

Drinking modulates monocyte migration in healthy subjects: a randomised intervention study of water, ethanol, red wine and beer with or without alcohol

ARMIN IMHOF, ROZA BLAGIEVA, NIKOLAUS MARX, WOLFGANG KOENIG

Abstract

Moderate alcohol consumption is associated with reduced cardiovascular mortality compared to non-consumption of alcohol and heavy drinking. Experimental data suggest a direct effect of alcohol on atherosclerotic lesion development. We assessed the effect of consumption of moderate amounts of alcoholic and non-alcoholic beverages on monocyte migration, a crucial step in atherogenesis.

Forty-nine healthy men and women (aged 22–56 years) were enrolled in this randomised controlled trial. After wash-out, participants were assigned to either ethanol (concentration 12.5%), beer (5.6%) or red wine (12.5%) equivalent to 30 grams of ethanol per day (g/d) for men and 20 g/d for women, or to the same amount of de-alcoholised beer or red wine, or to water. Monocyte migration was evaluated *ex vivo* using a modified Boyden chamber.

Intake of ethanol or de-alcoholised red wine significantly reduced monocyte chemoattractant protein-1 (MCP-1)-induced monocyte migration by 58% ($p < 0.05$; $n = 6$) and 36% ($p < 0.05$; $n = 7$) and FMLP (N-formyl-methionyl-leucyl-phenylalanine)-induced migration by 41% ($p < 0.05$) and 36% ($p < 0.05$), respectively. MCP-1 receptor expression was not affected by these interventions, as shown by flow cytometry.

Short-term intervention with moderate amounts of ethanol and de-alcoholised red wine inhibits monocyte migration *ex vivo*. This might represent one mechanism by which alcoholic beverages lower cardiovascular risk.

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Key words: alcohol, atherosclerosis, beer, intervention trial, monocytes chemotaxis, red wine.

Introduction

Moderate alcohol intake has consistently been shown to be associated with lower risk for fatal and non-fatal cardiovascular disease (CVD).¹ It has been suggested that alcohol in moderate doses – in addition to exerting favourable changes on blood lipids, the haemostatic profile and insulin resistance – may exhibit anti-inflammatory mechanisms, thus potentially modulating atherosclerosis development.² Atherosclerosis is an inflammatory disease, characterised by local inflammation in the vessel wall and a systemic immune response.^{3,4} Atherosclerotic lesions contain inflammatory cells in abundance; among them, monocytes/macrophages play a key role in the initiation and progression of atherosclerosis.⁵ Recruitment and migration of monocytes into the arterial wall is a crucial step in early atherogenesis.^{4,5}

Acute and chronic alcohol consumption are known to affect the innate immune system as well as the adaptive immune response.^{6–8} Observational studies have shown that moderate alcohol consumption is associated with lower concentrations of inflammatory markers such as C-reactive protein (CRP), fibrinogen, interleukin-6 (IL-6) and white blood cell (WBC) count.^{9–14} In experimental studies using cell cultures and mouse models, alcohol treatment inhibited leukocyte recruitment and endothelial cell activation during inflammation and infection in a dose-dependent manner.¹⁵ Moreover, in a short-term intervention trial in eight men randomised to either red wine (with high concentrations of polyphenols) or gin (containing low concentrations of polyphenols), Badia *et al.* demonstrated decreased tumour necrosis factor alpha (TNF- α)-induced adhesion of human monocytes to endothelial cells *ex vivo* with red wine and also, though to a lesser extent, pronounced inhibition of adhesion after intervention with gin. The authors hypothesised that ethanol and polyphenols may act synergistically.¹⁶ In *in vitro* studies, polyphenols from red wine down-regulated expression of endothelial cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on TNF- α -stimulated human umbilical cells as well as lipopolysaccharide (LPS)-stimulated human saphenous vein endothelial cells.¹⁷ The effect of alcohol

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Table 1. Clinical and biochemical characteristics of study participants at baseline

