Effects of alcohol and polyphenols from beer on atherosclerotic biomarkers in high cardiovascular risk men: a randomized feeding trial

Chiva-Blanch G[a,b], Magraner E[c], Condines X[c], Valderas-Martínez P[a,b], Roth I[a,b], Arranz S[a,b], Casas R[a,b], Navarro M[c], Hervas A[c], Sisó A[c], Martínez-Huélamo M[b,d], Vallverdú-Queralt A[b,d], Quifer-Rada P[b,d], Lamuela-Raventos RM[b,d], and Estruch R[a,b,*].

[a]Department of Internal Medicine, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain; [b]CIBER CB06/03 Fisiopatología de la Obesidad y la Nutrición, (CIBERobn) and RETIC RD06/0045; [c]Primary Care Research Group, IDIBAPS, Centre d'Assistència Primària ABS Les Corts, GESCLINIC, Barcelona, Spain and [d]Nutrition and Food Science Department, XaRTA, INSA, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain.

Corresponding author: Ramon Estruch, Department of Internal Medicine, Hospital Clinic, Villarroel 170, 08036 Barcelona, Spain. Telephone: +34 932275539. Fax: +34 93 2279236. E-mail: restruch@clinic.ub.es.

Present address for Gemma Chiva-Blanch: Cardiovascular Research Center (CSIC-ICCC), C/ Sant Antoni Mª Claret 167, 08025 Barcelona, Spain.

Word counts: 227 for the abstract and 3183 for the text.

Number of references: 28

Number of figures and tables: 1 and 4, respectively.

Keywords: beer, polyphenols, alcohol, adhesion molecules, inflammation, cytokines, cardiovascular disease, cardiovascular risk, atherosclerosis.

Abstract

Background and aims: Moderate alcohol consumption exerts a cardioprotective effect, but no studies have evaluated the alcohol-independent cardiovascular effects of the non-alcoholic components of beer. We aimed to evaluate the effects of ethanol and the phenolic compounds of beer on classical and novel cardiovascular risk factors.

Methods and results: Thirty-three high-risk, male volunteers were included in a randomized, crossover feeding trial. After a washout period, all subjects received beer (30 g alcohol/d, 660 mL), the equivalent amount of polyphenols as non-alcoholic beer (990 mL), and gin (30 g alcohol/d, 100 mL) for 4 weeks. All outcomes were evaluated before and after each intervention period. Moderate alcohol consumption increased serum HDL-cholesterol (~5%), ApoA-I (~6%), ApoA-II (~7%) and adiponectin (~7%), and decreased serum fibrinogen (~8%), and interleukin (IL)-5 (~14%) concentrations, whereas the non-alcoholic fraction of beer (mainly polyphenols) increased the receptor agonist of IL-1ra (~24%), and decreased lymphocyte expression of lymphocyte function-associated antigen-1 (~11%), lymphocyte and monocyte expression of Sialil-Lewis X (~16%) and monocyte expression of CCR2 (~31%), and tumor necrosis factor (TNF)-β (~14%) and IL-15 (~22%) plasma concentrations. No changes were observed in glucose metabolism parameters or in body weight and adiposity parameters.

Conclusion: The phenolic content of beer reduces leukocyte adhesion molecules and inflammatory biomarkers, whereas alcohol mainly improves the lipid profile and reduces some plasma inflammatory biomarkers related to atherosclerosis.

Trial registration number: ISRCTN95345245 (http://www.isrctn.org/).

Introduction

Atherosclerosis, the main cause of coronary heart disease (CHD), is considered a low-grade inflammatory disease mediated by the endothelial secretion of chemokines and adhesion molecules, such as integrins and selectins, which recruit circulating monocytes and T-cells to the endothelium and further migrate to the arterial wall triggering atherosclerotic lesions [1].

Moderate alcohol consumption is associated with a decreased cardiovascular risk and mortality independently of the type of alcoholic beverage consumed [2,3]. Nevertheless, red wine, a high polyphenolic fermented beverage, seems to confer greater cardioprotective effects than distilled beverages, which do not contain polyphenols [4], by down-regulating the expression of chemokines and adhesion molecules [5-8]. Recent meta-analyses suggest that beer, a fermented beverage with intermediate polyphenol content, could also confer greater cardioprotection than spirits [9-11], but the results of different trials are controversial, and this question is still under debate [12].

Therefore, we embarked on a randomized, crossover, controlled clinical trial to evaluate and compare the effects of moderate consumption of 30 g alcohol/d of gin, a non-polyphenolic alcoholic beverage, beer, an alcoholic beverage with a medium polyphenolic content, and the same polyphenolic amount of non-alcoholic beer, a medium polyphenolic non-alcoholic beverage, on several biomarkers related to the early stages of atherosclerosis in subjects at high risk for CHD.

Subjects and methods

Subjects

A total of 36 male moderate alcohol consumers between 55 and 75 years of age were recruited for the study in the outpatient clinic of the Internal Medicine Department of our institution. Subjects were at high risk for CVD (family history of premature CVD and/or the presence of diabetes, hypertension, dyslipidemia, and overweight/obesity). Exclusion criteria included documented CVD, human immunodeficiency virus infection, chronic liver disease, malnutrition, neoplastic or acute infectious diseases and customary use of vitamin supplements. Participants were offered free beverages but no monetary compensation. The Institutional Review Board of the hospital approved the study protocol, and all participants gave written consent.

Study design and diet monitoring

The study was an open, randomized, controlled, crossover trial with three intervention periods. Two weeks prior to the study, subjects were asked to maintain their usual diet and to refrain from any alcoholic beverage (run-in period). Baseline data were collected after this run-in period. Following this, participants were individually randomized in a crossover design among six sequences of interventions lasting 4 weeks each, in which the test beverages were provided. Randomization was based on a computer-generated random number table, resulting in six possible intervention sequences. Then, participants were instructed to consume beer (660 mL/day, containing 30 g of ethanol and 1209 mg of total polyphenols), non-alcoholic beer (990 mL/day, containing <1 g of ethanol and 1243 mg of total polyphenols) or gin (100 mL/day, containing 30 g of ethanol and no polyphenols). No washout periods were included in

the study. Therefore, the value of the previous intervention or the baseline value (run-in period) in the first intervention was considered as the starting value of each intervention.

The phenolic profile of the beer, non-alcoholic beer and gin used in the trial was determined by SPE-LC-ESI-MS/MS as previously reported [13,14]. No significant differences were observed in the phenolic content of the daily dose of beer and non-alcoholic beer, while gin contained no detectable phenolic compounds (**Supplemental Table 1**).

Throughout the study the participants were asked to maintain their usual dietary habits, physical activity level and medications, and to abstain from non-alcoholic beer or alcoholic beverages, except those provided by the investigators. Diet monitoring is explained in the **Supplemental Material**.

Clinical and laboratory measurements

After the run-in period (baseline) and the day after each intervention period, fasting blood, 24-h urine samples and anthropometric measurements were performed with standardized methods, and the blood pressure (BP) and heart rate were measured 3 times at 5-min intervals on the nondominant arm with an oscillometer (Omron 705 CP; Omron Matsusaka Co Ltd, Matsusaka City, Japan) after 15 minutes resting in a seated position. The mean of the second and the third measures was considered for statistical analysis.

Serum, EDTA-plasma, and urine samples were stored at –80°C until assayed.

Compliance with the test beers was assessed by measurement of urinary isoxanthohumol (IX), a biomarker of beer and non-alcoholic beer intake. Briefly, the last day of the run-in period and the last day of each intervention subjects were asked to

collect 24-h urine. IX was measured in 24-h urine by SPE-LC-MS/MS as previously described (14).

