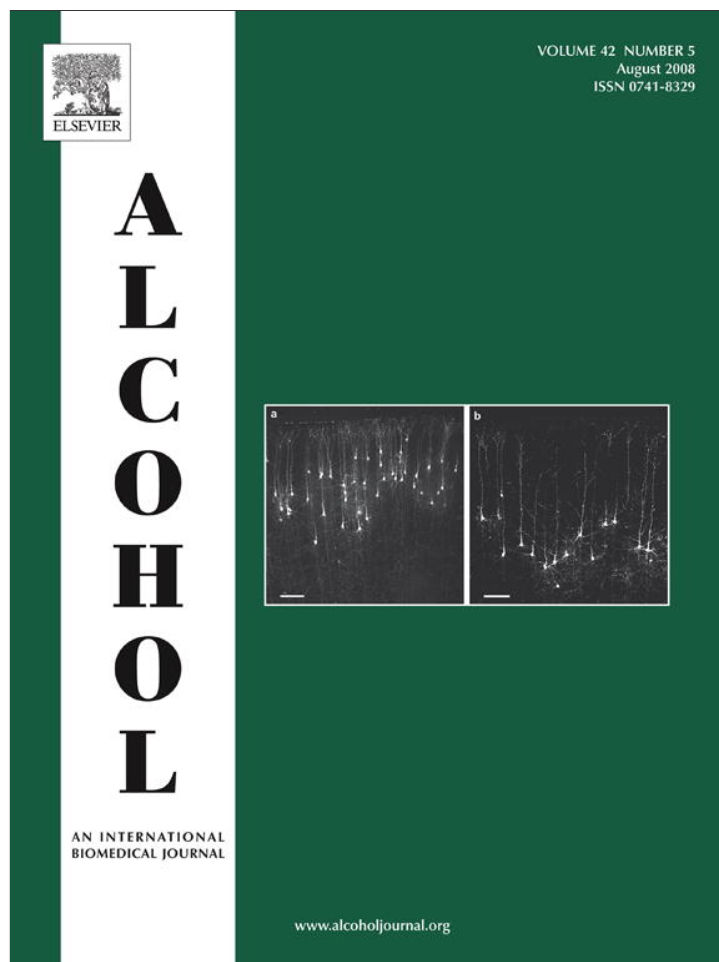


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**ALCOHOL**

## Alcohol reduces MMP-2 in humans and isolated smooth muscle cells

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### Abstract

Alcoholic beverages are known to exert a protective effect on atherosclerotic disease. This study aimed to assess the in vivo and in vitro effects of alcohol on matrix metalloproteinase (MMP) -2 and -9, known to determine atherosclerosis progression. Eighteen healthy volunteers, regular drinkers (two standard alcohol servings/day, on average) at first examination (baseline) were asked to abstain from any alcoholic beverage for one week (abstention), and then to assume two standard alcohol servings of beer daily for 1 week (re-exposure). Activity of MMP-2 and -9, total antioxidant activity (AOA), glutathione (GSH) plasma levels were carried out at baseline, at the end of abstention, and after 1 week of re-exposure. To validate the in vivo results, MMP-2 activity and expression, AOA, and GSH, were determined in human smooth muscle cells treated for 96 h with increasing concentrations (12.5–100 mM) of ethanol. MMP-2, but not MMP-9 plasma activity was higher at abstention than at baseline or re-exposure ( $P < .001$  and  $P \leq .005$ , respectively). Changes in AOA and GSH throughout the study were not significant. No correlation was found between MMPs and antioxidant activity. In vitro, ethanol at 25 mM reduced by around 10% MMP-2 activity ( $P = .003$ ) in smooth muscle cells, whereas MMP-2 expression, AOA, and GSH were unaffected. Alcohol reduces MMP-2 plasma activity in healthy humans and in isolated vascular smooth muscle cells. This in vitro reduction is unrelated to MMP-2 expression in vascular cells or to antioxidant levels changes. © 2008 Elsevier Inc. All rights reserved.

