Accepted Manuscript

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Esther Nova, Ismael San Mauro-Martín, Ligia E. Díaz-Prieto, Ascensión Marcos

PII: S0271-5317(18)30772-3
DOI: https://doi.org/10.1016/j.nutres.2018.12.007
Reference: NTR 7974

To appear in: *Nutrition Research*

Received date: 6 July 2018
Revised date: 24 October 2018
Accepted date: 13 December 2018

Please cite this article as: Esther Nova, Ismael San Mauro-Martín, Ligia E. Díaz-Prieto, Ascensión Marcos , Wine and beer within a moderate alcohol intake is associated with higher levels of HDL-c and adiponectin. Ntr (2018), https://doi.org/10.1016/j.nutres.2018.12.007

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Esther Nova, Ismael San Mauro-Martín, Ligia E. Díaz-Prieto, Ascensión Marcos.

Immunonutrition Research Group, Department of Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN) – CSIC. C/ Jose Antonio Novais, 10. 28040 Madrid. Spain.

Corresponding Author: Esther Nova, PhD. Mailing address: Institute of Food Science, Technology and Nutrition (ICTAN)-CSIC. C/ Jose Antonio Novais, 10. 28040 Madrid; Spain. Telephone number: +34 915492300 ext: 231209; e-mail: enova@ictan.csic.es

Ismael San Mauro-Martín (ismasmm@gmail.com), Ligia E. Díaz-Prieto (ldiaz@ictan.csic.es), Ascensión Marcos (amarcos@ictan.csic.es).

List of abbreviations

CVD: cardiovascular disease

DM: diabetes mellitus

HDL-c: high-density lipoprotein cholesterol

LDL-c: low-density lipoprotein cholesterol

MEDAS: Mediterranean diet adherence screener

MPV: mean platelet volume

sICAM-1: soluble intercellular adhesion molecule-1

sVCAM-1: soluble vascular cell adhesion molecule-1

Abstract

The scientific evidence available on the association between moderate alcohol intake and levels of blood cardiometabolic markers are still inconsistent and difficult to interpret for future disease prevention. However, we hypothesize that moderate consumption of alcohol is associated with lower levels of inflammation markers and higher levels of protective cardiometabolic markers. Thus, this work aimed to examine the associations of moderate alcohol intake and the type of alcoholic beverage, with metabolic and inflammatory biomarkers. An observational, cross-sectional study including 143 apparently healthy adults 55 years of age and older was performed. Interviewer-administered questionnaires were used to collect information on alcoholic beverage intake frequency, food frequency, physical activity, socioeconomic status, diseases and medications, and other health related habits. Three groups were established prior to recruitment: 1) abstainers and occasional consumers (ABS, N=54); 2) beer consumers (BEER ≥ 80% of total alcohol intake; N=40), and 3) mixed beverage consumers (MIXED; N=49). Univariate ANOVA models, adjusted for confounding factors and co-variables, were performed. HDL-c and P-selectin were significantly higher in the MIXED group than in the ABS group, and adiponectin was higher in the MIXED group compared to the BEER group. All alcohol consumers also had higher mean platelet volume (MPV) values compared to ABS. In linear regression analyses, HDL-c, P-selectin, and adiponectin were positively associated with wine intake (g/day) ($P<0.001$, $P=0.014$ and $P=0.017$, respectively) and MPV with beer intake ($P=0.017$). In conclusion, this cross-sectional study showed that moderate alcohol intake is associated with higher levels of HDL-c and adiponectin compared to those in abstainers, which is mainly explained by wine intake.

Keywords: alcohol, moderate drinkers, serum biomarkers, platelets, fermented beverages

1. Introduction

Observational, epidemiological studies have shown an advantage of moderate alcohol consumption compared to abstinence with regards to all-cause mortality [1], cardiovascular disease (CVD) [2], and diabetes risks [3]. One meta-analysis of interventional studies has also shown beneficial effects on several biomarkers that are intermediate end-points of those chronic diseases, including HDL cholesterol (HDL-c), fibrinogen, and adiponectin [4]. These and other mechanisms, such as reduced blood pressure, endothelial activation, coronary blood flow, platelet aggregation, and others, have been suggested to play a role on health outcomes related to moderate alcohol consumption [4-6]. However, controversies exist which could be explained by the influence of confounding factors (i.e: "sick quitters" considered as abstainers or differences in other lifestyle factors or health conditions) [5]. Moreover, the most recent combined analyses of multiple studies' data support the notion that, except for myocardial infarction, the lower the alcohol consumption, the better for any other measure of disease risk [7].