For the measurement of nitric oxide (NO), the release of NO_2^- and NO_3^-, the stable breakdown products of NO in thawed plasma samples, was determined by a chemiluminescence detector in a NO analyzer (Sievers Instruments, Inc., Boulder, CO). The following parameters were also determined in thawed samples of whole serum or plasma, as appropriate: blood glucose with the glucose oxidase method; cholesterol and triglycerides with enzymatic procedures; HDL cholesterol after precipitation with phosphotungstic acid and magnesium chloride; and homocysteine and vitamin B12 by an automated electrochimioluminiscence immunoassay system (Advia-Centaur, Siemens, Barcelona, Spain). ApoA-I, ApoA-II, ApoB, lipoprotein(a), insulin, adiponectin and leptin concentrations were quantified in whole serum samples by a customized Human Multi Analyte Profiling assay (Human MAP, Rules Based Medicine Inc., Austin, TX). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by multiplying fasting insulin concentrations (mIU/L) by fasting glucose concentrations (mM) and dividing by 22.5 [15].

In addition, platelet count, prothrombin time, partial thromboplastin time, and concentrations of factor VII, fibrinogen and plasminogen activator inhibitor-1 (PAI-1) were measured.

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 adhesion molecules on the surface of PBMC was analyzed via double direct

immunofluorescence with the use of commercial monoclonal antibodies following the manufacturer's instructions. The adhesion molecules 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 (CD11b/CD18) (Bender MedSystems), SLe^x (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 6.0-6.4μm (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 FACSCalibur Flow Cytometer (Becton-Dickinson, San Jose, CA) using the CellQuest software.

Quantification of soluble biomarkers of inflammation

The following serum soluble adhesion molecules and cytokines and other regulator molecules of adhesion and inflammation processes were quantified by customized Human Multi Analyte Profiling (Human MAP) (Rules Based Medicine Inc., Austin, Texas, USA) following the manufacturer's instructions: CD40 antigen (CD40a), CD40 Ligand (CD40L), C-Reactive Protein (CRP), E-Selectin, Intercellular Adhesion Molecule 1 (ICAM-1), Interleukin-1 receptor agonist (IL-1ra), IL-3, IL-5, IL-6 receptor (IL-6r), IL-10, IL-13, IL-15, Monocyte Chemotactic Protein 1 (MCP-1), Macrophage-Derived Chemokine (MDC), Monocyte interferon gamma inducing factor

(MIG), Regulated on Activation, Normal T cell Expressed and Secreted Protein (RANTES), Tumor Necrosis Factor alpha (TNF-α) Tumor Necrosis Factor beta (TNF-β) and Vascular Cell Adhesion Molecule-1 (VCAM-1).

Statistical analyses

Statistical analyses were performed using the SAS Statistical Analysis System (version 9.2, SAS Institute Inc, Cary, North Carolina). Descriptive statistics [mean ± SD or n (%)] were used to describe the baseline characteristics of the participants and the outcome variables. Variables with a skewed distribution [glucose, HOMA-IR, prothrombin time, CCR2, IL6r, and MCP-1] were transformed to their natural logarithms for analyses and are shown as antilogarithmic values to facilitate the interpretation of the results. To exclude the presence of a carryover effect for the three periods, the interaction between treatment (beer, non-alcoholic beer and gin) and period (1^{st}, 2^{nd} and 3^{rd}) was analyzed by the repeated measures analysis of covariance (ANCOVA) with the baseline values (the values of the previous intervention or the run-in period if the first intervention) as covariates. To analyze the changes within each treatment a Student's t test for paired samples was performed between the data obtained before and after each intervention. One-factor analysis of variance (ANOVA) for repeated measures and the Bonferroni post-hoc test were used to compare the differences of the changes in outcome variables between the interventions. P was considered significant when <0.05.

Results

Characteristics of study subjects and measures of compliance and dietary control

Of the 36 subjects included, three withdrew before completing the study. The reasons for withdrawal were work-related (n = 2) and need to travel (n = 1). Therefore, 33 subjects completed the study. The baseline characteristics are shown in **Table 1**. There were no individual deviations from the interventions according to the participants' dietary reports. Protocol adherence was optimum in all subjects according to their self reports. As a measure of intervention compliance, IX -a biomarker of beer consumption [13,14]- was determined in 24-h urine samples collected the last day of the run-in period and the last day of each intervention. After the consumption of beer and non-alcoholic beer, 24-h urinary excretion of IX increased to 7.2±3.3 and 7.5±2.9 μg, respectively (with no significant differences between the two values), whereas it was not detected at baseline and after the gin intervention ($P < 0.001$ between the two beer interventions and the gin and baseline interventions). According to these findings, compliance was excellent.

No significant differences were observed in energy and nutrient intake (**Supplemental Table 2**) or energy expenditure in physical activity before and after each intervention according to food records and physical activity questionnaires. No individual changes in drug intake were reported and no adverse effects were observed. No carryover effect was observed for any outcome. In addition, we compared the differences between the values obtained after each intervention period and the baseline value (after the run-in period) by repeated measures ANOVA and the results did not significantly change.

Effects on blood pressure and plasma nitric oxide concentration

As shown in **Figure 1**, systolic BP decreased a mean of 4 mm Hg after the non-alcoholic beer intervention (P=0.007), while no differences were observed after the beer and gin interventions. Diastolic BP, heart rate and plasma NO remained constant throughout the study.

Effects on anthropometric parameters, glucose metabolism, lipid profile and adipokines

Changes in anthropometric parameters, glucose metabolism, adipokines, lipid profile and other biochemical parameters are shown in **Table 2**. After the beer and gin interventions, HDL cholesterol, ApoA-I, ApoA-II and adiponectin increased by ~5, ~6%, ~7% and ~7%, respectively, and also compared to the non-alcoholic beer intervention. Apo A-I and Apo A-II also decreased by ~0.5 and ~6%, respectively, after the non-alcoholic beer intervention. Homocysteine concentration significantly decreased by ~6% and serum folic acid increased by ~9% only after the non-alcoholic beer intervention. No significant changes were observed before and after each intervention or among the three interventions for body weight, BMI, waist-to-hip ratio, and glucose metabolism parameters.

Effects on coagulation factors

Serum fibrinogen decreased by ~8% after the beer and gin interventions, compared to the non-alcoholic beer intervention (P=0.005, **Supplemental table 3**). No

differences were observed in platelet count, prothrombin and partial thromboplastin times, factor VII and PAI-1 concentrations in any of the interventions.

Expression of serum and cell adhesion molecules

As observed in **Table 3**, after the alcoholic and non-alcoholic beer interventions lymphocyte expression of LFA-1 and SLe^x decreased (~11 and 16%, respectively), as did monocyte expression of SLe^x and CCR2 (~16 and 31%, respectively). **Table 4** shows that serum concentrations of IL-1ra increased (~24%) and IL-5 decreased (~14%) after beer and gin interventions, whereas E-Selectin, IL-6r, IL-15, RANTES and TNF-β only decreased after the non-alcoholic beer intervention (~4, 3, 22, 13 and 14% respectively).

Discussion

Moderate beer intake may exert higher protection against CHD than spirits [9-11]. However, even in prospective cohort studies it is difficult to assess the type and amount of alcohol consumed by the subjects and to control important confounding factors such as diet and exercise. In fact, the issue of nutrition and physical activity can only be solved in well-designed randomized clinical trials. In the current study, we have carefully monitored food intake with a 7-d food recall questionnaire, exercise with the Minnesota Leisure Time Physical Activity Questionnaire and interventions by measurement of IX in urine. According to these results, the changes observed between interventions could be attributed to beer (constituted mainly by alcohol plus polyphenols), non-alcoholic beer (polyphenols) and gin (alcohol).

Low plasma levels of HDL cholesterol are a strong, independent risk factor for cardiovascular disease. As expected [3,16], in the current trial ethanol consumption increased plasma HDL cholesterol, ApoA-I, and ApoA-II concentrations and decreased serum fibrinogen concentrations since these changes were observed only after the beer and gin interventions, but not after the non-alcoholic beer intervention. Therefore, beer polyphenols do not affect HDL cholesterol secretion, in the line with Nicod *et al.,* who observed that red wine, cocoa and green tea polyphenols neither increased cholesterol secretion by intestinal cells nor enhanced HDL functionality [17].