**Keyword:** Matrix metalloproteinase-2; Smooth muscle cells; Antioxidant activity; Ethanol

### Introduction

A moderate regular consumption of alcoholic beverages is associated to the reduction of atherosclerotic lesion progression (Kiechl et al., 1998; Schminke et al., 2005), of the risk of cardiovascular events (Mukamal et al., 2003), and of mortality (Corrao et al., 2000; Muntwyler et al., 1998). Several studies concluded that there is no advantage of one kind of beverage over another (Mukamal et al., 2003), therefore ethanol is considered the driving force of these advantages (Belleville, 2002). Among the alcohol related mechanisms accounting for such protective activity, the increase of the antioxidant potential in plasma (Stoclet, 2001; Szmítko & Verma, 2005), the increase of nitric oxide by

endothelial cells and the increase in plasma HDL cholesterol are the most acknowledged (Vasdev et al., 2006).

Growth of atherosclerotic plaque, also known as negative remodeling, requires smooth muscle cell (SMC) proliferation and migration within the arterial wall; this progression is largely dependent on matrix metalloproteinases (MMP), in particular MMP-2 and MMP-9 (collectively named gelatinases) (Galis et al., 1994), that are capable to degrade reticular collagen (i.e., type IV and V collagen) surrounding SMCs. Recent in vitro evidences showed that ethanol reduces cell migration/proliferation (Cullen et al., 2005; Ghiselli et al., 2003; Hendrickson et al., 1999; Liu et al., 1997), inhibits MMP-2 and MMP-9 activity in isolated animal vascular SMCs when pulse pressure stimulated (Cullen et al., 2005), and that wine reduces MMP-2 in homocysteine stimulated SMCs (Guo et al., 2007). In general, all in vitro studies focused on stimulated cells, while MMP-2 is a constitutionally expressed protease and the in vivo effect is still unknown in humans.

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This study analyzes the changes of MMP-2, -9 activity in a group of moderate regular drinkers after suspension of ethanol intake and re-exposure to a moderate regular beer consumption. These changes were then related to the antioxidant activity (AOA) and glutathione (GSH) levels measured at the same time point. A second part of the study sought for confirmation of *in vivo* results in isolated human SMCs and assessment of MMP-2 expression after ethanol exposure.

## Materials and methods

### Human study

Volunteers of both sexes were recruited among the staff members, students or their relatives at the University of Udine (Italy). Inclusion criteria were to be clinically healthy and a moderate regular drinker, whereas exclusion criteria were alcoholism (potential or ascertained) and pregnancy.

In detail, general conditions and medical history were assessed at the beginning of the study, this time-point being defined as “Baseline.” Blood samples were obtained at baseline, when subjects had no restrictions or recommendations on drinking. The subjects were then asked to abstain from alcoholic beverages while drinking at least 375 ml of water per day for 1 week. The same clinical and laboratory assessments were carried out at the end of abstinence (time-point named Abstinence). Then, subjects had to drink, as the only alcoholic beverage, 375 ml/day of beer (around 30 g) for 1 week; at the end, a further complete assessment was performed (time point named Re-exposure). All subjects were recommended to keep constant their physical activity and diet habits during the whole observation.

Blood was withdrawn from antecubital vein, avoiding blood stasis, with Vacutainer® vials containing ethylenediaminetetraacetic acid or heparin as anticoagulants. Aliquots of whole blood and plasma (obtained after centrifugation at 1,000g) were frozen (at  $-20^{\circ}\text{C}$ ) to allow batch analysis. Plasma samples were used to assess gelatinase activity (with gelatin substrate zymography), AOA, reduced GSH. Blood specimen were collected in the afternoon, at least 2 h after the last meal and more than 15 h after the last alcohol intake. The collection of the samples was completed in 4 weeks. The study has been approved by the regional ethical committee, each participant gave his/her informed written consent.