Inflammation is implicated in the etiology of CVD, diabetes, and other age-related diseases. The inflammatory response of the arterial wall involves the activation of endothelial cells and platelets and increased expression of adhesion molecules [8,9]. Among these, intercellular adhesion molecule-1 (ICAM-1) and E-selectin are elevated in alcohol dependence syndrome [10]; however, lower levels of adhesion molecules have been described in observational studies when comparing moderate alcohol consumption with abstinence [11,12]. Very few published studies report outcomes of interventions with alcohol on adhesion molecules, and the results that are controversial

[13-16]. Similarly, the effect of moderate alcohol consumption on insulin sensitivity is not completely understood [17-20]; however, adiponectin has been identified as a possible mediator of this effect [18].

Another issue of debate is the relative contribution of alcohol to the beneficial changes in biomarkers compared to other bioactive compounds in alcoholic fermented drinks (e.g. the varying polyphenols in beer and wine) [21,22]. Nevertheless, a recent meta-analysis has shown that associations of baseline alcohol consumption with all-cause mortality are stronger in predominantly beer or spirit drinkers than in predominantly wine drinkers [7]. However, the former had higher baseline levels of smoking habits and other indicators of lower socioeconomic status (SES), suggesting the potential for confounding effects.

On the basis of the scientific evidence available so far, we hypothesized that consumption of fermented drinks in moderate amounts is associated, independently of other lifestyle factors, with lower levels of inflammation markers and higher levels of protective cardiometabolic markers. Thus, in the present work, we aimed to find out the associations of moderate alcohol intake with metabolic and inflammatory biomarkers, independently of other individual and health related factors. Then, we compared these biomarker levels in abstainers and the two most frequent drinking patterns in Spanish people who consumed alcohol in moderate amounts [23]: (1) predominantly beer consumers and (2) mixture consumers including wine, beer, and miscellaneous. Finally, we studied the contribution of different types of alcoholic drinks to the biomarker levels.

2. Methods and materials

2.1 Subjects

An observational cross-sectional study was carried out between April 2012 and June 2013, which included 240 Spanish subjects who were screened for the following inclusion criteria: 55-85 years of age, independent and free-living, BMI <30 kg/m^2, and moderate alcohol consumption. We assumed moderate alcohol intake as consumption < 25 g alcohol/day for women and < 40 g alcohol/day for men. This is consistent with an exclusion threshold for heavy drinking [23] and other published studies on moderate alcohol intake [24]. Exclusion criteria were the following: 1) any history of alcohol abuse, 2) higher than moderate alcohol intake, and/or 3) suffering any of the following pathologies: type I diabetes mellitus (DM), cancer, renal failure, liver disease, serious respiratory diseases (i.e., chronic obstructive pulmonary disease and respiratory support), and neurological diseases (i.e., Alzheimer's, Parkinson's, fibromyalgia, or multiple sclerosis). Subjects were recruited through advertisements in sociocultural centers and retired persons associations. All of the subjects provided written informed consent before entering the study. Ethical approval was obtained from the Ethics committee of the Puerta de Hierro University Hospital (Madrid, Spain) (ref. number: 283-2012), and the protocols used comply with the Spanish law on Biomedicine Research 14/2007, July 3rd. Sample size calculation was performed to demonstrate a 12% difference in HDL-cholesterol in subjects consuming alcohol compared to abstinence, with a power of 80% and a significance level of 0.05. Based on previous published work [25], a standard deviation of 10 mg/dL was assumed, and the resulting sample size was 50 subjects per group. In a blood test for biomarkers' analyses, 153

subjects were randomly selected to participate. The final number of subjects studied was 143, after exclusion of those who had suffered from a previous cardiovascular event (N=4) and those who had quit alcohol intake for health reasons (N=6) (Figure 1).

All participants presented themselves at the Institute of Food Science, Technology and Nutrition facilities twice, one for questionnaires completion and the second one for anthropometrical assessment and blood analysis. Height was measured using a stadiometer Seca 222 and weight with an electronic scale (Omron HBF-500 BIA device; precision of ± 1%). Interviewer-assisted self-estimation of the total capital owned was used for SES classification as follows: 1) "low-intermediate": between 10,000 to 200,000€, 2) "intermediate-high": between 200,000 to 600,000 € and 3) "high": above 600,000 €.