On the other hand, systolic BP, homocysteine and several biomarkers of inflammation decreased only after the non-alcoholic beer intervention, and these effects should be attributed to the non-alcoholic fraction of the beer, mainly polyphenols. Thus, no synergistic effects were observed between the alcoholic and the non-alcoholic fraction of beer in any of the outcomes studied, but to the contrary, in our study, alcoholic beer did not exhibit some beneficial effects observed after non-alcoholic beer, suggesting a possible antagonistic effect between alcohol and the non-alcoholic fraction of beer.

The effects of moderate alcohol consumption on BP are controversial and may be dependent on gender and grade of endothelial dysfunction. As we observed in a previous study [18], after one month of intervention moderate alcohol consumption did not affect BP in high cardiovascular risk subjects. By contrast, the systolic BP decreased 4 mmHg after non-alcoholic beer consumption in the study population, being of major clinical significance since this decrease has been associated with a 12% and 16% reduction in CHD and stroke risks, respectively [19]. This effect may be due to the vasodilator properties of polyphenols [20]. However, this reduction is approximately two-thirds the lowering effect observed after dealcoholized red wine intake [21]. In our

study moderate alcohol intake as beer or gin did not modify HOMA-IR, as reported previously [21-23]. Neither did the non-alcoholic beer intervention have any effect on the HOMA-IR, similar to the results of Beulens *et al*. [21], who, after comparison of the effects of beer and red wine, observed that beer does not improve HOMA-IR in high cardiovascular risk patients, while red wine intake showed a protective effect on insulin resistance [24-26]. Interestingly, adiponectin concentrations diminished after beer intake, similar to the findings obtained in the same study of Beulens *et al*. [21].

To our knowledge this is the first time that the effects of moderate beer consumption and its fractions on the expression of leukocyte adhesion molecules have been evaluated. We observed that the non-alcoholic fraction of beer was responsible for decreasing the leukocyte expression of adhesion molecules. Previous studies, based on the basis of the same daily amount of alcohol, have reported the inhibitory effects of wine intake on the expression of serum inflammatory and leukocyte adhesion molecules by increasing the serum concentration of IL-10 and decreasing ICAM-1,IL-1α, IL-6, E- and P-Selectin, CD40, MCP-1, MDC and VCAM-1, and the lymphocyte expression of VLA-4, LFA-1and SLex , and monocyte expression of Mac-1, SLex, MCP-1 and CCR2 [5,7,8,27]. However, although the cardioprotective effects of moderate beer intake observed in the current study (increased serum concentrations of IL-1ra, decreased IL-5, and decreased lymphocyte expression of LFA-1 and SLe^x and monocyte expression of SLe^x and CCR2) are higher than those of gin, when we compared these results with those observed after moderate wine intake [4,5], the protective effects of moderate wine intake (especially red wine) were higher than those observed after beer intake, suggesting that the non-alcoholic fraction of beer may be less cardioprotective than that of wine [9-11].

Xanthohumol and related prenylated flavonoids, polyphenols almost exclusively present in hop-derived products such as beer, have shown an anti-inflammatory effect *in vitro* [28]. *In vivo*, we observed an anti-inflammatory effect after beer and non-alcoholic beer interventions through the decrease in serum concentrations of inflammatory biomarkers, effects that should be related to the non-alcoholic components of beer, mainly polyphenols.

This study has some limitations. No washout periods were made between the interventions, because this would have prolonged the study 6 weeks more, making it difficult to ensure compliance, and the subjects would have been more inclined to withdraw from the study. However, since previous studies have shown that changes in cellular and soluble adhesion molecules are already observed after 2 weeks of intervention [8,27] and have also shown a lack of carryover effect [5], the presence of a washout period would probably not have changed the results obtained. Another limitation is that the dealcoholization process of beer provokes a significant loss of non-alcoholic compounds such as polyphenols and other bioactive compounds as a result of the different composition of the two beers. We tried to amend this inconvenience by equalizing the amount of polyphenols in both interventions (alcoholic and non-alcoholic beer), increasing 330mL the non-alcoholic beer dose with respect to the alcoholic beer. Additionally, gin may contain some substances derived from the aging process as juniper components. Given the low bioavailability of these compounds, and that gin contains no detectable polyphenols, it is possible to assume that gin contains no interfering substances other than ethanol. Furthermore, the consumption of alcohol was not blinded, which is difficult to achieve given the distinct taste of alcoholic beverages. In addition, our study sample was composed of older men at high cardiovascular risk, thus, the results may not be extrapolated to other populations. Lastly, the duration of

this study was of 4 weeks which may not represent the potential beneficial effects of long-term moderate alcohol consumption.

In conclusion, moderate beer and non-alcoholic beer intake confers greater protective effects on the cardiovascular system than distilled beverages probably because of their polyphenolic content.

Acknowledgments

We are grateful for the collaboration of the participants. We are indebted to the Asociación de Cerveceros de España for providing the non-alcoholic and regular beers, and Gin Xoriguer for providing the gin used in this study. CIBEROBN is an initiative from the Instituto de Salud Carlos III. This work was developed at the Centre de Recerca Biomèdica Cellex, Barcelona, Spain. Supported by grants from The European Foundation for Alcohol Research (ERAB) EA 11 17, CICYT (AGL2010-22319-C03), the Spanish Ministry of Science and Innovation (MICINN), and CIBEROBN, an initiative of the Instituto de Salud Carlos III. G C-B and P Q-R thank the Manuel de Oya fellowship program. P.V-M thanks APIF fellowship program from the University of Barcelona, S. A thanks the Sara Borrell postdoctoral program (CD10/00151) supported by the Instituto de Salud Carlos III and M M-H thanks the predoctoral program of MICINN.

Literature Cited

1. Badimon L, Storey RF, Vilahur G. Update on lipids, inflammation and atherothrombosis. Thromb Haemost 2011;105 Suppl 1:S34-42. http://dx.doi.org/10.1160/THS10-11-0717.
2. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. BMJ 2011;342:d671. doi: 10.1136/bmj.d671.
3. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. BMJ 2011;342:d636. doi: 10.1136/bmj.d636.
4. Chiva-Blanch G, Arranz S, Lamuela-Raventos RM, Estruch R. Effects of wine, alcohol and polyphenols on cardiovascular disease risk factors: evidences from human studies. Alcohol Alcohol 2013;48(3):270-7. doi: 10.1093/alcalc/agt007.
5. Chiva-Blanch G, Urpi-Sarda M, Llorach R, Rotches-Ribalta M, Guillen M, Casas R, Arranz S, Valderas-Martinez P, Portoles O, Corella D, et al. Differential effects of polyphenols and alcohol of red wine on the expression of adhesion molecules and inflammatory cytokines related to atherosclerosis: a randomized clinical trial. Am J Clin Nutr 2012;95(2):326-34. doi: 10.3945/ajcn.111.022889.
6. Djurovic S, Berge KE, Birkenes B, Braaten O, Retterstol L. The effect of red wine on plasma leptin levels and vasoactive factors from adipose tissue: a randomized crossover trial. Alcohol Alcohol 2007;42(6):525-8. doi: 10.1093/alcalc/agm056.
7. Sacanella E, Vázquez-Agell M, Mena MP, Antúnez E, Fernández-Solá J, Nicolás JM, Lamuela-Raventós RM, Ros E, Estruch R. Down-regulation of adhesion molecules and other inflammatory biomarkers after moderate wine consumption in healthy women: a randomized trial. Am J Clin Nutr 2007;86(5):1463-9.
8. Vázquez-Agell M, Sacanella E, Tobias E, Monagas M, Antúnez E, Zamora-Ros R, Andrés-Lacueva C, Lamuela-Raventós RM, Fernández-Solá J, Nicolás JM, Estruch R. Inflammatory markers of atherosclerosis are decreased after moderate consumption of cava (sparkling wine) in men with low cardiovascular risk. J Nutr 2007;137(10):2279-84.
9. Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, de Gaetano G. Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: a meta-analysis. Eur J Epidemiol 2011;26(11):833-50. doi: 10.1007/s10654-011-9631-0.
10. de Gaetano G, Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB. A meta-analysis of studies on wine and beer and cardiovascular disease. Pathophysiol Haemost Thromb 2002;32(5-6):353-5. doi:10.1159/000073598.
11. Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB, De Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation. 2002 Jun 18;105(24):2836-44. doi: 10.1161/01.CIR.0000018653.19696.01.
12. Di Castelnuovo A, Costanzo S, Donati MB, Iacoviello L, de Gaetano G. Alcohol consumption and cardiovascular risk: an epidemiological perspective. Nutr Metab Cardiovasc Dis 2007;17(8):561-4. doi:10.1016/j.numecd.2007.02.006.
13. Quifer-Rada P, Martinez-Huelamo M, Jauregui O, Chiva-Blanch G, Estruch R, Lamuela-Raventos RM. Analytical condition setting a crucial step in the quantification of unstable polyphenols in acidic conditions: analyzing prenylflavanoids in biological samples by liquid chromatography-electrospray ionization triple quadruple mass spectrometry. Anal Chem 2013;85(11):5547-54. doi: 10.1021/ac4007733.

