Effect of Moderate Red Wine versus Vodka Consumption on Inflammatory Markers Related to Cardiovascular Disease Risk: A Randomized Crossover Study

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Effect of moderate red wine versus vodka consumption on inflammatory markers related to cardiovascular disease risk: A randomized crossover study.

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Abstract

Objective

Few interventions have tested the effects of different alcohol types on cardiovascular risk biomarkers. The aim of this study was to investigate the effects of red wine versus vodka on inflammatory and vascular health-related biomarkers.

Methods

In a crossover study, participants were randomized to receive either red wine or vodka (3 units/ day) for 2 weeks. Following a 2-week washout period, participants then consumed the alternate alcoholic drink for 2 weeks. Fasting blood samples were collected just prior to and at the end of each 2-week period. A total of 13 inflammatory and vascular health biomarkers were assessed.

Results

A total of 77 of 85 recruited healthy men completed the study. Leptin levels were significantly raised after each intervention ($p \leq 0.01$). APO A1 significantly increased following vodka, but not red wine, intervention ($p \leq 0.01$). A significant difference between the interventions was noted for adiponectin only ($p \leq 0.01$), although neither of the within-group changes were statistically significant ($p > 0.01$).

Conclusions

The current study found significantly increased levels of leptin following both red wine and vodka consumption, increased levels of APO A1 following vodka consumption, and

significant difference between both interventions for adiponectin only. Further studies are needed to investigate the effects of longer-term alcohol consumption on inflammatory and vascular health biomarkers.

Keywords: alcohol consumption; intervention; cardiovascular disease; biomarkers.

Introduction

Epidemiological evidence has suggested that moderate alcohol consumption plays a protective role against cardiovascular disease (CVD) among general populations [1,2]. It was also shown to significantly reduce the incidence of pre-mature mortality among CVD patients [3]. The nature of this relationship appears to be J-shaped, with abstainers and heavy alcohol drinkers having greater cardiovascular mortality than moderate drinkers [4]. A recent viewpoint by Costanzo et al. [5], however, showed continuing scientific debate over the J-shaped relationship between alcohol intake and mortality. While some studies showed evidence of no protective effect of moderate alcohol on cardiovascular outcomes [6,7], others demonstrated significant protective effects [1,2]. Costanzo et al. [5] revealed that these conflicting results may be explained by potential confounders and selection bias (e.g. failure to correctly classify exposure- heavy vs. moderate drinkers- due to self-reporting issues) in different epidemiological studies.

Observational studies have suggested a reduction in inflammatory markers, such as C-reactive protein (CRP) [8], and an increase in anti-inflammatory modulators, such as adiponectin [1], in moderate alcohol drinkers compared with abstainers. The protective cardiovascular effects of alcohol may therefore be mediated through anti-inflammatory, or other vascular health-promoting mechanisms. Whilst likely mechanisms have been postulated for this possible protective effect, the exact mechanisms by which alcohol exerts protective effects are also yet to be elucidated [4], but may relate to type of alcohol consumed.

Red wine has been of particular interest in relation to cardiovascular protective effects [9]. Evidence for other alcoholic beverages also indicates some benefit, but to a lesser extent than for red wine [10,11]. In a meta-analysis of beer and wine studies, the vascular risk significantly decreased by 32 and 22% in wine and beer drinkers relative to non-drinkers,

respectively [10]. In addition to the alcohol content, red wine contains a number of other compounds including polyphenols, which may mediate cardiovascular protection via other mechanisms, yet their subtherapeutic concentrations in red wine pose a limitation to clinical trials [12]. Polyphenols may act by scavenging free radicals, reducing vascular tone and suppressing inflammatory mechanism which facilitate the development of CVD [12]. There is limited research, on the other hand, investigating the effect of vodka on cardio-vascular health. A randomized controlled 28-day vodka (30g/ day) trial by Joosten et al. [13] demonstrated some cardioprotective effects through significantly reducing interleukin-18 (IL-18) levels.

Given the lack of alcohol intervention studies in general, and specific testing of different alcoholic beverages, the current study aimed at assessing the effect of short-term moderate alcohol consumption on a panel of inflammatory and other vascular health markers. This study also aimed to determine whether the effects of intervention differed depending on type of alcohol consumed (red wine or vodka). Part of this study has already been published with respect to assessing total homocysteine (tHcy), folate, and vitamin B_{12} concentrations, where the 14-day alcohol intervention significantly decreased folate and vitamin B_{12} levels, and significantly raised plasma tHcy concentrations [14].