	Beer	Beer (-)	Wine	Wine (-)	Ethanol	Water	p value
Age [years]	40.5 (8.7)	35.3 (8.1)	39.7 (6.1)	40.3 (9.4)	38.0 (4.5)	34.8 (7.4)	ns
BMI [kg/m ²]	23.3 (3.9)	23.3 (5.0)	22.9 (2.0)	22.9 (2.4)	23.4 (2.3)	22.8 (3.5)	ns
GGT [U/L]	28 (15)	21 (12)	13 (4)	26 (13)	30 (19)	22 (11)	ns
AST [U/L]	26 (10)	22 (10)	18 (5)	21 (5)	23 (6)	27 (11)	ns
ALT [U/L]	30 (11)	25 (15)	19 (7)	31 (15)	35 (21)	32 (19)	ns

Key: BMI = body mass index; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; ns = not significant; Beer/Wine (-) indicates de-alcoholised beer/wine. All parameters are expressed as arithmetic means (standard deviation)

consumption on monocyte migration has not been examined yet.¹⁷ We sought to investigate the effect of short-term moderate consumption of low-concentration ethanol, red wine and beer with or without alcohol on monocyte chemotaxis *ex vivo*. Furthermore, to evaluate potential mechanisms by which monocyte migration might be modified, expression of monocyte chemoattractant protein-1 (MCP-1) receptor on monocytes and serum concentrations of ICAM-1 and E-selectin were measured.

Methods

Study subjects

Forty-nine healthy men and women, non-smokers aged 22–56 years, were recruited by placard and advertisement in local newspapers. They were consumers of moderate amounts of alcoholic beverages (from one drink per week up to two drinks every day) and had a family history free of alcohol dependencies. Pre-existing but unknown liver disease was excluded by measuring liver enzymes before inclusion in the study. All participants gave their written informed consent to all procedures. The study was approved by the local ethics committee.

Study design

After a wash-out period of at least two weeks, participants were randomly allocated – stratified by age and gender – to the following interventions over three weeks: ethanol (concentration 12.5%), beer (5.6%) or red wine (12.5%) equivalent to 30 grams of ethanol per day (g/d) for men and 20 g/d for women or the same amount of de-alcoholised beer or de-alcoholised red wine (same brand) or pure water (control group). The rationale for this study design was avoidance of selection bias caused by preferences in drinking behaviour. All participants were asked not to change their dietary habits or habitual physical activity during the study period. However, diet and physical activity were not predetermined since this might have led to substantial effects on monocyte migration which we would not have been able to control for. The overall polyphenol content, measured spectrophotometrically, of the beer and de-alcoholised beer was 169 mg/L and 171 mg/L, respectively. In the red wines, polyphenol content did not differ and was 275 mg/L as measured by high-performance liquid chromatography.

Clinical and laboratory measurements

At baseline, details of alcohol consumption, dietary habits, medical history and further sociodemographic parameters were obtained by standardised interview. From the body height (accurate to 1 cm) and body weight (accurate to 1 kg), body mass index (BMI) was calculated. At each visit, symptoms of concurrent inflammatory processes and infections such as fever and cough or antibiotic therapy were carefully assessed; if present, the participant was excluded from analyses.

Fasting blood was collected from the antecubital vein with the subject in a sitting position, with minimal suction and short-term occlusion. Plasma and serum were obtained and stored within 90 minutes at -80°C until analysis. All laboratory analyses were done in a blinded fashion. Monocytes were freshly isolated from drawn blood of each participant before and after intervention using serial Ficoll/Pecoll gradient centrifugation, as described previously.¹⁸ Cells were cultured for 16 hours in RPMI-1640 media supplement with 0.5% human serum to become quiescent after isolation. The purity of the cell type was > 95% as determined by flow cytometry analysis. Monocyte chemotaxis was assayed under serum-free conditions in a 48-well microchemotaxis chamber (Receptor Technologies Ltd., Adderbury, Oxon, UK). Wells in the upper and the lower chamber were separated by a polyvinylpyrrolidone-free polycarbonate membrane (pore size 5 µm; Costar). This membrane was coated with collagen type 1 and incubated in serum-free RPMI media for 1 hour at 37°C. MCP-1 (Monocyte chemoattractant protein-1) (1 nM) or FMLP (N-formyl-methionyl-leucyl-phenylalanine) (1 nM) were added into the lower chamber and monocytes at a density of 5x10⁵/ml were then added into the upper chamber. Cells were incubated for three hours; subsequently, migrated cells on the bottom face of the filter were stained and counted. Cells were counted in five random high-power fields per well and counting of migrated cells was blinded to the type of intervention. Monocyte CC chemokine receptor (CCR)-2 (MCP-1 receptor) expression was determined by standard flow cytometry, as described previously.¹⁹ Plasma concentrations of ICAM-1 and E-selectin were measured by ELISA (R&D Systems, Wiesbaden, Germany) before and after intervention. At each visit, liver enzymes were measured the same day

