Decreased tumor necrosis factor–induced adhesion of human monocytes to endothelial cells after moderate alcohol consumption1–3

Eva Badía, Emilio Sacanella, Joaquim Fernández-Solá, José Maria Nicolás, Emilia Antúnez, Domenico Rotilio, Giovanni de Gaetano, Álvaro Urbano-Márquez, and Ramon Estruch

ABSTRACT

Background: Moderate alcohol consumption protects against ischemic heart disease, possibly through an antiinflammatory effect. However, little is known about the mechanisms by which alcohol may interfere in the development of atherosclerosis.

Objective: We analyzed the effects of 2 alcoholic beverages with high (red wine) or low (gin) polyphenolic content on human monocyte adhesion to an endothelial cell line (Ea.hy926).

Design: This was a randomized, crossover trial with 8 healthy men. After a washout period, the subjects received 30 g ethanol/d as red wine or gin for 28 d. Before and after each intervention, a dietary survey and laboratory analysis were performed. Adhesion of human monocytes to endothelial cells was measured in basal and stimulated [by tumor necrosis factor α (TNF-α)] conditions. Adhesion molecules involved in monocyte-endothelium interactions were determined on the cell surface.

Results: The mean expression of very late activation antigen 4 on monocytes significantly decreased after red wine intake [by 18% (95% CI: 33%, 3%); P = 0.022]. Monocyte adhesion significantly increased after TNF-α stimulation of endothelial cells. This increase, however, was 39% less (95% CI: 48%, 35%; P = 0.049) after gin intake than after the respective washout period and was nearly abolished by red wine intake [96% less than after the respective washout period (95% CI: 142%, 76%); P < 0.001]. The reduction after red wine intake was significantly different from that after gin intake (P = 0.014).

Conclusions: TNF-α–induced adhesion of monocytes to endothelial cells was virtually abolished after red wine consumption but was only partially reduced after gin consumption. This effect may be due to the down-regulation of adhesion molecules on the monocyte surface.


KEY WORDS Endothelium, alcohol, monocyte adhesion, atherosclerosis, polyphenols, adhesion molecules

INTRODUCTION

Several epidemiologic studies have reported an association between moderate alcohol consumption and a lower risk of ischemic heart disease and ischemic stroke (1–3). Because this association was found to be independent of the type of alcoholic beverage consumed (liquor, beer, or wine), most researchers attributed these effects to ethanol itself. However, in addition to alcohol, most alcoholic beverages contain many nonalcoholic compounds, mainly polyphenols, which makes the comparison of the effects of either component on the basis of epidemiologic studies rather difficult (4, 5).

The beneficial effect of moderate drinking on atherosclerosis has usually been attributed to changes in the lipoprotein profile and the coagulation system (6–11). More recently, atherosclerosis has been considered a chronic low-grade inflammatory disease in which the adhesion of monocytes to endothelial cells, through the interaction of adhesion molecules expressed on both cells, plays a pivotal early event in pathogenesis (12, 13). In vitro studies have shown that some compounds, such as flavonoids, found in alcoholic beverages can modulate monocyte cell adhesion to the endothelium (14, 15). More recently, it was observed in ex vivo studies of healthy humans that cocoa flavonoids may reduce platelet function (16). However, few data are available on the effects of various alcoholic beverages with different polyphenolic content on the early phases of atherosclerosis in humans.

We therefore undertook a prospective, crossover study to evaluate the effects of moderate consumption of 2 alcoholic beverages with high (red wine) or low (gin) polyphenolic content on human monocyte adhesion to the endothelium. This trial was performed in healthy men with low cardiovascular disease risk in whom diet and exercise were carefully monitored.

SUBJECTS AND METHODS

Subjects

Healthy men aged 30–50 y who worked in our institution were eligible to participate in the study if they reported an average consumption1–3

1 From the Department of Internal Medicine, Hospital Clinic, Institut d’Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Spain (EB, ES, IF-S, JMN, EA, AU-M, and RE); the Consorzio Mario Negri Sud, Santa Maria Imbaro (DR); and the Università Cattolica, Campobasso, Italy (GdG)
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3 Address reprint requests to E. Sacanella, Department of Internal Medicine, Hospital Clinic, C/ Villarroel, 170 08036 Barcelona, Spain. E-mail: esacane@clinic.ub.es.
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daily ethanol intake of between 10 and 40 g over the past 5 y and did not smoke or have hypertension, diabetes mellitus, LDL-cholesterol concentrations > 160 mg/dL, HDL-cholesterol concentrations < 35 mg/dL, ischemic heart disease, a family history of premature ischemic heart disease, cerebrovascular disease, peripheral vascular disease, HIV infection, alcoholic liver disease, malnutrition, or neoplastic or acute infectious diseases. In addition, none of the subjects were receiving any medication or were taking vitamin supplements. All gave informed consent for the various procedures and none received any monetary compensation. The Institutional Review Board of the hospital approved the study protocol.