2.2 Alcohol intake assessment

Participants were interviewed by a trained nutritionist who administered an ad-hoc frequency recall questionnaire on alcoholic beverage consumption based on the SUN Study Questionnaire [26], which was based on the validated PREDIMED food frequency questionnaire [27]. The correlation coefficients for alcoholic drinks food group assessed by the self-administered FFQ and four 3-day dietary records was good (r = 0.70). Moreover, in the current study, the alcoholic drinks questionnaire was interviewer-administered, which improved the validity of the method. The questionnaire recorded the intake of wine, beer, champagne, cider, liquors, spirits, and all the mixtures by estimations over the last year. Reference drink sizes were considered. Frequency of intake was registered using a continuous scale as follows: never or almost never (0 to

once every two months); 1 to 3 times per month; 1, 2-4, 5-6 times per week or number of times per day. Habitual intake was recorded for working days and separately for weekends. Total alcohol intake (TAI) (grams/day) was calculated using average grams of alcohol content per 100 mL of each alcoholic beverage.

Subjects were classified into three groups: 1) abstainers and occasional consumers (< 4 alcohol drinks per month) (ABS); 2) predominantly beer consumers (BEER), (beer ≥ 80% TAI); and 3) mixed beverage consumers (MIXED), including wine, beer, and liquor. A recruitment table was designed in order to obtain a balanced distribution of subjects (N) in terms of age (55-64; 65-74; ≥75) and sex across the study groups. Upon review of subjects all that consumed alcoholic drinks were merged and formed the moderate alcohol consumption group (MOD).

2.3 Blood analysis

Blood was drawn in the early morning from the cubital vein after at least a 10-hour fast. After centrifugation at 1,200 *g* for 15 min, plasma and serum were collected in several aliquots and stored at -80ºC until analysis.

Blood cell count analysis, including platelet and mean platelet volume (MPV) determinations, was performed in an automated cell counter (ADVIA-2120, Siemens, Madrid). Glucose, lipid profile, iron, transferrin, ferritin, and high sensitivity C-reactive protein (hsCRP) were analyzed in serum by standard techniques in an accredited medical diagnostic laboratory (Unilabs, Madrid, Spain; Accredited Quality ISO 15189).

Plasma from the EDTA-tube was used for quantification of soluble vascular cell adhesion molecule (sVCAM)-1, sICAM-1, sP-selectin, sE-selectin, interleukin (IL)-1β, IL-6, IL-8, IL-10, leptin, and adiponectin. These determinations were performed by multiple analyte assays, using two commercial kits and the xMAP technology from Luminex® Corporation. sICAM-1, sVCAM-1, and sP-selectin were measured with the Human CVD 2 kit (Cat. # HCVD2-67K, Merck-Millipore), and cytokines were measured with the High Sensitivity Human Cytokine Magnetic Bead Kit (Cat. # HSCYTMAG-60SK, Merck-Millipore). Minimum detectable concentration (Min DC) was 0.01 pg/mL for IL-1β, 0.64 pg/mL for IL-6, 0.09 pg/mL for IL-8, and 0.65 pg/mL for IL-10. Low and high concentration quality controls were used in each of these assays.

Leptin and adiponectin were measured by ELISA using commercial kits (EMD Millipore, Missouri, USA), and sE-selectin was also measured by a commercial ELISA kit (Diaclone SAS, Besançon Cedex, France). Min DC was 0.2 ng/mL for leptin and adiponectin and 0.5 ng/mL for sE-selectin. All investigators were blinded to sample allocation while performing the analyses.

2.4 Health questionnaire and health behaviors

Information on disease and medication was obtained from the National Health Survey questionnaire [28]. Prevalence of chronic disease was defined as having a diagnosis for hypertension or type 2 DM; or alternatively, suffering from any combination of two of the following diseases: rheumatoid arthritis, asthma, bronchitis, gastric or duodenal ulcer, depression, migraine, constipation/hemorrhoids, osteoporosis, or anemia.

The Minnesota Leisure-Time Physical Activity Questionnaire (MLTPAQ, Spanish version) was employed for the assessment of physical activity. For each individual, METs (metabolic standard units) were estimated using the coefficients published in the Compendium of Physical Activities [29] and computed for weekly periods. The 14-item Mediterranean diet-adherence score (MEDAS), as used in the PREDIMED study [30], was also calculated. The question related to wine intake habits was excluded since this was a group-defining criterion in our study.