Figure 1. Effect of moderate consumption of alcoholic and corresponding non-alcoholic beverages on monocyte migration *ex vivo*. Data are expressed as mean fold induction before and after intervention using MCP-1 (1a) or FMLP (1b) to induce migration

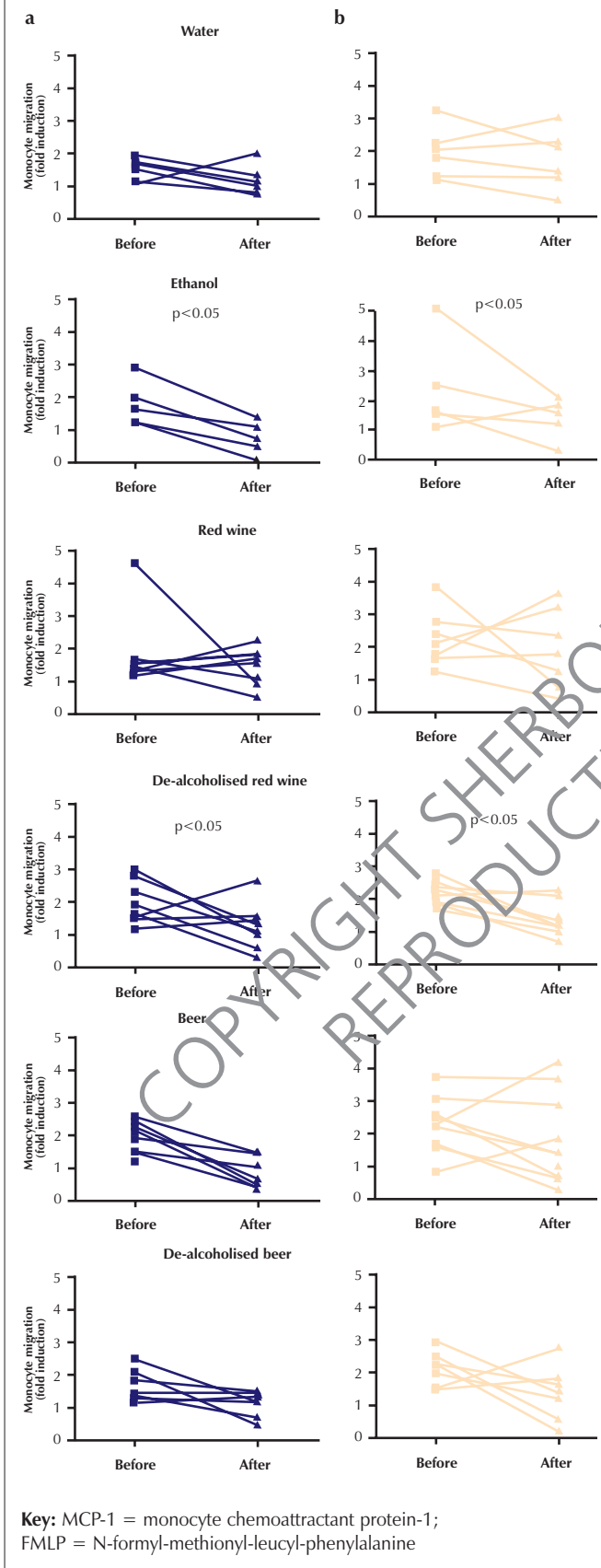
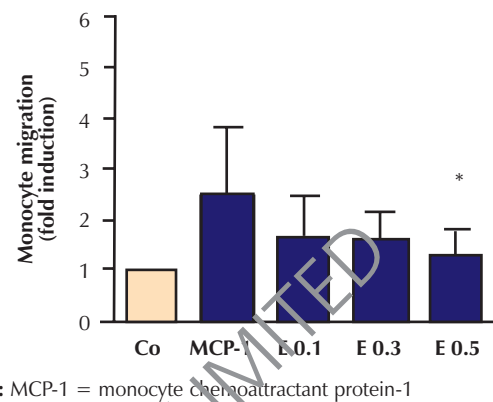


