88% participants were overweight or obese, 54% had dyslipidemia, 21% type-2 diabetes and nearly three-quarters had hypertension (73%). Moreover, 13% of the participants were smokers, 10% reported low physical activity and 11% a family history of premature CHD. The biochemical parameters (serum and intraerythrocytary folic acid, vitamin B_{12}, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyltranspeptidase (GGT) remained within the normal range throughout the study). None of the subjects reported any adverse effect related to the interventions.

No individual changes in drug intake were reported during the study period and no carryover effect was observed for any variable.

3.1. Gene expression changes at baseline and 4 h after the intervention

Table 3 shows the results of the expression of genes related to various stages of atherosclerosis. Intra-groups changes showed significant reductions for both interventions in the expression of TLR4, TLR6 and Caspase-1 genes ($p < 0.05$; all). However, AAW intake showed significant reductions in the gene expression of TLR2, IL1R1, CXCR3 and NLPN3 ($p < 0.05$; all), whereas gin intake only showed significant reductions in the gene expression of CCR5 ($p = 0.039$). NLPN3 increased, although not significantly, following gin intake ($p = 0.056$).

Comparison between groups showed statistically significant differences in the expression of the TLR2, IL1R1, CXR3 and NLPN3 genes in favor of AAW ($p < 0.05$; all).

Table 2
Baseline characteristics of the 39 subjects included in the study.

	Mean ± SD or %
Age (years)	68.7 ± 5.4[a]
High (cm)	168.9 ± 6.2
Bodyweight (kg)	82 ± 11.1
BMI (kg/m^2)	28.7 ± 3.2
BMI ≥ 25 kg/m^2 [n (%)]	35 [(88)][a]
Abdominal perimeter (cm)	103.2 ± 7.8
Abdominal circumference (cm)	105 ± 7
WHR	1 ± 0.5
Hypertension [n (%)]	27 [(73)]
Type 2 diabetes mellitus [n (%)]	8 [(21)]
Dyslipidemia [n (%)]	20 [(54)]
Current smokers [n (%)]	5 [(13)]
Sedentarism [n (%)]	4 [(10)]
Family history of premature CHD [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
Changes in medication [n (%)]	4 [(11)]
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 [(44)]
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
Intraerythrocytary folic acid (ng/ml)	719.9 ± 117.9
Vitamin B12 (pg/ml)	399.5 ± 175.4
APO1 (mg/dl)	113.7 ± 18.9
APOB(mg/dl)	79.3 ± 19.2
LpA (mg/dl)	37.1 ± 54.1

BMI, Body Mass Index; WHR, waist to hip ratio; CHD, Coronary Heart Disease; ACE, Angiotensin-Converting Enzyme; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, Gamma glutamyltranspeptidase; APO1, apolipoprotein 1; APOB apolipoprotein B; LPA Lipoprotein A.

[a] Values are mean ± SD or n (%), when indicated (n = 39) as appropriate.

3.2. Changes in the expression of cell adhesion molecules 4 h after the intervention

As shown in Table 4, after 4 h of intervention the expression of CD31 in T-lymphocytes was decreased for both alcoholic beverages ($p \leq 0.033$; both). AAW showed significant reductions in the expression of Mac-1 ($p = 0.004$) in lymphocytes and CD31, CD36 and CCR2 genes in monocytes ($p \leq 0.038$; all) compared to baseline. On the other hand, after gin intake the expression of monocytes was significantly reduced for Mac-1 ($p = 0.033$) and increased for CD15 ($p = 0.030$).

However, on analysis of inter-group differences, differences in the expression of LFA-1 and Mac-1 in T-lymphocytes and expression of CCR2 in monocytes were statistically significant, also in favor of AAW ($p = 0.031$, $p = 0.021$ and $p = 0.049$; respectively).

Table 3
Changes in gene expression at baseline and 4 h after the wine and gin interventions.