2.5 Statistical analyses

Prior to statistical analyses, all data were examined for normal distributions with the Kolmogov-Smirnov test, and those variables not fitting a normal distribution were logarithmically transformed. Basic characteristics of the study subjects were compared between alcohol consumption groups with ANOVA and Chi square test, depending on the type of variable (continuous or categorical, respectively). Univariate ANOVA models were used to analyze the relationship between alcohol consumption and the biomarkers. The first model was adjusted by age and sex, with a second model including lifestyle and health-related factors: SES, prevalence of chronic disease, smoking habit, BMI, amount of physical activity, and MEDAS (all of which were introduced sequentially and only retained if significantly improving the model). Pair comparison of estimated marginal means with Bonferroni correction was used to identify differences between groups. All analyses were performed on the three groups (ABS, BEER, and MIXED) and an additional two- group classification into ABS and "Moderate Drinkers" (MOD: BEER + MIXED). Finally, in order to determine the association between quantity of alcohol intake contributed by every specific alcoholic

drink and biomarkers, models 1 and 2 were repeated with quantitative data (g alcohol/day) simultaneously present in the model. Sensitivity analyses were conducted after sex stratification. All analyses were performed using the SPSS for Windows statistical software package version 22 (SPSS Inc., Chicago, IL, USA), and the significance level was set at $P<0.05$.

3. Results

3.1 Subjects' characteristics by alcohol consumption group

The demographic and health related characteristics of the study subjects are shown in Table 1. The mean daily dose of alcohol was similar in BEER and MIXED consumers and slightly superior to one standard dose. Differences were found among alcohol consumption groups in proportion of women, SES, and physical activity. The ABS group had more women, lower SES status, and number of smokers. The ABS group also performed less physical activity than the MIXED group. However, no group differences were observed for age, BMI, MEDAS score, and chronic diseases prevalence.

3.2 Biomarker analyses by alcohol consumption group

Regarding metabolic and inflammatory biomarkers (Table 2), HDL-c was higher in the MIXED and MOD groups than in the ABS group. As a consequence, the total cholesterol (TC)/HDL-c ratio was lower. Differences were also observed in sP-selectin, which was higher in MIXED and MOD groups than in the ABS group. MPV was significantly higher in MOD than ABS. No differences were found between

consumption groups in parameters related to iron status or inflammatory markers, such as CRP and cytokines. Regarding adipokines, a significantly higher concentration of plasma adiponectin was found in the MIXED group compared to both, BEER and ABS groups. However, since an almost significant interaction was found between alcohol consumption pattern and sex (P=0.059), a separate sex analysis was performed that showed significant results for women only.

Table 3 shows that sex is a significant contributor to HDL-c and adiponectin values, while age is a significant contributor to sP-selectin and MPV values. The rest of the confounding factors tested did not significantly improve the model, except for BMI's contribution to explain HDL-c values.

Since sP-selectin levels seemed to be significantly influenced by age, with a negative association (Table 3), an analysis of sP-selectin values by alcohol consumption pattern stratified by age was performed. The results showed that sP-selectin values were significantly higher in both MIXED and BEER than in ABS in the intermediate age group (Figure 1) as well as in MIXED vs. ABS in the oldest group.

3.3 Biomarker analyses by quantitative alcohol consumption and type of drink.

Subsequently, the statistical models for analysis were performed substituting alcohol consumption groups by the continuous variables (g/day) of wine, beer, and liquors intake, simultaneously. The results showed that the higher levels of HDL-c, sP-selectin, and adiponectin in alcohol consumers were related to a higher wine intake but not to beer intake; whereas the higher MPV values in alcohol consumers were explained by

increased beer intake but not by wine intake. Again, in sex stratified sensitivity analyses, adiponectin's relationship with wine intake was only significant in women (p=0.024) and not in men (p=0.256). The rest of the outcomes were similar in men and women. In order to assess whether these associations were driven exclusively by those subjects in the upper limit of moderate alcohol consumption, the analyses were repeated excluding 8 women consuming ≥ 15 g/day and 5 men consuming ≥ 30 g/day. The associations found between HDL-c and adiponectin with wine and between MPV and beer remained unaltered (P=0.001, P= 0.024 and P=0.026, respectively), while the relationship between sP-selectin and wine disappeared (P = 0.259) (data not shown).

4. Discussion

This study aimed to find the associations between moderate consumption of alcohol and inflammation markers in a convenience sample of adults 55 years and older. The higher proportion of women in the ABS group and the higher SES in the MOD group are in agreement with the results observed in a population-based study of elderly European subjects [31]. The results showed that HDL-c, adiponectin, sP-selectin, and MPV were positively associated with alcohol consumption. The higher HDL-c and adiponectin levels are in agreement with our hypothesis of higher protective cardiometabolic marker levels in moderate alcohol drinkers. However, we did not find an association between lower inflammation related biomarker levels and alcohol intake. Further analyses showed that wine consumption drove the association found between alcohol intake and the plasma levels of HDL-c, adiponectin, and sP-selectin; while beer consumption drove the association between alcohol intake and MPV.