Patients and Methods

Study Design

This randomized, crossover study included recruitment of 85 healthy male subjects aged 21-70 years from hospital workforce and the general population. Exclusion criteria included consumption of >21 units alcohol/week, detection of abnormal liver function, taking any vitamin and mineral supplements and any serious concurrent health conditions (e.g. cardiovascular disease, cancer). Randomization was conducted blind by a statistician

independent to the research team. Eligible participants were block randomized (using a computer-generated random-number sequence). Following a preliminary run-in period (2 weeks), with no alcohol consumption, subjects consumed either red wine (240 mL) (Jacob's Creek Shiraz Cabernet, South Eastern Australia; ABV 13.5% vol.) or vodka (80 mL) (Smirnoff vodka; ABV 37.5% vol.) for two weeks. This amount is equivalent to 24 g ethanol/ day [3]. After a 14-day washout period, subjects drank the alternate alcoholic beverage for a further 14 days (Fig. 1). No other alcoholic beverages were consumed during the study period. Before the study began, participants were subjected to a full clinical assessment; weight, height and blood pressure measurement. Participants were asked to avoid changing their levels of physical activity or dietary patterns during the study period. Participants were also advised to avoid mixing the vodka intervention with fruit juice. This study was approved by the Faculty of Medicine Research Ethics Committee at Queen's University Belfast, and all participants gave written informed consent.

Blood sampling

Fasting blood samples were collected before and after each treatment period in EDTA tubes and centrifuged at 4°C and 1590 g for 10 min for separation of serum and plasma (Sigma cooling centrifuge 4K15C; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Serum and plasma samples were stored at -80°C pending analysis.

Laboratory analysis

A total of 13 biomarkers were measured. Biomarkers measured in serum were CRP, interleukin-6 (IL-6), IL-18, intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), leptin, apolipoprotein A1 (APO A1) and apolipoprotein B (APO B). IL-6, IL-18, VCAM-1 and leptin were assayed using ELISA kits (R&D Systems, Abingdon, UK). ICAM-1 was also assayed using ELISA kits (Thermo Fisher, Swindon, UK).

APO A1, APO B and CRP levels were determined by Immunoturbidimetric assay (Architect c8000; Abott, UK).

Biomarkers measured in plasma were adiponectin, matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1), caspase-1 (CASP-1) and cystatin C. CASP-1 and adiponectin were assayed using ELISA kits (R&D Systems, Abingdon, UK). MMP-9 was assayed utilizing Enzyme immunoassay kits (Fuji Chemical Industries Co., Tokyo, Japan), TIMP-1 levels were determined by Chemiluminescent microparticle immunoassay (Architect i2000; Abbott, UK). Cystatin C concentrations were determined by Immunoturbidimetric assay (Architect c8000; Abott, UK). All intra and inter-assay coefficients of variation (CVs) were less than 7% and 17% respectively.

Statistical Analysis

The final analysis only included study subjects who completed the study. The Hills & Armitage test was initially exploited for doing analysis [15]. Given the absence of significant period or carry-over effects, paired samples t-tests were conducted for comparison of the intervention groups, as they provided a simpler presentation of results. In light of the multiple tests of significance performed with no prior hypotheses, the 1% significance level was used in all analyses. All statistical analyses were done using STATA release 14.0 (StataCorp, College Station, TX, USA) and SPSS version 22 (IBM Corp, Armonk, NY, USA).

Results

Of the 85 recruited study subjects, 77 subjects completed the entire study protocol. Table 1 shows the baseline characteristics. Participants randomized to receive red wine initially were older than those randomized to receive vodka, and there were more smokers initially randomized to red wine than to vodka.

Table 2 shows the changes in inflammatory markers, presented as either geometric mean ratio (post-intervention: pre-intervention) or difference in arithmetic means (post intervention – pre-intervention). Figure 2 presents the significant changes in the measured biomarkers. No significant changes were seen in the serum/plasma concentrations of CRP, IL-6, IL-18, VCAM-1, CASP-1, MMP-9, TIMP-1, APO B, Cystatin C or ICAM-1 following 2-week interventions with either vodka or red wine. Furthermore, no significant difference was seen between the vodka and red wine interventions for these markers. Although no significant increase in plasma adiponectin levels was observed following 14-day consumption of vodka [GM ratio (95% CI) 1.09 (1.02-1.17), p=0.013] nor red wine intervention [GM ratio (95% CI) 0.96 (0.91-1.03), p=0.24], comparison of change in adiponectin levels following the two interventions demonstrated a significant difference (p adjusted for period = 0.009). There were significant increases in serum leptin levels during both interventions [GM ratio (95% CI) vodka: 1.11 (1.04-1.17), p<0.001; red wine: 1.12 (1.05-1.19), p<0.001]; this increase did not differ between the interventions (p adjusted for period = 0.77). APO A1 levels were significantly increased following vodka intervention [mean difference (95% CI): 0.07 (0.05-0.09), p<0.001], however no significant change was identified following red wine intervention [mean difference (95% CI) 0.03 (-0.02-0.07), p=0.22], and the between-intervention difference was not significant (p=0.11).