### *In vitro* study

#### Cell culture and ethanol treatment

Primary cultures of human aortic SMCs (Promocell, Heidelberg, Germany) were grown in a medium containing one third Smooth Muscle Cell Basal Medium (Promocell, Heidelberg, Germany), one-third Nutrient Mixture F-12 and one-third Waymouth Medium (Invitrogen, Basel, Switzerland) supplemented with: 15% of heat-inactivated fetal

bovine serum, 100 U/ml penicillin, and 100  $\mu\text{g}/\text{ml}$  streptomycin (Euroclone, Celbio, Devon, UK). Smooth muscle cells were cultured at  $37^{\circ}\text{C}$  in humidified atmosphere with 5%  $\text{CO}_2$  in air.

Ethanol treated cells were seeded in complete medium at density of  $2 \times 10^4$  cells in duplicate wells of 24-well plate and incubated until they reached 70% confluence. Thereafter, the cells were serum deprived and cultivated in SMC medium supplemented with 5  $\mu\text{g}/\text{ml}$  of insulin (Novo Nordisk, Uppsala, Sweden). Ethanol (Sigma-Aldrich, St. Louis, MO, USA) was added directly to the medium to reach the final concentrations of 12.5, 25, 50, and 100 mM. Supernatants were collected 96 h after ethanol treatment and gelatin zymography was performed. The results are expressed as the median result of at least six experiments for each concentration.

Aliquots of plasma (ethylenediaminetetraacetic acid anticoagulated) from healthy donors have been incubated with the same concentrations of ethanol used for cell cultures for 6 h at  $37^{\circ}\text{C}$ , and MMP-2 activity has been assessed as described below.

#### Gelatin–substrate zymography

Plasma and culture media gelatinase activities (active MMP-2, MMP-9 and their proenzymes) were measured by gelatin–substrate zymography under nonreducing conditions. In brief, plasma samples were placed on 10% sodium-dodecyl sulfate polyacrylamide minigels copolymerized with gelatine (1mg/ml). Gels were washed in 2.5% Triton X-100 to remove sodium-dodecyl sulfate and allow renaturation of MMPs, after that transferred into developing buffer (50 mM of Tris pH 7.5,  $\text{CaCl}_2$  5 mM,  $\text{ZnCl}_2$  1  $\mu\text{M}$ ) and incubated overnight at  $37^{\circ}\text{C}$  under continuous shaking. Gels were stained with Coomassie Brilliant Blue solution (Bio-Rad Laboratories, Hercules, CA) and destained with water, methanol and acetic acid solution (45:45:10). A standard serum sample was used in each gel as molecular weight reference and (for active MMP-2 band) as standard for comparisons among gels. Gels were digitized using a digital camera, and a computer-based imaging program (NIH freeware Image-J) was used to quantify lyses areas. To minimize interassay variations, each subject and each *in vitro* experiment was performed in a single gel.

#### TIMP-1 and TIMP-2 measurements

Tissue inhibitors were determined using the immunoenzymatic method (Quantikine Human TIMP-1 and TIMP-2) according to the manufacturer's instruction. Lowest detectable concentration was 0.08 ng/ml and 0.01 ng/ml for TIMP-1 and TIMP-2, respectively. All determinations have been carried out in duplicate. In our hands both methods gave an intraassay an interassay CV  $<5\%$  and  $<9\%$ , respectively. This method was been used for both plasma determinations and surnatant of SMC culture.

### Total antioxidant activity determination

The details of the method for total antioxidant activity have been previously published (Tietze, 1969; Tubaro et al., 1998). Briefly, plasma samples were diluted 1:20 in sodium phosphate buffer (75 mmol/l, pH 7), an ABTS<sup>+</sup> radical cation solution (200 µl) was mixed with 20 µl of diluted plasma in 96 well plates and absorbance was read after 5–75 min in the ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA). Samples were analyzed in triplicate determinations. A fresh Trolox standard curve was prepared with each batch of plasma analysis, and Trolox equivalents in µmol/l were derived from the standard curve at 5 and 75 min incubation. The same method was also used for supernatant of SMC cell culture media.