14. Quifer-Rada P, Martínez-Huélamo M, Chiva-Blanch G, Jáuregui O, Estruch R, Lamuela-Raventós RM. Urinary isoxanthohumol is a specific and accurate biomarker of beer consumption. J Nutr. 2014;144(4):484-8. doi: 10.3945/jn.113.185199.
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.
16. Romeo J, Gonzalez-Gross M, Warnberg J, Diaz LE, Marcos A. Effects of moderate beer consumption on blood lipid profile in healthy Spanish adults. Nutr Metab Cardiovasc Dis 2008;18(5):365-72. doi:10.1016/j.numecd.2007.03.007.
17. Nicod N, Chiva-Blanch G, Giordano E, Dávalos A, Parker RS, Visioli F. Green tea, cocoa, and red wine polyphenols moderately modulate intestinal inflammation and do not increase high-density lipoprotein (HDL) production. J Agric Food Chem. 2014;62(10):2228-32. doi: 10.1021/jf500348u.
18. Chiva-Blanch G, Urpi-Sarda M, Ros E, Arranz S, Valderas-Martínez P, Casas R, Sacanella E, Llorach R, Lamuela-Raventos RM, Andres-Lacueva C, Estruch R. Dealcoholized red wine decreases systolic and diastolic blood pressure and increases plasma nitric oxide: short communication. Circ Res 2012;111(8):1065-8. doi: 10.1161/CIRCRESAHA.112.275636.
19. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, Prospective Studies C. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002;360(9349):1903-13. doi:10.1016/S0140-6736(02)11911-8.
20. Khoo NK, White CR, Pozzo-Miller L, Zhou F, Constance C, Inoue T, Patel RP, Parks DA. Dietary flavonoid quercetin stimulates vasorelaxation in aortic vessels. Free Radic Biol Med 2010;49(3):339-47. doi: 10.1016/j.freeradbiomed.2010.04.022.
21. Beulens JW, de Zoete EC, Kok FJ, Schaafsma G, Hendriks HF. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: a diet intervention study. Eur J Clin Nutr 2008;62(9):1098-105. doi:10.1038/sj.ejcn.1602821.
22. Riserus U, Ingelsson E. Alcohol intake, insulin resistance, and abdominal obesity in elderly men. Obesity (Silver Spring) 2007;15(7):1766-73.
23. Zilkens RR, Burke V, Watts G, Beilin LJ, Puddey IB. The effect of alcohol intake on insulin sensitivity in men: a randomized controlled trial. Diabetes care 2003 Mar;26(3):608-12. doi: 10.2337/diacare.26.3.608.
24. Chiva-Blanch G, Urpi-Sarda M, Ros E, Valderas-Martinez P, Casas R, Arranz S, Guillén M, Lamuela-Raventós RM, Llorach R, Andres-Lacueva C, Estruch R. Effects of red wine polyphenols and alcohol on glucose metabolism and the lipid profile: a randomized clinical trial. Clin Nutr 2013;32(2):200-6. doi: 10.1016/j.clnu.2012.08.022.
25. Kim SH, Abbasi F, Lamendola C, Reaven GM. Effect of moderate alcoholic beverage consumption on insulin sensitivity in insulin-resistant, nondiabetic individuals. Metabolism 2009;58(3):387-92. doi: 10.1016/j.metabol.2008.10.013.
26. Napoli R, Cozzolino D, Guardasole V, Angelini V, Zarra E, Matarazzo M, Cittadini A, Saccà L, Torella R. Red wine consumption improves insulin resistance but not endothelial function in type 2 diabetic patients. Metabolism 2005;54(3):306-13. doi:10.1016/j.metabol.2004.09.010.
27. Estruch R, Sacanella E, Badia E, Antúnez E, Nicolás JM, Fernández-Solá J, Rotilio D, de Gaetano G, Rubin E, Urbano-Márquez A. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial. Effects of wine on inflammatory markers. Atherosclerosis 2004;175(1):117-23. doi:10.1016/j.atherosclerosis.2004.03.006.

28. Peluso MR, Miranda CL, Hobbs DJ, Proteau RR, Stevens JF. Xanthohumol and related prenylated flavonoids inhibit inflammatory cytokine production in LPS-activated THP-1 monocytes: structure-activity relationships and in silico binding to myeloid differentiation protein-2 (MD-2). Planta Med 2010;76(14):1536-43. doi: 10.1055/s-0029-1241013.

Table 1. Baseline characteristics of the 33 subjects included in the study.

	Mean ± SD[a]
Age (years)	61 ± 6
Hypertension [n (%)]	21 (64)
Dyslipemia [n (%)]	23 (70)
Type 2 Diabetes Mellitus [n (%)]	7 (21)
Current smokers [n (%)]	8 (24)
Sedentarism [n (%)]	6 (18)
Family history of premature CHD [n (%)]	2 (6)
Medications [n (%)]	
ACE Inhibitors	16 (48)
Diuretics	4 (12)
Statins	15 (45)
Fibrates	1 (3)
Oral hypoglycemic drugs	6 (18)
Aspirin or antiplatelet drugs	4 (12)
Body weight (kg)	85.6 ± 12.8
BMI (kg/m2)	28.8 ± 4.1
BMI≥ 25 kg/m2 [n (%)]	28 (85)
Abdominal circumference (cm)	101 ± 10
WHR	0.95 ± 0.05
Systolic Blood Pressure (mmHg)	138 ± 16
Diastolic Blood Pressure (mmHg)	81 ± 8
Heart rate (beats/min)	68 ± 11
Glucose (mg/dL)	112 ± 27
Triglycerides (mg/dL)	99 ± 43
Total cholesterol (mg/dL)	185 ± 31
LDL cholesterol (mg/dL)	119 ± 26
HDL cholesterol (mg/dL)	44 ± 11
LDLc/HDLc ratio	3.03±0.88
Folic acid (serum) (ng/mL)	8.7 ± 3.7
Intraerythrocytary folic acid (ng/mL)	407 ± 94
Vitamin B12 (pg/mL)	432 ± 206
Albumin (mg/mL)	43 ± 2
ASAT (UI/L)	25 ± 12
ALAT (UI/L)	29 ± 16
GGT (UI/L)	29 ± 14

[a]Mean ± SD or *n* (%), when indicated (n=33).