In the few intervention studies that have been published regarding the effects of alcohol intake on adhesion molecule levels, findings mostly showed lower values of adhesion molecules after intervention with moderate doses of alcohol (red wine, cava, or gin) compared to baseline [13,14]. Specifically, red wine (30 g ethanol/d) decreased ICAM-1 and VCAM-1 in high CVD risk subjects [13] as well as in healthy female adults [14]. However, no effect of similar doses of alcohol provided by beer or gin were reported on ICAM-1 and VCAM-1 in subjects with risk factors for CVD [15], while elevated ICAM-1 and VCAM-1 levels were observed after an intervention with wine that provided 30g/day of alcohol in healthy young volunteers [16]. Since our results report associations in an observational, cross-sectional study, we are not able to make cause-effect interpretations. However, the finding of higher sP-selectin values with wine intake might help counteract a possible physiological reduction of sP-selectin associated with aging. In this sense, platelet number is known to decrease after 60 y of age in humans, but little is known about their function [reviewed 32]. P-selectin is expressed, under defined conditions, by both platelets and endothelial cells and has important roles in supporting platelet-leukocyte interactions, platelet aggregates, and some functions of an inflammatory nature on monocytes; with all of these possibly having a role in inflammation and atherogenesis [33,34]. Thus, its positive or negative role seems to depend on the balance between the coagulation and healing processes and the pro-atherogenic disturbances in these individuals over 55 years of age. In addition, our results indicate that only the highest level of moderate wine intake effectively led to the higher sP-selectin in wine consumers.

MPV is widely identified as an expression of metabolically and enzymatically active platelets and as a newly emerging biomarker of CVD [35]. In agreement with our

results, the study by Tozzi et al. [16] on healthy young subjects who were administered moderate amounts of red wine for 4 wk. also found a significant increase of MPV, which significantly correlated with IL-6 plasma levels. The average MPV values of the groups studied were within the normal range; thus, the true relevance of the increased MPV values associated with beer consumption is not clear. In this sense, the results of different studies measuring platelet responsiveness after ethanol exposure are not consistent. A decrease was observed in *in vitro*-induced platelet aggregation by some authors [36,37] but other authors found that only red wine and dealcoholized red wine decreased platelet aggregation [21,22]. In addition, other factors, such as a healthier drinking pattern and more favorable risk traits, in wine drinkers might confound the effect of beverage choice [38,39]. The beneficial, cardioprotective effects of elevated HDL-c levels with moderate alcoholic beverage consumption have been widely documented and might also result from the synergistic effects of ethanol and the polyphenol fraction of each drink [40]. In our study, this effect is suggested by the significant association found between wine intake (g/day) and HDL-c, which was not significant with beer intake.

In the Nurse's Health study, only adiponectin, among all inflammation and endothelial dysfunction biomarkers, showed a significant role in explaining the negative association observed between basal alcohol intake and incidence of diabetes assessed 10 years later [18]. In our study, adiponectin was increased in the MIXED group, which was characterized by a predominant intake of wine (70 % of TAI) and confirmed when wine intake (g alcohol/d) was used in the regression analysis. The fact that it only remained significant for women ($p= 0.024$), in a separate sex analysis ($p=0.256$ in men), is to be taken with caution since a lower proportion of men participated in the study and this

might affect power for this analysis. Other cross-sectional studies have also described similar results [19,20] but the potential role of adiponectin as a mediator of the beneficial effect of alcohol on diabetes prevention needs further study [20].

This study has some limitations. First of all, the sample size is scarce in the BEER group and the effect size on MPV values is also rather small, which makes the findings related to beer intake to be short of power (i.e. 67%). Secondly, the observational design does not allow cause-effect relationships, as mentioned above. However, the strength is the acquisition of alcohol intake information by a trained interviewer, which adds accuracy to the intake data.

In conclusion, this study showed that moderate alcohol intake, specifically wine, is related to higher levels of protective cardiometabolic markers, such as HDL-c and adiponectin (the last one only in women). It also showed higher sP-selectin with wine intake and higher MPV with beer intake; however, their effect size was rather small. A confirmatory study in a bigger population sample should be included in future studies.

Acknowledgment

The authors would like to thank all of the volunteers for taking part in this study and gratefully acknowledge Cristina López-Ruíz and Elizabeth González-Rubio for their help with questionnaire evaluation tasks. This work received intramural support and author ISM was awarded a scholarship from the Beer and Health information Centre (reference: XII call "Manuel de Oya)) to carry out this study. AM has been part of the scientific committee of the Beer and Health Information Centre. The authors declare no conflict of interest.