### Measurements of GSH

The enzymatic recycling method of (Tietze, 1969) was used to measure GSH. In brief, for measurements of “total GSH,” 200 µl of blood or extract from cultivated cells were diluted 1:2 with 5% (wt/vol) sulfosalicylic acid. The samples were immediately vortexed, and centrifuged for 10 min at RT at 5,000 rpm. Supernatants were transferred to new microcentrifuge tubes and assayed within 2 h. Two ml volume of 0.1M sodium phosphate buffer/0.6mM ethylenediaminetetraacetic acid, 30 µl of 10 mM 5,5'-dithio-bis(2-nitrobenzoic acid) and 50 µl of 10 mM NADPH were added to 30 or 60 µl of each sample, followed by the addition of 10 µl of 100 U/ml glutathione reductase. Formation of 5-thio-2-nitrobenzoic acid, by the reaction of glutathione and DTNB, was measured at 412 nm, 2 min before and 8 min after adding glutathione reductase versus a standard curve of glutathione ranging from 0.125 to 1.5 µg/ml. Spectrophotometric determinations were performed with Beckmann DU 650i spectrophotometer. The same method was also used for supernatant of SMC cell culture media.

### MMP-2 expression

Total RNA was extracted from the human aortic SMC cultures with the RNeasy Kit (Qiagen GmbH, Germany). RNA integrity and quality was evaluated by spectrophotometry and electrophoresis analysis. cDNA was synthesized from 1 µg of total RNA using the Murine Leukemia Virus (MuLV) Reverse Transcriptase following the producer's protocol (Applied Biosystem, CA, USA).

Real time polymerase chain reaction for quantitative analysis of mRNA expression was performed using the SYBR Green dye chemistry and the 7900/HT Sequence Detection System (Applied Biosystem, CA, USA). A 81-bp fragment of MMP-2 cDNA was amplified with human-specific primers (forward: 5' CCATGATGGAGAGGCA-GACA 3'; reverse 5' TCCGTCCTTACCGTCAAAGG 3'). In addition, primers to amplify a 83-bp fragment of the housekeeping gene 28S ribosomal RNA were used (forward: 5' TGGGAATGCAGCCCAAGG 3'; reverse 5' CCTTACGGTACTTGTGACTATGC 3'). Amplifications were performed in 25 µl of SYBR/Master Mix buffer

(Applied Biosystems) containing 0.3 µM primers and 1 µl of cDNA. The following amplification program was used: a predenaturation step at 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min, 72°C for 30 s, and a final extension at 72°C for 10 min. The dissociation stage involved three 15-s steps at 95°C, 60°C, and 95°C. The fluorescence of the green dye in the amplification reactions was detected and evaluated by the melting curve analysis. Standard curve was obtained using a dilution series of a standard mix of cDNAs. Polymerase chain reaction amplification reactions were performed in triplicate on material from two independent reverse transcription reactions. Values have been normalized for ribosomal 28S and reported as values standardized for 0 mM ethanol.

### Statistic analysis

Results for continuous variables are reported as median and, in parenthesis, interquartile values. Results for the human study were analyzed by Generalized Linear Model for repeated measurements and by Wilcoxon test with Bonferroni correction. Multivariate analysis was carried out to assess the variables influencing MMP-2 activity changes. The in vitro results were examined by analysis of variance. When significant differences were found in repeated measures, post hoc analysis (Bonferroni) was carried out to find the times or concentrations significantly different. Unless post hoc test was used, a *P*-value ≤ .05 was considered as statistically significant. Association among MMP-2 activity, expression, GSH, and antioxidant activity was carried out with Spearman's rank test. All statistics was carried out with SPSS 12.0 (SPSS Inc. IL, USA).