Gene	Before wine intake	4 h after wine intake	p*	Before gin intake	4 h after gin intake	p*	p**
	Mean ± SD⁺	Mean ± SD⁺		Mean ± SD⁺	Mean ± SD⁺		
TLR2	65.32 ± 13.91	47.18 ± 9.31	0.003	52.85 ± 4.45	53.81 ± 6.96	0.831	0.001
TLR4	49.01 ± 5.96	44.09 ± 8.90	0.032	49.60 ± 2.89	46.56 ± 9.71	0.034	0.407
TLR6	47.40 ± 15.83	41.89 ± 10.09	0.048	50.49 ± 10.31	46.86 ± 8.01	0.034	0.249
IL1R1	47.80 ± 5.19	43.68 ± 8.32	0.035	50.26 ± 3.11	49.66 ± 2.96	0.538	0.049
CASP-1	47.96 ± 4.67	42.14 ± 8.62	0.041	46.56 ± 3.52	43.26 ± 8.60	0.049	0.191
CCR2	51.41 ± 15.53	46.03 ± 16.92	0.266	52.99 ± 12.54	48.99 ± 7.78	0.124	0.586
CXCR3	46.56 ± 3.52	43.18 ± 8.57	0.049	47.28 ± 4.16	48.85 ± 2.56	0.084	0.010
CCR5	49.57 ± 13.48	47.02 ± 9.76	0.170	51.03 ± 6.86	48.43 ± 4.75	0.039	0.877
CXCR2	48.32 ± 7.16	45.45 ± 7.91	0.523	47.32 ± 3.32	48.22 ± 3.85	0.334	0.433
NLPR3	50.10 ± 9.54	43.47 ± 11.97	0.019	49.21 ± 4.85	50.72 ± 4.31	0.056	0.011

[a] Results are expressed as +mean ± standard deviation (SD) (n = 39).
[b]*(intra-group differences)∓mean differences (95% confidence interval [CI) before and after each intervention.
[c]** between group differences.
[d]P** of the paired samples Student's test for the differences before and after the intervention.

3.3. Changes in other cardiovascular risk factors

Following both AAW and gin intake, a significant decrease in systolic BP ($p = 0.001$ and $p = 0.007$, respectively), plasma glucose ($p < 0.001$ and $p = 0.003$, respectively) and LDL-cholesterol concentrations ($p < 0.001$ and $p = 0.028$, respectively) was observed. Conversely, triglyceride concentrations significantly increased after the intake of both beverages ($p < 0.001$; both). Only AAW intake showed a significant increase in AST ($p = 0.007$).

On the inter-group analysis, AAW intake showed a significant reduction in plasma glucose and LDL-cholesterol concentrations compared to gin intake ($p = 0.048$ and $p = 0.021$, respectively). No differences were observed in other biochemical parameters (Table 5).

4. Discussion

Atherosclerosis is a low-grade inflammatory process that occurs in the wall of the arteries. Leukocyte recruitment and migration from blood to endothelium are crucial in the early stages of atherosclerotic process and are mediated by cellular AM on T lymphocyte and monocyte surfaces that interact with the corresponding endothelial AM [1,2]. In the current study, we measured several genes as well as plasma and cellular AM implicated in the onset and progression of the atherosclerotic process at baseline and 4 h after ingestion of 0.5 g alcohol/kg weight as AAW (constituted mainly by ethanol plus other components such as polyphenols) and gin (mainly ethanol) in 39 subjects with high CVD risk. We observed that the expression of CCR2, CD36 and CD31 on monocyte surfaces, LFA-1 on lymphocytes and the expression of the TLR2, ILR1R, CXCR3 and NLPR3 genes related to the atherosclerosis process were reduced following AAW compared to gin intake. Nonetheless, changes in CD31 on lymphocytes and TLR4, TLR6 and Caspase-1 GE were similar after both interventions, which might be attributed to ethanol-related effects. On the other hand, CD15 expression was increased after the gin intervention. Overall, AAW intake showed greater changes in the expression of the genes studied which could be attributed to the phenolic content or other minor components included in this type of wine.