CHD, Coronary Heart Disease; BMI, Body Mass Index; WHR, waist-to-hip ratio; ACE, Angiotensin-Converting Enzyme; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; ASAT, Aspartate aminotransferase; ALAT, Alanine aminotransferase; GGT, Gamma glutamyl transpeptidase.

Table 2. Changes in glucose control, lipid profile and other cardiovascular risk factors after the three interventions in the 33 study subjects.

	Beer intervention			Non-alcoholic beer intervention			Gin intervention			
	Mean±SD[a]		Mean differences	Mean± SD[a]		Mean differences	Mean± SD[a]		Mean differences	
	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	*P*[c]
Antropometric Parameters										
Body weight (Kg)	85.4 ± 12.8	86.0 ± 12.1	0.62 (-0.19, 1.44)	86.0 ± 13.1	85.8 ± 13.0	-0.17 (-0.88, 0.53)	86.0 ± 13.0	85.9 ± 12.9	-0.1 (-0.79, 0.58)	0.39
BMI(kg/m^2)	28.8 ± 4.0	29.0 ± 3.8	2.06 (-0.01, 4.14)	28.9 ± 4.1	28.9 ± 4.0	0.81 (-2.58, 4.2)	29.0 ± 4.0	29.0 ± 4.0	-1.16 (-4.10, 1.78)	0.28
Waist-to-hip ratio	0.96 ± 0.05	0.96 ± 0.05	0 (-0.007, 0.006)	0.96 ± 0.05	0.95 ± 0.06	0 (-0.008, 0.001)	0.95 ± 0.05	0.96 ± 0.05	0 (-0.005, 0.009)	0.50
Glucose metabolism										
Glucose (mg/dL)	110 ± 25	112 ± 27	2.13 (-4.16, 8.41)	112 ± 28	109 ± 29	-3.47 (-9.33, 2.4)	114 ± 32	112 ± 27	-2.84 (-8.69, 3)	0.44
Insulin (µIU/mL)	3.31 ± 1.96	3.76 ± 2.49	0.44 (-0.26, 1.15)	3.6 ± 4	3.51 ± 1.99	-0.07 (-1.27, 1.13)	4.2 ± 3.63	3.98 ± 4.31	-0.22 (-1.05, 0.62)	0.63
HOMA-Insulin resistance	0.93 ± 0.68	1.13 ± 1.03	0.21 (-0.09, 0.5)	1.16 ± 2.01	0.98 ± 0.71	-0.18 (-0.76, 0.41)	1.33 ± 1.61	1.26 ± 2.09	-0.07 (-0.45, 0.32)	0.52
Lipids, lipoproteins and apolipoproteins										
Total cholesterol (mg/dL)	191 ± 32	189 ± 28	-2.19 (-8.62, 4.24)	189 ± 32	191 ± 31	2.66 (-3.15, 8.47)	187 ± 29	191 ± 30	3.66 (-2.49, 9.80)	0.43
Triglycerides (mg/dL)	100 ± 43	110 ± 50	10.41 (-1.83, 22.65)	107 ± 54	102 ± 42	-4.97 (-18.48, 8.55)	103 ± 41	107 ± 50	4.25 (-5.88, 14.38)	0.29
LDL-cholesterol (mg/dL)	123 ± 28	119 ± 27	-3.97 (-0.46, 8.39)	120 ± 25	124 ± 29	3.81 (-1.65, 9.28)	120 ± 27	123 ± 27	3.63 (-3.08, 10.33)	0.227
HDL-cholesterol (mg/dL)	44.1 ± 11.2	45.5 ± 10.8	1.44 (0.24, 3.11)*[,1]	43.7 ± 10.8	42.8 ± 10.4	-1.13 (-2.22, 0.03)[2]	43.2 ± 10.3	45.5 ± 11.2	2.22 (0.64, 3.79)*[,2]	0.009
Lipoprotein(a) (mg/dL)	547 ± 504	529 ± 440	-18.25 (-62.05, 25.56)	496 ± 442	515 ± 440	19.33 (-19.04, 57.71)	561 ± 537	514 ± 489	-46.17 (-95.39, 3.05)	0.13
Apolipoprotein A-I (mg/dL)	1.57 ± 0.5	1.79 ± 0.24	0.22 (0.02, 0.42)*[,1]	1.76 ± 0.27	1.67 ± 0.23	-0.09 (-0.16, -0.01)*[,2]	1.68 ± 0.25	1.77 ± 0.25	0.1 (0.02, 0.17)*[,1]	0.029
Apolipoprotein A-II (ng/mL)	256 ± 40	270 ± 41	14.68 (1.13, 28.23)*[,1]	274 ± 43	257 ± 44	-17.43 (-29.06, -5.80)*[,2]	255 ± 43	274 ± 52	18.71 (5.22, 32.34)*[,1]	0.004
Apolipoprotein B (mg/dL)	100 ± 20	99 ± 20	-1.06 (-5.43, 3.31)	99 ± 22	100 ± 19	0.91 (-4.13, 5.94)	98 ± 18	100 ± 19	1.53 (-2.65, 5.71)	0.76
Other cardiovascular risk factors										
Homocysteine (µmol/L)	10.0 ± 3.2	10.6 ± 2.6	0.3 (-0.11, 0.72)	10.9 ± 2.3	10.4 ± 2.1	-0.66 (-1.28, -0.05)*	10.7 ± 2.4	10.7 ± 2.5	0.11 (-0.66, 0.88)	0.11
Vitamin B_{12} (pg/mL)	404 ± 189	392 ± 243	-6.28 (-41.36, 28.81)	384 ± 221	381 ± 179	-8.17 (-49.86, 33.52)	409 ± 222	390 ± 213	-24.41 (-62.44, 13.61)	0.82
Folic acid, serum (ng/mL)	8.28 ± 3.73	8.22 ± 3.12	-0.02 (-0.97, 0.94)	8.45 ± 3.62	9.23 ± 4.21	0.77 (0.11, 1.56)*	8.00 ± 3.45	7.97 ± 3.5	-0.04 (-0.86, 0.78)	0.23
Adipokines										
Leptin (ng/mL)	9.61 ± 5.49	10.37 ± 5.97	0.86 (-0.11, 1.82)	9.04 ± 5.03	9.29 ± 5.17	0.25 (-0.72, 1.22)	10.24 ± 6.34	10.35 ± 6.29	0.11 (-0.73, 0.95)	0.47
Adiponectin (µg/mL)	3.26 ± 1.34	3.47 ± 1.47	0.21 (0.04, 0.38)*[,1]	3.28 ± 1.32	3.20 ± 1.33	-0.08 (-0.25, 0.11)[2]	3.08 ± 1.21	3.30 ± 1.31	0.22 (0.06, 0.38)*[,1]	0.017

Results expressed as [a]mean±SD (n=33) and [b]mean differences (95% CI) between after and before each intervention. Before each intervention is the value of the previous intervention or the baseline value (run-in period) in the first intervention. [c]P value of the repeated-measures ANOVA from the differences between interventions. *Significant differences (P<0.05) between after and before the intervention, measured by the Student's t test for paired samples. Values in a row with different superscript numbers are significantly different (P<0.05, Bonferroni post-hoc test). BMI, Body Mass Index; HOMA-Insulin resistance, Homeostasis Model Assessment of Insulin resistance; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein.

Table 3. Changes in the expression of adhesion molecules on the surface of T lymphocytes and monocytes after the three interventions in the 33 study subjects.