## Results

### Human study

Eighteen volunteers (six women/12 men; in the age range of 21–65 years old) were recruited. At baseline, subjects were consuming from one to three standard alcohol servings (Ellison et al., 2004) per day (50% wine, 45% beer, and 5% spirits). Compliance with the study was good: no relevant health or diet changes were observed and no changes in smoking habits and drug medication potentially interfering with alcohol occurred. All subjects concluded the protocol. A general description of the study subjects is provided in Table 1.

Gelatinase activity in plasma showed an important increase in the active form of MMP-2 at abstinence (Baseline vs. Abstinence *P* = .002) which reversed at re-exposure (Abstinence vs. Re-exposure *P* = .006, Generalized Linear Model *P* = .016), whereas such patterns were not observed for both MMP-2 proenzyme (proMMP-2) and MMP-9 (Table 2, Fig. 1). An increase in active MMP-2/proMMP-2 ratio was consequently observed at Abstinence; TIMP-1 and TIMP-2 levels did not change significantly

Table 1  
General characteristics of the study population

|                      |                         |
|----------------------|-------------------------|
| Number (M/W)         | 18 (12/6)               |
| Age (years)          | 28 (26–35); range 21–65 |
| Weight (Kg)          | 75 (58.5–90)            |
| Height (cm)          | 175 (169–183)           |
| BMI                  | 23 (20–26); range 18–33 |
| Overweight (BMI >25) | 6                       |
| SBP (mmHg)           | 120 (110–130)           |
| DBP(mmHg)            | 80 (70–85)              |
| Drinks/day           | 2 (1–2.5)               |
| Hypertension         | 1                       |
| Diabetes             | 0                       |
| Current smoker       | 2                       |

Continuous variables are reported as median and interquartile values.

during the observation. A marginally significant increase for AOA, was observed at both abstinence and re-exposure. At a lesser extent, a similar trend could be noticed with glutathione concentration (Table 2). To assess the in vivo relationships between MMP-2 changes and the other variables a multivariate analysis has been carried out considering age, gender, smoking habit, blood pressure, BMI, AOA, and GSH, as independent variables. The active MMP-2 decrease observed during beer consumption (i.e., Abstinence to Re-exposure) has been associated to gender, being higher in men (1,117; interquartile values 251–1,986) than in women (–88; interquartile values –314 to 212,  $P = .024$ ). Although not significant in multivariate analysis, also a positive linear correlation was found between MMP-2 decrease at beer consumption (i.e., Abstinence to Re-exposure) and glutathione at Abstinence (Fig. 2). Under a clinical point of view, also a moderate, but not significant, decrease in blood pressure was observed at abstinence: such decrease was not correlated either to MMPs activity changes in plasma or to antioxidant levels. In our study, we did not find any difference between MMP-2 levels of subjects previously consuming mainly wine or beer, nor between the first and the last observation (beer + wine vs. beer only or among mainly wine drinkers at Baseline and Re-exposure).

Table 2  
Changes in total antioxidant activity and glutathione at the different timepoints

| Zymography (arbitrary units) | Timepoints       |                  |                  | P    |
|------------------------------|------------------|------------------|------------------|------|
|                              | Baseline         | Abstinence       | Re-exposure      |      |
| Pro MMP-2                    | 187 (142–374)    | 159 (140–412)    | 131 (79–377)     | .356 |
| Active MMP-2                 | 1284 (1030–1659) | 2617 (1299–3034) | 1517 (770–2193)  | .016 |
| Pro MMP-9                    | 466 (308–521)    | 769 (400–821)    | 346 (250–444)    | .582 |
| Active MMP-9                 | 710 (260–1313)   | 485 (341–576)    | 438 (319–1048)   | .258 |
| TIMP-1 (ng/ml)               | 84 (72–98)       | 92 (82–98)       | 79 (77–97)       | .328 |
| TIMP-2 (ng/ml)               | 103 (93–137)     | 107 (100–117)    | 104 (95–120)     | .944 |
| Antioxidant( $\mu$ M)        | 0.84 (0.8–1.04)  | 1.09 (0.71–1.17) | 1.07 (0.9–1.27)  | .073 |
| Glutathione ( $\mu$ M)       | 3.46 (3.18–4.74) | 3.79 (3.37–4.07) | 3.87 (3.38–4.29) | .642 |
| Systolic BP (mm/Hg)          | 120 (110–130)    | 117 (110–130)    | 122 (120–130)    | .094 |
| Diastolic BP (mm/Hg)         | 80 (70–85)       | 80 (70–80)       | 85 (80–90)       | .174 |