**Study design**

The study was an open, prospective, randomized, crossover clinical trial. During the first 2 wk of the study, the subjects did not drink any alcoholic beverages (first washout period). During the following 4 wk, the subjects underwent the first intervention. During the following 2 wk, the participants again did not consume any alcoholic beverages (second washout period). Finally, during the last 4 wk, the subjects underwent the second intervention. All received 30 g ethanol/d as red wine (2 glasses of 160 mL each per day with dinner) and 30 g ethanol as an alcoholic beverage with a very low polyphenolic content (100 mL gin/d with dinner) in a random, crossover design. The diet followed and the exercise performed by the participants were monitored before and after each intervention period. In addition, laboratory analyses were performed before and after each intervention.

**Alcoholic beverages with high or low polyphenolic content**

The alcoholic beverage with a high polyphenolic content selected was red wine obtained from Merlot grapes. The alcoholic strength was 12.5° and contained 3.95 mg (−)-epicatechin gallate/L, <0.09 mg (−)-epicatechin/L, <0.1 mg coumarins/L, <0.15 mg cyanidin chloride/L, <0.15 mg malvidin chloride/L, <0.15 mg delphinidin chloride/L, 5.70 mg resveratrol/L, 624 mg anthocianins/L, and 2.93 g tannins/L. After we analyzed the phenolic content of several distilled alcoholic beverages, we selected gin for the study because its phenolic content was under the limits of detection. The polyphenolic content of the alcoholic beverages was determined as previously reported (17).

**Diet and exercise monitoring**

All subjects followed an isocaloric diet, which was designed according to each subject’s personal preferences. Because the participants ate on their own, detailed dietary information was provided to them and, if appropriate, to their partners. Foods such as onions, virgin olive oil, green and black tea, and brain were forbidden, and others with a high content of polyphenols, ascorbic acid, α-tocopherol, β-carotenes, cholesterol, or fat—such as chocolate, cocoa, orange and tomato juices, nuts, some fruit (oranges, lemons, strawberries, grapes, melon, apples, and apricots), some vegetables (spinach, turnips, carrots, parsley, peppers, garlic, and tomatoes), fatty meat and lamb, butter, margarine, soybean products, ice cream, custard, flan, and tripe (kidneys and livers)—were restricted. The consumption of antioxidant-containing foods, especially fruit and vegetables, was strictly controlled so that the diets of all the subjects had a similar antioxidant content.

A 3-d recall questionnaire of food and drink consumption that was previously validated in our population (18) was used to monitor the diet followed before and after each intervention. The reported food consumption was converted into nutritional data by using PROFESSIONAL DIET BALANCER software (Cardinal Health Systems Inc, Edina, MN). Exercise was monitored with the Minnesota Leisure Time Physical Activity Questionnaire, which has also been validated in Spain (19).