Similar to our results, in an ethanol preconditioning ischemia and reperfusion study in a murine small intestine model Yamaguchi et al. demonstrated that acute ingestion of a moderate dose of alcohol in mice (0.5 g/kg; ~300 μl vol.) completely prevented adhesive interactions between the endothelium and the circulating leukocytes in tissues exposed to ischemia/reperfusion [27,28]. As far as we know, no previous studies on this issue have been performed in humans.

The relevance of this study and others is that the results observed add plausibility to the scientific evidence on the protective effects of moderate alcohol consumption observed in epidemiological studies. In fact, several cohort studies have observed that regular moderate alcohol consumption reduces the risk of several diseases, whereas occasional consumption or abstention does not [29]. Thus, subjects who moderately consume alcohol (1–2 drinks a day) have a lower risk of CHD than those who sporadically drink or are abstainers. In most studies, the risk of CHD decreases in a downward linear trend with alcohol intake of up to two drinks day. These protective effects have mainly been attributed to the effects on lipids, glucose metabolism and hemostatic factors [30], but more recently several studies have pointed out the anti-inflammatory effects of alcoholic beverages [31], mainly attributed to their polyphenolic content [32]. The results of the current study demonstrate the acute effects of a single dose of an alcoholic beverage on several biomarkers related to atherosclerosis.

Since excessive alcohol consumption is one of the main causes of death in the world, recommendations to the population regarding the benefits of moderate alcohol consumption must be given with the greatest clarity and also with the greatest caution. However, we must bear in mind that the type of beverage consumed is also important, and the consumption of wine has been associated with a lower cardiovascular risk compared to other beverages. This protective benefit may be due in part to the presence of a high amount of polyphenols in wine compared to liquors and spirits which have smaller quantities or lack polyphenols [32–35].

For AAW, eight of the 10 genes studied showed differential expression: TLR2/4 and 6, ILR1R, Caspase-1, CXCR3, CCR5 and NLPR3. When the subjects followed-up the AAW intervention, the expression of TLR2, IL1R1, CXCR3 and NLPR3 differed from that when they were in the gin intervention. Previous studies in experimental animals and humans have suggested that IL1R1 and its ligand, IL-1β, could play a role in the development of CVD and obesity [36]. Thus, a reduction in the expression of these genes should have a beneficial effect on reducing CVD. In autoimmune and inflammatory diseases such as atherosclerosis and others associated to CVD, TLRs may contribute to tissue damage [37]. TLR activity increases with absorption and formation of intracellular crystals cholesterol and also provokes the activation of NLRP3 [38]. Interestingly, compared to gin AAW intake significantly reduced the expression of these genes. The only protective effect of gin on the expression of the genes evaluated was on CCR5, a gene that is related to key functions in macrophages and in the development of atherosclerosis [39,40]. However, the difference with the effects of AAW intake on the expression of this gene did not attain statistical significance (Table 3).

Table 4
Mean differences in T-lymphocyte and monocyte expression before and 4 h after wine and gin intake.