Discussion

This two-period crossover trial demonstrated a 2-week intervention with moderate alcohol consumption (red wine or vodka) was associated with a significant increase in leptin levels. Results also showed that a 2-week vodka period, but not red wine period, significantly raised APO A1 levels. A significant difference between the interventions was only noted for adiponectin, although no within group changes were observed for either red wine or vodka.

Adiponectin acts as an insulin-sensitising adipocytokine, shown to have an inverse relationship with development of coronary heart disease [16]. The protective effects are mediated by anti-atherogenic and ant-inflammatory mechanisms [16]. This study did not show any significant increase in plasma adiponectin following either vodka or red wine interventions, yet there was a significant difference between these interventions, with numeric differences suggesting an increase following vodka and decrease following red wine. In contrast, a study of 34 healthy men, who were randomized to receive either 450mL of red wine (40g alcohol) or 450mL of de-alcoholised red wine across a 4-week period, found that adiponectin levels were significantly increased following consumption of red wine when compared to de-alcoholised red wine [17]. These inconsistent results may be attributed to the differences in alcohol amounts consumed in the latter study and the current study (40 *vs* 24g, respectively). On the other hand, Imhof et al. [18] showed a significant increase in adiponectin levels among women, but not men, after consuming red wine in a randomized controlled crossover trial comparing the effect of 21-day consumption of ethanol, red wine, and beer, equivalent to 20 g ethanol/day for females, and 30 g/day for males. Later, the cross-sectional study conducted by Nishise et al. [19] in a Japanese population demonstrated a significantly inverse association between moderate alcohol intake (120-239 g/week) and adiponectin levels. Although only a cross-sectional study, this contradicts the results of studies conducted among European population and highlights the potential effect of ethnicity on adiponectin levels following alcohol consumption.

There are also conflicting results from the literature in relation to leptin and alcohol consumption. Leptin is a cytokine-type peptide hormone that is predominantly synthesized by adipocytes. It is best known for its involvement in energy homeostasis, including regulation of neuroendocrine function, feeding behavior and energy expenditure [20]. There is evidence that leptin acts as a pro-inflammatory hormone, and defective or reduced leptin signalling can

help to protect against atherosclerosis via anti-inflammatory mechanisms [20]. It is possible that different types of alcohol may affect leptin levels differently, yet the current study demonstrated a significant increase in leptin levels following both alcohol interventions. Likewise, Djurovic et al. [21] conducted a randomized crossover trial of 87 healthy individuals who received either a 3-week red wine intervention or no alcohol, where a significant increase in leptin levels was shown after the red wine intervention compared to no alcohol. When split by gender, the relationship remained significant for women, but not for men. In contrast, Beulens et al. [1] conducted an intervention study of a 21-day beer intervention (40g alcohol/ day) among 20 healthy men, which revealed no change in leptin levels. Similarly, Chiva-Blanch et al. [22] measured changes in leptin after consumption of beer, non-alcoholic beer and gin in 33 men at high risk of CVD and reported that there was no evidence for significant differences in leptin levels across the three interventions. Due to the contradictory evidence currently reported concerning alcohol's effect on leptin levels, there is a requirement for future research in the area to define the exact relationship and determine the mechanisms by which alcohol may exert its protective effects.

APO A1 and APO B have been reported to be early predictors of atherosclerotic diseases [23]. APO A1 is known for its protective effects against atherosclerosis through mediating reverse cholesterol transport, whereas APO B has atherogenic effects [23,24]. The current study showed a significant increase in APO AI levels following vodka, but not red wine intervention. APO B levels were not significantly raised following either intervention. An intervention study in 51 postmenopausal women by Baer et al. [23] showed similar results with regards to APO A1, which was significantly raised by 28-day alcohol consumption (30g/day), yet APO B levels exhibited a significant decrease.