**Clinical and laboratory measurements**

At baseline, a detailed clinical history of ethanol intake, smoking habits, and dietary habits was obtained by using a structured questionnaire. Before and after each intervention period, we obtained blood samples after the subjects had fasted overnight that were coded with random numbers and processed immediately. Serum lipoprotein analysis was performed and included total cholesterol, triacylglycerols, HDL cholesterol, LDL cholesterol, VLDL cholesterol, apolipoprotein A-I, and apolipoprotein B. Mononuclear cells were isolated from whole blood by the Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation method (20). Afterward, we performed a Percoll (Sigma-Aldrich, Steinheim, Germany) gradient centrifugation. With this method, the purity of the monocytes was >80% as assessed by flow cytometry (CD14+ cells), and cell viability was determined by the trypan blue (Sigma-Aldrich, Irvine, CA) dye-exclusion test. Adhesion molecule expression on monocytes was determined by double immunofluorescence staining as previously reported (20). Data analysis was performed with a FACscan Clinical Cytometer (Becton Dickinson, San Jose, CA) and the CELLQUEST software. The following adhesion molecules were analyzed: very late activation antigen 4 [VLA-4 (anti-CD49d); clone 44H6 (Cytogmos, Barcelona, Spain)], lymphocyte function-associated antigen 1 [LFA-1 (anti-CD11a); clone R7.1 (Bender Med Systems, Vienna)], and αMβ2 [Mac-1 (anti-CD11b); clone LM21 (Bender Med Systems)]. Expression of the monocyte chemotactic peptide 1 [MCP-1; clone SD3-F7 (Pharmingen, San Diego)] on the monocyte surface was also determined. Monocytes and T-lymphocytes were identified by using anti-CD14 (clone TUK4; Caltag Laboratories, Burlingame, CA) and anti-CD2 (clone G11; Caltag Laboratories) monoclonal antibodies, respectively. We also measured polyphenolic compounds in 500 μL plasma before and after each intervention by HPLC with the use of a Varian model instrument (Varian, Walnut Creek, CA) with a photodiode-array detector at wavelengths of 190–700 nm (21).

**Endothelial cell culture**

The endothelial cell hybridoma Ea.hy9.26 is a fusion product between the human umbilical vein endothelial cell line and the epithelial cell line A549. The permanent human Ea.hy9.26 endothelial cell line was obtained from the American Tissue Culture Collection (Rockville, MD) and was grown in RPMI-1640 medium supplemented with 2 mmol/L-glutamine/L (Biological Industries, Beit Haemek, Israel), 10% fetal bovine serum (Bio Whitaker, Verriers, Belgium), 40 μg gentamicin/L, and 0.04% HAT (hypoxantine, aminopterin, thymin) supplement (Biological Industries). This cell line has been shown to express factor VIII–related antigens with the same morphologic distribution observed in primary human endothelial cells (22). The cells were
seeded in 75-cm² culture flasks and were allowed to grow to confluence before experimental treatment. At confluence, the cells were subcultured at a 1:3 ratio.

**Monocyte adhesion assay**

Confluent endothelial cell monolayers grew in 96-well tissue culture plates (Becton Dickinson Labware, San José, CA). Half of the wells were stimulated with human recombinant tumor necrosis factor α (TNF-α; 1 × 10⁶ U/L) to obtain activated endothelial cells as previously described (23). After stimulation and washing, 1.5 × 10⁵ human monocytes per well were added and were allowed to attach for 30 min at 37 °C. After this process was completed, nonadherent cells were removed by aspiration, and the wells were washed once with warm, serum-free medium. Adherent cells were fixed and stained with 0.2% crystal violet in 20% methanol in phosphate-buffered saline for 20 min and were then washed repeatedly with distilled water. After solubilization with 1% sodium dodecyl sulfate, absorption was measured with a spectrophotometer at a wavelength of 600 nm (Becton Dickinson).

**Statistical analysis**

Standard statistical methods from SPSS STATISTICAL ANALYSIS SYSTEM 10.0 (SPSS Inc, Chicago) were used. Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to all variables to assess whether they showed a normal distribution. For variables such as β-carotenes, vitamin E equivalents, cholesterol, and vitamin C that did not follow a normal distribution, a logarithmic transformation was performed. After this transformation, these 4 variables followed a normal distribution. The two-tailed paired Student’s t test was then used to compare changes between baseline and stimulated conditions in all cases. We express the variables as means ± SDs, except for the transformed variables, which are expressed as geometric means ± SDs. The level of significance was set at P < 0.05. The effects of each intervention as well as the differences between the 2 interventions are expressed as mean changes (with 95% CIs).

**RESULTS**

**Patient characteristics, diet monitoring, and nutritional status**

Eight healthy men (mean age: 37.6 ± 7.4 y; range: 30–50 y) were included in the study and were randomly assigned to 1 of the 2 interventions. The first intervention for 4 subjects was red wine and the second intervention was gin. The remaining 4 subjects began drinking gin and afterward consumed red wine. The subjects’ reported daily intake of ethanol before the study was 23.4 ± 5.1 g over a period of 14.2 ± 5.5 y.