	Wine				Gin				
	Before wine intake	4 h after wine intake	Mean differences (95% CI)[‡]	p*	Before gin intake	4 h after gin intake	Mean differences (95% CI)[‡]	p*	p**
	Mean ± SD[+]	Mean ± SD[+]			Mean ± SD[+]	Mean ± SD[+]			
Lymphocytes (MFI)									
Lfa-1	117.52 ± 44.27	110.20 ± 38.18	−7.31 (−17.75 to 3.12)	0.164	120.65 ± 56.39	115.27 ± 43.56	−5.37 (−27.63 to 16.88)	0.626	0.031
Mac-1	81.58 ± 46.28	63.77 ± 39.34	−17.80 (−29.65 to −5.96)	0.004	91.63 ± 64.97	87.24 ± 82.43	−4.39 (−29.78 to 21.00)	0.727	0.021
VLA4	37.80 ± 14.14	37.58 ± 7.30	−0.22 (−3.99 to 3.54)	0.903	36.61 ± 12.94	35.40 ± 8.00	−1.21 (−6.33 to 3.91)	0.633	0.256
CD15	45.87 ± 21.95	44.19 ± 20.84	−1.67 (−9.71 to 6.35)	0.674	48.03 ± 34.99	45.26 ± 20.61	−2.77 (−13.23 to 7.69)	0.593	0.278
CD40	59.39 ± 24.26	55.91 ± 23.40	−3.48 (−8.27 to 1.30)	0.148	63.02 ± 48.93	51.23 ± 22.04	−11.78 (−30.58 to 7.00)	0.209	0.568
CD31	354.68 ± 286.61	299.92 ± 251.05	−54.75 (−160.05 to 50.54)	0.033	514.09 ± 740.69	215.70 ± 208.44	−298.39 (−544.81 to −51.97)	0.020	0.215
Monocytes (MFI)									
Lfa-1	75.26 ± 17.20	73.14 ± 17.20	−2.12 (−6.12 to 1.86)	0.286	76.69 ± 34.95	67.19 ± 21.78	−9.49 (−21.04 to 2.05)	0.103	0.979
Mac-1	51.90 ± 14.61	51.61 ± 15.07	−0.29 (−4.37 to 3.77)	0.883	56.19 ± 18.39	49.42 ± 16.81	−6.76 (−12.87 to −0.65)	0.031	0.206
VLA4	46.33 ± 19.80	45.71 ± 16.07	−0.62 (−5.40 to 4.17)	0.795	44.05 ± 14.22	42.60 ± 14.21	−1.45 (−6.41 to 3.50)	0.555	0.983
CD15	28.97 ± 6.71	30.47 ± 4.60	1.49 (−1.15 to 4.14)	0.254	30.06 ± 6.54	33.77 ± 7.58	3.70 (0.39–3.70)	0.030	0.638
CD40	32.02 ± 12.61	31.99 ± 9.81	0.02 (−2.57 to 2.52)	0.983	33.05 ± 10.93	32.04 ± 8.50	−1.00 (−5.97 to 3.97)	0.680	0.863
CD31	739.91 ± 612.81	527.45 ± 334.69	−212.46 (−409.18 to −15.73)	0.035	639.61 ± 527.62	543.93 ± 548.56	−95.68 (−252.29 to 60.92)	0.221	0.444
CD36	794.09 ± 610.81	574.64 ± 531.53	−219.45 (−421.65 to −17.25)	0.035	726.41 ± 596.52	426.99 ± 360.47	−299.42 (−485.47 to −113.36)	0.098	0.271
CCR2	122.77 ± 119.46	78.63 ± 50.74	−44.14 (−85.63 to −2.64)	0.038	76.74 ± 48.31	76.62 ± 50.01	−0.12 (−14.18 to 13.93)	0.985	0.049

MFI, median fluorescent intensity; CI, confidence interval; [a]Results are expressed as +mean ± SD (n = 39). [b]*(intra-group differences)ǂmean differences (95% CI) between after and before each intervention. [c]p** between groups differences.

Table 5
Mean differences in biomarkers before and 6 h after wine and gin intake.