Population cohort studies have reported that inflammatory mediators such as IL-6 and CRP are positively associated with development and progression of atherosclerosis [25]. This

study did not demonstrate a significant change in IL-6, IL-18 or CRP levels following red wine or vodka intervention; these findings are supported by a number of studies across alcohol types [26–29]. On the contrary, Joosten et al. [13] conducted a randomized controlled 28-day vodka (30g/ day) intervention study in 24 healthy males and showed a significant decrease in IL-18 levels. Furthermore, Vázquez-Agell et al. [8] reported significant reductions in IL-6 and CRP levels, following a 28-day sparkling wine intervention. In 2018, a cross-sectional study by Mangnus et al. [30] supported these findings, and revealed a significant J-shape relationship between CRP levels and alcohol consumption.

Secretion of endothelial adhesion molecules into the plasma acts as a sign of atherogenesis and endothelial dysfunction [31]. Our results did not show any significant changes in VCAM-1 nor ICAM-1 levels following either vodka or red wine interventions. In contrast, an intervention study by Estruch et al. [32] has demonstrated reductions in ICAM-1 and VCAM-1 levels following 28-day red wine (33g alcohol/day). This inconsistency in results may be linked to the difference in amount of alcohol consumed in our study and the latter study (24 *vs* 33g, respectively). Furthermore, moderate alcohol consumption was found to be inversely associated with serum levels and supresses endothelial expression of adhesion molecules [8,32,33]. Observational studies have shown a significant J-shape relationship between alcohol consumption and ICAM-1, as well as E-selectin, whereas a U-shape curve was detected for VCAM-1 [31].

Changes in inflammatory molecules by alcohol could be attributed to alterations in gene expression [26]. The alcohol consumed in the current study may have modulated the gene expression of the inflammatory and vascular health-related biomarkers examined. Our study showed that the higher polyphenol content of red wine did not show more protective effect against CVD relative to vodka. This is consistent with previous research findings that suggested that red wine contains subtherapeutic concentrations of polyphenols [12]. Longer

and larger prospective studies are needed to inform alcohol interventions for prevention/management of CVD.

Strengths and limitations

This study was a randomized crossover study, which is a robust design, aiming to determine the effect of two different alcoholic beverages consumed in moderation, on a range of inflammatory and vascular-related biomarkers. Such an approach enhances the largely epidemiological literature relating to alcohol, alcohol type and vascular health outcomes. One limitation in this study is the relatively short duration of the wine/vodka intervention to compare the effect of the two different alcoholic beverages on inflammatory biomarkers. Given the variations in polyphenol content between red wine products, our study findings are specific to the particular red wine brand used in this study (i.e. Shiraz-Cabernet). The participants of our study were advised to maintain their normal diet throughout the trial period to avoid confounding by a change in diet; however, the diet of the subjects was not strictly regulated or monitored throughout the trial period. Researchers encouraged compliance with the intervention, but there was no formal measurement of compliance, which was self-reported by participants. The weight/BMI of the volunteers were recorded at baseline but not re-measured post-intervention period, and we have not determined whether baseline BMI could have been associated with the influence of alcohol on inflammation. Furthermore, all individuals in this study were healthy volunteers without comorbidity and therefore, had low baseline risks. Our results, therefore, may not be representative of those who are most likely to benefit from the protective effects of alcohol.

Conclusions

Moderate consumption of either red wine or vodka had significant effects on leptin and APO A1. No significant difference in any other inflammatory and vascular biomarkers included in

the study were detected. Whilst leptin concentrations showed a significant increase following both interventions, APO A1 exhibited a significant increase after vodka only. A statistically significant difference between interventions was only observed for adiponectin, although within-group changes were not statistically significant. Further research with large cohorts is required to validate these findings in order to best develop and guide cardiologists on alcohol preventive measures against CVD. Prospective research will also assess the impact of longer-term alcohol use on inflammatory and vascular health biomarkers related to CVD risk.

Conflict of interest

The authors declare no conflict of interest.

Financial support

Author contributions

ISY, PCS and AE designed the original study and were responsible for study conduct. CCP was study statistician and conducted the statistical analysis. SB's laboratory conducted the biomarker analysis. MCM and JVW conceived and directed the current analysis. AW, SE, NMcA and KD contributed to the current analysis, literature review and paper draft. All authors approved the final manuscript.

Acknowledgements

References

1. Beulens JWJ, de Zoete EC, Kok FJ, Schaafsma G, Hendriks HFJ. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: A diet intervention study. Eur J Clin Nutr 62:1098–105, 2008.