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Table 1. Characteristics of the study participants

	ABS (n=54)	BEER (n=40)	MIXED (n= 49)	MOD (n=89)	P #	P^
Women (%)	72.2	55.0	53.1	53.9	0.093	0.030
Age	68.65±9.35	66.82±7.52	66.02±7.45	66.38±7.45	0.256	0.112
Alcohol consumption (g/d)	0.75±1.18 [a]	12.46±8.10 [b]	14.10±11.21 [b]	13.36±10.05	<0.001	<0.001
Wine (g/d)	0.33±0.70 [a]	1.41±2.53 [a]	9.96±8.43 [b]	6.12±7.74	<0.001	<0.001
Beer (g/d)	0.19±0.48 [a]	10.68±6.75 [b]	3.42±4.57 [c]	6.68±6.69	<0.001	<0.001
Liquor (g/d)	0.07±0.29 [a]	0.31±0.66 [a,b]	0.56±1.32 [b]	0.45±1.08	0.019	0.014
BMI (kg/m^2)	25.9±2.4	25.9±2.7	26.7±2.6	26.3±2.7	0.242	0.369
SES (%)						
Low-intermediate	22	13	16	15	0.052	0.025
Intermediate-high	52	35	33	33		
High	26	52	51	52		
Smoking habit (%)						
Smoke currently	15	20	25	23	0.097	0.044
Used to smoke	32	45	45	45		
Never smoked	54	35	30	33		
Physical activity (METs-min./wk)	2705±1236 [a]	3300±1420 [a,b]	3863±1836 [b]	3610±1677	0.001	0.001
MEDAS score [§]	4.48±1.44	4.80±1.30	4.96±1.54	4.89±1.43	0.233	0.103
Chronic disease prevalence (%)	53.7	50.0	55.1	52.8	0.886	0.917

Means ± SD; ABS (abstainers or occasional consumers); BEER: contribution ≥80% daily alcohol intake; MIXED: consumption of wine and either beer or liquor or all; MOD (moderate drinkers), all consumers of alcoholic drinks merged in a single group (BEER + MIXED).

#ANOVA or Chi-square tests for continuous and categorical variables, respectively, among three groups (ABS, BEER, and MIXED). Different letters indicate significant differences between the groups (ABS, BEER, and MIXED). Bonferroni test, $P<0.05$.

^ANOVA or Chi-square tests for continuous and categorical variables, respectively, between ABS and MOD.

§ Mediterranean diet adherence screener (MEDAS) score calculated without question #8 related to wine consumption habit.

Table 2. Metabolic and inflammatory biomarkers in moderate drinkers and abstainers

	ABS (n=54)	BEER (n=40)	MIXED (n=49)	MOD (n=89)	P#	P^
Glucose (mg/dL)	88±11	88±10	88±9	88±10	0.899	0.668
Total cholesterol (mg/dL)	204±32	206±32	202±29	203±30	0.853	0.820
LDL-c (mg/dL)	126±28	127±29	121±24	123±26	0.498	0.497
HDL-c (mg/dL)	55±15 [a]	60±14 [a,b]	62±18 [b]	61±16	**0.012**	**0.004**
Ratio TChol:HDL-c	3.9±1.0 [a]	3.6±0.9 [a,b]	3.4±0.8 [b]	3.5±0.8	**0.004**	**0.002**
Triglycerides (mg/dL)	108±55	95±43	92±34	93±38	0.125	**0.044**
sICAM-1 (ng/mL)	79±31	78±25	85±58	82±46	0.786	0.876
sVCAM-1 (ng/ mL)	672±199	735±187	749±171	742±177	0.216	0.083
sE-selectin (ng/mL)	30±17	28±14	30±19	29±17	0.480	0.277
sP-selectin (ng/mL)	59±43 [a]	75±38 [a,b]	82±28 [b]	79±33	**0.044**	**0.020**
Platelets ($x10^3/\mu L$)	231.8±49.1	236.9±56.8	233.3±55.4	234.9±55.8	0.919	0.898
MPV (fL)	9.64±1.20	10.13±1.14	10.06±1.23	10.09±1.18	0.074	**0.023**
Iron (µg/dL)	89±27	91±24	94±26	92±25	0.883	0.722
Transferrin (mg/dL)	257±34	262±30	257±32	259±31	0.767	0.842
Ferritin ((ng/dL)	116±95	117±95	124±93	121±93	0.652	0.332
Leptin (ng/mL)	8.73±6.10	8.79±7.72	8.03±5.40	8.39±6.56	0.501	0.262
Adiponectin (ng/mL)	12230±7366 [a]	10744±4305 [a]	13898±8396 [b]	12414±6931	**0.018**	0.218
C-reactive protein (mg/dL)	0.25±0.45	0.31±0.88	0.25±0.40	0.28±0.66	0.586	0.902
IL-1β (pg/mL)	0.69±0.76	0.76±0.64	0.62±0.38	0.68±0.52	0.609	0.952
IL-6 (pg/mL)	2.25±1.56	3.41±6.90	2.17±0.86	2.75±4.75	0.701	0.407
IL-8 (pg/mL)	2.20±1.02	2.36±1.08	2.03±0.71	2.19±0.91	0.301	0.709
IL-10 (pg/mL)	19.84±12.53	23.54±31.21	19.57±14.62	21.41±23.75	0.725	0.845