Biomarkers	Wine				Gin				
	Before wine intake	4 h after wine intake	Mean differences (95% CI)[‡]	p	Before gin intake	4 h after gin intake	Mean differences (95% CI)[‡]	p	p value
	Mean ± SD[+]	Mean ± SD[+]			Mean ± SD[+]	Mean ± SD[+]			
Systolic blood pressure (mmHg)	142.58 ± 15.71	132.88 ± 15.38	−9.71 (−14.90 to −4.52)	0.001	138.44 ± 16.82	132.18 ± 16.07	−6.25 (−10.68 to −1.84)	0.007	0.335
Diastolic blood pressure (mmHg)	75.86 ± 9.63	74.84 ± 10.15	−1.02 (−3.91 to 1.87)	0.479	72.75 ± 11.71	72.77 ± 9.56	0.013 (−2.90 to 2.92)	0.993	0.493
Heart rate (lpm)	65.17 ± 8.52	62.51 ± 9.88	−2.77 (−5.44 to −0.10)	0.042	65.60 ± 10.16	64.02 ± 9.39	−1.58 (−4.59 to 1.43)	0.295	0.266
Glucose (mg/dL)	112.71 ± 23.70	92.00 ± 17.75	−20.71 (−26.34 to −15.07)	0.001	114.55 ± 30.24	100.86 ± 30.91	−13.68 (−22.25 to −5.10)	0.003	0.048
Cholesterol (mg/dL)	171.51 ± 43.08	167.51 ± 44.57	−4 (−10.95 to 2.95)	0.252	169.23 ± 40.71	170.15 ± 40.07	0.92 (−3.04 to 4.89)	0.641	0.205
Triglycerides (mg/dL)	106.79 ± 56.98	136.64 ± 51.93	29.46 (15.22–43.28)	0.001	107.15 ± 52.97	136.64 ± 51.21	29.48 (16.67–42.29)	0.001	0.971
AST (U/L)	23.78 ± 6.51	25.25 ± 6.54	1.47 (0.42–2.51)	0.007	23.72 ± 12.25	24.90 ± 9.63	1.21 (−1.14 to 3.58)	0.304	0.863
ALT (U/L)	25.37 ± 11.52	26.29 ± 12.03	0.94 (−0.24 to 2.13)	0.115	28.32 ± 31.73	28.00 ± 25.82	−0.32 (−2.98 to 2.33)	0.806	0.346
Sodium (mEq/L)	141.64 ± 1.90	141.31 ± 2.43	−0.33 (−0.95 to 0.29)	0.286	141.87 ± 2.28	141.71 ± 2.76	−0.15 (−0.90 to 0.60)	0.680	0.221
Potassium (mEq/L)	4.23 ± 0.30	4.20 ± 0.39	−0.036 (−0.13 to 0.06)	0.458	5.18 ± 6.30	4.22 ± 0.55	−0.96 (−3.05 to 1.13)	0.358	0.376
LDL cholesterol (mg/dL)	105.31 ± 38.97	98.03 ± 38.58	−7.28 (−9.93 to −4.63)	0.001	106.30 ± 37.68	101.35 ± 35.12	−4.94 (−9.34 to −0.55)	0.028	0.021
HDL cholesterol (mg/dL)	40.07 ± 11.36	42.33 ± 12.79	0.25 (−1.36 to 1.87)	0.750	41.46 ± 11.43	43.38 ± 14.85	−1.92 (−5.78 to 1.94)	0.320	0.298
APOA (mg/dL)	114.03 ± 18.98	114.15 ± 19.97	0.12 (−2.80 to 3.06)	0.903	116.43 ± 16.71	116.94 ± 17.22	0.51 (−2.24 to 3.27)	0.709	0.841
APOB (mg/dL)	79.71 ± 20.63	77.92 ± 21.45	−1.79 (−4.49 to 0.90)	0.187	81.74 ± 20.11	80.12 ± 20.85	−1.61 (−4.00 to 0.77)	0.178	0.924
LpA (mg/dL)	31.57 ± 47.90	30.56 ± 47.25	−1.00 (−4.43 to 2.41)	0.548	32.27 ± 47.78	32.21 ± 51.78	−0.06 (−4.14 to 4.02)	0.976	0.746
prT (seg)	13.35 ± 1.76	13.47 ± 2.00	0.11 (−0.08 to 0.32)	0.239	13.19 ± 1.75	13.25 ± 1.79	0.06 (−0.11 to 0.24)	0.457	0.562
tplasT (seg)	29.32 ± 1.93	29.02 ± 2.41	−0.29 (−0.84 to 0.25)	0.282	29.12 ± 1.94	29.05 ± 2.31	−0.07 (−0.47 to 0.34)	0.737	0.425
Fibrinogen (g/L)	3.41 ± 0.90	3.65 ± 0.66	0.23 (−0.02 to 0.49)	0.077	3.40 ± 0.62	3.49 ± 0.68	0.09 (−0.00 to 0.19)	0.051	0.231