2. Bell S, Daskalopoulou M, Rapsomaniki E, George J, Britton A, Bobak M, Casas JP, Dale CE, Denaxas S, Shah AD, Hemingway H. Association between clinically recorded alcohol consumption and initial presentation of 12 cardiovascular diseases: Population based cohort study using linked health records. BMJ 356: j909, 2017.

3. Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, de Gaetano G. Alcohol consumption and mortality in patients with cardiovascular disease: A meta-analysis. J Am Coll Cardiol 55:1339–47, 2010.

4. De Gaetano G, Costanzo S. Alcohol and Health. J Am Coll Cardiol 70:923–5, 2017.

5. Costanzo S, de Gaetano G, Di Castelnuovo A, Djoussé L, Poli A, van Velden DP. Moderate alcohol consumption and lower total mortality risk: Justified doubts or established facts? Nutr Metab Cardiovasc Dis 29:1003–1008, 2019.

6. Knott CS, Coombs N, Stamatakis E, Biddulph JP. All cause mortality and the case for age specific alcohol consumption guidelines: Pooled analyses of up to 10 population based cohorts. BMJ. 350:h384, 2015.

7. Stockwell T, Zhao J, Panwar S, Roemer A, Naimi T, Chikritzhs T. Do "moderate" drinkers have reduced mortality risk? A systematic review and meta-analysis of alcohol consumption and all-cause mortality. J Stud Alcohol Drugs 77:185–98, 2016.

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 137:2279–84, 2007.

9. Haseeb S, Alexander B, Baranchuk A. Wine and cardiovascular health: A comprehensive review. Circulation 136:1434–48, 2017.

10. Di Castelnuovo A, Rotondo S, Iacoviello L, Donati M, de gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation 105:2836–2844,

2002.

11. 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 26:833–50, 2011.

12. Bonnefont-Rousselot D. Resveratrol and cardiovascular diseases. Nutrients 8:250, 2016

13. Joosten MM, van Erk MJ, Pellis L, Witkamp RF, Hendriks HFJ. Moderate alcohol consumption alters both leucocyte gene expression profiles and circulating proteins related to immune response and lipid metabolism in men. Br J Nutr 108:620–7, 2012.

14. Gibson A, Woodside J, Young I, Sharpe PC, Mercer C, Patterson CC, McKinley MC, Kluijtmans LA, Whitehead AS, Evans A. Alcohol increases homocysteine and reduces B vitamin concentration in healthy male volunteers - A randomized, crossover intervention study. QJM 101:881-887, 2008.

15. Hills M, Armitage P. The two-period cross-over clinical trial. Br J Clin Pharmacol 8:7–20, 1979.

16. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 29:1730–7, 2004.

17. Beulens JWJ, Beers RM, Stolk RP, Schaafsma G, Hendriks HFJ. The effect of moderate alcohol consumption on fat distribution and adipocytokines. Obesity 14:60–6, 2006.

18. Imhof A, Plamper I, Maier S, Trischler G, Koenig W. Effect of drinking on adiponectin in healthy men and women: A randomized intervention study of water, ethanol, red wine, and beer with or without alcohol. Diabetes care 32:1101-1103, 2009

19. Nishise Y, Saito T, Makino N, Okumoto K, Ito JI, Watanabe, H, Saito K, Togashi H, Ikeda C, Kubota I, Daimon M, Kato T, Fukao A, Kawata S. Relationship between alcohol consumption and serum adiponectin levels: The Takahata study—A cross-sectional study of a healthy Japanese population. J Clin Endocrinol Metab 95:3828–35, 2010.

20. Park H-K, Ahima RS. Physiology of leptin: Energy homeostasis, neuroendocrine

function and metabolism. Metabolism 64:24–34, 2015.

21. Djurovic S, Berge KE, Birkenes B, Braaten Ø, Retterstøl L. The effect of red wine on plasma leptin levels and vasoactive factors from adipose tissue: A randomized crossover trial. Alcohol Alcohol 42:525–8, 2007.

22. Chiva-Blanch G, Magraner E, Condines X, Valderas-Martínez P, Roth I, Arranz S, Casas R, Navarro M, Hervas A, Sisó A, Martínez-Huélamo M, Vallverdú-Queralt A, Quifer-Rada P, Lamuela-Raventos RM, Estruch R. Effects of alcohol and polyphenols from beer on atherosclerotic biomarkers in high cardiovascular risk men: A randomized feeding trial. Nutr Metab Cardiovasc Dis 25:36–45, 2015.

23. Baer DJ, Judd JT, Clevidence BA, Muesing RA, Campbell WS, Brown ED, Taylor PR. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am J Clin Nutr 75:593–9, 2002.