Significance = Generalized Linear Method, repeated measures for all three timepoints. Continuous variables are reported as median and interquartile values.

### In vitro study

A low grade (~10%) but consistent reduction of MMP-2 activity was observed in vitro at 96 h of incubation with ethanol at 25 mM ( $P = .003$ ; Table 3 and Fig. 3). MMP-2 activity reduction observed at ethanol concentrations of 12.5 or 50 mM was only marginally significant ( $P < .1$ ), whereas at 100 mM it was similar to 0 mM. In all in vitro experiments TIMP-1 and TIMP-2 were undetectable, and plasma incubation with the increasing concentrations of ethanol did not induce any change in MMP-2 activity (data not shown). MMP-2 expression did not show any significant change, compared to 0 mM.

Compared to plasma, very low AOA and GSH could be detected, and no significant/consistent changes were associated with ethanol incubation. More relevant, no correlation could be demonstrated between MMP-2 changes and antioxidation levels in vitro.

### Discussion

This study demonstrates that the moderate consumption of beer or alcoholic beverages in healthy subjects reduces MMP-2 plasma activity, and that an analogous effect is obtained incubating human aortic SMCs with ethanol in concentrations similar to those reached in vivo by moderate drinking (Brunelle et al., 2007; Savola et al., 2004). This in vitro reduction is posttranscriptional and independent from antioxidant activity.

### Human study

Aiming to unravel the mechanisms involved in alcohol related prevention of vascular diseases, the human study was designed to reproduce the pattern of alcohol drinking most likely associated to cardiovascular risk reduction. The amount of alcohol (30 g per day) and the number of drinking days per week closely reflects the drinking pattern of Southern Europe, and is associated in epidemiological studies, to the reduction of cardiovascular risk. Beer, as

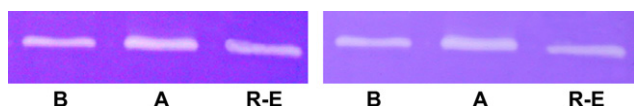


Fig. 1. Typical zymograms of volunteers showing active MMP-2 at Baseline (B), at Abstinence (A), and at Re-Exposure (R-E).

the beverage for re-exposure, was chosen considering that there is not a real advantage of wine over beer in prevention of vascular events, when confounders like socioeconomic and cultural status are considered (Mortensen et al., 2001; Nielsen et al., 2004). To our knowledge, this is the first demonstration that alcohol reduces MMP-2 activity in humans. This relationship between alcohol consumption and changes of MMP-2 in vivo has been demonstrated both after withdrawal and re-exposure, and might be due to a lower activation rate of MMP-2 in presence of ethanol. Of note, subjects were recommended to maintain constant all their own habits, such as diet and physical activity. The comparable MMP-2 activity measured at these two different time points accounts for the liability of the relationship. The present study also highlights the differences with in vitro studies, where a decrease in MMP-9 activity was also reported. Alcohol reduces intimal hyperplasia (Liu et al., 1996, 1997), SMC migration (Hendrickson et al., 1999), and acts on phenotype of SMC (Liu et al., 1996). Experimental evidence of MMP-2 role in vascular remodeling derives from knockout mice: double (i.e., MMP-2 and ApoE) knockout mice show a significant reduction in atherosclerotic burden and SMC accumulation, compared to single (apoE) knockout (Kuzuya et al., 2006). It seems plausible that the interplay between alcohol and MMP-2, both influencing SMC motility, can determine vascular remodeling and plaque progression in drinkers. This alcohol dependent reduction of MMP-2, could represent a driving force in protection from atherosclerosis although, in vivo, a role of ethanol-dependent inhibition of MMP-2 leading to changes in vascular remodeling is still missing.