Protocol adherence was optimum in the 8 subjects, and complete agreement was observed between the participants’ reports and the number of empty bottles returned. In addition, the nutrient content of the self-reported diets was close to that of the planned diets. None of the subjects were classified as being noncompliant, and no patient reported eating chocolate or other cocoa products during the study. As a measure of intervention compliance, epicatechin gallate (a marker of polyphenol constituents) was measured in plasma drawn after the subjects had fasted overnight, ∼10 h after the last ingestion of wine or gin. Plasma epicatechin gallate concentrations increased from 0.015 ± 0.004 to 0.055 ± 0.02 mg/L after red wine intake [255% (95% CI: 144%, to 366%); P < 0.001], whereas they did not change significantly during the gin intake period (0.016 ± 0.009 and 0.019 ± 0.007 mg/L).

Dietary intake data for the 2 intervention periods are shown in Table 1. No significant differences in nutrient intake were observed before and after each intervention. Interestingly, no significant differences were observed in the daily intake of antioxidants or fat before and after each intervention period. In addition, the daily average energy expended in physical activity was similar during the wine and gin intake periods. On the other hand, mean HDL-cholesterol concentrations increased by 7% after both gin and wine intake (52.8 ± 14.1 compared with 56.7 ± 14.8 mg/dL and 53.7 ± 13.8 compared with 57.7 ± 14.1 mg/dL, respectively; P < 0.001 for both). No significant changes were observed in the other nutritional indexes evaluated in the study.

**Table 1**

Reported dietary intake during the consumption of alcohol with a low (gin) or high (red wine) polyphenolic content

<table>
<thead>
<tr>
<th></th>
<th>Gin intervention (n = 8)</th>
<th>Red wine intervention (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Energy (kcal/d)²</td>
<td>2319 ± 638</td>
<td>2238 ± 580</td>
</tr>
<tr>
<td>Cholesterol (mg/d)³</td>
<td>300 ± 101</td>
<td>325 ± 89</td>
</tr>
<tr>
<td>SFA (mg/d)²</td>
<td>32.0 ± 10.3</td>
<td>31.6 ± 12.7</td>
</tr>
<tr>
<td>MUFA (mg/d)²</td>
<td>47.2 ± 19.3</td>
<td>45.6 ± 15.5</td>
</tr>
<tr>
<td>PUFA (mg/d)²</td>
<td>12.5 ± 3.9</td>
<td>13.4 ± 3.5</td>
</tr>
<tr>
<td>β-Carotenes (mg/d)⁵</td>
<td>961 ± 176</td>
<td>930 ± 154</td>
</tr>
<tr>
<td>Vitamin A (mg/d)²</td>
<td>999 ± 637</td>
<td>1061 ± 807</td>
</tr>
<tr>
<td>Vitamin E equivalents (mg/d)⁷</td>
<td>7.93 ± 3.11</td>
<td>9.36 ± 5.21</td>
</tr>
<tr>
<td>Vitamin C (mg/d)²</td>
<td>94.3 ± 54.6</td>
<td>95.2 ± 65.3</td>
</tr>
<tr>
<td>Polyphenols (mg/d)²</td>
<td>4.54 ± 3.12</td>
<td>3.53 ± 2.98</td>
</tr>
</tbody>
</table>

1 The food consumption reported on a 3-d recall questionnaire was converted into nutritional data by using PROFESSIONAL DIET BALANCER software (Cardinal Health Systems, Inc, Edina, MN). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. No significant differences were observed in any of the variables analyzed (two-tailed paired Student’s t test).

² x ± SD.

³ Geometric x ± SD.
Adhesion molecule expression on the monocyte surface

No significant changes in adhesion molecule expression (LFA-1, Mac-1, or VLA-4) or in MCP-1 expression on the monocyte surface were observed after consumption of gin. On the other hand, the mean expression of LFA-1, Mac-1, VLA-4, and MCP-1 on monocytes decreased after the consumption of red wine (Table 2), significantly so for VLA-4 (by 81.7% ± 2.27% units, 95% CI: 65.8%, 97%; P = 0.030) compared with baseline. Interestingly, monocyte adhesion to stimulated endothelial cells was quite different after the washout periods. As expected, monocyte adhesion to TNF-α–stimulated endothelial cells increased similarly by 27% (95% CI: 13%; 42%; P < 0.001) and 20% (95% CI: 11%, 29%; P < 0.001) compared with baseline after the 2 washout periods. Interestingly, monocyte adhesion to stimulated endothelial cells was quite different after the washout periods than after moderate alcohol consumption. After the consumption of gin, monocyte adhesion to TNF-α–stimulated endothelial cells increased by only 12% (95% CI: 6%, 19%; P < 0.001). After drinking red wine, however, no significant changes were seen in monocyte adhesion between baseline and the stimulated condition (1% (95% CI: –6%, 7%); P = 0.47). Compared with the data obtained after the corresponding washout period, gin and red wine intake decreased monocyte adhesion to TNF-α–stimulated endothelial cells by 39% (95% CI: 48%, 35%; P = 0.049) and 96% (95% CI: 142%, 76%; P < 0.001), respectively.