24. Smith JD. Apolipoprotein A-I and its mimetics for the treatment of atherosclerosis. Curr Opin Investig Drugs 11:989-96, 2010.

25. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population. Circulation 112:976–83, 2005.

26. Stote KS, Tracy RP, Taylor PR, Baer DJ. The effect of moderate alcohol consumption on biomarkers of inflammation and hemostatic factors in postmenopausal women. Eur J Clin Nutr 70:470–4, 2016.

27. 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 342:d636, 2011

28. Karlsen A, Retterstøl L, Laake P, Kjølsrud-Bøhn S, Sandvik L, Blomhoff R. Effects of a daily intake of one glass of red wine on biomarkers of antioxidant status, oxidative stress and inflammation in healthy adults. E Spen Eur E J Clin Nutr Metab 2:e127–33, 2007.

29. Romeo J, Wärnberg J, Nova E, Díaz LE, González-Gross M, Marcos A. Changes in the immune system after moderate beer consumption. Ann Nutr Metab 5:359–66,

2007.

30.   Mangnus L, van Steenbergen HW, Nieuwenhuis WP, Reijnierse M, van der Helm-van
        Mil AHM. Moderate use of alcohol is associated with lower levels of C reactive
        protein but not with less severe joint inflammation: A cross-sectional study in early
        RA and healthy volunteers. RMD Open 4:e000577, 2018.

31.   Sacanella E, Estruch R. The effect of alcohol consumption on endothelial adhesion
        molecule expression. Addict Biol 8:371–8, 2003.

32.   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
        175:117–23, 2004.

33.   van Bussel BCT, Henry R, Schalkwijk CG, Dekker JM, Nijpels G, Feskens JME,
        Stehouwer CDA. Alcohol and red wine consumption, but not fruit, vegetables, fish or
        dairy products, are associated with less endothelial dysfunction and less low-grade
        inflammation: the Hoorn Study. Eur J Nutr 57:1409–19, 2017.

Tables captions

Table 1. Baseline characteristics of study participants by intervention, during first treatment period

Table 2. Effect of 14-day vodka or red wine intervention on biomarkers in 77 healthy adult men who completed the study

Figures captions

Fig. 1. Crossover randomization of study subjects among the two alcohol interventions.

Fig. 2. Changes in the measured biomarkers following the two treatment periods.

Table 1: Baseline characteristics of study participants by intervention, during first treatment period

	Intervention group (during first intervention group)	
	Vodka	Red wine
n	41	36
Age (y)	39.9 (10.7)	47.7 (11.6)
BMI (kg/m^2)	25.4 (4.3)	25.3 (1.7)
Systolic BP (mmHg)	124.8 (11.0)	128.1 (14.8)
Diastolic BP (mm Hg)	76.8 (7.9)	81.5 (12.0)
Usual alcohol consumption (units/week)	12.2 (14.9)	11.5 (8.9)
Smoking	5%	17%
CRP (mg/L)	0.61 (0.29, 1.35)	0.64 (0.44, 1.09)
IL-6 (pg/mL)	1.24 (0.72, 1.99)	1.29 (0.86, 1.75)
IL-18 (pg/mL)	273 (233, 377)	270 (211, 378)
Adiponectin (ng/mL)	5051 (3132, 7271)	6132 (4657, 8128)
Leptin (pg/mL)	4825 (2861, 8177)	5353 (4306, 6855)
VCAM-1 (ng/mL) [a]	626 (525, 737)	628 (498, 795)
CASP-1 (pmol/L)	50.4 (37.2, 61.5)	39.7 (30.2, 56.6)
MMP-9 (mg/L)	122 (72, 155)	111 (70, 166)
TIMP-1 (ng/mL)	95 (79, 109)	99 (83, 116)
APO A1 (g/L)	1.32 (0.14)	1.37 (0.19)
APO B (g/L)	0.94 (0.24)	0.92 (0.22)
Cystatin C (mg/L)	0.74 (0.10)	0.76 (0.12)
ICAM-1 (ng/mL)	214 (38)	191 (45)

Data presented as mean (SD, geometric mean (25th, 75th percentile) or %. [a] based on n = 76.