	Beer intervention			Non-alcoholic beer intervention			Gin intervention			
	Mean±SD[a]		Mean differences	Mean± SD[a]		Mean differences	Mean± SD[a]		Mean differences	
	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	*P*[c]
T lymphocytes										
LFA-1 (MFI[d])	135.69 ± 41.34	120.56 ± 38.29	-17.29 (-30.11, -4.47)*,1	140.72 ± 43.96	127.39 ± 43.98	-16.17 (-25.43, -6.91)*,1	136.42 ± 47.07	134.11 ± 45.50	-1.42 (-8.40, 5.56)2	0.031
Mac-1 (MFI)	76.14 ± 34.36	74.17 ± 45.28	-3.81 (-22.4, 14.78)	81.88 ± 35.21	74.63 ± 39.44	-11.54 (-26.74, 3.66)	83.66 ± 43.82	79.64 ± 47.93	-0.13 (-13.7, 13.44)	0.637
VLA-4 (MFI)	44.78 ± 13.56	44.62 ± 13.79	0.03 (-3.96, 4.01)	45.4 ± 11.6	44.36 ± 12.62	-1.28 (-3.94, 1.37)	45.39 ± 12.51	44.68 ± 13.65	0.13 (-2.27, 2.52)	0.807
SLe[x] (MFI)	135.58 ± 82.62	109.21 ± 89.84	-28.68 (-54.972, -2.4)*	150.56 ± 104.13	123.72 ± 95.07	-23.59 (-52.367, -5.19)*	139.51 ± 85.81	121.37 ± 102.5	-17.59 (-39.623, 4.44)	0.865
CD40 (MFI)	95.57 ± 73.67	86.78 ± 86.62	-6.92 (-32.77, 18.93)	96.91 ± 80.15	90.71 ± 83.17	-2.82 (-28.33, 22.7)	108.51 ± 91.2	91.92 ± 84.38	-6.5 (-29.84, 16.85)	0.975
Monocytes										
LFA-1 (MFI)	70.89 ± 34.65	73.49 ± 21.25	0.7 (-8.52, 9.91)	77.14 ± 28.2	74.86 ± 23.17	-5.81 (-17.03, 5.41)	80.95 ± 24.4	75.97 ± 25.51	-4.98 (-12.47, 2.51)	0.638
Mac-1 (MFI)	40.46 ± 11.1	40.3 ± 13.99	-1.04 (-4.58, 2.49)	40.14 ± 10.09	40.27 ± 10.13	1.7 (-1.99, 5.39)	40.46 ± 10.17	40.06 ± 11.27	0.3 (-3.37, 3.97)	0.646
VLA-4 (MFI)	31.91 ± 9.82	32.4 ± 9.76	-0.19 (-2.79, 2.41)	31.29 ± 8.31	31.13 ± 10.22	0.2 (-2.15, 2.54)	33.43 ± 9.03	31.35 ± 10.03	-2.12 (-4.89, 0.65)	0.493
SLe[x] (MFI)	55.05 ± 23.16	51.19 ± 21.6	-4.15 (-9.495, -0.58)*	54.58 ± 21.92	50.45 ± 22.96	-3.53 (-8.063, -0.81)*	55.63 ± 19.67	51.61 ± 22.98	-3.79 (-9.085, 1.5)	0.989
CD40 (MFI)	33.45 ± 14.55	30.57 ± 15.14	-3.56 (-8.39, 1.27)	32.86 ± 16.3	30.09 ± 14.93	-2.90 (-9.38, 3.59)	33.14 ± 14.14	31.67 ± 16.01	0.49 (-3.58, 4.56)	0.583
CD36 (MFI)	860.05 ± 614.6	806.2 ± 572.6	-54.73 (-238.35, 128.88)	915.57 ± 641.43	784.79 ± 604.17	-130.77 (-272.10, 10.55)	897.34 ± 642.9	787.60 ± 639.61	-109.74 (-260.99, 41.51)	0.733
CCR2 (MFI)	94.96 ± 82.70	68.36 ± 44.49	-26.59(-54.62, -1.43)*,1	110.40 ± 82.54	70.61 ± 52.06	-39.79 (-60.30, -19.28)*,1	80.13 ± 58.04	97.71 ± 67.60	11.57 (-6.42, 29.56)2	0.011

Results expressed as [a]mean±SD (n=33) and [b]mean differences (95% CI) between after and before each intervention. Before each intervention is the value of the previous intervention or the baseline value (run-in period) in the first intervention. [c]P value of the repeated-measures ANOVA from the differences between interventions. [d]Mean Fluorescence Intensity (arbitrary units). *Significant differences (P<0.05) between after and before the intervention, measured by the Student's t test for paired samples. Values in a row with different superscript numbers are significantly different (P<0.05, Bonferroni post-hoc test). LFA-1, Lymphocyte Function-Associated Antigen-1; Mac-1, Macrophage-1 antigen; VLA-4, Very Late Antigen-4; SLe[x], Sialil-Lewis X.

Table 4. Changes in circulating inflammatory biomarkers after the three interventions in the 33 study subjects.

	Beer intervention			Non-alcoholic beer intervention			Gin intervention			
	Mean±SD[a]		Mean differences	Mean± SD[a]		Mean differences	Mean± SD[a]		Mean differences	
	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	*P*[c]
CD40a (ng/mL)	0.77 ± 0.14	0.81 ± 0.16	0.04 (-0.01, 0.1)	0.82 ± 0.19	0.82 ± 0.2	0.01 (-0.05, 0.07)	0.78 ± 0.18	0.81 ± 0.16	0.03 (-0.02, 0.08)	0.738
CD40L (ng/mL)	1.43 ± 1	1.69 ± 1.24	0.26 (-0.16, 0.68)	1.83 ± 1.4	1.89 ± 1.27	0.11 (-0.20, 0.42)	1.43 ± 1.08	1.7 ± 1.05	0.27 (-0.05, 0.6)	0.780
CRP (μg/mL)	2.93 ± 2.48	2.52 ± 2.62	-0.65 (-1.76, 0.46)	2.58 ± 2.52	2.53 ± 1.63	-0.01 (-0.77, 0.75)	3.34 ± 3.22	2.85 ± 2.99	0.17 (-0.61, 0.94)	0.496
E-Selectin (ng/mL)	7.59 ± 3.82	7.84 ± 3.72	0.24 (-0.05, 0.54)[1,2]	7.64 ± 3.59	7.3 ± 3.67	-0.33 (-0.71, 0.04)[2]	7.00 ± 3.24	7.36 ± 3.39	0.36 (0.01, 0.71)*[,1]	0.005
ICAM-1 (ng/mL)	127 ± 29	134 ± 36	7.11 (-2.37, 16.59)	134 ± 41	131 ± 29	-0.55 (-10.43, 9.32)	132 ± 33	131 ± 37	-1.68 (-8.23, 4.88)	0.399
IL-1ra (pg/mL)	75.1 ± 35.3	93.1 ± 38.4	17.97 (1.53, 34.4)*[,1]	90.7 ± 54.3	95.1 ± 47.9	5.89 (-18.31, 30.09)[1,2]	90.9 ± 46.1	81.4 ± 33.4	-10.61 (-26.31, 5.09)[2]	0.050
IL-3 (ng/mL)	0.09 ± 0.05	0.08 ± 0.04	-0.01 (-0.02, 0.01)	0.09 ± 0.05	0.09 ± 0.05	0.01 (-0.01, 0.02)	0.09 ± 0.04	0.08 ± 0.04	-0.01 (-0.02, 0)	0.338
IL-5 (pg/mL)	27.9 ± 16.5	23.8 ± 11.3	-4.02 (-7.56, -0.46)*[,1]	25.2 ± 15.6	27.2 ± 14.8	2.06 (-1.22, 5.34)[2]	24.6 ± 10.6	21.6 ± 12.6	-2.94 (0.73, 6.61)*[,1]	0.043
IL-6r (ng/mL)	30.3 ± 7.9	30.4 ± 8.7	0.12 (-1.15, 1.38)	30.5 ± 8.6	29.6 ± 7.9	-0.9 (-1.86, -0.06)*	29.9 ± 8.4	30.1 ± 8.3	0.18 (-0.80, 1.15)	0.387
IL-10 (pg/mL)	4.11 ± 1.42	4.32 ± 1.36	0.21 (-0.51, 0.93)	4.59 ± 1.25	4.2 ± 1.68	-0.38 (-1.12, 0.36)	4.21 ± 1.63	4.59 ± 1.97	0.38 (-0.30, 1.06)	0.521
IL-13 (pg/mL)	38.4 ± 17.0	36.6 ± 12.9	-1.89 (-5.85, 2.08)	37.0 ± 16.5	38.6 ± 16.5	1.56 (-1.75, 4.88)	37.1 ± 12.5	36.8 ± 16.1	-0.3 (-3.69, 3.09)	0.477
IL-15 (ng/mL)	0.24 ± 0.14	0.22 ± 0.11	-0.03 (-0.08, 0.03)[1,2]	0.23 ± 0.1	0.19 ± 0.08	-0.05 (-0.09, -0.01)*[,1]	0.21 ± 0.13	0.29 ± 0.14	0.07 (0.01, 0.13)*[,2]	0.043
MCP-1 (pg/mL)	487 ± 345	495 ± 360	7.97 (-27.42, 43.35)	493 ± 353	504 ± 345	12.34 (-21.95, 46.64)	508 ± 378	506 ± 377	-2.09 (-48.33, 44.15)	0.894
MDC (pg/mL)	507 ± 111	497 ± 106	-9.41 (-43.73, 24.92)	505 ± 116	511 ± 133	7.66 (-25.08, 40.39)	519 ± 112	511 ± 91	-7.88 (-38.56, 22.81)	0.774
MIG (pg/mL)	1265 ± 637	1445 ± 1190	183.5 (-173.0, 540.0)	1251 ± 536	1670 ± 839	196.6 (-60.6, 453.7)	1250 ± 629	1280 ± 534	30.5 (-146.0, 207.0)	0.518
RANTES (ng/mL)	23.8 ± 6.7	23.4 ± 7.6	-0.38 (-2.01, 1.24)	24.1 ± 7.1	21.1 ± 7.5	-3.20 (-5.66, -0.74)*	23.2 ± 6.0	22.7 ± 7.3	-0.51 (-3.13, 2.12)	0.212
TNF-α (pg/mL)	22.0 ± 11.1	23.6 ± 13.5	1.56 (-1.44, 4.57)	23.9 ± 15.4	24.0 ± 13.8	0.21 (-3.04, 3.47)	22.6 ± 12.9	22.4 ± 12.0	-0.2 (-3.49, 3.09)	0.764
TNF-β (pg/mL)	13.9 ± 7.5	11.1 ± 5.7	-3.2 (-7.31, 0.92)[1]	11.0 ± 8.7	11.1 ± 5.7	-1.61 (-5.11, 1.89)[1]	10.1 ± 6.4	13.7 ± 6.8	3.63 (0.61, 6.65)*[,2]	0.032
VCAM-1 (ng/mL)	461 ± 93	464 ± 96	2.38 (-10.63, 15.38)	451 ± 95	452 ± 91	0.93 (-13.04, 14.91)	466 ± 98	456 ± 107	-10.5 (-28.79, 7.79)	0.349