*In vitro study*

The vascular cells producing MMP-2 are mainly SMC, therefore, in vitro replica of the human study could provide some interesting insights on the relationship between alcohol and MMP-2, beyond the substantiation of the alcohol effect on the whole vascular system.

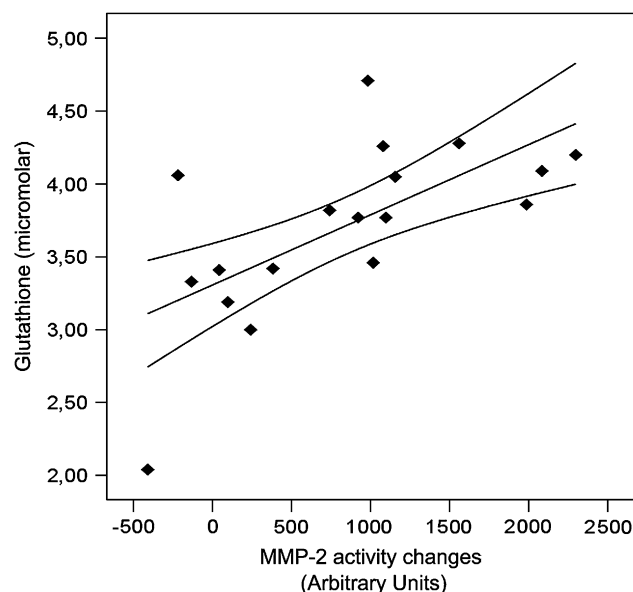


Fig. 2. Univariate analysis of active MMP-2 decrease and glutathione at Abstinence (Spearman's rho value 0.674,  $P = .002$ ).

The first is that alcohol itself can mimic the pattern of MMP-2 inhibition observed with alcoholic beverages in humans. In another study, a reduction of MMP-2 was obtained incubating animal homocysteine-stimulated SMCs with wine (Guo et al., 2007). The authors attributed such effect to Chinese yellow wine, but our experience suggests that ethanol itself can induce the same effect in unstimulated cells. The second evidence is that this effect was obtained independently of the well-known mechanisms alleged to prevent atherosclerosis effects in vivo. Epidemiological studies demonstrated that alcohol can prevent coronary heart disease; allegedly through the increase of HDL cholesterol plasma levels, endothelial cell fibrinolysis, nitric oxide synthesis, in antioxidant activity and the reduction of fibrinogen, platelet reactivity, advanced glycation products, and insulin resistance (Belleville, 2002; Szmítko & Verma, 2005; Vasdev et al., 2006). One or more, of these mechanisms might also account for MMP-2 reduction. Our in vitro results were obtained in serum free media, therefore, without any influence of circulating factors like lipids, nitric oxide, growth and coagulation factors. A reasonable doubt could be put forward for antioxidant level, provided the evidence that isolated SMC still retain the

Table 3

Ethanol and smooth muscle cell culture: MMP-2 activity and expression, antioxidant activity ( $\mu\text{M}$ ), and glutathione ( $\mu\text{M}$ )