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; APO1, apolipoprotein 1; APOB, apolipoprotein B; LPA, Lipoprotein A; prT, prothromobin time (seg); prot, prothrombin time rate; tplasT, thromboplastin time. [a] Results are expressed as +mean ± standard deviation (SD) (n = 39). [b]*(intra-group differences)ǂmean differences (95% confidence interval [CI]) before and after each intervention. [c]** between group differences. [d] p** of the paired samples Student's test from the differences before and after the intervention.

In the present study, we observed slight decreases in the gene expression of CXCR2 and CCR2, which are pro-inflammatory genes, but these differences were not significant after either AAW or gin intake. There was a correlation between gene and adhesion molecule expression for Caspase-1 and MAC-1, which are pro-inflammatory, after the intervention, with both being decreased and significantly correlated ($p = 0.01$) after gin intake. Our results are in concordance with the study by Di Renzo, in which the consumption of red wine changes the inflammatory gene expression [41].

In our study, gene expression was decreased after the intake of both beverages, and likewise, LDL-cholesterol, glucose and systolic BP significantly decreased. On the other hand, the heart rate significantly decreased after AAW intake but not after gin consumption. It was also observed that after the consumption of both beverages following 12 h of fasting, triglyceride levels increased 6 h after the intervention, with AST levels also increased after AAW intake.

The results of the present study may also be related to a possible synergistic or antagonistic effect of alcohol and polyphenols. The reductions observed in both wine and gin in gene expression of TLR4, TLR6 or Casp-1 could be due to the action of alcohol (ethanol). However, the reductions observed for TLR2, IL1R1, CXCR3 or NLPR3 could be explained by a possible alcohol-polyphenol synergy. While the decrease observed in CCR5 in gin could be explained by a possible alcohol-polyphenol antagonistic effect, in which polyphenols would counteract the effect of alcohol, these same results could be extended to the expression of the adhesion molecules and anthropometric parameters studied.

This study has limitations. While a 4 h intervention may not represent the potential effects of long-term consumption, we did observe favorable results following alcohol consumption, principally after wine intake. Another limitation is that the specific substances responsible for the effects observed were not identified and endothelial function was not measured.

In addition, during the 6 h after the intervention the participants did not consume any food which might explain the elevation in triglyceride concentration after the intake of both beverages. Lastly, while the alcohol intake in the present study might apparently be high compared with the current guidelines, these guidelines do not usually take into account the role of the whole pattern of alcohol consumption.

Conclusion

Epidemiologic studies have shown that moderate alcohol consumption is part of a healthy lifestyle behavior, significantly reducing all-cause mortality and cardiovascular mortality, among other benefits [30].

Our results are in accordance with previous published studies in which the consumption of wine showed greater cardioprotective effects on the initial stages of atherosclerosis compared to other alcoholic beverages that may be attributed to its higher anti-inflammatory effect. Although the changes observed in the expression of genes and leukocyte adhesion molecules may not be clinically or biologically relevant, they help to explain the mechanisms by which wine exerts its protective effects on the cardiovascular system. According to the results observed in the current study alcohol and polyphenols may have synergistic effects. However, the differences observed in gene expression following AAW and gin intake can most likely be attributed to the polyphenol content of AAW.

Conflict of interest

RE reports serving on the board of and receiving lecture fees from the Beer and Health Foundation, serving on the Scientific Board of 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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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, MR-C, MD, RML-R and RE: draft of the article; and IR, RC, MR-C, MD, RML-R and RE: critical revision and final approval. IR, RC, MR-C, RM, 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.

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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.2018.07.014.

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