Means ± SD. ABS (abstainers or occasional consumers); BEER, contribution ≥80% daily alcohol intake; MIXED, consumption of wine and either beer or liquor or all; MOD (moderate drinkers), all consumers of alcoholic drinks merged in a single group (BEER+MIXED); MVP (Mean Platelet Volume).

#,^Effect of the factor alcohol-consumption group in a factorial univariate analysis adjusted for age and sex. # Three alcohol-consumption groups: ABS, BEER, and MIXED; ^ Two alcohol-consumption groups: ABS and MOD. Values in the same row bearing different superscript letters indicate differences between groups by comparison of estimated marginal means with Bonferroni adjustment. $P<0.05$.

Table 3. Influence of individual characteristics and alcohol consumption on cardiometabolic markers.

Factors	P	Power (1-β)	Covariables	B	95% CI	P	Power (1-β)
HDL-c (mg/dL) (dependent variable)							
Alcohol consumption	**0.001**	0.930	BMI (kg/m^2)	-1.645	-2.548; -0.742	**0.003**	0.947
BEER vs. ABS[b]	0.095						
MIXED vs. ABS[b]	**0.001**						
BEER vs. MIXED[b]	0.504						
Sex	**<0.001**	0.999					
sP-selectin (ng/mL) (dependent variable)							
Alcohol consumption	**0.027**	0.671	Age (y)	-1.65	-2.36; -0.94	**<0.001**	0.996
BEER vs. ABS[b]	0.294						
MIXED vs. ABS[b]	**0.025**						
BEER vs. MIXED[b]	1.0						
Adiponectin (ng/mL) (dependent variable)							
Alcohol consumption	**0.020**	0.701					
BEER vs. ABS[b]	0.984						
MIXED vs. ABS[b]	0.059						
BEER vs. MIXED[b]	**0.036**						
Sex	**<0.001**	0.999					
MPV (fL) (dependent variable)							
Alcohol consumption			Age (y)	-0.055	-0.077; -0.033	**<0.001**	0.998
MOD vs. ABS	**0.037**	0.553					
Sex	**0.031**	0.581					

ABS, abstainers or occasional consumers; BEER, contribution ≥80% daily alcohol intake; MIXED, consumption of wine and either beer or liquor or all; MOD (moderate drinkers), all consumers of alcoholic drinks merged in a single group (BEER + MIXED); MPV (Mean Platelet Volume).

Univariate ANOVA, main effects model, of alcohol consumption on biomarkers. The basic model adjusted by sex and age was further adjusted stepwise with the confounding variables SES, chronic disease prevalence, smoking habit, BMI, METs/week, and Mediterranean diet adherence screener (MEDAS) score and only retained if contributing significantly to the model.

[b] Differences between each group pair by comparison of estimated marginal means with Bonferroni adjustment.

Table 4. Associations between alcohol intake from different drinks and biomarkers.

	HDL-c (mg/dL)			
	B	95%CI	P	Power (1-β)
Beer (g alcohol/day)	0.388	-0.007; 0.783	0.123	0.338
Wine (g alcohol/day)	0.637	0.289; 0.985	**<0.001**	0.949
Liquor (g alcohol/day)	-0.826	-3,555; 1.903	0.550	0.091
Sex	-13.1	-18.2; -8.0	**<0.001**	0.999
BMI (kg/m^2)	-1.561	-2.465;-0.654	**0.001**	0.924
	sP-selectin (ng/mL)			
	B	95%CI	P	Power (1-β)
Beer (g alcohol/day)	-0.179	-1.140; 0.782	0.713	0.065
Wine (g alcohol/day)	1.128	0.232; 2.023	**0.014**	0.696
Liquor (g alcohol/day)	-1.698	-8.534; 5.137	0.624	0.078
Age (y)	-1.773	-2.489; -1.057	**<0.001**	0.998
	MPV			
	B	95%CI	P	Power (1-β)
Beer (g alcohol/day)	0.037	0.007; 0.068	**0.017**	0.666
Wine (g alcohol/day)	-0.004	-0.032; 0.024	0.788	0.058
Liquor (g alcohol/day)	0.183	-0.037; 0.403	0.102	0.374
Age (y)	-0.055	-0.077; -0.032	**<0.001**	0.998
Sex	-0.556	-0.960; -0.152	**0.007**	0.771
	Adiponectin (ng/mL)			
	B	95%CI	P	Power (1-β)
Beer (g alcohol/day)	-42	-255; 142	0.653	0.073
Wine (g alcohol/day)	204	37; 370	**0.017**	0.671
Liquor (g alcohol/day)	-428	-1718; 862	0.513	0.100
Sex	-5963	-8384; -3542	**<0.001**	0.998