|   | Ethanol concentration (mM) |                     |                     |                     |                     |
|---|----------------------------|---------------------|---------------------|---------------------|---------------------|
|   | 0                          | 12.5                | 25                  | 50                  | 100                 |
| MMP-2 activity (%)                        | 100                        | 92 (87–106)         | 88 (82–93)          | 91 (84–99)          | 92 (89–105)         |
| MMP-2 expression<br>(relative to 28S RNA) | 1                          | 1.08 (.97–1.19)     | 1.35 (1.34–1.36)    | 1.17 (.97–1.37)     | 1.17 (1.13–1.22)    |
| Antioxidant activity ( $\mu\text{M}$ )    | 0.010 (0.005–0.016)        | 0.015 (0.011–0.032) | 0.007 (0.004–0.009) | 0.019 (0.016–0.026) | 0.013 (0.011–0.017) |
| Glutathione ( $\mu\text{M}$ )             | 0.016 (0.0–0.06)           | 0.002 (0.01–0.03)   | 0.02 (0.015–0.027)  | 0.01 (0.005–0.026)  | 0.01 (0.008–0.01)   |

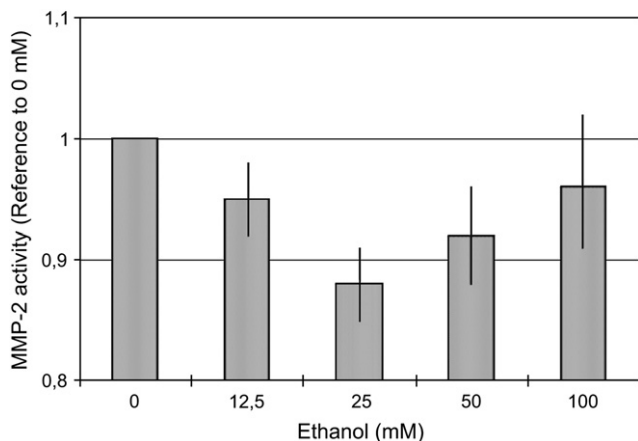


Fig. 3. Relative reduction of MMP-2 activity in surnatant of smooth muscle cells compared to 0 mg/mM. Data reported are median ( $\pm$  SD). The results derive from at least 6 different experiments for each concentration.

possibility to influence their antioxidant activities (Wassmann et al., 2006). Antioxidant activity observed in our cell cultures was very low and unrelated to MMP-2 activity changes. From an epidemiologic perspective, however, antioxidant activity plays a minor role in cardiovascular protection: alcoholic beverages consumption consistently reduced cardiovascular mortality whereas antioxidant therapy failed to achieve such a goal (Hercberg et al., 2004). In conclusion, this mechanism can be observed without many of the alleged alcohol dependent factors protecting from atherosclerosis. A third suggestion derives from the doses of ethanol required for MMP-2 reduction. In our study, low ethanol doses have definite effect, whereas the highest ethanol concentrations (100 mM) did not reduce MMP-2 activity, the final picture resembling a J-shaped curve. This evidence is in line with the reduced in vivo cardiovascular risk only for moderate alcohol intake and, in vitro, with an increased MMP-2 expression in cancer cells incubated with high ethanol concentration (Luo, 2006). A further support to the uniqueness and novelty of this effect is that simple incubation with increasing ethanol concentrations for up to 4 h did not change the MMP-2 activity in plasma or media (data not reported).

The last result, that MMP-2 reduction is not due to reduced gene expression, is in line with the reduced activation rate observed in vivo and has further support in the evidence that MMP-2 is constitutionally expressed and no inhibitory elements have been detected, so far, in its promoter. However, the mechanism(s) accounting for alcohol-dependent posttranscriptional negative regulation are still speculative. Alcohol has the capacity to influence expression of many genes (Uddin & Singh, 2006), but it is unknown if MMP-2 is among them. Such downregulation is also invoked for other genes (Wilke et al., 1994), particularly in those related to GABA receptors, involved in tolerance to alcohol and benzodiazepines (Klein & Harris, 1996), but the exact mechanisms still remain speculative.

In conclusion, it is becoming more clear that alcohol might prevent vascular diseases in humans through modification of vascular remodeling. It is desirable that this effect can be reproduced by other, less toxic, compounds leading to a more effective management of atherosclerosis.

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