Finally, when we analyzed the difference in the effects of the 2 interventions on the reduction in monocyte adhesion to stimulated endothelial cells, we found a significantly greater decrease after red wine intake than after gin intake (P = 0.014).

DISCUSSION

The present study showed that regular, moderate alcohol consumption (30 g/d) for 28 d reduces some of the inflammatory mechanisms involved in atheromatous plaque formation. Indeed, monocyte adhesion to TNF-α–stimulated endothelial cells after 1 mo of moderate alcohol drinking was significantly lower than that observed after a period of abstinence from alcohol. This effect was significantly greater when the subjects consumed an alcoholic beverage with a high polyphenolic content (red wine) than when they drank an alcoholic beverage with a low polyphenolic content (gin). The decreased monocyte–endothelial cell adhesion observed after wine intake was possibly due to down-regulation of some monocyte adhesion molecules, especially VLA-4, on the monocyte surface.

Increasing evidence suggests that moderate alcohol consumption reduces the incidence of ischemic heart disease and stroke (1–3). Increased HDL-cholesterol concentrations and fibrinolytic activity and decreased platelet aggregation have been proposed as the leading mechanisms to explain this beneficial effect on atherosclerosis (6–11), although other processes may be involved (12, 13). Today, atherosclerosis is considered a low-grade inflammatory disease in which monocyte adhesion to the endothelium is an early event (12, 13). Experimental and observational studies suggest that alcohol may act as an immunomodulator, thereby reducing the atherosclerotic process (24, 25). Local alcohol delivery or moderate alcohol feeding reduces neointimal hyperplasia after balloon injury in coronary arteries from pigs.

### TABLE 2
Mean fluorescence intensity (MFI) of adhesion molecules and chemokine expression on the monocyte surface before and after each intervention

<table>
<thead>
<tr>
<th></th>
<th>Gin intervention (n = 8)</th>
<th>Red wine intervention (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>LFA-1</td>
<td>149.5 ± 50.9</td>
<td>145.7 ± 43.1</td>
</tr>
<tr>
<td>Mac-1</td>
<td>68.9 ± 32.2</td>
<td>69.1 ± 25.1</td>
</tr>
<tr>
<td>VLA-4</td>
<td>46.2 ± 7.2</td>
<td>49.1 ± 13.3</td>
</tr>
<tr>
<td>MCP-1</td>
<td>35.6 ± 31.1</td>
<td>26.4 ± 15.9</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. LFA-1, lymphocyte function–associated antigen 1; Mac-1, αMβ2 (anti-CD11b); VLA-4, very late activation antigen 4; MCP-1, monocyte chemoattractant protein 1.

2 Significantly different from before intervention, P = 0.022 (two-tailed paired Student’s t test).

### TABLE 3
Monocyte adhesion to an endothelial cell line (Ea.hy926) at baseline and after stimulation with tumor necrosis factor α

<table>
<thead>
<tr>
<th></th>
<th>Baseline adhesion (n = 8)</th>
<th>Stimulated adhesion (n = 8)</th>
<th>Mean difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washout period</td>
<td>0.637 ± 0.179</td>
<td>0.763 ± 0.140</td>
<td>0.125 (0.071, 0.179)</td>
</tr>
<tr>
<td>Gin intervention</td>
<td>0.634 ± 0.053</td>
<td>0.711 ± 0.110</td>
<td>0.077 (0.037, 0.116)</td>
</tr>
<tr>
<td>Washout period</td>
<td>0.649 ± 0.087</td>
<td>0.771 ± 0.132</td>
<td>0.122 (0.071, 0.172)</td>
</tr>
<tr>
<td>Red wine intervention</td>
<td>0.625 ± 0.146</td>
<td>0.631 ± 0.115</td>
<td>0.005 (~0.030, 0.040)</td>
</tr>
</tbody>
</table>

1 x ± SD (all such values).