Results expressed as [a]mean±SD (n=33) and [b]mean differences (95% CI) between after and before each intervention. Before each intervention is the value of the previous intervention or the baseline value (run-in period) in the first intervention. [c]P value of the repeated-measures ANOVA from the differences between interventions. *Significant differences (P<0.05) between after and before the intervention, measured by the Student's t test for paired samples. Values in a row with different superscript numbers are significantly different (P<0.05, Bonferroni post-hoc

test). CD40a, CD40 antigen; CD40L, CD40 Ligand; CRP, C-Reactive Protein; ICAM-1, Intercellular Adhesion Molecule 1; IL, Interleukin; IL-1ra, Interleukin -1 receptor agonist; IL-6r, Interleukin -6 receptor; MCP, Monocyte Chemotactic Protein; MDC, Macrophage-Derived Chemokine; MIG, Monokine Induced by Gamma Interferon; RANES, Regulated on Activation, Normal T Cell Expressed and Secreted; TNF, Tumor Necrosis Factor alpha; VCAM-1, Vascular Cell Adhesion Molecule-1.

Figure 1. Changes in systolic and diastolic blood pressure in the 33 subjects studied after the three interventions.

*P= 0.007 (t test between before and after the intervention).

Diet monitoring

Natural foods rich in antioxidants, especially fruit and vegetables, were carefully monitored in order to achieve similar dietary antioxidant content during the three interventions. At the beginning of the study and after each intervention period, a medical record, a 7-d food record questionnaire (5 week-days and 2 week-end days) and the Minnesota Leisure Time Physical Activity Questionnaire were administered. The food records were used to assess nutrient intake and to monitor adherence to the study protocol. Foods were converted into nutrients using the Food Processor Nutrition and Fitness Software (*esha* Research, Salem, OR) adapted to local foods.

Supplemental Table 1. Phenolic composition of the beers used in the study.

	Beer mean ± sd (µg/L)	Non-alcoholic beer mean ± sd (µg/L)	Beer Daily intake (µg)	Non-alcoholic beer Daily intake (µg)	P[a]
Phenolic compounds[b]					
4hydroxybenzoic acid	79 ± 1	44 ± 1	52 ± 1	44 ± 1	0.149
Caffeic acid	72 ± 1	62 ± 1	47 ± 1	61 ± 1	0.401
Catechin	157 ± 2	165 ± 3	104 ± 1	163 ± 3	0.134
Chlorogenic acid	11.0 ± 0.2	2.3 ± 0	7.3 ± 0.1	2.3 ± 0	0.110
Epicatechin	21.2 ± 0.2	21.5 ± 0.2	14 ± 0.2	21.3 ± 0.2	0.456
Ferulic acid	555 ± 6	403 ± 5	366 ± 4	399 ± 5	0.504
Kaempferol-*O*-glucoside	10.3 ± 0.2	8.2 ± 0.1	6.8 ± 0.2	8.1 ± 0.1	0.403
p-Coumaric acid	235 ± 4	154 ± 2	155 ± 3	152 ± 2	0.871
Protocatechuic acid	37.4 ± 0.7	22.6 ± 0.3	24.7 ± 0.4	22.4 ± 0.3	0.282
Quercetin	16.6 ± 0.2	14.2 ± 0.3	10.9 ± 0.2	14.1 ± 0.3	0.106
Quercetin-3-*O*-glucoside	62.4 ± 1.1	36.4 ± 0.4	41.2 ± 0.7	36.1 ± 0.4	0.582
Rutin	16.8 ± 0.2	11.9 ± 0.3	11.1 ± 0.1	11.8 ± 0.3	0.683
Sinapic acid	129 ± 1	91 ± 1	85 ± 1	90 ± 1	0.919
Vanillic acid	28.4 ± 0.5	12 ± 0.3	18.7 ± 0.3	11.9 ± 0.3	0.339
Isoxanthohumol	552 ± 49	186 ± 8	365 ± 32	184 ± 8	0.674
8-Prenylnaringenin	32.6 ± 1.4	19.0 ± 1.2	21.5 ± 0.9	18.8 ± 1.2	0.331
Total (sum)	2016 ± 131	1253 ± 158	1330 ± 190	1240 ± 157	0.826

[a]Comparison between daily intake of beer and non-alcoholic beer polyphenols (Student's t test for independent samples).
[b]Results are expressed as mean ± SD (n=3).

Supplemental Table 2. Daily energy and dietary intakes in the 33 subjects studied before and after the three interventions*.