Figure 2. Ethanol solution inhibited MCP-1-induced monocyte migration *in vitro* in a dose-dependent manner. Data are expressed as mean fold induction and SEM. Inhibition of induction was statistically significant for ethanol 0.5%. (* $p < 0.05$)



photometrically from lithium-heparin plasma on a Dimension XL (Dade Behring, Marburg, Germany) for safety reasons and high-density lipoprotein cholesterol (HDL-C) was determined by routine enzymatic methods.

Statistical analyses

Each intervention group contained between six and eight subjects. Six individuals from the original sample were excluded from the analysis because migration of monocytes *ex vivo* before intervention was insufficiently inducible by MCP-1 and by FMLP. Descriptive data are expressed either as means together with their standard deviation or as numbers and proportions. Chemotaxis of monocytes induced either by MCP-1 or FMLP, MCP-1 receptor expression, concentration of TNF- α and HDL-C were compared before and after intervention by Student's paired *t*-test or Wilcoxon signed-rank test, as appropriate. All tests performed were two-sided, and a *p*-value < 0.05 was considered statistically significant. All computations were performed using SAS software, Release 8.2 (SAS Institute Inc., Cary, NC, US).

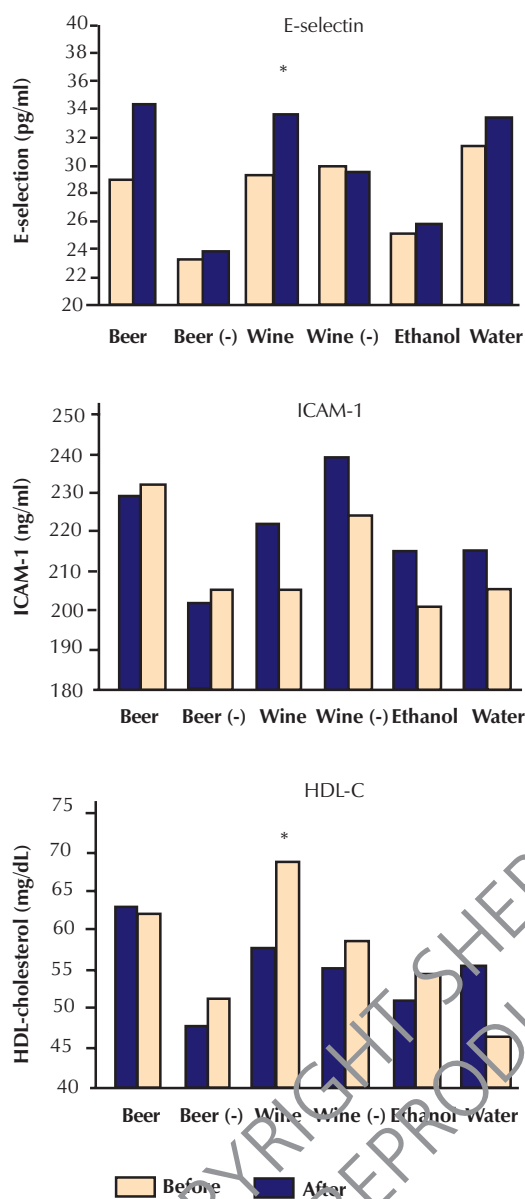
Results

Forty-two individuals completed the study protocol. Compliance was excellent according to self-report and counting of empty bottles returned. Baseline clinical and biochemical characteristics of the study participants are presented in table 1. Mean age, BMI and baseline concentrations of liver enzymes did not differ between groups.