Unstandardized regression coefficients (B) and confidence intervals (CI). MPV: (Mean platelet volume).

Univariate ANOVA, main effects model, with the biomarker as dependent variable and including alcohol intake from the different types of alcoholic drinks as covariates. The confounding variables SES, chronic disease prevalence, smoking habit, BMI, METs/week and Mediterranean diet adherence screener (MEDAS) score were included stepwise and only retained if contributing significantly to the model.

Figure 1. Selection of study participants

Figure 2. Plasma P-selectin by alcohol consumption groups at different age groups.

*Differences among groups assessed with the Kruskal-Wallis test and followed by Mann-Whitney U test for paired comparisons in the intermediate age group.

Figure 1

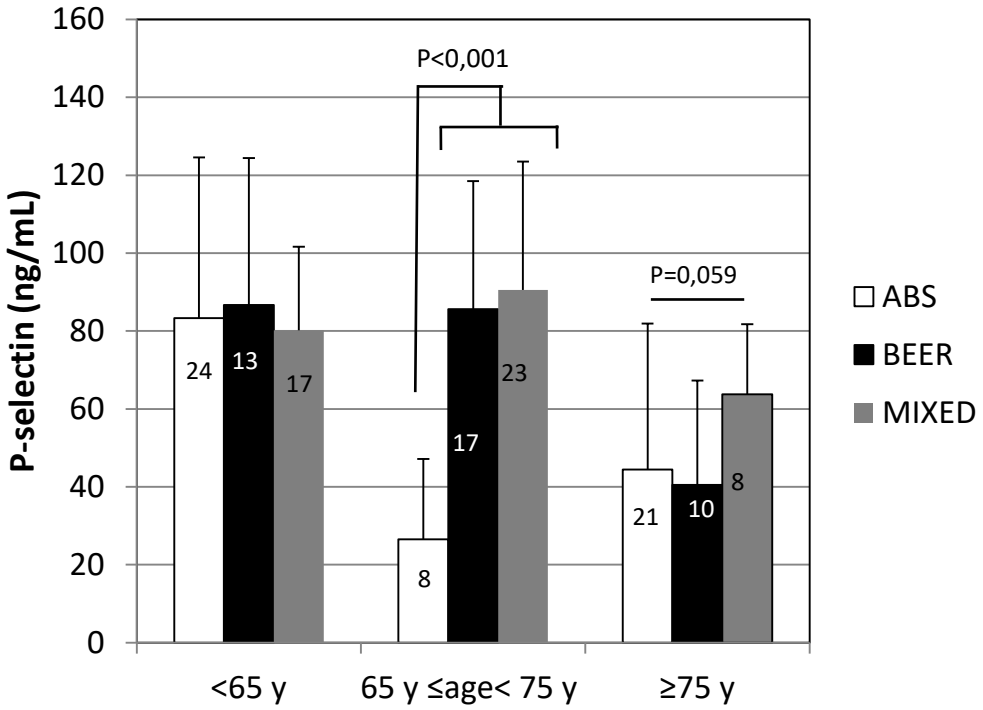

Figure 2

Highlights

Manuscript title: Associations of metabolic and inflammatory biomarkers with wine and beer intake in elderly subjects with a moderate consumption of alcohol. A cross-sectional study.

It is well acknowledge that alcohol intake is detrimental for general health but controversy exists regarding the risk for cardiovascular events and diabetes in human subjects with a moderate alcohol intake. Some blood biomarkers such as HDL-c and adiponectin have shown associations that suggest a potentially mediating role of beneficial effects. In this cross-sectional study, we have observed that:

Higher HDL-c and adiponectin levels are associated to a moderate wine intake in adults aged 55y and older.

For the first time, a positive association of P-selectin with wine intake and of MPV with beer intake was observed.