Table 2: Effect of 14-day vodka or red wine intervention on biomarkers in 77 healthy adult men who completed the study

| | Analysis of two period crossover trial including adjustment for period | | | | | | | | | |
| | Vodka intervention | | | | Red wine intervention | | | | Vodka vs. Red Wine | |
	GM [a] pre	GM [a] post	GM ratio [b] (95% CI [c])	p	GM [a] pre	GM [a] post	GM ratio [b] (95% CI [c])	p	p unadjusted	p adjusted for period
CRP (mg/L)	0.70	0.69	0.99 (0.85,1.15)	0.89	0.68	0.78	1.14 (0.92,1.42)	0.21	0.29	0.34
IL-6 (pg/mL)	1.38	1.55	1.12 (0.95,1.33)	0.17	1.35	1.43	1.06 (0.88,1.28)	0.53	0.68	0.69
IL-18 (pg/mL)	282	277	0.98 (0.94,1.03)	0.47	281	278	0.99 (0.93,1.05)	0.71	0.87	0.89
Adiponectin (ng/mL)	5008	5478	1.09 (1.02,1.17)	0.013	5586	5386	0.96 (0.91,1.03)	0.24	0.007	0.009[e]
Leptin (pg/mL)	5019	5547	1.11 (1.04,1.17)	<0.001[e]	4803	5374	1.12 (1.05,1.19)	<0.001[e]	0.78	0.77
VCAM-1 (ng/mL) [d]	631	634	1.00 (0.95,1.07)	0.87	628	628	1.00 (0.95,1.05)	0.98	0.88	0.91
CASP-1 (pmol/L)	48.8	46.3	0.95 (0.87,1.03)	0.19	44.7	44.4	0.99 (0.92,1.07)	0.88	0.40	0.38
MMP-9 (mg/L)	108	104	0.96 (0.87,1.06)	0.40	107	97	0.91 (0.82,1.01)	0.07	0.43	0.42
TIMP-1 (ng/mL)	96	94	0.98 (0.94,1.02)	0.27	96	93	0.97 (0.93,1.01)	0.10	0.67	0.70
	Mean pre	Mean post	Mean difference (95% CI [c])	p	Mean pre	Mean post	Mean difference (95% CI [c])	p	p unadjusted	p adjusted for period
APO A1 (g/L)	1.35	1.42	0.07 (0.05,0.09)	<0.001[e]	1.38	1.40	0.03 (-0.02,0.07)	0.22	0.09	0.11
APO B (g/L)	0.92	0.95	0.02 (-0.01,0.05)	0.16	0.92	0.95	0.03 (0.00,0.06)	0.03	0.69	0.70
Cystatin C (mg/L)	0.75	0.74	0.00 (-0.01,0.01)	0.71	0.81	0.74	-0.08 (-0.22,0.07)	0.29	0.30	0.33
ICAM-1 (ng/mL)	198	209	11 (2,20)	0.02	203	204	1 (-6,8)	0.81	0.10	0.10

[a] GM: Geometric mean; [b] GM Ratio: ratio of geometric means (post:pre); [c] CI: confidence interval. Results displayed as GM and GM ratio for logarithmically-transformed variables and mean and arithmetic difference for normally distributed variables. [d] based on n = 76. [e] $p \leq 0.01$.