Ex vivo and *in vitro* monocyte migration

The mean value of MCP-1-induced monocyte migration was 1.82-fold compared to unstimulated cells, with no significant differences between groups at baseline. After three weeks, ethanol consumption reduced MCP-1-stimulated migration from a 1.78-fold induction before to a 0.75-fold induction after intervention, $p < 0.05$ (58% decrease). Similarly, consumers of de-alcoholised red wine exhibited a significant reduction in MCP-1-induced monocyte migration, with a

Figure 3. Serum/plasma concentrations of E-selectin, ICAM-1 and HDL-cholesterol before and after intervention. (*p<0.05)



Key: ICAM-1 = intercellular adhesion molecule-1; HDL-C = high-density lipoprotein cholesterol

decrease from a 1.96-fold to a 1.25-fold induction (36% decrease, $p<0.05$). None of the other interventions significantly affected monocyte migration (figure 1a).

To determine to what extent the effects observed might be due to a reduction in monocyte MCP-1 receptor expression, we assessed CCR2 cell surface expression by flow cytometry in a subset of individuals. CCR2 expression was similar in all groups (data not shown).

Next, we examined whether the inhibitory effect of ethanol and de-alcoholised red wine depended on the stimulus employed and performed similar experiments using FMLP as an inducer of cell migration. The observed inhibition was also statistically significant for consumption of

ethanol (2.37-fold induction before and 1.40-fold induction after intervention, $p<0.05$) and de-alcoholised red wine (2.17/1.39, $p<0.05$) (figure 1b). After consumption of beer, de-alcoholised beer and wine, induction was also reduced but did not reach statistical significance. These data suggest that both ethanol and de-alcoholised red wine directly interfere with monocyte migration, independent of the inducing stimulus.

In vitro experiments using monocytes from healthy blood donors confirmed the direct inhibitory effect of ethanol on monocyte migration. *In vitro* treatment of human monocytes with ethanol significantly diminished MCP-1-induced cell migration in a concentration-dependent manner, with a maximal reduction at 0.5% ethanol ($n=10$) (figure 2).

Metabolic effects

Red wine consumption significantly increased HDL-C, while there was only a non-significant trend towards increased HDL-C levels in ethanol-consuming individuals (figure 3). Interestingly, beer did not affect HDL-C concentrations and in controls (consuming water) HDL-C substantially decreased, indicating an effective study design and good adherence of participants to the study protocol.

Adhesion molecules

Given that ethanol modulates inflammatory cell activation, we next examined the effect on inflammatory biomarkers representing endothelial function. Consumption of alcoholic and non-alcoholic beverages did not decrease E-selectin concentrations. In fact, after consumption of red wine, plasma concentrations of E-selectin increased significantly. After intervention with red wine, de-alcoholised red wine and ethanol, ICAM-1 concentrations substantially decreased but this fall was not seen in those drinking beer. ICAM-1 concentrations also decreased in the control group but the observed changes were not statistically significant (figure 3).

Moreover, serum concentrations of TNF- α , a known stimulus of monocyte adhesion to endothelial cells, tended to be lower after intervention compared to baseline among those consuming ethanol (mean difference -0.49 pg/ml), red wine (-1.38 pg/ml), de-alcoholised red wine (-0.27 pg/ml) and beer (-0.66 pg/ml) but increased among those consuming de-alcoholised beer (0.79 pg/ml). None of these changes reached statistical significance.

Discussion

In this open, randomised intervention study over three weeks we found a statistically significant inhibitory effect on *ex vivo* migration of monocytes after ethanol and de-alcoholised red wine. Monocyte MCP-1 receptor expression was not affected by any of the interventions, indicating that other factors, including modification of intracellular pathways, might be responsible for the effects seen. No significant changes in serum concentrations of ICAM-1, E-selectin and TNF- α , except for an increase of E-selectin in the red wine group, were seen after intervention compared to baseline.

Monocyte recruitment and migration into the arterial wall represent crucial steps in early atherosclerosis since these cells orchestrate the inflammatory response in the