	Beer intervention			Non-alcoholic beer intervention			Gin intervention			
	Mean±SD[a]		Mean differences	Mean± SD[a]		Mean differences	Mean± SD[a]		Mean differences	
	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	*P*[c]
Energy (kcal/day)	1705 ± 420	1700 ± 364	7.97 (-87.90, 103.84)	1747 ± 312	1715 ± 404	-27.95 (-177.80, 121.90)	1772 ± 399	1714 ± 339	-54.41 (-222.81, 13.99)	0.488
Total protein (g/day)	92.4 ± 19.1	90.8 ± 21.0	-0.95 (-9.49, 7.60)	95.4 ± 28.4	98.6 ± 20.6	2.96 (-5.79, 11.71)	96.5 ± 20.6	92.7 ± 20.1	-3.39 (-10.38, 3.59)	0.589
Carbohydrates (g/day)	213 ± 36	202 ± 49	-8.11 (-25.28, 9.05)	210 ± 45	223 ± 49	8.99 (-5.95, 23.93)	200 ± 54	197 ± 40.0	-0.75 (-16.82, 15.31)	0.404
Dietary fiber (g/day)	17.7 ± 6.8	19.8 ± 7.1	2.35 (-0.46, 5.178)	19.4 ± 6.4	19.5 ± 8.3	-1.09 (-4.04, 1.87)	18.5 ± 7.5	18.2 ± 5.9	-0.61 (-3.57, 2.34)	0.289
Sugars (g/day)	71.4 ± 22.6	72.6 ± 30.2	1.26 (-7.36, 9.89)	77.0 ± 27.9	76.0 ± 33.3	-1.01 (-7.62, 2.61)	71.8 ± 30.6	69.4 ± 21.1	-2.38 (-11.39, 6.62)	0.543
Total lipids (g/day)	77.3 ± 26.0	70.0 ± 29.8	-4.20 (-13.82, 5.41)	74.6 ± 27.4	76.3 ± 19.2	2.74 (-5.33, 10.81)	79.6 ± 24.9	75.7 ± 20.0	-3.85 (-11.50, 3.80)	0.490
SFA (g/day)	22.8 ± 6.9	21.8 ± 11.3	-0.48 (-4.40, 3.43)	23.1 ± 9.4	23.9 ± 9.6	1.18 (-2.49, 4.85)	23.5 ± 11.9	22.1 ± 7.1	-1.68 (-6.00, 2.64)	0.671
MUFA (g/day)	37.6 ± 11.9	36.0 ± 15.9	-1.24 (-6.04, 3.56)	38.3 ± 14.0	36.7 ± 10.1	-1.44 (-7.16, 4.27)	38.1 ± 10.9	36.5 ± 10.01	-2.12 (-5.52, 1.27)	0.969
PUFA (g/day)	11.5 ± 5.1	10.6 ± 3.4	-1.17 (-2.71, 1.05)	10.3 ± 4.7	10.8 ± 4.8	0.58 (-1.59, 2.44)	11.9 ± 4.6	10.6 ± 4.0	-0.71 (-2.13, 0.71)	0.335
Cholesterol (mg/day)	337 ± 119	327 ± 8	-9.50 (-70.09, 59.08)	317 ± 150	350 ± 111	34.49 (-30.00, 98.98)	323 ± 111	313 ± 126	-10.04 (-74.93, 54.85)	0.646
Vitamin C (mg/day)	117 ± 71	100 ± 63	-16.83 (-49.72, 16.07)	114 ± 58	116 ± 56	1.56 (-22.18, 23.29)	113 ± 55	127 ± 71	14.10 (-14.16, 42.36)	0.401
Vitamin A (µgRE/day)[d]	623 ± 425	681 ± 544	49.92 (-132.25, 231.09)	701 ± 556	581 ± 371	-98.27 (-310.41, 114.88)	647 ± 336	762 ± 544	96.39 (-12.86, 205.64)	0.314
Vitamin E (µg/day)	9.6 ± 3.6	9.8 ± 5.2	0.32 (-1.70, 2.34)	9.7 ± 3.1	10.1 ± 4.2	0.41 (-1.06, 1.88)	10.2 ± 4.0	9.6 ± 3.5	-0.76 (-2.05, 0.54)	0.574
Folic acid (µg/day)	419 ± 178	448 ± 210	31.79 (-28.21, 91.78)	448 ± 182	470 ± 273	21.34 (-19.16, 94.59)	413 ± 176	394 ± 158	-21.04 (-56.70, 14.61)	0.209
Total polyphenols (mg/day)	274 ± 127	269 ± 119	-5.21 (-37.10, 26.69)	270 ± 100	252 ± 102	-12.27 (-36.53, 11.99)	265 ± 87	268 ± 114	1.50 (-38.04, 41.04)	0.851

* Excluding the energy, nutrient and total polyphenol contributions from the tested beverages. Results are expressed as [a]mean ± SD (n=33) and [b]mean differences (95% CI) between before and after each intervention. Before each intervention is the value of the previous intervention or the baseline value in the first intervention. [c]P value of the repeated-measures ANOVA from the differences between interventions. No changes were observed between before and after each intervention, measured by a Student's t test for paired samples. [d]Retinol Equivalents.

Supplemental Table 3. Changes in coagulation factors after the three interventions in the 33 study subjects.

	Beer intervention			Non-alcoholic beer intervention			Gin intervention			
	Mean±SD[a]		Mean differences	Mean± SD[a]		Mean differences	Mean± SD[a]		Mean differences	
	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	Before	After	(95% CI)[b]	*P*[c]
Platelets (x10^9/L)	243 ± 49	240 ± 41	-3 (-16, 11)	240 ± 41	239 ± 37	0 (-8, 8)	247 ± 45	238 ± 49	-9 (-23, 5)	0.64
Prothrombin time (%)	95 ± 6	96 ± 5	1 (0, 2)	95 ± 6	95 ± 7	0 (-2, 2)	96 ± 6	95 ± 6	-1 (-3, 1)	0.33
Prothrombin time (sec)	13 ± 1	13 ± 1	0 (-0.1, 0.1)	13 ± 1	13 ± 1	0 (-0.2, 0.2)	13 ± 1	13 ± 1	0.1 (-0.1, 0.3)	0.44
Prothrombin time (ratio)	1.00 ± 0.06	1.00 ± 0.05	0.00 (-0.011, 0.01)	1.03 ± 0.15	1.37 ± 2.14	0.36 (-0.428, 1.14)	1.00 ± 0.06	1.06 ± 0.22	0.06 (-0.018, 0.13)	0.49
Partial thromboplastin time (sec)	29 ± 1	29 ± 2	-0.1 (-0.4, 0.2)	29 ± 1	29 ± 2	0.2 (-0.3, 0.7)	29 ± 2	28 ± 2	-0.4 (-0.9, 0.1)	0.18
Factor VII (ng/mL)	501 ± 180	534 ± 211	32.59 (-6.07, 71.25)	532 ± 209	513 ± 177	-18.47 (-53.447, 16.51)	505 ± 196	515 ± 215	9.81 (-25.08, 44.71)	0.21
Fibrinogen (g/L)	3.76 ± 0.59	3.46 ± 0.59	-0.28 (-0.455, -0.11)*[,1]	3.47 ± 0.47	3.57 ± 0.5	0.12 (-0.022, 0.26)[2]	3.79 ± 0.63	3.51 ± 0.77	-0.32 (-0.56, -0.08)*[,1]	0.005
PAI-1 (ng/mL)	173 ± 37	175 ± 35	2.41 (-8.995, 13.81)	175 ± 36	178 ± 42	2.18 (-7.993, 12.34)	172 ± 37	177 ± 36	5.42 (-5.412, 16.25)	0.92

Results expressed as [a]mean±SD (n=33) and [b]mean differences (95% CI) between after and before each intervention. Before each intervention is the value of the previous intervention or the baseline value (run-in period) in the first intervention. [c]P value of the repeated-measures ANOVA from the differences between interventions. *Significant differences (P<0.05) between after and before the intervention, measured by the Student's t test for paired samples. Values in a row with different superscript numbers are significantly different (P<0.05, Bonferroni post-hoc test).