2 Significantly different from baseline, P < 0.001 (two-tailed paired Student’s t test).

3,4 Significantly different from the corresponding washout period (two-tailed paired Student’s t test): 3 P = 0.049, 4 P < 0.001.

5 The reduction in monocyte adhesion to the stimulated endothelial cell line after red wine intake was significantly different from that after gin intake, P = 0.014 (two-tailed paired Student’s t test).
(26) and in abdominal aortas from rabbits (27), respectively. On the other hand, in vitro studies with polyphenols showed that resveratrol and quercetin down-regulate tissue factor expression by stimulated human vascular cells (28), and in healthy volunteers, red wine intake prevents the activation in mononuclear cells of nuclear factor κB, a transcription factor that is involved in the expression of adhesion molecules and cytokines related to early atherosclerosis (29). In addition, red wine intake has been associated with down-regulation of VLA-4, LFA-1, Mac-1, and MCP-1 expression on the monocyte surface (30). Finally, moderate alcohol drinkers show lower concentrations of soluble endothelial adhesion molecules [eg, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)] related to atherosclerosis than do abstainers or heavy drinkers (24). Although a recent study evaluated the effects of an acute ingestion of red wine on some immune functions (31), up to now, no ex vivo functional studies have evaluated the effects of moderate alcohol intake on early phases of atherosclerosis, such as monocyte adhesion to the endothelium.

Previous in vitro studies showed that red wine polyphenols at nutritionally relevant concentrations may down-regulate endothelial cell adhesion molecules (eg, ICAM-1 and VCAM-1) and decrease monocyte adhesion to the endothelium (14, 15, 23). In addition, a reduction of U937 cell (a monocytic cell line) adhesion to endothelial cells after incubation with different polyphenols was described in vitro. Ferrero et al (14) observed that resveratrol induces a significant reduction in the expression of ICAM-1 and VCAM-1 on lipopolysaccharide-stimulated human saphenous vein endothelial cells and a significant inhibition of U937 adhesion to endothelial cells. Similarly, Koga et al (15) observed that pretreatment of U937 cells or human aortic endothelial cells with catechin metabolites or quercetin reduces U937 cell adhesion to interleukin 1β-stimulated human aortic endothelial cells. The data obtained in our ex vivo study confirm previous in vitro observations (14, 15). Although TNF-α–induced adhesion of monocytes to endothelial cells was virtually abolished after red wine consumption, our results also suggest that ethanol itself may reduce monocyte adhesion to TNF-α–stimulated endothelial cells. Thus, it is possible that the ethanol and phenols present in some alcoholic beverages may have a synergistic effect, decreasing monocyte adhesion to stimulated endothelium.

The strengths of our study are its design (a randomized, crossover, clinical trial); the lack of difference in dietary composition throughout the study, as reported in the dietary surveys; and the excellent compliance of the participants, as assessed by close monitoring of diet and drinking and the measurement of epicatechin gallate in serum before and after each intervention. Therefore, the results of the current study can be attributed to the interventions administered, ie, red wine or gin. Two limitations of the study are the small number of participants and their low cardiovascular disease risk, which make it difficult to extend these results to subjects with high cardiovascular disease risk.

In summary, our results suggest that moderate alcohol consumption (especially as an alcoholic beverage with a high polyphenolic content, such as red wine) may reduce the adhesion of human monocytes to endothelial cells through down-regulation of adhesion molecules on the monocyte surface, mainly VLA-4. Because monocyte–endothelial cell adhesion is an early event in atheromatous plaque formation, a reduction in this process may be a mechanism by which moderate drinking exerts its beneficial effect on atherosclerosis.

EB, ES, and RE designed the study; JF-S, JMN, EA, AU-M, and RE collected the data; EB and ES performed the immunofluorescence analysis and adhesion assay; EB, ES, RE, and JMN analyzed the data; DR and GdG analyzed the polyphenolic content of the alcoholic beverages and measured epicatechin gallate in plasma; and EB, ES, RE, and GdG prepared the manuscript, which was reviewed by all of the authors. The authors had no conflicts of interest.

REFERENCES