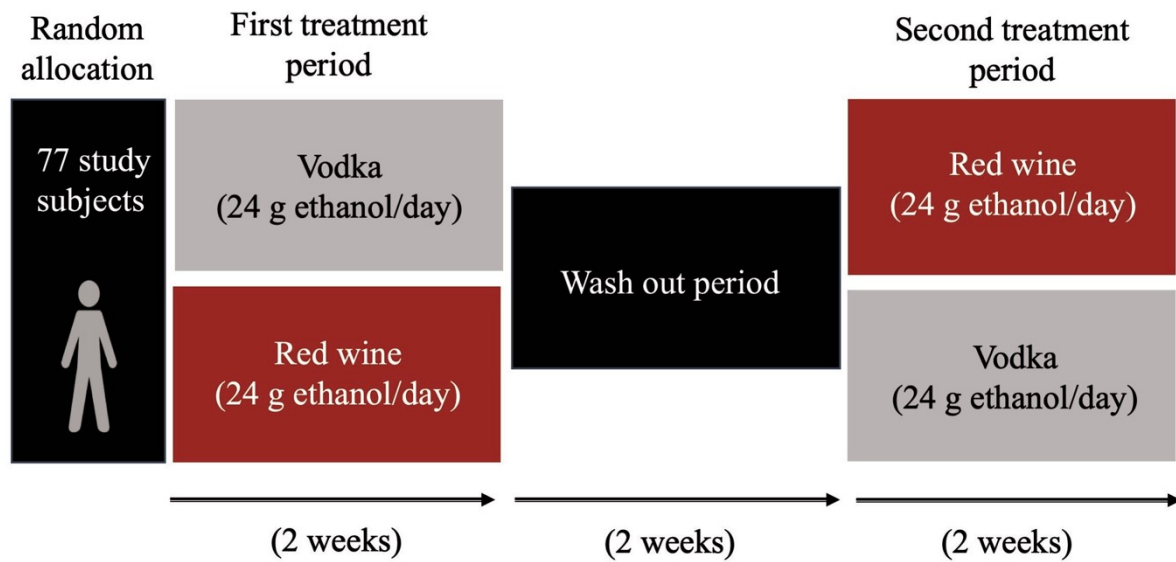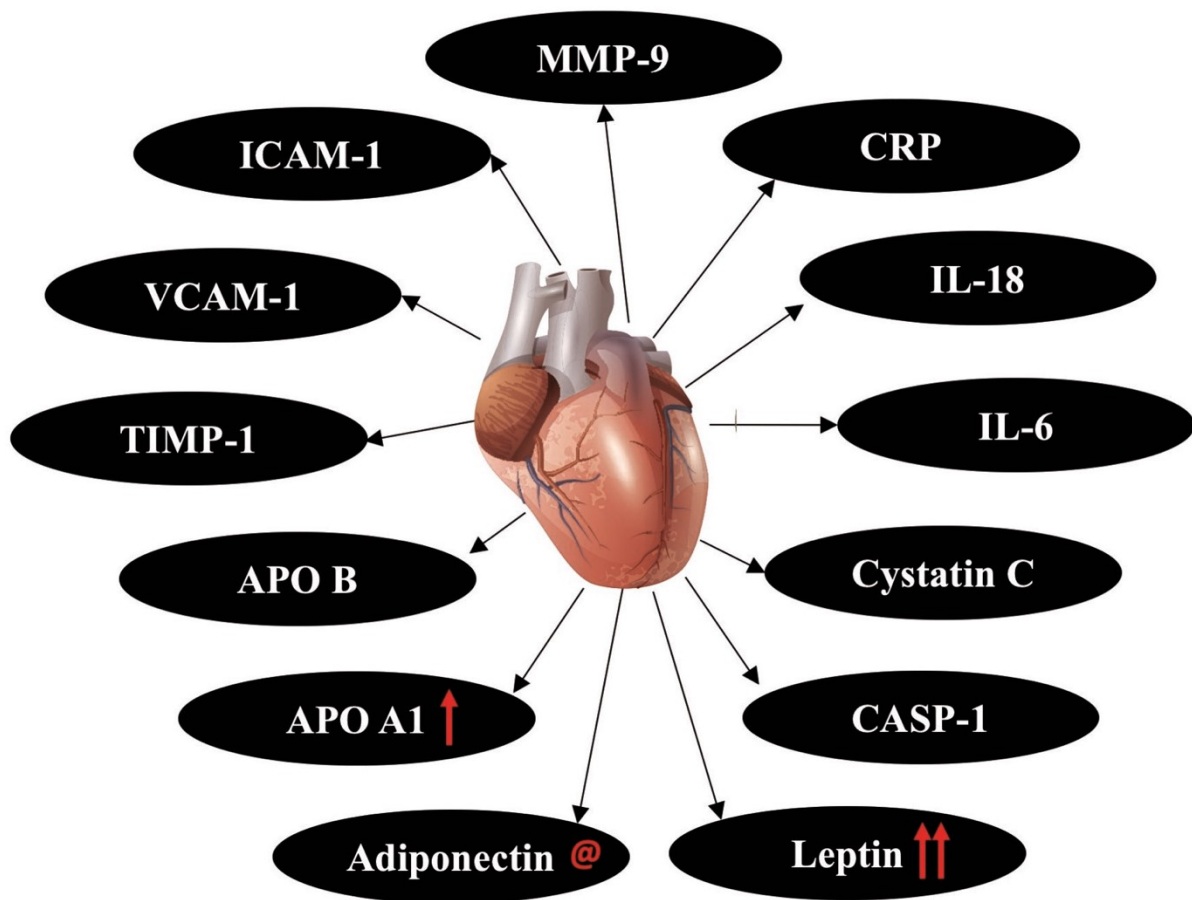**Fig. 1.** Crossover randomization of study subjects among the two alcohol interventions.

Fig. 2. Changes in the measured biomarkers following the two treatment periods.

↑ Significant increase after vodka only ($p \leq 0.01$)

↑↑ Significant increase after vodka & red wine ($p \leq 0.01$)

@ Significant difference between interventions ($p \leq 0.01$)